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How many endomyocardial biopsies are necessary in the first year after heart transplantation?

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Abstract Since 1989, the immunosuppressive regimen used in all heart transplant (HTx) patients at our center has consisted of a combination of cyclosporin, azathioprine, and prednisone. No prophylactic cytolytic agents have been given. One hundred consecutive patients were followed for periods of 4–56 months (mean 27 months). The incidence of rejection was so low in the initial 18 patients that we felt confident about reducing the number of routine endomyocardial biopsies (EMBs) that were performed. The mean number of EMBs in this subgroup was 10 (median 11). In the next 20 patients, EMB was performed routinely on only three occasions during the 1st post-transplant year (at 2, 4, and 8 weeks). In the subsequent 62 patients, EMB was performed on post-transplant days 10, 20, 30, and 60. Further EMBs were performed after acute rejection episodes had been treated. No noninvasive methods of diagnosing rejection were employed. In 82 consecutive patients, therefore, the mean number of EMBs within the 1st year was five per patient (median four), with 58 % undergoing fewer than five EMBs and 25 % requiring more than five EMBs. In the entire group of 100 patients, the mean number of EMBs was 5.9. The incidence of acute rejection requiring increased therapy was 24 %. Only 7 % required i. v. steroids, two of whom (2 %) also required ALG and/or OKT3, with 17 % requiring increased oral immunosuppression alone. Actuarial survival was 98 % at 30 days, 94 % at 1 year, and 92 % at 2 years. It is possible that we may have missed acute rejection episodes that resolved spontaneously. However, the excellent medium-term results would suggest that any such rejection episode did not progress to become hemodynamically significant. It may be, therefore, that when an effective immunosuppressive regimen is utilized, the number of EMBs performed at many centers is excessive.

Key words Heart transplantation, endomyocardial biopsy · Biopsy, endomyocardial · Endomyocardial biopsy, heart transplantation

Introduction

Endomyocardial biopsy (EMB) remains the most reliable and widely used method of diagnosing acute rejection in patients with heart transplants (HTx). At most centers, EMB is electively carried out at frequent intervals during the first 3-month period and less frequently thereafter. It is not unusual for a patient to undergo 7–12 EMBs during the 1st post-transplant year.

Until April 1990, our own policy was for patients to undergo EMB at weekly intervals for the 1st month, at biweekly intervals for the 2nd month, and then elec-

Table 1 Demographic data of 100 consecutive patients undergoing heart transplantation

Patients (*n*)	100
Sex	
Male	81
Female	19
Age (years)	
Mean	50
Range	15–68
Underlying cardiac pathology	
Ischemic	58
Idiopathic	39
Other	3
Unstable patients (in ICU at time of transplant)	
UNOS Status I	41
UNOS Status II	59
Pretransplant serum creatinine (mg/dl)	
Mean	1.3
Range	0.7–3.4
Length of post-transplant hospital stay (days)	
Mean	10
Range	4–32
Period of follow-up (months)	
Mean	27
Range	4–56

tively until 6 months had elapsed. Thereafter, no EMB was performed until the end of the 1st post-transplant year. This resulted in a total of 6–10 EMBs being performed electively during the first 6-month period.

In June 1989, however, we modified our immunosuppressive regimen significantly and began to follow a triple-drug regimen that had initially been utilized by the University of Minnesota group with excellent results [1, 3, 4]. Once we had experience with this regimen (in the first 18 patients), we made the decision to reduce the number of EMBs significantly, partly since patients generally do not like undergoing these procedures and partly to reduce the cost of HTx.

Initially, we decided to carry out EMBs only at 2, 4, and 8 weeks post-transplant and then not again until the end of the 1st year, totaling only three EMBs within the first 12-month period. One of our early patients, however, developed severe acute cellular rejection that was hemodynamically significant during the 3rd week, despite the absence of acute rejection on EMB at 2 weeks. Following this experience, we increased the number of biopsies so that EMBs were performed at 10, 20, 30, and 60 days post-transplant.

If all biopsies were negative for rejection, no further biopsy was performed until the end of the 1st year. If any biopsy had been positive for acute rejection, however, a further EMB was performed at 12 weeks. The total number of biopsies in these 82 consecutive patients, therefore, varied between three and five unless the development of severe acute rejection necessitated additional EMBs.

We report here on our experience with 100 consecutive patients who underwent orthotopic HTx at our center between June 1989 and November 1993, all of whom received induction and maintenance therapy with cyclosporin (CyA), azathioprine (AZA), and corticosteroids, without any prophylactic use of cytolytic agents. We have particularly reviewed (1) the number of EMBs performed and (2) the incidence of acute rejection requiring increased immunosuppressive treatment during the 1st post-transplant year.

Patients and methods

Patients

HTx was performed in 100 consecutive patients between June 1989 and November 1993. Demographic data are presented in Table 1. The period of follow-up – until March 1994 – ranged from 4 to 56 months (mean 27 months). Eighty-three patients were followed up for at least 1 year. Forty-one percent of the patients were in our intensive care unit at the time of transplantation receiving continuous supportive therapy in the form of inotropic agents ± intra-aortic balloon pump ± positive pressure ventilation [United Network for Organ Sharing (UNOS) status I].

Immunosuppressive therapy

All patients received a combination of CyA, AZA, and corticosteroids with no cytolytic agents. This regimen was based on that reported by the University of Minnesota [1, 3, 4].

CsA was begun before transplantation, as soon as a donor became available. The loading dose was 2–6 mg/kg, depending on the current renal function (serum creatinine) of the recipient. After HTx, a continuous infusion of intravenous (i.v.) CsA was begun, the rate of infusion (1–3 mg/h) again being modified depending on the patient's serum creatinine. The i.v. infusion was discontinued (within 2–4 days) when adequate whole blood trough levels could be sustained by oral CsA alone. After HTx, the daily oral (p.o.) dose consisted of the loading dose divided into two equal doses, the first of these being given (via a nasogastric tube) approximately 12 h after the loading dose. As the i.v. dose was reduced, the daily p.o. dose was modified to maintain a whole blood trough level of 300 ng/ml, as measured by monoclonal radioimmunoassay (INCSTAR). By 6 months, this blood level was allowed to fall to 250 ng/ml, and by 1 year to 150 ng/ml, after which it was maintained indefinitely at 125–150 ng/ml.

Mean creatinine levels were 1.3 mg/dl pretransplant, 1.4 mg/dl on day 7, 1.5 mg/dl at 30 days, and 1.8 mg/dl after 2 years [7]. Only one patient required temporary hemodialysis.

AZA was given p.o. in a loading dose of 2.5 mg/kg as soon as a donor became available and subsequently at 2.5 mg/kg per day as a single i.v. or p.o. dose. The dosage was not increased if the white blood cell (WBC) count remained high but was reduced or withdrawn if the WBC count fell below 5000 cells/mm^3 or if features of hepatic dysfunction developed.

Methylprednisolone (MP; 500 mg i.v.) was given during the operation and continued thereafter at a dose of 125 mg i.v. every 8 h for 24 h (a total of three doses). On the 2nd post-transplant day,

Table 2 Survival and incidence of acute rejection in 100 consecutive patients undergoing heart transplantation

	% of Patients
Actuarial survival	
Operative	99 %
30 days	98 %
1 year	94 %
2 years	92 %
Incidence of acute rejection requiring increased therapy	
Treated with increased oral steroids	17 %
Treated with i. v. steroids	5 %
Treated with i. v. steroids, ALG, and/or OKT3	2 %
Total	24 %

prednisone p. o. (or the equivalent dose of MP i. v.) was given at 1 mg/kg per day, divided into four equal doses. This dose was reduced at fixed intervals until a maintenance dose of 0.15 mg/kg per day was achieved at 6 months and of 0.1 mg/kg per day at 12 months. Only occasionally, and for exceptional reasons, were steroids eventually withdrawn from certain patients.

Diagnosis of acute rejection

In the initial 18 patients, EMB was performed according to our original protocol, with EMBs weekly for the 1st month, twice during the 2nd month, and then electively until 6 months had elapsed post-HTx.

In the next 20 patients, EMB was performed electively on only three occasions (at 2, 4, and 8 weeks). In the final 62 consecutive patients, EMB was performed on four occasions (at 10, 20, 30, and 60 days). A further EMB was carried out at 12 weeks in those patients who had required any increased immunosuppressive therapy during the first 8 weeks. Thereafter, EMB was performed annually in all patients. If acute rejection was suspected at any time on clinical grounds, an additional EMB was carried out.

No noninvasive methods of diagnosing rejection were employed.

Acute rejection was graded as recommended in the "Standardization of Nomenclature in the Diagnosis of Heart Rejection" [2].

Treatment of acute rejection

No increased immunosuppressive therapy was given unless acute rejection had been confirmed by histological examination of an EMB. We did not generally give increased therapy to patients with rejection of grades IA or IB (unless persistent or recurrent), though attention was paid to ensure the CyA dosage was sufficient to maintain the desired blood level. In some cases, a follow-up EMB was performed within 7–14 days. Grade 2 rejection (and some grade 3 A) was treated with a course of increased oral corticosteroids (prednisone, 125 mg daily × 3). Acute rejection episodes of grades 3 A and 3 B were treated with i. v. MP (250–1000 mg daily for 3 days, depending on the severity). In cases of persistent moderate rejection (confirmed by subsequent EMB), a further course of i. v. MP was administered with or without Minnesota antilymphocyte globulin (ALG). In severe rejection (grade 4), the course of MP was given together with a course of ALG. If this failed to reverse the rejection episode, a course of OKT3 was administered.

Adjunctive therapy

As prophylaxis against cytomegalovirus (CMV) infection [6], all patients, irrespective of recipient or donor CMV serology, received acyclovir p. o. at a dose of approximately 50 mg/kg per day (800 mg t. i. d. or q. i. d.); this was begun as soon as they started taking oral medication and continued for 3 months. In addition, the patients received commercially available i. v. gamma globulin (Sandoglobulin, Sandoz; Gamimune, Cutter; or Gamma-R, Armour, depending on availability in the hospital pharmacy) at a dose of 500 mg/kg on days 7 and 35 following HTx. (Details of patient CMV serological status and the incidence of CMV disease post-transplantation have been reported previously [6].)

All patients also received daily Bactrim (trimethoprim 160 mg, sulfamethoxazole 800 mg); this was begun as soon as they started taking p. o. medication and continued indefinitely (as prophylaxis against Pneumocystis pneumoniae) [6]. The dose and/or frequency of administration were reduced in patients with renal dysfunction.

Antihypertensive and cholesterol-lowering agents were given when indicated. The choice of antihypertensive agent was largely that of the transplant physician or cardiologist; we had no policy for prescribing calcium antagonists, such as diltiazem, that have been demonstrated to protect the kidneys from the toxic effect of CyA. Lovastatin, 20 mg daily or twice daily, was the therapy of choice for increased serum cholesterol levels that were not controlled by diet alone. In addition, all patients received certain dietary supplements, such as calcium and magnesium.

Results

Patient survival

Actuarial survival (Kaplan-Meier) of the 100 consecutive patients was 98 % at 30 days, 94 % at 1 year, and 92 % at 2 years (Table 2). At the end of the study period, 93 patients remained alive and well. The overall mortality was, therefore, 7 %.

The causes of death are listed in Table 3. EMB-proven acute rejection was not a cause of any death, although one late death (at 15 months) was related to cardiac failure of uncertain cause (Table 3). Infection accounted for three deaths, one of which was the direct consequence of the need for increased immunosuppressive therapy in the one patient who developed EMB-proven, severe acute rejection in this series.

If the three early deaths that were related to donor heart dysfunction or technical problems at the time of transplantation are excluded, there were no deaths in the first subgroup of 18 patients who underwent a mean of ten EMBs, and there were four deaths (of 82 patients) in the subgroup that underwent a mean of only five EMBs. (In view of the small numbers in the first subgroup, there is no significant difference between these mortality rates.) One of these four deaths was directly related to increased treatment for severe acute rejection, and one other was from late myocardial dysfunction of unknown cause. The remaining two deaths were from infection (Table 3).

Table 3 Causes of death in 100 consecutive patients undergoing heart transplantation

Cause	Post-transplant survival
1. Donor heart failure/recipient raised pulmonary vascular resistance	Operative death
2. Donor heart dysfunction	2 days
3. Hemorrhagic necrosis of the liver (ischemic injury)	33 days
4. *Aspergillus* septicemia (following therapy for severe acute rejection)	10 weeks
5. Atypical mycobacterial pulmonary infection	13 weeks
6. Disseminated cytomegalovirus infection	7 months
7. Myocardial dysfunction (uncertain cause)[a]	15 months

[a] Neither acute rejection (on repeated EMB) nor graft atherosclerosis (on coronary arteriography) could be confirmed. Autopsy was not obtained

Incidence of acute rejection

Acute rejection that required additional therapy (grades 2–4) was histologically confirmed in 24 patients (24 %; Table 2). Seventeen patients (17 %) were adequately treated with increased oral immunosuppression using prednisone, with evidence of resolution of acute rejection on follow-up EMB. Acute rejection was more severe in seven patients (7 %) and required the administration of i.v. MP; one of these patients also received ALG. Only one patient with severe (grade 4) rejection and hemodynamic instability required the combined i.v. administration of steroids, ALG, and OKT3. No patient died of EMB-proven acute rejection (Table 3).

Number of EMBs performed in the first year

Five patients died within the first postoperative 4 months (Table 3) and have been excluded. The maximum number of biopsies in any one of these patients was five (patient 5, Table 3). Seventy-eight of the remaining 95 patients have been followed for more than 1 year, with a minimum follow-up in the remaining 17 of 4 months.

The mean number of EMBs performed in the first subgroup of 18 patients (using our original EMB protocol) was 10. In the remaining patients, the mean number of EMBs performed in each patient within the first 12-month post-HTx period was 5 (median 4), with a minimum of 3 EMBs in six patients and a maximum of 13 in one patient. Only 25 % of patients required more than 5 EMBs with 58 % undergoing fewer than 5.

Discussion

Good patient survival (92 % at 2 years) and the low incidence of acute rejection episodes requiring increased therapy (24 %) clearly demonstrate the success of the triple immunosuppressive therapy regimen used in this group of patients. This experience supports that of the University of Minnesota group who introduced this immunosuppressive regimen [1, 3].

Early experience with CsA in clinical organ transplantation suggested that it is most efficacious when begun pretransplant [10]. In view of its potential nephrotoxic effect, however, many groups withhold it from HTx patients, both pretransplant and for the first few post-transplant days, until hemodynamic stability has been achieved and renal perfusion has been stabilized. Our experience, however, confirms that CyA can safely be given in the perioperative period without significant persisting renal dysfunction (data not shown).

The low incidence of rejection may be related in part to factors other than the immunosuppressive regimen used. By successfully reducing the incidence of CMV infection with acyclovir and gamma globulin [6], this may indirectly have reduced rejection by preventing upregulation of MHC antigens by the virus. Immunoglobulin may have other hitherto unknown or poorly understood effects that may be of importance in preventing an immune response. Recently, for example, it has been demonstrated that the administration of immunoglobulin can result in a fall in panel reactive alloantibodies [9] and can delay hyperacute rejection of discordant xenografts [5, 8].

Once confidence had been gained with this immunosuppressive program, patients underwent an average of only five EMBs during the 1st post-transplant year, which represents a considerable saving in costs, as well as an increase in the level of patient comfort. On the basis of the findings of this study, it could clearly be argued that the performance of an EMB at yearly intervals is unnecessary, particularly after the 1st year, unless the immunosuppressive regimen is likely to be modified significantly after this time.

It could be argued that the low incidence of acute rejection was directly related to the small number of EMBs that were performed during the first 6 months post-transplant. By not performing EMBs at frequent intervals, we may have missed acute rejection episodes that spontaneously resolved. Although we believe this is unlikely, the excellent medium-term results in this series would suggest that (1) any such rejection episodes did not progress to become hemodynamically significant and (2) the patient may actually have benefitted from the fact that the rejection episode remained undiagnosed and, therefore, the patient was not overimmunosuppressed, as suggested by the low incidence of infection in this series (data not presented). We believe

we can conclude that, given the efficacy of our management regimen, neither morbidity nor mortality appeared to be increased by not treating any possible acute rejection that spontaneously resolved. Whether there would be any difference in long-term cardiac function in patients in whom a mild acute rejection episode(s) was not detected remains to be ascertained. It may be, therefore, that the number of EMBs performed at most centers is excessive.

We believe we have demonstrated that a low incidence of acute rejection requiring increased immunosuppressive therapy can be achieved using induction immunosuppressive therapy with CyA without a high incidence of renal failure or infection. This has enabled us to reduce the number of EMBs significantly, thus reducing patient morbidity, discomfort, and cost. We would, therefore, strongly recommend this regimen to other centers, particularly those beginning new heart transplant programs.

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