Clinical experience in the management of pulmonary opportunist infection and rejection in recipients of heart-lung transplants

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ABSTRACT The main clinical problems that follow heart-lung transplantation are opportunist infections of the lungs and pulmonary rejection. Of 23 patients undergoing heart-lung transplantation, eight had opportunist infections and 12 had at least one episode of pulmonary rejection. Cardiac rejection occurred in only one patient, who did not need treatment. Of the 12 patients who had pulmonary rejection, nine recovered fully after augmented immunosuppression with high dose corticosteroids, although one patient required additional low dose corticosteroids for eight months before making a full recovery. Fatal opportunist lung infection followed treatment for rejection in two patients. One patient developed obliterative bronchiolitis. Of the eight patients with opportunist infections, five had primary cytomegalovirus pneumonitis, acquired from the donor. All three patients treated with acyclovir died, whereas the two treated with hyperimmune globulin and dihydroxy proxymethylguanine recovered fully. Two patients developed *Pneumoncystis carinii* pneumonia, which was treated successfully in one patient with intravenous sulphadimidine and trimethoprim. The other patient died after a further episode of rejection and aspergillus bronchitis. One patient developed a tuberculous empyema. The calculated actuarial survival at one year was 78% and at two years 67·2%. Although it is still in its innovative stage heart-lung transplantation appears to have complications and results similar to those of transplantation of other organs.

Introduction

Successful human heart and lung transplantation with cyclosporin to produce immunosuppression was first reported in 1982.[1] More than 300 operations had been performed worldwide by 1986.[2] Three main complications have been reported in long term survivors of the operation: opportunist infection of the lungs, acute pulmonary rejection, and chronic disabling obliterative bronchiolitis.[3][4] Infection is common and an important cause of early death.[4] The diagnosis and management of acute rejection had caused difficulty until recently because of the lack of a reliable diagnostic test,[5] but transbronchial biopsy has been shown to be adequate for histological diagnosis.[6]

Clinical and experimental work suggests that obliterative bronchiolitis is related to chronic rejection[5][7] and its incidence has fallen with greater experience in the regulation of immunotherapy.[4] We report our experience of these problems in 23 patients and describe our current management strategy for both opportunist infection and pulmonary rejection.

Patients and methods

Twenty three patients underwent heart-lung transplantation at Papworth Hospital from April 1984 to April 1987; one patient also received a liver transplant. The original diagnoses are shown in table 1 and details of perioperative and maintenance immunosuppression in table 2. The criteria for patient and donor selection and the operative procedure have been previously reported.[8][9] Antibiotic prophylaxis consisted of intravenous flucloxacillin (500 mg six hourly) and ceftazidime (1 g eight hourly) given at induction of

Table 1 *Diagnoses of 23 patients with heart-lung transplants*

Diagnosis	No of patients
Primary pulmonary hypertension	2
Pulmonary hypertension secondary to emboli	3
Eisenmenger's syndrome	9
Primary pulmonary hypertension and primary biliary cirrhosis	1
Emphysema	2
Sarcoidosis and pulmonary hypertension	2
Histiocytosis X	1
Cystic fibrosis	1
Bronchiectasis	1
Cryptogenic fibrosing alveolitis	1
Total	23

anaesthesia and for 48 hours postoperatively. Patients remained in intensive care during antithymocyte globulin treatment for an average of five days. They were discharged from the ward to a hospital flat in the third or fourth week after the operation and seen three times a week for a further fortnight before returning home. They were then seen every two weeks up to three months, every three months up to one year and six monthly thereafter.

POSTOPERATIVE ASSESSMENT
At routine follow up an electrocardiogram and chest radiograph were obtained. Pulmonary function assessment consisted of measurement of dynamic lung volumes (one second forced expiratory volume (FEV_1) and forced vital capacity (FVC), total lung capacity (TLC) measured by whole body plethysmography, and single breath gas transfer for carbon monoxide ($TLCO$)).

Cyclosporin concentrations were assayed in whole blood and plasma ("Cyclotrak" radioimmunoassay, Immunonuclear). Blood urea and serum creatinine concentrations and full blood counts were performed to monitor the effects of cyclosporin and azathioprine. Blood glucose and fasting lipids were also measured.

Serological tests for cytomegalovirus and toxoplasma were performed at intervals. Antibody to cytomegalovirus was detected by a complement fixation test and a competitive ELISA method.[10] Primary cytomegalovirus infection was diagnosed in patients who developed appreciable titres (a greater than fourfold rise in titre according to the complement fixation test or more than 50% inhibition in competitive ELISA), having had no detectable serum antibody before transplantation. Reactivation of cytomegalovirus infection was defined by a greater than fourfold rise in cytomegalovirus antibody according to the complement fixation test. In all cases infection was confirmed by detection of cytomegalovirus specific IgM by the μ capture ELISA method of Wreghitt *et al.*[11]

Routine endomyocardial biopsies were performed initially,[12] but this practice was discontinued in May 1987. It has been replaced by bronchoscopy and transbronchial lung biopsies, which are carried out routinely at four monthly intervals and then annually.

Further investigations were performed if patients complained of a cough, breathlessness, malaise, or fever, or had crackles or wheeze on auscultation of the chest. Abnormalities on routine chest radiographs and falls of 15% or more in FEV_1 or FVC from previous values were also used as indications for further investigations. Blood, sputum, and urine cultures were then performed together with serological tests for cytomegalovirus, respiratory viruses, and toxoplasmosis. Fibreoptic bronchoscopy was performed by standard techniques and transbronchial biopsy samples were taken with "alligator" forceps (Olympus, Keymed, Maidstone). Four to six samples were taken from the area of maximum radiographic shadowing if present, or from basal segments of one lower lobe.

Besides routine histological preparations, biopsy samples were fixed in formalin and stained for fungi and *Pneumocystis carinii*. Mycobacterial stains were performed if indicated clinically. Bronchial washings were cultured for fungi, mycobacteria, and bacterial pathogens. Culture of washings and biopsy material for viruses was not attempted for logistic reasons.

Pulmonary rejection was diagnosed histologically from transbronchial biopsy specimens.[6] *Pneumocystis carinii* pneumonia was diagnosed by the presence of a foamy exudate containing silver staining cysts, and cytomegalovirus pneumonitis was diagnosed histologically from the presence of typical inclusion bodies or on the basis of changes suggesting a "viral"

Table 2 *Immunosuppressive protocol used for patients with heart-lung transplants*

Drug	Perioperative dose	Maintenance dose
Cyclosporin	5–10 mg/kg daily	5–10 mg/kg^{-1} day^{-1}†
Methylprednisolone	500 mg IV × 2 125 mg IV ×3	— —
Equine antithymocytic globulin	5 mg/kg daily* (for 5 days)	—
Prednisolone	—	Started on 2nd perioperative day at 0·5 mg/kg daily, reducing to 0·2 mg/kg at 2 weeks; usually stopped at 1 month
Azathioprine	—	1–2 mg/kg daily after 1 month‡

*Dose adjusted to reduce the T cell fraction of the total lymphocyte count to approximately 10%.
†Cyclosporin dose adjusted according to renal function and plasma trough concentration (200–500 ng/ml).
‡Azathioprine dose adjusted to give a white cell count of 5·0 × 10^9/l.
IV—intravenously.

alveolitis in conjunction with a fourfold rise in cytomegalovirus IgM antibody. In all patients with cytomegalovirus pneumonitis the diagnosis was confirmed by the presence of inclusion bodies in repeated biopsy samples or by the histological findings at necropsy.

TREATMENT

During episodes of rejection patients were given daily intravenous boluses of 0·5 g methyl prednisolone for three days and oral prednisolone 1 mg/kg daily reducing to zero over a two week period. One patient was treated with 10 mg of prednisolone daily for eight months. *Pneumocystis carinii* pneumonia was treated with trimethoprim 15 mg/kg per day and sulphadimidine 2 g four times daily intravenously for 10 days followed by oral co-trimoxazole 3840 mg (Septrin eight tablets) daily for four weeks.[13]

The first three patients with cytomegalovirus pneumonia were treated with intravenous acyclovir. From September 1986 patients who were known to be cytomegalovirus "mismatches" (that is, seronegative recipients with organs from seropositive donors) were treated prophylactically with hyperimmune globulin postoperatively. They received a weekly intramuscular injection of cytomegalovirus specific gammaglobulin until they either developed disease, seroconverted without apparent disease, or were finally discharged from hospital. It was known whether the donor was cytomegalovirus seropositive within 48 hours of surgery and the first dose was given at that time. Cytomegalovirus negative blood products were not available for use in these patients as this diagnosis was made only after surgery. Later patients with cytomegalovirus pneumonitis received intravenous DHPG (9(1,3-dihydroxy-2-propoxymethyl) guanine) at a dose of 5 mg/kg for 14 days (Wellcome Foundation, Crewe).

Results

SURVIVAL AFTER TRANSPLANTATION

There were no operative deaths. Two early deaths occurred, one from tracheal dehiscence after 44 days

Table 3 *Opportunist infections in 23 patients with heart-lung transplants*

Infection	No of patients	No of deaths
Primary cytomegalovirus infection		
Ileum and stomach	1*	0
Lung	5	3
Pneumocystis carinii pneumonia	2	1
Tuberculous empyema	1*	0

*Same patient.

and one from cerebral infarction after 12 days. Three of the 23 patients died from primary cytomegalovirus pneumonitis in the first month. Eighteen patients were discharged from hospital, and 16 are well two months to three years after transplantation. One patient died at 14 months of respiratory failure after several rejection episodes and severe *Pneumocystis carinii* pneumonia. At necropsy there was evidence of obliterative bronchiolitis and pulmonary fibrosis, some rejection, and bronchial infection with *Aspergillus fumigatus*. One patient died 30 months after transplantation from obliterative bronchiolitis. The calculated actuarial survival of the 23 patients to one year was 78% and to two years 67·2%.

OPPORTUNISTIC INFECTION

Eight of the 23 patients developed pulmonary opportunist infection (table 3). One had a tuberculous empyema 18 months after transplantation, which responded to treatment with three antituberculous drugs. The patient had been treated for suspected tuberculosis in 1971 and had received long term corticosteroids for fibrosing alveolitis before transplantation in 1984. Two patients developed *Pneumocystis carinii* pneumonia at five and 12 months after operation. Both had had episodes of pulmonary rejection in the previous three months and had augmented immunosuppressive treatment (corticosteroids).

Nine of the 23 transplant recipients were seronegative for cytomegalovirus before operation and received organs from seropositive donors (table 4). One seroconverted without clinical disease and one

Table 4 *Outcome for nine patients with heart-lung transplants who were seronegative for cytomegalovirus (CMV) before operation and received organs from seropositive donors*

Postoperative complication	No of patients	CMV status	Outcome
Primary CMV infection			
Ileum	1	Seroconversion	Survived; well 3 y after transplantation
Lung	5	Seroconversion	3 deaths; 2 patients survived and are well 6 months after transplantation
Obliterative bronchiolitis	1	Seroconversion	Severely disabled; died awaiting retransplantation
None	1	Seroconversion	Well 18 months after transplantation
	1	Seronegative	Well 16 months after transplantation

Table 5 *Features of pulmonary rejection episodes in 12 heart-lung transplant patients*

Patient No*	Time after transplantation	Respiratory symptoms	% change in FEV₁	Chest radiograph abnormal	Transbronchial biopsy confirmed rejection	Outcome
1	21 m	+	−26	−	Not done	Recovered
2	18 m	+	−25	−	+	Recovered
	20 m	+	No change	−	+	(see fig 1)
	23 m	+	No change	−	+	
3	5 m	+	−24	+	+	Obliterative
	7 m	+	No change	−	+	bronchiolitis; died 30 m
6	1 m	+	−36	−	+	Recovered
8	14 m	−	−44	−	+	Recovered
9	3 w	+	−13	+	+	Died of cytomegalovirus infection at 4 w
10	5 m	+	−18	−	+	Died of pulmonary
	5½ m	+	No change	−	+	fibrosis, rejection
	9 m	+	No change	−	+	and *Aspergillus*
	11 m	+	No change	+	+	*fumigatus* bronchitis at 14 m
11	2 m	−	+9	+	Not done	Recovered
15	3 w	+	Not done	+	+	Recovered
		2 m	+	−	+	+
16	6 m	−	+5	−	+	Recovered
18	3 w	+	−33	+	−	Recovered
	5 w	+	No change	+	−	
21	3 w	+	−17	+	+	Recovered

*In order of transplantation.

has remained seronegative. The other seven patients acquired a primary cytomegalovirus infection within three months of transplantation. In one patient, whose infection of the stomach and small bowel was diagnosed by gastric mucosal biopsy, infection resolved without treatment. Five patients developed cytomegalovirus pneumonitis. The first three were treated with acyclovir, the diagnosis having been made by transbronchial biopsy, but all died. In the subsequent two patients cytomegalovirus infection was suspected at an early stage on clinical grounds and because they had seroconverted. Initial transbronchial biopsy samples showed extensive alveolar damage but no inclusion bodies; later biopsy samples contained inclusion bodies. Both patients were treated with DHPG (Wellcome) and recovered fully. The ninth patient seroconverted with minimal symptoms but subsequently had four episodes of pulmonary rejection, which failed to respond to augmented immunosuppression. She developed obliterative bronchiolitis and died 30 months after transplantation.

REJECTION

Twenty episodes of pulmonary rejection were diagnosed in 12 of the 23 patients. Eight episodes occurred in the first three months, but rejection could occur at any time during follow up. On three occasions patients were symptom free; two episodes were diagnosed by "routine" transbronchial biopsy and one because of an abnormal chest radiograph. The other patients complained of cough and breathlessness and there was often a low grade pyrexia. Wheezes were usually heard

on auscultation with late inspiratory crackles. The chest radiograph was abnormal in only eight of the 20 episodes. On five occasions concurrent endomyocardial biopsy samples were normal. Transbronchial biopsy, performed on 18 occasions, confirmed the diagnosis in 16 but there were two false negatives (these two episodes responded to treatment). One patient on one occasion showed evidence of mild to moderate cardiac rejection. The remaining 105 cardiac biopsies yielded normal specimens.

Table 5 gives the clinical details of these episodes of rejection. Pulmonary function was measured at the time of the first episode of rejection in 11 of the 12 patients. No measurements could be made in the other patient, in whom rejection occurred very early after surgery. There was a fall in FEV₁ and FVC in nine of the 11 patients, but only inconsistent changes in gas transfer were observed (table 6).

Of the 12 patients with rejection, two developed opportunist infections and died shortly after being given augmented immunosuppression; one (No 9) died of cytomegalovirus infection one month after

Table 6 *Changes in lung function at the first documented rejection episode in 11 patients with heart-lung transplants*

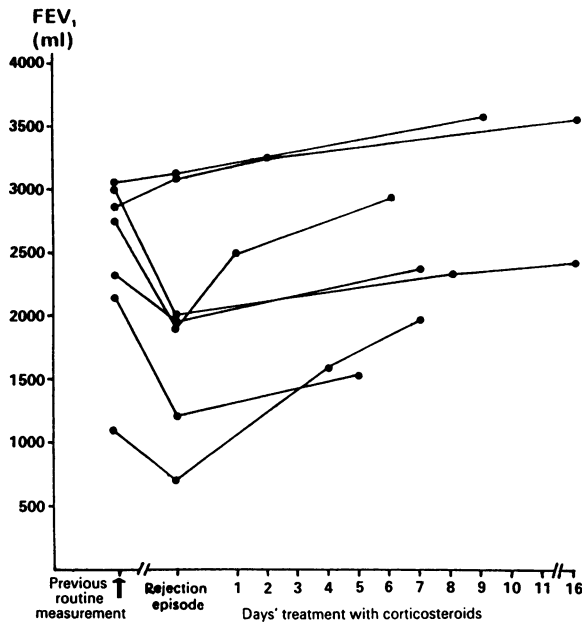| Lung function variable	Mean (SD) change as % of previous routine measurements
FEV₁	−21 (11·9)
FVC	−15·7 (10·3)
RV	+19 (25·6)
TLC	+4·5 (8·7)
KCO	+7·8 (38·5)

FEV₁
(ml)

Fig 1   *Forced expiratory volume in one second (FEV₁) of seven recipients of heart-lung transplants during their first documented episode of pulmonary rejection. The values are the best routine measurement before the episode of rejection, at the time of transbronchial biopsy, and during corticosteroid treatment.*

surgery; and one (No 10) never recovered from *Pneumocystis carinii* pneumonia, dying finally from invasive aspergillosis and obliterative bronchiolitis 14 months after transplantation (table 5). One patient (No 3) had an episode of rejection five months after operation, which initially responded to standard augmented immunosuppression; but she relapsed catastrophically when this was stopped. Her FEV₁ fell from 2·6 to 0·75 l in a few days and her FVC fell from 2·8 to 2·1 l, but there was no change in gas transfer. Further treatment with steroids had only marginal benefit. An open lung biopsy carried out later confirmed obliterative bronchiolitis; the patient died 30 months after transplantation.

Nine of the 12 patients who experienced rejection ultimately recovered after treatment and achieved normal lung function and exercise tolerance. Eight patients had improved considerably within a week (fig 1). One patient (No 2) responded poorly to treatment of a rejection episode 18 months after transplantation and a further biopsy two months later still showed active rejection. Another short course of corticosteroids had little effect. A third biopsy at 23 months showed active rejection, which was treated conventionally, but the patient was then given maintenance prednisolone 10 mg daily for eight months. He

recovered normal lung function and a repeat biopsy showed normal lung tissue.

OTHER HOSPITAL ADMISSIONS
After their initial discharge 10 patients were readmitted to hospital on 20 occasions for problems other than opportunist infection or rejection. On six occasions the problem was not infective; renal colic (1 case), steroid myopathy (1), vaginal bleeding (1), and non-specific abdominal pain and vomiting (3). The other 14 admissions were for infections not usually classified as opportunist. One patient had bilateral otitis media requiring surgical intervention and one a pyelonephrosis that required nephrectomy. The other 12 patients had infections of the respiratory tract, two with lobar pneumonia due to *Streptococcus pneumoniae* treated with intravenous penicillin and one with a mild influenza-like illness and dry cough, presumed to be caused by a virus, which resolved with no treatment. The other nine patients had a productive cough but no change on the radiograph and so were considered to have acute bronchitis. Organisms were grown from the sputum in six cases (*Staphylococcus aureus* (1 case), *H influenzae* 1, *Streptococcus pneumoniae* 4). Routine treatment was intravenous amoxycillin 500 mg 8 hourly with flucloxacillin 500 mg 6 hourly until culture results were available.

Discussion

The initial reports on combined heart-lung transplantation recorded a high perioperative mortality of 21–26%,[3,4] largely due to bleeding from the chest wall. Four of our patients had bleeding that necessitated further surgery, but all survived. Our predicted actuarial survival of 78% at one year compares favourably with that of the Stanford group (71%)[14] and is better than the worldwide experience of 50%.[2]

The most frequent cause of opportunist infection was cytomegalovirus, which resulted in symptomatic illness in six patients (26%). This is similar to the figures of 21% in recipients of heart-lung transplants,[15] and 34% in those receiving a heart transplant alone and those having heart-lung transplants.[16] The latter series, from Dummer *et al*,[16] included eight cases of cytomegalovirus pneumonia and showed a much greater incidence of pneumonia after combined heart-lung transplantation (four of five patients) than after transplantation of the heart only (four of 44 patients). They suggested that pulmonary rejection or greater immunosuppression might predispose to cytomegalovirus infection in the transplanted lung. In contrast to these reports, our patients with primary cytomegalovirus pneumonitis had all acquired it from the donor organs, compared with one out of three in the Standford series[15] and two out of four in the
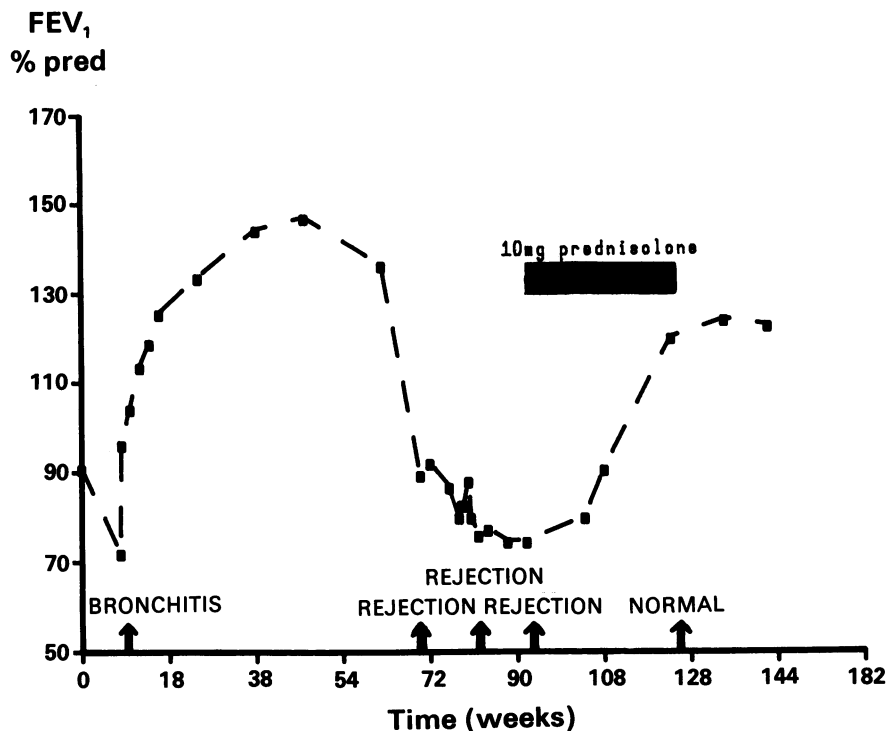
FEV₁
% pred

Fig 2   *Forced expiratory volume in one second (FEV₁) of patient 2, who experienced three episodes of pulmonary rejection and who received prolonged low dose prednisolone (10 mg daily). Arrows indicate transbronchial biopsy and high dose corticosteroids.*

Pittsburgh series.[16] Cytomegalovirus infection from the donor organ has been described in other forms of organ transplantation.[17 18]

We have assumed that the cytomegalovirus infections in these patients were acquired from the donor organs rather than blood products because of the severity of the disease. Unfortunately we do not have sufficient cytomegalovirus negative blood products to cover all operations. We did not know until after the operation and after blood transfusion had taken place whether the donor was cytomegalovirus seropositive. In the larger heart transplant programme at our institution 27% of cytomegalovirus negative recipients receiving organs from cytomegalovirus negative donors have developed primary infection with the virus, presumably from blood products. The disease was much milder in these patients and there were no deaths (TG Wreghitt, personal communication). Possibly blood products were the source of the infection in some of our patients, though none of our four patients who were cytomegalovirus seronegative and received matched negative organs and so were potentially at risk only from blood product infection has so far shown seroconversion.

The problem of cytomegalovirus infection from the donor can be avoided if the donor serotype is known before the operation and seropositive donor organs are used only in seropositive recipients. New, rapid methods for measuring cytomegalovirus antibody are now available[19] and our current practice is to "match" donors and recipients for cytomegalovirus status. When cytomegalovirus "mismatch" became apparent in three patients they were treated with prophylactic hyperimmune globulin after operation, but all developed cytomegalovirus pneumonitis. Experience with hyperimmune globulin in recipients of bone marrow transplants suggests that it is not very effective.[18]

The two most recent patients with cytomegalovirus infection were also treated with the antiviral agent DHPG, which has been shown to have some effect on cytomegalovirus infections in patients with bone marrow transplants and with AIDS.[20] The fact that these patients survived whereas the three treated with acyclovir died also suggests that this agent may be more effective in cytomegalovirus infections than is acyclovir. Dummer *et al*[16] found that acyclovir was ineffective. Cytomegalovirus infection invariably

occurred within the first three months after transplantation, as noted by others, and this offers a useful guide to diagnosis of pulmonary infection early after transplantation.

Pneumocystis carinii pneumonia was the most common infection reported by Griffiths *et al*[4] and these authors suggested that antibiotic prophylaxis should be given to patients undergoing heart-lung transplantation. Prophylaxis has been shown to be very effective in bone marrow transplantation.[21] We have not used co-trimoxazole prophylaxis routinely because of the additional risk of bone marrow suppression in patients also receiving azathioprine. Our two patients developed infection with *Pneumocystis carinii* shortly after a course of augmented immunosuppression and a similar association with immunosuppressive treatment was seen in our patients with heart transplants in 1986.[22] Our practice now is to give co-trimoxazole prophylaxis (Septrin, one tablet daily) selectively to patients who require augmented immunosuppression. The low dose of co-trimoxazole was selected because of the risk of marrow suppression. No cases of pneumocystis pneumonia in patients with either heart-lung or heart transplants have occurred in the six months since this practice was instituted. For acute pneumocystis pneumonia trimethoprim was given with sulphadimidine rather than sulphamethoxazole, to minimise the risk of further renal damage in patients receiving cyclosporin.[13]

Of this small series of 23 patients, 12 had one or more episodes of pulmonary rejection. This is similar to the incidence of rejection in recipients of renal transplants given cyclosporin A and azathioprine.[23] The incidence of pulmonary rejection has not been given in other series,[3 4] largely because of the lack of any method of diagnosis short of an open lung biopsy. It was initially thought that the heart and lungs would be rejected simultaneously, so that endomyocardial biopsy[8 12] could be used to monitor rejection. It is now known that the organs are rejected independently[4 24] and in our experience the lung is the more vulnerable organ. Transbronchial biopsy provides adequate material for diagnosis of rejection based on the histological appearance of perivascular lymphocytic infiltration.[6] We have now abandoned routine cardiac biopsy.

Recipients of transplants have an acute perception of dyspnoea, complaining of breathlessness with quite minor degrees of physiological impairment. In addition, the lung has an advantage over other transplanted organs in that physical signs can be detected and monitored easily. Any patient who develops respiratory symptoms or crackles or wheezes on auscultation undergoes lung function tests followed by bronchoscopy. A reduction in FEV_1 or FVC without symptoms or signs is also an indication for broncho-scopy and transbronchial lung biopsy. Our experience with patient 2 suggests that the treatment of rejection should be more prolonged if a good physiological response is not seen within a week. Careful monitoring of lung function may be used to assess the efficiency of augmented immunosuppression. We no longer give an arbitary course of two weeks of increased corticosteroids. Pulmonary function testing renders the management of heart-lung transplantation similar to that of other organ transplantation.

Obliterative bronchiolitis occurred as the sole pathological feature in only one patient in the present series and only one in that of Griffith *et al*.[4] It was, however, a major complication in the Stanford series, found in half of the patients who survived the operation at a mean of 11 months after surgery.[3] Only seven patients in the present series and eight in the Pittsburgh series have so far survived beyond one year, but from the Stanford experience we would have expected more cases of bronchiolitis. Close monitoring of lung function and the early diagnosis of pulmonary rejection by transbronchial biopsy may have contributed to our low incidence of obliterative bronchiolitis.

In our unit only cytomegalovirus "matches" are now accepted. Patients who experience rejection are treated more aggressively and earlier with longer courses of corticosteroids and they are also given prophylaxis against *Pneumocystis carinii* pneumonia. Heart-lung transplantation is in its infancy but as experience increases the mortality is being reduced.[5 14]

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