Journal of Korean Medical Science
Vol. 8, No. 3, 171-179 June. 1993

Early Allograft Function in Canine Single Lung Transplant

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An assessment of early graft function in canine single lung transplant recipients was made by analysing early postoperative radiographic progression, lung perfusion, bronchial patency and bronchial anastomotic wound healing and histopathology of the allografted lung. Eighteen mongrel dogs weighing 15kg on average were used. Donor lung bloc with a generous atrial cuff, the pulmonary artery and left bronchus were taken and flushed with Euro-Collins solution which implanted in the pneumonectomized recipient dog. Anastomosis was done with the atrium, pulmonary artery and bronchus in that order. To assess an early graft function, a protocol for a grading system was designed into the chest roentgenogram, lung perfusion scan, bronchial patency and histopathologic progression of the bronchial anastomosis and allografted lung (Table 1). The results were obtained as follows: Radiographically, clear to infiltrate was seen in 67% (8/12), 33% (5/15), 30% (3/10) and 33% (2/6) on postoperative day 0, 1, 2 and 3 respectively. Lobar to total opacification was 33% (4/12), 67% (10/15), 70% (7/10) and 67% (4/6) on days 0 to 3 (Table 2). Perfusion scan showed normal to mild defect in 43% (3/7) and moderate to severe defect in 57% (4/7) on day 0 and 100% (5/5) on day 2 (Table 3). The bronchial anastomotic site showed patent to mild stenosis in 100% (8/8) on day 0 and mild stenosis in 2/2 on day 9 bronchofiberscopically, and showed normal wound healing in 38% (3/8), cellular infiltration in 38% (3/8) and infarction in 25% (2/8) up to day 9 postoperatively. Histopathology of the allografted lung showed interstitial edema in 25% (4/16) up to day 3 and cellular infiltration in 19% (3/16) up to day 9 and infarction in 56% (9/16) up to day 9. In this study, radiograph and perfusion scans reflected early progressive deterioration in the allografted lung. Histopathology of the allografted lung showed early graft failure in half of the dogs while the bronchial anastomotic site showed relatively good wound healing.

Key Words: *Single Lung Transplantation, Allograft Function, Canine.*

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Tel: (02) 480-3170 FAX: (02) 473-4672
Supported by a Clinical Research Grant from the Korea Research Foundation. Read at the XIIth Meeting of the Asia Pacific Congress of Disease of the Chest, Seoul, Korea, October 7, 1992.

INTRODUCTION

Currently, single lung transplantation has become a successful therapy for selected patients with end-stage pulmonary disease. In Korea, at present, brain death has not yet been legally determined. However, it looks likely to be defined soon and then many advanced emphysema, COPD, idiopathic pulmonary fibrosis and

pneumoconiosis patients would be candidates for lung transplantation. Now, many of the technical problems have been resolved completely or partially by the pioneers (Cooper et al., 1987), but there still remain several factors including ischemia, reperfusion injury, infection and rejection to be solved. The authors wanted to resort to multiple modes of assessment in the early postoperative period to grade precisely lung allograft dysfunction and to grasp any dilemmas that may arise from the management of the transplant recipients. The purpose of this study is to assess the early graft function in canine single lung transplant recipients by analysing early postoperative radiographic progression, lung perfusion scan, bronchial patency and histopathology of the bronchial anastomosis and allografted lung.

MATERIALS AND METHODS

Eighteen mongrel dogs weighing 13kg to 20kg (average 15kg) were used in this study. Under general endotracheal anesthesia, the chest was entered through a left thoracotomy and then the left lung was pneumonectomized for donor lung with a generous atrial cuff, the pulmonary artery and left bronchus. The donor lung was flushed immediately after removal for about 10-15 minutes with approximately 600cc of 4°C Euro-Collins solution at a pressure of 60cm H_2O via a cannula placed in the left main pulmonary artery. Simultaneously, the recipient dog was also prepared, draped and opened the left chest in the same manner on another operating table. The main pulmonary artery and the base of the pulmonary veins down to the atrial wall were gently dissected and then clamped. The left main bronchus was divided with the inflation of a bronchial blocker (UNIVENT, Fuji System) proximally. Pneumonectomy was done with division of the pulmonary artery and atrium distal to the clamps. The donor lung was put into chest and then the atrial anastomosis was performed, followed by the pulmonary artery and the bronchus subsequently. Atrial and pulmonary artery anastomoses were performed with

Table 1. Grading System for the Assessment of Allografts

Radiographic Progression			
Clear	Infiltrate	Lobar opacification	Total opacification
Lung Perfusion Scan			
Normal (>40%)	Mild defect (40-30%)	Moderate (30-20%)	Severe (<20%)
Bronchial Patency of Anastomotic Site by Video-BFS			
Patent (>75%)	Mild stenosis (75-50%)	Moderate (50-25%)	Severe (<25%)
Histopathologic Observation			
Normal	Interstitial edema	Cellular infiltration	Infarction

BFS: bronchofiberscope

Table 2. Radiographic Progression

CXR/POD	#0	#1	#2	#3	#4	#5-9
Clear	4	3	2			
Infiltrate	4	2	1	2		
Lobar opacification	3	5	4	3	1	1
Total opacification	1	5	3	1	1	1
Total	12	15	10	6	2	2

CXR: chest roentgenogram
POD: postoperative day

4-0 polypropylene running suture, and the bronchial anastomosis with 3-0 or 4-0 vicryl running suture. After bronchial anastomosis, the lung was reexpanded and reperfused. The chest was then closed with sutures in layers after insertion of a chest tube. During the procedures and early postoperative period, electrocardiogram with hemodynamics (Physi-Control VSM 5) and arterial blood gas (NOVA biomedical STAT profile 5) were monitored continuously. Perioperative immunosuppression using cyclosporine (Sandimum®) 15mg/kg/day was administered intravenously on day 0 and 1 and orally thereafter. The longest survival was 12 days postoperatively.

To assess an early graft function in the allograft recipient, the authors designed the grading systems on the following findings: 1. radiographic progression, 2. the amount of lung perfusion, 3. bronchial patency and, 4. histopathologic features of bronchial anastomosis and allografted lung (Table 1).

Radiographic examination was devised as follows: preoperative control and postoperative chest roentgenograms of postero-anterior and lateral view were taken in each dog (Table 2). A lung perfusion scan of the allograft was carried out postoperatively using a Simens Micro-Delta (Table 3). Bronchial patency of the anastomotic site was grossly examined in the dogs at each postoperative day using video-bronchofiberscope (Pentax EPM-3000) (Table 4). Histopathologic examination was done at two sites: one at the bronchial anastomosis with focus on bronchial wound healing by grading normal, ischemia, cellular infiltration and infarction (Table 5). The other was allografted lung parenchyme and graded into four: normal, interstitial edema, cellular infiltration and infarction (Table 6). (An analysis was made according to these grading systems to the whole collected data base practically available.) Each test date and the number of animals tested can be found in Table 2, 3, 4, 5, and 6.

RESULTS

1. Radiographic Progression:

Radiologically, as is shown in Table 2, clear to infiltrate was 67% (8/12) on postoperative day 0, 33% (5/15) on day 1, 30% (3/10) on day 2 and 33% (2/6) on day 3. Lobar to total opacification was 33% (4/12) on day 0, 67% (10/15) on day 1, 70% (7/10) on day

Table 3. Lung Perfusion Scans of Allografts

Perfusion/POD	#0	#2	#4
Normal (>40%)	1 (57%/43%)		
Mild defect (40-30%)	2 (63%/37%) (68%/32%)		
Moderate defect (30-20%)	1 (71%/29%)	1 (73%/27%)	
Severe defect (<20%)	3 (91%/9%) (95%/5%) (98%/2%)	4 (84%/16%) (88%/12%) (98%/2%) (99%/1%)	1 (97%/13%)
Total	7	5	1

Table 4. Bronchial Patency of the Anastomotic Sites

Patency/POD	#0	#2	#3	#9
Patent (>75%)	7			
Mild stenosis (75-50%)	1	1		2
Moderate stenosis (50-25%)			1	
Severe stenosis (<25%)				
Total	8	1	1	2

Table 5. Histopathology of the Bronchial Anastomotic Sites

Histopathology	Postoperative day				
	#1	#2	#3	#4	#9
Normal			1	1	1
Ischemia					
Cellular infiltration		1	1	1	
Infarction		1	1		
Total		2	3	2	1

Table 6. Histopathology of the Allografted Lungs

Histopathology	Postoperative day				
	#1	#2	#3	#4	#5-9
Normal					
Interstitial edema	1		3		
Cellular infiltration			1	1	1
Infarction		3	3	1	2
Total	1	3	7	2	3

2 and 67% (4/6) on day 3. Also, radiographic progression was observed on days 4 to 9, which showed lobar to total opacification (4/4). The illustrating chest roentgenograms of the allografted lung by postoperative day 0, 1, and 2 are shown in Figure 1, 2, 3 in the same dog. A total opacification of the implanted lung in another dog is seen in Figure 4.

2. Perfusion Scan:

Perfusion scan of the allografted lung showed normal to mild defect in 43% (3/7) and moderate to severe defect in 57% (4/7) on day 0 and 100% (5/5) on day 2. As is seen in Table 3, perfusion defect was detected rather earlier up to day 4. The illustrating perfusion scintigrams of the implanted lung by postoperative day 0 are shown in Figure 5 and 6, which were normal to mild perfusion defects. The moderate and severe perfusion defects are seen in Figure 7 and 8.

3. Bronchial Patency:

Bronchial patency of the anastomotic site was observed, using video bronchofiberscopic examination. Patent to mild stenosis was seen in 100% (8/8) on day

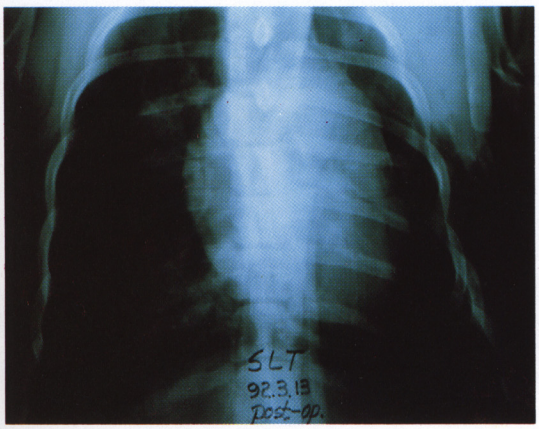

Fig. 1. P-A view of a chest roentgenogram shows a clear in left lung immediately after the allotransplantation.

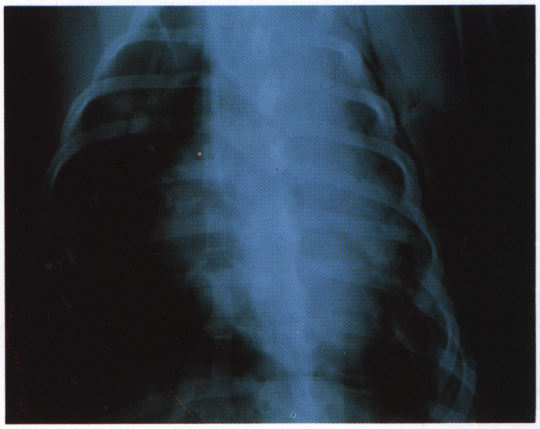

Fig. 2. Left allografted lung shown minimal infiltrate same dog on postoperative day 1.

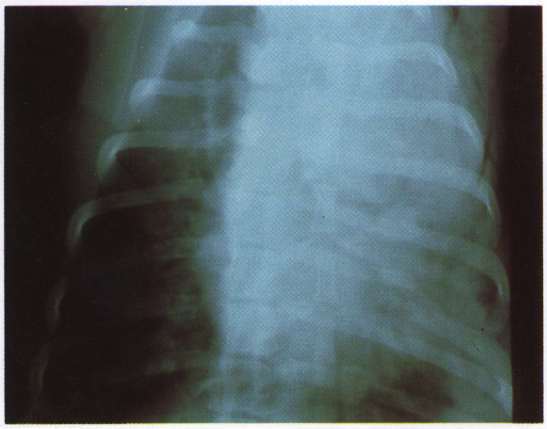

Fig. 3. Chest roentgenogram shows lobar opacification of the left in the same dog on postoperative day 2.

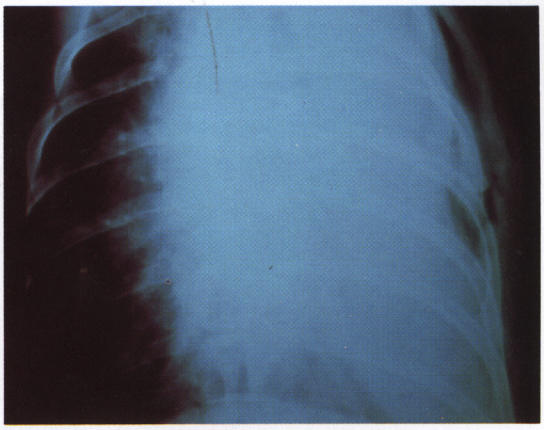

Fig. 4. A total opacification is seen in the allografted left lung of another dog on day 9.

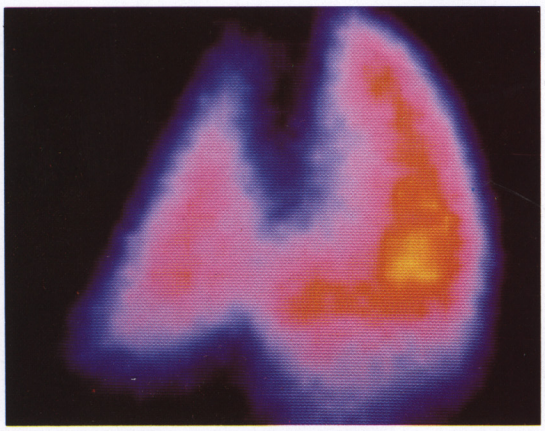

Fig. 5. Perfusion scan in the allografted lung on day 0, showing a normal perfusion (57% R/43% L).

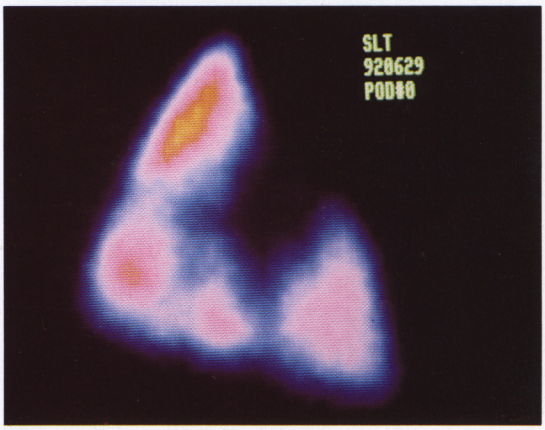

Fig. 6. Perfusion scan of another dog on day 0, showing a mild perfusion defect (63%/37%)

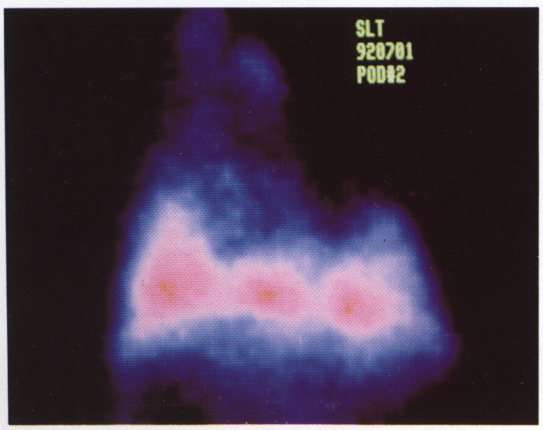

Fig. 7. A moderate perfusion defect was seen in the implanted lung (73/27%) on day 2.

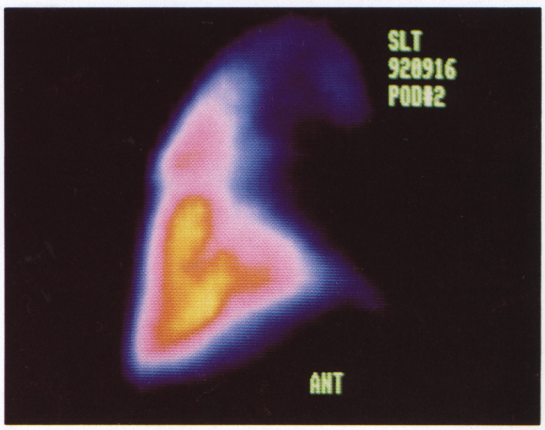

Fig. 8. A severe defect (98%/2%) was seen in another dog on day 2.

0 and mild stenosis in 2/2 dogs on day 9 (Table 4). The illustrating bronchofiberscopic views of the anastomotic site of the left main bronchus by postoperative day 0, 3 and 9 in each dog are shown in Figure 9, 10 and 11.

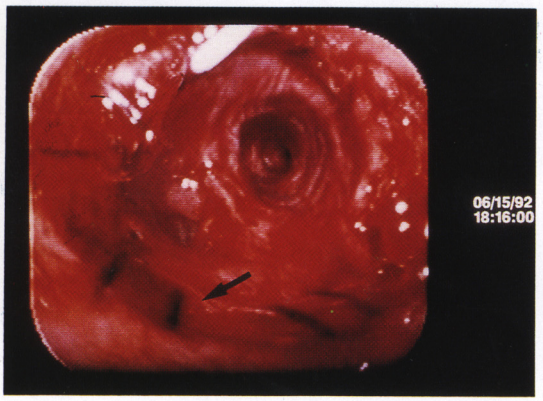

Fig. 9. A view showing good patency of the anastomotic site with evidence of sutures (arrow) immediately after the operation (day 0)

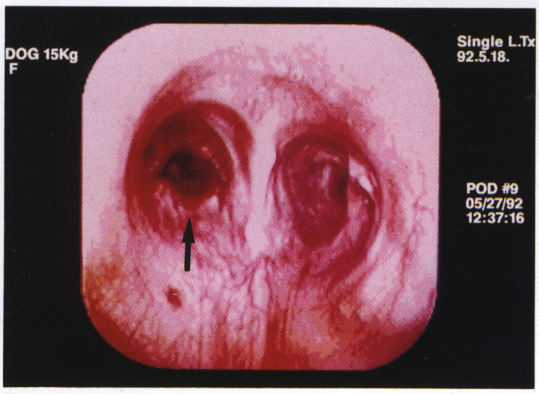

Fig. 11. An anastomotic site (arrow) of the left bronchus showing good patency on day 9.

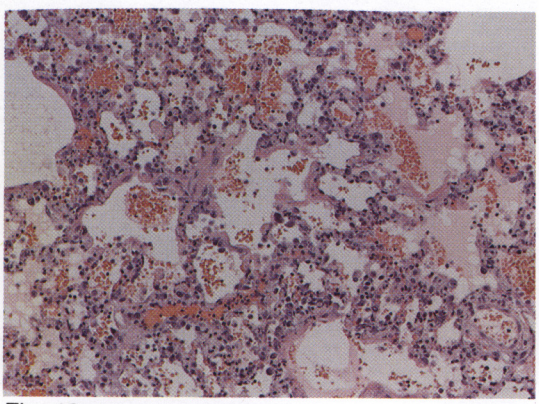

Fig. 13. Interstital edema and minimal cellular infiltration (on day 3). (H&E, ×200).

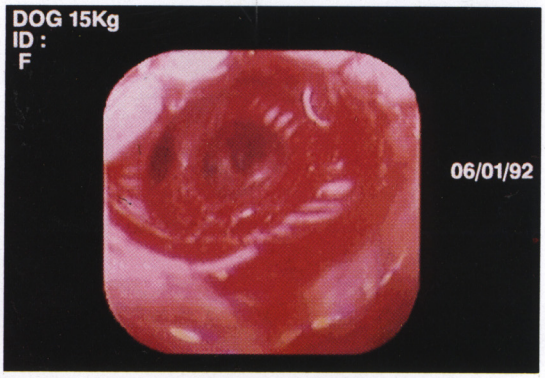

Fig. 10. A view showing just distal to good patent anastomotic site on day 3.

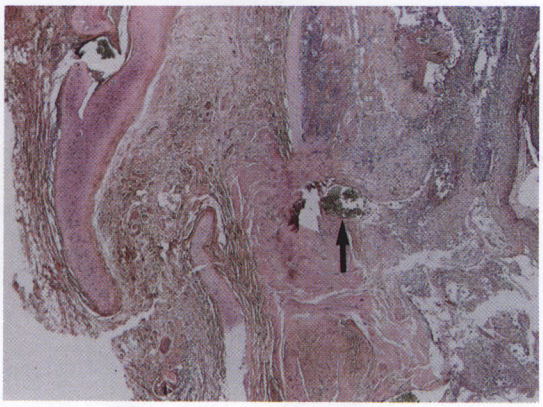

Fig. 12. A bronchial anastomotic site showing a preserved epithelium, peribronchial inflammatory cellular infiltration. Some suture materials seen in the middle (arrow) and lower part of the field. (H&E, ×100)

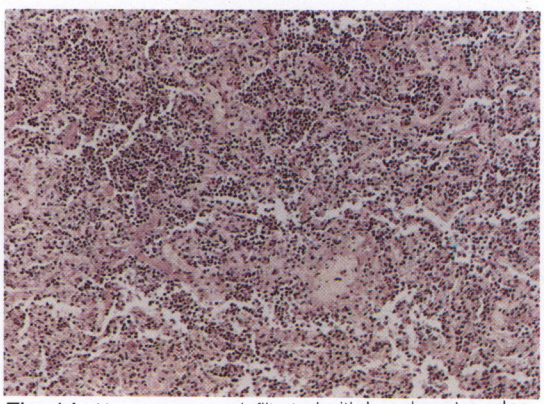

Fig. 14. Air spaces were infiltrated with lymphocytes, plasma cells and macrophages (on day 4) (H&E, ×200).

4. Histopathology of Bronchial Anastomotic Site:

The bronchial anastomotic site showed normal wound healing in 38% (3/8), cellular infiltration in 38% (3/8) and infarction in 25% (2/8) up to day 9 postoperatively. As the results indicate, the bronchial anastomotic site showed relatively good wound healing in the early postoperative days (Table 5). The illustrating histopathologic findings of the bronchial anastomotic site are shown in Figure 12.

5. Histopathology of the Allografted Lung:

Histopathology of the allografted lung was observed in 16 dogs. The specimens were obtained by open thoracotomy lung biopsies and autopsies. Interstitial edema was seen in 25% (4/16) up to day 3 and cellular infiltration-regarded as evidence of rejection-was seen in 19% (3/16) from day 3 up to day 9. Infarction was found in 56% (9/16), showing early graft failure (Table 6). The illustrating histopathologic findings of the allografted lung are shown in Figure 13, 14, 15, 16.

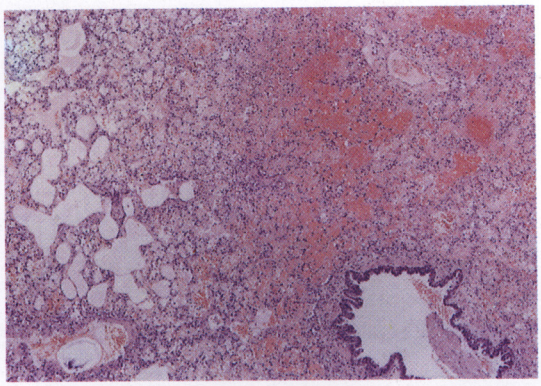

Fig. 15. Left side shows interstitial edema and right side shows hemorrhage with congestion (on day 5) (H&E, ×100).

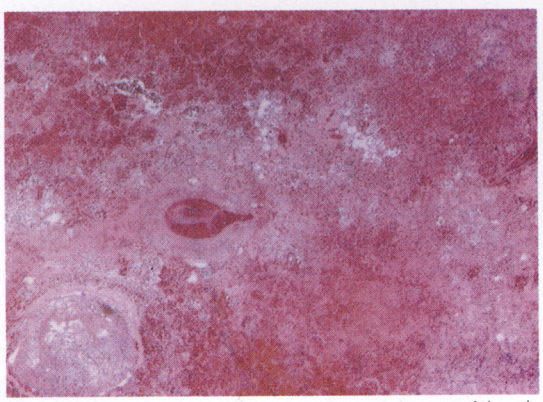

Fig. 16. Hemorrhagic infarction, where only part of the alveolar space can be seen (on day 3) (H&E, ×100).

DISCUSSION

A single reliable modality for the evaluation of allografted lung function has not yet to be obtained. Therefore, multiple modes of assessment are required. For the evaluation of the transplanted lung's function, a routine chest radiograph is essential, but this modality does not lead to an appropriate conclusion for differentiating rejection, infection or reimplantation response. Experimentally, Keshavjee et al (1990) studied radiographic correlation with early physiologic function of the transplanted canine lung by grading chest radiographs and concluded that a chest radiograph only partially reflects the function of the transplanted lung. Siegelman et al (1971) reported that chest radiographs in the early postoperative days are abnormal in all cases. According to that study, the abnomalities reached maximum on the third postoperative day, after which they resolved over 7 to 21 days. Dal Col et al (1990) compared radiographic findings with histologic grade of rejection from the transplanted lung and concluded that the chest roentgenogram was not an accurate predictor of early rejection. Clinically, Millet et al (1989) reported that while an abnormal chest radiograph was common during the first month after transplantation during acute rejection, it might alternatively be due to lung infection. In our study, abnormal findings in chest radiographs were seen mostly in the early postoperative days and deteriorated gradually up to day 9.

In other clinical studies for pulmonary flow using radionuclide perfusion scan (The Toronto Lung Transplant Group, 1988) and magnetic resonance imaging (Mohiaddin et al., 1991), the distribution of pulmonary flow to the implanted lung increased and that to the diseased native lung decreased with time. Therefore, the size of the physiologic shunting was limited and overall improvement in gas exchange and functional capacity was enhanced. But in our study as seen in table 3, the perfusion scan data of the transplanted lung decreased considerably rather earlier postoperatively, suggesting decreased pulmonary blood flow. This result was largely attributed to pulmonary arterial thromboembolism but it is very likely that infection, rejection and reimplantation response are also responsible. Compared with the radiographic findings as seen in table 2 and 3, a lung perfusion scan seems to be more sensitive in detecting early postoperative graft dysfunction.

Airway complications contributed significantly to morbidity and mortality after the lung transplantation. Historically, several methods to improve bronchial heal-

ing including wrapping the anastomosis with perihilar connective tissue (Blumenstock et al., 1961), telescoping the bronchial ends (Sinha et al., 1971), shortening the length of the donor bronchus (Pinsker et al., 1979), and bronchial artery revascularization (Schreinemakers et al., 1990) were developed. And the omental wrapping of the trachea or bronchial anastomotic site was established successfully by the pioneer of tracheobronchial surgery (Grillo, 1989). A pedicled omentum wrapped around the airway anastomosis has turned out to be a simple and effective means of revascularizing the donor bronchial vessels for several years by many authors (Lima et al., 1982, Morgan et al., 1983, and Cooper et al., 1987). Recently, Auteri et al (1992) reported in an experimental study that excellent bronchial healing could be obtained without wrapping the anastomoses with omentum or any other tissues, and that bronchial anastomotic wrapping with vascular tissue might not be essential. In our study, relatively good wound healing with simple end-to-end anastomosis, while preserving well peribronchial fibrous tissue, was observed bronchosopically and histopathologically. In a clinical allograft, factors such as lung preservation, ischemia, rejection, infection and hemodynamic instability of the recipient would undoubtedly play a role in enhancing bronchial healing.

Histopathologic findings after the lung transplantation resulted from infection, reimplantation response, rejection, pulmonary edema and infarction. Reimplantation response consists of alveolar edema and alveolar infiltrate. In animal models, these changes seem to reach their peak within three days after transplantation and then gradually regress over the next three weeks (Siegelman et al., 1973) and factors which exacerbate the functional defects produced by the reimplantation response seem to be intercurrent rejection, expanded blood volume and overhydration (Reitz et al., 1983). Pulmonary infarction can result from thrombosis and stenosis of the atrial anastomotic site, inappropriately flushed graft and no systemic heparinization (Lee J.S. et al., 1992, Lee D.Y. et al., 1992). In our study, all allografted lungs showed abnormal findings such as interstitial edema, cellular infiltration and infarction. Cellular infiltration regarded as acute rejection was seen from postoperative day 3 in spite of cyclosporine treatment, and infarction regarded as graft failure was considerably high in our study.

In conclusion, an assessment of early graft function in the canine single lung transplant recipient was made by grading postoperative serial chest roentgenograms, perfusion scintigrams, gross bronchial healing, histopathology of bronchial anastomotic wound healing and the allografted lung. The authors could summarize the study as follows: Roentgenograms and perfusion scintigrams showed progressive deterioration within the first week postoperatively. This result may reflect early allograft dysfunction. Bronchial patency was well maintained endoscopically and bronchial wound healing turned out to be satisfactory anatomically with simple end-to-end anastomosis while preserving the peribronchial fibrous tissues. Under cyclosporine treatment, the allograft lung showed interstitial edema to cellular infiltration of various degrees in half the dogs and infarction in to the other half, suggesting graft failure.

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