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Evaluation of the International Society for Heart Transplantation (ISHT) grading of pulmonary rejection in 100 consecutive biopsies

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Heart-lung and lung transplantation are accepted treatments for patients with end-stage pulmonary vascular disease or parenchymal lung disease [4, 5, 10]. Survival rates for heart-lung and lung transplantation are lower than those for heart transplantation alone. The 5-year actuarial survival for heart-lung transplantation has been 41% largely due to rejection and infection remaining as the limiting factors for long-term survival [3].

A standardized nomenclature for the histological grading of pulmonary rejection was formulated by the International Society for Heart Transplantation (ISHT) in July 1990 [12]. Infection, however, is a major problem in the histological assessment of lung recipient biopsies, potentially limiting the usefulnes of such a classification. In this study, 100 consecutive transbronchial biopsies (TBBs) from lung transplant recipients were analysed, together with microbiological and serological data, in order to evaluate the proposed ISHT grading system for pulmonary rejection and the importance of concomitant infections in the histological interpretation of TBBs.

Key words: Pulmonary rejection, ISHT grading – TBBs histology

Materials and methods

Patients

From September 1990 to March 1991, 100 consecutive TBBs were obtained from 43 patients during routine surveillance or clinically indicated procedures. The patients included four single lung and 39 heart-lung recipients and their ages ranged from 18 to 59 years (mean 34.1). The transbronchial procedure has previously been described [1].

Histology

The biopsies were fixed in 10% neutral buffered formalin and processed in a Shandon Hypercenter. Routine biopsies were processed overnight and clinically urgent specimens were processed using a short 2-hour cycle. The paraffin-embedded material was serially sectioned and stained with haematoxylin and eosin. Special stains performed on all cases included Perls'/elastic van Gieson for connective tissue, PAS and Grocott's methenamine silver method for fungal hyphae and the cysts of *Pneumocystis carinii*. Additional special stains were performed as appropriate, e.g. Ziel-Neelsen stains for acid fast bacilli in cases with granulomatous inflammation. Accompanying bronchioalveolar lavage (BAL) specimens were fixed in 100% alcohol and prepared slides stained with haematoxylin and eosin and by Grocott's methenamine silver method. Cytospin preparations were made if required. All the histological material was reviewed by S. S.

Microbiology

Specimens from either the BAL or transbronchial samples were sent for viral culture including cultures for HSV and CMV. In patients who were known CMV mismatches or seropositive, early detection

Table 1. Results of ISHT grading system applied to 76 TBBs

	(a)	(b)	(c)	(d)	Total
Grade A (acute rejection)					
1. Minimal	4	9	0	1	14
2. Mild	14	15	0	1	30
3. Moderate	7	6	0	0	13
4. Severe	0	0	0	0	0
Grade B (airway inflammation)					
B1. 0					
B2. 1					
Grade C (oblit. bronchiolitis)					
C1a 5					
C1b 2					
C2a 1					
C2b 0					
Grade 0 (no abnormality) 14					
Grade D (chronic vascular rejection) 5					
Grade E (vasculitis) 0					

Grade A, suffixes (a) with bronchiolar inflammation
(b) without bronchiolar inflammation
(c) with bronchial inflammation
(d) no bronchioles present
Grade B1, lymphocytic bronchitis; B2, lymphocytic bronchiolitis
Grade C1, sub total; C2 total; a active, b, inactive

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Table 2. Biopsies in which rejection could not be assessed due to histological evidence of infection

Nature of infection (histological)	Number of biopsies ($n = 22$)	Confirmed by appropriate culture serology ($n = 16$)
CMV Pneumonitis	12	11
Bacterial infections (bronchitis/organizing pneumonia)	4	3[a]
Asp – invasive	2	(1)[b]
– bronchocentric/ granulomatous	1	1
– pneumonia	1	1[c]
PCP	2	N/A

Asp, *Aspergillus fumigatus;* PCP, *Pneumocystis carinii pneumonia;* N/A, not applicable
[a] *Pseudomanas aeruginosa,* 2; *Haemophilus influenza,* 1
[b] One biopsy not sent for culture
[c] *Herpes simplex* virus also cultured

Table 3. Results of microbiological investigations in biopsies graded for rejection

Type of infection	Number of isolates ($n = 22$)
Pseud infections	11
CMV	3
Asp	2
Other bacteria[a]	4
Mixed infections[b]	2
	22

Pseud, *Pseudomanas aeruginosa;* Asp, *Aspergillus fumigatus*
[a] Includes: *Branhamella catarrhalis, Acinetobacter* spp., *Haemophilus influenzae, Staphylococcus aureus*
[b] Includes: Adenovirus/Pseud; CMV/Asp

Table 4. Rejection grades assigned to biopsies with microbiological evidence of concomitant infection

Rejection grade	Number of biopsies ($n = 22$)
0	2
A1a	2
A1b	4
A2a	5
A2b	4
A2d	1
B2	1
C1a	1
A1b/C1a/D[a]	1
Total	22

Multiple rejection grades assigned on one biopsy

of CMV was performed by the DEAFF (Direct early antigen fluorescent focus) test. Other infectious agents investigated by serological methods and according to clinical suspicion included toxoplasmosis, adenovirus, influenza virus, mycoplasma spp., legionella and Epstein-Barr virus. A review of all microbiological data from specimens that included sputa, blood cultures, throat swabs and BAL was undertaken for specimens taken 2 days prior to, and 2 days following, biopsy procedures.

Results

Gradable rejection was shown by 76 TBBs. The grades assigned at the time of biopsy are shown in Table 1. The majority of TBBs showed acute rejection, predominantly mild acute rejection, grade A2, and there were no cases of severe acute rejection. In six TBBs more than one grade was assigned, e.g. grade A2a (mild acute rejection) together with grade C1b (obliterative bronchiolitis) and grade D (chronic vascular rejection). Rejection could not be assessed in 24 TBBs. In the majority of cases (22), this was due to histological evidence of infection. In all but two biopsies this was confirmed by the appropriate microbiological cultures or serological investigation. The results are shown in Table 2. From only one procedure was the material inadequate for assessment, and in one biopsy the effects of a previous biopsy obscured the histology. In the two TBBs thought to be infective at histology (but 'culture negative'), one showed features suggestive of a viral pneumonitis and one showed a non-specific pneumonitis. In the 76 TBBs assessed as gradable for rejection, in 22 cases the caveat 'exclude infection' was included in the histological report. Analysis of the microbiological data showed eight of these cases to have significant positive cultures in addition to 14 out of the 54 cases graded confidently for rejection. Table 3 shows the patients culture/serological results. The rejection grades assigned to these biopsies at the time of reporting are shown in Table 4.

Discussion

This study validates the use of the TBB for the diagnosis of infection and rejection in heart-lung and single-lung transplant recipients, in keeping with previous reports [2, 8]. In only a single biopsy procedure was the material insufficient for a diagnosis. The number of biopsy specimens required to evaluate significant lung rejection is uncertain but the Lung Rejection Study Group [12] recommend a minimum of five transbronchial specimens containing lung parenchyma to be taken from the donor graft at each biopsy procedure. The ISHT Working Formulation Grading of Rejection can be applied to TBBS and in this study over three-quarters of the biopsies were assigned a grade to aid clinical management, most frequently Grade A, acute rejection. Perivascular infiltrates in TBBs are not specific for rejection, and the major differential diagnosis is infection [9]. Analysis of microbiological results showed evidence of a concomitant respiratory tract infection in 22 out of 76 biopsy procedures where a grade had been assigned, and emphasises the importance of reviewing all the data before assigning a final grade. Examination of the grades assigned in these cases, Table 4, shows no trend towards a particular grade and in particular no evidence of a preponderance of acute rejection with airways inflammation. The respiratory infections were presumably upper airways infections in these cases.

TBBs are useful for the diagnosis of complications in lung transplantation patients. The histological diagnosis of infection in this study was shown to be highly specific

and only two false positive histological diagnoses of infection were made on biopsy material when accompanying microbiological data were analysed. CMV is a major problem. Although there are special techniques available that increase the sensitivity of detection [7, 11], the diagnosis of CMV disease in the lung requires the demonstration of CMV inclusions together with an accompanying pneumonitis [6]. Culture and serological evidence of CMV indicates infection as seen in four out of 22 biopsies which had been graded but which also had evidence of a concomitant infection. CMV pneumonitis with inclusions and surrounding inflammation was not confused with rejection in our study.

References

1. Higenbottam T, Stewart S, Penketh A, Wallwork J (1988) Transbronchial lung biopsy for the diagnosis of rejection in heart-lung transplant patients. Transplantation 46:532–539
2. Higenbottam T, Stewart S, Wallwork J (1988) Transbronchial lung biopsy to diagnose lung rejection and infection of heart-lung transplants. Transplant Proc 20:767–769
3. Kriett JM, Kaye MP (1991) The Registry of the International Society for Heart and Lung Transplantation: Eighth Official Report – 1991. J Heart Lung Transplant 10:491–498
4. Penketh ARL, Higenbottam TW, Hakim M, Wallwork J (1987) Heart and lung transplantation in patients with end-stage lung disease. Br Med J 295:311–314
5. Reitz BA, Wallwork J, Hunt SA, Pennock JL, Billingham ME, Oyer PE, Stinson EB, Shumway NE (1982) Heart lung transplantation? Successful therapy for patients with pulmonary vascular disease. N Engl J Med 306:557–564
6. Sissons JGP, Borysiewicz LK (1989) Human cytomegalovirus infection. Thorax 44:241–246
7. Steinhoff G, Behrend M, Wagner TOF, Hoper MH, Haverich A (1991) Early diagnosis and effective treatment of pulmonary CMV infection after lung transplantation. J Heart Lung Transplant 10:9–14
8. Stewart S, Higenbottam TW, Hutter JW, Penketh ARL, Zebro TJ, Wallwork J (1988) Histopathology of transbronchial biopsies in heart-lung transplantation. Transplant Proc 20: 764–766
9. Tazelaar HD (1991) Perivascular inflammation in pulmonary infections: implications for the diagnosis of lung rejection. J Heart Lung Transplant 10: 437–441
10. Theodore J, Levington N (1990) Lung transplantation comes of age. N Engl J Med 332:772–774
11. Weiss LM, Movahed LA, Berry GJ, Billingham ME (1990) In situ hybridization studies for viral nucleic acids in heart and lung allograft biopsies. Am J Clin Pathol 93:675–679
12. Yousem SA, Berry GJ, Brunt EM, Chamberlain D, Hruban RH, Sibley RK, Stewart S, Tazelaar H (1990) A working formulation for the standardisation of nomenclature in the diagnosis of the heart and lung rejection: Lung Rejection Study Group. J Heart Transplant 9:593–601