Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Bronchoscopy in the diagnosis and surveillance of respiratory infections in lung and heart–lung transplant recipients

Juho T. Lehto,[1] Petri K. Koskinen,[2] Veli-Jukka Anttila,[3] Irmeli Lautenschlager,[4] Karl Lemström,[5] Jorma Sipponen,[6] Pentti Tukiainen[1] and Maija Halme[1]

1 Division of Respiratory Diseases, Department of Medicine, Helsinki University Central Hospital Helsinki, Finland
2 Division of Nephrology, Department of Medicine, Helsinki University Central Hospital and Transplantation Laboratory, University of Helsinki, Helsinki, Finland
3 Division of Infectious Diseases, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland
4 Department of Virology, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland
5 Department of Thoracic and Cardiovascular Surgery, Helsinki University Central Hospital and Transplantation laboratory, University of Helsinki, Helsinki, Finland
6 Department of Thoracic and Cardiovascular Surgery, Helsinki University Central Hospital, Helsinki, Finland

Keywords
bronchoscopy, infection, lung transplantation, *Pneumocystis carinii*.

Correspondence
Juho T. Lehto, Division of Respiratory Diseases, Department of Medicine, Helsinki University Central Hospital (c/o Susanna Hämäläinen), PO Box 340 (Haartmaninkatu 4), FIN-00029 HUS/Helsinki, Finland. Tel.: +358-9-4711; fax: +358-9-471 74049; e-mail: juho.lehto@hus.fi

Received: 24 May 2004
Revised: 25 October 2004
Accepted: 20 December 2004

doi:10.1111/j.1432-2277.2005.00089.x

Summary

Fiberoptic bronchoscopy (FOB) with bronchoalveolar lavage (BAL) and transbronchial biopsies (TBB) is a widely used method to detect respiratory infections and to differentiate them from other postoperative complications in lung transplant (LTX) recipients, but the usefulness of surveillance FOBs is not yet established. The aim of this study was to evaluate the usefulness of FOB in the diagnosis and surveillance of infections in LTX recipients. We reviewed all the consecutive 609 FOBs performed on 40 lung or heart–LTX recipients between February 1994 and November 2002. The overall diagnostic yield was 115/190 (61%) and 43/282 (15%) for clinically indicated and surveillance FOBs respectively ($P < 0.001$). Infection was established by bronchoscopic samples in 96/190 (50.5.%) of the clinically indicated FOBs and 34/282 (12.1%) of the surveillance FOBs ($P < 0.001$). The diagnostic yield of the clinically indicated FOBs was highest (72%) from 1 to 6 months post-transplant ($P = 0.04$). *Pneumocystis carinii* was detected in 23 (4.9%) of the bronchoscopic specimens and 15 (65%) of the *P. carinii* infections were detected during adequate chemoprophylaxis. To conclude, in LTX recipients clinically indicated FOB has a good diagnostic yield in detecting infections and other postoperative complications, whereas the information received from surveillance FOB has remained less significant. With current prophylaxis and screening strategies FOB is still required to detect *P. carinii* infections.

Introduction

Infections, rejection, and airway complications of the allograft are the major sources of morbidity and death in lung transplant (LTX) and heart–lung transplant (HLTX) recipients [1,2]. Fiberoptic bronchoscopy (FOB) with multiple transbronchial biopsies (TBB) and bronchoalveolar lavage (BAL) is a well-established procedure in the diagnosis of these major complications in the presence of radiographic infiltrates, respiratory symptoms, and/or functional deterioration of the allograft [3–5]. In contrast, the usefulness of performing surveillance FOBs in asymptomatic recipients remains controversial. Some authors have found a relatively low diagnostic yield of the procedure and no improvement in survival [6–9].

Bronchoscopy with BAL and/or TBB has an excellent diagnostic yield for significant opportunistic pathogens [e.g. *Pneumocystis carinii* and cytomegalovirus (CMV)]

[10,11]. The prophylaxis used against *P. carinii* weakens the role of this organism as pulmonary pathogen in LTX and HLTX recipients. However, breakthrough *P. carinii* infections during adequate chemoprophylaxis have been reported [12,13]. The surveillance of CMV by peripheral blood (PB) pp65 antigen detection has proved a useful method allowing the use of the pre-emptive therapy [14,15]. The additional value of TBB and bronchoalveolar lavage fluid (BALF) samples in the era of CMV prophylaxis and pre-emptive therapy has not been established.

The need for FOB to detect bacterial and fungal colonizations in asymptomatic LTX and HLTX recipients has not been widely studied. However, *Aspergillus* colonization has proved a risk factor for airway complications and invasive aspergillosis leading some authors to recommend the use of pre-emptive antifungal therapy [16–18]. The role of the bacterial airway colonization in LTX and HLTX recipients is not known, but detecting the *Pseudomonas* species from BALF is associated with increased inflammatory response of the allograft which may lead to active *Pseudomonas* infection or airway damage [19–21].

The aim of the present study was to evaluate the clinical significance and safety of FOB in the diagnosis and surveillance of respiratory infections with a special emphasis on *P. carinii* as well as other postoperative complications in LTX and HLTX recipients.

Patients and methods

Patients

We reviewed all the consecutive FOB performed on LTX and HLTX transplant recipients at Helsinki University Central Hospital between February 1994 and November 2002. The study was approved by the Ethics Committee of the Department of Medicine, Hospital District of Helsinki and Uusimaa. A total of 51 patients underwent transplantation during the study period. Eleven recipients died <30 days post-transplant because of graft failure (three), intestinal perforation/hemorrhagia (two), heart luxation (one), heart failure (one), pulmonary embolism (one), intracerebral hemorrhagia (one), brain anoxia (one) and stenosis of the trachea anastomosis (one). Forty patients surviving more than 30 days postoperatively were included in the study population. The patient characteristics are summarized in Table 1.

All patients were followed by a surveillance protocol consisting of spirometry, plain chest radiography, chest CT scans with high-resolution computed tomography (HRCT), routine blood tests combined with a CMV-antigen test, and bronchoscopy with BAL performed 1–4 weeks, 3, 6, 9 and 12 months after transplantation and once a year thereafter. Moreover, the tests were performed when clinical suspicion of rejection or infection was present. Transbronchial lung biopsy (TBB) was performed 1, 3, 6, 9 and 12 months after transplantation and when acute rejection (AR) was suspected. Only bronchoscopies with BAL (±TBB) were accepted for the analysis.

Table 1. The patient demographics.

No.	40
Gender (M/F)	19/21
Age, mean (range)	45 (18–61)
Indication for transplantation	
Emphysema	13 (32)
Congenital heart disease	9 (23)
Pulmonary hypertension	7 (18)
Idiopathic pulmonary fibrosis	5 (13)
Lymphangioleiomyomatosis	3 (7)
Other*	3 (7)
Type of transplantation	
Single lung	6 (15)
Double lung	15 (38)
Heart lung	19 (47)
Pretransplant CMV-serostatus	
R–/D–	3 (8)
R+/D– or R+/D+	36 (90)
R–/D+	1 (2)
Alive in November 2002	23 (58)
Died before November 2002	17 (42)
Time to death, mean (range)†	21 (2–82)
Causes of death	
OB or BOS	6
Invasive aspergillosis	2
Airway complication	2
Malignant lymphoma	2
Other‡	5

Percentage values are given in parentheses unless otherwise stated.
M, male; F, female; CMV, cytomegalovirus; R, recipient; D, donor; OB, obliterative bronchiolitis; BOS, bronchiolitis obliterans syndrome.
*Cardiomyopathy (one), Kartagener's syndrome (one), bronchiolitis obliterans (one).
†Months after transplantation.
‡Septic pneumonia (*S. epidermidis*), mycobacteriosis (*M. abscessus*), CMV pneumonia, pancreatitis, intestinal perforation.

Surveillance bronchoscopy was defined as a procedure performed according to a routine protocol in recipients with no new respiratory symptoms, radiographic infiltrate or functional deterioration [<10% decline in the forced expiratory volume in 1 s (FEV1)]. Bronchoscopy was classified as 'clinically indicated' when there were symptoms referring to infection or rejection (e.g. fever, purulent sputum production or dyspnea), new radiographic infiltrate or more than 10% decline in FEV1. In addition to 'clinically indicated' FOBs, there were follow-up bronchoscopies performed during the same episode of infection or rejection to assess the response to therapy or to receive more information about the

etiology of the allograft deterioration. These follow-up procedures were not analyzed in the diagnostic setting if they did not yield any additional diagnostic information.

Immunosuppressive medications and infection prophylaxis

Antithymocyte globulin (1.25–2.5 mg/kg/day) was given at induction and continued for 1–5 postoperative days (POD). The maintenance immunosuppressive regimen was initiated immediately after transplantation. It consisted of cyclosporine (200–400 ng/ml whole-blood trough level), azathioprine 1–2 mg/kg/day (WBC > 4000 cells/μl) or mycophenolate mofetil 2–3 g/day, and methylprednisolone starting with 1 g perioperatively and tapered down to 0.1 mg/kg/day. Rejection episodes of grade 1 with symptoms and rejection episodes of grades 2 or higher were treated with methylprednisolone 0.5–1 g daily for 3 days. One rejection episode not responsive to methylprednisolone was treated with OKT-3 (5 mg/day for 10 days).

Antibiotic prophylaxis (usually third generation cephalosporins and/or vancomysin) was given for the immediate perioperative period to prevent bacterial infections. The first line prophylaxis against *P. carinii* was 1–2 double strength tablets (160 mg of trimethoprim and 800 mg of sulfamethoxazole) of co-trimoxazole once daily 3 days a week. Inhaled pentamidine every 4 weeks was given if the patient did not tolerate co-trimoxazole. The prophylaxis was continued lifelong in all except six recipients operated during the first 2 years (the prophylaxis was interrupted after 8, 10, 13, 14, 36 and 37 months because of side effects).

Cytomegalovirus prophylaxis was used since January 1995. Twenty-nine CMV-seropositive recipients (R+/D+ or D−) received ganciclovir 5 mg/kg twice a day through POD 7–21 and then 5 mg/kg/day for 5 days a week through POD 22–28 continued with high-dose acyclovir (800 mg four times/day p.o.), valacyclovir (1 g three times/day p.o.) or oral ganciclovir (1 g three times/day) through POD 29–90. Oral ganciclovir (1 g three times/day) from POD 7 to 90 was given to two CMV-seropositive patients. The only CMV-seronegative recipient receiving a transplant from a CMV-seropositive donor (R−/D+) was given ganciclovir 5 mg/kg i.v. twice a day through POD 7–60 and then 5 mg/kg/day for 5 days a week through POD 61–90. Six patients were operated on before ganciclovir prophylaxis was instituted, and three CMV-seronegative recipients with CMV-seronegative donors (R−/D−) received acyclovir (200 mg three times/day) until POD 90 to prevent other herpes virus diseases.

Fiberoptic bronchoscopy, BAL and TBB

The bronchoscopic procedure was performed with a fiberoptic bronchoscope (Olympus model BF IT20 or BF 20, Olympus, Japan).

Before BAL bronchial brushing with a protected specimen brush (PSB) was performed. BAL was performed by wedging the tip of the bronchoscope into the segmental or subsegmental bronchus of the area with the greatest radiologic abnormality. In case of no new radiologic infiltrates or if the infiltrates were diffuse, BAL was usually performed in the lingula or in the right middle lobe. About160–200 ml of sterile physiologic saline warmed to the body temperature was instilled in 20 ml aliquots. Gentle manual suction was applied to retrieve the saline.

The TBBs were taken under fluoroscopic guidance by using alligator forceps from the areas of maximal parenchymal involvement detected by HRCT. In case of no parenchymal infiltration the samples were taken from every lobe of the allograft. Five to six TBB samples were collected per procedure. Endobronchial biopsies (EBB) were performed concomitantly with the TBBs and if any abnormalities were detected in the examination of the tracheobronchial tree.

BALF and TBB specimens and demonstration of CMV

Bronchoalveolar lavage fluid was collected in non-siliconized glass containers and carried on ice to the laboratory within 30 min. The following microbiological, cytological and histological specimens formed the routine BALF and TBB samples for LTX and HLTX transplant recipients at our institution during the study period. The BALF and PSB samples were cultured semiquantitatively for bacteria and fungi. Air-dried smears of BALF were examined by microscopy after Calcofluor white staining for fungal diagnostics. Cultures for mycobacteria, legionellae and viruses were also made from BALF. Varicella zoster, herpes simplex, and common respiratory viruses were identified by antigen detection. Direct immunofluoresence was employed for the detection of *Legionella pneumophilia*. Giemsa silver-methenamine stain and antigen detection with the immunofluorescent technique were used to detect *P. carinii*. CMV was demonstrated in BALF by detection of pp65CMV antigen-positive cells and/or typical viral inclusions in pneumocytes and alveolar macrophages and rapid shell vial culture. All the BALF left from the microbiological studies was used for cytological investigations. Papanicolaou-stained Millipore filter or Cyto-Tek preparations as well as native May–Grünwald–Giemsa-stained cytocentrifuge slides were utilized to determine the total and differential cell count, viral inclusion bodies, microbes, and atypical cells.

The TBB specimens were fixed in 10% buffered formalin, processed routinely, and examined histologically after sectioning and staining with Mayer's hematoxylin–eosin, toluidine blue, Masson's trichrome, and Unna Pappenheim to detect any evidence of rejection or infection. CMV-pp65 and *P. carinii* antigen expressions were also investigated from TBB specimens.

The demonstration of CMV antigen in PB was based on the standard CMV pp65 antigen test [22].

Diagnostic criteria

The bacteria in BALF or PSB samples were considered significant if they were known respiratory pathogens and the sample was taken from a patient with new respiratory symptoms (productive cough, dyspnoea, and/or fever) and a new or increasing radiologic infiltrate (pneumonia) or bronchoscopic appearance of purulent secretions and/or membranes (bronchitis). *Staphylococcus epidermidis* was accepted as a causative agent only if cultured repeatedly from BALF and/or PSB samples during the same infectious episode without any other pathogenic bacteria present. The bacteria cultured from the BALF/PSB samples and blood/pleural fluid at the same time were always considered significant. Isolated growth of bacteria belonging to the mouth flora (e.g. *Streptococcus viridans*) in the BALF or PSB specimens was not considered of diagnostic value. The bacteria cultured from the surveillance bronchoscopic samples were considered significant if the patient later developed a symptomatic infection caused by the same organism. Colonizations with *Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia* were considered clinically significant if an antibiotic therapy was initiated by the clinician.

Aspergillus airway colonization was defined as isolation of the *Aspergillus* species from BALF or PSB specimens without evidence of an invasive disease or tracheobronchitis. If characteristic membranes and ulcerations were detected together with *Aspergillus* airway colonization without an alternative diagnosis or invasive parenchymal disease, the diagnosis was *Aspergillus* tracheobronchitis. Invasive aspergillosis was defined as a histopathologic or cytopathologic finding showing hyphae in the transthoracic needle aspiration or biopsy specimen with associated tissue damage [23]. The *Candida* species cultured from bronchoscopic samples were not considered significant unless histopathologic evidence of invasive fungal pneumonia or positive blood culture was present.

Pneumocystis carinii and non-CMV viruses were considered clinically significant pathogens when detected in BALF or TBB specimens.

The CMV pneumonia was defined as the presence of respiratory symptoms and radiologic infiltrate combined with the detection of characteristic intracellular inclusion bodies on BALF or TBB or CMV antigen-positive cells on the TBB specimens.

The histologic diagnosis of AR was based on the presence of perivascular mononuclear infiltrates on the TBB specimens. The severity of AR was graded from 0 to 4 according to the International Society for Heart and Lung Transplantation criteria [24].

The airway complication was defined as endobronchial stricture or granulation tissue resulting in symptoms or functional impairment of the recipient and necessitating an intervention (e.g. laser, balloon dilatation or stent placement).

Statistical methods

The diagnostic yield and complication rate of bronchoscopy in different situations were compared using the chi-square test. A statistical significance was accepted for $P < 0.05$.

Results

During the study period 40 transplant recipients underwent a total of 609 FOBs. The number of FOBs analyzed in the diagnostic setting was 472 (190 clinically indicated and 282 surveillance FOBs). The remaining 137 FOBs were follow-up procedures analyzed only to detect any potential complications of the procedure.

Utility of bronchoscopies

The overall diagnostic yield was 115/190 (61%) and 43/282 (15%) for clinically indicated and surveillance FOBs, respectively ($P < 0.001$). Infection was established by bronchoscopic samples in 96/190 (50.5%) of the clinically indicated FOBs and 34/282 (12.1%) of the surveillance FOBs ($P < 0.001$). The diagnoses in both FOB groups are presented in Table 2. The procedure caused a change in

Table 2. Diagnoses established by bronchoscopic specimens.

Diagnoses	Clinically indicated FOBs (% of FOBs)	Surveillance FOBs (% of FOBs)
Infection	96 (50.5)	34 (12.1)*
AR	21 (11.1)	8 (2.8)*
Airway complication	12 (6.3)	1 (0.4)*
Other†	4 (2.1)	–
No diagnosis	75 (39.5)	239 (84.8)*

FOB, fiberoptic bronchoscopy; AR, acute rejection.
Eighteen of the clinically indicated FOBs established two diagnoses [infection + AR (11), infection + airway complication (six) and AR + airway complication (one)].
*$P < 0.001$.
†Eosinophilic pneumonia (two), COP (one) and bronchiolitis obliterans (one).

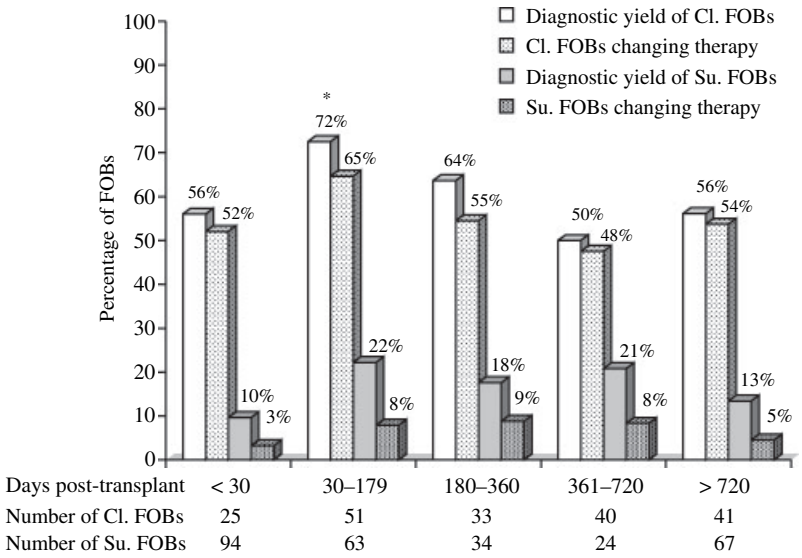

Figure 1 Utility of bronchoscopies at different time intervals after transplantation. (Cl. FOB, clinically indicated fiberoptic bronchoscopy; Su. FOB, surveillance fiberoptic bronchoscopy). *$P = 0.04$ when compared with all the other time intervals.

the medical therapy in 105/190 (55%) of the clinically indicated FOBs, while 16/282 (6%) of the surveillance FOBs altered the previous treatment of the patient. The utility of FOBs at different time intervals after transplantation is presented in Fig. 1. The diagnostic yield was highest from 1 to 6 months post-transplant when compared with other time intervals, although the difference was statistically significant only in the group of clinically indicated FOBs ($P = 0.04$).

Bacteria

The infectious agents detected in the bronchoscopic specimens are presented in Table 3. Bacteria were the most frequently detected microbes by the clinically indicated FOBs, and 20/57 (35%) of the bacterial infections occurred <6 months after transplantation. In five cases (9%) the bacteria were also cultured from blood [*S. epidermidis* (three), *Staphylococcus aureus* (one), *Enterococcus faecalis* (one)]. The bacteria cultured from the surveillance bronchoscopic samples were clinically significant in nine cases. Three patients had the same bacteria [*P. aeruginosa* (one), *S. maltophilia* (one), and *Haemophilus influenzae* (one)] cultured from the bronchoscopic samples preceding (surveillance FOB) and during the symptomatic respiratory infection (clinically indicated FOB). Six bacterial colonizations (*P. aeruginosa* (four) and *S. maltophilia* (two)] detected by surveillance FOB were treated with antibiotic therapy.

Fungi

Aspergillus was isolated in the BALF collected from 16 recipients and 17 bronchoscopies [*Aspergillus fumigatus*

Table 3. Significant microbes detected in bronchoscopic specimens.

Pathogens	Clinically indicated FOBs (% of FOBs)	Surveillance FOBs (% of FOBs)
Bacteria	57 (30.0)	9 (3.2)
P. aeruginosa	11	5
P. aeruginosa + *S. pneumoniae*	1	
S. maltophilia	7	3
S. aureus	10	
S. aureus + *S. pneumoniae*	1	
S. pneumoniae	6	
H. influenzae	3	1
M. catarrhalis	4	
Nocardia	3	
S. epidermidis	3	
Klebsiella oxytoca	2	
L. pneumophila	2	
Other*	4	
Aspergillus	7 (3.7)	10 (3.5)
P. carinii	18 (9.5)	5 (1.8)
CMV†	27 (14.2)	7 (2.5)
Viruses other than CMV	11 (5.8)	6 (2.1)
HSV	1	2
VZV		1
RSV	4	
RSV + VZV	1	
Influenzavirus	2	2
Parainfluenzavirus	2	
Parainfluenzavirus + HSV	1	
Adenovirus		1
Mycobacteria‡	2 (1.1)	

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella zoster virus; RSV, respiratory syncytial virus.
**Enterococcus faecalis* (one), *Escherichia coli* (one), *Serratia marcescens* (one), Mycoplasma (one).
†pp65 antigen or viral inclusions.
‡*Mycobacterium abscessus* (one), *Mycobacterium tuberculosis* (one).

(13), *Aspergillus ustus* (three) and *Aspergillus flavus* (one)]. Two patients with *Aspergillus* detected by clinically indicated FOB had a histologically proven invasive *Aspergillus* infection (*Aspergillus pneumonia* confirmed by autopsy specimens and *Aspergillus* tracheobronchitis diagnosed by EBB). All the other cases revealed *Aspergillus* airway colonization four of them occurring <6 months after transplantation. Anastomotic complication [stricture (three) or dehiscence (one)] occurred in the airways of three of 16 (19%) of the patients with *Aspergillus* cultured from BALF. None of the *Candida* species cultured from the bronchoscopic samples were related to invasive *Candida* infection.

Pneumocystis carinii

Pneumocystis carinii was detected in 23/472 (4.9%) of the bronchoscopic specimens in 11/40 (28%) of the patients. The characteristics of the episodes with *P. carinii* detected in bronchoscopic samples are presented in Table 4. *Pneumocystis carinii* was detected in five asymptomatic recipients (surveillance FOBs), while all the remaining patients had symptoms or radiographic infiltrate. Two recipients had *P. carinii* detected in the BALF before the initiation of prophylaxis during the first postoperative week and another two after the first postoperative year because of interrupted chemoprophylaxis. Of the infections 19/23 (82%) were detected during *P. carinii* prophylaxis. Three episodes occurred during the first 7 days after the initiation of co-trimoxazole and one before the recipient had received two doses of inhaled pentamidine (i.e. during the first 8 weeks of prophylaxis). Thus, 15/23 (65%) of the cases were breakthrough infections during adequate chemoprophylaxis (eight during co-trimoxazole and seven during inhaled pentamidine). When calculated within the whole study population, the frequency of *P. carinii* breakthrough infection was one infection in 91 months for patients receiving co-trimoxazole and one infection in 67 months for patients receiving inhaled pentamidine.

There were no precise treatment protocol for the breakthrough *P.carinii* infections. The treatment regimen chosen for the patient depended on the severity of the infection. Seven of the eight breakthrough infections during co-trimoxazole prophylaxis were treated with high doses of parenteral or oral sulfamethoxazole–trimethoprime for 2–3 weeks. One asymptomatic recipient recovered without therapy, but had a recurrent infection after 10 months. The seven breakthrough infections detected during inhaled pentamidine chemoprophylaxis were treated with high doses of sulfamethoxazole–trimethoprime (two cases), parenteral or daily inhaled pentamidine for 2 weeks (two cases), doubling the frequency of inhaled pentamidine prophylaxis (two cases) or oral primaquine + clindamycine for 3 weeks (one case). The eight recipients without adequate chemoprophylaxis and *P. carinii* infection received sulfamethoxazole–trimethoprime and pentamidine in four cases each. All recipients demonstrated a good clinical and microbiological response to therapy, but six of 11 recipients had recurrent *P. carinii* infections and two of them had even several recurrences despite adequate therapy and secondary prophylaxis.

In seven of 23 (30%) episodes a concomitant pathogen together with *P. carinii* was detected in BALF [bacteria (three), CMV (two), respiratory syncytial virus (one) and *A. fumigatus* (one)], and in three of 23 (13%) of the cases an AR (grades 2–3) was present.

Table 4. *Pneumocystis carinii* detected in bronchoscopic specimens.

	P. carinii prophylaxis			Total (% of *P. carinii* infections)
	TMP–SMZ	IP	None	
No.	11	8	4	23 (100%)
Type of FOB				
Clinically indicated FOB	9	5	4	18 (78)
Surveillance FOB	2	3	0	5 (22)
Infiltrate on HRCT or chest radiograph				
Infiltrate	7	4	2	13 (57)
No infiltrate	4	4	2	10 (43)
Time after transplantation				
<30 days	3	0	2	5 (22)
31–360 days	5	3	0	8 (35)
361–720 days	2	3	1	6 (26)
>720 days	1	2	1	4 (17)
P. carinii detected in				
BALF+/TBB not done	7	4	3	14 (61)
BALF+/TBB+	1	1	1	3 (13)
BALF+/TBB −	1	3	0	4 (17)
BALF−/TBB+	2	0	0	2 (9)

TMP-SMZ, co-trimoxazole; IP, inhaled pentamidine; FOB, fiberoptic bronchoscopy; HRCT, high-resolution computed tomography; BALF, bronchoalveolar lavage fluid; TBB, transbronchial lung biopsy.

In seven of 23 (30%) episodes a concomitant pathogen together with *P. carinii* was detected in BALF [bacteria (three), CMV (two), respiratory syncytial virus (one) and *A. fumigatus* (one)], and in three of 23 (13%) of the cases an AR (grades 2–3) was present.

Non-CMV viruses

Seventeen non-CMV virus infections were detected by FOB. Two herpes simplex virus infections and one varicella zoster virus infection developed during the immediate postoperative period. All the patients with non-CMV virus infection survived over 30 days, and none of the later deaths was related to the viral infection.

Cytomegalovirus

The CMV was demonstrated by a positive antigen test and culture in 32/472 (6.8%) and 135/472 (28.6%) of the BALFs respectively (Table 5). The positive predictive values for CMV pneumonia (the proportion of BALFs with a positive

Table 5. CMV detected by antigen test and culture from BALF.

	Clinically indicated FOBs			
	CMV-Pn. ($n = 7$) (%)	No CMV-Pn. ($n = 183$) (%)	Total ($n = 190$) (%)	Surveillance FOBs ($n = 282$) (%)
CMV antigen test in BALF				
Positive	6 (86)	19 (10)	25 (13)	7 (3)
Negative	1 (14)	161 (88)	162 (85)	269 (95)
NA	–	3 (2)	3 (2)	6 (2)
CMV culture* in BALF				
Positive	7 (100)	65 (36)	72 (38)	63 (22)
Negative	0 (0)	107 (58)	107 (58)	208 (74)
NA	–	11 (6)	11 (6)	11 (4)

CMV, cytomegalovirus; CMV-Pn., cytomegalovirus pneumonia; BALF, bronchoalveolar lavage fluid; NA, not available.
*Shell vial culture.

test result and CMV pneumonia present) by CMV antigen test and CMV culture in BALF from symptomatic recipients (clinically indicated FOB) were 24.0% and 9.7% respectively. Of the seven asymptomatic recipients with CMV demonstrated by a positive antigen test in BALF (surveillance FOB) five recovered without therapy, one was already receiving pre-emptive ganciclovir treatment, and the remaining patient developed CMV pneumonia despite the initiation of i.v. ganciclovir therapy because of the positive CMV antigen test in PB. The latter patient had also positive CMV culture from BALF, while none of the remaining asymptomatic recipients with CMV cultured from BALF developed CMV pneumonia in 30 days.

TBBs

Altogether 192 TBBs were performed (70 clinically indicated, 92 surveillance, and 30 follow-up TBBs).

The CMV-pp65 and *P. carinii* antigen were detected in three and five of the clinically indicated TBBs, respectively. However, all CMV pneumonias and three of five *P.carinii* infections were also diagnosed by BALF samples. Rejection was detected in 22/70 (31%) and eight of 92 (9%) of the clinically indicated and surveillance TBBs, respectively. All eight asymptomatic rejections diagnosed by surveillance TBBs were of grade 1 and none of the recipients received therapy. Two diagnoses were achieved by one TBB sample in four cases [*P. carinii* + AR (three) and CMV + AR (one)].

Thus, the overall diagnostic yield was 26/70 (37%) and eight of 92 (9%) for clinically indicated and surveillance TBBs, respectively ($P < 0.001$). None of the surveillance TBBs, but 19/70 (27%) of the clinically indicated TBBs directly changed the therapy chosen for the recipient.

Airway complications

Airway complications necessitating endobronchial intervention were detected in seven of 40 (18%) of the recipients and 13/472 (3%) of the bronchoscopies [anastomotic stricture (eight), endobronchial granulation tissue (three) and anastomotic dehiscence (two)]. Endobronchial obstruction was dilated with balloon and/or lazer in 10 cases and a stent was inserted in four occasions. *Aspergillus* was detected in the bronchoscopic samples of three of seven (43%) and 13/33 (39%) of the patients with and without anastomotic complications, respectively.

Complications

There were no fatalities associated with the FOBs. The complication rate for all the FOBs was 2.1% (13/609). Five recipients suffered from transient respiratory insufficiency demanding ventilatory assistance after the procedure and one patient had severe hypoxemia disturbing the FOB. Two pneumothoraces were detected after TBB (complication rate 1%), but both healed spontaneously without insertion of a chest tube. Four FOBs were prematurely interrupted because of bleeding and one local pulmonary hemorrhage was detected in CT scan after TBB. All bleeding episodes resolved without interventions and none led to hypotension or blood transfusion.

Discussion

We report data from all the consecutive bronchoscopies performed on LTX and HLTX recipients in a single institution over an 8-year period. The primary goal of this study was to evaluate the usefulness of FOB in the diagnosis and surveillance of respiratory infections. The procedures were classified as surveillance or clinically indicated FOBs based on a clinical, radiographic (CT scan) and functional (spirometry) evaluation of the recipients.

Clinically indicated FOB had a relatively high overall diagnostic yield of 61% and majority of the diagnoses were respiratory infections (50.5% of the FOB). This is in concordance with previous studies in which FOB with TBB and BAL samples revealed the diagnosis in 48–67% of LTX recipients with clinical suspicion of infection or rejection [3,4]. In contrast, only 15% of the surveillance FOBs established a specific diagnosis. None of the surveillance TBBs established grade ≥2 AR or respiratory infection. In the studies by Hopkins *et al.* [9], Baz *et al.* [4], and Guilinger *et al.* [25] the diagnostic yield for surveillance FOBs varied between 19% and 26%. The utility of FOB was highest from 1 to 6 months after transplantation. It can be explained by the high occurrence of

opportunistic respiratory infections as well as by ARs during this time interval. However, the diagnostic yield of surveillance FOB remained low in all time intervals.

Bacterial pneumonia is reported to be the most common infection after transplantation and its prevalence has been highest during the first 6 months after transplantation [19,26]. In our material bacteria were causative agents in 57/96 (59%) of all the symptomatic respiratory infections and 20 (35%) of them occurred <6 months after transplantation. *Pseudomonas aeruginosa* and *S. aureus* were the most common microbes detected in the present study. This is in line with the international reports [26].

Aspergillus was cultured from BALF and/or PSB samples in 10 of the surveillance FOBs and in seven of the clinically indicated FOBs, while only two of the isolates were associated to a proven invasive disease. Anastomotic complications occurred slightly more frequently in recipients with *Aspergillus* colonization, but did not reach statistical significance in our material. However, previous studies have shown that sole *Aspergillus* airway colonization without invasive aspergillosis is a risk factor for airway/anastomotic complications and possibly also for an invasive disease especially when present within 6 months after transplantation [16–18]. This may warrant the surveillance of bronchoscopic samples by fungal cultures in LTX recipients at least in the first six postoperative months for the early detection of *Aspergillus* and initiation of pre-emptive antifungal therapy.

Pneumocystis carinii was detected by bronchoscopic samples with a surprisingly high frequency (28%) in LTX and HLTX recipients, and the organism was often detected even in patients with adequate chemoprophylaxis. While *P. carinii* pneumonia (PCP) may occur in up to 80% of LTX recipients not receiving prophylaxis, co-trimoxazole, and to a lesser extent, inhaled pentamidine have been accepted as highly effective in preventing PCP [27–30]. However, a previous study by Halme *et al.* [12] from our own institution and a case report by Faul *et al.* [13] have shown that breakthrough infections may occur in LTX and HLTX recipients. *Pneumocystis carinii* was detected in the BALF from surveillance FOB in five asymptomatic recipients with no radiographic infiltrate present. The clinical significance of detecting *P. carinii* in asymptomatic patients is not known. It could be argued that these cases represented the result of airborne transmission of the organism and/or colonization of the recipient's airways rather than infection. All the remaining patients with *Pneumocystis carinii* detected in the bronchoscopic samples had respiratory symptoms and/or radiographic infiltrates and in nine of these episodes *P. carinii* was the only organism detected. Thus it is likely that most of the cases represented PCP. Significant mortality rates in PCP have been reported in patients without prophylaxis [31]. In contrast, all patients in our study responded to therapy suggesting that previous *P. carinii* prophylaxis may modify the clinical picture of PCP. *Pneumocystis carinii* was detected from the immediate postoperative period to more than 2 years post-transplantation. The result is in line with the recent recommendation to use lifelong *P. carinii* prophylaxis in LTX and HLTX recipients [27]. The prophylaxis should be initiated immediately after the transplantation. Breakthrough infections occurred more frequently in recipients with inhaled pentamidine. As co-trimoxazole has been shown to be the most effective agent in preventing PCP also in other immunocompromised patients, co-trimoxazole should be the first-line agent for *P. carinii* prophylaxis in LTX and HLTX recipients [27,30].

The role of community-acquired respiratory virus infections in LTX recipients is not clear, but some of the previous studies have shown a significant mortality and morbidity related to these infections [32–34]. None of the community-acquired respiratory virus infections in our study was fatal and three of them were asymptomatic. Isolation of these viruses in the BALF from surveillance FOBs may provide valuable information, as it is possible that there is an association to the development of bronchiolitis obliterans syndrome [19].

Detecting CMV by antigen test or culture in BALF had a poor positive predictive value for CMV pneumonia. CMV screening from PB by the antigen test or with other techniques (e.g. PCR) has allowed early diagnosis of CMV infection and initiation of pre-emptive antiviral therapy without invasive procedures [35]. When CMV antigen screening from PB is used, surveillance of CMV from BALF in asymptomatic recipients seemed to be of little additional value also in our material.

Only 13 (2.1%) clinically significant complications of FOB occurred. In the reports by Hopkins *et al.* [9] and Baz *et al.* [4] the complication rate was 4.4% and 6.4%, respectively, and bleeding was the most common complication in both studies. We did not routinely calculate the bleeding volume after the FOB in the study period, which may underestimate the complication rate in the present study compared with previous reports. However, bleeding leading to premature cessation of the procedure is included and none of the bleeding episodes led to major complication.

To conclude, FOB with BAL and/or TBB is a useful and safe tool in diagnosing infection in symptomatic LTX and HLTX recipients. The significance of surveillance FOBs remains controversial. Surveillance TBBs in particular, yield only little clinically significant information to support the performing of them. *Pneumocystis carinii* infections still occur despite the chemoprophylaxis justifying the use of FOB in the diagnosis and the surveillance of this organism.

Acknowledgements

This work was supported by grants from the Helsinki University Central Hospital Research Fund (EVO).

References

1. Trulock EP. Lung Transplantation. *Am J Respir Crit Care Med* 1997; **155**: 789.
2. Arcasoy SM, Kotloff RM. Lung transplantation. *N Engl J Med* 1999; **340**: 1081.
3. Chan CC, Abi-Saleh WJ, Arroliga AC, *et al.* Diagnostic yield and therapeutic impact of flexible bronchoscopy in lung transplant recipients. *J Heart Lung Transplant* 1996; **15**: 196.
4. Baz MA, Layish DT, Govert JA, *et al.* Diagnostic yield of bronchoscopies after isolated lung transplantation. *Chest* 1996; **110**: 84.
5. Sibley RK, Berry GJ, Tazelaar HD, *et al.* The role of transbronchial biopsies in the management of lung transplant recipients. *J Heart Lung Transplant* 1993; **12**: 308.
6. Kesten S, Chamberlain D, Maurer J. Yield of surveillance transbronchial biopsies performed beyond two years after lung transplantation. *J Heart Lung Transplant* 1996; **15**: 384.
7. Swanson SJ, Mentzer SJ, Reilly JJ, *et al.* Surveillance transbronchial lung biopsies: implication for survival after lung transplantation. *J Thorac Cardiovasc Surg* 2000; **119**: 27.
8. Valentine VG, Taylor DE, Dhillon GS, *et al.* Success of lung transplantation without surveillance bronchoscopy. *J Heart Lung Transplant* 2002; **21**: 319.
9. Hopkins PM, Aboyoun CL, Chhajed PN, *et al.* Prospective analysis of 1,235 transbronchial lung biopsies in lung transplant recipients. *J Heart Lung Transplant* 2002; **21**: 1062.
10. Nusair S, Kramer MR. The role of fibre-optic bronchoscopy in solid organ, transplant patients with pulmonary infections. *Respir Med* 1999; **93**: 621.
11. Baughman RP. Use of bronchoscopy in the diagnosis of infection in the immunocompromised host. *Thorax* 1994; **49**: 3.
12. Halme M, Lautenschlager I, Mattila S, Tukiainen P. Breakthrough *Pneumocystis carinii* infections in lung and heart-lung transplant patients with chemoprophylaxis. *Transplant Proc* 1999; **31**: 197.
13. Faul JL, Akindipe OA, Berry GJ, Doyle RL, Theodore J. Recurrent *Pneumocystis carinii* colonization in a heart-lung transplant recipient on long-term trimethoprim-sulfamethoxazole prophylaxis. *J Heart Lung Transplant* 1999; **18**: 384.
14. Egan JJ, Barber L, Lomax J, *et al.* Detection of human cytomegalovirus antigenaemia: a rapid diagnostic technique for predicting cytomegalovirus infection/pneumonitis in lung and heart transplant recipients. *Thorax* 1995; **50**: 9.
15. Egan JJ, Lomax J, Barber L, *et al.* Preemptive treatment for the prevention of cytomegalovirus disease: in lung and heart transplant recipients. *Transplantation* 1998; **65**: 747.
16. Nathan SD, Shorr AF, Schmidt ME, Burton NA. *Aspergillus* and endobronchial abnormalities in lung transplant recipients. *Chest* 2000; **118**: 403.
17. Nunley DR, Gal AA, Vega D, Perlino C, Smith P, Lawrence C. Saprophytic fungal infections and complications involving the bronchial anastomosis following human lung transplantation. *Chest* 2002; **122**: 1185.
18. Cahill BC, Hibbs JR, Savik K, *et al. Aspergillus* airway colonization and invasive disease after lung transplantation. *Chest* 1997; **112**: 1160.
19. Speich R, Van der Bij W. Epidemiology and management of infections after lung transplantation. *Clin Infect Dis* 2001; **33**(Suppl. 1): S58.
20. Nunley DR, Grgurich W, Iacono AT, *et al.* Allograft colonization and infections with pseudomonas in cystic fibrosis lung transplant recipients. *Chest* 1998; **113**: 1235.
21. Wilson R, Dowling RB. *Pseudomonas aeruginosa* and other related species. *Thorax* 1998; **53**: 213.
22. The TH, van den Berg AP, Harmsen MC, van der Bij W, van Son WJ. The cytomegalovirus antigenemia assay: a plea for standardization. *Scand J Infect Dis Suppl* 1995; **99**: 25.
23. Ascioglu S, Rex JH, De Pauw B, *et al.* Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7.
24. Yousem SA, Berry GD, Cagle PT. Revision of the 1990 working formulation for the classification of pulmonary rejection: lung rejection group. *J Heart Lung Transplant* 1996; **15**: 1.
25. Guillinger RA, Paradis IL, Dauber JH, *et al.* The importance of bronchoscopy with transbronchial biopsy and bronchoalveolar lavage in the management of lung transplant recipients. *Am J Respir Crit Care Med* 1995; **152**: 2037.
26. Trulock EP. Flexible bronchoscopy in lung transplantation. *Clin Chest Med* 1999; **20**: 77.
27. Fishman JA. Prevention of infection caused by *Pneumocystis carinii* in transplant recipients. *Clin Infect Dis* 2001; **33**: 1397.
28. Gordon SM, LaRosa SP, Kalmadi S, *et al.* Should prophylaxis for *Pneumocystis carinii* pneumonia in solid organ transplant recipients ever be discontinued? *Clin Infect Dis* 1999; **28**: 240.
29. Nathan SD, Ross DJ, Zakowski P, Kass RM, Koerner SK. Utility of inhaled pentamidine prophylaxis in lung transplant recipients. *Chest* 1994; **105**: 417.
30. Schneider MME, Hoepelman IM, Schattenkerk JKME, *et al.* A controlled trial of aerosolized pentamidine or trimethoprim-sulfamethoxazole as primary prophylaxis against *Pneumocystis carinii* pneumonia in patients with

human immunodeficiency virus infection. *N Engl J Med* 1992; **327**: 1836.
31. Roblot F, Godet C, Le Moal G, *et al.* Analysis of underlying disease and prognosis factors associated with *Pneumocystis carinii* pneumonia in immunocompromised HIV-negative patients. *Eur J Clin Microbiol Infect Dis* 2002; **21**: 523.
32. Palmer SM, Henshaw NG, Howell DN, Miller SE, Davis RD, Tapson VF. Community respiratory viral infection in adult lung transplant recipients. *Chest* 1998; **113**: 944.
33. Holt ND, Gould FK, Taylor CE, *et al.* Incidence and significance of noncytomegalovirus viral respiratory infection after adult lung transplantation. *J Heart Lung Transplant* 1997; **16**: 416.
34. Wendt CH, Fox JMK, Hertz MI. Paramyxovirus infection in lung transplant recipients. *J Heart Lung Transplant* 1995; **14**: 479.
35. Feinstein MB, Mokhtari M, Ferreiro R, Stover DE, Jakubowski A. Fiberoptic bronchoscopy in allogenic bone marrow transplantation. findings in the era of serum cytomegalovirus antigen surveillance. *Chest* 2001; **120**: 1094.