Surveillance bronchoscopy in children during the first year after lung transplantation: is it worth it?

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Thorax 2007;62:57-61; originally published online 23 Aug 2006;
doi:10.1136/thx.2006.063404

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Lung transplantation has become an accepted treatment option for end-stage lung disease even in children, but the overall survival remains poorer compared with other solid organ transplants.\(^1\) Most early deaths are related to overwhelming infection in the immunocompromised host, accounting for almost 50% of deaths in the first year after transplant.\(^2\) The International Society of Heart and Lung Transplantation (ISHLT) Eighth Official Registry Report 2005 showed that infection remains a major cause of mortality throughout the entire follow-up period, even if cytomegalovirus (CMV) infection is excluded. Acute cellular rejection (ACR) accounts for 4% of deaths up to 1 year after transplant. Bronchoscopies were classified according to whether subjects had symptoms, defined as the presence of cough, sputum production, dyspnoea, malaise, decrease in lung function or chest radiograph changes.

**Results:** Results of biopsies and BAL were collected, and procedural complications recorded. 23 lung-transplant operations were performed, 12 DLTx, 10 heart–lung transplants and 1 SLTx (15 female patients). The median (range) age of patients was 14.0 (4.9–17.3) years. 17 patients had cystic fibrosis. 95% of the birth group received transbronchial biopsy has been performed in all paediatric recipients of lung and heart–lung transplants at the Great Ormond Street Hospital for Children, London, UK, using a newly revised treatment protocol.

**Methods:** Surveillance bronchoscopies were performed at 1 week, and 1, 3, 6 and 12 months after transplant. Bronchoscopies were classified according to whether subjects had symptoms, defined as the presence of cough, sputum production, dyspnoea, malaise, decrease in lung function or chest radiograph changes.

**Conclusions:** Many children have silent rejection or subclinical infection in the first year after lung transplantation. Routine surveillance bronchoscopy allows detection and targeted treatment of these complications.
demographics (sex, age), patients’ underlying diagnosis and type of transplant. All transplants were matched for blood group, but not for human leucocyte antigen. This study was approved by the Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health Research Ethics Committee.

Transplant surgery was performed in standard fashion. All patients received basiliximab as induction therapy, then triple immunosuppression with tacrolimus, azathioprine and prednisolone according to our standard protocol (available on request). We aim for tacrolimus trough concentrations of 12–15 ng/ml in the first 3 months after transplant, followed by target concentrations of 10–12 ng/ml for up to 1 year after transplant, and 8–10 ng/ml thereafter. Prednisolone is tapered down to 0.5 mg/kg/day in the first 6 weeks after transplant, then down to 0.25 mg/kg/day for 6 months, and maintained on 0.1–0.15 mg/kg/day indefinitely. A minimum of two anti-pseudomonal antibiotics was used in transplant recipients with cystic fibrosis, based on the recipient’s most recent sputum culture results before transplant. Intravenous liposomal amphotericin was used in all transplant recipients with cystic fibrosis for the first week after transplant. Patients with cystic fibrosis and isolation of atypical mycobacteria before transplant were started on appropriate anti-mycobacterial therapy perioperatively. In addition, all transplant recipients received teicoplanin on induction of anaesthesia and soon after transplant. All patients were started on anti-infective prophylactic treatment with co-trimoxazole and aciclovir, which were then down to 0.25 mg/kg/day for 6 months, and maintained on 0.5 mg/kg/day in the first 6 weeks after transplant, followed by target concentrations of 10–12 ng/ml for up to 1 year after transplant, and 15 ng/ml in the first 3 months after transplant, followed by grade A2 = mild; grade A3 = moderate; and grade A4 = severe.2 In addition, further aspects of pulmonary allograft rejection were also graded according to the above ISHLT criteria: grade B0 = active airway damage without scarring; grade B1 = lymphocytic bronchiolitis; and grade B2 = lymphocytic bronchiolitis.12

BAL was performed by wedging the tip of the bronchoscope in the right upper lobe, right middle lobe or in the lingula. A volume of 10–20 ml of sterile 0.9% sodium chloride was instilled as one aliquot and retrieved by manual suction. BAL fluid was collected in a sterile container and processed. BAL fluid was examined microscopically as a direct wet preparation and after gram and Zielh–Neelsen staining. BAL fluid was cultured semiquantitatively for bacteria and fungi including specific culture for mycobacteria and Legionella pneumophila. The isolation of coagulase negative staphylococci on BAL culture was considered to be of doubtful clinical significance unless the pathogen was simultaneously cultured on peripheral blood culture. Isolation of viridans streptococci (normal mouth flora) was not regarded as of clinical relevance. Candida albicans and Candida species (non-albicans) were considered clinically relevant if there was additional evidence of invasive fungal infection (positive histopathology or blood culture). Blood cultures were taken only from children with clinical signs of sepsis, defined as pyrexia (peripheral body temperature >37.5°C), raised blood inflammatory markers or malaise.

The immunofluorescence technique was used for detecting adenovirus, influenza virus type A and B, and influenza virus type 1, 2 and 3, respiratory syncytial virus and Pneumocystis carinii in the BAL fluid. The early antigen fluorescent foci (DEAFF) test was used for detecting CMV in this study. Supplementary testing for viral DNA (CMV, Epstein–Barr virus, adenovirus) by PCR was performed if indicated, using qualitative PCR in the early part of the study (until 2002) and quantitative real-time PCR thereafter (since 2003).

All procedural complications were recorded.

Statistical analysis

The diagnostic yield of bronchoscopies in the two groups of subjects was compared using the χ² test, incorporating Yates’ correction for continuity. Significance was accepted for p<0.05.

RESULTS

Study population

A total of 23 lung-transplant operations were performed between January 2002 and December 2005, of which 12 (52%) were DLTx, 10 (43%) heart–lung transplants and 1 (4%) SLTx. The SLTx was a boy with cystic fibrosis who had previous pneumonectomy. The median (range) age at transplant was 14.0 (4.9–17.3) years; there were 15 (65%) female and 8 (35%) male patients.

In all, 17 (74%) patients had cystic fibrosis, of which 14 were ΔF508-homozygotic. Five (22%) patients had primary or...
secondary pulmonary hypertension and 1 (4%) had end-stage lung disease secondary to post-adenosirus lung damage and chronic aspiration pneumonia in infancy. Six patients with cystic fibrosis had chronic lung infection with *Pseudomonas aeruginosa* only three had chronically grown *P. aeruginosa* and *Staphylococcus aureus*, one had *S. aureus* only, one had methicillin-resistant *S. aureus* and one had chronic lung infection with *P. aeruginosa* and *Stenotrophomonas maltophilia*. Specimens from three transplant recipients with cystic fibrosis were found to have atypical mycobacteria before transplant on sputum culture, of which one had isolated Mycobacterium avium intracellulare and two had isolated Mycobacterium abscessus. One transplant recipient with cystic fibrosis had chronic lung infection with *Burkholderia multivorans* before transplant.

No patient died within the first year after transplant. However, one male patient with cystic fibrosis died at the age of 17.2 years due to fungal sepsis, 23 months after left SLTx and one female patient aged 16.7 years died 27 months after DLTx for cystic fibrosis due to chronic graft failure.

Transbronchial biopsy and BAL results

A total of 95 bronchoscopies with biopsies and BAL were performed as routinely scheduled surveillance procedures, of which 69 (73%) were performed in subjects classified as asymptomatic and 26 (27%) in those with symptoms. A median of five biopsies were performed per patient (range 2–5).

ACR ≥ A2 was diagnosed in 3 of 69 (4%) biopsies of asymptomatic subjects, and in 3 of 26 (12%) biopsies of subjects with symptoms (table 1). These episodes of ACR were treated with a minimum 3-day course of high-dose intravenous methylprednisolone (10 mg/kg/day). In addition, ACR = A1 was diagnosed in 5 of 69 (7%) biopsies of asymptomatic children, and in 4 of 26 (15%) of biopsies of children with symptoms. In response to these results, maintenance immunosuppression was tapered down more slowly. In one case, a grade A1 rejection in an asymptomatic patient 1 week after transplant progressed to a grade A2 rejection on surveillance biopsy follow-up 1 month after transplant. At that time, the child presented with respiratory symptoms and decreased lung function.

In nine patients, biopsy samples showed lymphocytic bronchitis/bronchiolitis (grade≥B1). However, immunosuppression was not augmented in these patients.

Due to small biopsy sample size, no grading (ISHLT Lung Rejection Study Group classification of pulmonary allograft rejection) was given for 11 patients. However, there was no histological evidence of ACR in any of these patients. Seven of the subjects were classified as asymptomatic and four as having symptoms. If these 11 patients were excluded from the analysis, ACR= A2 was diagnosed in 3 of 62 (5%) biopsies in asymptomatic subjects and in 3 of 22 (14%) of biopsies subjects with symptoms. In only one of these cases was a paediatric bronchoscope used.

Bacterial, viral or fungal pathogens were detected in 20 of 69 (29%) asymptomatic children and in 18 of 26 (69%) children with symptoms (table 1). More than one pathogen was detected in 11 patients.

Bacteria were the most frequently isolated pathogens in BAL fluid. Bacterial pathogens were detected in 16 of 69 (23%) asymptomatic children, and in 17 of 26 (65%) children with symptoms. Table 2 lists the different bacterial pathogens. Of the bacterial pathogens, >95% were detected in lung transplant recipients with cystic fibrosis; no bacteria were detected in blood cultures taken simultaneously. All children with isolation of bacterial pathogens in BAL fluid were treated with either an appropriate oral or intravenous antibiotic course even if infection was subclinical.

Viral pathogens were detected in 4 of 69 (6%) asymptomatic children, and in 4 of 26 (15%) children with symptoms. Table 2 lists the viral pathogens. All children detected with CMV in BAL fluid were donor–recipient CMV mismatches; two children had a breakthrough CMV infection despite oral prophylactic ganciclovir or valganciclovir. All patients detected with CMV received a 2–3-week course of intravenous ganciclovir followed by oral ganciclovir or valganciclovir for a further 3 months. CMV monitoring in peripheral blood was performed up to twice weekly in these patients until CMV DNA was undetectable.

Fungi were detected in four patients: two asymptomatic children and two children with symptoms. Table 2 lists the fungal species. None of the fungal pathogens were related to invasive fungal infection.

*P. carinii* was detected in only one case of an asymptomatic child 6 months after transplant; this female patient with pulmonary hypertension received a sequential bilateral lung transplant at the age of 4.9 years. In this case, co-trimoxazole prophylaxis had been withheld for 2 months due to drug-induced neutropenia. The patient was treated with high-dose co-trimoxazole (120 mg/kg/day) for 3 weeks after detection of *P. carinii* in the BAL fluid. Airway complications that needed intervention therapy were detected in two patients (one anastomotic airway stenosis and one bronchomalacia of the left main bronchus). Balloon dilatation was successfully performed to overcome the anastomotic stenosis; a stent insertion was required to improve the airway obstruction due to bronchomalacia.

### Table 1 Results of transbronchial biopsy and bronchoalveolar lavage in asymptomatic subjects (group 1) and subjects with symptoms (group 2)

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute rejection (≥A2)</td>
<td>3 (4)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Acute rejection (≥A1)</td>
<td>5 (7)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Isolation of pathogen</td>
<td>20 (29)</td>
<td>18 (69)</td>
</tr>
<tr>
<td>ACR/isolation of pathogen</td>
<td>24 (35)</td>
<td>22 (85)*</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>26</td>
</tr>
</tbody>
</table>

ACR, acute cellular rejection. *p<0.001.

### Table 2 Bacterial, viral and fungal pathogens detected in the bronchoalveolar lavage specimens of all 95 bronchoscopies performed in the study

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>Pandoraer species</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Achromobacter xylosidans</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Raltinia pickettii</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Burkholderia multivorans</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Morganella morgani</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas species</em></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>Viridans streptococci</em></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>Cytomegalovirus</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>Parainfluenza virus 2</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Influenza virus A</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Influenza virus B</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans/non-albicans</em></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Including two methicillin-resistant *Staphylococcus aureus*. 

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The overall diagnostic yield was 35% (24/69) for asymptomatic children and 85% (22/26) for children with symptoms (p<0.001).

No lethal complications occurred due to surveillance bronchoscopies. One patient had moderate bleeding after biopsy (estimated blood loss 100–200 ml), but no blood transfusion was indicated. A pneumothorax requiring chest tube insertion occurred in one case. One patient had a minor aspiration after anaesthesia in the recovery room while sitting upright. A chest radiograph showed radiological features compatible with right lower lobe aspiration pneumonia in this case, but no treatment was required. The overall complications rate was 3.2%.

**DISCUSSION**

To our knowledge, this is the first study comparing the prevalence of ACR and subclinical graft infection in asymptomatic and symptomatic paediatric lung transplant recipients in the era of modern immunosuppression in a single centre applying a uniform management approach. This retrospective analysis has shown that many children have silent rejection or subclinical infection in the first year after lung transplantation. Surveillance bronchoscopies have been used as a therapy for end-stage lung disease since the early 1980s, and has by now become an accepted therapeutic option, even in the paediatric age group. Despite improved short-term survival due to improved surgical techniques, organ preservation and intensive care, long-term outcome after lung or heart–lung transplantation remains poor. The major obstacles to improved long-term survival are ACR and infection, and the subsequent development of bronchiolitis obliterans. The underlying mechanism of this graft deterioration is unknown, but many risk factors are reported. The single, most important factor for the development of bronchiolitis obliterans is the frequency and severity of ACR. However, non-alloimmunological factors such as bacterial, viral and fungal infections may also have a role. It remains uncertain whether ACR in children is more frequent compared with adults. It is important to recognise that differentiation between ACR and infection in children and adults is nearly impossible, as clinical signs and symptoms are non-specific in both groups. However, it is unknown whether children report symptoms less frequently compared with adults. As there is a lack of a valuable surrogate marker to reliably distinguish ACR from infection, bronchoscopy with biopsy and BAL has remained the gold standard.

By means of routine surveillance bronchoscopies, we aim to reduce the incidence of bronchiolitis obliterans due to earlier detection of ACR and subsequent augmentation of immunosuppression. However, the role of surveillance bronchoscopies with biopsy and BAL in asymptomatic lung transplant recipients has remained a controversial issue up to now. Valentine et al estimated that >50% of adult lung transplant centres in the ISHLT perform regularly scheduled bronchoscopies. According to a recent informal survey of paediatric lung transplant centres participating in the International Paediatric Lung Transplant Collaborative, three quarters of the centres perform routine surveillance bronchoscopies for at least the first year after transplant. Three centres continue surveillance bronchoscopies for an extended period >1 year (A Faro, personal communication, 2005).

To our knowledge, there are 11 published studies (four prospective, seven retrospective) regarding the role of bronchial biopsies in lung transplant recipients. The studies have different study designs, end points and durations of follow-up. Taking these studies together, surveillance biopsies detected ACR (grade A2) in approximately 13.6% of clinically silent cases, the majority within the first 4–6 months after transplant. Previous studies by Valentine et al and Tamm et al reported the success of lung transplantation in adult recipients without surveillance biopsies, although these studies have limitations. Valentine et al used pooled data from the ISHLT Registry as their control group and therefore it is not a homogeneous cohort. Tamm et al used historical controls instead.

There is only one published study investigating the role of surveillance bronchoscopies in detecting ACR in children after lung transplantation. Visner et al from the University of Florida reported the incidence of ACR (grade A2) in 393 biopsies performed in paediatric lung transplant recipients. Only 25% of biopsies were performed for surveillance, but 38% as ACR treatment follow-up and 37% in patients with symptoms. ACR was found in 24% of surveillance procedures. Results of BAL to rule out infection were not reported. The incidence of complications in this study was 1.8%.

We found an incidence of ACR (grade A2) of 12% in children with symptoms compared with 4% in asymptomatic children. In addition, the incidence of ACR (grade A1) was 15% in patients with symptoms and 7% in asymptomatic patients.

The importance of grade A1 ACR remains controversial, although a previous prospective study from Toronto reported that 22% of grade A1 rejection episodes progressed to a higher-grade ACR within 3 months. We found progression of grade A1 ACR to a higher-grade ACR in only one case.

In our study, we detected lymphocytic bronchitis/bronchiolitis (grade B1) in nine biopsies, just more than half in asymptomatic children. The relevance of lymphocytic bronchitis/bronchiolitis is unknown. Previously, Yousem reported the likelihood of ACR following the development of lymphocytic bronchitis, which responds to augmented immunosuppression, but further details are not known.

We report evidence of infection (bacterial, viral or fungal) in more than two thirds of children with symptoms, and also isolation of pathogens in nearly one third of asymptomatic children. Without the use of routine surveillance bronchoscopies with BAL, subclinical infection would have remained undetected in many cases.

Apart from CMV, infection accounts for 37% of deaths during the first year after transplant, and for 19% between 3 and 5 years post transplant. The risk of infection is much higher after lung transplantation compared with any other type of solid organ transplantation. In addition, the potential role of pathogens in the pathogenesis of bronchiolitis obliterans remains unclear. Bacterial and fungal infections are not known directly to contribute to the pathogenesis of bronchiolitis obliterans, although it may increase the risk of ACR. P. aeruginosa is often isolated from lung transplant recipients. It has been suggested that infections caused by pathogens such as P. aeruginosa may be linked to bronchiolitis obliterans, but this remains uncertain. In contrast, CMV pneumonitis was correlated with the development of bronchiolitis obliterans in previous studies.

Our study has some limitations. The sample size of our study population is small, and there is a lack of long-term follow-up. Despite this, our study offers the unique opportunity to show the positive effect of modern immunosuppression and a uniform management approach including routine surveillance bronchoscopy on long-term results in children after lung transplantation. The effect of routine surveillance bronchoscopy on long-term graft survival remains unproved though. Only 13 bronchoscopies with BAL and biopsies were performed in children at 12 months after transplant. Most subjects were asymptomatic (10/13). Nevertheless, five asymptomatic children had silent rejection or subclinical infection. The small number of subjects precludes statistical analysis, but these data suggest that surveillance bronchoscopy at 12 months after...
transplant is justified. It could also be argued that the role of surveillance bronchoscopy in monitoring children >12 months after transplant should be re-examined.

In this study, the DEAFF test was routinely used for detecting CMV in BAL fluid, and for supplementary testing of quantitative viral load by real-time PCR in plasma, if indicated. However, Westall et al. recently showed that quantitative PCR analysis of CMV load in BAL fluid and the application of a diagnostic threshold are better predictors for the presence of histologically proved CMV infection, compared with detection of viral load in plasma. In our institution, quantitative measurements of CMV load by real-time PCR in BAL fluid are now routinely performed.

In conclusion, our data suggest that many children have silent rejection or subclinical infection in the first year after lung transplantation. Routine surveillance bronchoscopy allows detection and targeted therapy of these complications, and may therefore improve the long-term outcome after transplant.

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Funding: Research at the Institute of Child Health and Great Ormond Street Hospital for Children National Health Service Trust benefits from Research and Development funding received from the National Health Service Executive.
Competing interests: None.

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