Clinical outcome following lung transplantation in patients with cystic fibrosis colonised with *Burkholderia cepacia complex*: results from two French centres

V Boussaud, R Guillemain, D Grenet, N Coley, R Souilamas, P Bonnette, M Stern

ABSTRACT

**Background:** Infection with *Burkholderia cepacia* complex (BCC) is a life threatening complication of cystic fibrosis (CF), often seen as a contraindication for lung transplantation.

**Methods:** A long term retrospective study was conducted of all patients with CF undergoing lung transplants from January 1990 to October 2006 in two French centres allowing transplantation in patients colonised with BCC.

**Results:** 22 of the 247 lung transplant patients with CF were infected with BCC (*B. cepacia* genomovar III (*n* = 8), *B. multivorans* genomovar II (*n* = 11), *B. vietnamiensis* genomovar V (*n* = 2) and *B. stabilis* genomovar IV (*n* = 1)). BCC colonisation was not associated with any significant excess mortality (HR 1.5, 95% CI 0.7 to 3.2; *p* = 0.58). However, early mortality rates tended to be higher in the BCC group than in the non-BCC group (3 month survival: 85% vs 95%, respectively; log rank *p* = 0.05). Univariate analysis showed that the risk of death was significantly higher for the eight patients infected with *B. cepacia* than for the other 14 colonised patients (HR 3.2, 95% CI 1.1 to 5.9; *p* = 0.04). None of the other risk factors tested—primary graft failure, late extubation, septicaemia—had a significant effect. The 5 year cumulative incidence rate of bronchiolitis obliterans syndrome was not significantly higher in the BCC group than in the non-BCC group (38% vs 24%, respectively; *p* = 0.35).

**Conclusion:** Our results suggest that BCC infection with a non-genomovar III organism may not be associated with excess mortality after lung transplantation in patients with CF and should not be seen as sufficient reason to exclude lung transplantation. However, colonisation with *B. cepacia* remains potentially detrimental.

Cystic fibrosis (CF) is the most common inherited lung disease. It frequently progresses to respiratory failure and death and the treatments currently available aim to slow progression through respiratory therapy, good nutritional support and the treatment of pulmonary exacerbations. In patients with end stage CF, lung transplantation may help to prolong survival and ensure that patients have an acceptable quality of life. Survival rates 1, 3 and 5 years after transplantation of 76%, 60% and 49%, respectively, have been reported.1 Organ shortages have led some physicians to try to select the “best candidates” for lung transplantation.2 One of the criteria used in pretransplantation selection is the absence of infection with *Burkholderia cepacia* complex (BCC). This gram negative bacterium causes serious opportunistic infections and has emerged as a major pathogen in patients with CF, with the clinical outcome of infection varying from long term bronchial colonisation to life threatening necrotising pneumonia and sepsis, known as “cepacia syndrome”. Global mortality rates for BCC colonisation may reach 50%,3 depending on genomovar type. However, these rates were determined for patients who had not yet undergone transplantation. Conflicting results have been published concerning post-transplantation outcome.10–14 Nonetheless, BCC colonisation remains a contraindication in many transplantation centres worldwide, including the USA, which performs the largest number of transplant operations of any country worldwide.

The purpose of this study was to determine whether patients infected with BCC had a worse outcome after transplantation than those harbouring other common bacteria, to confirm the influence of BCC genomovar and to identify factors predictive of outcome.

**METHODS**

**Study population**

We retrospectively reviewed the medical records of all patients with CF undergoing lung transplantation at Georges Pompidou European Hospital (HEGP) and Foch Hospital (HF), Paris, France, from January 1990 to October 2006. These centres allow transplantation in BCC colonised patients: all patients identified as being chronically infected with BCC were included in the transplantation programme. These two French centres are responsible for almost all lung transplant operations in patients with CF and BCC infection in France.

**Graft procedure and monitoring**

Lung transplantation procedures were systematically performed under cardiopulmonary bypass (CPB) for patients at HEGP, whereas CPB was carried out selectively in patients at HF. Methylprednisolone was administered during the operative period (500 mg during the preoperative phase then 500 mg during CPB), and was subsequently replaced by prednisolone (1 mg/kg/day for the first month, with gradual tapering to 5–10 mg/day). Calcineurin inhibitors (ciclosporin or tacrolimus) were initially given intravenously on day 1, and were then administered orally between days 1 and 8. Daily doses were adjusted to obtain plasma concentrations of between
200 and 350 ng/ml for ciclesporin and between 8 and 13 ng/ml for tacrolimus. Azathioprine was administered at a dose of 1.5–2 mg/kg/day. After 2000, azathioprine was replaced by mycophenolate at HEGP. Cytolytic induction therapy with rabbit antithymocyte globulin was systematically administered during the first 5 days. Induction therapy was discontinued after 2001 at HF but is still administered at HEGP. Despite the use of these slightly different approaches, the results from the two centres were pooled.

Patients with CF generally harbour bacterial strains with multiple antibiotic resistance. They therefore receive prophylaxis with two or three antibiotics for 10–15 days. All patients colonised with BCC received at least three intravenous antibiotics (beta lactam + aminoside + quinolone) for 10–30 days after surgery, according to postoperative bacteriological documentation. Aerosolised colomycin and tobramycin were administered after extubation. Intravenous implantable devices were systematically removed at the time of surgery for all BCC infected patients. For CMV mismatch patients (donor positive/recipient negative), gancyclovir was administered after extubation. Intravenous implantable devices were systematically removed at the time of surgery for all BCC infected patients. For CMV mismatch patients (donor positive/recipient negative), gancyclovir was administered intravenously at a dose of 5 mg/kg twice daily and then orally for 3 months. Since 2001, this gancyclovir treatment has been replaced by valgancyclovir (900 mg/day) for at least 3 months. Prior or postoperative colonisation with Aspergillus species was treated with itraconazole or voriconazole (after 2003) for up to 6 months. Finally, cotrimoxazole or inhaled pentamidine was administered for an indefinite period after day 14.

Surveillance bronchoscopy, with bronchoalveolar lavage (BAL) and transbronchial biopsy, was routinely carried out on day 7 and after 1, 3, 6, 9, 12 and 24 months. After 2000, HEGP patients systematically underwent transbronchial biopsy in the first month and thereafter only if acute rejection was suspected. BAL and sputum samples were sent for bacteriological examination and testing for susceptibility to antimicrobial agents.

### Data collection

Epidemiological data on *Burkholderia cepacia* were obtained from the National Observatory for Cepacia database. All BCC isolates were identified by amplified rDNA restriction analysis. All *B. cenocepacia* genomovar III strains were *cbl*-negative. These strains, with no cable pili, are different from the ET-12 clone responsible for a North American outbreak. In this group of strains, phylogenetic analysis of the recA cluster revealed a predominance of III-A strains, with only one III-B strain. Clinical data were obtained from the patients’ medical records. Follow-up data were obtained from medical charts.

Sputum, BAL fluid and blood samples were systematically cultured, using selective media for BCC. Cefazidime, tobramycin, ciprofloxacin, aztreonam, imipenem, colomycin and cotrimoxazole were tested, along with other antibiotics, such as chloramphenicol, temocillin, meropenem and doxycycline, if required. BCC was defined as panresistant if no antibiotic was active against the strain, and multiresistant if it was resistant to all beta lactams (including temocillin and meropenem).

Infectious complications, primary graft failure (PGF), incidence of bronchiolitis obliterans syndrome (BOS), survival and causes of death were analysed for all Patients with CF undergoing lung transplantation, and patients with and without BCC infection were compared.

### Statistical analysis

Continuous data were summarised by calculating the mean (SD) or median (25% percentile; 75% percentile) as appropriate. Data for recipients with and without BCC colonisation were then compared by univariate analysis. Survival analysis was carried out by plotting Kaplan–Meier curves and comparing distributions by means of the log rank test; Mantel–Haenszel hazard ratios and associated 95% confidence interval (CI) were calculated. We also estimated the BOS specific cumulative incidence rate, considering death as a competing risk. We

### Table 1 Characteristics of patients infected with *Burkholderia cepacia* complex (BCC)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>BCC</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Susceptibility</th>
<th>Induction therapy</th>
<th>AR (days)</th>
<th>BO (months)</th>
<th>Status</th>
<th>Survival (y)</th>
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<td>–</td>
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AR, acute rejection; ARF, acute respiratory failure; BO, bronchiolitis obliterans; MOF, multiple organ failure; MR, multiresistant; NA, not available; PGF, primary graft failure; PR, panresistant; Retranspl, retransplantation.
assessed the equivalence of subdistributions between patients with and without BCC colonisation, using Gray’s test. Statistical analysis was carried out with Graphpad and R software. A p value less than 0.05 was considered statistically significant.

RESULTS

Demographics

Between January 1990 and October 2006, 495 patients underwent lung transplantation; 247 of these patients had CF (126 men and 121 women, mean age 21.5 (7.7) years) and were included in the study. Surgical procedures were as follows: 213 bilateral lung transplantations, 24 combined lung–liver, six combined heart–lung–liver and four heart–lung transplant procedures. There were nine retransplantation procedures: two heart–lung–liver and four heart–lung transplantations. One patient developed uncontrollable B cepacia sepsis and died in intensive care within 2 months of transplantation. One patient developed B cepacia empyema after 1 month and recovered after surgical treatment. All patients with recurrences of BCC colonisation were treated only if infection was confirmed by clinical, radiological and bacteriological data.

Outcome

In total, 109 recipients with CF died. There were 97 deaths in the non-BCC group and 12 in the BCC group. Three of the 12 deaths in the BCC group were directly related to BCC whereas the other nine were caused by PFG in one case, unknown causes (7 months after transplantation) in one case, complications of BOS in five cases and acute respiratory failure, 10 months and 2 years after transplantation, in two cases (table 1). Median survival time was 79 months for non-BCC patients and 43 months for BCC patients. Overall mortality risk was similar in the two groups (hazard ratio (HR) for death of BCC patients 1.4, 95% CI 0.7 to 3.2; log rank test p = 0.53) but early mortality rates tended to be higher in the BCC group (3 month survival 85% in the BCC group vs 95% in the non-BCC group; log rank test p = 0.05).
DISCUSSION

Chronic infection with multiresistant BCC is associated with poor outcome in patients with CF and may represent a threat to recipients on high doses of immunosuppressive treatment. This large study suggests that patients infected with BCC are at no greater risk than uninfected patients of death following lung transplantation. Excess mortality was observed in the genomovar III subgroup with respect to genomovars II, IV and V. Nonetheless, some patients in this subgroup survived for prolonged periods and died from causes other than cepacia complications (one patient died after nearly 10 years from multiple organ failure after retransplantation). However, this study is subject to several limitations: differences in mortality between BCC and non-BCC patients must be interpreted with caution because of the small number of patients in the BCC group; we cannot exclude the possibility that our study has insufficient power. Multivariate analysis was not possible. In addition, the two centres differed in terms of the treatment given, particularly with regard to cytolytic induction therapy and the duration of antibiotic treatment. Furthermore, 90 day mortality rates in the non-BCC group were particularly high because the study was retrospective and included data from the first lung transplantation procedures for which postoperative mortality rates were particularly high.

The prevalence of BCC infection is 5% in France and 3.1% in North America. However, some regions of North America have a particularly high prevalence, reaching 20% in the USA and 15% in Canada. Revisions of *Burkholderia* taxonomy have led to the definition of nine different species, the virulence and clinical impact of which remain unclear. *B cepacia* (genomovar III) accounts for more than half of all isolates worldwide—83% of all isolates in Canada, 50% in the USA and the UK—and has been associated with higher mortality and morbidity. *B multivorans* (genomovar II) accounts for one-third of US isolates and is believed to be less virulent. In France, 16S rRNA analysis has shown these genomovars to have similar prevalences: 49.5% for genomovar III and 41.1% for genomovar II.

Several putative virulence factors other than genomovar have been identified: expression of a cable-like pilus, interleukin 8 stimulation and proinflammatory activity, production of haemolytic toxin, proteases and escape from host cell microbicidal activity by means of delayed phagosome maturation. The promotion of biofilm development may also increase the resistance and tolerance of BCC to antibiotics. Several studies have investigated the impact of *Burkholderia cepacia*, with conflicting results. In 1993, Snell et al reported their results for patients with CF with positive cultures for *B cepacia*. 25% of such patients died from *B cepacia* pneumonia within 2 months of transplantation. UK centres gave more promising results, with no differences in mortality observed. In Toronto, where the incidence of *B cepacia* is high (52% of the 53 patients undergoing transplantation), Chaparro et al found evidence for excess mortality (68% vs 36%) within 3 months of transplantation (60% of all deaths) and an excess risk of BOS. These findings were consistent with other North American data establishing a link with genomovar type. The work of De Soya et al supported these findings, with 100% mortality observed in patients infected with *B cepacia* (genomovar III). In France, 120 patients were recorded on the Observatory for Cepacia register in 2004. Mean age at first colonisation was 14.7 (7.9) years, with a higher proportion of men (70 vs 50), particularly for *B cepacia* isolates (genomovar III). Three cases of spontaneous septicaemia were observed, with one death.

**Specificity of *B cepacia* genomovar III**

Patients infected with *B cepacia* (n = 8) had higher mortality rates than patients infected with other strains of BCC (n = 14) (HR for death of cepacia infected patients vs others 3.2 (95% CI 1.1 to 15.9); p = 0.04) (figs 1, 2). Six of the eight patients died, with three of these deaths directly linked to *B cepacia* infection, during the postoperative period. Surgery was difficult in two of these patients. One patient had a thoracic malformation causing technical problems, suffered bleeding and received a graft from a borderline donor with condensation of the left lower lobe, leading to grade 4 PGF. She developed immediate pulmonary and pleural infection and septicaemia refractory to combinations of multiple antibiotics. The second patient suffered from cardiac arrest, air embolism, severe pulmonary hypertension and grade 4 PGF. She developed pulmonary opacities, pleural effusion and septicaemia within 10 days of transplantation. The third patient suffered from grade 3 PGF, underwent tracheotomy because of phrenic paralysis and developed non-septicaemic pulmonary cepacia infection that progressed despite combined antibiotic treatment.

**BOS**

We analysed the association between BCC and BOS in the 212 patients of the cohort alive 90 days after transplantation. Sixty of these patients developed BOS. If death was considered as a competing event, the 5 year BOS specific cumulative incidence rate was 38% for the BCC group compared with 24% for the non-BCC group (p = 0.85).
within a year. BCC mortality rates remain low in the French CF population, with three deaths in 2004, a figure comparable with the postoperative results reported here for lung transplant patients. However, long-term survival was also recorded in some members of this population, which is encouraging. Prolonged treatment with a combination of antibiotics seems to be a reasonable approach to the treatment of these patients. We frequently used a combination of antibiotics (ceftazidime or temocilllin or meronem + tobramycin + ciprofloxacin), particularly in cases of panresistant bacteria, and no BCC infection was observed other than in the three patients with B cepacia. Cotrimoxazole or chloramphenicol could also be used if the strains were sensitive. Triple associations are known to be effective against BCC. However, the question of combination therapy against multiresistant or panresistant organisms in CF has recently been addressed: Aaron et al demonstrated that antibiotic treatment directed by susceptibility testing for combinations of antibiotics gave no improvement in clinical and bacteriological outcomes over treatment directed by standard culture and susceptibility techniques. Little is known about the non-bactericidal effect of antibiotic treatment, but such effects may play an important role in determining the improvement of patients with CF, particularly in terms of susceptibility to Pseudomonas or B cepacia infections. Greater insight into inflammatory trigger receptors might help to increase our understanding of defences against bacteria.

Liu et al recently refined the selection of potential candidates for lung transplantation. This statistical analysis of the UNOS (United Network for Organ Sharing) database and the CFFP Registry (CF Foundation Patient Registry) suggest that the outlook is gloomy for BCC infected patients, but this analysis did not distinguish between genomovars. The authors suggested the restriction of lung transplantation in BCC infected patients, despite the improvements in quality of life and survival provided by such treatment. However, we feel that extending lifespan is not the only relevant goal. CF is the leading indication for lung transplantation in France, accounting for 35.8% of all cases, followed by emphysema and chronic obstructive pulmonary disease (19.8%). The benefits of transplantation for patients with chronic obstructive pulmonary disease, in terms of prolonged survival, have not been clearly demonstrated, but no one would question the validity of this indication. Physical and psychological functioning is also a key issue for patients with CF who have had to cope with overwhelming frustration for years, particularly in cases of BCC colonisation that might lead to the patient being excluded from a transplantation programme. Survival is clearly a matter of concern, but we feel that decisions relating to the allocation of resources for lung transplantation should not be restricted to this dimension. Changes in the organisation of organ procurement in France, with re-evaluation of existing lung donor pool, should be informed of the higher risk of an unfavourable outcome. Early mortality rates are higher in cases of B cepacia genomovar III colonisation, so patients and their relatives should be informed of the higher risk of an unfavourable outcome.

Acknowledgements: The authors wish to acknowledge the assistance provided by Ludovic Trinquart (Epidemiology and clinical research, INSERM CIE4, HEGP, Paris), Clément Picard, Christine Segonds and Isabelle Sermet-Gaudelus in reviewing the manuscript and all the members of Anaesthesiology and Intensive Care Unit in Foch Hospital and HEGP for their dedication to patient care.

Competing interests: None.

REFERENCES
Early routine follow-up chest radiographs for pneumonia are not useful

This study investigated the clinical value of chest radiographs during the 4-week period after diagnosis of severe community acquired pneumonia (CAP) in immune competent hospitalised patients. The time to resolution of pulmonary infiltrates and other radiographic abnormalities caused by infection was compared with resolution of clinical parameters, and factors associated with delayed resolution were identified.

Two hundred and eighty-eight hospitalised patients were followed up between July 2000 and June 2003. Severe pneumonia was defined as a pneumonia severity index score of >90 or according to the American Thoracic Society definition. Patients were treated according to the Dutch guidelines for CAP management. The time for clearance of chest radiographic abnormalities was estimated from chest radiographs taken at 0, 7 and 28 days. Clearance of chest radiographic abnormalities lagged considerably behind clinical improvement at all the assessment time intervals. Raised C-reactive protein levels >200 mg/ml, multilobar infiltrates, dullness on percussion, raised urea levels and Streptococcus pneumoniae infection on admission were independent factors associated with delayed radiographic resolution. Age and co-morbidities did not influence the rate of radiographic clearance.

This study was based on secondary data from a prospective randomised trial on the cost-effectiveness of an early switch from parenteral to oral treatment for severe CAP, which may not be the standard clinical practice in many settings. However, it addressed a pertinent question and might influence the way physicians manage CAP.


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Lung alert
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*Thorax* 2008 63: 732-737 originally published online April 11, 2008
doi: 10.1136/thx.2007.089458

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