Outcome of *Burkholderia cepacia* colonisation in an adult cystic fibrosis centre

M J Ledson, M J Gallagher, M Jackson, C A Hart, M J Walshaw

Background: Colonisation with *Burkholderia cepacia* is a poor prognostic indicator in subjects with cystic fibrosis (CF), but outcome prediction is impossible since patients are colonised by different strains with differing pathogenicity. The clinical course of a large cohort of CF patients colonised with UK epidemic (ET12) *B cepacia* was followed for 5 years and compared with that of the remaining patients in the clinic.

Methods: Pulmonary function, nutritional state, and lung pathogen colonisation were recorded for 5 years before December 1997 or death for all 107 patients who had attended the Liverpool adult CF clinic since 1993. For each patient a time line from study entry to date of death or 1997 was constructed. In 1993 potential risk factors including age and sex were subjected to Cox proportional hazards analysis using the end point of mortality as the outcome variable. The analysis was supplemented by time varying covariates that described the change in FEV1, BMI, and colonisation status across time, and the excess risk associated with *B cepacia* colonisation was calculated. Subsequently, in those patients who died between 1993 and 1997, predictive factors for death were compared within groups using complete 5 year data.

Results: Thirty seven patients had been colonised by epidemic *B cepacia* and these patients had four times the mortality of the remainder (*p<0.01*). In 1993 univariate predictors of mortality were age (alive 19.6 (0.64) v dead 23.8 (1.44); *p<0.005*) and baseline FEV1 (alive 68.6 (2.5)% predicted v dead 43.2 (4.8)%; *p<0.001*) with a trend for BMI (*p=0.07*). However, following time varying covariates Cox proportional hazards analysis, only lower FEV1 (hazards ratio 1.1, 95% confidence limits 1.06 to 1.14; *p<0.001*) and colonisation with *B cepacia* (hazards ratio 7.92, confidence limits 2.65 to 23.69; *p<0.001*) were identified as significant factors for death. Surviving *B cepacia* patients had similar initial lung function to the remaining surviving patients but had an accelerated loss of lung function over the study period (colonised –1.9% predicted per year v non-colonised –0.3% predicted per year; *p<0.05*). Deceased patients colonised with *B cepacia* had better spirometric results than the remaining deceased patients 5 years before death (*p<0.05*) but lost lung function at a greater rate than non-colonised patients (colonised –6.2% predicted per year v non-colonised –1.9% predicted per year; *p<0.05*).

Conclusions: This study confirms the excess mortality associated with epidemic *B cepacia* colonisation and shows that those with poor spirometric values are at the greatest risk.
(SD) 21.2 (0.9) years, non-colonised 19.9 (0.8) years, p=NS) nor in the proportion of patients with diabetes mellitus or liver disease, and only two patients were pancreatic sufficient.

Microbiology
Sputum samples were cultured on chocolate, MacConkey, and B. cepacia agar (Oxoid UK, Mast UK) for 48 hours. Mucoid colonies of P. aeruginosa were confirmed using the C390 test (Roscoe Scientific UK). B. cepacia colonies were strain typed using polymerase chain reaction (PCR) ribotyping, pulsed field gel electrophoresis (PFGE), and cable pilus typing by PCR as previously described. Strain types were regularly reviewed using the above methods to document any changes. In practice, once patients became colonised by ET12 B cepacia, all their subsequent sputum samples grew this strain. Chronic colonisation with an organism was defined as three or more positive sputum cultures separated by a 6 month period.

Statistical analysis
Descriptive data are presented as means with standard errors. For each patient a time line from study entry to date of death or 1997 was constructed. Potential risk factors for all patients at 1993 (FEV1, BMI, age, sex, colonisation status) were subjected to Cox proportional hazards analysis using the end point of mortality as the outcome variable. The analysis was supplemented by time varying covariables that described the change in FEV1, BMI, and colonisation status across time. Variables achieving significance at <0.05 were considered as risk factors for death. Given the importance of age, this was included as a covariate in the final model.

Subsequently, those factors shown to be predictors of mortality were analysed using complete 5 year data for all patients. Thus, in those patients who were deceased, this included data points prior to 1993. The changes in FEV1, the first 5 years preceding death or 1997 were analysed using the summary measures approach recommended by Matthews et al. A separate linear regression line was fitted to the FEV1 data for each subject and the slope of this line was used as an estimate of the mean rate of change in FEV1, in that subject over the 5 years preceding death or 1997. Statistical analysis was performed using the Wilcoxon signed rank and rank sum tests as appropriate.

Finally, the excess risk associated with B. cepacia colonisation was calculated using the $\chi^2$ test and relative risk calculated with 95% confidence intervals.

RESULTS
Thirteen of 37 patients (35%) (five men) colonised by UK epidemic ET12 B. cepacia died during the study period, and six of the remaining 70 patients (9%) (four men) died during the study period, all of whom were colonised by P. aeruginosa. Univariate predictors of mortality included age at 1993 and baseline FEV1, with a trend towards nutritional status (table 1). Following the time varying covariate Cox proportional hazards analysis, only a lower FEV1, and colonisation with B. cepacia were identified as significant factors for death (table 2) but, given the significant univariate effect of age, this variable was included in the model to adjust risk factors for age. The relative risk of mortality from B. cepacia colonisation was 4.0 (95% CI 1.6 to 11.2) compared with non-colonised patients.

In the survivor group we compared the effect of B. cepacia colonisation on FEV1 over the 5 year study period (fig 1). No difference was seen in spirometric parameters between patients colonised with B. cepacia and the remainder in 1993. Those patients colonised with B. cepacia had an accelerated loss of lung function with time (~1.9 (0.7)% predicted FEV1/year) compared with the remaining patients (~0.3 (0.4)% predicted FEV1/year; p<0.05) over the 5 year study period.

In the deceased group the effect of colonisation with B. cepacia on spirometric data over the 5 year period before death was studied (fig 2). At baseline B. cepacia colonised patients had a significantly higher FEV1 than the remaining patients (mean 53.3% predicted v 34.6% predicted, p=0.007). However, while both subgroups had worsening pulmonary function with time

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Table 1  Demographic characteristics of the study group stratified by death

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (n=88)</th>
<th>Dead (n=19)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonised with B cepacia in 1993 (%,[n])</td>
<td>22 (19)</td>
<td>39 (7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Colonised with B cepacia (%,[n])</td>
<td>28 (24)</td>
<td>68 (13)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Mean (SE) age in 1993</td>
<td>19.6 (0.6)</td>
<td>23.8 (1.4)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Sex (% male,[n])</td>
<td>53 (46)</td>
<td>47 (9)</td>
<td>0.82</td>
</tr>
<tr>
<td>Mean (SE) baseline FEV1 in 1993 (% predicted)</td>
<td>68.6 (2.5)</td>
<td>43.2 (4.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SE) baseline BMI in 1993 (% predicted)</td>
<td>20.6 (0.3)</td>
<td>19.5 (0.5)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

FEV1 = forced expiratory volume in 1 second; BMI = body mass index.

Table 2  Time varying Cox proportional hazards analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1 (decreasing) per unit % predicted change</td>
<td>1.10 (1.06 to 1.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colonisation (present in 1993 or acquired)</td>
<td>7.92 (2.65 to 23.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years) in 1993 (covariate)</td>
<td>1.08 (0.99 to 1.18)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Figure 1  Subgroup FEV1 data for surviving patients from 1993 to 1997 showing that B. cepacia colonised patients lose lung function at an accelerated rate (~1.9% predicted/year) compared with non-colonised patients (~0.3% predicted/year; p<0.05). PSA = Pseudomonas aeruginosa; Bc = Burkholderia cepacia.

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patients were first described in the USA in 1984, cepacia strains which can be easily transmitted between CF already 31 years. This, in our centre we still have a prevalence rate of 35%, rapid fulminant pneumonia and death.

Outcomes, varying from relatively asymptomatic carriage to a non-colonised patients but had a greater loss over time (−6.2% predicted per year versus −1.9% predicted per year; p<0.05). Psa = Pseudomonas aeruginosa; Bc = Burkholderia cepacia.

(p<0.01), the patients colonised with B cepacia had a greater rate of decline in spirometric data (−6.2 (1.3)% predicted FEV1/year) than those not colonised with B cepacia (−1.9 (1.0)% predicted FEV1/year; p<0.05) such that, at death, both subgroups had similar lung function.

DISCUSSION

There has been a marked improvement in survival in CF and over 80% of patients now survive childhood. Indeed, it is estimated that those patients born in 1990 can expect to live until at least 40 years of age, and the median survival is already 31 years. While there is little doubt that patients cared for in specialist centres receive optimum care, 95% of patients still die from respiratory failure caused by chronic pulmonary sepsis, usually due to Pseudomonas aeruginosa. Previous workers have shown that increasing bacterial lung colonisation in CF is associated with increasing morbidity and mortality; and for this reason intensive antibiotic treatment regimes are advocated. While in some paediatric CF centres B. cepacia strains have been rare, in adult colonisation rates are still over 60%. More recently an aggressive new multisistant respiratory pathogen in the form of B cepacia has appeared in the CF population. This was first described as an opportunistic organism in CF in 1972, and its prevalence increased in North America following this. Epidemic B cepacia strains which can be easily transmitted between CF patients were first described in the USA in 1984, and by 1989 such a strain (ET12) had entered the UK CF population. We have recently shown that this strain is capable of cross-colonising CF patients already infected with B cepacia, often with fatal consequences, and can also colonise non-CF relatives causing serious morbidity. In 1993 this epidemic strain was present in up to 50% of CF centres in the UK, although in many centres only a few patients were infected. Since then the introduction of strict segregation policies and the deaths of colonised patients have decreased the prevalence of this organism, and recent epidemiological surveys have revealed that 3.2% of American, 5% of European, and 6% of UK patients with CF are colonised with B cepacia. Despite this, in our centre we still have a prevalence rate of 35%, although we have had no new cases for 3 years.

Colonisation with B cepacia can have several clinical outcomes, varying from relatively asymptomatic carriage to a rapid fulminant pneumonia and death. While it is recognised that colonisation with B cepacia is a poor prognostic indicator in CF, previous studies have given a confused picture of the consequences of such colonisation for individual patients. For example, while some workers have suggested that patients with poorer lung function are more likely to be colonised with B cepacia, others have suggested that when lung function before acquisition undergo a greater decline following colonisation. Furthermore, other workers have suggested that B cepacia colonisation has much less impact on patients if they survive the first year of colonisation.

These conflicting results may be due to the fact that most previous studies were short term or contained few patients. Furthermore, it is now known that different strains of B cepacia vary not only in their transmissibility but also in their pathogenicity. Previous studies almost certainly looked at multiple strains of B cepacia since reliable strain typing has only recently become available. Thus, predicting the outcome of B cepacia colonisation in the CF population has not previously been possible.

For the first time, therefore, we have been able to assess the effect of a single epidemic B cepacia strain on the clinical course of a substantial group of CF patients over a prolonged time period. We did not attempt to ascertain the date of first B cepacia colonisation of our patients for two reasons. Firstly, most of these patients were already colonised when they first came to our unit, and it may not be possible to determine colonisation status reliably prior to this. In the early 1990s many laboratories were unaware that this organism was a potential pathogen and routine culture for B cepacia was not available in many hospitals. Furthermore, reliable B cepacia culture requires expertise and inexperienced microbiology laboratories may misreport samples. Indeed, a recent survey of US CF centres found that 20% of samples labelled as B cepacia were, in fact, other organisms. Thus, in our patients a positive B cepacia culture at first attendance at our unit in 1993 is unlikely to represent the date of colonisation. Secondly, it has been shown that patients may be colonised with B cepacia for a variable length of time before the organism can be reliably grown from sputum, such that the date of acquisition even where B cepacia is suspected is impossible to determine. However, since the Liverpool adult CF unit opened in 1993, we have assiduously looked for B cepacia in the sputum of our patients and genotyped every strain, demonstrating that since this time their colonisation status is assured.

While on univariate analysis it appears that age and possibly nutritional status may be important factors determining mortality, when subjected to time related multivariate analysis the contribution of these factors as predictors of death was subsumed. In our patients this technique confirmed that a time related deterioration in lung function together with colonisation with epidemic B cepacia are the only independent predictors of death. By looking back at these factors for 5 years in our patients who subsequently died, we have shown that, although B cepacia colonised patients had a greatly higher baseline spirometric values than non-colonised patients, their FEV1 was still much lower than either group of survivors at 1993. This suggests that the level of pulmonary function at B cepacia acquisition may be an important predictor of survival in patients with CF. Furthermore, these patients had a greatly accelerated loss of lung function over the 5 year period, confirming the pathogenicity of this organism. The apparent wide variation in clinical outcome that occurs in response to colonisation with B cepacia remains unclear, but possible explanations include host immunological response or interaction with other colonising organisms.

As expected, the non-colonised patients who died appear to have had long standing poor pulmonary function with a mean FEV1 of less than 40% predicted. It has always been assumed that even patients with CF who are well cared for would have a steady decline in lung function, and it is therefore reassuring to find that, with modern treatment, stable adult patients can be maintained with minimal loss of lung function.

While it has been suggested that nutrition plays an important part in maintaining the health of patients with CF, there
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is little evidence that weight gain diminishes the number of respiratory exacerbations or improves spirometric parameters or survival.4 In keeping with this, univariate analysis showed that BMI had only a weak correlation with survival. This suggests that the most important factor indicating survival in CF patients is lung function, and that nutritional state merely reflects this.

Thus, in our clinic most non-B cepacia colonised patients remain stable with little deterioration in pulmonary function and a constant nutritional status. Only six of these patients died during the study period compared with 13 in the epidemic B cepacia group, giving a fourfold excess risk of mortality from ET12 B cepacia colonisation. Conversely, while our results show the risks associated with epidemic (ET12) B cepacia colonisation, the effect of colonisation by non-epidemic B cepacia strains is unknown. Several European studies have suggested that B cepacia colonisation is of less importance,33–35 possibly because these patients were largely colonised by other (non-ET12) B cepacia strains which may be less harmful.

Our results illustrate the threat posed to patients with CF from epidemic ET12 B cepacia and confirm the need to segregate these patients from the remainder of the CF population.

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Authors’ affiliations

M J Ledson, M J Gallagher, M Jackson, C A Hart, M J Walsh, Regional Adult Cystic Fibrosis Unit, The Cardiothoracic Centre, Liverpool L14 3PE, UK

REFERENCES