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Case-control study of *Stenotrophomonas maltophilia* acquisition in cystic fibrosis patients

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Case-control study of Stenotrophomonas maltophilia *acquisition in cystic fibrosis patients. V. Marchac, A. Equi, C. Le Bihan-Benjamin, M. Hodson, A. Bush. ©ERS Journals Ltd 2004.*

ABSTRACT: The aims of this case-control study were to describe the characteristics of cystic fibrosis (CF) patients who isolated *Stenotrophomonas maltophilia* in sputum, to determine risk factors for acquisition, to assess persistence of the organism and clinical outcomes postacquisition.

Data were collected from 1991–1999. CF patients and controls (who had never isolated *S. maltophilia*) were matched for age (±1 yr), sex and forced expiratory volume in one second (±10%). Data were collected from the year prior and for 2 yrs postacquisition of *S. maltophilia*.

The prevalence of *S. maltophilia* increased from 3.3% to 15%. Factors associated with *S. maltophilia* acquisition were more than two courses of intravenous antibiotics, isolation of *Aspergillus fumigatus* in sputum and oral steroid use. The effect of *A. fumigatus* was independent of steroid use. Clinical status did not change postacquisition. The majority of patients cleared the organism from the sputum.

Long-term infection or an accelerated deterioration in lung function or nutrition is not likely post-*Stenotrophomonas maltophilia* acquisition in cystic fibrosis. This is the first documentation of an association between *Aspergillus fumigatus* isolation and *Stenotrophomonas maltophilia* acquisition in cystic fibrosis, independently of steroid therapy.

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Cystic fibrosis (CF) is an autosomal recessive condition characterised by chronic bronchopulmonary infection and inflammation, and in most cases, pancreatic insufficiency. The median survival has improved from <1 yr in the 1940s, to >30 yrs now, with a predicted 40-yr median survival for babies born after 1990 [1]. Most of the morbidity and mortality is still related to pulmonary infection despite the aggressive use of oral, intravenous and aerosolised antibiotics. Aggressive antibiotic use, as well as other advances in treatment, has made a major contribution to the improved prognosis, however, it has also been associated with a change in the pattern of infecting organisms over the decades. When CF was first described, *Staphylococcus aureus* was the major pathogen [2]. Subsequently, *Pseudomonas aeruginosa* became increasingly common, and now >80% of CF patients eventually become chronically infected with this organism [3]. The use of ciprofloxacin and nebulised colistin at first isolation, followed by regular nebulised and intravenous antibiotics if the patient becomes chronically infected, has been associated with improvement in lung function and prognosis [4, 5]. The price may have been the emergence of new pathogens, including *Burkholderia cepacia*, and increasing prominence of previously known but less usual pathogens, such as *Aspergillus fumigatus* and atypical *Mycobacteria* sp. Among the emerging pathogens has been *Stenotrophomonas maltophilia* [6–8]. The clinical significance of this microorganism is unclear, and there is limited evidence to direct treatment [9]. The aims of this study were to describe the characteristics of CF patients infected with *S. maltophilia*, and then to assess risk factors for acquisition of *S. maltophilia* and determine the clinical outcomes postacquisition including the persistence or otherwise of the organism and its effect on clinical status.

Subjects and methods

Patients were identified from the CF paediatric and adult clinics at the Royal Brompton Hospital between January 1991–30 June 1999. CF was diagnosed with standard criteria duplicate sweat chloride >60 mM on adequate quantities of sweat and/or genotype [10]. Sputum samples were obtained from all productive patients at each clinic visit, however, the number samples obtained per patient per year were variable. Sputum samples were cultured for the common CF pathogens using the following media: heated blood agar, MaConkey, *B. cepacia*, Difco Pseudomonas, Sabaurauds Dextrose and mannitol salt agar. The plates were incubated at 37°C and assessed at 24 and 48 h. Colonies were identified as *S. maltophilia* using the Analytical Profile Index system (Bio Merieux UK Ltd, Basingstoke, UK). Definition of a case was a CF patient who had at least one sputum isolate of *S. maltophilia* during the study period. The patterns of isolation were categorised into four groups as follows: 1) group A, single isolate or cluster of positive isolates for <1 month, then cleared completely (n=26); 2) group B, several isolates during a period of ⩽1 yr, then cleared (n=8); 3) group C, recurrent isolates for >1 yr, then cleared (n=5); and 4) group D, regular isolates of *S. maltophilia* that never cleared, or recurrent

isolates and death occurring <1 yr after first isolation, never clearing before death (n=13).

For each patient, a control CF subject was selected who had never isolated *S. maltophilia* from sputum. Controls were matched as far as possible for age (±1 yr), sex and forced expiratory volume in one second (FEV1) ±10% for the values in the year prior to the first isolation of *S. maltophilia* (defined as Y-1). The FEV1 measurement was taken from the annual assessment if performed within 6 months of isolation of *S. maltophilia*. Otherwise, the FEV1 was calculated as the mean of the FEV1 results from the previous three clinic visits in Y-1. For all patients the following data were collected: 1) lung function (FEV1, forced vital capacity (FVC)); 2) height, weight and hence, body mass index (BMI); 3) sputum culture for isolation of other microrganisms at $>10^5$ colony-forming units (cfu)·mL^{-1} from the lower airway during Y-1 and at the time of initial isolation; 4) use of deoxyribonuclease (DNase), steroids (nebulised or oral); and 5) inhaled and intravenous antibiotic use. Results were stratified by severity of lung function impairment at the time of *S. maltophilia* isolation; the bandings were FEV1 <40% (severe), ⩾40–<60% (moderate), and ⩾60% (mild).

To study the effect of *S. maltophilia* acquisition on clinical status, the following clinical parameters were noted over the study period: number of respiratory exacerbations, number and type of antibiotic courses, oral corticosteroid treatment and DNase use. Antibiotic sensitivity patterns for *S. maltophilia* were described, and whether treatment with antibiotics with a favourable sensitivity pattern was associated with eradication of the organisms from lower airways culture. Eradication, in this study, was defined as no further isolates of *S. maltophilia* from sputum within 2 yrs of original isolation.

Statistical analysis

Comparison between cases and controls were initially made using univariate analysis with unpaired t-tests for continuous data and Chi-squared for categorical data. A stepwise logistical regression model was used to determine risk factors for *S. maltophilia* acquisition. Survival was compared using Kaplan-Meier plots and the Cox proportional hazards model for multivariate analysis. For significant variables, on stepwise logistic regression, odds ratios (OR) with 95% confidence intervals (CI) were calculated. A p-value <0.01 was considered statistically significant. The variables used were oral steroid use, *A. fumigatus* in sputum, and intravenous and nebulised antibiotics use. Adverse events were considered to include a >5% decrease in FEV1, death or heart/lung transplantation.

Results

Details of patients and controls are given in table 1. Between January 1991–June 1999, 63 patients with CF had at least one sputum sample positive for *S. maltophilia*. Of these, 22 patients were children. Prevalence and antibiotic sensitivity data are based on all 63 patients. The other organisms isolated from sputum included: *S. aureus*, *P. aeruginosa*, *B. cepacia*, atypical mycobacteria and *A. fumigatus*. Controls were found for 52 patients. For 11 patients, controls could not be found for the following reasons: age (too young n=6), severity of disease (n=3), and infrequency of attendance (n=2). Median age at time of *S. maltophilia* acquisition was 22.5 yrs (range 0.75–52.0 yrs). There was no significant difference between cases and controls for BMI, FEV1 and FVC in the year preceding the first isolation of *S. maltophilia*. This is true for any of the individual groups (A–D) compared with controls, for group A compared with groups B–D, or when stratifying into groups for FEV1 (⩽40%, 40–⩽60% and >60%). There was no significant effect on data analysis by the inclusion or exclusion of data from the six subjects with *B. cepacia*.

Patients were separated into groups as previously defined. Initially the groups were compared individually, however, the numbers were too small in each group for significant statitistical analysis so groups B, C and D were combined to become group 2, and group A became group 1. The control group was named group 0.

Incidence and prevalence of Stenotrophomonas maltophilia

The prevalence of *S. maltophilia* increased from 3.3% in 1991 to 15% by mid-1999 with a peak of 23% in 1998. The

Table 1. – Details of patients and controls studied

	Group A	Group B	Group C	Group D	Group B–D
Patients					
n	26	8	5	13	26
M/F	17/9	2/6	2/3	6/7	10/16
Mean±SD age yrs T0	24.1±10.3	20.5±5.4	18.6±1.9	26.3±10.2	23.0±8.4
Median age yrs	21.9	20.9	18.3	26.8	25.1
Age range yrs	0.8–48.0	12.5–29.2	6.0–20	9.0–52.0	6.0–52.0
Mean±SD FEV1 % pred Y-1	53±24	53±26	58±11	32±15	40±18
Mean±SD BMI Y-1	19.5±4.0	18.4±2.5	21.3±6.1	17.4±2.6	18.5±3.4
Controls					
n	26	8	5	13	26
M/F	17/9	2/6	2/3	6/7	10/16
Mean±SD age yrs T0	24.6±9.4	19.8±5.5	21.3±6.5	26.5±11.0	25.1±9.2
Median age yrs	24.0	19.9	20	27.1	26.3
Age range yrs	8.5–42.0	13.2–27.2	14.0–30.9	10.0–55.8	10.9–55.8
Mean±SD FEV1 % pred Y-1	51±23	55±23	55±16	34±15	40±18
Mean±SD BMI Y-1	19.4±3.2	18.2±3.3	19.7±2.2	19.4±2.4	19.2±2.5

Group A: single isolate or cluster of positive isolates for <1 month, then cleared completely (n=26); Group B: several isolates during a period of ⩽1 yr, then cleared (n=8); Group C: recurrent isolates for >1 yr, then cleared (n=5); Group D: regular isolates of *Stenotrophomonas maltophilia* that never cleared, or recurrent isolates and death occurring <1 yr after first isolation, never clearing before death (n=13); M: male; F: female; T0: time of acquisition of *S. maltophilia*; FEV1: forced expiratory volume in one second; Y-1: year prior to acquisition; BMI: body mass index. FEV1 and BMI were measured in the year prior to first isolation of *S. maltophilia* for the patients.

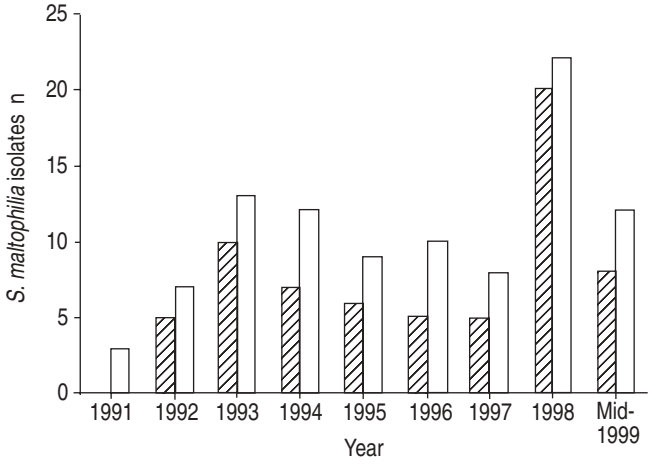

Fig. 1. – Incidence (□) and prevalence (▨) of *Stenotrophomonas maltophilia* in cystic fibrosis patients from 1991 to June 1999.

incidence varied from 2–5% per year over the period studied (fig. 1).

Risk factors associated with Stenotrophomonas maltophilia *acquisition*

Univariate analysis was performed to assess the features significantly different between patients and controls in Y-1 (table 2). The number of intravenous antibiotic courses was significantly different between the two groups. Patients with *S. maltophilia* were highly likely to have had more than two courses of intravenous antibiotics in Y-1 compared with controls (OR 6.8, 95% CI 1.7–26.8). All subjects in group 2 and most in group 1 had isolated *P. aeruginosa* in Y-1. Three patients in group 1 and two control patients did not have isolated *P. aeruginosa*.

A. fumigatus was much more frequently isolated in the *S. maltophilia* patients: 51% cases *versus* 9% controls (OR 5.9, CI 2.0–17.8; p=0.001). This effect of *A. fumigatus* was independent of oral steroid use. Allergic bronchopulmonary aspergillosis was diagnosed in five of 17 (30%) patients with *A. fumigatus* in the sputum and taking oral steroids. Oral steroid use was more frequent in cases than controls (34% *versus* 9%, respectively; p=0.003).

Table 2. – Risk factors for isolation of *Stenotrophomonas maltophilia* in sputum (univariate analysis)

Data in Y-1	Patients	Controls	p-value
Subjects n	52	52	
BMI			
⩽20	24 (63.4)	23 (69.7)	NS
>20	14 (36.8)	10 (30.3)	
Number of *i.v.* courses			
0	12 (25.5)	24 (52.2)	0.004
⩽2	19 (40.6)	18 (39.1)	
>2	16 (34.1)	4 (8.7)	
Nebulised antibiotics	43 (84.3)	36 (70.6)	0.10
Oral steroids	17 (34.0)	4 (8.9)	0.003
Other sputum isolates			
Pseudomonas aeruginosa	48 (94.1)	43 (95.6)	0.75
Staphylococcus aureus	35 (68.6)	30 (66.7)	0.84
Burkholderia cepacia	9 (17.7)	4 (8.9)	0.21
Aspergillus fumigatus	26 (51.0)	4 (8.9)	0.001

Data are presented as n (%) unless otherwise stated. Y-1: year prior to *S. maltophilia* acquisition; BMI: body mass index; *i.v.*: intravenous.

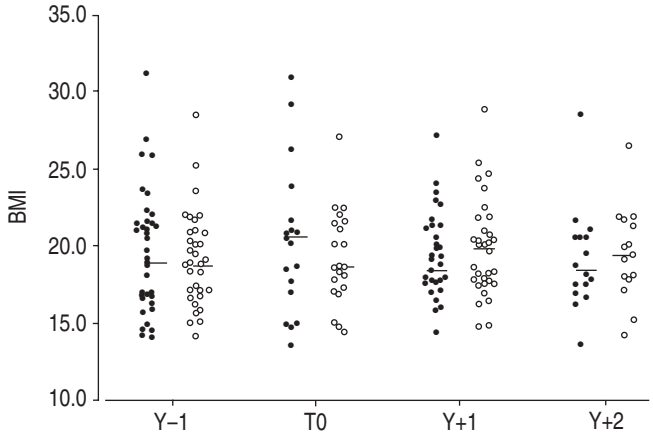

Fig. 2. – Body mass index (BMI) evolution in all patients (●) compared with matched controls (○). Y-1: year prior to *Stenotrophomonas maltophilia* acquisition; T0: time of acquisition; Y+1: 1 yr postacquisition; Y+2: 2 yrs postacquisition. The horizontal lines indicate the median BMI.

There was no statistical difference in detection of other microrganisms in respiratory secretions in Y-1: *S. aureus* cases 68.6% *versus* 66.7% controls, p=0.84; *P. aeruginosa* cases 94.1% *versus* 95.6% controls, p=0.75; *B. cepacia* cases 17.7% *versus* 8.9% controls, p=0.21. There was also no relationship with the use of nebulised antibiotics (cases 84.3% *versus* 70.6%, p=0.10). BMI data was incomplete from the patient cohort, however, from the available data there was no statistical difference between cases and controls (fig. 2).

Multivariate analysis showed that the CF patients most likely to acquire *S. maltophilia* required more intravenous antibiotic courses (more than two courses) and were more likely to have had *A. fumigatus* isolated (table 3)

Antibiotic resistance pattern

Multiresistance to antibiotics was common. Four patients had *S. maltophilia* isolates that were resistant to all antibiotics tested. Of 63 isolates, 34 (54%) were multiresistant *i.e.* sensitive to one or two antibiotics only. In descending order, antibiotic sensitivity was chloramphenicol (70% isolates sensitive), colistin (30% isolates sensitive), cotrimoxazole (14.5%) and ciprofloxacin (6.5%). *S. maltophilia* was sensitive to the prescribed antibiotics in 23 cases. In 24 cases, either no treatment was prescribed or *S. maltophilia* was resistant to the treatment prescribed. The choice or length of therapy with antibiotics did not affect the likelihood of eradication of *S. maltophilia* from the sputum.

Respiratory exacerbations

Of 63 cases, 24 (38%) had a respiratory exacerbation reported, defined as the requirement for antibiotics. Thirteen

Table 3. – Risk factors for isolation of *Stenotrophomonas maltophilia* in sputum (multivariate analysis)

	OR	95% CI
Number of *i.v.* courses Y-1		
1–2	1.7	0.6–5.1
>2	6.8	1.7–26.8
Aspergillus fumigatus	5.9	2.0–17.8

OR: odds ratio; CI: confidence interval; *i.v.*: intravenous.

(22%) were admitted to hospital for intravenous antibiotic treatment. Of 63 patients, 31 (49%) did not have a reported respiratory exacerbation. Of 63 cases, eight had incomplete information. In the 24 patients who were treated for a respiratory exacerbation, four isolated *S. maltophilia* only in the sputum, whereas 13 had coinfection with other bacteria (*S. aureus*, *P. aeruginosa* and/or *B. cepacia*). In those patients with a respiratory exacerbation, 37% had *S. maltophilia* $>10^6$ cfu·mL^{-1} from sputum. There was insufficient data to determine the prevalence of viral infections in these patients.

Impact on lung function and survival

For the 2 yrs studied postacquisition of *S. maltophilia*, there was no significant deterioration in lung function (FEV1) in any of the groups or controls (figs 3 and 4). Survival data were available for 30 of 52 patients due to insufficient data from matched controls. Although the numbers are small in each group, there was no statistically significant difference in mortality or transplantation rates between the groups post-acquisition of *S. maltophilia*.

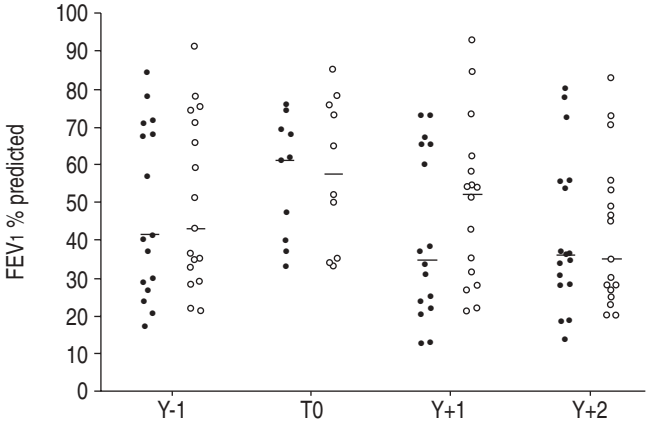

Fig. 3. – Forced expiratory volume in one second (FEV1) evolution in group 2 (●) compared with matched controls (○). Y-1: year prior to *Stenotrophomonas maltophilia* acquisition; T0: time of acquisition; Y+1: 1 yr postacquisition; Y+2: 2 yrs postacquisition. The horizontal lines indicate the median FEV1.

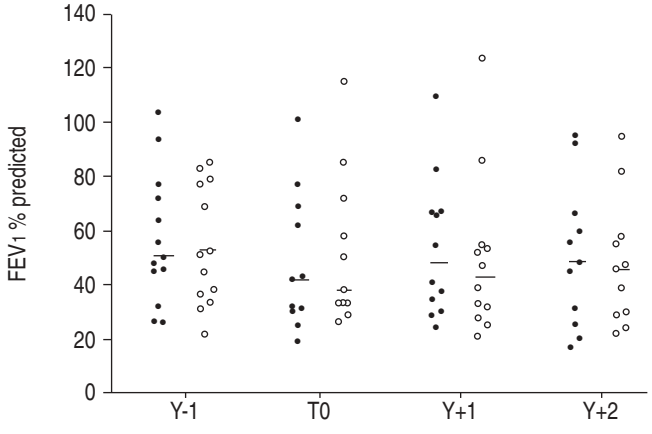

Fig. 4. – Forced expiratory volume in one second (FEV1) evolution in group 1 (●) compared with matched controls (○). Y-1: year prior to *Stenotrophomonas maltophilia* acquisition; T0: time of acquisition; Y+1: 1 yr postacquisition; Y+2: 2 yrs postacquisition. The horizontal lines indicate the median FEV1.

Discussion

The data presented in this case-control study lead to the following five conclusions. First, there is no trend suggesting that *S. maltophilia* isolation *de novo* is increasing in the CF clinic. Secondly, *S. maltophilia* acquisition is strongly associated with the isolation of *A. fumigatus* in sputum, with oral steroid use and also with previous use of intravenous antibiotics. This is the first documentation of an association with *A. fumigatus* and *S. maltophilia* isolation. Since >90% of the cases and controls were using inhaled antibiotics, it was not possible to determine whether or not this treatment was also associated with *S. maltophilia* acquisition. Thirdly, long-term infection with *S. maltophilia* is unusual; most common is a single isolate, and even after a cluster of isolations, *S. maltophilia* may disappear from sputum; 50% cleared within 1 month and two-thirds cleared by 1 yr. *S. maltophilia* acquisition does not seem to be associated particularly closely with poor previous nutritional state. There was no correlation between FEV1 or nutritional state and whether the patient had a single or multiple isolate of this microorganism. Fourthly, there is no change in nutritional state, lung function or use of intravenous antibiotics in the cases compared with controls after first isolation of *S. maltophilia*, whether categorised as single *versus* multiple isolation, or when stratified for lung function. Finally, antibiotic resistance *in vitro* is common, but >70% of strains are sensitive to chloramphenicol.

Due to the retrospective design of this study, there are a number of factors that inevitably weaken the conclusions of this study. There was a variable number of sputum samples obtained per patient. Bronchoalveolar lavage was not performed routinely, which may have affected the microbiology results. However, in expectorating older patients, sputum is reflective of lower airway secretions. It was not possible to obtain data on the use of oral antibiotics because they are used so liberally in the clinic, and self-medication is common. Furthermore, the clinic population varied over the period studied, with the referral of new patients, and others moving out of the area or dying.

A further important criticism is the matching of controls for the cases studied. The authors elected to match for FEV1 to look at rate of change of lung function after first isolation, and whether prior high utilisation of intravenous antibiotics was associated with *S. maltophilia.* This design meant that the authors were unable to assess whether acquisition of *S. maltophilia* was more likely in those with poor lung function, but inspection of the baseline data (figs 3 and 4) suggests that there is no particular bias towards those with poorer lung function. FEV1 performed during Y-1 was chosen rather than the actual time of first isolation because sputum tests are more likely to be performed at the time of an infective exacerbation, when a drop in FEV1 is to be anticipated.

During the time period of the data reported here, there was no uniform treatment policy after initial *S. maltophilia* acquisition, so acquisition may have resulted in a change in intravenous antibiotics, the prescribing of oral antibiotics, or no action. As a result, any recommendations on treatment can only be tentative, however, from this study, it would seem reasonable not to treat a single isolate of *S. maltophilia* in an otherwise well CF patient. It is well known that within the airway of the CF patient is an intense and damaging inflammatory response, yet to the best of the authors' knowledge there are no published studies characterising the inflammatory response to *S. maltophilia*. It may be argued

that these reassuring findings do not support a damaging inflammatory response, but the measures made are relatively crude, and perhaps measurements of inflammatory mediators such as neutrophils or interleukin-8 would be useful.

This study is the first to report on an association between the isolation of *A. fumigatus* and subsequent *S. maltophilia* infection. The effect of *S. maltophilia* acquisition on prognosis is controversial, with one group reporting transient isolation as common, and no effect on prognosis [11]. The present findings are more reassuring as to prognosis and so the main importance of the study is that it provides information with which to reassure patients with CF. CF patients who isolate this organism can be reassured that long-term chronic infection is unusual, and that an accelerated deterioration in lung function or nutrition is also unlikely. No sign of an epidemic of *S. maltophilia* was detected within the clinic, however, it is important to maintain strict microbiological surveillance in order to detect any increase in prevalence, and also evidence of nosocomial acquisition.

There are still many unanswered questions that need to be addressed with prospective studies, with any treatment effects studied in randomised trials, possibly with more sophisticated surrogate markers, such as sputum cytokine or chemokine levels. In the meantime, careful clinical observation is mandatory, but reassurance to cystic fibrosis patients who have acquired *Stenotrophomonas maltophilia* can be given.

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