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Review

Bordetella species in children with cystic fibrosis: What do we know? The role in acute exacerbations and chronic course

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Abstract

Despite vaccination, pertussis is still endemic in the Netherlands. A literature search was performed to verify what is known about the role of *Bordetella* species in children with cystic fibrosis, with regard to the incidence of *Bordetella* infections, the involvement in pulmonary exacerbations and the influence on chronic course.

Little is known about the frequency of *Bordetella* infections and the involvement of *Bordetella* species both in relation to the chronic course of cystic fibrosis and to pulmonary exacerbations. Since it is difficult to detect *Bordetella* species in cultures and few sputum cultures investigated have been obtained during an exacerbation, it is likely that the frequency of *Bordetella* species in CF patients is underestimated. Identification of *Bordetella* species in these patients may have serious consequences for the treatment of exacerbations in CF.

Future research investigating the role of *Bordetella* species in cystic fibrosis should use specific techniques to detect *Bordetella* in cultures.

Keywords: Cystic fibrosis; Bordetella; Pertussis

Contents

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1. Introduction

Cystic fibrosis (CF) is the most common inherited autosomal-recessive disease in the Caucasian population. CF is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) and primarily affects the respiratory tract and gastro-intestinal system. The respiratory disease is characterized by chronic airway inflammation with periodic exacerbations due to respiratory tract infections, presenting as an increase of productive and often paroxysmal cough and dyspnoea. These pulmonary exacerbations seriously affect morbidity and mortality of patients as they may deteriorate the lung function [1,2].

It is well known that several bacterial species as well as viruses are involved in both chronic infections and pulmonary exacerbations in individuals with cystic fibrosis. Frequently involved pathogens are *Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae* and *Burkholderia cepacia* complex [2]. Recently, an increasing number of other pathogens have been reported in patients with CF such as *Stenotrophomonas maltophilia,* non-tuberculous mycobacteria, MRSA, *Ralstonia pickettii*, *Alcaligenes xylosoxidans*, *Burkholderia gladioli* and *Aspergillus fumigatus* [2]. However the exact role of these microorganisms in the course of CF lung disease has not been completely established yet [2].

There have also been anecdotal reports on a possible role for another microbe in these patients, namely *Bordetella* species [2–7]. The most well known member of this family, *B. pertussis,* causes whooping cough or pertussis. Despite vaccination, the incidence of pertussis has been rising over the past decennia [8]. Studies of whooping cough in chronic pulmonary disease in children are scarce and have focussed merely on asthma.

The objective of this literature review is to verify what is known about the role of *Bordetella* species in children with cystic fibrosis.

The following questions were investigated:

- What is the incidence of *Bordetella* species infections in CF patients?
- Are *Bordetella* species involved in pulmonary exacerbations of CF?
- Do infections with *Bordetella* species influence the chronic course of CF?

2. Methods literature study

We identified studies searching PUBMED, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) from inception through July 2010 using the following search terms: (1) “whooping cough”, “bordetella” or “pertussis” (2) “cystic fibrosis” or “mucoviscidosis”. Predefined inclusion criteria were: 1) identification of *Bordetella* species in CF patients; 3) language: English; 4) species: humans; 5) type of article: clinical studies, case–control studies, or case reports. Date of the most recent search of PUBMED: July 30th 2010.

Three reviewers (AB, TW, BA) independently screened titles and abstracts for eligibility. Every article, considered useful by at least one of the authors, was included. Also reference lists of all the included studies and review articles were reviewed for additional studies. The abstracts of all these studies were read to determine if they should be included.

3. Results

The search strategy yielded a total of 92 articles. PUBMED identified 47 articles, Cochrane 14 and EMBASE 46 articles of which 15 were corresponding to articles identified in PUBMED. After independently screening the titles and abstracts, seven publications (all retrievable from PUBMED), met the inclusion criteria and were considered for further review. Most important reasons for exclusion were: 1) no involvement of *Bordetella* species, 2) not CF patients, 3) only description of CFTR functioning, 4) only description of pertussis toxin in cell lines and 5) only reference *Bordetella* to differential diagnosis.

After reading the full articles, one case-report did not contribute to any of our questions; therefore six publications were included in this literature review.

Of these, three publications were case-reports of CF patients in whom *Bordetella* species were involved (Ner 2003 [3], Wallet 2002 [5], Funke 1996 [6]). Two articles evaluated sputum cultures in CF patients for several bacterial species, including *Bordetella* species (Spilker 2008 [4], Coenye 2002 [2]). One study examined the clinical impact of bacterial infections, including *Bordetella*, on lung function (Olesen 2006 [9]). No clinical studies were found.

4. What is the incidence of *Bordetella (pertussis)* infections in CF patients?

Four studies evaluated either sputum samples or blood samples of CF patients for the presence of *Bordetella* species (Table 1). Incidence is dependent on the reliability of tests used. Direct and indirect diagnostics are available. The two direct tests consist of culture and real time polymerase chain reaction (PCR) [10]. Nasopharyngeal aspirates, nasopharyngeal swabs or sputum samples are needed for these tests. The indirect tests measure anti-pertussis toxin antibodies in oral fluid or sera using enzyme-linked immunosorbent assays (ELISA) or multiplex immunoassays [11].

Spilker et al. [4] used a combination of the two direct diagnoses, bacterial genotyping and 16S rDNA sequencing (PCR), to recognize *Bordetella* species in sputum cultures of 874 CF patients receiving care in 183 treatment centers. In these patients, *Bordetella* species were found in 43 samples from 43 patients during a 6 year period. This results in a 5% positivity rate at a time for all patients and means an annual incidence of 7/874 patients/year. Although not reported, the number of samples will have been around 500–1000 per year, i.e., if all samples have been investigated for *Bordetella* then the percentage of positive cultures must have been around 1%. Their analysis of the 43 sputum samples revealed *B.*

Table 1
Studies evaluating the presence of *Bordetella* species in CF patients.

Author	# CF patients	Age	# sputum samples	# *Bordetella* (%)	Species
Spilker et al. [4]	N=874	No age limitation. CF patients: 3–53 years old 25 (58%) aged <18 years	Not known	43 cultures from 43 patients (5%) during a 6 year period	*B. bronchiseptica/parapertussis* *B. hinzii, B. petrii, B. avium, unidentified B.* spp.
Olesen et al. [9]	N=59	<16 years old	206 blood samples	5 patients (ages 4–13 years) (7.6%/year)	*B. pertussis*
Ner et al. [3]	N=532	0–18 years old	Not known	2 lung transplant recipients with CF (ages 10 and 15 years) (3%) 2 CF patients (ages 2 and 14 years) (0.4%) during a 9 year period	*B. bronchiseptica*
Coenye et al. [2]	N=47	Age not known	51	1 (2%), period not known	*B. hinzii*

Number of (#), number of CF patients (N).

bronchiseptica/parapertussis in 23 samples, five were *B. hinzii*, four were *B. petrii*, three were *B. avium*, and eight were unidentified *Bordetella* species. Importantly, bacterial cultures were positive for *Bordetella* species in only 11 (25%) of these 43 patients. Others were originally misclassified but identified correctly by genotyping. This suggests that the recovery of *Bordetella* is influenced by the presence of other species that overgrow *Bordetella* in culture, causing an underestimation of the prevalence of *Bordetella* within the CF population if only bacterial cultures are used.

Olesen et al. [9] retrieved serum of 59 CF patients every 2–4 months for 13 months. These blood samples were analyzed using ELISA for IgM and IgG antibodies against *Mycoplasma pneumonia, Chlamydia pneumoniae* and *Bordetella pertussis.* They found a *M. pneumonia* infection in 11 patients (17.2%/year), *C. pneumoniae* infection in 5 (7.9%/year) and *B. pertussis* in 5 patients (7.6%/year), who had a rise in antibodies against *B. pertussis* that could not be explained by vaccination.

Ner et al. [3] conducted a retrospective medical record review at one children's hospital over a 9 year period and reported all cases with documented *B. bronchiseptica* infection. Data collected of 60 lung transplant recipients and 532 CF patients showed a total of 4 patients with positive respiratory cultures for *B. bronchiseptica.* The diagnosis was made using culture of sputum samples, except for one case where bronchoalveolar lavage was used. Out of the 60 patients who received a lung transplant, 2 with a primary diagnosis of CF (3%) had documented *B. bronchiseptica* infection and 2/532 other CF patients (0.4%) were positive.

Coenye et al. [2] describe the use of both biochemical and microbiological techniques (including gel electrophoresis, PCR and fatty acid analysis) to characterize bacterial isolates in 51 sputum cultures of 47 CF patients attending 16 different treatment centers in the United States. These isolates carried pathogens that had not previously been reported from CF patients. One of these unusual species was *B. hinzii,* detected in 1 isolate.

5. Are *Bordetella* species involved in pulmonary exacerbations of CF?

None of the studies discuss *B. pertussis* as the etiological agent of an exacerbation in patients with CF. Two case-reports [5,6] describe pulmonary exacerbations in CF patients in which other *Bordetella* species are involved (Table 2).

One case-report describes eight pulmonary exacerbations in a 51-year old CF patient during a 3-year period. [6] In all exacerbations, *B. hinzii* was isolated from sputum samples with culture. In six out of these eight episodes *S. aureus* was cultured in parallel in the sputum samples. However, *B. hinzii* was the only bacterium detected during two pulmonary exacerbations. Despite mild exertional dyspnoea, no deterioration in pulmonary disease was seen during a follow up period of three years.

The other case-report describes three exacerbations and two episodes of pneumonia in a 27-year-old female [5]. In all the episodes *S. aureus* and *B. bronchiseptica* were isolated in the sputum with culture and PCR. Her lung function significantly decreased due to the infections.

Table 2
The involvement of *Bordetella* species in pulmonary exacerbations.

Author	# of exacerbations	Clinical features	Spirometry	Species in sputum samples	Progression of CF pulmonary disease
Funke et al. [6]	N=8 (in 1 patient)	• ↑ Cough • Occasional fever • Exertional dyspnoea	Mild deterioration (percentages not known)	6× *S. aureus* and *B.hinzii*, 2× *B. hinzii*	No radiologic changes in the lung, mild exertional dyspnoea
Wallet et al. [5]	N=3 And 2 episodes of pneumonia (in 1 patient)	• ↑ Sputum • Fever of 38 °C • Dyspnoea	FEV-1 1.2 liter, versus 1.4 l 6 months earlier, 15% decrease from baseline	5× *S. aureus* and *B. bronchiseptica*	FEV-1 decreased to 0.95 l versus 1.4 l 3 years earlier.

Number of (#); number of CF patients (N); FEV-1, Forced Expiratory Volume in One Second.

6. Do infections with *Bordetella* species influence the chronic course of CF?

None of the studies specifically address the consequences of a *B. pertussis* infection for the progression of CF pulmonary disease. Three studies [5,6,9] discuss the effect of other *Bordetella* infections on pulmonary function of CF patients (Table 3).

In the study of Olesen et al. [9] the clinical impact of atypical bacterial infections, including *B. pertussis*, on lung function in CF patients was investigated, using spirometry or sRaw measurement. The patients, aged <16 years at enrollment, were followed for 12 months at regular clinic visits and the lung function was tested at every visit. Out of 59 CF patients analyzed, 5 had a significant increase in antibodies against *B. pertussis,* reflecting a natural infection.

The specific effect of the *B. pertussis* infection on lung function is not described in this study, but for all patients with atypical bacterial infections, consisting of 21 patients of which 5 had a *B. pertussis* infection, FEV-1 was significantly decreased compared to the rest of the year (mean 79% with and 82% without infection, $p=0.039$). To study the more long-term impact on lung function, they compared the maximum FEV-1 and FEF_{25-75} in the 4 months prior to and the 4 months following the study period. No significant difference was found between the change in FEV-1 for negative patients and positive patients.

The two case-reports [5,6] report a mild deterioration in spirometry results during the infection with *B. hinzii*, though the infection did not affect the pulmonary function, measured over a 3 year period. Results of lung function tests are not reported, nor is the frequency of measurements [6]. During the *B. bronchiseptica* infection, the forced expiratory volume in 1 s (FEV1) significantly decreased to 1.2 l. Compared to a FEV-1 of 1.4 l 6 months earlier, this is a 15% decrease of baseline. After 2.5 years, her condition was stabilized. However, her pulmonary function had significantly decreased to a FEV-1 of 0.95 l during this period [5]. In both case-reports, patients were co-infected with *S. aureus*.

7. Discussion and conclusions

Our search revealed only a small number of publications specifically addressing our main objectives for this literature study.

Little is known about the frequency of *Bordetella* infections and the involvement of *Bordetella* species both in relation to the chronic course of cystic fibrosis and to pulmonary exacerbations.

The frequency with which *Bordetella* species are detected in CF patients is low as it varies from 0.4% till 7.6%. However, these numbers are based on small numbers of CF patients and none of the articles discussed Dutch patients. Pertussis is still endemic in the Netherlands with a higher incidence compared to the years before the epidemic of 1996–1997 [12]. Annually, 4000 to 8000 cases of whooping cough are reported [8]. While the incidence in children receiving the preschool booster vaccination has sharply decreased, the incidence in adolescents and adults on the other hand has increased [8,12]. This increase in incidence in adults is seen in other countries as well [13], though in most of these countries, unlike the Netherlands, booster vaccination is introduced for the population [14]. The frequency of *Bordetella* species within CF patients in the Netherlands may well be higher, due to transmission from adolescents and adults to children with CF.

It is interesting to see that not only the causative agents of whooping cough in humans, *B. pertussis* and *B. parapertussis,* are seen in CF patients, but also the pathogens primarily found in animals. For instance, most of the time, *B. bronchiseptica* is transmitted by domestic or wild animals [3] and *B. hinzii* and *B. avium* are transmitted by poultry or birds [6,7].

In none of the publications nasopharyngeal aspirates or swabs have been used as samples for culture or PCR, even though this seems to be the most reliable method [10]. In most studies sputum samples have been used for the diagnosis. Sputum samples may contain *Bordetella*, but the sensitivity of detecting *Bordetella* DNA in sputum still needs to be validated [10]. As throat swabs have been found to be less suitable [15], the studies used materials that probably contain less *Bordetella* DNA than if nasopharyngeal samples had been used. This might have led to underestimation of the incidence of *B. pertussis.* Furthermore, the timing of the collection of samples has been less favorable. Even though the publications describing case-reports [3,5,6] expressed obtaining the sputum cultures during exacerbations, the larger studies all collected sputum cultures from CF patients without exacerbations [2,4,9].

Currently, *Bordetella* species are not included in the standard procedures of evaluating CF sputum cultures during an exacerbation [4]. In addition, even when included in the evaluation, several studies have shown that the identification of CF pathogens, including *Bordetella* species, is difficult [2,4].

Table 3
Effect of *Bordetella* infections on pulmonary function of CF patients.

Author	Type of infection	FEV-1 during the period of infection	Long-term effect on pulmonary function
Olesen et al. [9]	Atypical bacterial infection (*C. pneumoniae, M. pneumoniae, B. pertussis*) 5/21 being *B. pertussis*	Significant decrease of FEV-1 (79% versus 82%)	None
Funke et al. [6]	Infection with *S. aureus* and *B. hinzii*	Mild deterioration (percentages not known)	No radiologic changes in the lung, mild exertional dyspnoea
Wallet et al. [5]	Infection with *S. aureus* and *B. bronchiseptica*	Significant decrease of FEV-1 (1.2 liter versus 1.4 liter) (15% decrease from baseline)	FEV-1 decreased to 0.95 l versus 1.4 l 3 years earlier.

FEV-1, Forced Expiratory Volume in One Second.

The recovery of *Bordetella* in cultures is probably influenced by an overgrowth of other CF-related pathogens [4]. The species will only be detected in sputum cultures when specific techniques are used, what makes misidentification of these organisms common using the regular microbial identification methods [2,4]. This results in an underestimation of the prevalence of *Bordetella* species, also (and maybe especially because of abundant microbial presence in the sputum) in CF patients.

An etiological role of *Bordetella* species in pulmonary exacerbations cannot be excluded, based on the two case-reports describing exacerbations in CF patients. Although in 6 out of 8 exacerbations *S. aureus* was detected as well, in two episodes *B. hinzii* was the only microbe detected, suggesting an association with the exacerbation. Another association might be suggested for *B. bronchiseptica*. The decline in pulmonary function seen in this patient is unusual for patients with long-term isolated *S. aureus* colonization [5].

In daily practice pediatricians notice that more and more exacerbations are difficult to treat when the regular antibiotic treatment for CF exacerbations is used.

This suggests involvement of other atypical pathogens in these exacerbations. Specific techniques to detect *Bordetella* species in sputum cultures could identify its role in CF exacerbations. A comparable lack of clinical response to antibiotics was seen for the therapies initially used in the case reports mentioned above [5,6]. To enable physicians to start the right treatment in exacerbations, correct identification of CF pathogens is important.

Macrolide antibiotics are used more and more in the maintenance treatment of CF patients [16]. These antibiotics are mainly used in CF patients with chronic *Pseudomonas* colonization and also for their anti-inflammatory effects. No evidence of a pattern of emerging macrolide resistance has been seen, but resistant isolates have been identified [17].

None of the articles specifically addressed the consequences of a *B. pertussis* infection for the progression of CF pulmonary disease. Three studies discussed the effect of other *Bordetella* infections on pulmonary function of CF patients and showed unequivocal results. Although during exacerbations (almost by definition) lung function decrease is seen, long term consequences appear to be mild if any.

Are there pathophysiological explanations for a possible negative effect of *Bordetella* infection in CF patients? This remains unclear, but several mechanisms could be involved. These mechanisms are especially based on animal studies.

Robay et al. [18] showed that the normal increase in CFTR activity in normal human lung epithelium cells, caused by a β_3-agonist is nullified by pre-treatment with pertussis toxin. This indicates that *Bordetella* infections might further impair CFTR function in CF patients, who already show loss in CFTR function due to the mutation in the CFTR gene. This could increase all known effects of CFTR dysfunction and make these patients even more susceptible to bacterial infections.

Another mechanism of increased susceptibility for infections with many pathogens could arise from reduced antimicrobial activity of CF airway surface fluid (ASF) [19]. Yim et al. [19] showed that treatment of CF cell lines with vitamin D content, increased cathelicidin content in ASF, which induced antimicrobial activity against *B. bronchiseptica*. This suggests that the reduced antimicrobial activity of CF airway surface fluid, combined with the vitamin D deficiency which is common in CF patients [19], makes them even more susceptible for microbial airway colonization with, e.g., *B. bronchoseptica*.

Could we learn something from studies in other respiratory disease, e.g. asthma?

For asthmatics, most articles focus on the effect of previous pertussis infection on the development of asthma and other atopic disorders. Suffering from pertussis rather than having pertussis antibodies seems to increase the risk of atopic disorders such as asthma, eczema or hay fever [20–26].

In a murine model, it was demonstrated that *B. pertussis* infections exacerbate allergic asthma [27]. In addition, adults with stable asthma reported asthma symptoms more frequently and had a decrease in lung function, when positive for *B. pertussis* [28]. This indicates that *Bordetella* species might exacerbate allergic asthma in humans, but has not yet been reported. If one would specifically look for *Bordetella* species in sputum cultures of patients with exacerbated chronic lung disease, one might identify this microbe more often.

In conclusion, it remains unclear if there is a role for *Bordetella* species in the chronic morbidity and/or acute exacerbations of cystic fibrosis. However, there are no reasons to suppose that the rising incidence of pertussis is restricted to non-CF patients.

Since it is difficult to detect *Bordetella* species in cultures and since few sputum cultures investigated have been obtained during an exacerbation, it is likely that the frequency of *Bordetella* species in CF patients is underestimated. Identification of *Bordetella* species in these patients may have serious consequences for the treatment of exacerbations in CF.

Therefore, a prospective study investigating the role of *Bordetella* species in cystic fibrosis using specific techniques to detect *Bordetella* in cultures should yield further information about this possibly important aspect of CF lung disease. The development of a Bordetella specific PCR could contribute to quicker, better and more specific determination of this new, known pathogen.

In the meantime, if clinicians are confronted with a sudden change in cough in CF children, our advice is to perform culture and/or PCR of nasopharyngeal aspirates in the first three weeks after the beginning of the “new symptoms of cough” and to follow up with serology after three weeks of cough. In addition we recommend a pertussis vaccine booster for this population of CF children at risk.

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