

The Role of *Bordetella* Infections in Patients with Acute Exacerbation of Chronic Bronchitis

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Abstract

Background: Acute exacerbations of chronic bronchitis (AECB) are associated with a variety of viral and bacterial infectious agents, some of which are potentially preventable by immunization. *Bordetella pertussis*, which causes whooping cough, has not been studied in this context. We aimed to assess the role of *Bordetella* infections in patients with AECB.

Patients and Methods: Patients with AECB, who presented to participating private practices in Basel, Switzerland, between October 2000 and June 2002, were evaluated by a standardized questionnaire, nasopharyngeal swabs for culture (*Bordetella* spp.), and PCR (*Bordetella* spp. and selected other respiratory pathogens) and paired blood samples for serologic diagnosis of *Bordetella* infection.

Results: A total of 26 patients (34–86 years of age) were recruited. All culture and PCR samples were negative. Serology revealed *Bordetella* infection in eight (31%) patients. Duration of cough was shorter in patients with *Bordetella* infection compared to those without *Bordetella* infection (mean 15 days vs 41 days, $p = 0.04$). Cough ≥ 21 days duration was present in three (43%) of seven patients with evidence of *Bordetella* infection compared to 17 (94%) of 18 controls ($p = 0.012$). Progression to convalescence from initial to follow-up visit after 4–6 weeks was comparable between both groups.

Conclusion: *Bordetella* infections appear to play a significant role in AECB and preventive measurements such as immunization with acellular pertussis vaccines should be considered. Extended investigations are necessary to confirm our preliminary and provocative findings.

of debate. New data indicate the importance of recently acquired strains of bacteria for the induction of AECB [1]. The potential role of *Bordetella pertussis* in AECB has not been studied so far.

Pertussis (or whooping cough) is caused by the gram-negative bacterium *B. pertussis* or, less commonly, *Bordetella parapertussis* [2]. The disease not only occurs in unimmunized children and in infants too young to be immunized but also in adolescents and adults with waning immunity [3, 4]. Complications such as encephalopathy may affect patients at any age [5, 6]. Although most countries have implemented pertussis component vaccines in their immunization programs for children, booster doses in adolescence are recommended only in a few countries [2]. The concept of general booster doses in adults is currently under discussion [7]. As an alternative, booster immunizations could be considered for selected adults at high risk for pertussis. The aim of the present study was to assess the role of *Bordetella* infections in adults with chronic bronchitis, who suffer from frequent exacerbations and therefore form a potential target group for booster immunization.

Patients and Methods

Study Population

Patients with an acute exacerbation of AECB, presenting to one of 17 general practitioners or internists in Basel, Switzerland, were eligible to participate in this study. The diagnosis of chronic bronchitis was based on a history of cough with expectoration for ≥ 3 months/year in two consecutive years [8].

Exacerbation was defined as increasing expectoration and/or change of sputum color with or without increased frequency or severity of cough and elevated body temperature ($\geq 38^\circ\text{C}$) [9]. Patients were excluded in the presence of known HIV infection,

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Introduction

The mechanisms leading to acute exacerbations of chronic bronchitis (AECB) have been controversially discussed. While viral agents may initially play an important role, colonizing bacteria then proliferate and aggravate symptoms. The use of antibiotics in this context remains a topic

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if they had received any immunoglobulins within 3 months prior to the date of inclusion or during the study period, or in case of participation in any other study. The study protocol was approved by the medical faculty's ethics committee, University of Basel, Switzerland, and was performed in accordance with good clinical practices (GCP) guidelines. Written informed consent was obtained from all patients.

Clinical Information

At three visits (initial presentation, 7–14 days and 4–6 weeks later) the severity of AECSB was assessed by a standardized questionnaire including known exposition to *B. pertussis*, clinical variables of chronic bronchitis such as the date of first diagnosis, active smoking, number of pack-years (average packs/day multiplied by years of smoking), current number of cigarettes per day, number of exacerbations in the last 12 months, use of antibiotics in the last 3 months, and current medication. To assess the clinical features of the current exacerbation, increased cough, duration of cough, paroxysms, productive cough, increased expectoration, change in sputum color, whooping, post-tussive vomiting, and similar previous exacerbations were recorded. Dyspnea was assessed by the conventional performance scale in which level 1 represents the least and level 5 the most severe impact on everyday performance [10].

Specimen Collection and Laboratory Procedures

Two nasopharyngeal swabs (NPS) and a venous blood sample were taken at the initial visit and a second blood specimen was obtained at the 4–6 week follow-up visit. One NPS (in modified Regan-Lowe transport medium) was used for culture of *B. pertussis* or *B. parapertussis* in Stainer-Scholte broth and on Regan-Lowe agar in the microbiological laboratory of the University Children's Hospital in Basel. Conventional bacterial cultures were not performed. The second NPS (in sterile 0.9% NaCl) was used for PCR analyses, which were performed in laboratory facilities separate from those for bacterial cultures. The methods have been described in detail [11–13]. In addition, aliquots of NPS for PCR analyses were sent to the microbiological laboratory, University Children's Hospital Kiel, Germany, for single-tube multiplex reverse transcription PCR [14]. Primers for DNA sequences of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, adeno-, entero-, parainfluenza type 1, parainfluenza type 3, influenza-A, influenza-B, and respiratory-syncytial virus were used.

Serological analyses were performed at the German National Reference Center for *Bordetella* Infections (Institute of Hygiene and Laboratory Medicine, Krefeld). IgG and IgA antibodies against filamentous hemagglutinin (FHA) and pertussis toxin (PT) were determined by a well-established, in-house ELISA [15, 16]. Paired sera were analyzed in the same test. An internal reference was used for constructing the reference line, where Food and Drug Administration – Center for Biologics Evaluation and Control (FDA-CBER) reference sera 3 and 4 served as controls. The minimal level of detection was 1.9–3.0 U/ml and was arbitrarily set as 2.0 U/ml for all methods. Intra-assay serology varied from 3.0% (anti-FHA IgA) to 14.0% (anti-PT IgA) [16, 17]. No other serological tests were performed.

Case Definitions

A diagnosis of pertussis was made in the presence of a positive bacterial culture and/or PCR and/or serological evidence of *B. pertussis* infection. Serological evidence of infection was assumed in the presence of a significant increase or decrease in at least two of the four antibody assays of which at least one had to be directed

against PT (IgG and/or IgA). Alternatively, antibody concentrations above the 95th percentile (established by use of a healthy control group) in at least two of the four assays were considered diagnostic, where at least one had to be directed against PT in an acute or convalescent serum specimen. The control group consisted of healthy unvaccinated adults, i.e. regular blood donors and military staff.

A diagnosis of parapertussis was made in the presence of a positive culture and/or a positive PCR for *B. parapertussis* and absence of a significant antibody response to PT.

If culture and PCR were negative and significant antibody responses were directed at FHA only, it was assumed that a recent contact to either *B. pertussis* or *B. parapertussis* was possible.

A twofold increase or decrease of antibody concentrations was considered significant. Single high antibody concentrations were defined as follows (U/ml): IgG-PT \geq 38, IgA-PT \geq 12, IgG-FHA \geq 86, IgA-FHA \geq 42.

Statistics

Statistical analysis was performed using SPSS 11.0.0 (SPSS Inc. 2001, Chicago, IL). Independent proportions were compared by Fisher's exact test. Student's t test was used for comparison of means. Friedman test was used for comparison of ordinal data in dependent groups. $P < 0.05$ was considered significant.

Results

Basic Characteristics

26 patients with 26 episodes of AECSB were enrolled between October 2000 and June 2002 and 25 (96%) of them completed follow-up. One patient was not available for the 4–6 week visit because of poor compliance. The mean age of patients was 68 years and 25 of 26 patients were 40 years of age or older (median 71, range 34–86). Mean duration of chronic bronchitis was 12 years (median 10, range 2–36) with a mean of two acute exacerbations (median 1, range 0–5) during the last 12 months. Twelve patients (46%) were active smokers with a mean of 35 pack-years (median 40, range 1–70). None of the patients had a history of known recent exposure to *B. pertussis*. The mean interval between onset of cough and presentation was 7 days (median 5, range –21). 20 patients (77%) recalled at least one prior similar cough episode. Eight patients (35%) had received antibiotic treatment within the last 3 months. The mean interval between last dose of antibiotic and study enrolment was 20 days (median 21, range 1–38).

Laboratory Findings

None of the 26 NPS cultures revealed growth of *B. pertussis* or *B. parapertussis*. In addition, all 26 PCR samples were negative for *Bordetella* spp. or any other infectious agent identified by multiplex PCR. For ELISA, 25 paired serum specimens and a further single acute-phase serum specimen were available. The results are shown in table 1. As can be seen, five (19%) episodes were classified as *B. pertussis* infection and for three (12%) episodes a diagnosis of "either *B. pertussis* or *B. parapertussis* infection" was made. In all of these eight episodes, *Bordetella* infection was detected by elevated antibody concentrations in either the

Table 1
Antibody concentrations^a demonstrating *Bordetella* infection in eight patients with acute exacerbation of chronic bronchitis.

Patient	Acute serum ^b				Convalescent serum ^c				Interpretation of serology results
	PT ^a		FHA ^a		PT		FHA		
	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	
1	9	3	149^d	135	12	6	243	115	Pertussis or parapertussis
2	10	< 2	179	94	12	< 2	186	112	Pertussis or parapertussis
3	38	2	146	55	-	-	-	-	Pertussis or parapertussis
4	31	16	199	133	32	13	188	138	Pertussis
5	10	40	73	113	7	33	79	111	Pertussis
6	79	3	42	39	68	5	52	43	Pertussis
7	371	90	118	607	288	38	124	571	Pertussis
8	12	16	106	34	17	20	72	47	Pertussis

^a U/ml; FHA: filamentous hemagglutinin; PT: pertussis toxin; ^b venous blood sample taken at the initial presentation of the patient with cough illness; ^c venous blood sample taken 4–6 weeks after the initial presentation of the patient with cough illness; ^d figures in bold indicate significant high concentrations (U/ml): IgG-PT ≥ 38, IgA-PT ≥ 12, IgG-FHA ≥ 86, IgA-FHA ≥ 42

acute or convalescent serum specimen, whereas a significant increase or decrease of antibody concentrations was not observed in any of the paired specimens (Table 1).

Clinical Findings

There was no significant difference between patients with or without evidence for *Bordetella* infection concerning age, characteristics of underlying disease and clinical presentation of the current exacerbation. However, total duration of cough (documented in 25 patients) was shorter in patients with *Bordetella* infection compared to those without *Bordetella* infection: mean 15 days (95% CI: 7–23) vs 41 days (95% CI: 17–64), $p = 0.04$. Furthermore, a total duration of cough ≥ 21 days during AECB was present in three (43%) of seven patients with evidence of *Bordetella* infection compared to 17 (94%) of 18 patients without *Bordetella* infection ($p = 0.012$; Table 2). Interestingly, four of five patients (80%) with a duration of cough between 7 and 21 days during AECB had *Bordetella* infections, whereas only three of 20 patients (15%) with cough > 21 days duration had evidence for *Bordetella* infection.

Mean values of the dyspnea performance scale decreased from visit to visit in both groups, indicating similar progression to convalescence: *Bordetella* infection vs no *Bordetella* infection: initial visit 3.0 (CI: 1.9–4.0) vs 2.5 (CI: 1.95–3.05); 7–14 days 2.4 (CI: 1.4–3.4) vs 2.0 (CI: 1.6–2.4); 4–6 weeks 2.1 (CI:

1.2–3.0) vs 1.8 (CI: 1.3–2.4). There were no hospital admissions or other complications reported in any study subject.

Discussion

Here we present findings from a pilot study on the role of *Bordetella* infection in patients with acute exacerbations of chronic bronchitis. To our knowledge, this has not been studied before and our results indicate that *Bordetella* infections may play a significant role in these patients.

We found evidence of *Bordetella* infection in eight (31%) of 26 AECB by demonstration of elevated antibody concentrations in acute serum specimens. In three patients the antibody pattern (i.e. response to FHA only) did not allow us to discern *B. pertussis*

from *B. parapertussis* infection. Furthermore, we cannot rule out that antibody responses against FHA were caused by cross-reaction to antigens from microorganisms other than *Bordetella* [18]. However, there were five patients (19%) with a significant antibody response to PT, which is specific for *B. pertussis* infection. Interestingly, in a recent study of 155 health-care workers at a German children's hospital with regular contact to patients, 6% were found to have elevated IgG and/or IgA antibodies against PT or FHA by using the same serological methods [17]. Applying the definitions by DeMelker et al. [19] (relying on a single IgG anti-PT > 100 EU/ml) or Birkebaek et al. [20] (relying on a single Ig Ganti-PT > 123 EU/ml) to our data,

Table 2
Presence of clinical findings in 26 patients^a with acute exacerbation of chronic bronchitis depending on evidence for infection with *B. pertussis* or *B. parapertussis*.

Finding	Pertussis/parapertussis N/total (%)	Others N/total (%)
Any increased cough	8/8 (100)	18/18 (100)
Productive	8/8 (100)	17/18 (94)
Paroxysms	8/8 (100)	15/18 (83)
Whooping	3/8 (38)	3/18 (17)
Post-tussive vomiting	1/8 (12)	6/18 (33)
Any prior similar cough	7/8 (87)	13/18 (72)
Duration of cough ≥ 21d	3/7 (43) ^b	17/18 (94) ^b
Duration of paroxysms ≥ 21d	2/7 (28)	7/18 (39)
Dyspnea	3/8 (38)	13/18 (72)
Fever	3/8 (38)	5/18 (28)

^a One patient with evidence for infection with *B. pertussis* or *B. parapertussis* was lost for follow-up at 4–6 weeks; ^b $p = 0.012$

only one patient would have fulfilled the serological criteria for acute *B. pertussis* infection [19, 20]. In contrast to this and other previously published serological definitions for recent exposure to *B. pertussis*, which are based solely on IgG antibody analyses [19–21], our definition includes IgG and IgA antibody tests. Importantly, four of the five patients fulfilling our definition of *B. pertussis* infection do this based on elevated anti-PT IgA antibodies.

Also, we cannot exclude the possibility that our reference values, deriving from adults without chronic bronchitis, are different from those in patients with chronic bronchitis. Overall, however, the proportion of *B. pertussis* infections in our study is in line with the 20–25% found among otherwise healthy adults with cough illnesses [3, 22–24].

Antibody determinations in convalescent specimens demonstrated little changes compared to those in acute specimens and did not contribute to the diagnostic sensitivity. This is a plausible finding, because adults are likely to have had previous infection with *B. pertussis* (or *B. parapertussis*) and secondary immune responses cause a rapid increase in antibody concentrations [25]. Furthermore, specific IgA and IgG serum antibodies do significantly decrease again within a few months after acute infection [26]. Thus, our data confirm that a single acute serum sample probably is sufficient for serological diagnosis of pertussis in adults.

It is not surprising that direct demonstration of *Bordetella* spp. by PCR or culture was not possible in any of the eight *Bordetella* infections in this study. In a recent study from Canada, 442 adolescents and adults > 12 years of age with cough of 7–56 days duration were evaluated. Only four (0.9%) of them were positive for *B. pertussis* by culture or PCR, whereas 84 (19.1%) had serologically diagnosed *B. pertussis* infection [27]. This means that *B. pertussis* was detected in only one of 20 patients with pertussis. Also, in other previous investigations on pertussis in adults, 21–65% of serologically diagnosed *Bordetella* infections were associated with positive PCR and/or culture [28–32]. This considerable variability in direct demonstration of the organism from nasopharyngeal specimens may be due to inhomogeneous sampling techniques among different investigators, which may also have contributed to the negative finding in our study.

Thus, our data confirm that serological analysis is of paramount importance for diagnosis of pertussis not only in otherwise healthy adults, but also in those with AECB, whereas culture and PCR lack sensitivity.

To our surprise, patients with *Bordetella* infection had a shorter duration of cough compared to those with cough of unknown etiology. This is a new and provocative finding. It can be explained by the fact that illness caused by *B. pertussis* in adults is usually due to reinfection, which seems to occur frequently in otherwise healthy adults [4, 33]. Our findings indicate that this is probably also the case in patients with chronic bronchitis. We offer the hypothesis that partial immunity acquired from prior infection with *B. pertussis* leads to comparatively mild symptoms during rein-

fections. The atypical presentation of *Bordetella* infections under these circumstances makes specific microbiological investigations necessary to establish the diagnosis. This is of clinical relevance, because (1) treatment with macrolides should be initiated to avoid transmission to other susceptible individuals, especially young infants [34], and (2) no further diagnostic procedures are usually necessary.

The overall similar clinical presentation of AECB associated with *Bordetella* infection compared to non-*Bordetella* infection in our study is of no surprise and has also been observed in patients without underlying pulmonary disease [24, 35, 36]. However, given the frequency of cough exacerbations in patients with chronic bronchitis, our finding of *Bordetella* infection in up to 30% of these episodes is relevant. In light of these data, pertussis immunization could significantly decrease the morbidity of patients with chronic bronchitis. Acellular pertussis vaccines, with proven excellent immunogenicity and safety in adolescents and adults, would be available for this purpose [2, 37].

Our study has limitations. Most importantly, despite our repeated efforts to remind participating physicians of the purpose and goal of this study, recruitment of patients with AECB was lower than expected. Obviously, tight schedules in their offices caused prolonged intervals of enrollment inactivity. One could argue that therefore our study sample might be biased towards recruiting patients with suspected pertussis. However, based on the unspecific clinical findings in our patients and the fact that exposure to *B. pertussis* was denied by all patients, we believe there is no indication for such a bias.

Therefore, this present study should be considered as a pilot project which provides preliminary and provocative evidence on the role of *Bordetella* infection in adults with acute exacerbations of chronic bronchitis. Confirmative, extended investigations are necessary.

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