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Burkholderia cepacia Is Associated with Pulmonary Hypertension and Increased Mortality among Cystic Fibrosis Patients

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The aim of the study was to evaluate the impact of *Burkholderia cepacia* on cardiovascular status and mortality in cystic fibrosis. Seven patients infected with *B. cepacia* were matched with 31 patients not infected with this organism for gender, age, height, weight, genotype, and percent predicted forced expiratory volume in one second, partial arterial oxygen pressure, and pancreatic sufficiency status. The pulmonary artery systolic pressure, as assessed by transthoracic echocardiography, was significantly higher in patients infected with *B. cepacia* (61.3 ± 17.2 mm Hg) than in controls (37.3 ± 13.9 mm Hg; $P = 0.02$), and the mean acceleration time was significantly lower (77 ± 33 ms versus 108 ± 25 ms; $P = 0.02$). The 6-month mortality was significantly higher in patients infected with *B. cepacia* (57% versus 16%; $P = 0.02$). Six of the seven patients infected with *B. cepacia* harbored the same ribotype (genomovar II, *B. multivorans*). Pulmonary hypertension was significantly more frequent in patients infected by *B. cepacia* and could contribute to the increased mortality rate.

Lung disease in cystic fibrosis (CF) is characterized by an early, persistent inflammatory process and chronic bacterial colonization. The most common organisms are *Staphylococcus aureus* and *Pseudomonas aeruginosa* (15). *Burkholderia cepacia* is less frequently cultured from sputum in CF patients (15). *B. cepacia* has a broad spectrum of disease states from chronic asymptomatic carriage, accelerated decline in pulmonary function to a rapid, and often fatal, deterioration of lung function with "cepacia syndrome" (23). However, the cause for these differences in clinical presentation and the mechanisms by which *B. cepacia* causes pulmonary deterioration is unclear, but possible explanations include host immunological response or interaction with other colonizing organisms (19, 25). An increased mortality associated with *B. cepacia* has been reported more than two decades ago (39) but is observed mainly after lung transplantation (1, 6, 9) and can occur at an earlier stage of the disease (11). In fact, *B. cepacia*-related sepsis, bacterial pneumonia and lung abscesses have all been observed in lung transplant recipients (1, 6, 9).

A recent observation in our clinic of a 13-year-old girl, chronically infected with *B. cepacia*, who died because of severe pulmonary hypertension (PHT) promoted our focus on a possible link between *B. cepacia* and pulmonary inflammatory vascular response. The pathophysiological mechanism of PHT in chronic lung disease is assumed to be the consequence of progressive destruction of the lung parenchyma and pulmonary vasculature, causing hypoxemic pulmonary vasoconstriction. Lung destruction with amputation of the vascular bed is also observed in CF, but it is not clear if other factors, such as bacterial infection of the airways, or the level or type of inflammation, contribute to the development of PHT. Furthermore, PHT in CF seems important to document since previous studies have demonstrated the negative impact of even a moderate level of PHT on survival (12). At present, assessment of right-sided cardiac function is a relatively easy process since the pulmonary artery systolic pressure (PASP) can be measured from the tricuspid regurgitant jet velocity using noninvasive Doppler echocardiography (38). More recent studies have demonstrated the usefulness of this noninvasive technique in the assessment of PHT in adult patients with CF and severe lung disease (10, 12, 22).

Thus, the aim of the present study was to compare echocardiographic measures of PHT and outcome in patients chronically infected with *B. cepacia* and patients not infected with this organism.

MATERIALS AND METHODS

Patient population. The studied population was all of the patients with CF who had been infected with *B. cepacia*. *B. cepacia* had to be isolated from routine sputum samples by a reference laboratory in our CF center for a minimum of 6 months, on at least two occasions (*B. cepacia*-positive cases, $n = 7$). These patients were matched with 31 consecutive patients who attended the same CF Clinic (with a total population of 180 patients) at Armand Trousseau Hospital for routine evaluation between May and November 2001. In these patients, at least two sputum samples were obtained during the same period, and were all negative for *B. cepacia*. Infection with *S. aureus* and *P. aeruginosa* was also defined as at least two positive sputum samples.

For the two groups of patients, CF was diagnosed based on sweat chloride levels of >60 mmol/liter and relevant cystic fibrosis transmembrane conductance regulator (*CFTR*) genotype. Inclusion criteria were as follows: (i) patients who had available bacteriological, radiological, lung function, and echocardiographic data during a clinically stable period for at least one month and (ii) patients who had at least a 6-month follow-up. Patients were excluded if they had an exacerbation in the previous month, were unable to perform reproducible forced

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TABLE 1. Characteristics, bacterial infection, and radiological severity score of patients infected (*B. cepacia*-positive cases) and not infected (*B. cepacia*-negative cases) with *B. cepacia*

Parameter	*B. cepacia*		*P*
	Positive cases ($n = 7$)	Negative cases ($n = 31$)	
Male/female (n) ratio	2/5	16/15	0.34
Mean age (yr) ± SD	14.6 ± 3.5	14.6 ± 3.9	0.99
Mean wt (kg) ± SD	38 ± 8	40 ± 12	0.61
Mean height (cm) ± SD	148 ± 13	152 ± 15	0.49
Mean BMI[a] z-score ± SD	−0.70 ± 1.02	−0.80 ± 0.95	0.79
CFTR mutation genotype (%)			
Homozygote ΔF 508	72	58	0.52
Heterozygote ΔF 508	14	29	
Other mutation	14	13	
Sputum cultures (n)	*B. cepacia* alone (4) *B. cepacia* + *P. aeruginosa* (2) *B. cepacia* + *S. aureus* (1) *B. cepacia* + *P. aeruginosa* + *S. aureus* (0)	No bacteria (3) *P. aeruginosa* alone (9) *S. aureus* alone (8) *P. aeruginosa* + *S. aureus* (11)	

[a] BMI, body mass index.

expiratory flows, or had a primary cardiac or other respiratory disease that was not related to CF.

B. cepacia-positive cases were matched with *B. cepacia*-negative cases for gender, age (±1 year), height (±5 cm), weight (±5 kg), *CFTR* genotype, percent predicted forced expiratory volume in 1 s (FEV_1 %predicted value of ±20%), partial arterial oxygen pressure (PaO_2 ± 5 mm Hg), and pancreatic sufficiency status.

Bacteriological status. Bacterial isolates cultured from sputum were determined as previously described (15). For *B. cepacia*, the ribotypes were determined. The isolates were grown in 10 ml of nutrient broth (Sanofi Diagnostic Pasteur) for 18 h, and DNA was prepared from the bacterial pellet as previously described (2, 3). DNA was digested with EcoRI (Boehringer, Mannheim, Germany) according to the manufacturer's instructions and analyzed by Southern blotting with a chemiluminescent ribosomal probe (2). Identification of genomovars was determined as previously described (20).

Outcome parameters. Data were compiled from the patient's medical charts. Clinical data was obtained for each patient and included nutritional status with the calculation of the body mass index z-score (BMI z-score) (34).

The importance of the lung destruction was rated on a computed tomographic scan performed in the two last years by two independent observers according to a modified four-scale rating score: 1, no bronchiectasis; 2, mild bronchiectasis; 3, moderate bronchiectasis; and 4, severe bronchiectasis (32, 33). Indeed, in order to evaluate the radiological extent of the lung destruction, only the severity of the bronchiectasis was rated.

Current treatments were recorded including nasogastric or gastrostomy feeding, long-term oxygen therapy (LTOT), and noninvasive mechanical ventilation (NIMV).

Arterialized capillary blood gases and spirometry were measured within 24 h of the study according to European Respiratory Society guidelines (31). Vital capacity (VC), FEV_1, and the FEV between 25 and 75% of maximal flow (FEF_{25-75}) were expressed as a percentage of the published values (31). Pulse oximetry saturation (SaO_2) was measured with a finger pulse oximeter.

After the inclusion in the study and the realization of the lung function tests and echocardiography, the duration of the follow-up lasted 6 months. The mortality during this period was recorded.

Echocardiographic study. All subjects were examined in a semisupine, left lateral position by the same senior physician who was blinded to the clinical status of the patient. The electrocardiogram was recorded continuously. Patients were studied with a Challenge 7000 ESAOTE scanner interfaced with 5- to 3.3-mHz and 3.5- to 2.5-mHz transducers. The pulsed Doppler mode was used to detect pulmonary flow with a left parasternal position at the level just proximal to the pulmonary valve without angle correction. A continuous Doppler wave was used to detect tricuspid regurgitation and to calculate PASP. The velocities in the tricuspid regurgitation jet were obtained from the apical four-chamber view and from the left lower parasternal position. PASP was estimated from the tricuspid regurgitation gradient, added to the right atrial pressure. The gradient between right atrium and right ventricle (RV) was measured from the continuous wave Doppler signal of the tricuspid regurgitation by the simplified Bernoulli equation $\Delta P = 4v^2$, where ΔP is the peak pressure difference between RV and right atrium, and v is the peak flow velocity of the tricuspid regurgitant jet and added to the right atrial pressure, which was assumed to be 10 mm Hg, in the absence of right inferior vena cava dilatation. From the pulmonary forward flow trace, the pulmonary acceleration time was calculated as the time interval between the onset of flow and the peak flow velocity. The RV dimension was measured at end diastole from M-mode traces and by using the recommendations of the American Society of Echocardiography (35).

Statistical analysis. All data are expressed as mean ± the standard deviation (SD). Differences between the patients with or without *B. cepacia* infection were evaluated by using the Mann-Whitney test for comparison of the mean and corrected χ^2 test for comparison of percentages (Statview; SAS Institute, Inc., Cary, N.C.). The Yates correction for small numbers was used. Correlation between variables were assessed by using simple linear regression analysis. A P value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics. Over the 6-month period of inclusion, seven *B. cepacia*-positive patients were identified. All of these patients had been infected consistently with *B. cepacia* for the 2 to 4 years prior to the study. Six *B. cepacia* isolates harbored the same ribotype and belonged to genomovar II (*B. multivorans*). No subculture could be obtained for the genomovar determination of the last patient. For the purpose of the study, 31 matched controls were individualized. A comparison of *B. cepacia*-positive cases and *B. cepacia*-negative cases on matched variables is provided in Table 1 and shows that there were no differences observed in age, sex, anthropometrical variables, and *CFTR* genotype between the two groups. Bacterial colonization with *S. aureus* was significantly more common in the *B. cepacia*-negative patients (with 14 and 61% of the *B. cepacia*-positive and *B. cepacia*-negative patients having *S. aureus*, respectively; $P = 0.03$). Although *P. aeruginosa* was

TABLE 2. Lung function parameters, radiological score, and echocardiographic parameters of patients infected (*B. cepacia*-positive cases) and not infected (*B. cepacia*-negative cases) with *B. cepacia*

Parameter	*B. cepacia*[a]		*P*
	Positive cases ($n = 7$)	Negative cases ($n = 31$)	
Lung function parameters			
VC (% predicted)	46 ± 20	64 ± 25	0.08
FEV_1 (% predicted)	31 ± 22	50 ± 24	0.07
$FEF_{25–75}$ (% predicted)	20 ± 17	32 ± 25	0.23
SaO_2 (%)	90.5 ± 4.6	93.6 ± 4.2	0.11
PaO_2 (mm Hg)	65.3 ± 16.2	68.9 ± 13.6	0.54
$PaCO_2$ (mm Hg)	41.4 ± 6.6	40.9 ± 5.3	0.83
Computed tomography scan severity score	3.7 ± 0.8	3.0 ± 1.1	0.10
Echocardiographic indices			
PASP (mm Hg)	61.3 ± 17.2	37.3 ± 13.9	<0.0001
Patients with tricuspid regurgitation (*n* [%])	3 (43)	11 (35)	0.79
Acceleration time (ms)	77 ± 33	108 ± 25	0.02
RV dimension (mm)	22.9 ± 6.1	20.7 ± 5.5	0.38
Pulmonary flow (m s^{-1})	0.90 ± 0.36	0.87 ± 0.25	0.56

[a] Values are means ± the SD except as otherwise noted.

also more frequently isolated from *B. cepacia*-negative patients, this difference was not statistically different.

Lung function, gas exchange and radiological severity. No significant differences were observed between *B. cepacia*-positive and the *B. cepacia*-negative patients with regard to lung function parameters, SaO_2, arterial blood gases, and CT scan severity score (Table 2).

Echocardiographic findings. Tricuspid regurgitation was present in 14 patients (37%), with no significant difference between the two groups (Table 2). In the patients who had tricuspid regurgitation and a measurement of pulmonary blood flow acceleration time ($n = 11$), a significant negative correlation was observed between the level of PASP and acceleration time ($r = -0.85$, $P = 0.0001$). Transtricuspid gradient was significantly higher, and acceleration time was significantly lower in *B. cepacia*-positive cases than in the *B. cepacia*-negative patients (Table 2). Only one *B. cepacia*-positive patient had an acceleration time of >75 ms, and she was the patient in whom the genomovar has not been identified. The mean values of the RV dimensions and pulmonary blood flow were similar in the two groups (Table 2). Regression analysis showed that for the *B. cepacia*-positive patients as a whole, there was a negative correlation between the pulmonary blood flow acceleration time and the RV dimension ($r = -0.70$; $P = 0.02$).

Outcome. During the 6-month follow up, four of the seven *B. cepacia*-positive patients died. All of these four patients were infected with *B. multivorans*. The 6-month mortality was thus significantly higher in the *B. cepacia*-positive patients compared to the *B. cepacia*-negative patients (57% versus 13%; $P = 0.02$). One *B. cepacia*-positive patient died suddenly because of an acute exacerbation of her PHT, and in two other patients the rise in PASP during the few days preceding death may have precipitated the fatal outcome. Bacteremia, pneumothoraces, or major hemoptysis did not occur in the patients infected with *B. cepacia*. None of the four *B. cepacia*-negative patients who died during the 6-month follow-up had echocardiographic signs of PHT, despite the existence of an end-stage lung disease. All *B. cepacia*-positive patients received nutritional support by a gastrostomy, LTOT, and NIMV. Of the *B. cepacia*-negative patients, 19% received nutritional support ($P = 0.002$), 42% were prescribed LTOT ($P = 0.04$), and 26% were prescribed NIMV ($P = 0.003$).

Correlation of cardiovascular status with lung function parameters and gas exchange. Regression analysis demonstrated a positive correlation between the acceleration time and the different lung function parameters (with $r = 0.525$, $P = 0.0007$ for VC, $r = 0.550$, $P = 0.0006$ for FEV_1, and $r = 0.496$, $P = 0.0024$ for $FEF_{25–75}$), PaO_2 ($r = 0.472$, $P = 0.004$), and SaO_2 ($r = 0.354$, $P = 0.04$). Also, we observed that acceleration time correlated negatively with $PaCO_2$ ($r = -0.335$, $P = 0.049$). The opposite was observed for RV dimension, which correlated negatively with the lung function parameters (with $r = -0.398$, $P = 0.018$ for VC, $r = -0.490$, $P = 0.003$ for FEV_1, and $r = -0.487$, $P = 0.003$ for $FEF_{25–75}$), PaO_2 ($r = -0.530$, $P = 0.001$), and SaO_2 ($r = -0.445$, $P = 0.008$) and positively with $PaCO_2$ ($r = 0.428$, $P = 0.01$).

DISCUSSION

The main finding of this study was that chronic infection with *B. multivorans* in young patients with CF was associated with PHT. Indeed, this cardiovascular complication was only observed in the patients infected with this genomovar. To our knowledge, this is the first report to observe such an association in young patients with CF. Furthermore, the evidence of right heart dysfunction in these patients could have a significant impact on short-term survival.

Infection with *B. multivorans* and mortality rate. The 6-month survival was reduced in patients infected with *B. cepacia*. Although this observation confirms previous data, both within and outside the transplant context (5, 6, 9, 11, 37), the current data have shown that survival was reduced in patients infected with *B. cepacia* and in particular in patients infected with *B. multivorans* compared to patients not infected with this

organism, despite being matched for *CFTR* genotype, lung function, gas exchange, and nutritional status. *B. cepacia* was cultured from all of the sputum samples of the 7 patients over the past 2 to 4 years.

Infection with *B. cepacia* and PHT. We observed in our study that patients with chronic *B. multivorans* infection had higher PASP. In recent years, infection with *B. cepacia* in CF patients has become a clinical issue of increasing concern. Certain strains of this bacteria are easily transmitted from person to person (8), and all are resistant to many antibiotics and seem to be associated with a significantly worse outcome. However, the exact pathogenic mechanisms remain unclear. One explanation could be the ability of *B. cepacia* lipopolysaccharides (LPS) to stimulate tumor necrosis factor alpha (TNF-α) from human monocytes. Indeed, this ability seems to be significantly greater for LPS extracted from *B. cepacia* than for LPS extracted from *P. aeruginosa* and *Stenotrophomonas maltophilia* and also greater for LPS extracted from *B. cepacia* from patients with CF than from patients without CF (41).

The excessive inflammatory burden in the lungs of patients with CF is a feature that distinguishes CF from all other forms or chronic lung disease. CF is associated with increased circulating levels of inflammatory mediators such as interleukin-6 (IL-6), IL-1 and TNF-α, which are generated as the result of severe pulmonary inflammation, which is, at least in part, the direct consequence of the expression of mutated *CFTR* in bronchial cells (4, 27, 28). These cytokines have been implicated in different models of PHT. Synthesis and release of TNF-α have been shown to be an important event influencing the development of sustained PHT and respiratory failure induced by endotoxemia in an animal model (30). Moreover, overexpression of TNF-α produces an increase in lung volume and PHT in mice (13). In addition, increased IL-1 and IL-6 concentrations in serum have been reported in patients with severe primary PHT, and an association between some inflammatory disorders and PHT has been observed (21).

Taxonomic studies have shown that bacteria previously identified as *B. cepacia* by conventional tests actually belong to several distinct species or genomovars, referred to collectively as the *B. cepacia* complex (40). Although all *B. cepacia* complex species are capable of human infection, the distribution of species in CF indicates a differential capacity for pulmonary colonization, interpatient spread, and clinical outcome. Genomovar III strains have been associated with epidemic spread, chronic colonization, or "*cepacia* syndrome" (16).

In order to improve the understanding of the relationship between *B. cepacia* and PHT, the ribotypes and the genomovars of the isolated *B. cepacia* were identified. Six *B. cepacia* isolates were all genetically related and belonged to genomovar II, named *B. multivorans*. All of these patients had severe PHT. The single strain obtained from the only patient without PHT (acceleration time of 130 ms) was not related to the latter strain, but its genomovar has not been identified. Several reports have shown that *B. multivorans* (II) is a less-severe infection than *B. cenocepacia* (III). However, the results of the present study do not seem to support this observation with a significant greater short-term mortality in patients infected with *B. multivorans* compared to those not infected with this organism. Interestingly, recent data demonstrate that strains belonging to genomovar II can be as invasive as those belonging to genomovar III and can cause fatal septicemia (24, 36). There is also an intragenomovar variation in invasiveness, with 37.5 and 77.8% of genomovars II and III being considered invasive in an in vitro invasion assay (7). Thus, some *B. multivorans* strains could be associated with a poor prognosis in patients with CF.

PHT in young patients with CF. PHT develops secondary to primary diseases of the lung as a consequence of the pulmonary vasoconstriction caused by chronic hypoxia (18). Supporting the other studies of patients with CF, we observed a strong inverse correlation between signs of PHT (pulmonary blood flow acceleration time and RV dimension) and PaO_2 and SaO_2 (10, 12). In previous studies in patients with CF, the correlation between PHT and $PaCO_2$ has been inconsistent (12), but we observed that $PaCO_2$ was correlated negatively with pulmonary blood flow acceleration time and positively with the RV dimension. Although Fraser et al. (12) found no correlation between PHT and daytime $PaCO_2$ despite a similar degree of lung impairment in the two studies, the patients in the current study were younger and this, combined with the fact that $PaCO_2$ is more closely correlated with FEV_1 in children and young adults than in adults, can explain, in part, the disparity in the correlation between PHT and $PaCO_2$ (17). Furthermore, since FEV_1 is the most sensitive indicator of the severity of lung disease in CF, it is not surprising that this parameter correlates also with RV dysfunction and direct and indirect signs of PHT (12, 22, 26).

An additional factor contributing to PHT in CF is the loss of the peripheral vasculature as a result of the persisting inflammatory and infectious processes that cause destruction to the lungs. This could explain the observation of the correlation between the signs of PHT and the radiological severity of pulmonary CF disease (14, 29).

In conclusion, PHT, assessed by echocardiography, was significantly more common in patients infected with *B. multivorans* than in patients not infected with this organism, despite the similar levels of oxygenation, lung function, and lung destruction. Thus, PHT could contribute to the reduced survival observed in CF patients infected with *B. multivorans*. The results of our study need to be examined in a larger group of patients, harboring different *B. cepacia* strains in order to verify whether the association between this microorganism and PHT and mortality is strain specific or a general characteristic of *B. cepacia* infection in CF. Our study also suggests that, in light of the poor outcome of these patients, PHT in patients with CF who are chronically infected with *B. cepacia* should be systematically assessed not only by echocardiography but also by cardiac catheterization.

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