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ORIGINAL ARTICLE
CYSTIC FIBROSIS

Chest physiotherapy enhances detection of *Pseudomonas aeruginosa* in nonexpectorating children with cystic fibrosis

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ABSTRACT Lung damage in cystic fibrosis (CF) is strongly associated with lower airway infections. Early treatment of *Pseudomonas aeruginosa* is recommended. Pathogen detection requires sampling of lower airway secretions, which remains a challenge in nonexpectorating patients. Our hypothesis was that chest physiotherapy would improve the quality of airway secretion samples and increase the rates of pathogens detected in nonexpectorating patients.

This prospective multicentre study compared three successive methods for sampling airway secretions applied through the same session: 1) an oropharyngeal swab (OP), 2) a chest physiotherapy session followed by a provoked cough to obtain sputum (CP-SP) and 3) a second oropharyngeal swab collected after chest physiotherapy (CP-OP). *Haemophilus influenzae*, *Staphylococcus aureus* and *P. aeruginosa* growth cultures were assessed. Accuracy tests and an equivalence test were performed to compare the three successive methods of collection.

300 nonexpectorating children with CF were included. *P. aeruginosa* was detected cumulatively in 56 (18.9%) children, and according to the different collection methods in 28 (9.8%), 37 (12.4%) and 44 (14.7%) children by using OP, CP-OP and CP-SP, respectively. Compared with OP, the increased detection rate was +22% for CP-OP (p=0.029) and +57% for CP-SP (p=0.003). CP-SP had the best positive predictive value (86.3%) and negative predictive value (96.0%) for *P. aeruginosa* compared with the overall detection.

The results of this adequately powered study show differences in the rates of pathogens detected according to the sampling method used. Chest physiotherapy enhanced detection of *P. aeruginosa* in nonexpectorating children with CF.

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Sputum collection after a chest physiotherapy session strongly enhances the detection of *P. aeruginosa* in nonexpectorating CF children compared with the commonly used oropharyngeal swab method. Oropharyngeal swab after physiotherapy may be an acceptable alternative. https://bit.ly/3757ewq

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Introduction

Cystic fibrosis (CF) is a genetic disease characterised by altered lower airway clearance and recurrent lower airway infections, which both lead to CF-related lung disease progression. Patient follow-up should target the early detection of lower airway colonisation by potentially pathogenic microorganisms. Thus, current guidelines recommend sampling airway secretions for microbiological culture and analysis at least four times per year and proposing adapted antibiotic treatment [1].

Early detection of *Pseudomonas aeruginosa* in CF is crucial as early treatment is a key to delaying *P. aeruginosa*-related chronic bronchial infection [2]. *P. aeruginosa* colonisation may be present in young children who are not yet able to expectorate spontaneously, *i.e.* 17% of children aged 4 years [3]. The accuracy of the evaluation of the microbiological status of CF patients depends on the quality of both airway samples and microbiological procedures. Thus, it is crucial to optimise sampling of lower airway secretions in CF. Currently, bronchoalveolar lavage (BAL) is considered as the gold standard; however, it cannot be performed repeatedly. Moreover, a 5-year BAL- *versus* oropharyngeal swab-directed therapy trial has been conducted for treating exacerbations in young CF children, showing similar clinical and radiological outcomes in both groups [4]. When patients are able to expectorate, results obtained in spontaneous sputum are considered to reflect the microbiological status of the lower airways. However, many children cannot expectorate as they are not able, do not want or do not have enough secretions to expectorate [5, 6].

Currently, oropharyngeal swab is a common sampling method. Nevertheless, available validation studies show notable differences in positive predictive values (PPVs) and, to a lesser extent, in negative predictive values (NPVs) compared with other methods [7–11]. Moreover, sputum cultures are likely to be better indicators of the bronchial microbiological flora than oropharyngeal swab samples, as reported by at least two studies in expectorating patients [10, 12] and one study in nonexpectorating patients [13]. Since these earlier studies, induced sputum collection by nebulised hypertonic saline solution has been proposed [13–21] with a good microbiological yield, but it is a time-consuming procedure. In contrast, chest physiotherapy has rarely been reported [16] as a reliable method for obtaining sputum in nonexpectorating children, although it is already used on a daily basis to improve airway clearance in CF.

In the present study, we focused on the methods used to sample airway secretions and formulated the hypothesis that sputum collected after a chest physiotherapy course provided more accurate samples than the oropharyngeal swab method in nonexpectorating children, as previously suggested in CF patients with productive cough [22].

Patients and methods

This prospective multicentre study was conducted in 16 French tertiary CF centres from January 1, 2006 to January 1, 2008. The included patients fulfilled the following criteria: 1) confirmed diagnosis of CF, 2) regularly followed up in a tertiary paediatric CF centre, 3) aged ⩽18 years and 4) unable to spontaneously expectorate either routinely or during a pulmonary exacerbation. The study was approved

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by the Ethics Committee of Haute-Normandie (CCP-SPHN), and informed consent was obtained from all parents and children when relevant.

Study design

Data on demographics, history of microbiological status and ability to expectorate were collected. During the appointment at the CF centre, the chest physiotherapist collected three airway secretion samples during the dedicated session by using three successive methods: 1) an oropharyngeal swab (OP method), 2) a chest physiotherapy session followed by a provoked cough to obtain sputum (CP-SP method) and 3) a second oropharyngeal swab collected after chest physiotherapy (CP-OP method).

Airway secretion sampling

Chest physiotherapists applied a standardised operating procedure across all participating CF centres. The child was maintained in a sitting position and nasal lavage was first performed carefully by flushing 5 mL isotonic saline serum in each nostril until the liquid returned clear. The nasal fluid was evacuated either through the other nostril or was aspirated by introducing a suction catheter (6 Fr; Vygon, Ecouen, France) through each nostril as far as the nasopharynx. Oral lavage was performed in older children with sterile water. Oropharyngeal swabs were rubbed on the tonsils and pharynx without touching the buccal mucosa. Then, the chest physiotherapist applied at least four series of 15 expiratory flow ventilations to the child's chest with an empty stomach and drained the bronchial secretions to the lower pharynx. Cough at the end of chest physiotherapy was either spontaneous or gently provoked by pressing the thumb on the trachea in younger patients in order to collect samples for CP-SP spontaneously whenever possible or quickly suctioned into a sterile vial (catheter AM10610P; Cair LGL, Lissieu, France).

Microbiological analyses

The airway secretion samples were transported to the microbiology laboratory within 2 h and each sample was processed separately. A standardised operating procedure based on French Standard Operating Procedures was used in all CF centres [23]. Pure and diluted liquefied sputum samples as well as oropharyngeal swabs were inoculated and incubated onto several nonselective and selective isolation media, notably for *Haemophilus influenzae*, *Staphylococcus aureus* and *P. aeruginosa* detection and quantification. All media were incubated aerobically at 37°C for 5 days and monitored daily. All different morphotypes of bacterial colonies were identified. For sputum samples only, quantification was conducted based on the $CFU{\cdot}mL^{-1}$ counts and the dilution ratio of the plates. The study of antibiotic resistance was carried out according to Comité de l'Antibiogramme de la Société Française de Microbiologie/European Committee on Antimicrobial Susceptibility Testing guidelines [24]. Quantitative cultures were only done from sputum obtained after chest physiotherapy. Other identified bacteria were noted as recommended, but only *H. influenzae*, *P. aeruginosa* and *S. aureus* counts were determined for the comparison of the three methods.

The number of patients needed was calculated according to an expected prevalence of 20% of *P. aeruginosa* in the studied population, and a true detection of 87.5% of *P. aeruginosa* and *S. aureus* and 69.2% of *H. influenzae* [10]. The expected difference between the methods of sputum collection was set to 10% [14, 15]. In total, 300 nonexpectorating patients were required to achieve a power of 90% for equivalence between the studied methods.

Statistical analysis

The results of the three methods were compared using the McNemar test (Cochran–Mantel–Haenszel for dichotomous variables). An equivalence test was performed: the three methods for collecting secretions were considered as equivalent if the difference of true detection was $\leqslant$10%, *i.e.* the 90% confidence intervals were within −10% to +10%. The analyses were also displayed with 95% confidence intervals. Then, accuracy tests (predictive values, specificity and sensitivity) were performed with reference to sputum collection (CP-SP) and according to positive results (OP+CP-SP+CP-OP). All tests were performed at a bilateral risk of α=0.05 using SAS version 8.2 (SAS Institute, Cary, NC, USA).

Results

300 children were included, but the collection of secretions failed in one child. Therefore, data from 299 children were analysed (table 1). Nasal and oral lavage were performed prior to sampling in 216 (72.2%) and 198 (66.2%) children, respectively. The acceptability of the procedures is reported in table 2 and was comparable between the three methods. No respiratory distress occurred. The results for sampling airway secretions and microbiology cultures are displayed in table 2. *S. aureus*, *H. influenzae* and *P. aeruginosa* were detected in at least one sample in 188 (63.7%), 77 (26.1%) and 56 (19.0%) children, respectively. The results of growth cultures were concordant for the three methods in 176 (58.9%) patients (n=106 for positive cultures and n=70 for negative cultures).

TABLE 1 Characteristics of the studied population

Patients	299
Age years	7.2±5.7
Male (n=298)	162 (54.4)
Age at diagnosis months	11.7±24.9
Diagnosis circumstances	
Neonatal screening (n=298)	151 (50.7)
Meconium ileus	48 (16.0)
Symptoms	110 (37.0)
CFTR genotype	
ΔF508/ΔF508	148 (49.5)
ΔF508/other	110 (36.7)
Other	41 (13.7)
History of microbiological status	
Haemophilus influenzae	176 (59.1)
Pseudomonas aeruginosa	136 (45.6)
Frequency of detection	
Only once	64 (47.1)
Intermittent	51 (37.5)
Chronic	21 (15.4)
Staphylococcus aureus	243 (81.5)
Frequency of detection	
Only once	31 (13.0)
Intermittent	104 (43.5)
Chronic	103 (43.1)
Clinical status at visit	
Exacerbations	54 (18.1)
Routine control	244 (81.9)
Under antibiotic at visit	119 (39.8)
Azithromycin	51 (43.2)
Antibiotic targeting *H. influenzae*	10 (8.5)
Antibiotic targeting *P. aeruginosa*	58 (28.8)
Antibiotic targeting *S. aureus*	34 (49.2)

Data are presented as n, mean±SD or n (%). CFTR: cystic fibrosis transmembrane conductance regulator.

Equivalence test for the three methods

The results for the three bacterial species are presented for both 90% confidence intervals (primary end-point) and 95% confidence intervals (table 3). Positive cultures obtained after CP-SP and CP-OP *versus* OP differed according to the studied bacteria. Although close to the 90% confidence intervals equivalence for *P. aeruginosa*, CP-SP appeared the most efficient method for the three bacteria. Conversely, OP and CP-OP as well as CP-SP and CP-OP appeared to be equivalent methods. The only difference was a better identification of *S. aureus* with CP-OP compared with OP. The results for 95% confidence intervals indicated that CP-SP is a better method than CP-OP, which is a better method than OP for detecting these three bacteria.

Accuracy of analyses of microbiological growth obtained by the three methods

CP-SP detected the three bacterial species more frequently than OP (p=0.013) (table 1). *P. aeruginosa* was detected in 56 (18.9%) children. Among those children, the use of CP-SP, CP-OP and OP contributed to detecting *P. aeruginosa* infection in 44 (78.6%), 37 (66%) and 28 (50%) children, respectively. Thus, the use of CP-SP and CP-OP augmented the overall detection of *P. aeruginosa* by +57% (p=0.003) and +22% (p=0.029), respectively, compared with OP. In detail, CP-SP and CP-OP enabled the additional identification of 22 and 13 infected children, respectively, who were not identified by OP (figure 1a). Conversely, CP-SP compared with OP or CP-OP failed to detect positive *P. aeruginosa* growth in 11 (19.6%) children. *P. aeruginosa* was detected with both OP and CP-OP in three samples, with OP only in three samples, and with CP-OP only in five samples. All *P. aeruginosa* strains detected by both OP and CP-SP had similar antibiotic susceptibilities.

H. influenzae was detected in 77 (26.1%) children. In samples collected after CP-SP and CP-OP, *H. influenzae* was cultured in 59 (20.0%) and 50 (17.1%) children, respectively, compared with 43 (14.6%) children with OP. Therefore, the use of CP-SP identified 75.3% of the infected children, leading to an additional detection rate of 34.9% (p=0.01) for *H. influenzae* compared with the use of OP. *H. influenzae*

TABLE 2 Collection of airway secretions and growth results by three different methods

	OP	CP-SP	CP-OP
Successful collection of samples	299 (100.0)	296 (98.9)	299 (100.0)
Aspiration		160 (54.1)	
Expectoration		127 (42.9)	
Missing data		9	
Occurrence of cough	105 (36.3)	299# (100.0)	113 (39.4)
Missing data	10		12
Safety			
Overall acceptability	212 (72.9)	210 (72.4)	206 (71.5)
Nausea/vomiting	20 (25.3)	13 (16.7)	21 (25.9)
Crying	51 (66.7)	68 (86.1)	57 (71.3)
Agitation	40 (51.3)	44 (55.7)	34 (42.5)
Other		2 (2.7)	
Microbiological analyses			
Negative for the three bacteria	86	49	66
Positive for at least one bacteria	209	246	227
Haemophilus influenzae			
Positive	43 (14.6)	59 (20.0)	50 (17.1)
Negative	252 (85.4)	236 (80.0)	243 (82.9)
Missing data	4	4	6
Pseudomonas aeruginosa			
Positive	28 (9.5)	44 (15.0)	37 (12.6)
Negative	267 (90.5)	249 (85.0)	257 (87.4)
Missing data	4	6	5
Staphylococcus aureus			
Positive	144 (48.8)	156 (52.9)	152 (51.5)
Negative	150 (50.8)	139 (47.1)	143 (48.5)
Missing data	5	4	4

Results are expressed as n (%) or n. OP: oropharyngeal swab; CP-SP: chest physiotherapy session followed by a provoked cough to obtain sputum; CP-OP: second oropharyngeal swab collected after chest physiotherapy. #: provoked cough is part of CP-SP to obtain airway secretions.

carriage was detected with only OP and only CP-OP sampling in 11 (14.2%) and 15 (19.4%) infected children, respectively (figure 1b).

S. aureus grew in 181 (61.1%) children and no significant difference was seen in the detection of additional patients irrespective of the chosen methods (figure 1c).

TABLE 3 Equivalence test for the three methods and comparison of the paired results (McNemar test)

	p-value (McNemar test)	Difference	90% CI	95% CI
Haemophilus influenzae				
OP/CP-SP	0.014	−5.4	−10.5 to −0.3#	−11.5 to 0.7#
OP/CP-OP	0.144	−2.5	−7.4 to 2.5	−8.4 to 3.4
CP-SP/CP-OP	0.194	2.9	−2.3 to 8.2	−3.3 to 9.2
Pseudomonas aeruginosa				
OP/CP-SP	0.003	−5.5	−10.0 to −1.1#	−10.8 to −0.2#
OP/CP-OP	0.029	−3.1	−7.3 to 1.1	−8.1 to 2.0
CP-SP/CP-OP	0.144	2.4	−2.2 to 7.1	−3.1 to 8.0
Staphylococcus aureus				
OP/CP-SP	0.159	−3.7	−10.5 to 3.0#	−11.8 to 4.3#
OP/CP-OP	0.262	−2.4	−9.1 to 4.4	−10.4 to 5.7#
CP-SP/CP-OP	0.466	1.4	−5.4 to 8.1	−6.7 to 9.4

Differences, 90% confidence intervals and 95% confidence intervals are presented as %. OP: oropharyngeal swab; CP-SP: chest physiotherapy session followed by a provoked cough to obtain sputum; CP-OP: second oropharyngeal swab collected after chest physiotherapy. #: confidence interval outside −10% to +10% (*i.e.* 90% CI) or −5% to +5% (*i.e.* 95% CI) signifying that the methods are not equivalent. p<0.05 indicates statistical significance.

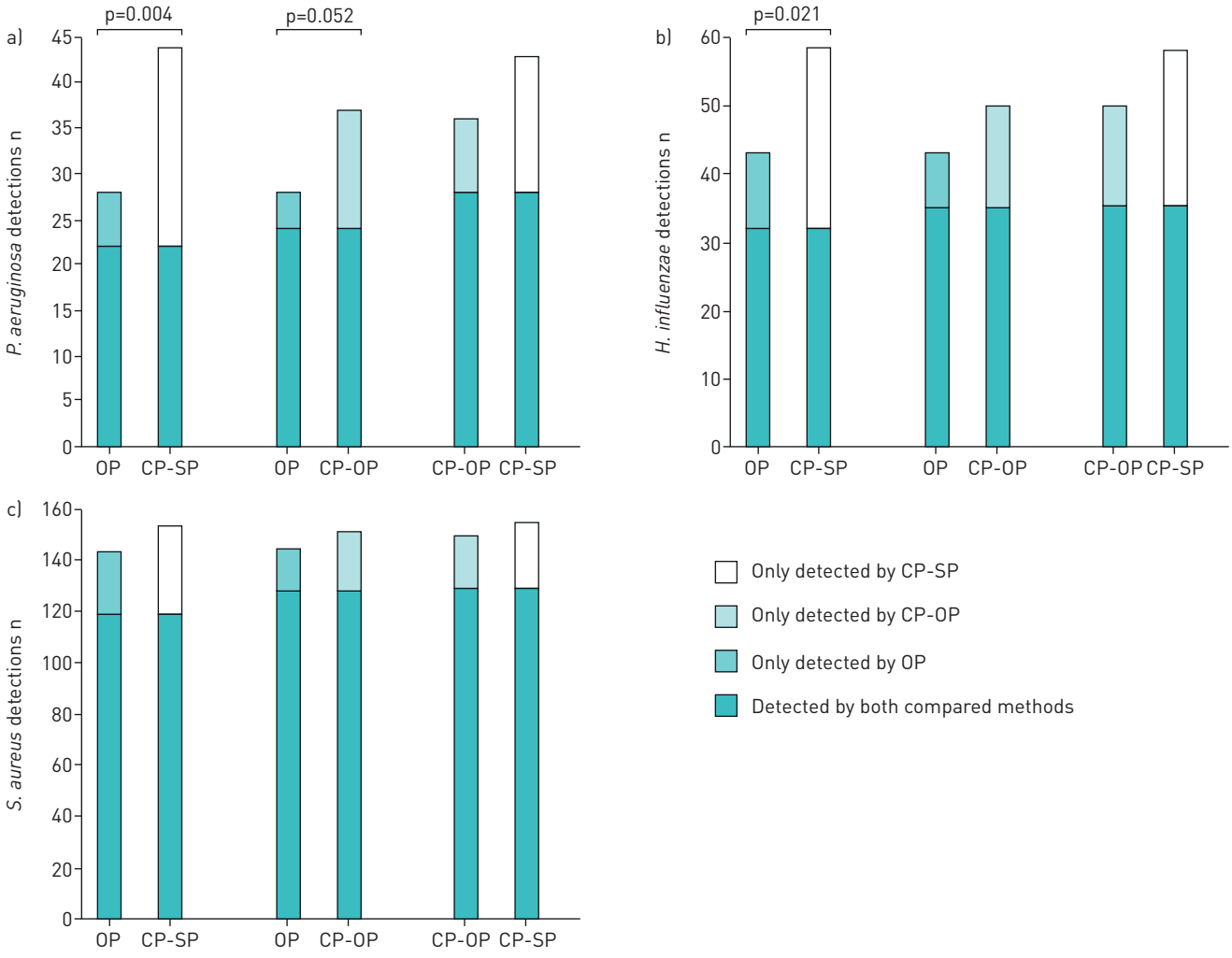

FIGURE 1 Number of a) *Pseudomonas aeruginosa*, b) *Haemophilus influenzae* and c) *Staphylococcus aureus* detections in the airway secretions of 295 nonexpectorating cystic fibrosis patients using three methods: OP (oropharyngeal swab), CP-SP (a chest physiotherapy session followed by a provoked cough to obtain sputum) and CP-OP (a second oropharyngeal swab collected after chest physiotherapy). The results of positive airway secretion cultures collected by each method are compared by pairs. CP-SP and CP-OP significantly increased the detection of patients with *P. aeruginosa* compared with OP. CP-SP significantly increased the detection of patients with *H. influenzae* compared with OP.

Sensitivity, specificity, predictive values and accuracy of OP and CP-OP were calculated by referring to the CP-SP results (table 4). OP and CP-OP had a weak sensitivity for *P. aeruginosa* identification compared with CP-SP. The predictive values of each method were then analysed by referring to all bacterial isolations obtained from all three sampling methods (table 5). CP-SP provided the best PPV and NPV for *P. aeruginosa* and *S. aureus*.

Additional effects of age, cough/symptoms and current antibiotics were analysed. None of them had any relationship with the results of the current analyses.

Acceptability of chest physiotherapy and sampling was determined. Overall, chest physiotherapy was well accepted in 75.5% of in this young population, 55% were agitated and 86.1% cried mostly before starting the technique. The acceptability of sampling was also good (72.4%) and nausea was observed in 13 (16.7%) children. No respiratory worsening or distress was reported.

Discussion

In this present study, we focused on the methods used to sample airway secretions and two different statistical approaches were applied. The first considered equivalence testing at both 90% and 95% confidence intervals; the second tested the accuracy of the methods used and reflected the assessment at an individual level. Equivalence testings showed that oropharyngeal swab before chest physiotherapy (OP method) and sputum collected after chest physiotherapy (CP-SP method) provided different results. Our results clearly sustained the hypothesis that sputum provided a higher yield for the three studied bacteria.

TABLE 4 Accuracy of bacteria detection by the OP and CP-OP methods *versus* the CP-SP method

	Specificity	Sensitivity	PPV	NPV	Accuracy
Haemophilus influenzae					
OP *versus* CP-SP	95.3	55.0	74.4	89.5	87.3
CP-OP *versus* CP-SP	93.6	60.3	70.0	90.6	87.1
Staphylococcus aureus					
OP *versus* CP-SP	76.6	81.7	82.5	76.0	79.1
CP-OP *versus* CP-SP	84.8	83.2	86.0	81.9	84.0
Pseudomonas aeruginosa					
OP *versus* CP-SP	97.6	50.0	78.6	91.7	90.4
CP-OP *versus* CP-SP	96.8	65.1	77.7	94.1	92.1

Data are presented as %. OP: oropharyngeal swab; CP-SP: chest physiotherapy session followed by a provoked cough to obtain sputum; CP-OP: second oropharyngeal swab collected after chest physiotherapy; PPV: positive predictive value; NPV: negative predictive value.

Considering *P. aeruginosa* results alone, one-third of the children infected with this pathogen would not have been identified by using oropharyngeal swab cultures alone.

Few studies have validated methods for sampling airway secretions in nonexpectorating children and those have mainly compared them with BAL, considered as the gold standard. Existing data in the literature present the results for the three common potentially pathogenic bacteria in CF [7–10]. Oropharyngeal swab sensitivity, specificity, PPV and NPV values varied widely between studies; the ranges for *P. aeruginosa* were 46–75%, 80–97%, 55–83% and 70–97%, respectively. The variations in these results might depend on various parameters, mainly on the size of the tested population for each bacteria and consequently on the relevant number of positive and negative growths for statistical analyses. For instance, an overwhelming number of negative culture growths will overestimate the NPV and consequently bias correct estimation of the PPV. The up-to-date largest Australian study compared 690 paired oropharyngeal swab and BAL sampling cultures in 181 young children with a prevalence of 7.8% *P. aeruginosa* infections [11]. Oropharyngeal swab compared with BAL had a very low sensitivity and PPV for detecting *P. aeruginosa*: 23.0% and 18.2%, respectively.

We powered our study to prevent any errors related to the size of the populations, according to the expected results of lower powered studies which compared sputum growth samples with samples obtained using other methods. Moreover, sputum analyses allowed quantification of pathogens and the detection limit of culture growth was 10^2 CFU·mL^{-1}. In expectorating patients, BAL was better correlated with sputum than oropharyngeal swab culture growth [10] and sputum has been shown to provide more sensitive airway material for microbiological cultures than oropharyngeal swab [12]. Seven studies have explored the benefit of induced sputum cultures in smaller sized populations (19–125 children) with a range from 29 to 167 samples [13–15, 17, 18, 20, 21]. The additional yield of induced sputum compared with oropharyngeal swab or spontaneous sputum collection varied from 0% to +175% pathogen growths, and conversely enabled the

TABLE 5 Predictive values of each method with reference to pooled positive cultures

	Haemophilus influenzae		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
	PPV	**NPV**	**PPV**	**NPV**	**PPV**	**NPV**
Total	n=77		n=181		n=56	
OP	n=43		n=141		n=28	
	55.8	86.5	77.9	74.0	51.9	90.1
CP-OP	n=50		n=150		n=36	
	64.9	89.7	82.8	79.2	66.7	92.7
CP-SP	n=59		n=153		n=44	
	76.6	92.4	84.5	80.4	86.3	96.0

Predicative values are presented as %. PPV: positive predictive value; NPV: negative predictive value; OP: oropharyngeal swab; CP-SP: chest physiotherapy session followed by a provoked cough to obtain sputum; CP-OP: second oropharyngeal swab collected after chest physiotherapy. The values were calculated in the children who underwent the complete procedure (all three sampling methods).

identification of new pathogens in the seven studies. This underlined the risk of false-negative cultures from oropharyngeal swabs. The recent large study by Ronchetti *et al.* [21] compared the results of 169 paired samples of oropharyngeal swabs by coughing and induced sputum from 103 CF children. They identified at least one pathogen in 38% of induced sputum compared with 14% of swab samples. 92% of the pathogens were isolated from sputum compared with 31% from samples collected with oropharyngeal swabs by coughing. Their findings were independent of the presence of symptoms and age, as in our own study. In a molecular study, Zemanick *et al.* [25] observed a marked underestimation of the detected bacterial strains in oropharyngeal swab compared with sputum samples. Few studies focused on nonexpectorating children. In one study [14], 42% of 20 nonexpectorating children were shown to carry new bacteria. Zampoli *et al.* [20] reported a higher yield of induced sputum (+81%) than oropharyngeal swab growth cultures in infants. However, the low prevalence of positive growth samples in these previous studies prevented specific conclusions according to identified bacteria. The present study supports the use of the CP-SP method for detecting additional *P. aeruginosa*, which is known as a prognostic factor in CF. The benefit of induced sputum to detect additional *P. aeruginosa* was not found to be relevant in three cohorts [18, 20], although it increased the screening by 54% in another small study [19]. Our results also suggest that a swab applied after chest physiotherapy (CP-OP method) is an acceptable alternative method. The qualitative effect of chest physiotherapy on swab culture was previously suggested, with a two-fold increase achieved in the detection of *P. aeruginosa* [26] and doubling sensitivity of sputum compared with BAL [16].

Although microbiological analyses of collected sputum during a chest physiotherapy session allowed screening of 78.5% of the infected children with *P. aeruginosa*, the PPVs of sputum growths did not achieve 100%. In fact, 2% of *P. aeruginosa*-infected children were screened solely with oropharyngeal swabs. The lack of concomitant positive sputum growths has previously been underlined and remains a topic of discussion. With the growing interest in upper airway infection and the need to treat it [27], it is thought that isolated upper airway *P. aeruginosa* growths might reflect sinus infection [28], considered as a bacterial reservoir [29, 30]. Others defined them as false-positive results with no treatment necessary [11, 31].

This study has a number of strengths and limitations. The strengths are the adequately powered study on the number of detected *P. aeruginosa* for preventing bias in the measures of test accuracy. These results are those expected through the routine follow-up of a CF paediatric cohort, as 96% of the collections were performed during routine visits. Attention was given to providing training and guidelines in all CF centres for a similar application of identical physiotherapy practices and microbiological methods, with detection of bacteria at a low level of 10^2 CFU·mL^{-1}. No centre effect was identified through the analyses. There are also some limitations. The techniques of chest physiotherapy for CF patients have progressed. The airway clearance technique currently used in France is autogenic drainage, or the concept of flow and breathing level modulation, as recommended by the European CF Society [32]. A similar acceptability was reported for each sampling method. Most crying started before the session in this young population. Thus, no specific adverse effect might be attributed to chest physiotherapy, a routine home care practice in CF. The aim of this study was to test the growth results in real life and, unfortunately, no molecular analysis of *P. aeruginosa* strains was performed. However, antibiotic susceptibility testing was constantly similar for an individual patient. This suggests that the same strains were found in the upper and lower airways, as previously demonstrated [33]. Not all of the study population benefitted from nasal lavage or oral washing, although no difference in the proportion of the identified pathogens was observed. Since this study, the matrix assisted laser desorption ionisation-time of flight mass spectrometry identification tool has become available, although this would not significantly improve the identification of *P. aeruginosa*, *H. influenzae* and *S. aureus* in CF compared with the usual phenotypic method [34].

In conclusion, this prospective, powered, multicentre study has demonstrated that sputum collected after chest physiotherapy in nonexpectorating CF children provides the most sensitive samples for *P. aeruginosa* screening. Oropharyngeal swab applied after chest physiotherapy remains an acceptable alternative. These results may enhance both patient care and end-points for research trials. A study using induced sputum, a method of a growing interest, and/or PCR diagnosis will be further clinically useful [21, 35, 36]. Given the microbial diversity and the emergence of other pathogenic species (*i.e. Achromobacter* spp. and *Stenotrophomonas* spp.) it would be interesting to extend the study to a larger panel of biomarker bacteria or even to make the comparison by metagenomics [21].

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