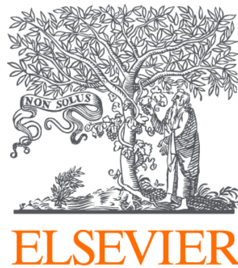

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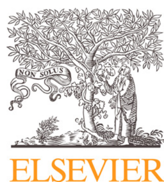

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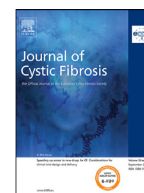

Sputum versus nasopharyngeal samples for the molecular diagnosis of respiratory viral infection in cystic fibrosis: A pilot study☆

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ABSTRACT

Viruses are important agents in lung function deterioration in Cystic Fibrosis (CF). To date, no standard operating procedures (SOPs) have been established to determine which sampling method is the most effective for an optimal virological diagnosis of respiratory viral infections in CF. Here we investigated the performances of two sampling sites, sputum samples versus nasopharyngeal (NP) swabs, for thirty participants from three CF centres presenting an acute respiratory infection. Sputum and NP samples were simultaneously collected and multiplex PCR targeting 16 to 18 viruses were performed. Viruses were detected for 18/30 patients (60%). A high concordance between the sputum and NP samples was observed in 25 (83%) paired samples of which 13 tested positive and 12 tested negative. These results highlighted the relevance of sputum sampling for diagnostic of respiratory viruses in CF, which is less invasive and better accepted by CF patients than NP, and allows accurate bacterial detection.

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1. Introduction

Lung function deterioration is a common event in cystic fibrosis (CF). Pulmonary deterioration has a multifactorial origin with chronic inflammation and polymicrobial infections having a major impact on the respiratory tract. While the role of bacterial pathogens in CF is well known, respiratory viruses are now also considered as major players in CF lung morbidity. Viruses have been detected in 28 to 50% of exacerbations, with a significant impact on the clinical outcome, an increased risk of hospitalisation and longer antibiotic therapy [1–4]. Viruses could facilitate bacterial colonisation of the CF airways, affecting innate immunity, bacterial adhesion, impairing mucociliary clearance, which facilitates persistence of pathogens in the lung and intensify inflammatory reaction to the infection [5,6]. Viral infection screening can be seen as essential and systematically advisable in the clinical context of acute respiratory syndrome in CF. Many recent commercial multiplex PCRs are available allowing for the simultane-

☆ CF, Cystic Fibrosis. NP, Nasopharyngeal. SOPs, Standard Operating Procedures

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Table 1
Viral screening spectra in the three participant centres.

Centre	Multiplex Technique	Viral screening	patients	Age range (years)
A	*Multi Well System MWS R-GENE® bioMerieux* *	*ADV, EV, RV, IVA, IVB, PIV 1-4, RSV A and B, HCoV 229E, NL63 and OC43, and HKU1, HMPV, HBoV*	*5*	*6-43 3 childs 2 adults*
B	*RespiFinder® SMART 22 Fast v2.0, PathoFinder***	*ADV, EV/RV, IVA, IVA(H1N1) vpdm09, IVB, PIV1-4, RSV A and B, HCoV 229E, OC43, NL63 and HKU1, HMPV, HBoV*	*13*	*2-29 2 childs 11 adults*
C	*Anyplex II RV16® Seegene****	*ADV, EV, RV, IVA,IVB, PIV1-4, RSV A and B, HCoV229E, OC43, NL63, HMPV, HBoV*	*12*	*12 adults*

ADV: Adenovirus, EV: Enterovirus, RV: Rhinovirus, IVA: Influenza virus, IVB: Influenza virus B, PIV: Parainfluenza virus, RSV: Respiratory syncytial virus, HCoV: Human Coronavirus, HMPV: Human Metapneumovirus, HBoV: Human Bocavirus
The multiplex technique used by centres A and C detected rhinoviruses and enteroviruses separately although centre B's multiplex technique could not differentiate rhinoviruses from enteroviruses, which gave a combined RV/EV result.
* *Paba P, Farchi F, Mortati E, Ciccozzi M, Piperno M, Perno CF, et al. Screening of respiratory pathogens by Respiratory Multi Well System (MWS) r-gene assay in hospitalized patients. The new microbiologica. 2014;37:231-6.*Resa C, Bertrand M, Bes J, Vignoles M, Magro S, Barranger C, et al. Respiratory multi well system (mws) r-gene™: Simultaneous detection of infectious agents involved in respiratory diseases. France2010.*
** *Dabisch-Ruthe M, Vollmer T, Adams O, Knabbe C, Dreier J. Comparison of three multiplex PCR assays for the detection of respiratory viral infections: evaluation of xTAG respiratory virus panel fast assay, RespiFinder 19 assay and RespiFinder SMART 22 assay. BMC infectious diseases. 2012;12:163.*
*** *Kim HK, Oh SH, Yun KA, Sung H, Kim MN. Comparison of Anyplex II RV16 with the xTAG respiratory viral panel and Seeplex RV15 for detection of respiratory viruses. Journal of clinical microbiology. 2013;51:1137-41.*

ous detection of a wide spectrum of known human respiratory viruses [7,8].

Nasopharyngeal (NP) sampling is considered as a reference for respiratory viral screening in general population, but this technique is invasive and sometimes painful and is therefore not systematically carried out, especially in CF patients. Furthermore, epidemics of virulent respiratory virus as H1N1 or SARS-CoV-2 highlight difficulties in obtaining a large number of good quality NP specimens [9,10]. Regardless, bacterial analyses are routinely performed on sputum samples taken from CF patients. As it is a non-invasive method, it seems relevant to define whether sputum sampling could be recommended for the molecular viral diagnosis of acute respiratory infections in CF in order to improve patient comfort while maintaining a sensitive viral diagnosis. Few monocentric studies previously reported scattered data when comparing the two sampling sites for virus detection and did not lead to a change in routine practices [1,3,11].

In this context, the aim of this study was to compare sputum and NP sampling in CF patients for respiratory virological diagnosis.

2. Materials and methods

This study was a retrospective multicentre investigation hosted by the MucoMicrobes working group and was approved by the ethics committee at the Brest Teaching Hospital (CHRU of Brest, France). CF patients were recruited from three French CF centres: CHU of Angers (centre A); Roscoff (centre B), and Foch Hospital, Paris-Suresnes (centre C). These patients were retrospectively enrolled between February 2016 and February 2017 based on the following inclusion criteria: patients with a confirmed diagnosis of CF, adults or children regardless of their age, able to expectorate, with signs of acute lower or upper respiratory tract infection, during a consultation or while hospitalised.

To comply with the limited number of included participants inherent to a pilot study, NP swabs and sputum samples from 30 CF patients were simultaneously collected and nucleic acids were purified (Supplementary Material) [12]. In order to screen for respiratory viruses, a multiplex PCR (16 to 18 viruses) was performed according to the technique available in each centre on NP and sputum pair (Table 1). The Kappa index as well as the associated 95% confidence interval (CI) were calculated to determine the degree of agreement between both investigated methods [13].

3. Results and discussion

Thirty participants were included in this study, 25 adults (18 to 55 years old) and 5 children (2 to 15 years old). Sputum and NP samples were collected in a context of acute respiratory infection during a flu epidemic period. Clinical data were retrospectively collected for 23/30 patients. The thirty pairs of sputum-NP samples were collected within a maximum of 48 hours after the onset of the symptoms. Five, 13 and 12 participants were included in centres A, B, and C, respectively. A virus was detected for 60% of the CF patients (n=18); the most frequently identified viruses were influenza viruses and enteroviruses/rhinoviruses. Two viral co-infections were also reported (Table 2).

A perfect congruency for viral detection was observed in 25 paired-samples (83%), including 13 paired virus-positive and 12 virus-negative samples. The Kappa index, κ=0.67 [95%CI: 0.40; 0.93] indicated a substantial concordance for viral molecular diagnosis between sputum and NP samples, arguing for equivalence between the two types of sampling methods. Furthermore, there was no discordance regarding the identification of the virus type (Table 2).

CF is a difficult disease to manage and patient comfort should be given priority while limiting invasive investigations for patients. Our results showed agreement between the two types of samples in terms of molecular virus detection and position CF sputum as an optimal sampling technique for respiratory virus diagnosis with the advantage of being non-invasive and routinely taken for bacterial monitoring. The positioning of the sputum sample as the one single respiratory sample that provides the combining viral, bacterial and fungal investigations could be both relevant for health monitoring and well accepted by patients.

Viral detection discordance was described for five paired-samples. NP flocked swabs allowed the release of high viral loads present in respiratory epithelial cells**.** Sputum quality is routinely checked with epithelial respiratory cell counts [14]. In our study, the quality of the NP and sputa samples from CF patients was not verified as multiplex kits provided no cellular control. Thus, we could hypothesize that the quality of these NP samples was not optimal in the three discordant pairs, which could explain the loss of viral detection sensitivity compared with sputa as it has been well illustrated in diagnosis of COVID-19 and other viral respiratory infections [9,10]. This observation underlines the importance of implementing cellular control in future prospective studies (i.e. through the quantification of housekeeping genes such as RNAse, NADPH or HPRT1).

In our study, all samples were obtained within a maximum of 48 hours after the onset of the symptoms. The two discordant pairs with positive NP and negative sputum could be explained by samples obtained early after the onset of symptoms.

All three centres used different multiplex approaches for respiratory viral detection. These techniques are essential to improve CF patient care: if only virus is detected, an antibiotic cure could

Table 2
Viral detection concordances and discordances between sputum samples and nasopharyngeal (NP) swabs.

n=30	Sputum	NP	sexe	Age (years)	centre	Hospitalisation	Clinical symptoms
	Concordances (n=25)						
n=4	*Influenza virus B*	*Influenza virus B*	F	19	B	X	fever, bronchial obstruction, FEV1↓
			M	35	C	NA	NA
			M	19	C	NA	NA
			M	28	C	NA	fever
n=2	*Influenza virus A*	*Influenza virus A*	F	9	A	/	fever
			F	22	C	NA	fever
n=1	*Influenza virus A + respiratory syncytial virus*	*Influenza virus A + respiratory syncytial virus*	M	13	A	/	fever, bronchial obstruction, Cough
n=1	*Rhinovirus*	*Rhinovirus*	F	28	C	/	NA
n=3	*Rhinovirus/Enterovirus*	*Rhinovirus/Enterovirus*	M	29	B	/	bronchial obstruction, bronchiectasis, fever, rhinitis
			F	20	B	/	bronchial obstruction, exacerbation bronchiectasis dyspnea, thick expectoration
			M	25	B	X	fever, rhinitis, FEV1↓
n=2	*Human coronavirus OC43*	*Human coronavirus OC43*	M	24	B	/	fever, rhinitis,
			F	33	C	NA	fever, rhinitis
n=12	no virus detected	no virus detected	M	6	A	/	NA
			F	43	A	/	NA
			M	26	B	X	exacerbation,bronchial obstruction,cough, FEV1↓
			M	18	A	/	bronchial obstruction, thick expectoration, FEV1↓
			M	28	B	/	fever
			F	20	B	/	fever
			F	15	B	/	NA
			M	40	C	NA	rhinitis
			F	21	C	NA	fever
			M	21	C	NA	fever
			F	34	C	NA	fever
			M	55	C	NA	exacerbation
	Discordances (n=5)						
n=1	*Adenovirus*	*no virus detected*	F	20	B	/	Fever, FEV1↓
n=1	*Human Bocavirus*	*no virus detected*	M	29	B	/	Fever
n=1	*Influenza virus B + rhinovirus*	*Influenza virus B*	M	22	C	NA	NA
n=1	no virus detected	*Influenza virus B*	M	2	B	X	Fever, exacerbation, dyspnea, cough, thick expectoration, FEV1↓
n=1	no virus detected	*Rhinovirus/Enterovirus*	F	28	B	/	Fever, cough, thick expectoration, FEV1↓

FEV: Forced expiratory volume in 1 s
NA: data Not Available

be avoided and thus resistance antibiotic can be delayed; if a non-influenza virus is detected, oseltamivir will not be prescribed. Moreover, in the early future, new anti-VRS and anti-rhinovirus will be available, allowing a drug adapted to the viral etiology [15,16]. This might decrease secondary bacterial infections. Viral molecular detection and concomitant bacterial or fungal co-infections were not evaluated in this work but will be explored as secondary objectives in a future larger prospective study. This is valuable information as bacterial populations have been identified for 40% of viral infections requiring hospitalisation [2]; This may justify the initiation of early empirical antibiotic therapy for patients with severe viral illness [17,18].

In this study, most of the enrolled participants were adults. However, the prevalence of the viruses detected was high, with 60% of the sample pairs testing positive for a virus even though it has been reported that viruses are more frequent in children <5 years old than adults. For children, one limitation for recruitment and viral diagnosis with sputa could reside in non-expectorating patients, mostly <2 years old [19,20].

In conclusion, this pilot study highlighted the relevance of sputum sampling, and paved the way for a larger prospective study including CF viral screening to adjust the sampling recommendations in CF.

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Ethical approval

The study was approved by the ethics committee at the Brest Teaching Hospital (CHU) and was registered on the Clinical Trials.gov website.

Authors' contribution

Emilie Cardot-Martin: Investigation, Methodology, Formal analysis. **Hélène Le Guillou-Guillemette:** Investigation, Methodology, Formal analysis. **Rozenn Le Berre:** Conceptualization, Formal analysis, Visualization; Writing. **Sophie Ramel:** Investigation, Formal analysis, Resources. **Jean Le Bihan:** investigation. **Dominique Grenet:** Investigation. **Eric Farfour:** Methodology, Formal analysis. **Françoise Troussier:** Investigation. **Thierry Urban:** Investigation. **Lisa Billard:** Writing, Formal analysis. **Léa Pilorgé:** Formal analysis; Methodology, Writing. **Adissa Minoui-Tran:** Formal analysis.

Christopher Payan: Formal analysis. **Marie-Reine Munk:** Investigation. **Genevieve Hery-Arnaud:** Original draft, Formal analysis; Writing. **Sophie Vallet:** Original draft, Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Writing, Validation. All authors critically reviewed the manuscript and provided valuable comments.

Supplementary Material

Samples collection and nucleic acids purification

In order to ensure optimal sample quality, nasopharyngeal swabs and nasopharyngeal sputum were taken at the inclusion of each patient by the same experienced medical staff. For NP, a sterile flexible flocked tip with a nylon fibre swab (Copan®) was inserted into each nostril to sample the posterior nasopharynx in a rotating motion, and finally cut into a universal transport medium. Collection of sputa (spontaneous or induced sputa) was obtained as recommended for routine bacteriological cultures [12], using standardised procedures. All samples were tested by multiplex PCR within 4 days of collection when stored at 2-8°C, and within 90 minutes after thawing when stored at -80 C.

The pre-treatment protocol for sputa was standardized and applied accordingly in each participant centre. A mucolytic agent (Sputagest Selectavial MAST®, Merseyside, UK) was used at 1:20. If the sputum was still viscous, 200 μL were digested with 20-25 µl proteinase K (Qiagen®, Hilden, Germany) between 20 min and 3 h at 56°C. For sputum and NP samples, nucleic acids from 200 μL were extracted in a biosafety level 2 laboratory to avoid contamination of molecular PCR assays using an automated NUCLISENS® easyMAG^{TM} (bioMérieux S.A., Marcy l'Etoile, France) and eluted in 100 µL of RNase-DNase-free water.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2020.09.003.

References

[1] Asner S, Waters V, Solomon M, Yau Y, Richardson SE, Grasemann H, et al. Role of respiratory viruses in pulmonary exacerbations in children with cystic fibrosis. J Cystic fibros: Off J Eur Cystic Fibros Soc 2012;11:433–9.

[2] Falsey AR, Becker KL, Swinburne AJ, Nylen ES, Formica MA, Hennessey PA, et al. Bacterial complications of respiratory tract viral illness: a comprehensive evaluation. J Infect Dis 2013;208:432–41.

[3] Flight WG, Bright-Thomas RJ, Tilston P, Mutton KJ, Guiver M, Morris J, et al. Incidence and clinical impact of respiratory viruses in adults with cystic fibrosis. Thorax 2014;69:247–53.

[4] Wat D, Gelder C, Hibbitts S, Cafferty F, Bowler I, Pierrepoint M, et al. The role of respiratory viruses in cystic fibrosis. J Cystic Fibros: Off J Eur Cystic Fibros Soc 2008;7:320–8.

[5] Billard L, Le Berre R, Pilorge L, Payan C, Hery-Arnaud G, Vallet S. Viruses in cystic fibrosis patients' airways. Crit Rev Microbiol 2017;43:690–708.

[6] Rossi GA, Fanous H, Colin AA. Viral strategies predisposing to respiratory bacterial superinfections. Pediatr Pulmonol 2020;55:1061–73.

[7] Weiss ZF, Cunha CB, Chambers AB, Carr AV, Rochat C, Raglow-Defranco M, et al. Opportunities revealed for antimicrobial stewardship and clinical practice with implementation of a rapid respiratory multiplex assay. J Clin Microbiol 2019;57.

[8] Pillet S, Lardeux M, Dina J, Grattard F, Verhoeven P, Le Goff J, et al. Comparative evaluation of six commercialized multiplex PCR kits for the diagnosis of respiratory infections. PLoS One 2013;8:e72174.

[9] Abu-Diab A, Azzeh M, Ghneim R, Ghneim R, Zoughbi M, Turkuman S, et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. J Clin Microbiol 2008;46:2414–17.

[10] Jiang G, Ren X, Liu Y, Chen H, Liu W, Guo Z, et al. Application and optimization of RT-PCR in diagnosis of SARS-CoV-2 infection. medRxiv 2020;2020:20027755 02.25.

[11] Jones AM, Flight W, Isalska B, Cullen M, Mutton K, Bowling A, et al. Diagnosis of respiratory viral infections in cystic fibrosis by PCR using sputum samples. Eur Respir J 2011;38:1486–7.

[12] Mucomicrobes SG. Infections broncho-pulmonaires et mucoviscidose. In: Microbiologie SFd, editor. REMIC Référentiel en Microbiologie Médicale; 2018. p. 213–20. 6ème ed.

[13] Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychol Bull 1968;70:213–20.

[14] Wong LK, Barry AL, Horgan SM. Comparison of six different criteria for judging the acceptability of sputum specimens. J Clin Microbiol 1982;16:627–31.

[15] Rappo U, Schuetz AN, Jenkins SG, Calfee DP, Walsh TJ, Wells MT, et al. Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adult patients. J Clin Microbiol 2016;54:2096–103.

[16] Behzadi MA, Leyva-Grado VH. Overview of current therapeutics and novel candidates against influenza, respiratory syncytial virus, and middle east respiratory syndrome coronavirus infections. Front Microbiol 2019;10:1327.

[17] Kiedrowski MR, Bomberger JM. Viral-bacterial co-infections in the cystic fibrosis respiratory tract. Front Immunol 2018;9:3067.

[18] Etherington C, Naseer R, Conway SP, Whitaker P, Denton M, Peckham DG. The role of respiratory viruses in adult patients with cystic fibrosis receiving intravenous antibiotics for a pulmonary exacerbation. J Cystic Fibros: Off J Eur Cystic Fibros Soc 2014;13:49–55.

[19] Miro-Canis S, Capilla-Rubio S, Marzo-Checa L, Fontanals-Aymerich D, Sanfeliu-Sala I, Espasa-Soley M, et al. Multiplex PCR reveals that viruses are more frequent than bacteria in children with cystic fibrosis. J Clin Virol: Off Public Pan Am Soc Clin Virol 2017;86:1–4.

[20] Deschamp AR, Hatch JE, Slaven JE, Gebregziabher N, Storch G, Hall GL, et al. Early respiratory viral infections in infants with cystic fibrosis. J Cystic Fibros: Off J Eur Cystic Fibros Soc 2019;18:844–50.