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Pathogenicity of individual rhinovirus species during exacerbations of cystic fibrosis

To the Editor:

Pulmonary exacerbations in patients with cystic fibrosis (CF) accelerate deterioration of lung function and cause increased morbidity and mortality [1]. Viruses have been identified in up to 60% of CF related pulmonary exacerbations with a higher prevalence in children [2–5]. FLIGHT *et al.* [4] identified viruses in 40% of exacerbations in adult patients with CF; viral PCR was performed on nasal, throat, and sputum specimens. We routinely undertake viral throat swabs in all adult patients receiving intravenous antibiotics as part of prospective clinical surveillance, with data automatically uploaded to the electronic patient record (EPR). Patients with self-limiting exacerbations are not screened. In our selected cohort, viral PCR was positive in 9.2% of cases and a positive result is associated with longer duration of treatment, increased inflammatory response, and less recovery of lung volumes [3].

In both studies human rhinovirus (HRV) accounted for >70% of the viruses identified [3, 4].

HRVs are common respiratory pathogens that traditionally, it has been felt, result in self-limiting and mild upper respiratory symptoms. HRVs are members of the *Enterovirus* genus from the family *Picornaviridae*. With the advent of molecular methods of detecting HRV a great diversity of HRVs has been identified [6]. This includes HRV species C, which is unculturable and failed to be identified by previous viral isolation methods. Three species of HRV are now known, these are HRV A, HRV B, and HRV C. Within each species are many types, which are classified according to sequence divergence in the VP1 region that codes for a capsid protein [6].

Recent studies have implicated certain species of HRV as being more pathogenic. In childhood asthma, exacerbations due to HRV-C have been associated with more severe exacerbations than other HRV groups [7, 8]. In stable children with CF the presence of HRV-C or HRV-A2 is associated with an increased frequency of exacerbations [9]. Studies in adult patients with CF have investigated the role of individual HRV species, although, they have not been specifically designed to assess the effect on clinical outcomes following exacerbations. FLIGHT *et al.* [4] performed typing on HRV positive samples from 42 clinic visits involving 27 patients; HRV-A was most frequently identified and appeared to induce more upper respiratory symptoms than HRV-B. The group identified HRV-C in only 4.8% of patients. In contrast, GOFFARD *et al.* [5] detected HRV-C in 44% of pulmonary exacerbations, although the numbers were small.

The aim of this study was to investigate the relative prevalence of HRV species in throat swabs taken from a large cohort of adult CF patients, who were starting on *i.v.* antibiotics for pulmonary exacerbations, and to explore the differences in the clinical outcomes between the HRV species.

Between December 2009 and May 2012 all throat swabs positive for HRV were stored at -80°C and later typed in more detail. Only post lung-transplant patients were excluded from the study. Date matched non-CF samples that were positive for HRV were used as controls in order to evaluate the HRV species present in the general population. For the control samples no patient identifiable data was available, other than the location and date the sample was taken. The study was approved by the Leeds Central Research Ethics Committee and written consent was obtained from all patients.

Samples underwent RNA extraction using the Qiagen Virus Mini Spin Kit (Qiagen, Manchester, UK) according to the manufacturer's instructions. Extracted RNA was reverse transcribed into cDNA using the Promega reverse transcription system (Promega, Southampton, UK), as per manufacturer's instructions. The resulting cDNA underwent two rounds of nested PCR reaction to produce amplicons of the VP4/VP2 region, which were sequenced using the Big Dye Terminator kit (Applied Biosystems, Paisley, UK). Sequences were aligned in SSE sequence editor v2.0 [10], and phylogenetic trees constructed in the MEGA 4 software [11], to allow for genetic typing of the specimens.

The HRV species were compared with demographic and clinical outcome data on the EPR. Electronic clinical text entries were reviewed to establish the number of patients who the attending clinician believed to have symptoms consistent with the upper respiratory viral infection. The Mann-Whitney U test and the Wilcoxon signed-rank test were used for unpaired and paired data, respectively (Minitab14.0; Minitab, Coventry, UK). Proportions were compared using Chi-squared test. $p < 0.05$ was considered significant.

A total of 1800 pulmonary exacerbations in 360 adults were assessed during the study period. HRV was identified in 126 (7%) of exacerbations; further detailed typing was technically possible in 104 of the samples. HRV-A was identified in 57 (55%) isolates, HRV-B in 23 (22%), and HRV-C in 24 (23%). No HRV species appeared more pathogenic, no differences were found between HRV species and hospitalisation, length of treatment, levels of inflammatory markers, recovery of lung function or the time to the next exacerbation (table 1).

54 (52%) out of 104 HRV-positive patients had documented viral symptoms at presentation. Symptoms included new nasal discharge (74%), sore throat (43%), fever (19%), myalgia (11%), and headache (6%).

TABLE 1 Comparison of human rhinovirus (HRV) species and clinical outcomes

	HRV-A	HRV-B	HRV-C	p-value
Total	57	23	24	
Male	25 [44]	7 [30]	18 [75]	0.006
Sputum				
Non- <i>Pseudomonas</i>	10	1	2	0.54
Intermittent <i>Pseudomonas</i>	10	2	4	
Chronic <i>Pseudomonas</i>	36	19	17	
<i>Burkholderia cepacia</i>	1	1	1	
Cystic fibrosis related diabetes	37 [65]	15 [65]	19 [79]	0.43
Location of exacerbation treatment				0.73
Hospital	21	6	11	
Home	11	11	8	
Both	6	6	5	
Viral symptoms	32 [56]	13 [57]	9 [38]	0.31
Length of treatment days	14 [6–100]	14 [9–32]	14.5 [1–31]	0.67
Baseline CRP $\text{mg}\cdot\text{L}^{-1}$	26.5 [0–176]	24.0 [0–263]	21.5 [0–154]	0.43
Post CRP $\text{mg}\cdot\text{L}^{-1}$	10.4 [0–144]	7.1 [0–35]	6.5 [10–53]	0.52
FEV₁ best 6 month prior % pred	51 [16–103]	52 [20–91]	45.5 [15–126]	0.47
FEV₁ best 6 month post % pred	49 [11–108]	48 [24–89]	40 [15–118]	0.51
Change in FEV₁ % pred	–3 [–32–9]	–4 [–13–14]	–3 [–11–5]	0.54
Time to next exacerbation days	44 [0–725]	46.5 [0–271]	47 [7–211]	0.70
Exacerbations 1 year prior	4 [0–14]	3 [0–8]	3 [1–6]	0.95
Exacerbations 1 year post	4 [0–18]	4 [0–10]	3 [0–8]	0.25

Data are presented as n, n (%) or median [range], unless otherwise stated. FEV₁: forced expiratory volume in 1 s; CRP: C-reactive protein.

No significant difference in clinical outcome was identified in the presence or absence of viral symptoms. Furthermore no HRV species was found to be significantly associated with viral symptoms.

While the study was not designed to assess viral persistence, five patients were identified as having an identical HRV type on a subsequent exacerbation. One patient with persistent symptoms remained positive for the same HRV C15 type 5 weeks later. The remaining four patients remained positive for between 2 and 4 weeks.

A group of 126 non-CF patients positive for HRV were used as controls; further typing was possible in 76 samples. 33 samples were collected in outpatients and 43 from hospitalised patients. HRV-A was identified in 35 (46%) isolates, HRV-B in 17 (22%), and HRV-C in 24 (32%). The frequencies of HRV types in non-CF controls were similar to patients with CF ($p=0.63$).

In conclusion, viral throat swabs are frequently positive for HRV in adult patients with CF receiving antibiotics for acute pulmonary exacerbations. HRV-A is most common and represents 55% of all HRV isolated. Interestingly, HRV-C accounted for 23% of the HRV species identified. There was no difference in clinical outcome between HRV species and the variation in species is likely to reflect the community reservoir.

The proportion of patients identified as being HRV positive was lower than other recently published data [4, 5]. This may be, in part, due to the absence of additional nose and sputum samples collected in other studies [4]. Throat swabs when used alone may miss patients with lower viral loads. An alternative explanation is that the lower prevalence may reflect the different study cohort. In this study throat swabs were only obtained when patients needed *i.v.* antibiotics and excluded those with self-limiting episodes.

A small number of patients were found to have the same HRV type on a subsequent exacerbation. The duration of HRV PCR positivity in patients with CF is unknown, although, there is a single case report describing the persistence of HRV-A33 for >2 years [12]. HRV-RNA positivity can persist for up to 6 weeks in asthma; although most patients clear it in <2 weeks [13, 14].

Key factors that influence the effectiveness of the lungs to clear HRV are complex and poorly understood. Studies have reported a link with the use of immunosuppression and the presence of impaired interferon response [15]. Future research is needed to investigate the influence of viral infection on the CF airway and to correlate the immune response and viral loads with clinical recovery.

@ERSpublications

Rhinovirus associated pulmonary exacerbations: no differences in clinical outcomes between individual species <http://ow.ly/KJatw>

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Referred shoulder pain (C4 dermatome) can adversely impact diaphragm pacing with intramuscular electrodes

To the Editor:

Diaphragm pacing is an alternative to mechanical ventilation in patients with high cervical cord damage (>C4 dermatome). It brings clinical benefits and reduces health costs [1–3]. It is also indicated in certain cases of central hypoventilation [4]. Two types of diaphragm pacing devices are commercially available. With intrathoracic phrenic stimulation, electrodes are implanted around the phrenic nerves in the thorax (Avery Biomedical, Commack, NY, USA; and Atrotech, Tampere, Finland). Nerve dissection during a video-assisted mini-thoracotomy allows intimate contact of the stimulating electrodes with the nerve [5]. Stimulating currents typically range between 0.3–3.5 mA [6]. With intradiaphragmatic stimulation, hook wire electrodes are laparoscopically implanted in the diaphragm in the vicinity of the phrenic nerve termination (Synapse Biomedical, USA) [in 5]. Stimulating currents typically range between 5–20 mA. In quadriplegic patients, intradiaphragmatic phrenic stimulation is effective [7] and has been advocated as easier to implement and less expensive than its intrathoracic counterpart [5]. In patients who retain spontaneous diaphragm activity, this approach is appealing because it is devoid of the theoretical risk of procedure-induced phrenic nerve damage associated with the intrathoracic route. For these reasons, we implanted intradiaphragmatic phrenic stimulators in four hypoventilation patients after the device was authorised in France in 2010. In France, both intrathoracic and intradiaphragmatic diaphragm pacing are authorised and reimbursed by social security in quadriplegia and central hypoventilation and are managed at a single multidisciplinary centre nationwide.

This report is motivated by the fact that, in these four cases, diaphragm pacing efficiency was compromised and clinical management complicated by phrenic stimulation associated pain. We did not observe this in comparable patients implanted intrathoracically.

We describe nine patients (table 1) who all had central hypoventilation, documented diaphragm responses to phrenic stimulation and preserved sensitivity on clinical examination (present and symmetrical pin-prick and light-touch sensations on routine systematic neurological examination). They reported normal pain perception in daily life. Phrenic stimulator implantations were performed over a 15-year period. Patients 1–5 were implanted intrathoracically (single surgeon, group 1) and patients 6–9 were implanted intradiaphragmatically (single surgeon, group 2). Patients 1–3 were implanted first, then patients 6–9 and then patients 4–5. Patients 1–3 were implanted in the context of an externally approved research [2]. The other patients were implanted on clinical indications. They gave consent to the anonymous use of their clinical data, which was approved by the Institutional Review Board of the Société de Pneumologie de Langue Française (decision #2014-048). The reversal of hypoventilation-related pulmonary hypertension by intradiaphragmatic phrenic stimulation in patient 6 has been described elsewhere [8].

Phrenic stimulation-induced pain was defined as pain: 1) reported spontaneously or in response to oriented questioning, 2) appearing upon start of pacing and disappearing more or less rapidly upon its discontinuation, 3) involving a pathophysiologically logical territory (upper abdominal quadrants, inferior