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Short communication

Upper respiratory virus detection without parent-reported illness in children is virus-specific

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Abstract

Background: Viral upper respiratory tract infection (vURI) may or may not present with a cold/flu-like illness (CFLI).

Objectives: For common upper respiratory viruses that cause vURIs, to determine the relative frequencies of virus detection by PCR in subjects with and without CFLIs.

Study design: Prospective follow-up of 170 children aged 1–8.6 years through the CFLI season by daily parental diary for CFLI episodes and nasal secretion sampling using PCR assays for adenovirus, coronavirus (types 229E and OC43), influenza virus (types A and B), parainfluenza (types 1–3) virus, rhinovirus, and respiratory syncytial virus (RSV).

Results: Virus was detected in 415 of 956 independent assays: 425 CFLI episodes and 531 non-CFLI periods were sampled; samples from 270 (64%) CFLI episodes and 145 (27%) non-CFLI periods contained virus detected by PCR. Rhinovirus was most frequently detected at 64%, followed by mixed viruses at 12%, RSV at 7%, and the other viruses at 3–5% of all detections. About 85% of RSV, influenza A and adenovirus detections were associated with a CFLI, whereas less than 62% of other virus detections were associated with CFLI.

Conclusions: The frequency of PCR virus detection without CFLI was different among viruses. This introduces virus-specific biases to estimating the frequencies of specific complications attributable to a vURI when ascertained by CFLI identification.

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1. Introduction

Viral upper respiratory tract infections (vURIs), which are extremely common, may be subclinical or cause a cold/flu-like illness (CFLI) (Rakes et al., 1999; Nokso-Koivisto et al., 2002; Harju et al., 2006). A CFLI is usually diagnosed by the presence of a set of symptoms and/or signs that are culturally accepted, but modified by past experiences. The known complications of a vURI include sinusitis, otitis media, bronchiolitis, asthma exacerbation and pneumonia (Wald et al., 1991; Alho et al., 2001; Greenberg, 2003; Heikkinen and Jarvinen, 2003; Fleming et al., 2005).

Many studies focused on estimating the coincidence of these complications and vURIs for purposes of identifying individual factors that define levels of risk for a specific vURI (Doyle and Alper, 2003). Because vURIs without CFLIs are not apparent, coincidence estimates for vURI complications are usually calculated as events/CFLI episode, which introduces an ascertainment bias that underestimates the true frequency of a complication that is attributable to a vURI (Doyle et al., 1999; Winther et al., 2006, 2007; Doyle et al., 1994 #15). This study determined if that ascertainment bias is virus-specific by defining the relative frequencies of subclinical vURIs for the most common viruses causing vURIs. The hypothesis tested is that subclinical vURIs are rare and that their relative frequency is not different among viruses.

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2. Methods

Families with at least two children aged 1–5 years were recruited at two study sites (Pittsburgh, Charlottesville) in each of 3 years. After acquisition of informed consent, families were entered in October, followed through April of that year, and reimbursed \$100/month for participation. The study was approved by the IRBs at the Universities of Pittsburgh and Virginia.

The general protocol included acquiring demographic information on the children, followed by daily parental assignment of their children's status with respect to the presence/absence of a CFLI and the presence/absence of seven illness signs (i.e. runny nose, nasal congestion, sore throat, cough, fever, irritability and earache), and periodic collection of nasal secretions for virus identification by PCR throughout the "typical cold season". Because there is no generally accepted criteria for assigning a CFLI in infants and children, CFLI presence/absence for each day was not based on pre-defined illness criteria, but rather on assignment for that day as determined by the caretaker. The initial plans were to collect secretion samples at regular intervals with supplemental sampling during CFLIs in the child or a family member but this was not possible for a variety of reasons. Consequently, a convenience sampling strategy was adopted that concentrated on CFLI periods, periods when the child's sib had a CFLI and other non-CFLI periods when acceptable to the children. For all years, the number of samples processed in each month from October through April was 149, 229, 219, 181, 211, 195 and 115 with some variability in the frequency of monthly samples among years.

Parent-reported CFLI days were cast as a temporal string extending from entry to 30 April and CFLI episodes were defined by two or more CFLI days separated from other episodes by at least three non-CFLI days. Nasal secretions

collected from the children were frozen at -70 and assayed in batch using PCR to detect adenovirus, coronavirus (types 229E and OC43), influenza virus (types A and B), parainfluenza (types 1–3) virus, rhinovirus/picornavirus and respiratory syncytial virus (RSV). These viruses were sought using a protocol adapted from the Hexaplex procedure (Prodesse, Inc., Waukesha, WI) and previously described primers and probes (Winther et al., 2007). Reported sensitivities of the PCR assays were ≤ 1 tissue culture infectious dose₅₀/0.1 ml for picornavirus, 0.01 TCID₅₀/0.1 ml for coronavirus, 0.28 TCID₅₀/280 μ l for RSV and < 1 TCID₅₀/0.1 μ l for adenovirus (Winther et al., 2007). For time periods with no CFLI or a single identified CFLI, individual virus/no virus detections were reduced by linking identical virus/no virus detections within a 20-day period as one detection/no detection.

For each child, virus results were mapped onto the corresponding CFLI present/absent string. Virus detection within 5 days before, during or within 5 days after a CFLI episode was associated with the episode, +PCR+CFLI and operationally defined as a clinical vURI. A CFLI episode sampled for virus but with absent detection, –PCR+CFLI, was defined as UR inflammation of undermined etiology. Virus detection not associated with a CFLI episode, +PCR–CFLI, was defined as a subclinical vURI. For each assayed virus, the relative frequency of subclinical vURIs was calculated as +PCR–CFLI/+PCR. Pairwise comparisons of these frequencies were done using Fisher's exact test.

3. Results

170 children (86 male; 147 white) aged 1–8.6 (3.7 ± 1.6) years, distributed as 60 children in year 1, 56 in year 2, and 54 in year 3 were studied. A total of 956 independent virus assays was done (5.7 ± 2.6 samples/child); 415 (43%) were

Table 1
Distribution of independent virus detections and their association with CFLI episodes for each assayed virus

Virus	Type	# +PCR detections ^a	% All +PCR detections ^b	# +PCR and +CFLI ^c	# +PCR and –CFLI ^d	% PCR+ and –CFLI ^e
Rhinovirus		267	64.3	164	103	38.6
RSV		29	7.0	25	4	13.8
Influenza	A	16	3.9	14	2	12.5
	B	8	1.9	4	4	50.0
Adenovirus		13	3.1	11	2	15.4
Coronavirus	OC43	2	0.5	1	1	50.0
	229E	19	4.6	11	8	42.1
Parainfluenza	1	2	0.5	2	0	0.0
	2	10	2.4	5	5	50.0
	3	0	ND	0	0	ND
Mixed viruses		49	11.8	33	16	32.7

ND: not defined.

^a Number of independent detections for each listed virus.

^b Percent of all virus detections attributed to each listed virus.

^c Number of virus detections that were associated with CFLIs for each listed virus.

^d Number of virus detections that were independent of a CFLI for each listed virus.

^e Percent of virus detections that was independent of a CFLI for each listed virus.

and 541 (57%) were not +PCR for virus detection. Of the 425 CFLI episodes sampled, 270 (64%) were and 155 (36%) were not +PCR for virus detection ($P < .001$). Of the 531 non-CFLI periods sampled, 145 (27%) were and 386 (73%) were not +PCR for virus detection ($P < .001$). Overall, the proportion of +PCR–CFLI was 35% for all +PCR detections (i.e. subclinical infections).

Of all +PCR detections for CFLI and non-CFLI periods, rhinovirus/picornavirus was most frequent, followed by mixed viruses, RSV and then influenza, adenovirus, coronavirus and parainfluenza viruses (see Table 1). About 85% of RSV, influenza A and adenovirus detections were associated with a CFLI (+PCR+CFLI), but that association for the other viruses was less than 62% for the other viruses. Pairwise comparison of that frequency between rhinovirus and each assayed virus was significant for RSV ($P = .015$) and approached significance for influenza A ($P = .067$).

4. Discussion

Past studies showed that experimental vURIs with rhinovirus, influenza A, and RSV provoke a variable illness expression that qualifies as a CFLI only in a subset (Buchman et al., 1994, 2002; Doyle et al., 1994) and that the frequencies of otologic complications were independent of CFLI expression (Doyle et al., 1999). Similar results documenting illness-free virus detection with complications were reported for clinical studies of infants, children and adults (Rakes et al., 1999; Nokso-Koivisto et al., 2002; Harju et al., 2006; Winther et al., 2006).

In a preliminary report we analyzed the data for the first year of this study and found that the frequency of +PCR–CFLI/+PCR for all assayed viruses was 34%. However, there were insufficient numbers of virus detections to determine if this rate was virus-specific (Winther et al., 2007). In that analysis, 26% of CFLIs were –PCR and the current results approximate that value (i.e. 36%). Because we did not assay all viruses that can cause CFLIs (e.g. metapneumovirus, human bocavirus), procedural failures with our assay may have occurred, and the assay sensitivities depend on the unmeasured dilution of aspirated nasal secretions and may be virus-specific, a viral etiology cannot be ruled out for those episodes. Also, those episodes may be associated with bacterial infection (e.g. Strep Throat) or nasal atopy and, thus, these presentations are best classified as UR inflammation of undetermined etiology.

The data reported in the table do not support our hypothesis. Specifically, +PCR–CFLI episodes are not rare (35% overall) and the relative frequencies of +PCR–CFLI/+PCR episodes were different among viruses. Assuming that +PCR virus detection represents a true vURI, the differences in the relative frequencies of subclinical infections among viruses introduces an additional difficulty in estimating the frequency

of complications attributable to a vURI when estimated based on CFLI presentations.

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References

- Alho OP, Ylitalo K, Jokinen K, Laitinen J, Suramo I, Tuokko H, et al. The common cold in patients with a history of recurrent sinusitis: increased symptoms and radiologic sinusitislike findings. *J Fam Pract* 2001;50(1):26–31.
- Buchman CA, Doyle WJ, Pilcher O, Gentile DA, Skoner DP. Nasal and otologic effects of experimental respiratory syncytial virus infection in adults. *Am J Otolaryngol* 2002;23(2):70–5.
- Buchman CA, Doyle WJ, Skoner D, Fireman P, Gwaltney JM. Otolgic manifestations of experimental rhinovirus infection. *Laryngoscope* 1994;104(10): 1295–9.
- Doyle WJ, Alper CM. Prevention of otitis media caused by viral upper respiratory tract infection: vaccines, antivirals, and other approaches. *Curr Allergy Asthma Rep* 2003;3(4):326–34.
- Doyle WJ, Alper CM, Buchman CA, Moody SA, Skoner DP, Cohen S. Illness and otological changes during upper respiratory virus infection. *Laryngoscope* 1999;109(2 Pt 1): 324–8.
- Doyle WJ, Skoner DP, Hayden F, Buchman CA, Seroky JT, Fireman P. Nasal and otologic effects of experimental influenza A virus infection. *Ann Otol Rhinol Laryngol* 1994;103(1):59–69.
- Fleming DM, Pannell RS, Elliot AJ, Cross KW. Respiratory illness associated with influenza and respiratory syncytial virus infection. *Arch Dis Child* 2005;90(7):741–6.
- Greenberg SB. Respiratory consequences of rhinovirus infection. *Arch Intern Med* 2003;163(3):278–84.
- Harju TH, Leinonen M, Nokso-Koivisto J, Korhonen T, Raty R, He Q, et al. Pathogenic bacteria and viruses in induced sputum or pharyngeal secretions of adults with stable asthma. *Thorax* 2006;61(7):579–84.
- Heikkinen T, Jarvinen A. The common cold. *Lancet* 2003;361(9351):51–9.
- Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkaranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. *J Med Virol* 2002;66(3):417–20.
- Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med* 1999;159(3):785–90.
- Wald ER, Guerra N, Byers C. Upper respiratory tract infections in young children: duration of and frequency of complications. *Pediatrics* 1991;87(2):129–33.
- Winther B, Alper CM, Mandel EM, Doyle WJ, Hendley JO. Temporal relationships between colds, upper respiratory viruses detected by polymerase chain reaction, and otitis media in young children followed through a typical cold season. *Pediatrics* 2007;119(6):1069–75.
- Winther B, Doyle WJ, Alper CM. A high prevalence of new onset otitis media during parent diagnosed common colds. *Int J Pediatr Otorhinolaryngol* 2006;70:1725–30.