Is chronic rhinosinusitis caused by persistent respiratory virus infection?

Andrew James Wood, BA (Oxon)[1–3], Hanna Antoszewska, MSc[4], John Fraser, PhD[3], Richard George Douglas, MD[1,2]

Background: **Many chronic rhinosinusitis (CRS) patients recall an upper respiratory tract infection as the inciting event of their chronic illness. Viral infections have been shown to cause obstruction of the osteomeatal complex, which is likely to be a critical step in the development of CRS. There is clear overlap between the pathogenesis of CRS and asthma. Infections with respiratory viruses in childhood increase the risk of subsequently developing asthma. Viral infections in established asthmatics are associated with acute exacerbations. We sought to determine whether respiratory viruses could be detected within the sinonasal mucosa of CRS patients using polymerase chain reaction (PCR) techniques.**

Methods: **Sinus mucosa was sampled from 13 patients with CRS and 2 patients with normal sinuses. PCR was used to look for common respiratory viruses (parainfluenza 1, 2, and 3; respiratory syncytial virus [RSV]; human metapneumovirus [hMPV]; adenovirus [ADV]; rhinovirus; coronavirus; bocavirus [BoV]; cytomegalovirus [CMV]; and influenza A and B).**

Results: **No respiratory viruses were detected in any of the samples.**

Conclusion: **Persistence of respiratory viruses within the sinonasal mucosa is unlikely to be a cause of ongoing inflammation in CRS. The possibility remains that a transient viral infection provides the initial inflammatory stimulus.** © 2011 ARS-AAOA, LLC.

Key Words:
adult; chronic disease; humans; infection; nasal mucosa; nasal polyps; polymerase chain reaction; rhinitis; sinusitis; virology

How to Cite this Article:
Wood AJ, Antoszewska H, Fraser J, Douglas RG. Is chronic rhinosinusitis caused by persistent respiratory virus infection? *Int Forum Allergy Rhinol*, 2011; 1:95–100

Despite being 1 of the most common chronic diseases in the Western world the pathogenesis of chronic rhinosinusitis (CRS) remains poorly understood. As a consequence of this, the significant morbidity and financial burden associated with this disease persist.[1] A wide variety of pathogenic mechanisms, mostly related to microorganisms, have been investigated extensively, including bacterial and fungal biofilms,[2,3] intracellular bacteria,[4] and aberrant immune responses to both fungal allergens[5] and staphylococcal superantigens.[6]

Two of the key events in the development of CRS are thought to be obstruction of the osteomeatal complex[7] and the development of epithelial cell dysfunction.[8] It is common for CRS patients to report that their symptoms initially developed after a viral infection and it is noteworthy that viral infections have been shown to cause obstruction of sinus ostia,[9] production of inflammatory mediators by nasal epithelial cells,[10] and damage to epithelial cells and cilia.[11] Rhinovirus has also been shown to induce persistent changes in the local cytokine milieu[12] and to increase bacterial adhesion to nasal epithelial cells,[13] with both mechanisms potentially providing a lasting effect following transient infection.

The link between asthma and CRS, particularly in those with nasal polyps, is well-established[14] and potential parallels in pathogenesis of these conditions exist. Childhood infection with respiratory syncytial virus (RSV) increases

[1]*Department of Otolaryngology–Head and Neck Surgery;* [2]*Department of Surgery;* [3]*Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand;* [4]*Department of Virology & Immunology, Auckland City Hospital, Auckland, New Zealand*

Correspondence to: Dr Richard Douglas, Department of Surgery, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; e-mail: richarddouglas@xtra.co.nz

Funding sources for the study: Garnett Passe and Rodney Williams Memorial Foundation; Green Lane Research and Education Fund; University of Auckland Faculty Research Development Fund.
Presented at The American Rhinologic Society 56th Annual Meeting, Saturday, September 25, 2010.
Potential conflict of interest: None provided.

Received: 28 August 2010; Revised: 2 November 2010; Accepted: 9 November 2010
DOI: 10.1002/alr.20030
View this article online at wileyonlinelibrary.com.

the probability of an affected child developing asthma for at least a decade afterward.[15] It may be that the virus induces a persisting change in the mucosa before it is cleared or that viral particles persist within the mucosa, there being clear evidence that respiratory viruses are capable of establishing latent infections in human tissue.[16] It has also been demonstrated that many acute exacerbations of asthma relate to infection with respiratory viruses.[17]

Several studies have used polymerase chain reaction (PCR) techniques to look for the presence of respiratory viruses in samples from CRS patients. Ramadan et al.[18] reported that 20% of patients had evidence of RSV in their mucosa but no patients had evidence of adenovirus (ADV). They did not, however, have a control group nor report whether samples were collected from patients during the winter months when a significant proportion of the general population are affected by respiratory viruses.[19] Jang et al.[20] published a similar study that avoided these problems, finding rhinovirus in 21% of epithelial cell samples from CRS patients and none in controls during the summer months. However, the number of virus species sought was limited and samples were collected from the inferior turbinates rather than sinus mucosa.

Hypotheses concerning a role for viruses in the pathogenesis of CRS appear to fall into 3 groups. Viruses have been considered as potentially causative in the initial development of inflammation, the ongoing stimulus of inflammation, or the cause of acute exacerbations in symptoms.

This study was designed to evaluate whether evidence could be found to implicate respiratory virus persistence in the ongoing inflammation seen in CRS.

Patients and methods

Patients

A total of 15 adult patients who were undergoing endoscopic sinus surgery in the tertiary practice of the senior author (R.G.D.) either for CRS or for access to skull-base lesions were prospectively recruited. CRS patients fulfilled agreed diagnostic criteria for CRS[21] and had failed a prolonged trial of medical therapy.[1] All had sufficiently extensive disease to merit dissection of all their paranasal sinuses and there were no exclusion criteria. Normal sinonasal mucosa was sampled from 1 patient with a nonfunctioning pituitary adenoma and 1 patient with a malignant lesion of the pterygopalatine fossa. Neither of these patients had symptoms of CRS or evidence of CRS on endoscopy or imaging. Patients were recruited during the southern hemisphere summer and early autumn months (February-April, 2010). Recruitment was ceased at an agreed time when in previous years the rate of detection of respiratory viruses in the general population had been seen to rise. The regional ethics committee (Northern Regional Ethics Committee Ref: NTX/08/12/126) and the hospitals involved gave prior approval of the study and informed written consent was given by all patients.

Clinical data

Patient demographics as well as relevant past medical and surgical history were recorded. Patients were also asked to quantify how long they had had symptoms from their nose and sinuses (Table 1). Patients were classified on the basis of the presence (CRSwNP) or absence (CRSsNP) of nasal polyps as per published guidelines.[21] No patents had aspirin exacerbated respiratory disease. Subjective and

TABLE 1. Patient details

No.	Diagnosis	Age (years)	Sex	Ethnicity	Comorbidities	Duration of symptoms (years)	Revision surgery
1	CRSwNP	46	F	European		35	Yes
2	CRSwNP	41	M	Tongan	Bronchiectasis	15	No
3	CRSwNP	54	M	European		15	No
4	CRSwNP	69	F	Maori	Asthma	5	No
5	CRSwNP	38	M	Algerian		4	No
6	CRSsNP	18	F	Maori	Bronchiectasis	10	No
7	CRSsNP	53	F	European		2	No
8	CRSsNP	39	M	European		4	No
9	CRSsNP	55	F	European	Asthma	2.5	No
10	CRSsNP	18	M	European	Cystic fibrosis	2	Yes
11	CRSsNP	44	F	European	Asthma	4	No
12	CRSsNP	19	F	European		2	No
13	CRSsNP	52	F	European		5	Yes
14	Normal	33	F	European		N/A	N/A
15	Normal	65	M	European		N/A	N/A

TABLE 2. Primers and probes

Primers and probes	Oligonucleotide sequence (5′-3′)	Target gene
Inf A F	GAC CRA TCC TGT CAC CTC TGA C	M
Inf A R	AGG GCA TTY TGG ACA AAK CGT CTA	
Inf A P	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ1	
Inf B F	TCC TCA AYT CAC TCT TCG AGC G	NC
Inf B R	CGG TGC TCT TGA CCA AAT TGG	
Inf B P	6FAM-CCA ATT CGA GCA GCT GAA ACT GCG GTG-BHQ1	
PIV1 F	GTT GTC AAT GTC TTA ATT CGT ATC AAT AAT T	HN
PIV1 R	GTA GCC TMC CTT CGG CAC CTA A	
PIV1 P	FAM-TAG GCC AAA GAT TGT TGT CGA GAC TAT TCC AA-BHQ1	
PIV2 F	GCA TTT CCA ATC TTC AGG ACT ATG A	HN
PIV2 R	ACC TCC TGG TAT AGC AGT GAC TGA AC	
PIV 2 P	CAL FO560-CCA TTT ACC TAA GTG ATG GAA TCA ATC GCA AA-BH	
PIV 3 F	CCA GGG ATA TAY TAY AAA GGC AAA A	HN
PIV 3 R	CCG GGR CAC CCA GTT GTG	
PIV 3 P	FAM-TGG RTG TTC AAG ACC TCC ATA YCC GAG AAA-BHQ1	
ADV F	GCC CCA GTG GTC TTA CAT GCA CAT C	Hexon
ADV R	GCC ACG GTG GGG TTT CTA AAC TT	
ADV P	FAM-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-BHQ1	
RSV F	AAT ACA GCM AAA TCT AAC CAA CTT TAC A	L
RSV R	GCC AAG GAA GCA TGC AAT AAA	
RSV P1	FAM-TGC TAT TGT GCA CTA AAG-BHQ1	
RSV P2	CAL FO560-CAC TAT TCC TTA CTA AAG ATG TC-BHQ1	
hMPV F	CATATAAGCATGCTATATTAAAAGAGTCTC	NS
hMPV R	CCTATTTCTGCAGCATATTTGTAATCAG	
hMPV P	FAM-TGY AAT GAT GAG GGT GTC ACT GCG TGG G-BHQ1	
CMV F	CCG GCA AGC TCT TTA TGC A	Phosphoprotein 65
CMV R	TGG GAC ACA ACA CCG TAA AGC	
CMV P	FAM-CCG CAA CCC TTC AT-BHQ1	
RV F	GCA CTT CTG TTT CCC C	5′ noncoding region
RV R	GGC AGC CAC GCA GGC T	
RV P1	FAM-AGC CTC ATC TGC CAG GTC TA-BHQ1	
RV P2	CAL FO560-AGC CTC ATC CAC CAA ACT A-BHQ1	
hBoV F	TGC AGA CAA CGC YTA GT TGT TT	NS1
hBoV R	CTG TCC CGC CCA AGA TAC A	
hBoV P	6FAM-CCA GGA TTG GGT GGA ACC TGC AAA-BHQ1	
OC43 + HKU1		Polymerase 1b
CoV F1	TGG TGG CTG GGA CGA TAT GT	
CoV R1	GGC ATA GCA CGA TCA CAC TTA GG	
CoV P1	6-FAM-ATA ATC CCA ACC CAT RAG-BHQ1	
NL63		Polymerase 1b
CoV F2	TTT ATG GTG CTT GGA ATA ATA TGT TG	
CoV R2	GGC AAA GCT CTA TCA CAT TTG G	

(Continued)

TABLE 2. Continued

Primers and probes	Oligonucleotide sequence (5′-3′)	Target gene
CoV P1	FAM-ATA ATC CCA ACC CAT RAG-BHQ1	
229E		Polymerase 1b
CoV F3	TGG CGG GTG GGA TAA TAT GT	
CoV R3	GAG GGC ATA GCT CTA TCA CAC TTA GG	
CoV P2	CAL FO560-ATA GTC CCA TCC CAT CAA-BHQ1	
RnaseP F	AGA TTT GGA CCT GCG AGC G	Human ribonuclease P
RnaseP R	GAG CGG CTG TCT CCA CAA GT	
RnaseP P	FAM-GAG CGG CTG TCT CCA CAA GT-BHQ1	

F = forward; HN = hemagglutinin-neuramidase; L = RNA polymerase large subunit; M = matrix; NC = nucleocapsid; NS = nonstructural gene; P = probe; R = reverse.

objective measures of disease severity in the form of the Lund-MacKay score[22] and preoperative symptom scores[23] were recorded. Our practice is to ask patients to give the 5 main symptoms of CRS (obstruction, anterior rhinorrhea, posterior rhinorrhea, hyposmia, and midface congestion) a score from 0 to 5 based on their severity in the preceding 2 weeks.

Sample collection

Representative mucosal samples were collected from the ethmoid or sphenoid sinuses and immediately placed into sterile normal saline. They were then transferred to the laboratory where analysis was undertaken by a technician blinded to clinical details.

Nucleic acid extraction

Tissue pieces approximately 5 mm in diameter were predigested in 50 μL proteinase K 20 mg/μL and 150 μL of tissue lysis buffer at 55°C until completely dissolved (Roche High Pure PCR Template Preparation kit; Roche, Mannheim, Germany), then extracted using MagNA Pure LC automatic extractor and Total Nucleic Acid High Performance kit according to the manufacturer's recommendations. Total nucleic acid was eluted with 100 μL elution buffer. Extracted samples yielded on average 50–200 ng/μL nucleic acids.

For each patient, between 2 and 6 tissue pieces were processed.

Respiratory panel

A total of 15 independent PCR assays were designed with identical assay protocols and PCR platforms. These included influenza A, influenza B, parainfluenza 1, multiplex parainfluenza 2 and 3, multiplex RSV A and B, human metapneumovirus (hMPV), ADV, cytomegalovirus (CMV), bocavirus (BoV), multiplex rhinovirus (RV) 1 and 2, coronavirus OC43 and HKU1, coronavirus NL63, coronavirus 229E, and human RnaseP (which acted as the extraction control).

Sequences were adapted from the Centers for Disease Control and Prevention Protocol for Detection and Characterization of Influenza (www.cdc.gov) and from published reports.[24–31] The CMV assay was designed in-house. All assays used TaqMan hydrolysis probes labeled with fluorophore FAM or CAL Fluor Orange 560 at the 5′ end and no fluorescent Black Hole Quencher 1 (BHQ1) at the 3′ end. All primers and probes were synthesized by Biosearch Technologies (Novato, CA); sequences are listed in Table2.

Reagents

Reactions were carried out in 25-μL reaction mixtures containing 1 × reaction mix (Invitrogen SS III Platinum One-Step Quantitative RT-PCR System; Invitrogen, Carlsbad, CA) and 0.5 μL enzyme mix Superscript III/Platinum Taq Polymerase; in case of ADV, CMV, and BoV, Platinum Taq Polymerase was used, 0.8–0.9 μM forward and reverse primers, 0.2 μM fluorescent probe, and 5 μL extracted ribonucleic acid (RNA)/DNA.

PCR mixes without enzyme were prepared in large volumes and stored in single use aliquots at −20°C. Before use, aliquots for each PCR were thawed, mixed with enzyme, and aliquotted onto a reaction plate. RNA/DNA was then added. Positive controls were aliquotted last to minimize possible contamination.

Controls

Each run contained a no template control (NTC) water, extraction blank controls, and positive controls.

Positive controls consisted of RNA extracted from confirmed tissue culture isolates. ADV type 5 (Ad-5) strain and CMV AD169 were obtained commercially from Advanced Biotechnologies (Columbia, MD). BoV control consisted of a cloned amplified isolate, confirmed by sequencing.

All these assays (except BoV) are subjected to annual quality control programs as distributed by Quality Control for Molecular Diagnostics (Glasgow, Scotland) and RCPA Australia.

Amplification

Assays were carried out on the Roche Light Cycler 480 using a 96-well microplate format. Amplification conditions were 50°C for 20 minutes (reverse transcription), 95°C for

2 minutes (initial DNA polymerase activation), then 45 cycles of denaturation at 95°C for 15 seconds, followed by 45 seconds at 55°C (annealing/extension).

Results were analyzed in the FAM channel and Cal Orange 560 separately. Assays were considered valid if RnaseP amplification was positive.

Results

In the CRS patients, the median duration of symptoms reported was 4 years (range, 2–35 years). The median preoperative symptom score was 17 out of 25 (range, 10–21). The median Lund-MacKay score was 16 out of 24 (range, 10–22).

No respiratory viruses were detected in any of the samples.

Since conventional respiratory viruses were not found, further assays were performed looking for viruses known to be capable of establishing persistent infection, namely human herpes-6 (HHV-6) and Epstein-Barr virus (EBV) using PCR techniques with published primers.[32,33] Low-titer HHV-6 was found in samples from 3 of 8 CRSsNP patients, 4 of 5 CRSwNP patients, and 1 of 2 normal subjects. Low-titer EBV was found in 1 of 8 CRSsNP patients, 4 of 5 CRSwNP patients, and 0 of 2 normal subjects. The low titers of virus present suggest latent rather than active infection.

Conclusion

It seems likely that CRS has a multifactorial pathogenesis, and that the inflammatory stimuli differ at different stages of the disease and in different subgroups. Respiratory viruses can however cause florid sinonasal symptoms and may generate a long-lasting effect on respiratory mucosa. Respiratory viruses have also been shown to be capable of establishing latency in human tissue.[16] The role of respiratory viruses in the ongoing inflammation seen in CRS therefore warrants further investigation.

In this series of patients all CRS patients had active inflammation and despite using sensitive tests for an extensive panel of respiratory viruses we did not identify any evidence of respiratory viral presence in our patients. It may be that CRS is a spectrum of disease and our small sample size may not therefore include all variants but our clearly negative results do suggest that persistent respiratory virus infection is not responsible for the chronic inflammation seen in the major variants of this disorder. Evidence of latent EBV infection was seen in CRS mucosa in 42% of cases but in the small number of patients examined, EBV was not found in the controls. The significance of this is unclear.

Despite not finding respiratory viruses in our series it is noted that animal and in vitro models have shown that the deleterious effect of respiratory virus infection may persist well beyond the time when the virus particles have been cleared.[12,34]

Our rate of detection of viruses in CRS is lower than in other published series. This may in part be due to methodological differences such as the collection of sinus mucosa rather than inferior turbinate samples[20] and collection of specimens during the summer months when respiratory viruses are far less prevalent.

A study of whether viral infections are responsible for acute exacerbations in CRS symptoms could be designed in a similar fashion but undertaken during the winter months. A large group of negative control patients would be required to establish what the background rate of viral infection is at that time of year.

The chronic nature of CRS implies that presentation to specialist care is remote from the development of the condition, which makes study of the initiating factors more difficult. Transient respiratory virus infection causing damage to the epithelial surface could be the process that allows bacterial biofilms to form and adhere to the mucosal surface or could cause long-lasting changes in the inflammatory milieu to occur that are critical to the subsequent development of CRS. We found, however, no evidence of persisting respiratory virus infection in diseased sinus mucosa.

References

1. Wood AJ, Douglas RG. Pathogenesis and treatment of chronic rhinosinusitis. *Postgrad Med J*. 2010;86:359–364.
2. Psaltis AJ, Ha KR, Beule AG, et al. Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. *Laryngoscope*. 2007;117:1302–1306.
3. Healy DY, Leid JG, Sanderson AR, et al. Biofilms with fungi in chronic rhinosinusitis. *Otolaryngol Head Neck Surg*. 2008;138:641–647.
4. Clement S, Vaudaux P, Francois P, et al. Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis. *J Infect Dis*. 2005;192:1023–1028.
5. Shin SH, Ponikau JU, Sherris DA, et al. Chronic rhinosinusitis: an enhanced immune response to ubiquitous airborne fungi. *J Allergy Clin Immunol*. 2004;114:1369–1375.
6. Bachert C, Zhang N, Patou J, et al. Role of staphylococcal superantigens in upper airway disease. *Curr Opin Allergy Clin Immunol*. 2008;8:34–38.
7. Ha KR, Psaltis AJ, Tan L, et al. A sheep model for the study of biofilms in rhinosinusitis. [Erratum: *Am J Rhinol*. 2007;21:519]. *Am J Rhinol*. 2007;21:339–345.
8. Tieu DD, Kern RC, Schleimer RP. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. *J Allergy Clin Immunol*. 2009;124:37–42.
9. Gwaltney JM Jr, Phillips CD, Miller RD, Riker DK. Computed tomographic study of the common cold. *N Engl J Med*. 1994;330:25–30.
10. Wang J, Watanabe S, Matsukura S, Suzaki H. Double-stranded RNA poly(I:C) enhances matrix metalloproteinase mRNA expression in human nasal polyp epithelial cells. *Acta Otolaryngol Suppl*. 2009;(562):105–109.
11. Pedersen M, Sakakura Y, Winther B, Brofeldt S, Mygind N. Nasal mucociliary transport, number of ciliated cells, and beating pattern in naturally acquired common colds. *Eur J Respir Dis Suppl*. 1983;128(Pt 1):355–365.
12. Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol*. 2007;179:1080–1087.
13. Wang JH, Kwon HJ, Jang YJ. Rhinovirus enhances various bacterial adhesions to nasal epithelial cells simultaneously. *Laryngoscope*. 2009;119:1406–1411.
14. Bachert C, Patou J, Van Cauwenberge P, Bachert C, Patou J, Van Cauwenberge P. The role of sinus disease in asthma. *Curr Opin Allergy Clin Immunol*. 2006;6:29–36.
15. Sigurs N. Epidemiologic and clinical evidence of a respiratory syncytial virus-reactive airway disease link. *Am J Respir Crit Care Med*. 2001;163(3 Pt 2):S2–S6.
16. Neumann R, Genersch E, Eggers HJ. Detection of adenovirus nucleic acid sequences in human tonsils in the absence of infectious virus. *Virus Res*. 1987;7:93–97.
17. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ*. 1995;310:1225–1229.
18. Ramadan HH, Farr RW, Wetmore SJ. Adenovirus and respiratory syncytial virus in chronic sinusitis using polymerase chain reaction. *Laryngoscope*. 1997;107:923–925.
19. Sullivan KM, Monto AS, Longini IM Jr. Estimates of the US health impact of influenza. *Am J Public Health*. 1993;83:1712–1716.

20. Jang YJ, Kwon HJ, Park HW, Lee BJ. Detection of rhinovirus in turbinate epithelial cells of chronic sinusitis. *Am J Rhinol*. 2006;20:634–636.
21. Fokkens W, Lund V, Mullol J. EPOS 2007: European position paper on rhinosinusitis and nasal polyps. *Rhinology*. 2007;45:1–139.
22. Lund VJ, Mackay IS. Staging in rhinosinusitus. *Rhinology*. 1993;31:183–184.
23. Wabnitz DA, Nair S, Wormald PJ, Wabnitz DAM, Nair S. Correlation between preoperative symptom scores, quality-of-life questionnaires, and staging with computed tomography in patients with chronic rhinosinusitis. *Am J Rhinol*. 2005;19:91–96.
24. Deffernez C, Wunderli W, Thomas Y, Yerly S, Perrin L, Kaiser L. Amplicon sequencing and improved detection of human rhinovirus in respiratory samples. *J Clin Microbiol*. 2004;42:3212–3218.
25. Gunson RN, Collins TC, Carman WF. Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. *J Clin Virol*. 2005;33:341–344.
26. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol*. 2003;70:228–239.
27. Kuypers J, Wright N, Morrow R. Evaluation of quantitative and type-specific real-time RT-PCR assays for detection of respiratory syncytial virus in respiratory specimens from children. *J Clin Virol*. 2004;31:123–129.
28. Kuypers J, Martin ET, Heugel J, Wright N, Morrow R, Englund JA. Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics*. 2007;119:e70-e76.
29. Lu X, Chittaganpitch M, Olsen SJ, et al. Real-time PCR assays for detection of bocavirus in human specimens. *J Clin Microbiol*. 2006;44:3231–3235.
30. Maertzdorf J, Wang CK, Brown JB, et al. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *J Clin Microbiol*. 2004;42:981–986.
31. Watzinger F, Suda M, Preuner S, et al. Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. *J Clin Microbiol*. 2004;42:5189–5198.
32. Croxson MC, Taylor G, Nisbet M, Nagappan R, Ellis-Pegler R, Van de Water N. Human herpesvirus 8-associated spinal cord lymphoma in an HIV-positive subject. *Pathology*. 2006;38:356–358.
33. Gautheret-Dejean A, Manichanh C, Thien-Ah-Koon F, et al. Development of a real-time polymerase chain reaction assay for the diagnosis of human herpesvirus-6 infection and application to bone marrow transplant patients. *J Virol Methods*. 2002;100:27–35.
34. Klemens JJ, Thompson K, Langerman A, Naclerio RM. Persistent inflammation and hyperresponsiveness following viral rhinosinusitis. *Laryngoscope*. 2006;116:1236–1240.