Rhinovirus Viremia in Patients Hospitalized With Community-Acquired Pneumonia

Xiaoyan Lu, Centers for Disease Control and Prevention
Eileen Schneider, Centers for Disease Control and Prevention
Seema Jain, Centers for Disease Control and Prevention
Anna M. Bramley, Centers for Disease Control and Prevention
Weston Hymas, University of Utah
Chris Stockmann, University of Utah
Krow Ampofo, University of Utah
Sandra R. Arnold, Le Bonheur Children’s Hospital
Derek J. Williams, Vanderbilt University
Wesley H. Self, Vanderbilt University

Only first 10 authors above; see publication for full author list.

Journal Title: Journal of Infectious Diseases
Volume: Volume 216, Number 9
Publisher: Oxford University Press | 2017-11-01, Pages 1104-1111
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1093/infdis/jix455
Permanent URL: https://pid.emory.edu/ark:/25593/s6vv7

Final published version: http://dx.doi.org/10.1093/infdis/jix455

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Accessed December 18, 2018 10:53 AM EST
Rhinovirus Viremia in Patients Hospitalized With Community-Acquired Pneumonia


1Centers for Disease Control and Prevention, and 2Emory University School of Medicine, Atlanta, Georgia; 3University of Utah Health Sciences Center, Salt Lake City; 4Le Bonheur Children's Hospital, Memphis; 5University of Tennessee Health Science Center, Memphis; 6Vanderbilt University School of Medicine, Nashville, and 7St. Jude Children's Research Hospital, Memphis, Tennessee; and 8Northwestern University Feinberg School of Medicine, Chicago, Illinois

Background. Rhinoviruses (RVs) are ubiquitous respiratory pathogens that often cause mild or subclinical infections. Molecular detection of RVs from the upper respiratory tract can be prolonged, complicating etiologic association in persons with severe lower respiratory tract infections. Little is known about RV viremia and its value as a diagnostic indicator in persons hospitalized with community-acquired pneumonia (CAP).

Methods. Sera from RV-positive children and adults hospitalized with CAP were tested for RV by real-time reverse-transcriptase polymerase chain reaction. Rhinovirus species and type were determined by partial genome sequencing.

Results. Overall, 57 of 570 (10%) RV-positive patients were viremic, and all were children aged <10 years (n = 57/375; 15.2%). Although RV-A was the most common RV species detected from respiratory specimens (48.8%), almost all viremias were RV-C (98.2%). Viremic patients had fewer codetected pathogens and were more likely to have chest retractions, wheezing, and a history of underlying asthma/reactive airway disease than patients without viremia.

Conclusions. More than 1 out of 7 RV-infected children aged <10 years hospitalized with CAP were viremic. In contrast with other RV species, RV-C infections were highly associated with viremia and were usually the only respiratory pathogen identified, suggesting that RV-C viremia may be an important diagnostic indicator in pediatric pneumonia.

Keywords. rhinovirus; community-acquired pneumonia; viremia.

Rhinoviruses (RVs), together with enteroviruses (EVs), are members of the Enterovirus genus, family Picornaviridae, and are further divided into 3 species (RV-A, -B, and -C) with >160 types [1]. Although RVs and EVs share a common genomic organization and both can cause acute respiratory disease, they exhibit significant differences in disease pathology. A hallmark of EV infections is their capacity for viremia and systemic infections [2], which has rarely been demonstrated for RVs using classical culture-based diagnostic methods [3, 4]. However, recent studies using sensitive molecular methods have reported detection of RV in the blood of children presenting with respiratory illness [5–7].

Although more often associated with mild upper respiratory tract infections, RVs are now recognized as a major cause of exacerbations of asthma and other chronic pulmonary diseases [8, 9] and a cause of severe lower respiratory tract infections, including pneumonia [10–13]. In the Centers for Disease Control and Prevention (CDC) Etiology of Pneumonia in the Community (EPIC) study, RV was found to be the most commonly detected pathogen in the upper respiratory tract of patients hospitalized with community-acquired pneumonia (CAP) [12, 13]. However, RVs were also detected in a high proportion of asymptomatic pediatric controls [12, 14] and were often codetected with other respiratory pathogens [12], complicating determination of their causal relationship with CAP. Thus, the causality of RV in CAP is unknown but may correlate with viremia as defined by detection of RV RNA in the blood. Nested within the EPIC study, we determined the prevalence of RV viremia in RV-infected patients hospitalized with CAP and assessed correlations between viremia and corresponding virologic, demographic, and clinical features.

METHODS

Patient and Specimens
From 1 January 2010 to 30 June 2012, children and adults admitted to 8 hospitals located in Chicago, Memphis, Nashville, and Salt Lake City were enrolled in the EPIC study [12, 13]. Enrollment criteria included evidence of CAP based on clinicians’ initial interpretation of chest radiographs obtained within 72 hours before or after hospital admission, and patients who were severely immunocompromised were excluded. Whereas

Received 1 August 2017; editorial decision 24 August 2017; accepted 29 August 2017; published online August 29, 2017.


Correspondence: X. Lu, MS, Division of Viral Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd, NE, MS G04, Atlanta, GA 30329 (xal9@cdc.gov).

The Journal of Infectious Diseases® 2017;216:1104–11

Published by Oxford University Press for the Infectious Diseases Society of America 2017. This work is written by (a) US Government employee(s) and is in the public domain in the US. DOI: 10.1093/infdis/jix452
patients enrolled at the 3 children’s hospitals was initially per-
molecular typing of RVs detected from NP/OP specimens of
Rhinovirus Molecular Typing

patients had previously been tested for multiple respiratory viral,
bacterial, and fungal pathogens by molecular methods, serology,
and culture at the study sites and CDC [12, 13]. For this study,
we tested NP/OP specimens and corresponding acute serum
specimens from RV-positive patients. Total nucleic acids were
extracted from both serum and NP/OP specimens (200 µL) on
a NücliSens EasyMAG (BioMerieux). The extracts were tested
extracted from both serum and NP/OP specimens (200 µL) on
a NücliSens EasyMAG (BioMerieux). The extracts were tested
by a real-time reverse transcription-polymerase chain reaction
(rRT-PCR) assay [15] with a modified forward primer
(5’-CYA\textsubscript{INA}GCCT\textsubscript{INA}GGTGGY-3’) to expand RV-C type cov-
verage and using the AgPath-ID One-Step RT-PCR Kit (Thermo
Fisher Scientific). Cycle threshold (Ct) values <40 Ct were consid-
ered positive and were used as a proxy for virus load. The NP/OP
and serum specimen extractions and rRT-PCR were performed
on separate days to minimize potential for cross-contamination.

Rhinovirus Molecular Typing

Molecular typing of RVs detected from NP/OP specimens of
patients enrolled at the 3 children’s hospitals was initially per-
formed by the University of Utah Health Sciences Center (method
available on request); RV genotypes were subsequently confirmed
by the CDC for children with viremia. For adult patients, RV
typing from NP/OP and serum specimens was performed at the
CDC. Rhinovirus typing was based on RT-PCR and sequenc-
ing of the VP4/VP2 capsid gene spanning nucleotide positions 626–1046 (numbering according to reference strain
RV-A1, GenBank accession no. FJ445111.1). In brief, 5 µL of total
nucleic acid extract was subjected to RT-PCR amplification with
SuperScript III One-Step RT-PCR System with Platinum Taq
DNA Polymerase (Thermo Fisher Scientific) in a final volume
of 25 µL following manufacturer’s protocol using external for-
ward primer (5’-GGCCCCGTTGAATGGGCATAA-3’) and reverse primer
(5’-GCATCIGGYARTTCCACCCACCC-3’). The
RT-PCR cycling conditions were 55°C for 30 minutes and 94°C
for 2 minutes followed by 40 cycles of 94°C for 30 seconds, 52°C
for 30 seconds, and 68°C for 50 seconds, with a final extension
of 68°C for 5 minutes. One µL of the RT-PCR product was then
added to a seminested reaction using internal forward primer
(5’-ACTACTTTGGGTGTCCGTGTTTC-3’) and reverse primer
in a final volume of 50 µL using Taq DNA Polymerase (Roche
Applied Science). The PCR cycling conditions were 95°C for 2
minutes followed by 30 cycles of 95°C for 30 seconds, 55°C for
30 seconds, and 72°C for 40 seconds, with a final extension of
72°C for 5 minutes. Seminested amplicons were resolved on 1%
agarose gels and purified using ExoSAP-IT (USB Corporation).
Ambiplex sequencing was performed in both directions using
BigDye Terminator v1.1 Cycle Sequencing Kit on a 3730XL
Genetic Analyzer (Thermo Fisher Scientific) using internal for-
ward and reverse primers. Sequencher version 5.3 software (Gene
Codes) was used for sequence assembly and editing. VP4/VP2
nucleotide sequences were aligned using Clustal X version 1.83 as
implemented in BioEdit version 7.2.5. (Ibis Biosciences), and phy-
logenetic trees were constructed by the neighbor joining method
using a maximum composite likelihood substitution model with
pairwise deletion of gaps and missing data as implemented in
MEGA7: Molecular Evolutionary Genetics Analysis version 7.0
[16]. Rhinovirus species and provisional genotype assignments
were based on phylogenetic congruence of VP4/VP2 sequences
and pairwise nucleotide divergence criteria developed by McIntyre
et al [1]. Sequences generated in this study were deposited in
GenBank under the accession numbers MF579447–MF579503.

Statistical Analysis

Statistical analyses were performed using SAS version 9.3
(SAS Institute). Chi-square and Wilcoxon rank-sum tests were
performed to assess clinical and demographic associations of
children with and without RV viremia. The Mann–Whitney U
test was used for pair-wise comparison of Ct values as a proxy
for viral loads in respiratory specimens among RV species. A P
value < .05 was considered statistically significant.

RESULTS

Study Population

Of 2638 children enrolled in the EPIC study, 723 (27.4%) had
RV detected in their NP/OP specimens, among whom 416
(57.5%) had acute serum available; of these, 356 (85.6%) had
radiographically confirmed pneumonia. Of 2488 enrolled
adults, 209 (8.4%) had RV detected in their NP/OP specimens,
among whom 154 (73.7%) had acute serum available; of these,
146 (94.8%) had radiographically confirmed pneumonia.

Rhinovirus NP/OP and Viremia-Associated Species/Genotypes

Of the 570 RV-NP/OP–positive patients from whom serum
was available, RVs were all successfully typed from the NP/OP
specimens. Rhinovirus A was identified in 278 (48.8%), RV-B
in 39 (6.8%), and RV-C in 253 (44.4%) (Table 1). Rhinovirus
viremia was detected by rRT-PCR in the acute serum of 57

Human Rhinovirus Viremia • JID 2017:216 (1 November) • 1105
Rhinovirus Viremia and Age Group and Rhinovirus Species

<table>
<thead>
<tr>
<th>Age group</th>
<th>RV-A n/N (%)</th>
<th>RV-B n/N (%)</th>
<th>RV-C n/N (%)</th>
<th>Total RV n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6 mo</td>
<td>0/27 (0.0)</td>
<td>0/14 (0.0)</td>
<td>2/20 (10.0)</td>
<td>2/51 (3.9)</td>
</tr>
<tr>
<td>6–11 mo</td>
<td>0/25 (0.0)</td>
<td>0/2 (0.0)</td>
<td>5/28 (17.9)</td>
<td>5/55 (9.1)</td>
</tr>
<tr>
<td>12–23 mo</td>
<td>1/25 (4.0)</td>
<td>0/3 (0.0)</td>
<td>20/52 (38.5)</td>
<td>21/80 (26.3)</td>
</tr>
<tr>
<td>≥18 y</td>
<td>0/104 (0.0)</td>
<td>0/19 (0.0)</td>
<td>0/31 (0.0)</td>
<td>0/154 (0.0)</td>
</tr>
<tr>
<td>6–11 mo</td>
<td>0/25 (0.0)</td>
<td>0/2 (0.0)</td>
<td>5/28 (17.9)</td>
<td>5/55 (9.1)</td>
</tr>
<tr>
<td>&lt;6 mo</td>
<td>0/27 (0.0)</td>
<td>0/4 (0.0)</td>
<td>2/20 (10.0)</td>
<td>2/51 (3.9)</td>
</tr>
<tr>
<td>24–59 mo</td>
<td>0/38 (0.0)</td>
<td>0/2 (0.0)</td>
<td>20/58 (34.5)</td>
<td>20/98 (20.4)</td>
</tr>
<tr>
<td>5–9 y</td>
<td>0/31 (0.0)</td>
<td>0/7 (0.0)</td>
<td>9/53 (17.0)</td>
<td>9/91 (9.9)</td>
</tr>
<tr>
<td>10–17 y</td>
<td>0/28 (0.0)</td>
<td>0/2 (0.0)</td>
<td>0/11 (0.0)</td>
<td>0/41 (0.0)</td>
</tr>
<tr>
<td>≥18 y</td>
<td>0/104 (0.0)</td>
<td>0/19 (0.0)</td>
<td>0/31 (0.0)</td>
<td>0/154 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>1/278 (0.4)</td>
<td>0/39 (0.0)</td>
<td>56/253 (22.1)</td>
<td>57/570 (10.0)</td>
</tr>
</tbody>
</table>

Abbreviations: RV, rhinovirus.

*RV-C detected in the serum of 1 patient, and RVA detected in the corresponding respiratory specimen.

(10%) of these patients. Overall, 56 of 57 (98.2%) viremias were associated with RV-C, and the remaining 1 viremia was associated with RV-A. Identical VP4/V2 sequences were obtained from both the respiratory and acute serum specimens of all viremic patients except for one 6-month-old child with RV-A identified in the respiratory specimen and RV-C in the serum. Convalescent sera collected a median of 27 days (range, 14–51 days) after the acute specimen from 29 viremic patients were all rRT-PCR negative for RV. Phylogenetic analysis of the RV-C VP4/V2P sequences identified >25 different genotypes (Figure 1). In addition, a cluster of closely related RV-C sequences obtained from 4 patients enrolled at EPIC study sites in 3 different states diverged by >23% from other known RV-C genotypes, which exceeded the 10% threshold for new genotype assignment in the VP4/V2 region [1] and therefore may represent a new genotype.

Rhinovirus NP/OP and Age Group

Most RV detections from NP/OP specimens (65.8%) were in children aged <10 years, and proportionately more detections of RV-C (n/N = 211/253; 83.4%) were in this age group than those without viremia. There were no differences in intensive care admission, mechanical ventilation, deaths, length of hospital stay, or chest radiographic features within 48 hours of admission between patients with and without RV viremia (median Ct, 26.5; IQR, 23.3–29.0) with RV viremia also had another pathogen detected compared with 107 of 315 (34.0%) without viremia (P = .001). Interestingly, all codetections were viruses, including respiratory syncytial virus (n = 3), influenza virus (n = 2), parainfluenza virus (n = 1), and respiratory syncytial virus adenovirus (n = 1).

Rhinovirus Viremia and Codetection With Other Respiratory Pathogens

We compared proportions of codetections in patients with and without RV viremia to determine whether patients with viremia were less likely to be coinfected with another respiratory pathogen that could account for the illness. Among 566 (99.3%) patients with RV-positive respiratory specimens and available test results for bacterial and other viral pathogens, 143 (25.3%) had another viral or bacterial respiratory pathogen infection detected. Among 372 patients aged <10 years with RV detected from the respiratory specimen and available test results for other pathogens, 7 of 57 (12.3%) with RV viremia also had another pathogen detected compared with 107 of 315 (34.0%) without viremia (P = .001). Interestingly, all codetections were viruses, including respiratory syncytial virus (n = 3), influenza virus (n = 2), parainfluenza virus (n = 1), and respiratory syncytial virus adenovirus (n = 1).

Rhinovirus Viremia and Clinical Features

We compared the clinical features of 375 patients aged <10 years with RV-positive NP/OP specimens with and without RV viremia. Patients with viremia were significantly more likely to have chest retractions (61.4% vs 45.9%; P < .05) or wheezing (86.0% vs 61.3%; P < .001) at enrollment and to have preexisting asthma/reactive airway disease (59.7% vs 41.2%; P < .05) or wheezing (86.0% vs 61.3%; P < .001) at enrollment and to have preexisting asthma/reactive airway disease (59.7% vs 41.2%; P < .05) or have preexisting asthma/reactive airway disease (59.7% vs 41.2%; P < .05) or have preexisting asthma/reactive airway disease (59.7% vs 41.2%; P < .05) or have preexisting asthma/reactive airway disease (59.7% vs 41.2%; P < .05).

DISCUSSION

In this large, multisite study of CAP, we detected RV viremia in 10% of 570 hospitalized patients with RV detected from upper respiratory tract specimens. Our findings were similar to several international reports of RV viremia detected by RT-PCR in children with acute respiratory RV infections [5–7]. A study from Greece detected RV viremia in 10 of 88 (11.4%) children presenting with acute respiratory symptoms ranging from common colds to pneumonia [5]; a study from Italy detected RV viremia in 6 of 50 (12.0%) children with fever and acute respiratory symptoms [6]; and a study from Japan detected RV viremia...
in 30 of 243 (12.3%) children hospitalized in the Philippines with severe lower respiratory tract infections [7]. Although these studies examined RV respiratory tract infections of varied clinical severity among mostly young children, our study was larger, included all age groups, and focused on hospitalized patients diagnosed with CAP, of which nearly 90% of pneumonias were subsequently confirmed on radiographic review.

Before the advent of sensitive molecular diagnostic tests, RV infections were thought to remain localized to the mucosal epithelium of the upper respiratory tract [17]. Using culture-based detection methods, RV viremia had only been reported from a few children with sudden and unexpected death [3, 4]. Past failures to culture RV from blood might be explained by the predominance of RV-C in viremia (see below), which is known to not grow well in conventional cell lines [18]. With some recent exceptions [19, 20], acute RV infections have rarely been associated with disseminated disease, unlike EVs, where viremia can lead to infection of multiple sites with varied clinical manifestations [21]. However, blood detection of RV by RT-PCR may only represent identification of RV RNA and not true viremia with viable virus.

Routes of RV entry into the bloodstream are unknown. Experimental studies have shown that RV can infect lower respiratory tract airways [22, 23], and individual case reports have
documented RV infection of the lung [24, 25]. Direct infection and viral-mediated injury of the lung might hypothetically lead to direct viral seeding of the blood. However, RV viremia has also been found in children presenting with only upper respiratory tract symptoms [4, 6], suggesting that infection of the lower respiratory tract may not be required for bloodstream access. Rhinovirus RNA and infected cells have been found in tonsils and adenoid tissues that facilitate antigen capture and immune cell recruitment [26, 27]. These tissues are drained by efferent lymphatic vessels that may serve to convey RV to the bloodstream.

Although there were more RV-A detections from respiratory specimens of study patients than RV-C detections, RV-C accounted for almost all viremias (98.2%). Differences in virus load in the upper respiratory tract did not appear to explain this finding; median Ct values were similar for RV-A and RV-C, although lower median Ct values (representing higher virus loads) for RV-C were associated with viremia. Our findings are consistent with other studies that compared proportions of viremias among children with acute RV respiratory tract infections by virus species [6, 7]. Of the 6 children with RV viremia detected by Esposito et al [6], 4 (66.7%) were identified as having RV-C. Of the 30 children with RV viremia detected by Fuji et al [7], 26 (86.7%) were found to have RV-C; interestingly, these authors found that the 4 patients with RV-A viremia all possessed RV-C-like 5’ non-coding regions (NCRs) acquired through recombination, leading the authors to speculate that the RV-C 5’ NCR might mediate viremia. However, the 5’ NCR of the RV-A that we identified from a single viremic patient did not show evidence of recombination (data not shown). Like Fuji et al [7], we found a wide range of RV-C genotypes without obvious phylogenetic clustering, suggesting that viremia is not genotype-specific.

We found young age to be strongly associated with RV viremia. Rhinovirus viremia peaked in children aged 1–2 years, with lower prevalence in infants aged <6 months and declining prevalence with increasing age after 2 years; viremia was not detected among patients aged >10 years. Clearance of RV from blood by maternal antibodies may explain the lower prevalence in infants aged <6 months [7, 28]. Because RV infections peak in early childhood [29], with reinfections occurring with increasing age, immune memory and cross-protective RV immunity may lead to more rapid virus clearance from blood and explain the subsequent decline in viremia. A further contributing factor may be that RV-C infections were proportionately more common in young children as compared with other RV species and more commonly associated with viremia [6, 7].

Multiple epidemiologic studies that compared RV species found RV-C to be more frequently associated with severe respiratory infections, particularly in children with asthma [8, 9, 30–32]. Although we found no clear differences in severity of RV infections between patients with and without viremia,
patients with viremia were more likely to have chest retractions or wheezing symptoms at enrollment and were more likely to have preexisting asthma/reactive airway disease. In those studies of RV viremia in children hospitalized with severe respiratory illnesses, viremia was more often associated with increased severity measures suggestive of lower respiratory infection, including decreased oxygen saturation levels [6, 7] and required oxygen therapy [6]. Personal history of asthma and proportion of asthma exacerbations were significantly associated with RV viremia [5], as was wheezing [7]. Together, these findings suggest that the pathophysiology of infection with RV-C may differ from other RV species. A unique feature of RV-C is its use of the cadherin-related family member 3 (CDHR3) protein for host cell entry [33], whereas RV-A and RV-B use intercellular adhesion molecule 1 or low-density lipoprotein receptor family members. Interestingly, CDHR3 has been identified as a susceptibility locus for early childhood asthma [34]. Moreover, cells expressing genetic variants of CDHR3 show enhanced binding efficiency for RV-C and increased virus production, suggesting that these variants could be a risk factor for RV-C-associated childhood wheezing and asthma hospitalization [33, 35]. Further studies will be needed to determine the relationship between receptor utilization and pathophysiology of RV-C infection and whether viremia might be an indicator of or contributor to this process.

Rhinovirus infections in young children are often subclinical or are codetected with other respiratory pathogens, complicating disease attribution [36]. In the original EPIC study [12], >42% of all RV-positive pediatric patients had another respiratory pathogen detected, which could account for patient illness. Some studies that compared RV species in persons with acute respiratory infections have found codetection prevalence to be significantly lower in those with RV-C [10, 31]. We found significantly fewer codetections among RV-C-infected patients with RV viremia, suggesting that viremic RV-C infections may be more indicative of pediatric pneumonia than nonviremic episodes.

Our study had several limitations. First, not all RV-positive patients had acute serum specimens available for testing, and specimen collection times from onset of patient reported symptoms varied and may have been suboptimal in some patients for viremia detection. We were restricted to specimens collected from the upper respiratory tract, preventing assessment of possible correlation of RV viremia with lower respiratory tract viral loads. Others have shown that sample collection timing and sample type (serum vs whole blood) might impact the ability to detect virus [5, 7]. Together, these findings might impact the detectability of virus [5, 7].

In conclusion, we detected RV viremia in hospitalized CAP patients aged <10 years with RV-positive upper respiratory tract specimens. Compared with other RV species, RV-C was almost exclusively associated with viremia and was more often the only respiratory pathogen identified in viremic patients. Although causality cannot be conclusively inferred, these findings strongly suggest that RV-C is an important cause of pediatric pneumonia and that viremia may be a useful diagnostic indicator.
Acknowledgments. We thank the patients who graciously enrolled in the EPIC study.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

Financial support. This work was supported by funds from the CDC.

Potential conflicts of interest. All reported support was received outside of the submitted work. W. H. S. reports grants from BioMerieux, Affinium Pharmaceuticals, Astute Medical, BRAHMS GmbH/Thermo Fisher, Pfizer, Rapid Pathogen Screening, Venaxis, BioAegis Inc, Sphingotec GmbH, and Ferring Pharmaceuticals, and personal fees from BioFire Diagnostics, Venaxis, Abbott Point of Care, and Cemptra Pharmaceuticals. E. J. A. reports personal fees from AbbVie, grants and nonfinancial support from MedImmune, and grants from Regeneron and Novavax. C. G. G. reports serving as a consultant for Pfizer. R. G. W. reports grants from the CDC, funds to conduct clinical research from BioMerieux and Vertex Pharmaceuticals, and personal fees from BioMerieux and Visterra Inc. D. J. W. reports grants from the CDC. K. M. E. reports serving on a data and safety monitoring board for Novartis for which her institution receives funds to conduct clinical research from BioMerieux and Vertex Pharmaceuticals, and personal fees from BioMerieux and Visterra Inc. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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