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BRIEF REPORT

Midturbinate Swabs Are Comparable to Nasopharyngeal Swabs for Quantitative Detection of Respiratory Syncytial Virus in Infants

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Nasopharyngeal (NP) swabs are generally used to detect respiratory syncytial virus (RSV) in infants. However, midturbinate (MT) swabs may provide comparable results. In this study, we enrolled hospitalized infants aged <24 months with RSV and collected NP and MT swabs. The resulting viral loads measured by real-time reverse-transcription quantitative polymerase chain reaction were similar. Most parents preferred MT swabs over NP swabs.

Keywords. bronchiolitis; diagnostics; infants; nasal swab; PCR; RSV.

Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis, pneumonia, and hospitalizations in infants and young children [1]. RSV diagnosis is confirmed by detecting viral antigen or viral nucleic acid in respiratory secretions [1]. Molecular-based assays use the real-time reverse-transcription quantitative polymerase chain reaction (real-time RT-qPCR) technique, which enables quantification of viral particles. Quantification of RSV in nasal secretions by real-time RT-qPCR might help in the evaluation of severity of disease [2] and in assessing the effect of novel antiviral therapeutics [3].

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Nasopharyngeal (NP) wash or swab samples have been studied and validated for the detection and quantification of respiratory viruses [4, 5]. However, NP sampling is invasive and uncomfortable for the patient and distressing for the caregiver, and it requires a trained provider to perform the collection. Studies have found that testing of anterior nares or midturbinate (MT) samples from infants has a sensitivity similar to that of NP swab testing for qualitative detection of RSV [6, 7]. It is unknown, however, how quantitative RSV viral loads between these 2 sample types (NP vs MT) compare. We sought to determine whether less-invasive MT swabs are comparable to NP samples for quantifying RSV viral loads in infants. Our secondary objectives included assessing the correlation between viral load and parent-reported symptoms and symptom days among hospitalized infants with RSV and determining parent preference of swabbing method for their child.

METHODS

This study was approved by the institutional review boards of the University of Utah and Primary Children's Hospital (Salt Lake City).

Study Design and Patient Selection

Ours was a prospective study of infants admitted to Primary Children's Hospital with symptomatic RSV infection enrolled over 2 consecutive RSV seasons in 2016 and 2017 (January through April 2016 and December through April 2017). Infants were included in the study if they were aged <24 months with a gestational age at birth of ≥28 weeks and an adjusted gestational age (actual gestational age + chronologic age) of ≥40 weeks. Upper or lower respiratory tract infection diagnosed by the infant's treating physician and a confirmed RSV diagnosis (PCR or rapid antigen testing based) was required. Time between hospital admission and study sample collection could be no greater than 72 hours. Infants were excluded if they were mechanically ventilated, could not provide the study samples, or had used an investigational medicinal product in the 28 days before screening or had ever received an investigational RSV vaccine. Infants were not excluded for having used palivizumab. Parents/guardians were given the option to have MT and NP swabs collected from their child for up to 7 days for serial testing if they remained hospitalized.

Study Procedures

At the time of enrollment and before swabbing, each infant's parent/guardian was asked to rate the severity of their child's RSV infection with the single-question Patient Global

Impression of Severity (PGIS) score, which was modified for caregivers of infants with RSV disease [8]. After scoring, the study coordinator collected both an MT and an NP swab (FLOQSwab, Copan Diagnostics, Inc, Murrieta, California) from the infant. Order of collection (NP or MT swab first) and side (left vs right nostril) were assigned randomly to account for possible differences in collection techniques according to the order of swabbing or collection site [9]. The second swab was taken from the opposite nostril so that the initial swab did not affect the amount of biological material available for sampling with the second swab. If the parent/guardian directly observed both swabbing procedures, they were asked to assess (using a 5-point Likert scale) which technique they would prefer for their child in the future.

Viral Load Measurements

MT and NP swabs were stored at −70°C after collection and shipped on dry ice to DDL Diagnostic Laboratory (Rijswijk, Netherlands) for RSV type A (RSV-A) and RSV-B viral load testing. Dry NP and MT swabs were resuspended in 1.5 mL of easyMAG lysis buffer (bioMérieux, Durham, North Carolina), and total nucleic acid was isolated with the NucliSENS easyMAG robot with on-board lysis (protocol, Generic 2.0.1) using 500 µL of the homogenized sample suspension. Viral RNA was quantified with a real-time RT-qPCR assay aimed at the N gene, modified from the assay as described by Hu et al [10]. RSV-A and RSV-B viral loads were calculated on the basis of quantified dilution series of in vitro–transcribed RSV-A and RSV-B RNA tested in each RT-qPCR run. Adequacy of the collected material was assessed by a parallel qPCR assay for human DNA (RNase P gene). As yet, it seems that no consensus in the field has been reached on the utility of "normalizing" raw viral load data on the basis of quantification of human DNA in the sample and thus normalization was not performed [9, 11]. Additional details of the PCR methods are provided in Supplementary Methods.

Statistical Methods

Correlation between the NP and MT viral loads was examined. A linear mixed-effect model was used to evaluate the mean difference in viral loads obtained from the 2 swab types. The order in which NP vs. MT swabbing was performed, and the nostril used for each swab, were included as fixed effects, and subject was used as a random effect.

A Bland–Altman plot was used to quantify the agreement of viral loads obtained from the NP and the MT swabs by creating a scatterplot that depicts the differences in viral load values obtained from the NP and MT swabs as a function of their average. The Bland–Altman plot provided the average difference between the NP and MT swabs and limits of agreement (average difference, ±1.96 standard deviations of the difference). A further exploratory analysis was conducted to evaluate the relationship between symptom days and viral load and parental assessment of severity and viral load.

RESULTS

We enrolled and collected swabs from 84 infants during 2 RSV seasons. Demographic and clinical data are shown in Table 1. The median age was 4 months (range, 0–23 months). We collected swabs from 17 infants on multiple consecutive days. The median (quartile 1 [Q1] and Q3) duration of symptoms before enrollment was 5 days (4 and 7 days). The median (Q1 and Q3) hospital stay length was 2 days (1 and 4 days). We had complete data (with no missing viral loads) for 78 infants. Of those 78 infants, 77 had at least one study swab positive for RSV at baseline; for 1 infant, the real-time RT-qPCR results from both the MT and NP swabs were negative for RSV.

The mean (SD) viral loads were similar (7.34 [1.26] and 7.09 [1.25] $\log_{10}$ copies/mL for 77 paired NP and MT swabs with positive results, respectively) (Figure 1A). The correlation coefficient between the paired viral loads was high (0.82). The Bland–Altman plot (Figure 1B) represents every difference between 2 paired viral loads against the average of the 2 viral loads. We found a small (−0.24 ± 0.18) but consistent bias toward lower viral load with the MT swab. Median viral loads, as measured by both NP and MT swabs decreased over the course of hospitalization for infants who underwent repeated swabbing (Figure 1A).

Before swabbing, parents were asked to rate their child's disease using the single-question PGIS score [8]. Most (63 of 78 [81%]) parents rated their child's illness as mild (29 of 78 [37%]) or moderate (34 of 78 [44%]). Only 13 (17%) parents rated their child's illness as severe, and only 2 (2%) rated their child's illness as very severe. Mean viral loads measured by both NP and MT swabs were positively correlated with PGIS score (Spearman correlations, 0.37 [NP swabs] and 0.41 [MT swabs]; $P < .001$ for both) (Figure 1C). The correlation between symptom days and viral load was weak (NP correlation coefficient r = 0.09; MT correlation coefficient r = −0.27; Figure 1D).

Most (53 of 65 [82%]) parents who watched the swabbing of their infant definitely (48 of 65 [74%]) or somewhat (5 of 65 [8%]) preferred the MT to the NP swab; 17% (11 of 65) of them had no preference.

DISCUSSION

Initial validation studies for molecular diagnostics to detect and identify respiratory viruses used NP aspirate (NPA) samples as the gold standard [12]. Since then, several studies have compared NPA samples and NP swab samples for

Table 1. Demographics, Clinical Information, and RSV Diagnosis for Enrolled Infants

Demographic or Clinical Data	Values
Age at enrollment (mean [SD]) (months)	6.8 (6.5)
Gestational age at birth (n [%])	
Full term (>37 wk)	68 (81)
Preterm (28–36 wk)	15 (18)
Unknown	1 (1)
Sex (n [%])	
Male	44 (52)
Female	40 (48)
Ethnicity (n [%])	
Hispanic or Latino	10 (12)
Not Hispanic or Latino	71 (84)
Not provided	3 (4)
Race (n [%])	
White	71 (84)
Asian	3 (4)
Black or African American	4 (5)
Native Hawaiian or other Pacific Islander	6 (7)
Weight (mean [SD]) (kg)	
At enrollment	7.05 (2.81)
At birth	3.17 (0.62)
Clinical data	
Duration of RSV symptoms before enrollment (mean [SD (Q1, Q3)]) (days)	6.1 (6.6 [4, 7])
Duration of hospitalization before enrollment (mean [SD (range)]) (days)	2.4 (0.67 [1, 4])
Length of hospital stay (mean [SD (range)]) (days)	3.1 (2.4 [1–16])
Required ICU care (n [%])[a]	
No	62 (74)
Yes	21 (25)
Required mechanical ventilation (n [%])[b]	
No	75 (89.3)
Yes	7 (8.3)
Required supplemental oxygen (n [%])[a]	
No	9 (11)
Yes	74 (88)
Required supplemental fluids (n [%])[b]	
No	27 (32.1)
Yes	55 (65.5)
RSV diagnosis according to swab type (n [%])	
RSV type with MT swab	
RSV-A only	64 (76)
RSV-B only	15 (18)
Negative	2 (2)
RSV type with NP swab	
RSV-A only	64 (76)
RSV-B only	15 (18)
Negative	1 (1)
Patients with RSV-negative results	
Concordant	1 (1)
Patients with RSV-positive results	
Concordant	77 (92)
Discordant	1 (1)
Patients with missing data[c]	5 (6)

Abbreviations: ICU, intensive care unit; MT, midturbinate; NP, nasopharyngeal; RSV, respiratory syncytial virus; SD, standard deviation; Q, quartile.

[a]Data unknown for 1 patient.

[b]Data unknown for 2 patients.

[c]Patients for whom 1 or more swabs for analysis were missing.

the molecular detection of respiratory viruses and concluded that these sample types are similar for diagnostic purposes, particularly when flocked swabs are used [4, 5]. Although NP swabs are now used preferentially over NPAs, NP swab collection is still uncomfortable and requires trained providers. Several studies have considered the less-invasive MT swab for qualitatively diagnosing respiratory viruses in adults and concluded that this type of swab might be adequate [13, 14]. Other studies have reported similar findings for children [7, 15]. It should be noted that for most Food and Drug Administration–cleared tests, using the swabs for this purpose would constitute use outside the manufacturer's instructions.

We have compared the quantitative detection of RSV from NP swabs and that from MT swabs in young infants. Our comparison revealed that although viral loads were consistently slightly lower with the MT swab approach, viral load data from both NP and MT swabs were strongly correlated. Differences in viral loads were small relative to the standard deviation and unlikely to be clinically relevant. More than three-fourths of the parents preferred MT swabs over NP swabs, and 99% of those with a preference would choose the MT swab for their child. Our results suggest that clinically, MT swabs are preferable for parents for the routine detection of RSV and should be reliable for measuring nasal viral loads for research and clinical trials. Being able to offer a more comfortable approach to acquiring these samples could positively influence future clinical trial participation.

Monitoring viral load in children with RSV disease is typically required for clinical trials, because the results of previous studies, albeit small, suggest that RSV viral load correlates with symptom severity and recovery [2, 16]. Although baseline viral load and parent-reported disease severity were correlated in our study ($P < .001$), we observed only a weak correlation between baseline viral load and days of symptoms before admission and between viral load change and symptom recovery during hospitalization. Although these results might have been a result of lack of standardization for collection of exact volumes or quantities of biologic material, these real-world data and the variability they reflect are important to recognize and consider as clinical trials are designed.

The results of our study confirm that a less-invasive sampling procedure, using an MT swab, provides adequate data for not only the clinical management of RSV in infants but also quantitative viral load monitoring in research settings. The difference in mean viral loads between the NP and MT swabs was small and unlikely to affect clinical decision-making or clinical trial results. Use of the less-invasive MT swab is preferred by parents, and with further study, MT swabs have the potential to replace NP swabs as the gold standard for quantitative respiratory viral load measurement.

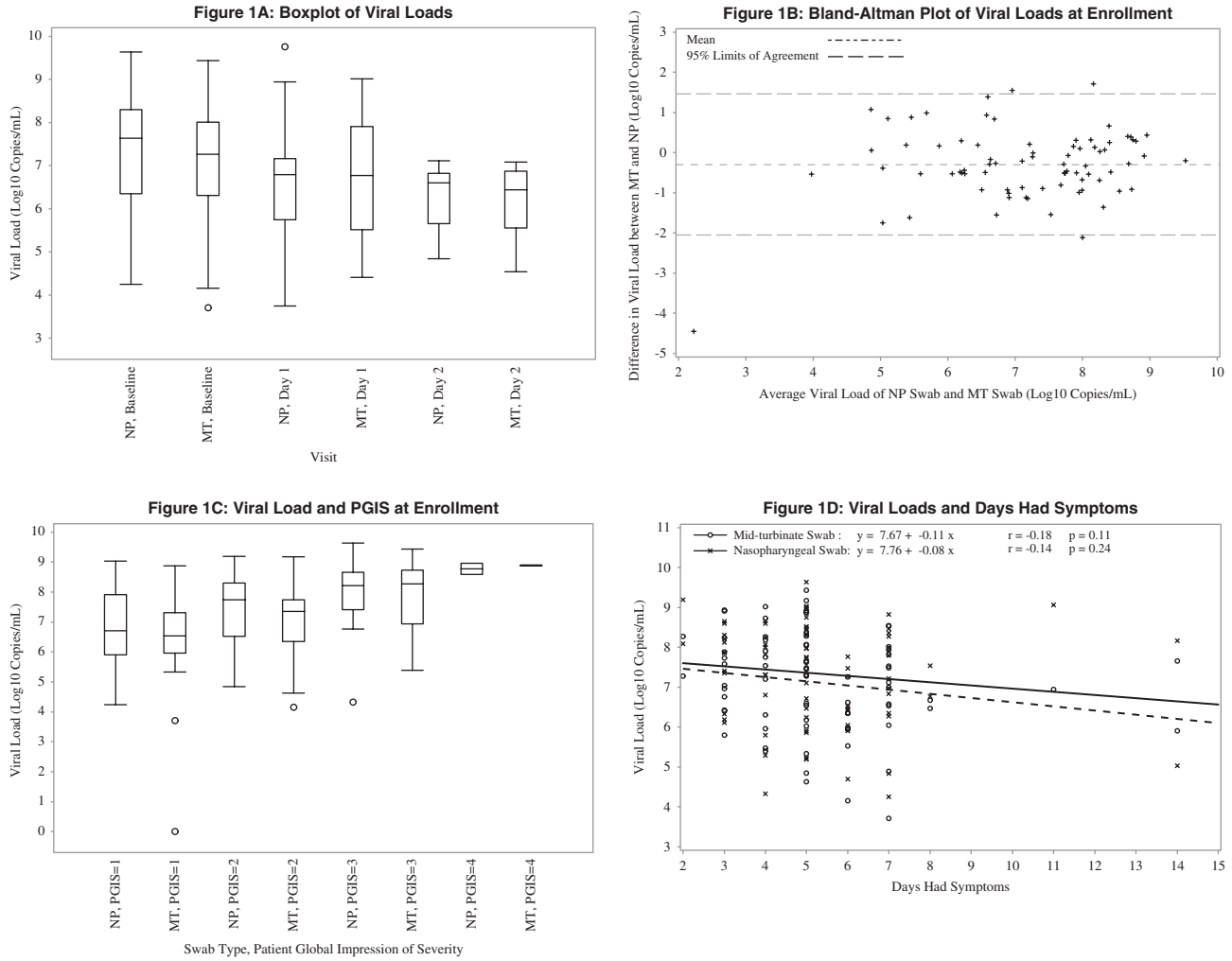

Figure 1. (A) Box plot of viral loads from nasopharyngeal (NP) and midturbinate (MT) swabs according to day after enrollment (baseline = day 1). Boxes show the first and third quartiles, and the whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range. The median is indicated by the bold line in the box. In the baseline (Day 1) analysis, paired swabs from 77 infants were included; 18 paired swabs were collected and included in the Day 2 analysis and 6 paired swabs were collected and included in the Day 3 analysis. The mean (standard deviation [SD]) viral loads were similar (7.34 [1.26] and 7.09 [1.25] $\log_{10}$ copies/mL for 77 paired NP and MT swabs with positive results at baseline, respectively). Median viral loads, as measured from both NP and MT swabs, decreased over the course of hospitalization for infants who underwent repeated swabbing. Mean (SD) viral loads for respiratory syncytial virus type A (RSV-A) and RSV-B according to swab type were 7.40 (1.28) $\log_{10}$ copies/mL (NP/RSV-A), 6.90 (1.33) $\log_{10}$ copies/mL (NP/RSV-B), 7.18 (1.24) $\log_{10}$ copies/mL (MT/RSV-A), and 6.74 (1.23) $\log_{10}$ copies/mL (MT/RSV-B). RSV-A vs. RSV-B data are not shown on the graph. (B) Bland–Altman plot showing every difference between 2 paired viral loads against the average of the 2 viral loads. We found a small (–0.29) but consistent bias toward lower viral loads with the MT swab. However, the 95% limit of agreement covers 0 with a large margin (–2.05 for lower limit, 1.46 for upper limit), which shows overall agreement between the viral loads obtained from the NP and MT swabs despite the small difference. (C) Box plot showing viral loads from NP and MT swabs compared with Parental Global Impression of Severity (PGIS) scores, which were modified for caregivers of infants with RSV disease [8]. The question asks "On average, how would you describe your child's RSV symptoms right now?" Parents could respond with a score of 1 (mild), 2 (moderate), 3 (severe), or 4 (very severe.). The boxes show the first and third quartiles, and the whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range. The median is shown by the bold line in the box. Mean viral loads, as measured by both NP and MT swabs, were positively correlated with PGIS scores (Spearman correlation, 0.37 and 0.41 for NP and MT swabs, respectively; $P < .001$ for both). (D) Scatter plot comparing NP and MT viral loads with parent-reported days of symptoms before swabbing. Trend lines are included, and slopes represent the correlation coefficients. The correlation between symptom days and viral load was weak (correlations, –0.14 [NP] and –0.18 [MT]) and insignificant ($P = .24$ and 0.11 for NP and MT swabs, respectively).

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

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Potential conflicts of interest. A. J. B. and K. A. received funding for this investigator-initiated research from Gilead Sciences, Inc. A. J. B. collaborates with BioFire Diagnostics, LLC, on federally funded studies and has intellectual property licensed to BioFire Diagnostics through the University of Utah for which she receives royalties through the University of Utah and has received research funding from BioFire Diagnostics for investigator-initiated research and has acted as a paid advisor to BioFire

Diagnostics. M. M., H. C., and Y. G. are employees of Gilead Sciences, Inc, and hold stock in Gilead Sciences, Inc. At the start of the study, S. T. and J. W. C. were employees of Gilead Sciences, Inc, and owned stocks in Gilead Sciences, Inc. S. T. is a current employee of bioMérieux and no longer hold stock in Gilead Sciences, Inc. J. W. C. is a current employee of Janssen Biopharma, Inc, and still hold stock in Gilead Sciences, Inc. All other authors: No reported conflicts of interest. 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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