Impact of Nasal Saline Irrigations on Viral Load in Patients with COVID-19

NCT04347538

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1.0 Background

The novel coronavirus known as SARS-CoV-2 and the associated disease process COVID-19 (coronavirus disease 2019) was first seen in late 2019 in Wuhan, China. Over the following months, it quickly spread across the continent and, in short order, the globe, making an impact that hasn't been seen in generations. Although coronaviruses have been prevalent for millennia, this version is immunologically novel, and thus there is no natural immunity to the virus. This has been a major reason for its rapid spread across the world.

Previous members of the coronavirus family have typically caused upper respiratory symptoms such as the common cold, though there have also been more virulent versions of this virus seen in the recent past, such as SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome). Similarly named, SARS-CoV-2 also causes upper respiratory symptoms but has varied from the previous viral syndromes in a number of ways including how quickly it has been able to transmit within a population. This is a disease that does not segregate and can affect all ages, genders, and ethnicities. Everyone is susceptible to this virus.

New diagnostic and therapeutic approaches for respiratory viruses are also being rapidly developed and polymerase chain reaction-based (PCR) diagnostics and multiplex assays are increasingly used in clinical laboratories for SARS-CoV-2 clinical detection and subtyping. Rapid antigenic and genetic evolution has been expected for SARS-CoV-2 strains, and a better understanding of SARS-CoV-2 evolutionary dynamics is needed to establish an effective vaccine.

Our present understanding of the nature and extent of the upper respiratory track (URT) microbiome in humans is limited. Furthermore, we have little understanding of how acute viral respiratory infections of SARS-CoV-2 influence the URT microbiome, or how genotypic differences in the virus influence the URT microbiome and vice versa. Innate immune responses to pathogens, along with dysregulation of inflammation, are key factors involved in pathogenesis, and different viral pathogens activate different types of inflammatory responses. Respiratory viral infection i.e., SARS-CoV-2 infection is expected to activate TLR2, TLR3, TLR4 and TLR7 responses and this is likely to modulate commensal microbiota populations. It is not yet known if the severity of SARS-CoV-2 disease in older adults is due to a biased host response, SARS-CoV-2 virulence determinants, or the impact infection has on commensal microbiota.

Up to this point, there is no unanimously approved treatment for the disease nor is there a vaccine or antiviral drugs available for the public. The primary methods for treatment of this deadly virus have been supportive in nature including intubation in severe cases with respiratory failure.

While a unanimous treatment has yet to be discovered, there has been a great amount of knowledge garnered over the last few months about the virus and the disease that accompanies it. Several studies have demonstrated high viral titers within the nasopharynx and oral cavity and many have posited that this is the primary source of

infection and viral replication. Additionally, a high nasal/nasopharyngeal viral load has been associated with increased symptoms and higher severity of the disease.

Interestingly, there have been a number of studies recently looking at the effect of nasal saline irrigations in the setting of viral URIs, including coronaviruses (not including SARS-CoV-2). One of the major takeaways from these studies was decreased viral shedding in patients treated with saline irrigations compared to the control group. Nasal saline irrigations are available over the counter and widely viewed as both safe and affordable. Could these irrigations have a similar effect on the novel SARS-CoV-2 that they have on other viral respiratory infections?

2.0 Rationale and Specific Aims

While many systemic medications and treatments have been proposed, there has not yet been a study looking at targeted local intervention to the nasal cavity and nasopharynx where the viral load is the highest. Studies have shown that the use of simple over the counter nasal saline irrigations can decrease viral shedding in the setting of viral URIs, including the common coronavirus (not SARS-CoV-2). Further, as SARS-CoV-2 is an enveloped virus, mild-detergent application with nasal saline would neutralize the virus further. It is our hypothesis that nasal saline or nasal saline with baby shampoo irrigations may decrease viral shedding/viral load and viral transmission, secondary bacterial load, nasopharyngeal inflammation in patients infected with the novel SARS-CoV-2.

Specific Aim 1. Define the longitudinal SARS-CoV-2 mucosal immune response, microbial load, and viral load in the nasopharynx over the course of COVID-19 infection.

Specific Aim 2. Assess the impact of nasal saline or nasal saline with baby shampoo on SARS-CoV-2 viral load, mucosal inflammation and secondary bacterial load in nasal cavity.

Specific Aim 3. Identify the longitudinal symptom burden and temperature during the course of the disease

3.0 Animal Studies and Previous Human Studies

Nasal saline irrigations are a safe and commonly used mechanism to treat a variety of sinonasal diseases including sinusitis, rhinitis, and upper respiratory tract infections. When used properly, these irrigations are a safe and easy intervention available over the counter without a prescription. Additionally, baby shampoo has been found to be a safe additive functioning as a surfactant when a small amount is added to the saline rinses which may help augment clearance of the sinonasal cavity.

There have been reports of infections secondary to improper use and thus it is important to use filtered water or previously boiled water when performing rinses. Apart from these issues related to improper use, there are no substantial adverse effects associated with nasal saline irrigations noted in prior human studies. Typically, nasal saline irrigations have been a treatment in prior studies as a part of the control arm assessing intranasal medications, but there are no discrete Phase 1, 2, or 3 studies looking at nasal saline as an independent intervention, as nasal saline rinses have for many years been used as a standard care adjunct for intranasal/sinus disease. There have been a small number of studies showing a transient loss of smell when adding surfactant to nasal saline rinses, though this appears to resolve when surfactant is discontinued.

4.0 Inclusion/Exclusion Criteria

List the criteria:

- Inclusion
    - Patients testing positive for COVID-19 at Vanderbilt University Medical Center or VUMC-associated testing centers
    - Age of 18 years or greater
    - Patients must be planning self-quarantine after infection in the greater Nashville area within a 30-mile radius of Vanderbilt University Medical Center

- Exclusion
    - Requiring hospitalization – only outpatient COVID-19 cases are eligible for the study
    - Current use of nasal saline irrigations or other intranasal medications
    - Inability to perform saline irrigations/nasal swabs in separate bathroom away from household contacts

5.0 Enrollment/Randomization

Patients enrolled will be randomized to one of three treatment groups (control- no intervention, intervention 1- nasal saline irrigations BID, intervention 2- nasal saline irrigations with ½ teaspoon surfactant (Johnson's baby shampoo) BID). Patients identified as COVID-19 positive through VUMC testing centers will be contacted and given information regarding the study. Randomization, enrollment, and registration will take place via REDCap (*see telephone script*).

The address for the home of the study is:

Department of Otolaryngology – Head and Neck Surgery
Vanderbilt University Medical Center
7209 Medical Center East – South Tower
1215 21st Ave South
Nashville, TN 37232-8605

6.0 Study Procedures

Day 0: Patient tests positive for COVID-19 via nasal swab which is identified through Epic and is given information about the trial via telephone call. At that time, they may determine whether or not they would like to be enrolled in the study. Those enrolled in the study will be randomly assigned into one of 3 groups (control, intervention 1, intervention 2) using REDCap and delivered a package containing: nasal swabs with premeasured depth for insertion, saline rinse bottle with saline packets and detailed instruction guide (intervention 1, 2), Johnson's Baby Shampoo (Johnson & Johnson Inc., New Brunswick, NJ) for use with saline rinses (intervention 2), and symptom questionnaire/temperature sheets.

Days 1-21: Patients in intervention group 1 and 2 will perform nasal saline irrigations +/- ½ teaspoon of Johnson's Baby Shampoo in the morning and the evening. All patients will perform nasal swabs every 2-3 days (*See Instruction Sheet*). In the intervention arms, these swabs should be taken at least 4 hours after saline rinses are performed. Patients will also check temperature (at same time each day) and fill out a symptom questionnaire (*See Symptom Questionnaire*). At the completion of week 3, patients will call our team to collect swabs and questionnaire/temperature sheets. The paper questionnaires will be transferred to REDcap for data collection and analysis.

*The timing of the study is based on when patient initially tests positive for COVID-19 and is a rolling enrollment rather than simultaneously performed.

Once 90 (~30 per group) patients have completed their 21 days of swabs/temperature checks/questionnaires, the study trial will be completed and data analysis will be performed. Interim analysis will be performed at 45 total patients.

As these patients will be under quarantine for COVID-19 infection, they will receive and provide their swabs, temperature and symptom scoring sheets from their home address at the beginning and at the conclusion of the 21-day study period. The swabs will be collected using OMR 110, DNA Genoteck Inc, Canada, that will immediately neutralize virus due to isotonic detergents present in the buffer, at the same time stabilizing the DNA and RNA for further analysis. Study administrators will wear appropriate personal protective equipment when retrieving the above-mentioned specimens.

Laboratory and Analytic Methods
qPCR Analysis to asses viral copy number: Viral RNA will be extracted using a standard Qiagen viral RNA isolation kit. After viral RNA extraction, qPCR will be utilized to analyze viral titers.

SARS-CoV-2 Complete Genome Sequencing and analysis: Viral RNA will be extracted using a standard Qiagen viral RNA isolation kit. We have established a high-throughput CoV genome sequencing pipeline, where we will perform overlapping long-range RT-PCR across the viral genome for each viral genome proposed in this project.

The sequencing will be performed using the Illumina MiSeq platforms. We will 1) generate the long-range amplicons; 2) create libraries; 3) perform high-throughput sequencing; 4) deconvolve and assemble sequencing reads; 5) close genomes using the appropriate finishing methods; and 6) submit the assembled sequence files and metadata to NCBI. We will apply phylodynamic analyses that are being developed as part of our analytic pipeline toward a comprehensive study of SARS-CoV-2 evolutionary dynamics. Genome sequences from these samples will allow us to assess the evolution and extent of co-circulation of the different SARS-CoV-2 subgroups; we will also assess whether there are genetic associations with different clinical signs and symptoms of disease severity using the vast amount of clinical metadata that are being collected. Because all sequence information will be made publicly available, this data will be of use for the entire SARS-CoV-2 research community and will serve as references for viral diversity for all subsequent studies in this field.

16S Sequencing for microbiome analysis: DNA/RNA isolation and 16S sequencing will be conducted as described before (2, 3). Microbial sequence data will be evaluated in the context of SARS-CoV-2 infection status to determine taxonomic profiles and their distributions within and between samples, including taxonomic classification and abundance, clustering, and measures of within sample (alpha diversity) and between sample (beta diversity) variation (4). Our goal is also to apply hypothesis testing for differences in community structure using multivariate statistics such as non-parametric multivariate analysis of variance (NP-MANOVA) and to estimate the significance of changes in the diversity, intragroup variation, and specific proportions of taxonomic classifications and OTUs. We will determine if specific microorganisms correlate with viral SNPs, disease severity, and/or host gene expression, especially genes important to innate immune response.

Metatranscriptomics Sequencing: To simultaneously sequence the viral, host, and microbial transcriptomes, RNA-seq libraries will be created from total RNA extracted from NP and oropharyngeal swabs and treated to remove all ribosomal RNA (Ribo-Zero™, Epicentre). Forty-eight samples will be multiplex per lane on an Illumina NovaSeq V4 sequencing lane to producing ~10GB of 150-bp paired-end reads/sample. Based on our current experience with *de novo* metatranscriptomics assembly, we favor Trinity assembly software (5) for this project because it consistently predicted the longest and fewest number of contigs and had the highest rate of ORFs that align to known genes. Transcripts will be mapped onto risk-active genomes and their relative abundance determined from the various samples and time points. To validate RNA-seq data, we will perform qRT-PCR on selected genes that show specific differential expression patterns. Transcripts that correlate with host and microbiome responses in the samples selected for RNA-seq will be investigated across the entire sample set using qRT-PCR. The innate immune response data will be validated by examining the levels of cytokines and chemokines using Luminex assays.

Data integration. We will interrogate the taxonomic composition of the URT microbiome across a wide range of donors (from adults vs. infants) for which we will know the genome sequence of the infecting virus, the disease-severity phenotype, and the microbial diversity. These data will inform us as to the presence and relative abundance of key microbial species that reflect healthy versus disease states.

Incorporating RNA-seq data will provide insights into the active functions of the host and the microbiome (i.e., both taxonomy and function), including potential changes in expression caused by the infecting virus. Correlations between biomarker diversity profiles (e.g., 16S) and functional profiles (RNA-seq), along with disease phenotypes and relevant patient metadata, will be identified using statistical approaches. Distance matrices will be computed among the phenotypes that are ordinal and matrix regressed in conjunction with the distance matrices between the samples. Significance and effect sizes of the regression coefficients will be reported. Multiple regression will then be performed with all the biomarker and RNA-seq data types simultaneously, using each -omics distance matrix as a predictor variable against the phenotype distance matrix as the response variable. The integration of these data will allow us to draw a more concrete picture of host-microbiome interactions, particularly as they relate to immune status and disease during SARS-CoV-2 infection.

7.0 Risks

Because of the reported risk of aerosolizing the virus with rinses, no hospitalized patients will be enrolled in the study. Additionally, patients in the study will be strictly directed to perform nasal saline irrigations in a separate bathroom/room in their house to prevent exposure to household contacts. If they are unable to accommodate these instructions, they will be excluded/removed from the trial. All patients who test positive for COVID-19 at VUMC are instructed to strictly self-quarantine and isolate themselves from friends, family, and acquaintances. Therefore, the restrictions of this study are not a substantial imposition on the ability of patients to keep themselves and their families safe.

There are reports of transient anosmia (loss of smell) in patients undergoing nasal saline irrigations with surfactant, though larger scale studies have not evaluated this and patients typically recovered once irrigations were discontinued.

While an absolute percentage is unknown, estimates have reported an average of 16 deaths per year in the United States secondary to amoebic meningitis, though saline irrigation or CPAP use in this population is also unclear. Many of the deaths related to amoebic meningitis in the United States are presumed secondary to swimming and diving. There are extremely rare and isolated reports of amoebic meningitis in patients using nasal saline irrigations or continuous positive airway (CPAP) masks. Because of this risk, the FDA has recommended using boiled water (3-5 minutes) or water passed through a filter with an absolute pore size of 1 micron or smaller (either purchased or filtered at home). Use of appropriately treated water in saline rinses renders the risk of infectious meningitis vanishingly small. Johnson's Baby Shampoo is often added as an adjunct to rinses in the dosage of 0.5 teaspoons per rinse bottle. There are no known significant toxicities to this common adjunct to nasal saline, although patients have been noted in prior studies (PMID: 17405780, 23710951) to complain of the taste of shampoo in the back of the nose.

The medical records of the patients in this study will be accessed to ensure that they have not undergone inpatient hospitalization at VUMC during the course of the study. All data will be stored on encrypted, HIPPA compliant VUMC Box accounts for management and analysis. Data will not be exported in an unencrypted format. All KSP for the study have been trained in data safety and data management.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Any adverse risks will be reported immediately to Vanderbilt IRB and the Institution Biosafety Committee. If any AEs occurred, they would be reported to the Vanderbilt HRPP as required per institutional policy. Contact information for HRPP is as follow:

Human Research Protections Program
3319 West End Ave., Suite 600
Nashville, TN 37203-1059
On campus: 600 CT (8547)
(615) 322-2918

9.0 Study Withdrawal/Discontinuation

Any patients that wish to be withdrawn from the study can do so at any time. Any patients that initiate other intranasal medications or therapies outside of the study protocol will also be withdrawn. Additionally, any patient hospitalized during the trial period will have that hospitalization as their individual end point for the study.

10.0 Statistical Considerations

As this is a pilot study for the use of nasal saline irrigations in the COVID-19 patient population, a true power analysis is not possible. This study will use a truncated initial sample size of 90 patients spread across 3 intervention arms to ensure that exclusion of true outliers is possible. Data will be analyzed with routine statistical software, with a planned 95% confidence interval for statistical testing.

11.0 Privacy/Confidentiality Issues

The medical records of the patients in this study will be accessed to ensure that they have not undergone inpatient hospitalization at VUMC during the course of the study, as well as to ensure positive COVID-19 testing. All data will be stored on encrypted, HIPPA compliant VUMC Box accounts for management and analysis. Data will not be exported in an unencrypted format. All KSP for the study have been trained in data safety and

data management. There will be no protected health information used for analysis or publication.

12.0 Follow-up and Record Retention

The estimated study duration is 12 months including data retrieval and data analysis. Following completion of the study, the data stored within the encrypted VUMC Box files will be deleted.