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BRIEF REPORT

Tracheal Aspirate as an Alternative Biologic Sample for Pharmacogenomics Testing in Mechanically Ventilated Pediatric Patients

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Patients in the pediatric intensive care unit are exposed to multiple medications and are at high risk for adverse drug reactions. Pharmacogenomic (PGx) testing could help decrease their risk of adverse reactions. Although whole blood is preferred for PGx testing, blood volume in this population is often limited. However, for patients on mechanical ventilation, tracheal secretions are abundant, frequently suctioned, and discarded. Thus, the aim of this pilot study was to determine if tracheal aspirates could be used as a source of human genomic DNA for PGx testing. We successfully extracted DNA from tracheal secretions of all 23 patients in the study. The samples were successfully genotyped for 10 clinically actionable single nucleotide variants across 3 cytochrome P450 genes (*CYP2D6*, *CYP2C19*, and *CYP3A5*). Using DNA from whole blood samples in 11 of the patients, we confirmed the accuracy of the genotyping with 100% concordance. Therefore, our results support the use of tracheal aspirates from mechanically ventilated children as an adequate biospecimen for clinical genetic testing.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The current preferred biologic samples for pharmacogenomics testing are whole blood, salvia, or buccal swab. In pediatric patients, blood sampling is conserved and in mechanically ventilated patients, tracheal secretions are abundant and discarded.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Are tracheal aspirates a viable source of human genomic DNA for pharmacogenomic testing?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Genomic DNA was successfully extracted from tracheal secretions and were successfully genotyped for ten clinically actionable single nucleotide variants across three cytochrome P450 genes with 100% concordance with blood samples.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Tracheal aspirates from mechanically ventilated children as an adequate biospecimen for clinical genetic testing.

Children admitted to the pediatric intensive care unit (PICU) receive an average of 10 different medications per day and receive an average cumulative 20 medications during their stay.[1] Many of these have clinical guidelines for using pharmacogenomic (PGx) testing to guide the drug choice and/or dose, including voriconazole, selective serotonin reuptake inhibitors, tricyclic antidepressants, and codeine.[2–5] The US Food and Drug Administration (FDA) has placed genetic testing recommendations and black box warnings on over 100 drug labels, including guidelines on gene-drug pairs. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published guidelines for 35 medications with plans for writing over 100 more.[6] These initiatives are indicative of the importance of PGx to prescribing practices. The use of PGx has not been routinely utilized in the PICU, but as more evidence and guidelines for PGx-guided dosing emerges, this could play an important role in the safe delivery of medications to patients in the PICU.

The preferred biospecimen for PGx testing is whole blood but saliva and buccal swabs have also been validated and used as an alternative source of DNA.[7–11] However, in small patients, blood volumes can be limited and mechanical ventilation or sedation can impair the collection of saliva or buccal swabs. In pediatrics, most current blood sampling guidelines are not evidence based; so, it is generally

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recommended to use alternatives to blood to ensure patient safety and maximize clinical benefit.[12,13] In addition, blood samples cannot be used for genotyping from children that have had stem cell transplants or recent blood transfusions. Therefore, there is a need to identify an alternative source of DNA that can be used for validating biomarkers in clinical trials and conducting clinical PGx testing.

Many patients admitted to the PICU are mechanically ventilated. When they are vented, the patient's respiratory secretions are frequently suctioned and discarded. As this biological sample is plentiful, easily accessible, and collection is noninvasive, we hypothesized that tracheal aspirates from mechanically ventilated patients could be used as a source of DNA for clinical genotyping. Although one might assume that this would work, there could be too little DNA or it could be to degraded to use for genotyping; so, the feasibility needs to be supported by laboratory data. Thus, the aim of this study was to determine if tracheal aspirates from mechanically ventilated pediatric patients can be used as a source of DNA for PGx testing.

METHODS

This study was submitted for institutional review board approval and was granted an exempt approval for patient data as all sample collections were from medical waste. Mechanically ventilated patients were randomly selected from the PICU and tracheal aspirates were collected from any patient that required suctioning as part of his or her routine clinical care. Samples were collected by attaching a sterile specimen trap to the ventilator circuit to obtain tracheal secretions during routine suctioning. Patients with very thick secretions required administration of normal saline for the respiratory therapist to successfully suction these secretions. Once the sample trap contained at least 1 mL secretions, the respiratory therapist removed the trap from the circuit and the sample was transferred into an Oragene DNA kit (DNA Genotek, Ottawa, Ontario, Canada). Upon collection, the viscosity of the sample and timing of collection with relationship to previous suctioning was noted. Sample viscosity was determined by subjective visualization when transferring the samples into Oragene containers and categorized as, thin, medium thick, thick, and very thick. When available, residual whole blood samples after clinical testing were obtained and used as patient-specific controls.

Due to the thick viscosity of the tracheal samples, all samples were incubated in the Oragene solution at 56°C for a minimum of 24 hours (range 1–10 days) prior to DNA extraction. Extraction from tracheal aspirates and whole blood was performed using the Qiagen EZ1 automated instrument. Following DNA extraction and quantification, 10 ng of sample per assay were used to genotype for 10 clinically actionable single nucleotide variants in 3 cytochrome P450 genes using custom designed TaqMan Assays and TaqMan Universal PCR Master Mix, without UNG on the QuantStudio 12K Flex platform (ThermoFisher Scientific, Waltham, MA) according to manufacturer's instructions. Patient genotype was determined using TaqMan Genotyper software version 1.5.0 (ThermoFisher Scientific, Waltham, MA). There were not enough samples to conduct a meaningful Hardy–Weinberg analysis on the results.

Due to small sample size and non-normal distribution, nonparametric tests were used to determine significant correlations between DNA and trachea samples using SAS 9.4 (SAS Institute, Cary, NC) with a significance of $P = 0.05$. Significant correlations between the concentration of DNA extracted from trachea samples and sample viscosity, patient airway, and patient age were determined in separate analyses. The Kruskal–Wallis test was used to determine correlations between concentration and sample viscosity (thin, medium, thick, and very thick), and concentration and patient airway (tracheostomy, endotracheal tube, and nasotracheal tube). Spearman correlation was used to determine correlation between concentration and patient age.

RESULTS

Tracheal aspirates were collected from 23 patients in the PICU of which, 69.6% were boys, 39.1% were African American, 47.8% white, 0.04% Hispanic or Latino, and 0.09% did not report race. The mean age was 31.65 ± 54.08 months (range 3 months to 17 years). Patient demographics and sample information are reported in **Table 1**. Blood samples were available for 11 of these patients and served as patient-specific controls. DNA was successfully isolated from all tracheal aspirates and blood samples, irrespective of aspirate volume and viscosity. The DNA yield from the tracheal aspirates and blood samples were 73.5 ± 77.2 ng/μL and 121.9 ± 31.3 ng/μL, respectively. The genotyping results from the 11 paired trachea and blood samples showed 100% concordance on all 10 variants (i.e., 110 data points). Patient genotyping results are shown in **Table 2**.

As expected, we observed differences in DNA concentrations extracted from tracheal aspirates based on the viscosity of the sample (thin, medium, medium thick, and thick); this was presumably due to the increasing cellularity or free DNA of samples causing the increased viscosity. The Kruskal–Wallis test showed there was a significant difference in DNA concentration between the different trachea sample viscosities ($P = 0.01$) with a mean concentration of 8.4 ng/μL for samples with a thin viscosity and 9.00, 18.1, and 18.8 ng/μL for medium, medium thick, and thick viscosities, respectively. DNA concentrations significantly differed between samples with thin viscosities compared with those with thick viscosities ($P = 0.035$ using Tukey adjustment for multiple comparisons), indicating patients producing thicker tracheal secretions yield higher DNA concentrations following extraction. There was a direct correlation with sample viscosity and DNA concentration, where the visually observed viscosity increased with an increase in DNA concentration ($P = 0.01$). Significant correlations were not found between concentration and airway type or patient age. Removing the patient with a nasotracheal tube airway did not improve significance between concentration and patient airway.

DISCUSSION

To our knowledge, this is the first report to show that human genomic DNA suitable for genotyping assays can

Table 1 Patient demographics and sample information

Patient	Age	Sex	Race	Indication for mechanical ventilation	Airway	Physical characterization of sample viscosity	DNA concentration, ng/uL	Trach aspirate and blood with matching PGx
1	9 months	Male	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Thin, clear[a]	24.4	No blood
2	17 years	Female	White (AA father)	Chronic respiratory failure	Tracheostomy	Thick, vicious, opaque	46.4	No blood
3	6 months	Male	White	Chronic respiratory failure due to prematurity	Tracheostomy	Thin, clear[a]	14.3	No blood
4	10 months	Male	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Thin, clear[a]	30.8	No blood
5	8 months	Female	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Thin, clear[a]	47.5	No blood
6	11 years	Male	White	Irretractable seizures	Endotracheal tube	Very thick, viscous	99.7 95.8[b]	Yes
7	23 months	Male	White	Subdural hematoma, multiple strokes	Endotracheal tube	Very thick, viscous	56.8 203.8[b]	Yes
8	6 years	Male	African American	Septic shock – respiratory distress	Endotracheal tube	Very thick, viscous	128.3 106.6[b]	Yes
9	7 months	Male	White	Chronic respiratory failure due to prematurity	Tracheostomy	Thin, clear[a]	41.4 108.4[b]	Yes
10	2 years	Male	African American	Acute hypoxic respiratory failure	Nasotracheal tube	Thin	45.9	No blood
11	15 months	Female	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Thin	89.6	No blood
12	4 months	Male	White	Acute respiratory failure	Endotracheal tube	Medium thick with chunks	63.8 100[b]	Yes
13	6 months	Male	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Thin	12.7 120.7[b]	Yes
14	4 months	Female	White	Chronic respiratory failure due to prematurity	Tracheostomy	Thick	154.6 123.1[b]	Yes
15	7 months	Male	Not reported	Chronic respiratory failure due to prematurity	Tracheostomy	Very thick	260.1 152.6[b]	Yes
16	4 months	Male	White	Chronic respiratory failure due to prematurity	Tracheostomy	Thin	15.0	No blood
17	12 years	Female	White	Pneumonia	Endotracheal	Medium thick	16.6 108.2[b]	Yes
18	22 months	Male	White	Obstructive hydrocephalus from newly diagnosed disseminated atypical teratoid rhabdoid tumor	Endotracheal	Medium thick	61.0 103.6[b]	Yes
19	5 months	Female	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Medium thick	35.1 118.7[b]	Yes
20	7 months	Male	Not reported	Chronic respiratory failure due to prematurity	Tracheostomy	Thick	72.4	No blood
21	6 months	Male	Hispanic or Latino	Chronic respiratory failure due to prematurity	Tracheostomy	Thin	46.4	No blood
22	3 months	Male	White	Chronic respiratory failure due to prematurity	Tracheostomy	Medium thick	11.0	No blood
23	6 months	Female	African American	Chronic respiratory failure due to prematurity	Tracheostomy	Thick	317.6	No blood

PGx, pharmacogenomic.
[a]Diluted with normal saline to suction.
[b]Blood sample.

Table 2 Patient genotype of pharmacological single nucleotide variants

Patient	rs4244285, CYP2C19*2	rs776746, CYP3A5*3	rs1135840, CYP2D6*2	rs35742686, CYP2D6*3	rs3892097, CYP2D6*4	rs5030655, CYP2D6*6	rs1065852, CYP2D6*10	rs28371706, CYP2D6*17	rs59421388, CYP2D6*29	rs28371725, CYP2D6*41
1	GA	TT	GG	TT	CC	AA	AA	AA	CC	CC
2	GA	CC	CC	TT	CC	AA	AA	GG	CC	CC
3	GG	TC	CC	TT	CC	AA	AA	GG	CC	CC
4	GA	TC	GG	TT	CC	AA	AA	AA	CC	CC
5	GA	TC	GC	TT	CC	AA	AA	GG	CC	CC
6	GG	CC	GC	TT	CC	AA	AA	GG	CC	CC
7	GG	CC	GG	TT	TT	AA	GG	GG	CC	CC
8	GG	CC	GG	TT	TC	AA	AG	GG	CC	CC
9	GA	CC	GC	TT	CC	AA	AA	GG	CC	CC
10	GA	CT	CC	TT	CC	AA	AG	GG	CC	CC
11	GG	TC	GG	TT	CC	AA	AA	AA	CC	CC
12	GG	CC	GC	TT	TC	AA	AG	GG	CC	CC
13	GA	TT	GG	TT	TC	AA	AG	GA	CC	CC
14	GA	CC	GG	TT	CC	AA	AA	GG	CC	TT
15	GA	CC	GG	TT	TC	AA	GG	GG	CC	CC
16	GA	CC	CC	TT	CC	AA	AA	GG	CC	CC
17	GG	CC	GC	TT	CC	AA	AA	GG	CC	CC
18	GG	CC	GC	TT	CC	AA	AA	GG	CC	CT
19	GG	CT	GG	TT	CC	AA	AA	GA	TC	CC
20	GG	CT	GC	TT	CC	AA	AG	GG	CC	CC
21	GG	CC	GC	TT	CC	AA	AA	GG	CC	CC
22	GA	CC	GG	TT	CC	AA	AA	GG	CC	TT
23	GG	CT	GC	TT	TC	AA	AG	GG	CC	CC

be successfully isolated from tracheal secretions. Although this is a small pilot feasibility study, our 100% success rate and concordance with blood controls suggest that tracheal secretions can serve as a suitable alternative to blood for genotyping in critically ill children. Advantages of sampling from trachea secretions include abundance, convenience, and conservation of blood. Additionally, we did not find that trachea volume or viscosity affected the usefulness of this sample as all samples yielded usable DNA. Use of tracheal secretions alleviates the need for blood sampling for both research and clinical purposes, reducing the risk of collecting more than current guideline recommendations of 1–5% total blood volume in 24 hours.[12] This is particularly important for pre-terms, neonates, and infants with very small blood volumes, those at risk of anemia, patients requiring high frequency of clinically necessary blood sampling, patients without a central line, and very small infants who have received multiple blood transfusions. Reducing blood sampling in patients in the PICU decreases daily blood loss through phlebotomy, likelihood of requiring transfusion, transmission of infection, and puncture burden. These risks highlight the benefit of using tracheal secretions for genotyping in the PICU.

The utility of this alternative biological sample is dependent on the patient requiring mechanical ventilation and having tracheal secretions available for collection. Although this is not available in all patients in the PICU, ~ 30–51% of patients admitted in the PICU do require mechanical ventilation.[14–16] These patients will often have frequent clinical laboratory monitoring, therefore, putting them at an increased risk of critical illness associated anemia and requiring blood transfusions.[17] That makes this alternative biologic sampling even more appealing in this population.

Limitations of our study include not being able to collect blood controls for all patients and the limited number of variants that we tested. However, the cytochrome P450 genes are notoriously challenging to accurately genotype due to multiple family members and pseudogenes with high homology. In addition, they are variants that are commonly used for clinical pharmacogenetic testing, so they represent clinically important variants. Thus, the genes that we chose were good candidates for rigorously testing the use of the tracheal aspirates for genetic testing. We would also like to acknowledge the potential for interaction of bacterial genomic material in our DNA samples, despite successful and concordant genotyping of our samples.

In summary, we have shown that tracheal aspirates are an acceptable biological alternative to blood in pediatric patients in this pilot study. In validating this biologic sample, we have demonstrated the feasibility of using these samples for conducting pharmacogenetics research and clinical testing with high accuracy. Although this study focused on pediatric patients, we expect that tracheal aspirates can be collected from mechanically ventilated patients of any age and could be a suitable alternative to blood and especially useful for patients where blood volume sparing is desired.

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1. Dai, D., Feinstein, J.A., Morrison, W., Zuppa, A.F. & Feudtner, C. Epidemiology of polypharmacy and potential drug-drug interactions among pediatric patients in intensive care units of U.S. Children's Hospitals. *Pediatr. Crit. Care Med.* **17**, e218–e228 (2017).
2. Hicks, J.K. *et al.* Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. *Clin. Pharmacol. Ther.* **102**, 37–44 (2017).
3. Crews, K.R. *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450 2D6 genotype and codeine therapy: 2014 update. *Clin. Pharmacol. Ther.* **95**, 376–382 (2014).
4. Moriyama, B. *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C19 and voriconazole therapy. *Clin. Pharmacol. Ther.* **102**, 45–51 (2017).
5. Hicks, J.K. *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. *Clin. Pharmacol. Ther.* **98**, 127–134 (2015).
6. Clinical Pharmacogenomics Implementation Consortium (CPIC). [Internet] <https://cpicpgx.org/>. Accessed May 2, 2020.
7. Ang, J.S. *et al.* Evaluation of buccal swabs for pharmacogenetics. *BMC Res. Notes* **11**, 382 (2018).
8. Woo, J.G. *et al.* Quality assessment of buccal versus blood genomic DNA using the Affymetrix 500 K GeneChip. *BMC Genet.* **8**, 79 (2007).
9. Hu, Y. *et al.* Genotyping performance between saliva and blood-derived genomic DNAs on the DMET array: a comparison. *PLoS One* **7**, e33968 (2012).
10. Trost, B. *et al.* Impact of DNA source on genetic variant detection from human whole-genome sequencing data. *J. Med. Genet.* **56**, 809–817 (2019).
11. Yao, R.A., Akinrinade, O., Chaix, M. & Mital, S. Quality of whole genome sequencing from blood versus saliva derived DNA in cardiac patients. *BMC Med. Genomics* **13**, 11 (2020).
12. Howie, S.R. Blood sample volumes in child health research: review of safe limits. *Bull. World Health Organ.* **89**, 46–53 (2011).
13. Veal, G.J. Blood volumes in pediatric clinical trials: a review of current regulations and guidance for research studies. *Clin. Invest.* **4**, 1005–1011 (2014).
14. Balcells Ramírez, J., López-Herce Cid, J. &, Modesto Alapont, V. Prevalence of mechanical ventilation in pediatric intensive care units in Spain. *Ann. Pediat.* **61**, 533–541 (2004).
15. Khemani, R.G., Markovitz, B.P. & Curley, M.A.Q. Characteristics of children intubated and mechanically ventilated in 16 PICUs. *Chest* **136**, 765–771 (2009).
16. Mukhtar, B., Siddiqui, N.R. & Haque, A. Clinical characteristics and immediate-outcome of children mechanically ventilated in PICU of Pakistan. *Pak. J. Med. Sci.* **30**, 927–930 (2014).
17. Bateman, S.T. *et al.* Anemia, blood loss, and blood transfusions in North American children in the intensive care unit. *Am. J. Respir. Crit. Care Med.* **178**, 26–33 (2008).