Volatile Biomarkers of Influenza Infection in the Breath

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Background

While the severity and duration of influenza can be reduced by early detection and treatment, laboratory testing for influenza has historically been of questionable value due to limited test sensitivity, lengthy turnaround times, and the need to develop new assays due to emergence of novel strains. For example, available point of care viral antigen detection assays performed very poorly during the influenza A(H1N1)pdm09 pandemic of 2009-10, with sensitivities in the 12-66% range. Assay of exhaled breath for volatile organic compounds (VOCs) via gas chromatography-mass spectroscopy (GC-MS) is an emerging diagnostic modality with characteristics ideally suited to fill the gap in influenza diagnostics. VOCs are produced systemically in humans as a result of varied physiological states such as oxidative stress and are excreted through the lungs. Available benchtop and clinical data suggest that breath testing may be a useful diagnostic modality for influenza infection.[1-3]

Methods

Patients with influenza like illness (ILI) presenting to the Troop Medical Clinic (TMC) on JBSA Fort Sam Houston, TX, between March 2017 and March 2019, were asked to submit a single 2-minute breath sample in addition to the usual nasopharyngeal swab collected for polymerase chain reaction (PCR) assay for influenza virus at the time of enrollment in the ongoing Infectious Disease Clinical Research Program (IDCRP) Acute Respiratory Infection Consortium (ARIC) natural history study. ILI was defined as temperature > 100.4^{0}F AND respiratory symptoms like cough, sputum production, chest pain and/or sore throat.

We assayed breath VOCs with GC-MS and identified breath VOC biomarkers that discriminated between ILI patients with and without an influenza positive PCR assay (the gold-standard test for influenza infection) with greater than random accuracy. Multiple Monte Carlo simulations were performed, and 20 chromatogram segments exhibited greater than random diagnostic accuracy, thereby fulfilling the requirements of true biomarkers.

These candidate VOC biomarkers of influenza infection were entered into a multivariate predictive algorithm using multivariate weighted digital analysis (WDA) to determine the sensitivity and specificity of the breath test and displayed in a receiver operating characteristic (ROC) curve. Test accuracy was determined from the area under curve of the ROC curve. Candidate VOCs were tentatively identified by matching their mass spectral signatures to a library of mass spectra (NIST 2.0, Gaithersburg, MD 20899-1070).

Results

Demographic, clinical, PCR and breath data were available for 237 episodes of ILI among 235 unique patients (2 patients presented twice with ILI during the study period). PCR was positive for influenza for 32 episodes of ILI (30 influenza A and 2 influenza B) and negative for 205. The median age of participants was 21 (IQR 19, 23) and 69% were male. There were no differences in age, gender, education level, race, smoking status, or military affiliation (branch of service), between the influenza positive and negative groups (Table). Likewise, there was no difference in days of limited activity, days of missed work, or symptoms at presentation, between the groups.

The algorithm achieved near maximal predictive accuracy of 78% with four biomarkers. (74% sensitivity and 70% specificity) (Figures 1 and 2). Based on their mass spectra these biomarker VOCs were tentatively identified as 2-amino-1-propanol, 2-butanamine, n-nitro, 3-methyl-hexanal, and heptane. These VOCs are consistent with products of oxidative stress, comprising either straight-chain n-alkane hydrocarbons (e.g. heptane) or their methylated derivatives (e.g. 3-methyl-hexanal).

Table. Characteristics of participants with and without influenza

	Influenza (N=32)	No influenza (N=203)	p value
Median age (IQR)	21 (19, 23.5)	21 (19, 23)	**0.95***
Gender			**0.45†**
Male	24 (75%)	138 (68%)	
Female	8 (25%)	64 (31.5%)	
Missing	0 (0%)	1 (0.5%)	
Race			**0.7‡**
Black	2 (6.2%)	28 (13.8%)	
Hispanic	6 (18.8%)	40 (19.7%)	
Unknown/Other	4 (12.5%)	21 (10.3%)	
White	20 (62.5%)	114 (56.2%)	
Military affiliation			**0.59‡**
Army	23 (71.9%)	126 (62.1%)	
Navy	6 (18.8%)	56 (27.6%)	
Air Force	3 (9.4%)	21 (10.3%)	
Smoking status			**0.2‡**
Current	2 (6.2%)	4 (2%)	
Former	4 (12.5%)	19 (9.4%)	
Never	26 (81.2%)	180 (88.7%)	
Education level			**0.4‡**
Associate's degree	7 (21.9%)	33 (16.3%)	
Bachelor's degree	4 (12.5%)	13 (6.4%)	
High school	21 (65.6%)	152 (74.9%)	
Higher degree	0 (0%)	5 (2.5%)	

*Kruskal-Wallis test. †Chi squared test. ‡Fisher's exact test.

Results (continued)

Figure 1. Accuracy, sensitivity, and specificity of the breath test.

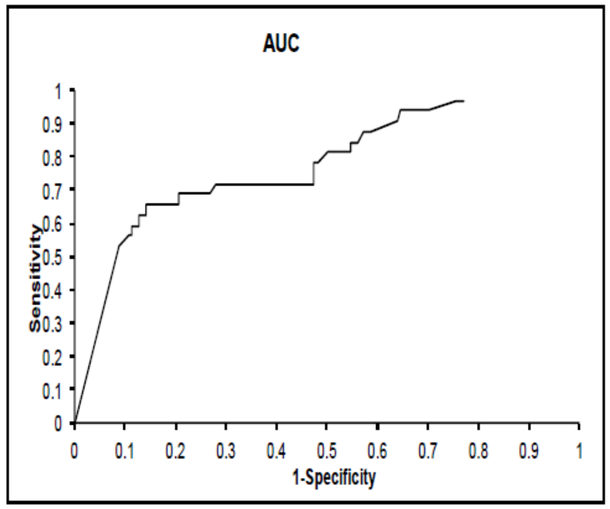
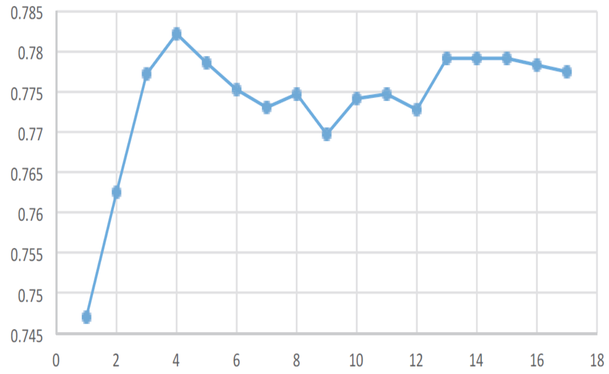


Figure 2. Effect of number of VOCs in algorithm (x-axis) on predictive accuracy (y-axis). It is apparent that the algorithm achieves its near maximal predictive accuracy (~78%) with only four VOCs (chromatogram segments) in the model, and then reaches a plateau. Addition of more VOCs to the predictive algorithm did not significantly improve its performance.



Conclusions

Our findings bolster available benchtop and clinical data suggesting that breath testing may be a useful diagnostic modality for influenza infection.[1-3] The next step will be to study the predictive algorithm developed in this protocol in a blinded validation cohort. If the predictive algorithm performs well in a validation study, adaptation for its use in a portable, tabletop GC would be warranted to allow for a rapid, accurate, universal point-of-care influenza diagnostic test which would be especially useful in the early stages of a pandemic due to a novel influenza virus.

References

1. Aksenov AA. Cellular scent of influenza virus infection. Chembiochem 2014;15(7):1040-8. doi: 1.1002/cbic.201300695.
2. Phillips M. Effect of influenza vaccination on oxidative stress products in breath. J Breath Res 2010;4:026001. doi: 10.1088/1752-7155/4/2/026001.
3. Mashir A. Effect of the influenza A (H1N1) live attenuated intranasal vaccine on nitric oxide (FENO) and other volatiles in exhaled breath. J Breath Res 2011;5:037107. doi: 10.1088/1752-7155/5/3/037107.

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The investigators have adhered to the policies for protection of human subjects as prescribed in 45CFR46.

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