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# Noninvasive Diagnosis of Pancreatic Cancer Through Detection of Volatile Organic Compounds in Urine

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This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e16. Learning Objective: Upon completion of this CME activity, successful learners will be able to revise the importance of early diagnosis of pancreatic adenocarcinoma and the role of biomarkers, including demonstrate understanding of volatile organic compounds. In so doing learners will be able to recognize its utility in cancer diagnostics.

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With its incidence approaching mortality, and with >300,000 new cases diagnosed worldwide in 2013, pancreatic ductal adenocarcinoma (PDAC) is currently the fourth leading cause of cancer-related death and predicted to become the second by 2030.[1] More than 80% of patients with PDAC are diagnosed late,[2] with locally invasive and/or metastatic disease, resulting in negligible 5-year survival. Thus, the quest for a simple, inexpensive, and noninvasive test to detect PDAC early, while it is still amenable to surgical resection, continues. Detection of volatile organic compounds (VOCs) has come to the fore offering a novel approach for the detection of disease. It uses the odors that emanate from urine, breath, and feces and is akin to canine "sniffing." These compounds are metabolic products and/or consequence of bacterial dysbiosis produced by the disease state.[3,4] We thus postulate that either altered cellular physiology or even alteration in the microbial milieu in patients with PDAC will alter the individual's metabolome profile, such that the resultant VOC patterns that are emitted provide a characteristic signature that can be detected.

## Description of Technology

Urinary VOC was analyzed using an Owlstone (Lonestar, Cambridge, UK) instrument based on ion mobility spectrometry (IMS). Patient information is summarized in Table 1. Midstream urine samples were collected and frozen at −80°C within 4 hours of collection for long-term storage. Before analysis, each sample was defrosted and heated to 40°C for 10 minutes. The air above the sample (known as the headspace) was then passed into the Lonestar unit and the ion mobility measured. Each sample typically takes 5 minutes to analyze (60 seconds per sample to scan, with 5 repeats). Once all the samples were analyzed, an existing data processing pipeline with 4 different classifiers was applied (sparse logistic regression, random forest, gaussian process classifier, and support vector machine). Age was not deemed to be a confounder based on our previous published work (see the Supplementary Material). PDAC urine samples were detected with a sensitivity of 0.91 (95% confidence

**Table 1.** Demographic Information

| | Healthy Controls (n = 81) | Pancreatic Cancer (n = 81) |
|---|---|---|
| Age (mean ± SD) | 51.4 ± 10.6 | 64.3 ± 23.7 |
| Male sex (%) | 30.9 | 53.1 |
| Stage (n)* | | |
| I | N/A | 4 |
| IIA | N/A | 5 |
| IIB | N/A | 35 |
| III | N/A | 24 |
| IV | N/A | 12 |

N/A, not applicable; SD, standard deviation.
*One case could not be assessed.

***Authors share co-senior authorship.**

***Abbreviations used in this paper:*** **AUC, area under the curve; CI, confidence interval; PDAC, pancreatic ductal adenocarcinoma; VOC, volatile organic compound.**

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interval [CI], 0.83-0.96) and specificity of 0.83 (95% CI, 0.73-0.90), with an area under the curve (AUC) of 0.92 (95% CI, 0.88-0.96), using a support vector machine algorithm (Figure 1*C*). The results were validated by random data splitting into the training and test set achieving similar results with AUC of 0.92 (95% CI, 0.85-0.98), and sensitivity and specificity of 0.90 (95% CI, 0.74-0.98) and 0.81 (95% CI, 0.63-0.93), respectively. We also compared early stage disease (I/II) with healthy individuals as well as early stage I/II with late stage disease (III/IV) using the same analysis pipeline. These analyses revealed a sensitivity of 0.91 (95% CI, 0.78-0.97) and specificity of 0.78 (95% CI, 0.69-0.86), with an AUC of 0.89 (95% CI, 0.83-0.94), a sensitivity of 0.82 (95% CI, 0.67-0.92), and a specificity of 0.89 (95% CI, 0.75-0.97) with an AUC of 0.92 (95% CI, 0.86-0.97), respectively, again using a support vector machine algorithm (Figure 1*D*, *E*).

## Video Description

The Lonestar is a special type of IMS, based on a method called field asymmetric ion mobility spectroscopy. It has the advantage of being reproducible and far more sensitive than electronic noses, but without the limitations of mass spectrometry, which requires specialized and expensive infrastructure. Field asymmetric ion mobility spectroscopy is based on measuring the way that ionized molecules move in very high electric fields and, owing to the differences in this movement, the instrument is able to separate complex mixtures of chemicals that are found in biological samples. To improve separation, the magnitude of the electric field is raised through a series of values to create a 3-dimensional map of a urine sample. These samples are then analyzed using our in-house data processing pipeline, as described previously.[5] In brief, first a data compression wavelet and threshold is applied to reduce the size of the dataset

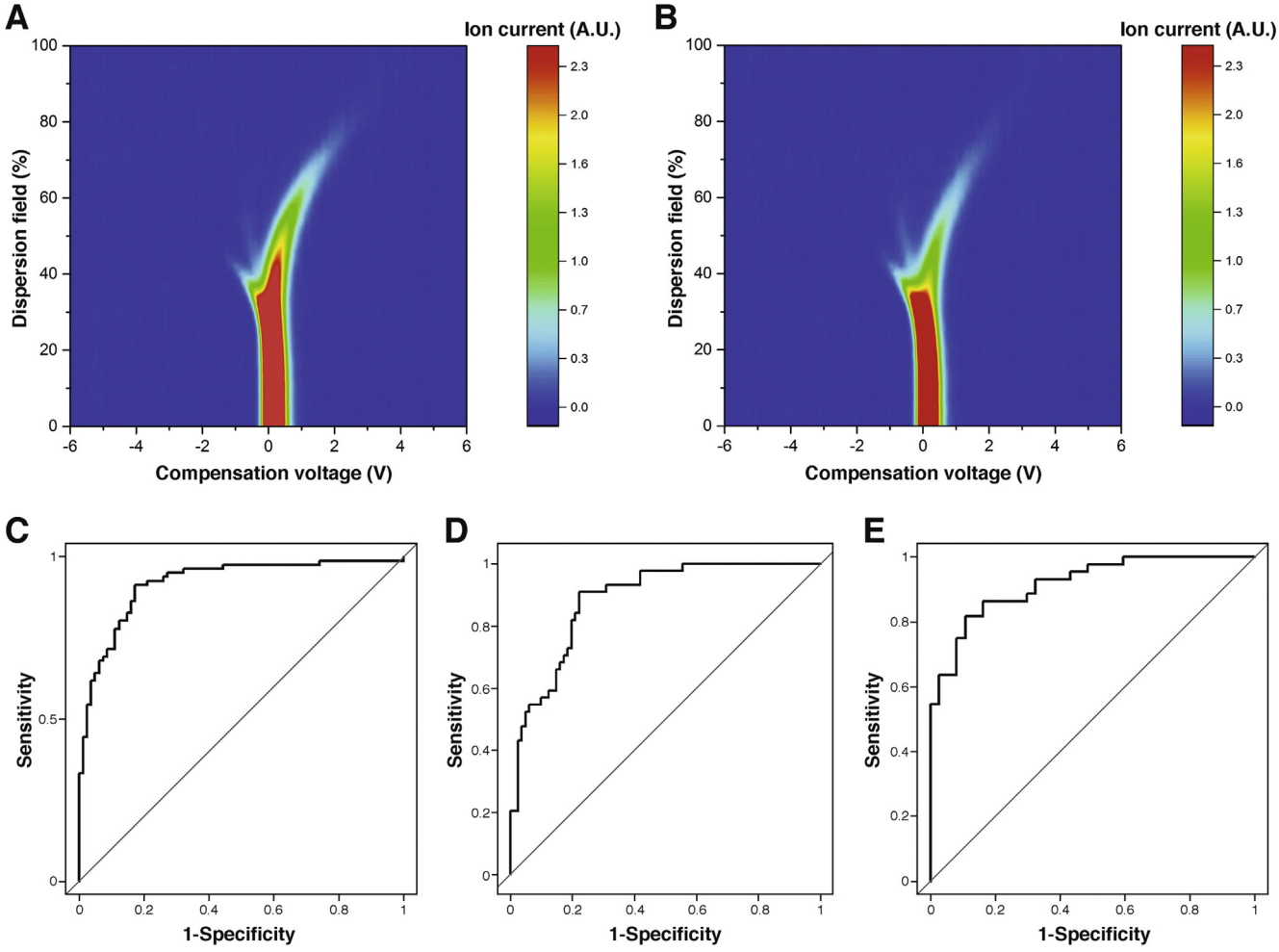


**Figure 1.** *A*, Representative image of field asymmetric ion mobility spectroscopy output showing the plume of ion dispersion from a single urine sample of a healthy control. A.U., arbitrary units.[5] *B,* A similar plume of ion dispersion from a single urine sample of a patient with PDAC. *C,* Receiver operating characteristic curve showing performance of volatile organic compounds in differentiating healthy individuals from PDAC with an AUC of 0.92 (95% CI, 0.88-0.96), sensitivity of 0.91 (95% CI, 0.83-0.96), and specificity of 0.83 (95% CI, 0.73-0.90). *D*, Healthy individuals from early stage I/II with an AUC of 0.89 (95% CI, 0.83-0.94), a sensitivity of 0.91 (95% CI, 0.78-0.97), and a specificity of 0.78 (95% CI, 0.69-0.86). *E*, Early stage PDAC (I/II) could also be successfully separated from advanced stage PDAC (III/IV) with an AUC of 0.92 (95% CI, 0.86-0.97), sensitivity of 0.82 (95% CI, 0.67-0.92), and specificity of 0.89 (95% CI, 0.75-0.97).

(which in our case is >50,000 data points per sample). Once completed, important features are sought within the compressed dataset, using a Wilcoxon rank-sum test, with a 10-fold cross-validation step. Such identified features can then be used with different classification algorithms to calculate clinically relevant values, such as the AUC, sensitivity, and specificity of unknown samples.

## Take Home Message

We have shown previously that urine is a useful biofluid for the detection of early stage, resectable pancreatic cancer.[6,7] In this proof-of-concept study, we demonstrate for the first time the usefulness of urine specimens to discriminate healthy individuals from patients with PDAC through the detection of VOCs. Moreover, we have shown the ability to separate healthy from early stage and early stage versus advanced disease. This distinction was achieved using only a 5-mL urine sample and applying novel IMS technology, which offers a rapid and more cost-effective analysis compared with other gas analysis technologies. These results demonstrate that urine analysis using IMS platform shows promise as an additional noninvasive approach for identification of patients with pancreatic cancer.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2017.09.054.

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**Reprint requests**
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**Conflicts of interest**
The authors disclose no conflicts.

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## Supplementary Material

The instrument used in this study was a commercial field asymmetric ion mobility spectroscopy instrument (Lonestar, Owlstone, Cambridge, UK), which is fitted with an ATLAS (Owlstone) sampling system. It is highly sensitive (detecting ions up to parts per billion) and based on distinguishing different ions based on its physical properties, specifically its mobility; it does not identify per se the specific volatile organic compounds.

The samples were frozen at –80°C within 4 hours of collection and 5-mL aliquots were transported on dry ice from Barts Cancer Institute to the School of Engineering at the University of Warwick. The samples were thawed overnight at 4°C in a laboratory fridge. The vial with the urine was placed in the ATLAS sampling system for 10 minutes at 40°C to maximise volatile organic compound chemical release. After 10 minutes, clean dry air is pushed over the surface of the urine and into the Lonestar. The flow rate over the sample was 500 mL/min with an additional 1500 mL/min of makeup air (to make 2 L/min) pushed into the Lonestar. Each sample was tested in triplicate.

In practice, ionized molecules are passed between 2 plates, where an asynchronous electric field is applied. This attracts, repels, or has no effect on these ions. If an ion touches one of the plates it loses its charge, and thus only ions that exit the plates without touching either side are detected. To the asynchronous electric field, an additional fixed "compensation voltage" is added that counteracts the movement. Thus, by scanning through a range of different compensation voltages, we are able to measure a large range of different mobilities.

The machine was set with a dispersion field of 0% to 100% in 51 steps and –6 V to +6 V compensation voltage in 512 steps, with both positive and negative fields applied to the samples. This created a dataset of 52,224 points per sample, with each sample taking <60 seconds to capture.

The field asymmetric ion mobility spectroscopy data were processed using a well-established pipeline, which we have developed over a number of years for this type of study.[1] In brief, we use a preprocessing data compression step in the form of a 2-dimensional wavelet transform (using Daubechies D4 wavelets). This step has the effect of improving and simplifying subsequent analysis steps. A threshold is then applied to remove data with little or no discriminatory power (based on our previous work). This was followed by a 10-fold cross-validation, using 80% of the data as a training set, and the remaining 20% as a test set. Within each fold, important features were identified using a Wilcoxon rank-sum test from the training set. From this, 44 statistically important features were used to predict the result of the test set. Four different classifiers were used for prediction: sparse logistic regression, random forest, gaussian process classifier and support vector machine. Furthermore, the data was split into a training set of 100 samples and a test set of 62 samples. The classifiers were trained on the 100 samples and then applied to the 62 unknown samples to get a better measure of accuracy. Of note, our previous studies have determined that age, diet, smoking, and sex are not confounders and no correction to the analyses was required.[2–4] Equally given the significant discrimination between those with PDAC and healthy controls, the data processing tools are designed such that it would disregard volatile organic compound signals that may be affected by timing of sample collection.

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