Gastroenterology In Motion

Title: Non-invasive Diagnosis of Pancreatic Cancer Through Detection of Volatile Organic Compounds in Urine

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With its incidence approaching mortality, and with over 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 PDAC patients are diagnosed late, with locally invasive and/or metastatic disease, resulting in negligible 5-year survival. Timely detection of PDAC is hampered by several factors: lack of specific clinical symptoms in the early stages, insufficient sensitivity of current imaging modalities and a lack of accurate, clinically utilisable biomarkers (2). Thus the quest for a simple, inexpensive and non-invasive test to detect PDAC early - whilst 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 odours that emanate from urine, breath and faeces 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

For this study, we used a commercial gas analysis instrument (Owlstone Lonestar), based on ion mobility spectrometry (IMS) to analyse the VOCs emanating from patients' urine samples. Patients' information is summarised in **Table 1**. Midstream urine samples were collected and frozen at -80^{o}C within 4 hours of collection for long-term storage. Prior to analysis, each sample was defrosted and heated to 40^{o}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 five minutes to analyse (60 seconds per sample to scan, with five repeats). Once all the samples were analysed, an existing data processing pipeline with four 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 – supplementary material. PDAC urine samples were detected with a sensitivity (SN) of 0.91 (CI: 0.83-0.96) and specificity (SP) of 0.83 (CI: 0.73-0.90), with an area under the curve (AUC) of 0.92 (CI:0.88-0.96), using a support vector machine algorithm – **Figure 1c**. The results were validated by random data splitting into the training and test set: the machine algorithm system was trained using the first 100 samples and the results of the remaining 62 test samples were then predicted. This achieved similar results with AUC of 0.92 (CI: 0.85-0.98), and SN and SP of 0.90 (CI: 0.74-0.98) and 0.81 (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 revealed a sensitivity (SN) of 0.91 (CI: 0.78-0.97) and specificity (SP) of 0.78 (CI: 0.69-0.86), with an area under the curve (AUC) of 0.89 (CI: 0.83-0.94), and SN of 0.82 (CI: 0.67-0.92), SP of 0.89 (CI: 0.75-0.97) with AUC of 0.92 (CI: 0.86-0.97), respectively, again using a support vector machine algorithm (**Figures 1d** and **e**).

Video Description

The Lonestar is a special type of Ion Mobility Spectrometer (IMS), based on a method called FAIMS, (Field Asymmetric Ion Mobility Spectroscopy). Developed and used extensively by the security services, it has recently been employed for the detection of VOCs in medicine. It has the advantage of being more reproducible and far more sensitive than electronic noses, whilst at the same time, it does not have the limitations of mass spectrometry, which requires specialised and expensive infrastructure. FAIMS is based on measuring the way that ionised molecules move in very high electric fields and due to the differences in this movement, the instrument is able to separate complex mixtures of chemicals that are found in biological samples. It practice, this is achieved by passing ionised molecules between two plates, where an asynchronous electric field is applied. This attracts, repels or has no effect of 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. To improve separation, the magnitude of the electric field is raised through a series of values to create a 3D map of a urine sample. These are then analysed using our in house data processing pipeline, previously described when analysing urine samples with different disease groups (5). In brief, first a data compression wavelet and threshold is applied to reduce the size of the dataset (which in our case is more than 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 e.g. AUC, SN and SP of unknown samples.

Take Home Message

We have already showed that urine is a useful bio-fluid for detection of early stage, resectable pancreatic cancer (6,7). In this proof of concept study, we demonstrate for the first time, utility of urine specimens to discriminate healthy individuals from PDAC patients also through detection of volatile organic compounds. Moreover, we have also shown the ability to separate healthy from early stage and early stage vs advanced disease. This was achieved using only 5 ml of urine sample and applying novel IMS technology, which offers a rapid, and more cost effective analysis compared with other gas analysis technologies. The obtained results demonstrate that urine analysis using IMS platform shows promise as an additional non-invasive approach for identification of patients with pancreatic cancer.

Table 1. Demographic information

	Healthy controls (n = 81)	Pancreatic Cancer (n=81)
Age (mean ± SD)	51.4 ± 10.6	64.3 ± 23.7
Sex (male) %	30.9	53.1
Stage*		
I	n/a	4
IIA	n/a	5
IIB	n/a	35
III	n/a	24
IV	n/a	12

*One case could not be assessed

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Figure 1Legend

Figure 1a) Representative image of FAIMS output showing the plume of ion dispersion from a single urine sample of a healthy control (A.U. is arbitrary units). The mobility of ions determines the shape of the plume (5). **Figure 1b)** shows similar plume of ion dispersion from a single urine sample of a patient with PDAC. Note the difference in the shape of the plume as reflected by the different ion mobility shown in red. **Figure 1c)** Receiver Operating Characteristic (ROC) curve showing performance of VOCs in differentiating healthy individuals from pancreatic cancer samples with AUC of 0.92 (CI: 0.88-0.96); SN=0.91 (CI: 0.83-0.96); SP=0.83 (CI: 0.73-0.9); **Figure 1d)** Healthy individuals from early stage I/II (AUC =0.89; CI: 0.83-0.94); SN=0.91 (CI: 0.78-0.97); SP=0.78 (CI: 0.69-0.86). **Figure 1e)** shows early stage PDAC(I/II) could also be successfully separated from advanced stage (III/IV) pancreatic cancer with AUC 0.92 (CI: 0.86-0.97); SN=0.82 (CI: 0.67-0.92); SP=0.89 (CI: 0.75-0.97).