Relationship between exhaled volatile organic compounds and lung function change in idiopathic pulmonary fibrosis

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To cite: Hayton C, Ahmed W, Terrington D, *et al*. *Thorax* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/thorax-2024-222321

ABSTRACT

Volatile organic compounds (VOCs) in exhaled breath have shown promise as biomarkers in idiopathic pulmonary fibrosis (IPF). We analysed breath from 57 people with IPF using thermal desorption–gas chromatography–mass spectrometry to identify VOCs related to lung function change over 12 months. A LASSO regression model selected 63 VOCs associated with relative change in forced vital capacity (8 with correlation coefficient (CC) ≥0.20 on Spearman's rank analysis), and 28 associated with relative change in diffusion capacity of the lung for carbon monoxide % predicted (12 with CC ≥0.20). Secondary analyses demonstrated a correlation between VOCs and baseline lung function parameters and association with survival. This study suggests that there may be a volatile signature of prognosis in IPF that merits further validation.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease associated with morbidity and premature death. It is recognised that IPF displays heterogeneity in disease progression.[1] Currently, few tools exist to aid prognostication and predict individual disease behaviour.

There is growing interest in the use of volatile organic compounds (VOCs) as biomarkers. VOCs are a diverse group of metabolites present in exhaled breath. It has been demonstrated using electronic nose technology that VOCs in breath can distinguish IPF from healthy controls and other interstitial lung diseases with a high level of accuracy.[2] However, this analysis method does not allow individual VOCs to be identified or links to underlying pathophysiology to be explored.

The aim of this study was to explore the relationship between individual VOCs present in exhaled breath and longitudinal changes in lung function in IPF.

METHODS

Study design and participants

Eligible participants were recruited to an observational cohort study (IPF VOC; ISRCTN1806574) at two UK centres. Demographic, clinical and lung function data, including forced vital capacity (FVC), FVC % predicted, diffusion capacity of the lung for carbon monoxide (D_{LCO}) and D_{LCO} % predicted, were collected at baseline and at 3, 6 and 12 months.

Breath sampling

Exhaled breath was collected from participants in clinic using the ReCIVA device (Owlstone Medical, Cambridge, UK) onto two TenaxGR (Markes International, Bridgend, UK) stainless steel sorbent tubes according to a locally derived protocol.[3] The tubes were sealed and transferred to the laboratory where they underwent thermal desorption–gas chromatograph–mass spectrometry (TD-GC-MS). Breath samples were collected at baseline and at each subsequent visit. Further details about participant eligibility, TD-GC-MS and data preprocessing are provided in the online supplemental file 1.

Statistical analysis

Untargeted analysis was performed on baseline breath samples to identify VOCs associated with lung function change. This was assessed in two ways: relative change in FVC % predicted and relative change in D_{LCO} % predicted over 12 months. A least absolute shrinkage and selection operator (LASSO) regression model was used to select VOCs associated with either parameter. Relative change in FVC and DLCO % predicted was estimated using a linear mixed effects model. Correlation of relative concentration of individual VOCs selected was tested using Spearman's rank correlation with adjustment for false discovery using the Benjamini-Hochberg procedure. VOCs with a correlation coefficient ≥0.20 were reported. These VOCs were retained for secondary analyses including correlation with baseline FVC % predicted, DLCO % predicted, University San Diego Shortness of Breath Questionnaire (UCSD-SOBQ) score and Medical Research Council (MRC) dyspnoea score, association with survival and progression-free survival and change in VOC relative concentration in response to antifibrotic treatment. A Cox proportional hazards model was used to test associations between VOC relative concentration with survival and progression-free survival. HRs were adjusted for gender, age and physiology (GAP) stage and antifibrotic use within the model. Progression was defined as a ≥10% relative decline in FVC % predicted, or ≥15% relative decline in D_{LCO} % predicted at 12 months, or death, as previously

Table 1 Demographic and clinical data of cohort

Demographics	n=57
Age (years)	75.1 (±6.5)
Sex male	46 (80.7%)
BMI (kg/m^2)	28.4 (±4.9)
Ex-smoker	43 (75.4%)
Pack years	25.5 (±16.3)
Years since stopping	26.7 (±16.7)
FVC (L)	2.77 (±3.15)
FVC % predicted	79.7 (±18.8)
D_{LCO} (mmol/min/kPa)	3.71 (±1.21)
D_{LCO} % predicted	41.6 (±19.2)
GAP stage Stage 1 Stage 2 Stage 3	15 (26.3%) 28 (49.1%) 14 (24.6%)
MRC 1 MRC 2 MRC 3 MRC 4 MRC 5	3 (5.3%) 24 (42.1%) 18 (31.6%) 7 (12.3%) 5 (8.8%)
UCSD-SOBQ total	40.5 (±27.2)
Supplementary oxygen use at baseline	2 (3.5%)
Antifibrotic use at baseline Pirfenidone Nintedanib None	 3 (5.3% 1 (1.8%) 53 (92.9%)

Data are presented as mean (±SD) or number (percentage of total).
BMI, body mass index; D_{LCO}, diffusion capacity of the lung for carbon monoxide; FVC, forced vital capacity; GAP, gender, age and physiology stage; MRC, Medical Research Council Dyspnoea Score; UCSD-SOBQ, University of California San Diego Shortness of Breath Questionnaire.

described.[4] Further details of the statistical analysis can be found in the online supplemental file 1.

RESULTS

Study cohort

88 patients were recruited with 57 included in final analysis (online supplemental figure S1). Baseline demographics are shown in table 1 with further details provided in online supplemental figures S2 and S3.

Untargeted VOC analysis

A total of 180 VOCs were identified across baseline samples. LASSO regression selected 63 VOCs that were associated with relative change in FVC % predicted and 28 with relative change in D_{LCO} % predicted at 12 months (online supplemental table S1). Table 2 lists VOCs with a correlation coefficient ≥0.2 for each lung function parameter.

Secondary analysis

Significant negative correlation was observed between baseline D_{LCO} % predicted and *m*-cymene (R=−0.34, 95% CI −0.58 to −0.10, p=0.01), 2-chloro-*p*-xylene (R=−0.27, 95% CI −0.51 to −0.03, p=0.03) and 4,6-dimethylundecane (R=−0.26, 95% CI −0.50 to −0.02 p=0.04 (online supplemental table S2). 4,6-dimethylundecane correlated with baseline UCSD-SOBQ (R=0.31, 95% CI 0.07 to 0.55, p=0.01 (online supplemental table S3) and MRC dyspnoea score (R=0.36, 95% CI 0.13 to 0.60, p=0.003 (online supplemental table S4). No significant correlation was noted between VOCs and baseline FVC % predicted or change in symptom scores at 12 months (online supplemental able S5–S9). Increasing concentration of 4,6-dimethylundecane was associated with reduced survival after adjusting for GAP stage and antifibrotic use (HR 4.14, 95% CI 1.24 to 13.74, p=0.02), while increasing concentration of d-limonene was associated with improved survival (HR 0.25, 95% CI 0.08 to 0.80, p=0.02) (figure 1 and online supplemental table S10). No association was observed with progression-free survival (online supplemental table S11). Three VOCs (octanal, 3-methylfuran and *m*-cymene) demonstrated a significant change in relative concentration post-treatment with antifibrotics (online supplemental table S12).

DISCUSSION

We identified individual VOCs in exhaled breath which may be related to lung function change in IPF. Correlations between VOC concentration and lung function change were generally weak (r=0.2–0.5), although a moderate negative correlation (r>0.5) was observed between 2-methyltetrahydrofuran and relative change in D_{LCO} % predicted at 12 months. *m*-cymene, a monoterpenoid, also demonstrated a negative correlation with relative change in D_{LCO} % predicted as well as baseline D_{LCO} % predicted. In addition, treatment with nintedanib led to a reduction in *m*-cymene concentration on repeated sampling. An isomer, *p*-cymene, has been shown to discriminate IPF from healthy controls and correlate with measures of disease severity.[5] We previously observed an increased concentration of monoterpenes in the headspace of lung cells stimulated with the profibrotic cytokine transforming growth factor-β.[6] Many detectable VOCs appear to be exogenous and may indicate ambient air pollution exposure, which is associated with both the incidence and progression of IPF.[7 8] We found increased 4,6-dimethylundecane, a suspected exogenous VOC, correlated with decline in D_{LCO} % predicted, increased symptom scores and was associated with reduced survival. Exposure to air pollution is known to alter the composition of VOCs on exhaled breath,[9] but the exact interplay between the exposome and volatilome remains uncertain.

This study was limited by a small sample size, impacted by a significant batch effect as a consequence of drift in analytical performance over the timeframe of the study. This limited the number of baseline and follow-up breath samples we could use. In addition, in the absence of echocardiography data, we cannot exclude the impact of emerging pulmonary hypertension, which is known to alter VOC composition,[10] on change in D_{LCO} rather than progressive fibrosis.

The results of this study support previous work that VOCs may be altered in lung fibrosis,[2 5] although a targeted validation study is required to confirm this and further work is needed to establish the pathophysiological link between identified VOCs and IPF. Breath analysis using GC-MS offers the opportunity to perform biomarker discovery; however, the utility of this technique in clinical practice is uncertain given analysis is performed offline. Ultimately, online devices developed to target specific VOCs are likely to represent the best solution as clinically useful breath biomarkers in respiratory disease.

Table 2 Volatile organic compounds with a correlation coefficient ≥0.20 between relative concentration and relative change in FVC % predicted or DLCO % predicted at 12 months

VOC	IUPAC name	CAS	Class	Correlation coefficient	95% CI	P value
Relative change in FVC % predicted at 12 months						
4-cyclopentene-1,3-dione	Cyclopent-4-ene-1,3-dione	930-60-9	Unsaturated cyclic dicarbonyl	0.36	0.10 to 0.57	0.02*
1-chloropentane	1-chloropentane	543-59-9	Haloalkane	0.33	0.08 to 0.62	0.03*
2-chloro-*p*-xylene	2-chloro-1,4-dimethylbenzene	95-72-7	Aromatic hydrocarbon	−0.29	−0.58 to −0.04	0.06
trans-3,3,5-trimethylcyclohexanol	(1S,5R)3,3,5-trimethylcyclohexan-1-ol	767-54-4	Secondary alcohol	0.26	−0.002 to 0.47	0.10
o-cymene	1-methyl-2-propan-2-ylbenzene	527-84-4	Monoterpenoid	−0.25	−0.52 to 0.02	0.12
Mesitylene	1,3,5-trimethylbenzene	108-67-8	Aromatic hydrocarbon	0.25	−0.02 to 0.53	0.12
Cyclopentadiene	Cyclopenta-1,3-diene	542-92-7	Alicyclic hydrocarbon	0.23	−0.03 to 0.46	0.15
2-phenyl-2-propanol	2-phenylpropan-2-ol	617-94-7	Benzyl alcohol	−0.22	−0.42 to 0.04	0.18
Relative change in D_{LCO} % predicted at 12 months						
2-methyltetrahydrofuran	2-methyloxolane	25265-68-3	Cyclic ester	−0.51	−0.74 to −0.28	<0.001*
m-cymene	1-methyl-3-propan-2-ylbenzene	535-77-3	Monoterpenoid	−0.40	−0.67 to −0.16	0.002*
3-methylfuran	3-methylfuran	930-27-8	Heteroaromatic compound	−0.32	−0.59 to −0.07	0.01*
1,1,3-trimethylcyclohexane	1,1,3-trimethylcyclohexane	3073-66-3	Cyclic alkane	0.32	0.05 to 0.51	0.02*
D-limonene	(4R)-1-methyl-4-prop-1-en-2-ylcyclohexene	5989-27-5	Monoterpene	−0.31	−0.59 to −0.05	0.02*
o-cymene	1-methyl-2-propan-2-ylbenzene	527-84-4	Monoterpenoid	−0.30	−0.57 to −0.04	0.02*
4,6-dimethylundecane	4,6-dimethylundecane	17312-82-2	Branched alkane	−0.30	−0.56 to −0.04	0.03*
1,1,1-trichloroethane	1,1,1-trichloroethane	71-55-6	Haloalkane	−0.29	−0.58 to −0.04	0.03*
2-butoxyethanol	2-butoxyethanol	111-76-2	Glycol ether derivative	−0.29	−0.56 to −0.03	0.03*
1-butanol	Butan-1-ol	71-36-3	Primary alcohol	−0.27	−0.52 to −0.01	0.04*
Octanal	Octanal	124-13-0	Saturated fatty aldehyde	−0.27	−0.52 to −0.01	0.04*
1,4-benzoquinone	Cyclohexa-2,5-diene-1,4-dione	106-51-4	Unsaturated cyclic dicarbonyl	−0.26	−0.52 to 0.003	0.04*

*p<0.05.
CAS, Chemical Abstracts Service; D_{LCO}, diffusion capacity of the lung for carbon monoxide; FVC, forced vital capacity; IUPAC, International Union of Pure and Applied Chemistry; VOC, volatile organic compound.

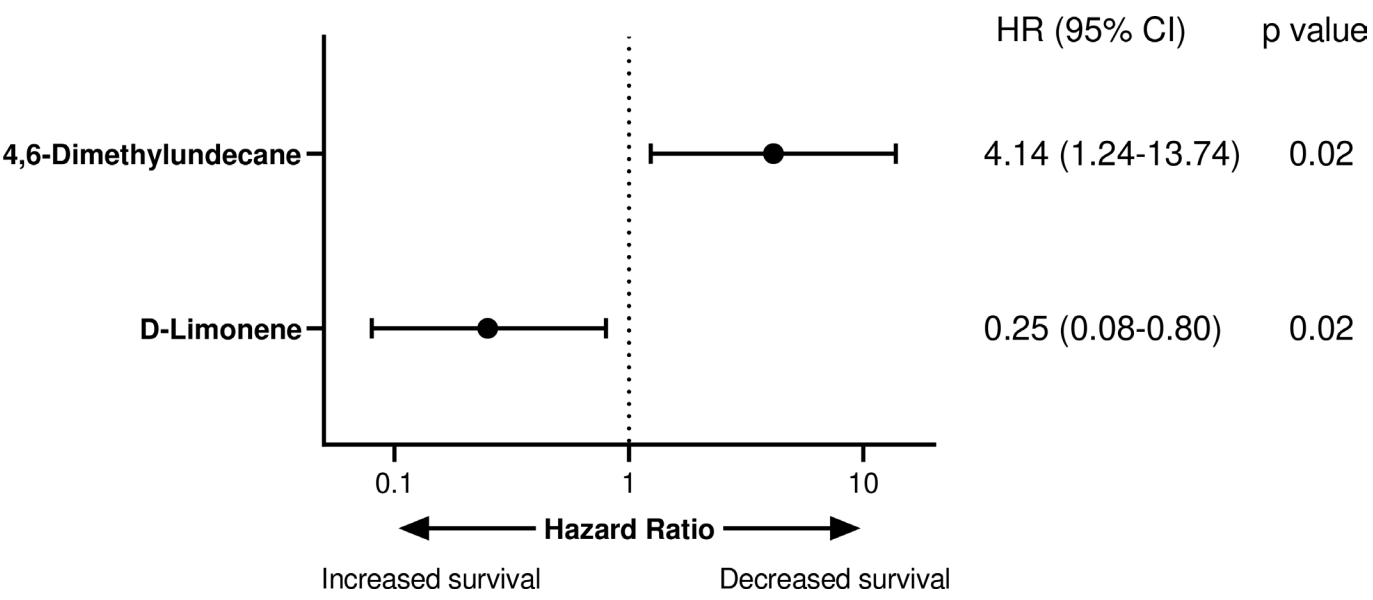

Figure 1 Forest plot showing significant associations between relative volatile organic compound concentration and survival. Adjusted HRs are reported.

In conclusion, we identified VOCs in exhaled breath that may be related to measures of lung function decline in IPF. These results support further studies to investigate the possibility of a volatile signature for prognosis in IPF.

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Acknowledgements WA and SJF are supported by the National Institute for Health and Care Research (NIHR) Manchester Biomedical Research Centre (BRC) (NIHR203308). IRW is supported by the Slovenian Research Agency (ARIS program No. P1-0034).

Contributors CH, WA, DT, IRW, NC, CL, AMW and SJF were involved in project conceptualisation and method development. SJF and NC secured funding. CH and DT performed data collection. WA and IRW performed sample analysis. CH and WA performed data analysis. CH prepared the manuscript first draft. WA, DT, IRW, NC, CL, AMW and SJF reviewed manuscript drafts. CH is the guarantor of the manuscript.

Funding This study was funded by an investigator-initiated grant from Boehringer Ingelheim (Award number: 199.0311)

Competing interests CH has received an honorarium from Boehringer Ingelheim for providing educational seminars. AMW has received grants from Brainomix, Celgene and Glaxosmithkline, consulting fees from Vicor Pharma and Bridge Biotherapeutics, speaker fees from Chiesi and Boehringer Ingelheim and educational support from Chiesi. WA, DT, IRW, NC, CL, and SJF have no relevant conflict of interests.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Wales REC 6 (17/WA/0339). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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