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Early View

Research letter

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Breathomics to monitor interstitial lung disease associated with systemic sclerosis

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Take Home message

Exhaled breath analysis, utilizing VOCs, offers promise in identifying SSc-ILD patients, aiding targeted treatment. Further multicentric studies are crucial for validation and exploring longitudinal VOC changes for comprehensive disease management.

Systemic sclerosis (SSc) is an auto-immune disease of unknown origin characterized by an inflammatory process associated with vascular damages and collagen deposition. Interstitial lung disease (ILD) highly prevalent in SSc (SSc-ILD) is known to be the leading cause of death and its treatment requires aggressive multimodal therapy[1]. In this context, there is a major clinical need to identify significant SSc-ILD at the earliest stage, especially for patients at risk to develop a progressive form of the disease. Nowadays, few biomarkers can classify patients at risk to develop SSc-ILD, most of them are blood-based and detected in the last clinical stage of the disease. Previously, we have demonstrated that SSc patients exhibit a specific signature of volatile organic compounds (VOCs) compared to healthy subjects (HS)[2]. In this prospective study, we aimed to identify the potential of VOCs to predict the ILD phenotype.

METHODS

The study presented was conducted on a cohort composed of 42 patients, *i.e.*, 21 patients suffering from systemic sclerosis (SSc) and 21 suffering from interstitial lung disease associated with systemic sclerosis (SSc-ILD). Patients suffering from SSc and SSc-ILD were prospectively recruited both in University Hospital of Liège (CHU), Belgium, and Maastricht University Medical Center (MUMC+), the Netherlands during a period of six month starting in July 2021 and ending in September 2021. SSc was diagnosed according to 2013 ACR/Eular. SSc-ILD was define by a specific interstitial lung involvement confirmed through a multidisciplinary discussion as recommended by ATS/ERS guidelines[3]. The protocol was locally approved by ethical committees, Belgian number: B707201422832, reference Liege 2014/302 and Dutch number NL57351.068.17, reference Maastricht 172021 respectively. All subjects gave written informed consent before participating to the study. All breath samples were systematically collected within same room at the two medical facilities, to minimize the effect of variation in background air. As established in our SOPs, breath sampling was conducted before any pulmonary function test, and patients were not required to fast[4]. The exhaled breath samples were collected into inert 5 L Tedlar® bags. The content of the sampling bag was subsequently concentrated under standardized conditions onto Tenax® GR/ Carbopack™ B TD tubes (Markes International Ltd., Llantrisant, UK). Following the collection process, the tubes were hermetically sealed using specific caps for preservation before being analyzed. The exhaled air was finally analysed by thermal desorption comprehensive two-dimensional gas chromatography high-resolution time-of-flight mass spectrometry (TD-GC×GC-HRTOFMS, Leco Corporation) at the OBiAChem laboratory in Belgium[2]. Statistical analyses were performed using RStudio (2022.12.0) and MetaboAnalyst online 5.0 (Quebec, Ca)[5]. For more detailed technical information, see previous research conducted[2,4].

RESULTS

A total of 42 patients were recruited from two expert centres. The patients' demographics are presented in Figure 1.A.

In our study, we compared the exhaled breath composition between SSc and SSc-ILD patients using TD-GC×GC-HRMS. This technique allowed us to detect around 800 features. We developed a statistical model based on Partial Least Square Discriminant Analysis (PLS-DA). This model was then subsequently employed to select nine significant markers based on their variable importance score (Figure 1.B-C.). This model achieved an AUC (Area Under the Curve) of 0.82, accuracy of 85%, sensitivity of 77% and a specificity of 100% (Figure 1.D.) for identifying ILD phenotype. Furthermore, the achieved metrics were similar to the DLco-based univariate model, which achieved an AUC of 0.83 (Figure 1.D.).

For robustness evaluation, we tested potential confounding factors such as smoking habits, treatments, and gender, included in the metadata (Figure 1.A.). We did not identify any interference on the predictive ability of VOCs regarding potential confounders. A correlation was observed between

the functional respiratory parameters (i.e., DLco and FVC% predicted value) and the VOCs. A positive correlation was observed between the DLco and the probability of classification of the VOCs based model.

DISCUSSION

We have identified a breath-based model able to discriminate SSc-ILDs with a high sensitivity confirming its potential in patient management. Four markers are in line with our previous study, reaffirming the potential of VOCs in disease classification (*i.e., two Terpineol isomers, Menthone I and Menthone II)* with their potential metabolic pathways discussed in our earlier work[2]. A key focus of this research lies in the discovery of nine volatile organic compounds present in the exhaled breath of patients that exhibit discriminatory capabilities in classifying between SSc and SSc-ILD. These nine markers demonstrated significant classification performance in comparison to conventional lung physiological markers and functional parameters[6]. Furthermore, we validated methodological SOPs to conduct breath-based multi-centric studies, a determinant step toward validating our classification across several clinical centres. Multi-centric breath studies are major improvement for this emerging monitoring strategy.

Moreover, this finding contributes to an enhanced understanding of the disease and the associated metabolic pathways. For instance, *1,4-Pentadiene*, a hydrocarbon, emerges as a potential biomarker of several lung pathologies. We previously demonstrated that chemically and biologically induced inflammation in lung epithelial cells can lead to increased hydrocarbons level due to inflammation-associated oxidative stress[7]. Another compound, *1-Propanol,* have been proposed as a potential marker for lung cancer, detected in the breath of individuals suffering from the disease and in the headspace of cancer cells[8]. The presence of this alcohol might stem from the cytochrome P450 enzymes that hydroxylate lipid peroxidation biomarkers, generating alcohols. Notably, this last marker has also been observed in the exhaled breath of asthmatic patients and has discriminatory capabilities, along with other VOCs, in distinguishing between neutrophilic and eosinophilic asthma[4].

The constant exposure of humans to exogenous compounds through various sources such as diet and environment can lead to the direct secretion of these volatile compounds in breath. Additionally, volatile downstream products stemming from these compounds could potentially serve as medical probes[9]. *Limonene (D-Limonene),* another terpene regarded as an exogenous marker, is found to be elevated in the breath of patients with liver cirrhosis[10]. Following entry into the blood stream, limonene is metabolized by the P450 enzymes CYP2C9 and CYP2C19. This represents the second instance in this study where cytochrome P450 enzymes play a role. Conversely, *Carvone* and *chlorobenzene* have yet to be associated with disease markers based on current knowledge. As Limonene, these compounds could be considered as a probe which assess metabolism performances. It is worth noting that an increased amount of altered extracellular matrix components destroys alveolar architecture and disrupts gas exchange equilibrium[11]. Therefore, elevated volatile concentrations could be also attributed to the thickening of alveolar walls and subsequent impairment of gas exchange, influencing concentrations.

The accurate and sensitive statistical model presented in this study showed the potential of VOCs in exhaled breath to identify SSc-ILD patients in an SSC cohort. In addition, our study is corroborating the potential of four terpenes to discriminate SSc patients. Exhaled breath could help clinicians to rapidly provide targeted treatment to patients suffering from ILD. Nevertheless, prospective multicentric studies to further validate the potential of exhaled breath analysis for the management of SSc-ILD patients would be needed. Future studies would include SSc-ILD at early stages to evaluate longitudinal changes of VOCs compared to disease progression and treatment response.

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Authors' Contributions: T.M. takes the responsibility for the content of the manuscript. T.M., J.G., P-H.S. wrote the initial draft of the manuscript; T.M., J.P., D.Z., J.G., P-H.S. conceptualized the research question and contributed substantially to the study design, data interpretation, and the writing of the manuscript; T.M., J.P., R.T., F.G., D.Z., M.H., M-S.N., T.D., G.G., L.G., F.G., F.G., B.A., C.R., J.G., P-H.S. contributed substantially to recruitment, data collection and manuscript writing; T.M., R.L., P.V.P., J.G., P-H.S. supervised the study, wrote, reviewed the manuscript, and administrated the project; T.M., J.P., D.Z., B.A., C.R., P.V.P., R.L., J.G. Reviewed and provided significant insights to the manuscript. All authors approved the final draft of the manuscript.

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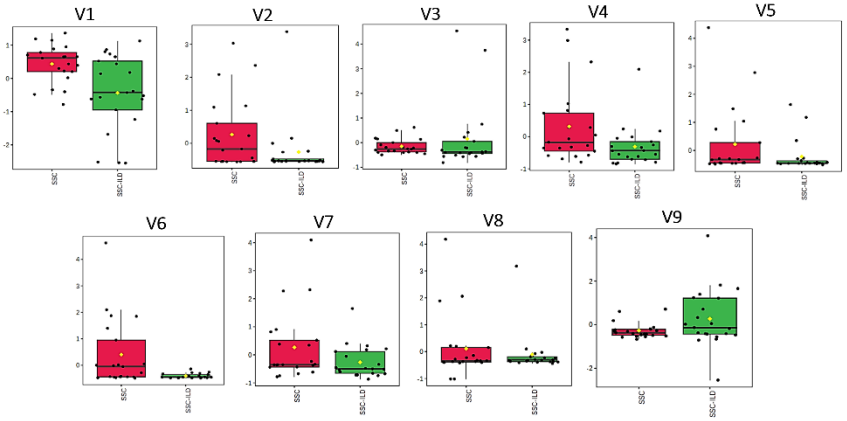
A.

Characteristics	SSc	SSc-ILD	*p*-value
N.	21	21	
Age, yr	59 ± 12	67 ± 13	0.04
Gender, F, %	76 (n=16)	81 (n=17)	0.71
BMI, Kg/m²	24 ± 6	25 ± 6	0.44
Patients who smoke, yes%	5 (n=1)	24 (n=5)	0.08
FEV_1, % predicted	90 ± 13	80 ± 19	0.09
FVC, % predicted	96 ± 11	79 ± 19	0.01
DLco	73 ± 14	53 ± 20	<0.01
Immunosuppressive therapy, yes%	24 (n=5)	76 (n=16)	<0.01
Oral corticosteroids therapy, yes%	14 (n=3)	43 (n=9)	0.04
Antifibrotic therapy, yes%	0	10 (n=2)	
Pulmonary hypertension, yes%	0	24 (n=5)	

B.



C.

ID	Identification	PLS-DA (VIP score)	*p*-value	^1t_R (s)	^2t_R (s)	CAS	Library Match (similarity)	Probability (%)	Observed ion (m/z)	Mass accuracy (ppm)
V1	1,4-Pentadiene[b]	100	<0.01	262.2	1.09	591-93-5	900	43.2	68.0620	-0.63
V2	Terpineol isomer[c] ($C_{10}H_{18}O$)	62	0.07	1827	3.255	/	/	/	154.1356	2.49
V3	Terpineol isomer[c] ($C_{10}H_{18}O$)	49	0.26	1827	0.14	/	/	/	154.1356	2.49
V4	1-Propanol[a]	48	0.03	441	1.44	71-23-8	874	97.4	60.0570	-0.13
V5	Carvone[b]	45	0.11	1974	0.33	99-49-0	919	42.1	150.1038	-0.58
V6	Menthone I[b]	41	<0.01	1795.2	2.14	491-07-6	949	35.3	154.1351	-0.77
V7	D-Limonene[b]	33	0.07	1462.8	1.44	5989-27-5	938	43.3	136.1247	0.03
V8	Menthone II[b]	24	0.47	1886.4	2.99	5948-04-9	931	41.4	152.1195	-0.76
V9	Benzene, chloro-[b]	22	0.08	1095	0.55	108-90-7	971	96.8	112.0074	-0.30

D.

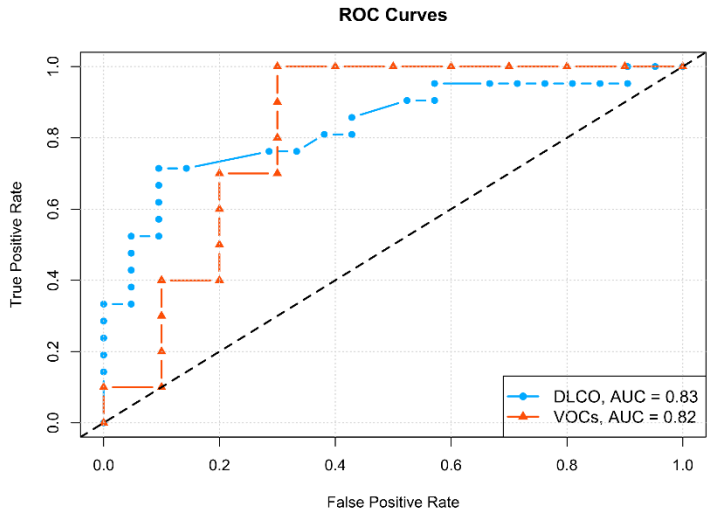


Figure 1