TD-GC×GC-HRTOFMS to investigate pulmonary fibrosis in patients

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Key points

- **Systemic sclerosis (SSC) & Interstitial lung disease (ILD)**
- **SSC-ILD diagnostic tool development**
- **TD-GC×GC-HRTOFMS for targeted and untargeted breath analysis**

Introduction

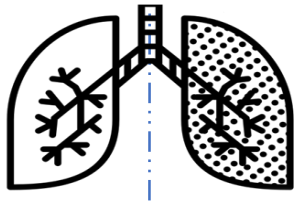
Systemic sclerosis (SSc), is a chronic and heterogenous auto-immune disease characterized by several disorders (inflammation, fibrosis, etc.) involving multiple internal organs.

Furthermore, interstitial lung disease (ILD) is one of the most common types of pulmonary involvement responsible for the disease severity and leading to high morbidity and mortality. ILD is frequently associated with SSc (SSc-ILD). Therefore, it is essential to diagnose patients suffering from SSc-ILD at an early stage. In fact, the reference treatment of SSc-ILD relies on corticosteroids and immunosuppressive therapy to reduce the inflammation-associated ILD. This treatment should therefore exclusively be administered to high-risk SSc-ILD patients.

In a previous research, we demonstrated that a combination of sixteen volatile metabolites in breath could reliably discriminate SSc patients and matched controls (healthy). Furthermore, thermal desorption (TD) coupled with comprehensive two-dimensional gas chromatography – high resolution time-of-flight mass spectrometry (TD-GC×GC-HRTOFMS) stands out as the perfect tool for exhaled breath analysis regarding its peak capacity and its ability to correctly identify biomarkers.

Workflow & preliminary results

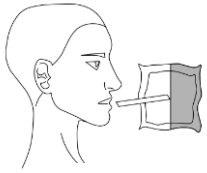
1. Patients



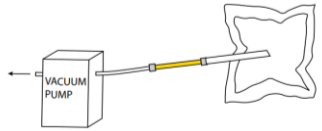
50 SSC patients | 50 SSc-ILD patients

The patients have been recruited and diagnosed at the medical center of the University of Maastricht and the hospital of the University of Liège.

2. Sampling

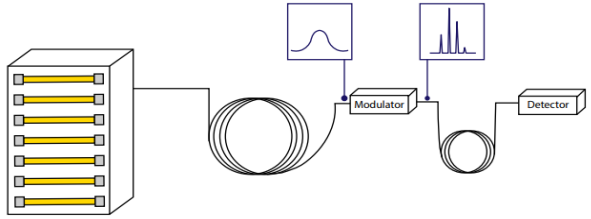


A. The patients exhale the air contained in their lungs in a tedlar bag.



B. A low-flow pump is used to transfer all the VOCs onto a sorbent tube (Tenax®GR/Carbopack™B).

3. Samples analysis



All the VOCs trapped onto the sorbent tubes are finally released and separated into a Pegasus GC-HRT 4D.

GC×GC :

Columns : ^{1}D Rxi-624Sil MS (30m x 0.25 µm x 1.4 µm d_f)
^{2}D Stabilwax (3m x 0.25 µm x 0.5 µm d_f)

Temperature prog. : 40°C (5 min), ramped 5°C/min to 235°C (3 min)

Modulator : Quad jet dual-stage, P_M=3.5 s, hot pulse time : 1.05 s

HR-TOFMS :

Acquisition delay : 210 s

Acquisition frequency : 200 Hz

Electron ionization energy : 70 eV

Mass range : 29-450 m/z

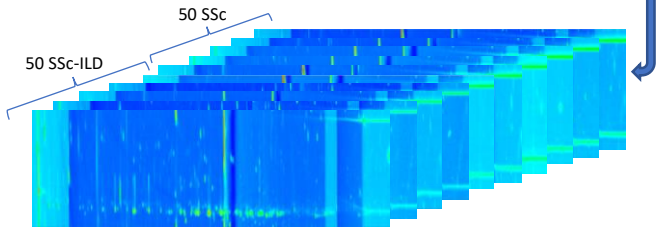
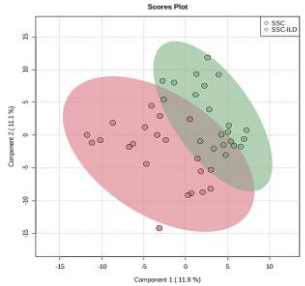


Figure : Representation of the large number of chromatograms acquired during this study (one for each patient).

4. Data treatment/ Results



After a preliminary data curation on 42 chromatograms (21 SSC vs 21 SSC-ILD), multivariate statistics were applied. Whereas no separation was observed using a PCA, PLS-DA allowed us to statistically separate the two groups of patients and to ascertain important features (biomarkers). Among these biomarkers, some of them have already been observed in our previous research.

Name (based on library research)	VIP
l-Menthone	2.567
Butanoic acid	2.5435
Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	2.5079
Caryophyllene	2.4516
Acetone	2.2813
Cyclohexanol, 1-methyl-4-(1-methylethyl)-	2.2375
Isopropyl Alcohol	2.2004
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate, (1α,2β,5β)-	2.1969
Pyrazine	2.1784
α-Terpineol	2.1638
Heptane, 2-methyl-	2.1135
Benzofuran, 4,5,6,7-tetrahydro-3,6-dimethyl-	2.0397
m-Chloroaniline	2.0375
Linalyl acetate	2.0331
(-)-β-Bourbonene	2.0307
1-Nonanol	2.0166

Figure (top): PLS-DA scores plot based on the training set (21 SSc & 21 SSc-ILD). Figure (bottom): Most important features based on their VIP scores.

Conclusion

We are convinced that breath VOCs analysis is an efficient approach to diagnose SSC-ILD at an early stage. Therefore, following these preliminary results, a robust statistical model will be constructed in order to correctly diagnose patients.

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