# SYSTEMIC SCLEROSIS: CAN BREATHOMICS MONITOR INTERSTITIAL LUNG DISEASE?

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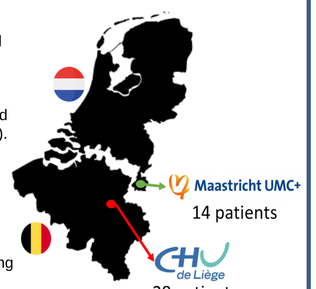
## Key points

- **This is the first multicentric study for SSc vs SSc-ILD breath screening.**
- **A PLS-DA model based on 9 specific features allowed us to discriminate SSc patients from SSC-ILD patients.**
- **The VOCs-based model correlates with clinical ILD parameters.**
- **This study confirms a set of biomarkers that have already been observed in our previous research.**

## Introduction

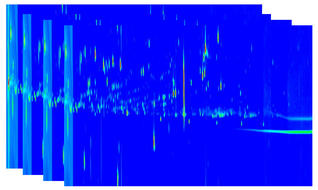
**Systemic sclerosis (SSc)** is a chronic and heterogeneous auto-immune disease characterized by several disorders (inflammation, fibrosis, etc.) involving multiple internal organs. Furthermore, **interstitial lung disease (ILD),** highly prevalent in SSc (referred to as **SSc-ILD**), is known to be **the leading cause of death**. Therefore, there is a significant clinical need to **identify SSc-ILD at the earliest stage of the disease** in order to propose an aggressive multimodal therapy. In a previous study, Zanella et al. identified that SSc patients exhibit a specific signature of volatile organic compounds (VOCs) compared to healthy subjects (HS). **In this multicentric prospective study, our aim was to determine the potential of VOCs profiles in predicting the ILD phenotype (SSc-ILD).**

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 months starting in July 2021 and ending in September 2021.




## Clinical application

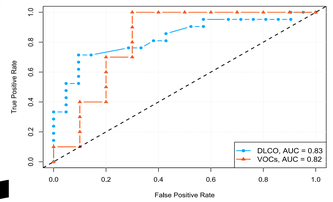
### Data curation



- After injecting exhaled air samples, a two-dimensional (2D) chromatogram is produced for each patient. This method uses two different GC columns to improve peak resolution. Each colored dot represents a specific compound in the sample, allowing for clearer identification of substances.

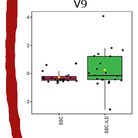
### Classification model

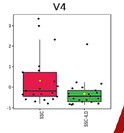
PLS-DA stat. model




- Good performances have been reached compared to conventional lung physiological markers and functional parameters.
- Positive correlation between Diffusing Capacity Of The Lungs For Carbon Monoxide (DLCO) and the probability of classification.

### Data assimilation




| ID | Identification | CAS | Library Match (similarity) | Mass accuracy (ppm) |
|---|---|---|---|---|
| V1 | 1,4-Pentadiene | 591-93-5 | 900 | -0.63 |
| **V2** | **Terpineol isomer ($C_{10}H_{18}O$)** | **/** | **/** | **2.49** |
| **V3** | **Terpineol isomer ($C_{10}H_{18}O$)** | **/** | **/** | **2.49** |
| V4 | 1-Propanol | 71-23-8 | 874 | -0.13 |
| V5 | Carvone | 99-49-0 | 919 | -0.58 |
| **V6** | **I-Menthone** | **14073-97-3** | **877** | **-0.55** |
| V7 | D-Limonene | 5989-27-5 | 938 | 0.03 |
| **V8** | **II-Menthone** | **14073-97-3** | **931** | **-0.76** |
| V9 | Benzene, chloro- | 108-90-7 | 971 | -0.30 |

- Data selection based on VIP Scores.

- **Better metabolic pathways understanding.**
- **Features confirmation (V2, V3, V6 and V8) based on our preliminary study.**

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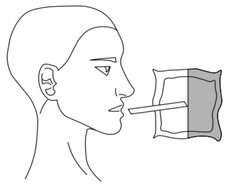
## Analytical approach

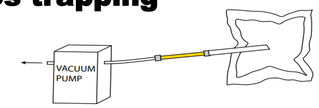
- Established protocol implemented at the clinical level for several years.

### 1 Sampling



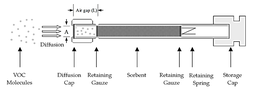
Exhaled breath samples were collected from 21 SSc patients and 21 SSc-ILD patients into 5L Tedlar® bags at the two medical centers.

### 2 VOCs trapping

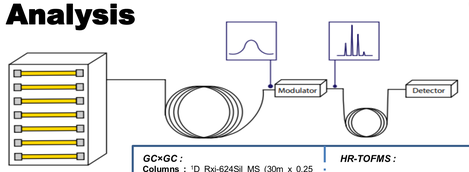



The content of the sampling bag was concentrated onto Tenax®GR/Carbopack™B TD tubes under standardized conditions. A low-flow pump was used to transfer all the VOCs through the sorbent tube, where they were trapped due to their affinity with the sorbent material.

- **Non-invasive technique**




### 3 Analysis




***GC×GC :***
**Columns :** $^1$D Rxi-624Sil MS (30m x 0.25 mm x 1.4 µm $d_f$)
$^2$D Stabilwax (3m x 0.25 mm 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 mu

All the VOCs trapped onto the sorbent tubes are finally released and separated using a GC×GC-HRTOFMS.

## Published paper

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## Conclusion

**To the best of our knowledge, this is the first multicentric study aiming to develop a VOCs-based model to classify SSc patients based on the presence of SSc-associated ILD.**

**In line with our previous study, we identified four features that further confirm the potential of VOCs in disease classification. A significant aspect of this research is the identification of nine VOCs that demonstrate discriminatory properties in classifying SSc and SSc-ILD. These nine specific features have shown promising performance in terms of classification. However, this study deserves further prospective multicentric validation to confirm the potential of a VOCs-based model for diagnosing SSc-ILD and predicting disease progression. Additionally, evaluating treatment response as a monitoring tool is crucial for better disease management. This point will be considered in future perspectives.**

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