TOWARDS CLINICAL IMPLEMENTATION: A MULTICENTRIC STUDY FOR THE DIAGNOSIS AND STRATIFICATION OF SYSTEMIC SCLEROSIS USING TD-GC×GC-HRTOFMS

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Key points

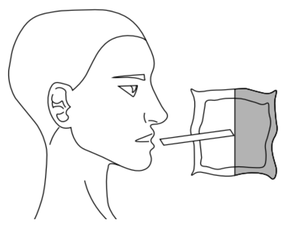
- **Multicentric 2-phase study for Healthy vs SSc vs SSc-ILD breath screening.**
- **A PLS-DA model based on 9 specific features allowed us to discriminate SSc patients from SSC-ILD patients.**
- **The VOCs-based model correlates with clinical ILD parameters.**
- **This study confirms a set of biomarkers 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 this multicentric prospective study, we investigated the potential of volatile organic compound (VOC) profiles as predictive biomarkers of the ILD phenotype in SSc. The study was designed in two phases: (I) an exploratory and feasibility phase; and (II) a subsequent phase dedicated to generating clinically relevant data to support SSc-ILD monitoring.**

Multicentric Clinical Implementation

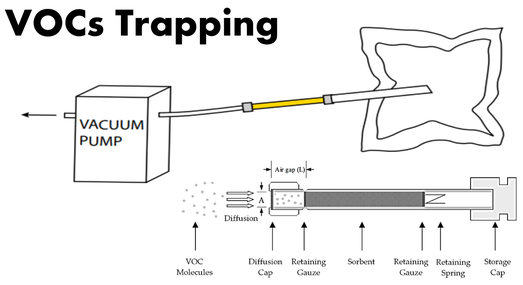
1 Breath Sampling



Exhaled breath samples are collected using 5L Tedlar® bags at the two medical centers.

- **Simplicity and patient comfort**

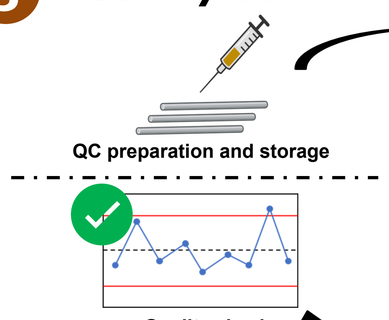
2 VOCs Trapping



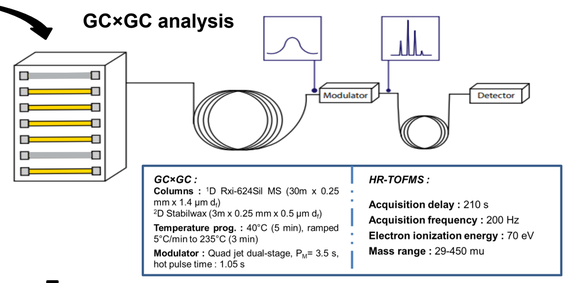
Tenax®GR/Carbopack™B TD tubes

- **Comprehensive VOCs trapping and storage**

3 Quality Conrol

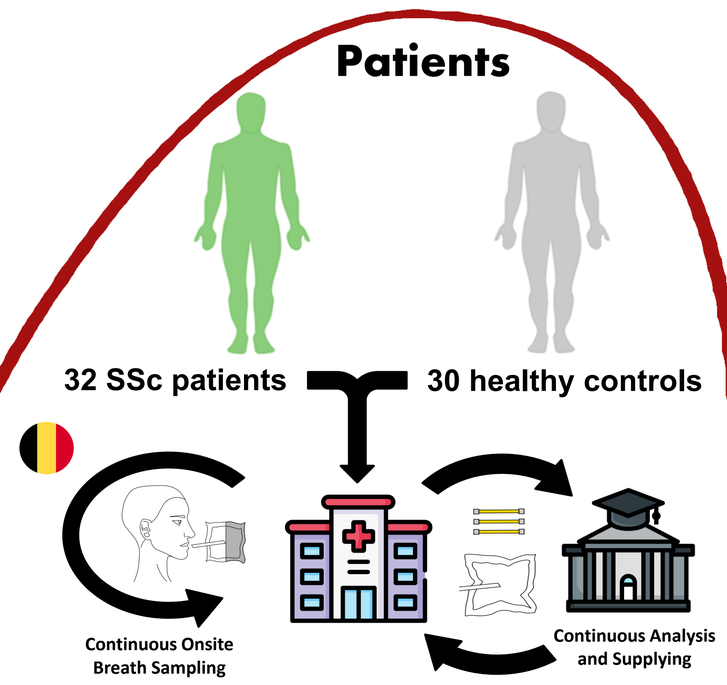


4 Sample Analysis

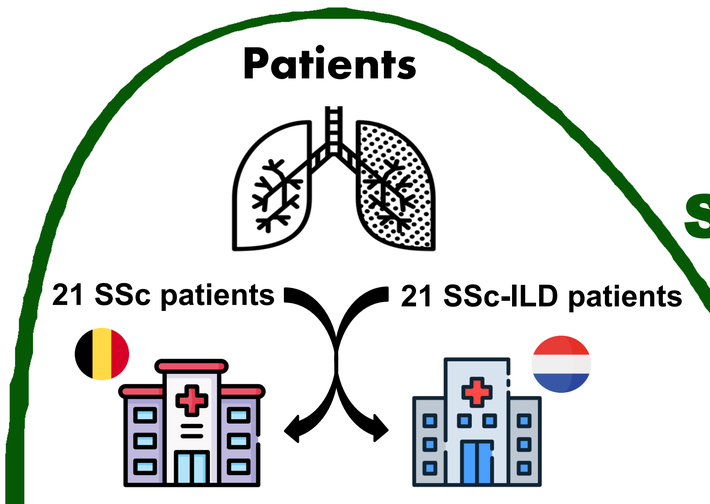


- **Sensitivity and Comprehensiveness**

Phase I Disease Diagnosis

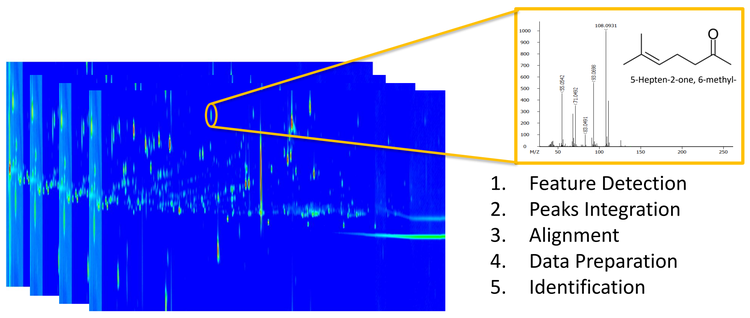


Phase II Disease Stratification



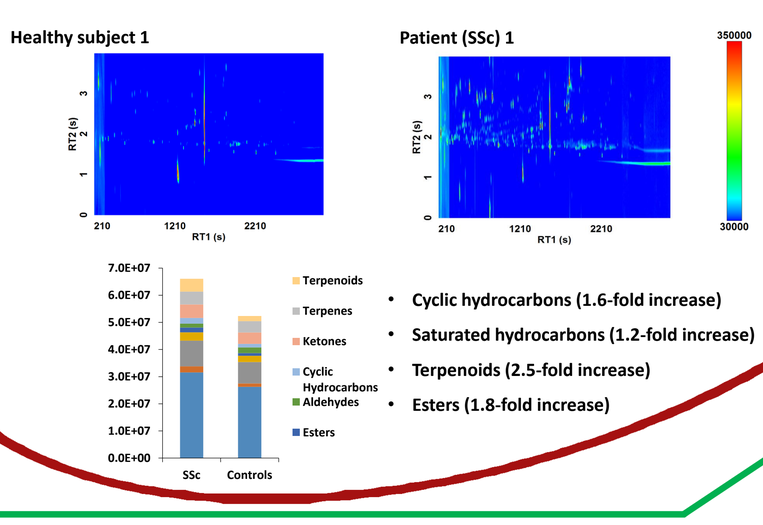
- Multicentric design to increase robustness. Patients were sampled in dedicated rooms in each centre using ready to use kits.

Data Treatment and Evaluation



1. Feature Detection
2. Peaks Integration
3. Alignment
4. Data Preparation
5. Identification

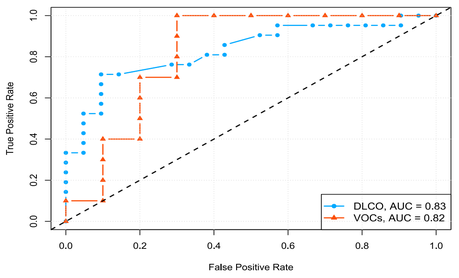
- For each patient, a two-dimensional (2D) chromatogram is generated. Each colored dot corresponds to a specific compound, enabling clear identification of substances.



- Cyclic hydrocarbons (1.6-fold increase)
- Saturated hydrocarbons (1.2-fold increase)
- Terpenoids (2.5-fold increase)
- Esters (1.8-fold increase)

Classification Model

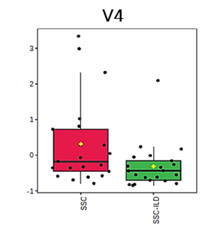
PLS-DA stat. model

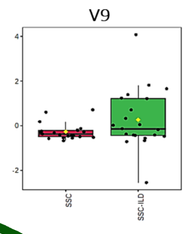


- Good performances has been reached compared to conventional lung physiological markers and functional parameters.
- Positive correlation between Diffusing Capacity Of The Lungs For Carbon Monoxide (DLco) and the probability of classification.

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	**l-Menthone**	**14073-97-3**	**877**	**-0.55**
V7	D-Limonene	5989-27-5	938	0.03
V8	**ll-Menthone**	**14073-97-3**	**931**	**-0.76**
V9	Benzene, chloro-	108-90-7	971	-0.30





- Data selection based on Variable Importance Scores (VIPs).
- **Better metabolic pathways and interactions understanding.**
- **Features confirmation (V2, V3, V6 and V8) based on our preliminary study.**

Conclusion

- **Multicentric study aiming to develop a VOCs-based model to classify SSc patients based on the presence of SSc-associated ILD.**

A significant aspect of this research is the identification of nine volatile organic compounds that demonstrate discriminatory properties in classifying SSc and SSc-ILD. These nine specific features have shown promising performance compared to DLco, a conventional parameter. However, this study requires further prospective multicentric validation to confirm the potential of a VOC-based model for diagnosing SSc-ILD and predicting disease progression. Additionally, assessing treatment response as a monitoring tool is crucial for better disease management. This aspect will be addressed in future perspectives.

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