Organic and Biological Analytical Chemistry Group

LIÈGE université MolSys Molecular Systems

CHIMIC CHEMICAL INFORMATION MINING

CHU de Liège

GC×GC-TOFMS for in vitro and ex vivo Characterization of Lung Inflammation Mechanisms

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5TH SBSE INTERNATIONAL MEETING
23 & 24 SEPTEMBRE 2019 - NOVOTEL PARIS-SUD
SBSE Technical Meeting

Why Lung Inflammation ? E.g. Asthma

200% increase over ten years

334 million people worldwide

14% of children < 18y

€19.3 billion per year in Europe

20% of patients on a wrong medication

Clinical Diagnosis of Asthma

Regular diagnosis approach

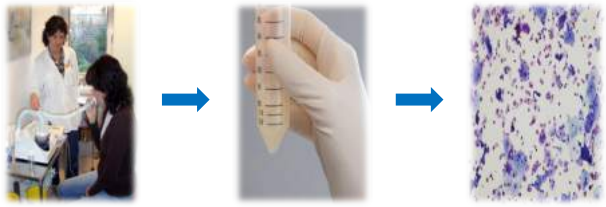
- Pre & post-bronchodilator spirometry
- FeNO measurement
- Methacholine challenge (brochoprovocation)


www.kidshealth.org

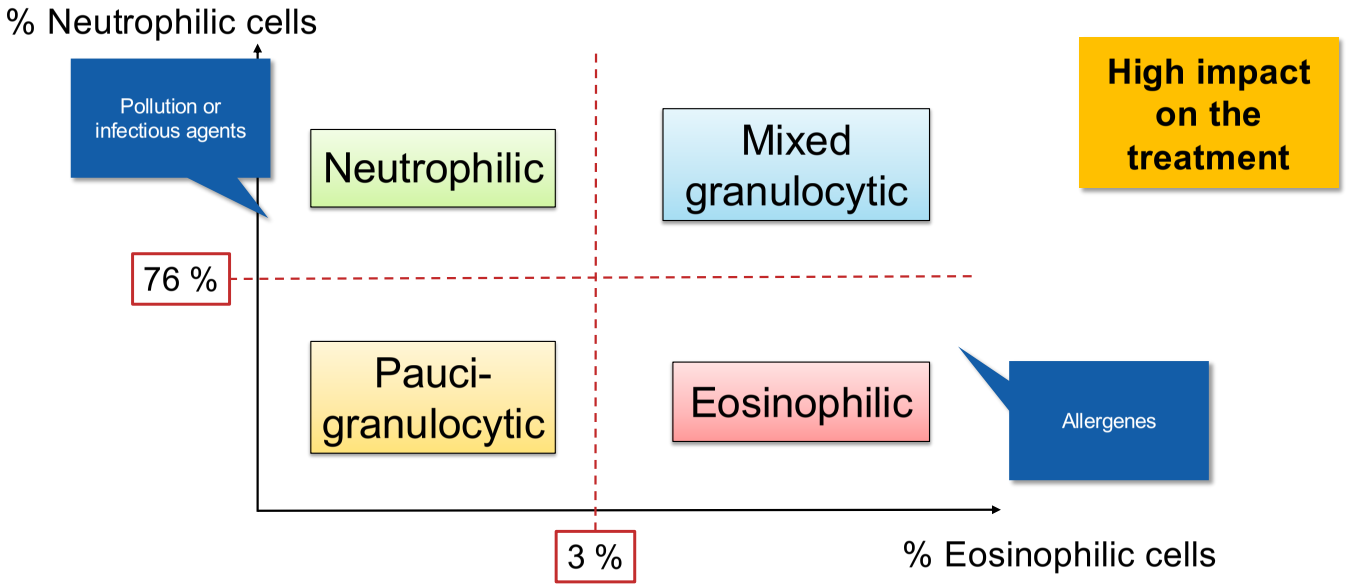
www.niox.com

Induced sputum (in addition to blood eosinophil count)

- Count of the cells
- Characterization of the cells
- Morphology classification



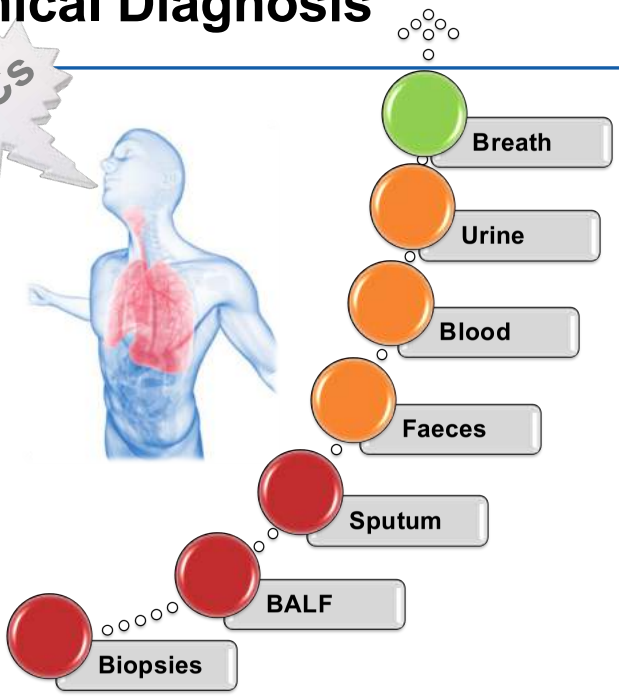
Asthma Inflammatory Phenotypes: Induced Sputum



Breath as Alternative Clinical Diagnosis

Advantages of Breath

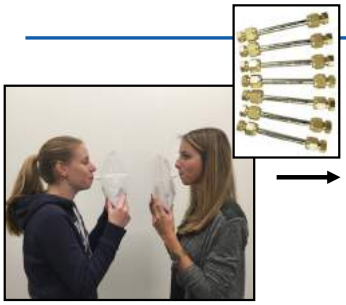
- Patient information-rich
- Accessible and non-invasive
- Cost-efficient
- Patient compliant



BreathPrint Study Design

Maastricht University

LIÈGE université



Classification	Features
Eosinophilic	VOC Peak Areas
Neutrophilic	
Paucigranulocytic	

Discovery study (n: 276)

Training Set

- CIFs Model building on VOCs
- Feature selection
- CIFs Model using selected features

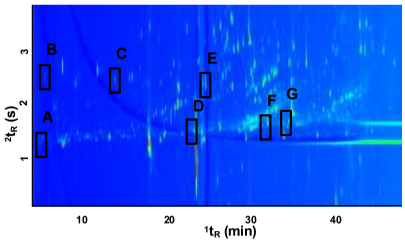
Test Set

- CIFs Model predictions
- AUROC calculations
- Accuracy measures

Validation study (n: 245)

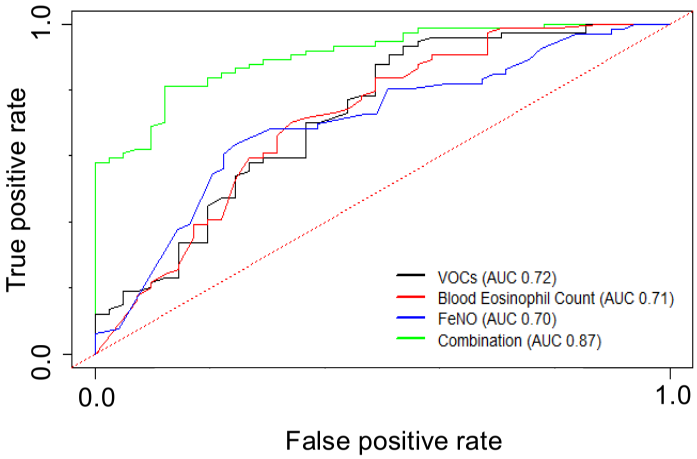
Compound Name	Molecular Weight (amu)	Similarity	Mass Accuracy (ppm)
Hexane	**86.1090**	**904**	**1.16**
1-Propanol	**60.0570**	**901**	**1.51**
2-Hexanone	**100.0883**	**926**	**0.03**
Undecane	**156.1873**	**934**	**-0.29**
Nonanal	**142.1352**	**942**	**0.10**
Tetradecane	196.2186	934	N/A
Pentadecene	210.2342	939	N/A

Target VOCs



7 potential biomarkers

Eosinophilic Asthma

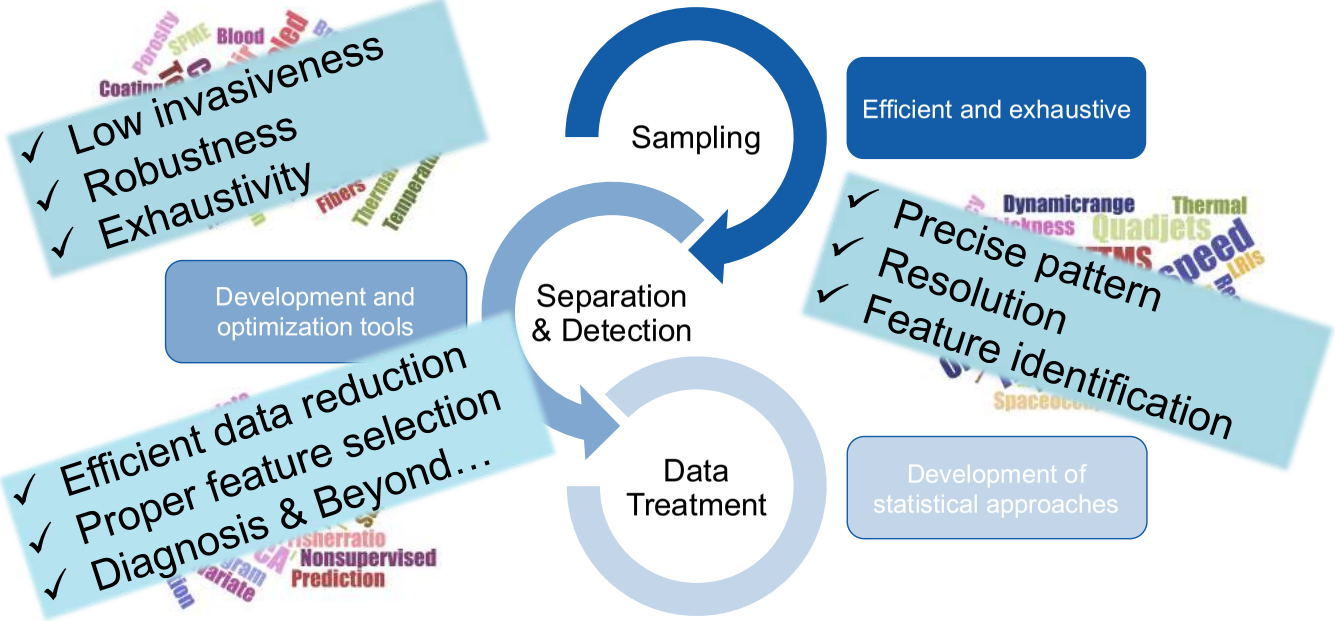


	Accuracy	Sensitivity	Specificity
FeNO	67 %	44 %	83 %
Blood Eos%	72 %	54 %	83 %
VOCs	72 %	76 %	66 %
Combination	76 %	79 %	78 %

Schleich FN *et al.*, Am. J. Respir. Crit. Care Med. (2019) 200 (4), 444-453.

➡ Best diagnostic approach so far…

The Big Picture (Clinical Diagnosis)



Towards Ex-Vivo & In-Vitro Approaches

A better understanding of the human volatilome is desirable

- What are the 'end-reaction' VOCs ?
- Is therapeutic monitoring of oxidative stress possible ?
- Can we elucidate the biochemical background of VOCs ?
- Can we measure the impact of ROS on biological systems ?

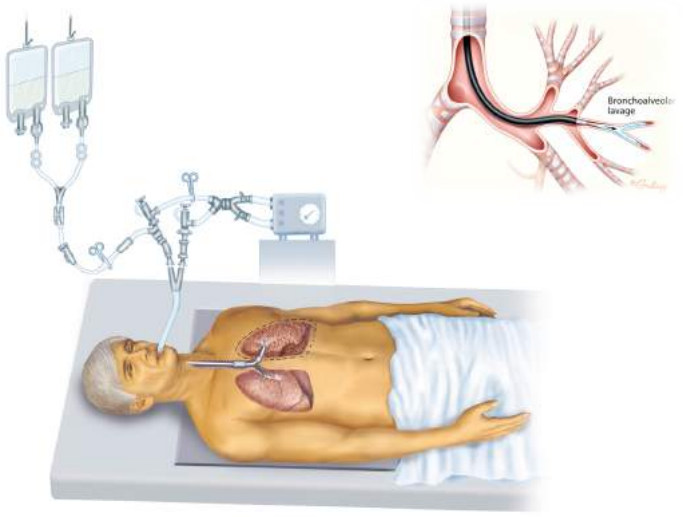
We curently investigate two main axes

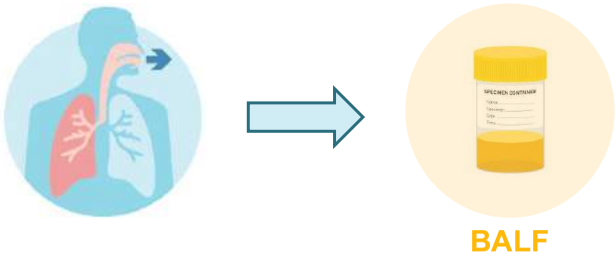
- Ex-vivo consideration on bronchoalveolar lavage fluids (BALFs)
- In-vitro consideration of cell cultures

High emphasis on QA/QC aspects

BALF APPROACH

Bronchoalveolar Lavage Fluids (BALFs)





Liquid samples:
→ Easy handling
→ Storage and multiple injections
→ Possibilities of derivatization

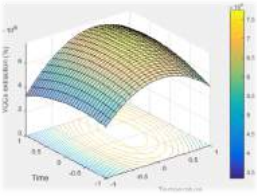
Method (BALFs)

1. Comparison of normal and reverse columns combination
2. Optimization of the GC×GC conditions

Final conditions:
- Columns: Rxi-5ms (30 x 0.25 x 0.25) coupled to Rxi-17MS (1 x 0.25 x 0.25)
- P_M 2 s

Oven	40 C	3 min
8 C/min	270 C	3 min

3. Comparison of trapping capacity of different SPME fiber coating



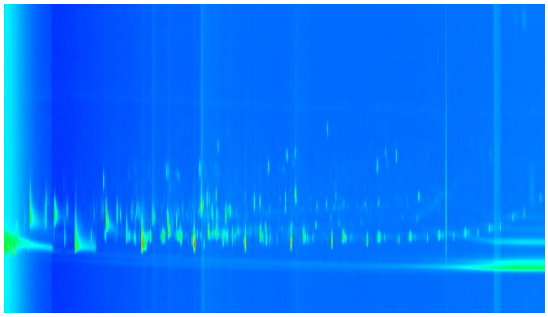
MilliporeSigma

RESTEK

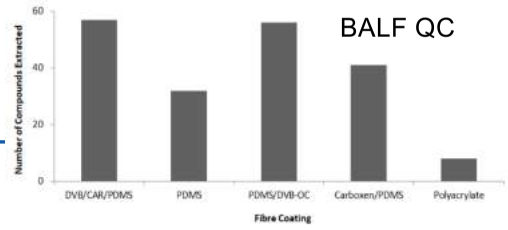
Samples (BALFs)

5 mL from 10 patients

50 mL of BALF QC for method control and optimization

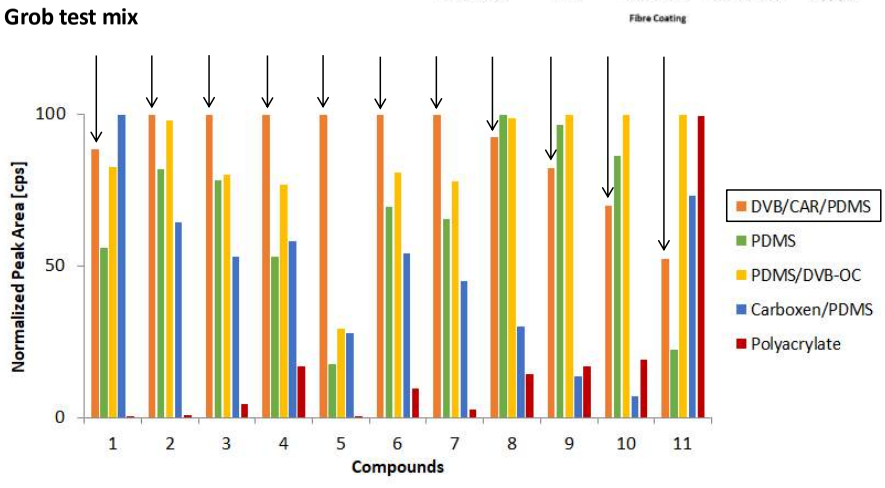


SPME Optimization (BALFs)



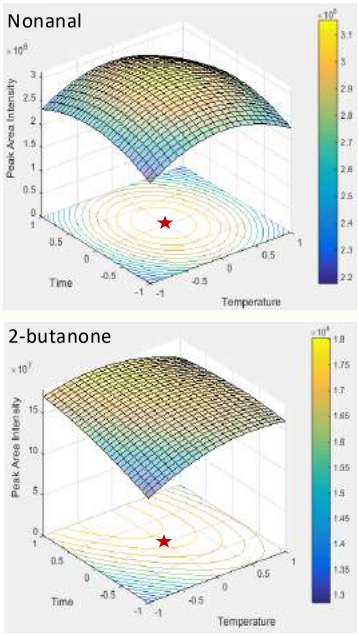
Headspace SPME

Number given	Compound's Name
1	Decane
2	Undecane
3	2,6-Dimethylaniline
4	2,6-Dimethylphenol
5	2,3-Butanediol
6	1-Octanol
7	Nonanal
8	Methyl decanoate
9	Methyl undecanoate
10	Methyl dodecanoate
11	Dicyclohexylamine



→ Selection of DVB/CAR/PDMS

SPME Optimization by DoE (BALFs)

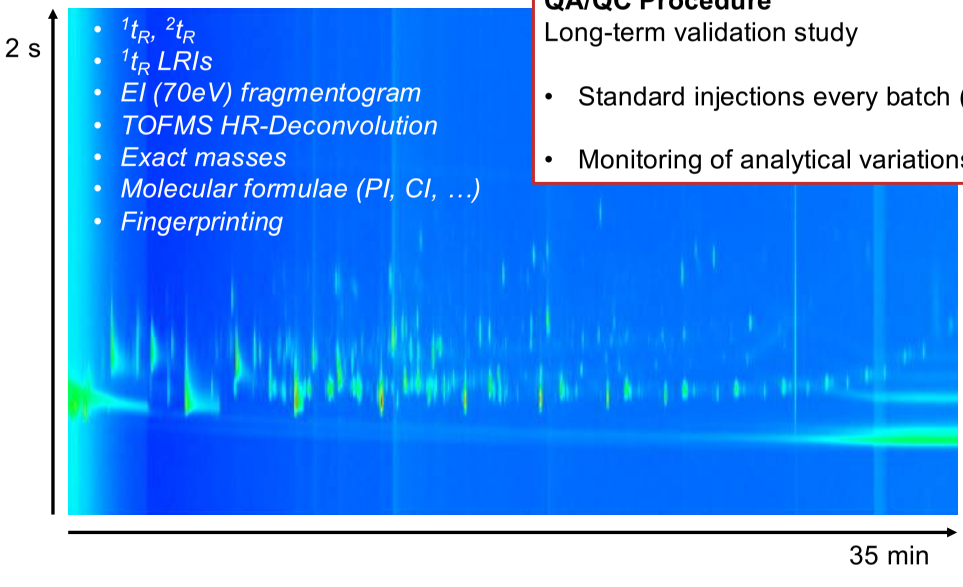


Incubation temperature and extraction time

Chemical Family		Compounds – BAL QC	
Hydrocarbons	Aliphatic	Nonane	2,6-dimethylheptadecane
	Naphthenic	1,2,4-trimethylcyclohexane	
	Aromatic	1,2,4-trimethylbenzene	
Aldehydes		Nonanal	Hexanal
Ketones		2-butanone	
Alcohols		2-ethylhexan-1-ol	3-methylbutan-1-ol
Others		Methoxyphenyloxime	
		1,2-nonadecanediol	
		2,3-octanedione	

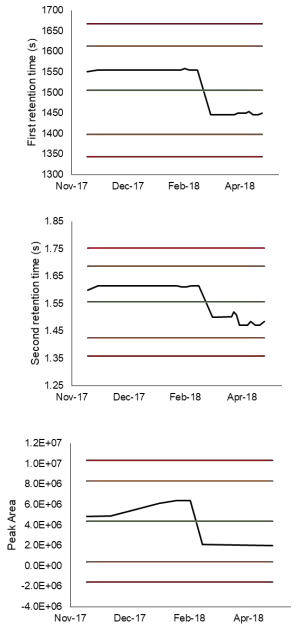
→ Selection of 40 °C - 30 min as the best compromise

Quality Control (BALFs)

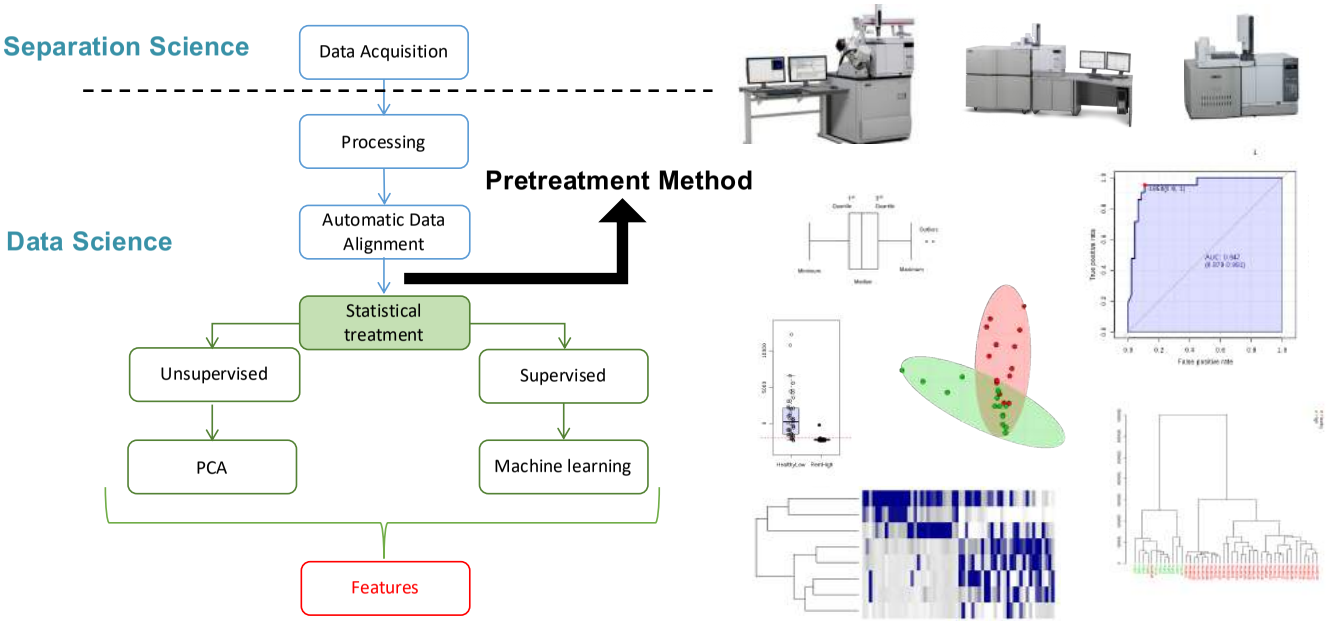


QA/QC Procedure
Long-term validation study

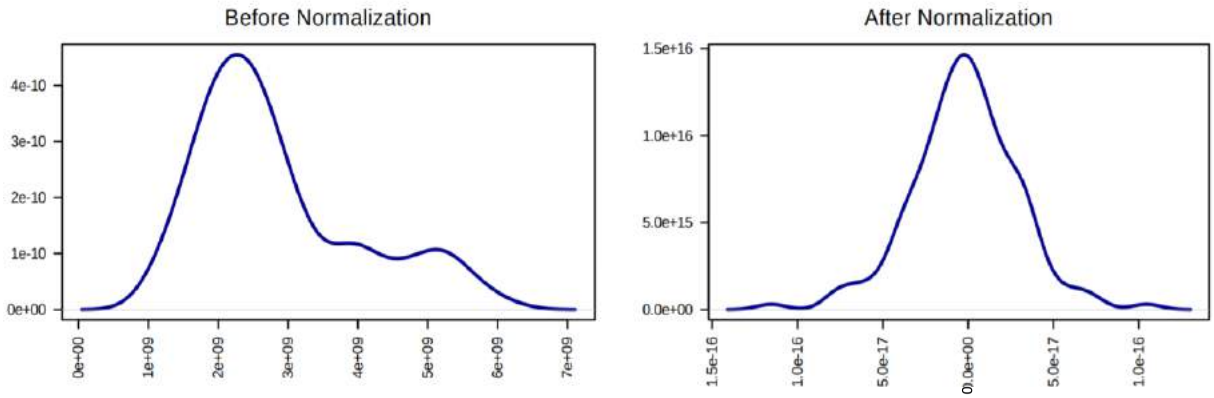
- Standard injections every batch (10)
- Monitoring of analytical variations



Data Science: Object of all Attentions



Pretreatment Method (BALFs)

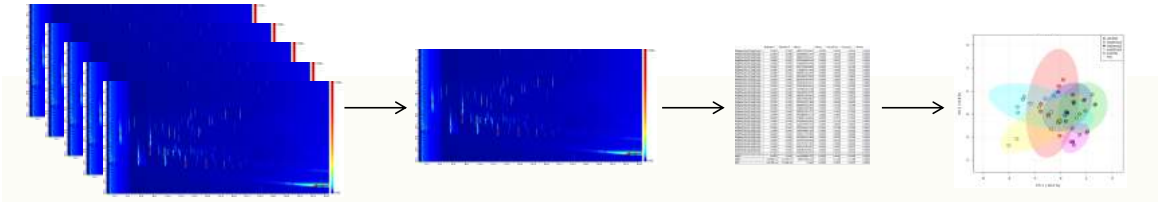


1. Normalization
2. Scaling
3. Transformation

Various Pathologies (BALFs)

Patient	Pathology	Abbreviation	Number of replicates
1	Chronic Obstructive Pulmonary Disease	COPD	7 x 500 µL
2	Asthma	Asthma1	10 x 500 µL
3	Asthma	Asthma2	9 x 500 µL
4	Asthma/COPD Overlap Syndrome	ACOS	5 x 500 µL
5	Asthma	Asthma3	10 x 500 µL
6	Pulmonary Sarcoidosis	PS	10 x 500 µL

51 samples

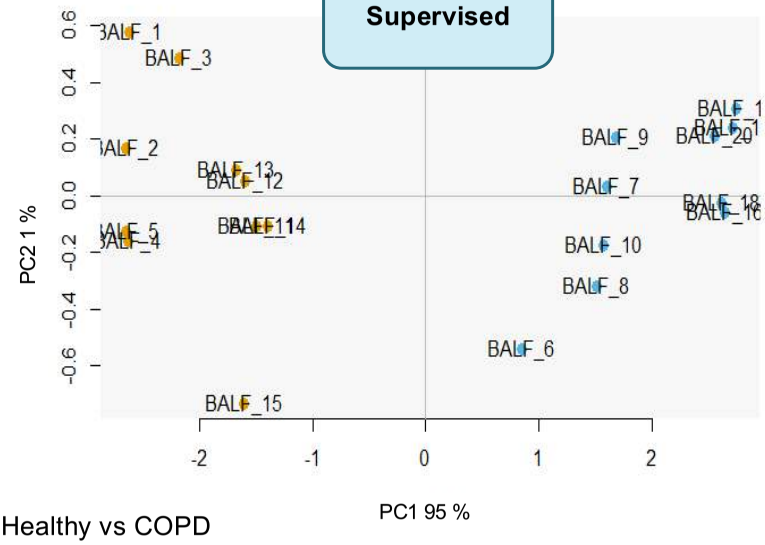


51-chromatogram superimposition → Cumulative image → Peaks table → Pattern recognition

Compounds extraction | Statistical analysis

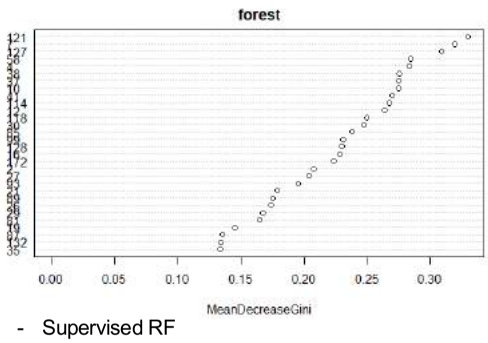
Robustness & Repeatability (BALFs)

Supervised



Healthy vs COPD

Features selection

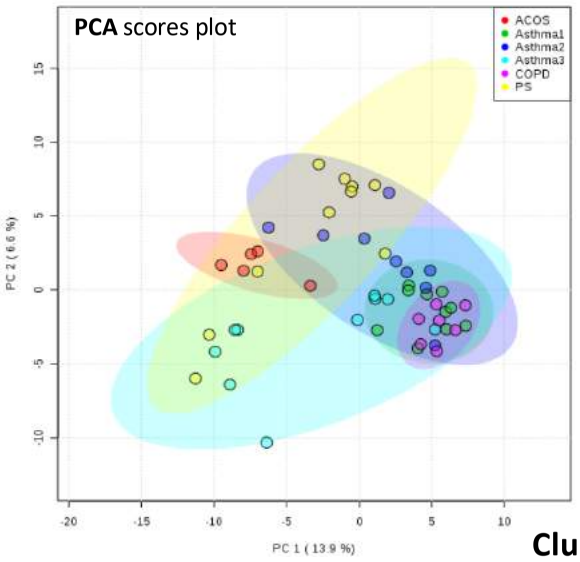


- Supervised RF
- BALF only

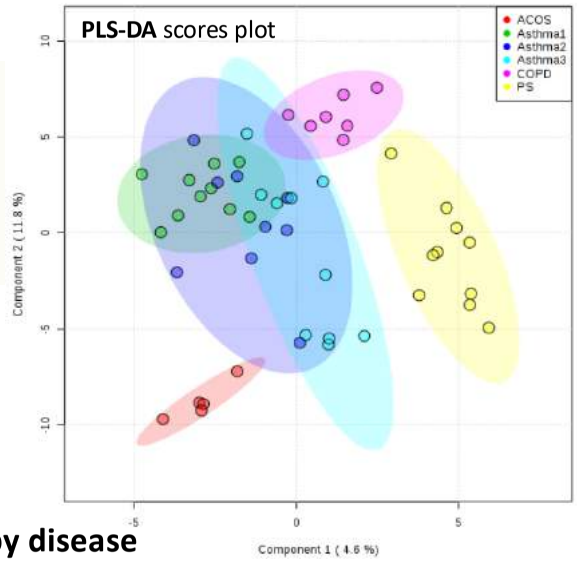
```
Type of random forest: classification
Number of trees: 500
No. of variables tried at each split: 14
OOB estimate of error rate: 0%
```

Various Pathologies (BALFs)

Unsupervised Pattern Recognition

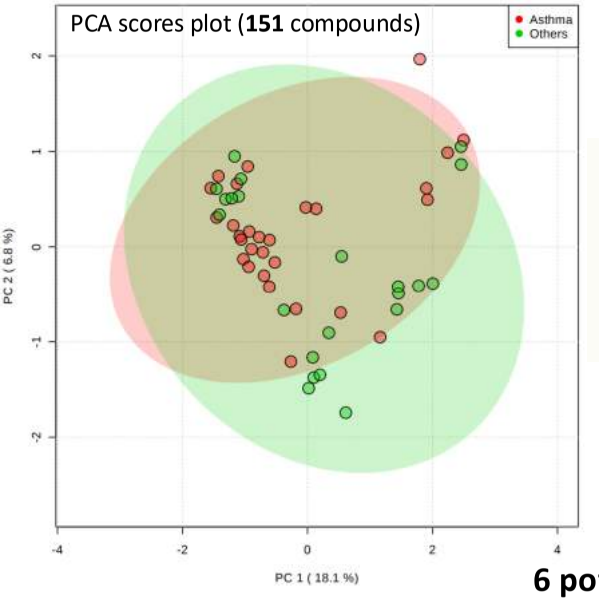


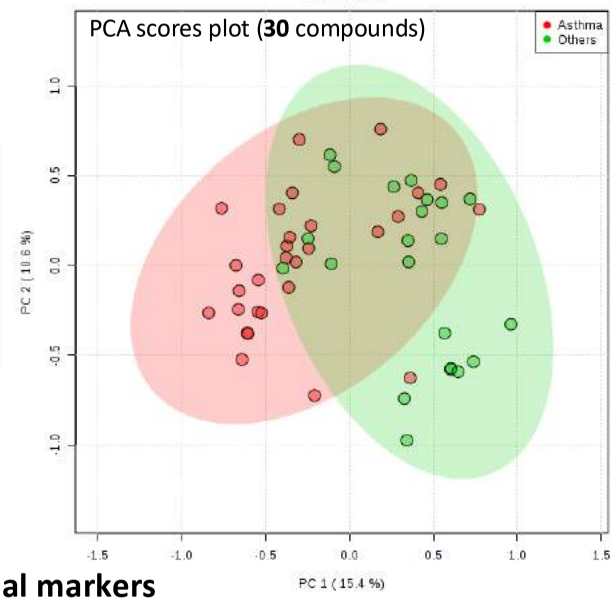
Supervised Pattern Recognition



Clustering by disease

Feature Selection (BALFs)



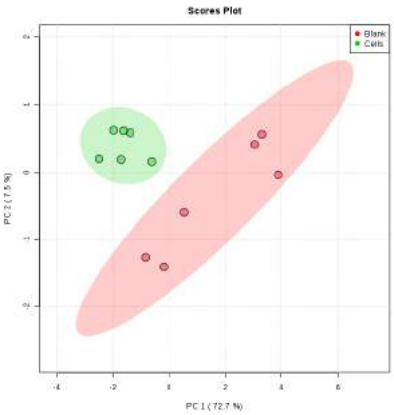


6 potential markers

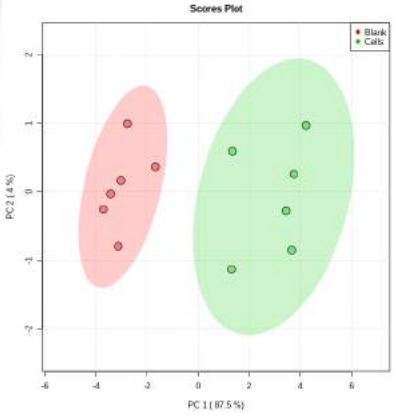
CELL CULTURE APPROACH

Cell Cultures – Proof of Concept

Differentiation between headspaces of fresh media and cultured media (A549 Epithelial Cells)

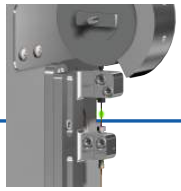


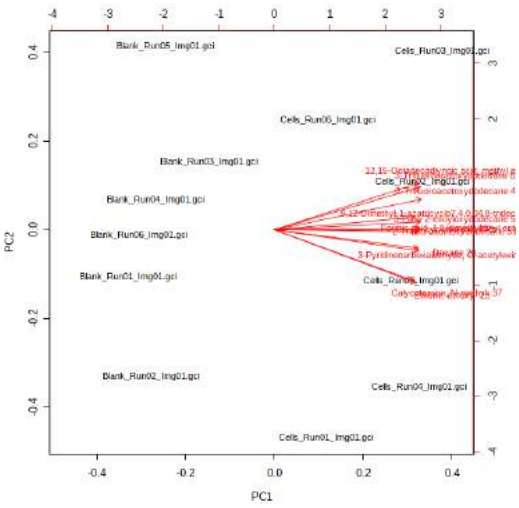
PC's : 80.2%
Features : 44 VOCs

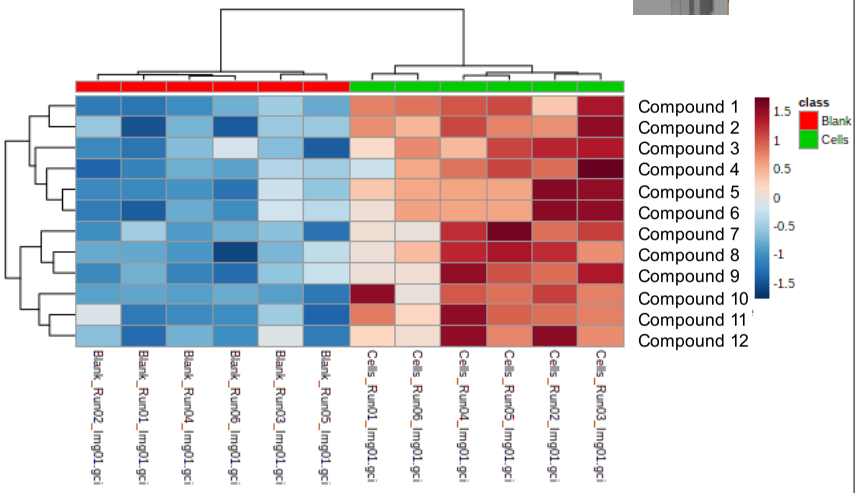


PC's : 91.5%
Features : 12 VOCs

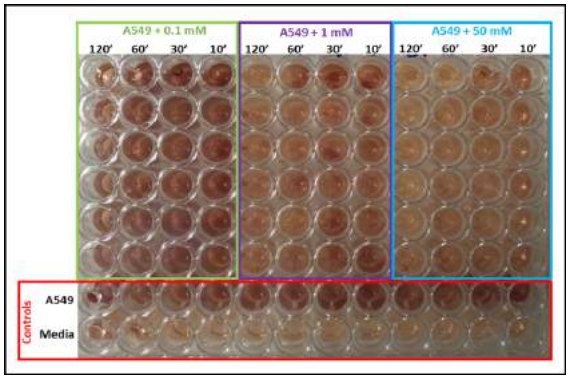
Focus on A549 Epithelial Cells

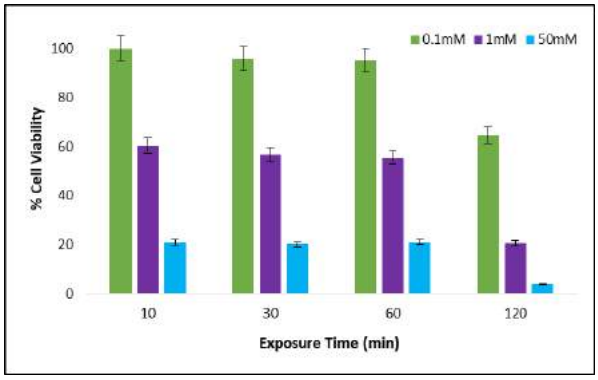






Effect of Oxidative Stress on A549 (H_2O_2)





The viability of A549 cells remains unchanged when using low concentration, *i.e.*, 0.1 mM of H_2O_2 for 1 h,

→ Conditions to explore metabolic changes of the cells under oxidative stress

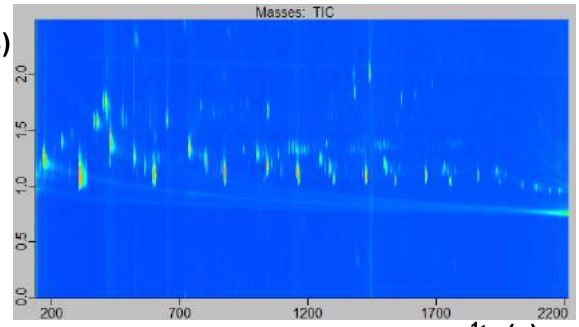
Effect of Oxidative Stress on A549 (H_2O_2)

In the headspace of the media

- ✓ SPME fiber : DVB/CAR/PDMS
- ✓ Temperature of extraction : 40 °C
- ✓ Extraction time : 30 min
- ✓ Desorption of the fiber : 3 min
- ✓ Inlet temperature : 250 °C

(Optimized conditions using a DoE)

Column Set :
^{1}D : non-polar Rxi-5MS (30 m × 0.25 mm × 0.25 µm d_f)
^{2}D : mid-polar Rxi-17SilMS (2 m × 0.25 mm × 0.25 µm d_f)
Modulation Period P_M : 2,5s
GC Temperature Ramp : 40°C to 270°C at 5°C/min

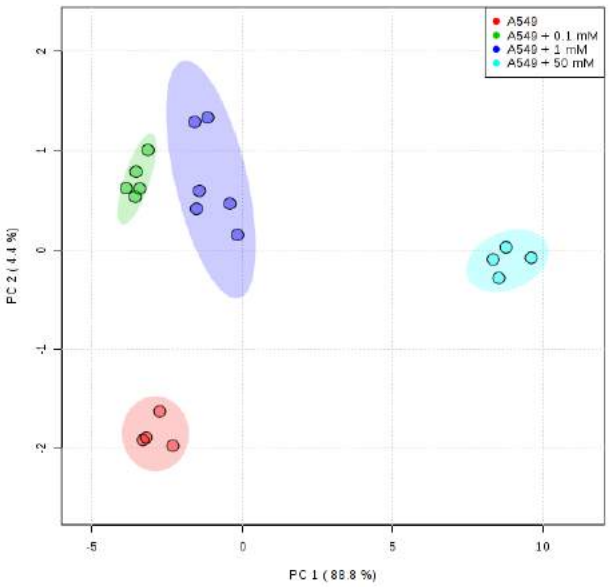


Stability of the Unstrumentation

ID#	Compounds	Chemical formula	CAS#	^1t_R (min)	^2t_R (s)	m/z	RSD (%)
1	2,3-Butanediol	$C_4H_{10}O_2$	6982-25-8	4.83	1.949	45	5.6
2	Decane	$C_{10}H_{22}$	124-18-5	9.832	1.289	57	6.0
3	1-Octanol	$C_8H_{18}O$	111-87-5	11.299	1.671	56	6.6
4	Undecane	$C_{11}H_{24}$	1120-21-4	11.899	1.304	57	6.4
5	Nonanal	$C_9H_{18}O$	124-19-6	11.999	1.728	41	6.1
6	2,6-Dimethylphenol	$C_8H_{10}O$	576-26-1	12.099	0.253	107	6.6
7	2,6-Dimethylaniline	$C_8H_{11}N$	87-62-7	13.299	0.52	106	6.3
8	Methyl caprate	$C_{11}H_{22}O_2$	110-42-9	16.032	1.663	74	7.2
9	Methyl undecanoate	$C_{12}H_{24}O_2$	1731-86-8	17.698	1.663	74	9.6
10	Dicyclohexylamine	$C_{12}H_{23}N$	101-83-7	17.732	1.863	138	12.3
11	Methyl laurate	$C_{13}H_{26}O_2$	111-82-0	19.232	1.677	74	11.1

Injection of 10 Grob mix successively to evaluate the repeatability and the stability of the BT 4D

Effect of Oxidative Stress on A549 (H_2O_2)

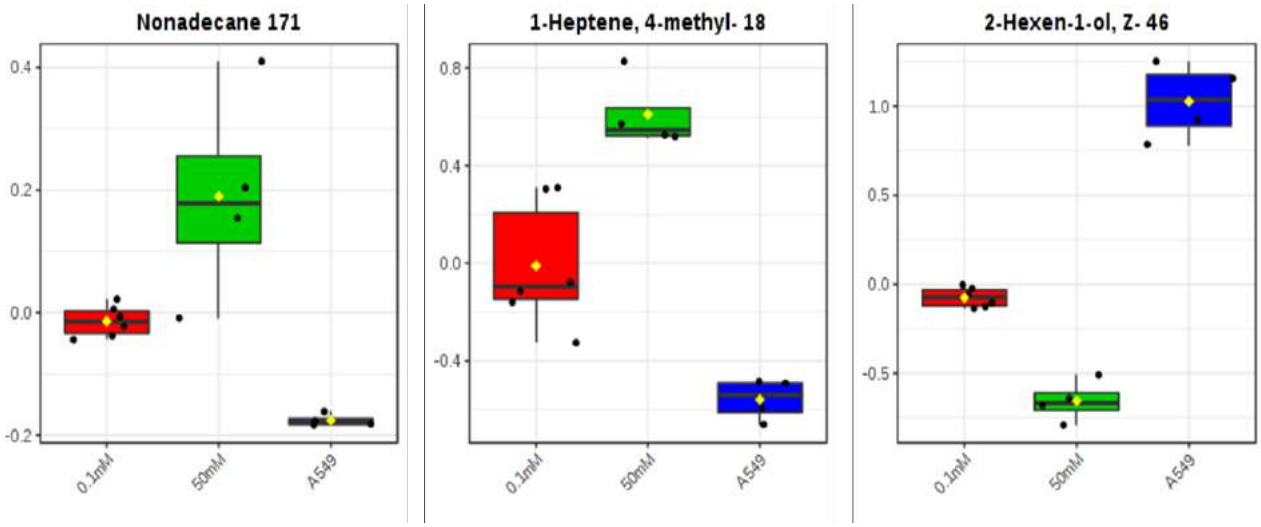


Obvious clustering tendency between H_2O_2 treated cells and non-treated cells is highlighted

Significant metabolic changes of the cells after H_2O_2 treatment for 1 h.

Features…

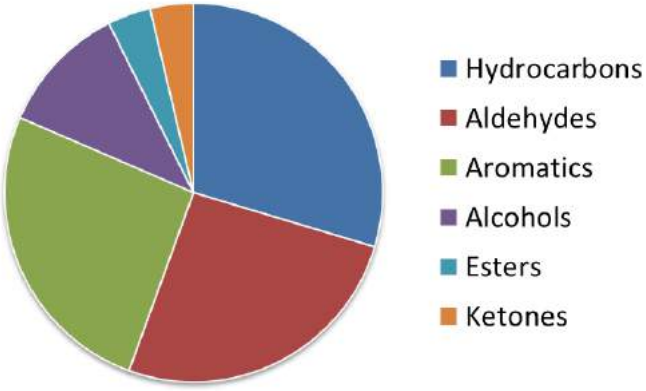
Effect of Oxidative Stress on A549 (H_2O_2)



Effect of Oxidative Stress on A549 (H_2O_2)

26 VOCs were tentatively identified:

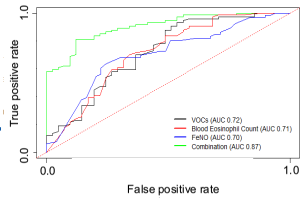
- Hydrocarbons
- Aldehydes
- Aromatic compounds



H_2O_2-treated A549 cells appear to be producing these 'suspected' families of VOCs

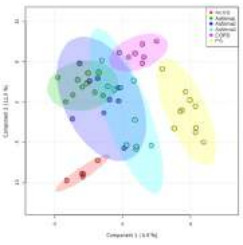
Oxidative stress markers found in both breath and cell media? (Under investigation)

Overall Conclusions

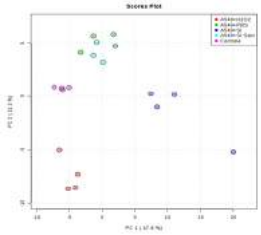


- GC×GC-(HR)TOFMS (& SIFTMS) is a powerful tool for asthma phenotyping)

- Ex-vivo BALF samples offers valuable headspace information on the volatilome of inflammation



- In-vitro cell cultures allow the study of the impact of oxidative stress on the volatilome



- Deeper investigation is undergoing…

Acknowledgments

P-H Stefanuto
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L M Dubois
T Dejong

CHU Liège – ULiege
Prof. R. Louis
Dr. F Schleich

GiGA I³
M Henket
V Paulus

1 post-doctoral position open at the moment

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