Differentiating asthma from chronic obstructive pulmonary disease via their metabolomic signatures – Preliminary work

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Introduction

Chronic obstructive pulmonary disease (COPD) diagnosis and subsequent management is largely based on clinical assessment which may lead to misdiagnosis that occurs, in particular, in primary care settings.[1] Clinical assessment does not always accurately differentiate COPD from asthma or from asthma-COPD overlap leading inadequate treatment and increased risk of morbidity and mortality.

Exhaled breath condensates (EBCs) are obtained by condensation of gases and droplets released during exhalation. Traditionally, pulmonologists focused on respiratory gases in these plumes, but it has been recently demonstrated the EBCs are rich sources of biomarkers and can reflect inflammation processes that are occurring within the lungs. However, biomarkers are often found a very low concentration.

This study aims to establish the metabolomics signature of COPD and asthma patients using liquid chromatography hyphenated to high-resolution mass spectrometry. Such untargeted analysis will rely on a computer-assisted MS analysis tool (CAMSAT) called Finnee to mine for features of very low intensities (peaks) and measure key chromatographic parameters (intensities, migration time and accurate masses).[2]

Methods

Figure 1: Schematic representation of the methodology developed for this work

The following methodologies were devised for this preliminary work:

- **EBC samples and clinical assessment**
  EBCs from 15 patients (5 healthy, 5 with asthma symptoms and 5 with COPD symptoms, as assessed by the Serviço de Imunoalergologia, Hospital Dona Estefânia) have been collected.

- **Separation parameters**
  Liquid chromatography: HALO C18, 2.7 µm, 90 Å, 0.5x50 mm column using a 30 min gradient from 5-95% B (solvent A: water + 0.1% FA; solvent B: Acetonitrile + 0.1% FA).
  Mass spectrometry: MS analysis was performed using a Sciex TripleTOF6600 with the DuoSpray Ion source. MS spectra accumulation time of 210 msec was used.

- **Data post-processing**
  Data were further analysed using *Finnee* (https://finneeblog.wordpress.com/) a Matlab toolbox that aims in mining chromatographic peaks in LC-HRMS datasets in an untargeted approach. The following steps were used:
  1. The original files were *converted to the mzML* format using msConvert.
  2. mzML files were then transformed to Matlab objects, corrected for *baseline drift and the background noise* removed.[3]
  3. All profile MS scans were transformed *to centroid MS scans.*
  4. Chromatographic *peaks were reconstructed* from the raw data and chromatographic parameters calculated (migration time, intensity and accurate mass) and summarised in a peaks table for each datasets.
  5. Resulting peak tables were *aligned* and the intensity of all peaks input in a matrix.

Results

Description of steps	Running Time	Size
Original file (wiff file)		*NA*
Conversion to mzML	15 min	*NA*
Conversion to Matlab Object	25 min	2.7 GB
Baseline correction	35 min	1.0 GB
Noise removal	15 min	8 MB
Conversion of profile MS scans to centroid scans	0.5 min	800 KB
Mining for chromatographic features and characterization	5 sec	*NA*

See Figure 2

See Figure 3

Table 1: Running time for the different computerised step for a single dataset (CPU: Intel Core i7 7700HQ @ 2.80GHz with 16.0GB of RAM)

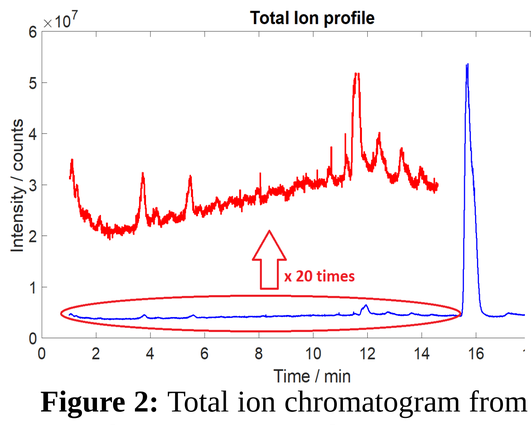

Figure 2: Total ion chromatogram from an asthmatic EBC sample

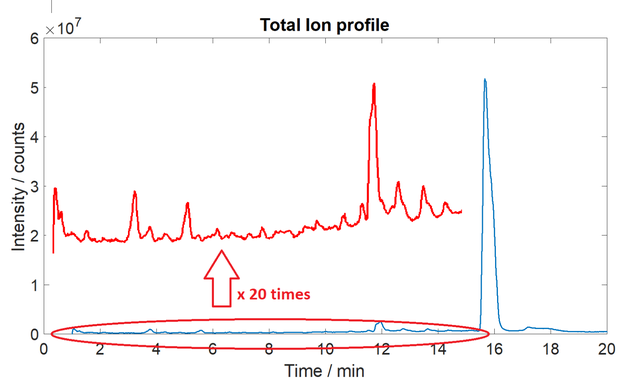

Figure 3: Total ion chromatogram after correcting for baseline drift and background noise

Main achievements:

- ✓ **1200 to 1500 peaks per dataset**
- ✓ **Intensities ranging from 500 to 5,000,000**
- ✓ **After peaks matching, 1173 selected peaks (4 to 15 occurrences in all datasets)**

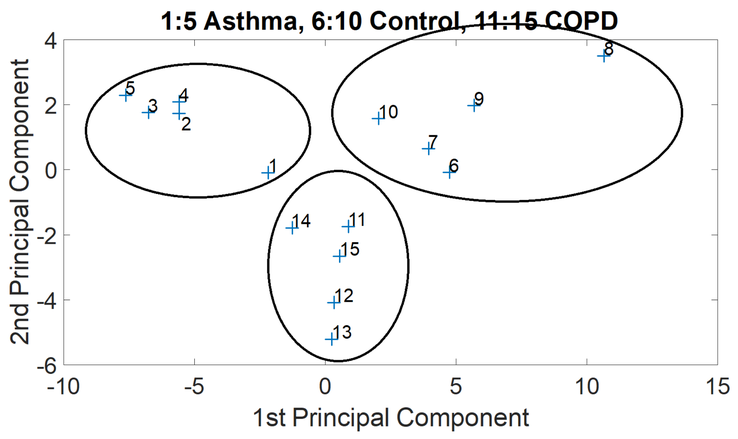

Figure 4: Variance weighted principal component analysis (PCA) using an intensity matrix with 15 observations (5 controls, 5 asthma and 5 COPD) and 1173 variables (peak intensities). Intensities were normalised before the PCA by the sum of intensities for each observation.

References

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Conclusions

- ✓ **Finnee allows extracting peak of very low intensity, ~1200 peaks whose intensities range four order of magnitudes were recover from each dataset;**
- ✓ **After PCA, asthma, COPD and control EBCs samples were accurately differentiated, without any samples preparation techniques;**
- ✓ **Simple and inexpensive sample pre-treatment techniques should be investigated to improve the quantity and quality of information;**
- ✓ **Specific biomarkers should now be identified and fully characterised;**
- ✓ **The study should be extended to include various asthma phenotypes and endotypes.**