Targeted eicosanoid lipidomics of induced sputum (IS) as compared to exhaled breath condensate (EBC) in asthmatic subjects

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Introduction
Eicosanoids are produced from arachidonic acid and rapidly inactivated. Induced sputum (IS) is a non-invasive material from the lower airways; its collection and processing is well standardized. Exhaled breath condensate (EBC) has the advantage of shorter collection time and lower protein content, but this is compromised by the extreme dilution and lack of standardized methods of measurement. Both methods have been introduced for assessment of inflammatory mediators in the asthmatic lung.

Aims and objectives
To compare concentration of several eicosanoids in IS and EBC samples collected according to the current guidelines and to estimate post-sampling redistribution of eicosanoids and their metabolites as a result of sample processing.

Methods
EBC was collected from asthmatic subjects (n=11) using Jaeger ECO Screen I; IS according to the most recent ERS Task Force recommendations. The same validated quantitative mass spectrometry platform was used for eicosanoids measurements organic phase extracted from both matrices. Random IS samples (n=6) were split following collection to inhibit enzymatic activity during solubilisation in non-physiological pH (normal saline).

Results
Twenty nine eicosanoids were measured, including all major prostaglandins, leukotrienes, and their metabolites. Average concentration of eicosanoids was 42 times lower in EBC than in IS. Important differences between EBC and IS included: absence of leukotriene C4, conversion of leukotriene B4 into 5-oxo-LTB4 and higher concentration of tetranor-PGEM in IS. IS solubilisation in non-physiological pH prevented these redistributions only partially.

Conclusions
Although processing of IS shifts eicosanoid profile toward their metabolites, significant amounts of these mediators are present within detection levels of common immunoassays. A strict adherence to the recommended IS collection and handling protocols is required to avoid a pre-analytical bias.
Most of eicosanoids indirect immunoassays have a limited sensitivity, around 7.5 pg/mL. To avoid biological matrix effects and to improve specificity, an extraction using solid phase adsorption is recommended. We recently validated our eicosanoids measurement platform according to the guidelines described by Honour (2011). The organic phase recovery varied between 88.9% for hydroxyeicosatetraenoic acids to 54.8% for prostanoids, 76.9% for prostaglandins metabolites, and 89.4% for leukotrienes. Stability of extracted samples was acceptable (z-score < |−1; 1|) within 48 hours before analysis if stored in 4°C. Lowest limits of detection and lowest limits of quantification were: 0.32 – 1.2, 0.48 – 1.8, 0.70 – 3.0, and 0.77 – 3.1 pg/mL respectively. Excellent linearity ($R^2 > 0.99$) was obtained in the range of concentration from 1 to 10 000 pg/mL for all tested eicosanoids except prostaglandins, which measurements were linear within the range from 1 to 250 pg/mL, and their higher concentrations could be estimated either from calibration curves or by a serial dilution of the material. The accuracy of assays was better than 99.2% for tested eicosanoids in the range characterizing biological samples (5 – 1 000 pg/mL), except PGE$_2$ and PGD$_2$ which accuracy was 98.7%. Finally, average intra-assay coefficient of variance (repeatability) was 5.42% and inter-assay reproducibility was 7.98%, in none analyte exceeding 10% or 15% respectively. We summarize, that chromatography – mass spectrometry assays for eicosanoids, especially combined with a stable isotope dilution method of internal standards, are suitable for targeted quantitative lipidomics of asthmatic patients.

Honour J.W. Development and validation of quantitative assays based on tandem mass spectrometry. 