UNTARGETED METABOLOMICS IN URINE TO INVESTIGATE SMOKING EXPOSURE

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Background: Although thousands of different chemicals have been identified in cigarette smoke, the characterization of urinary metabolites derived from those compounds is still not completely achieved. The aim of this work was to perform an untargeted metabolomic experiment on a pilot cross-sectional study conducted on subjects with different smoking habits.

Methods: Urine samples were collected from 67 adults; including 38 non-smokers, 7 electronic cigarette smokers, and 22 traditional tobacco smokers. Samples were analyzed by liquid chromatography/time-of flight mass spectrometer operating in data dependent mode. Data were processed using the R-packages IPO and XCMS to perform feature detection, retention time correction and alignment. The ANOVA test was used to detect significant features among groups. The software BEAMS (University of Birmingham) was implemented for grouping adducts and isotopes, and to perform a first annotation. Annotation was completed by comparing fragmentation patterns with on-line databases as Metlin, and using the software MS-FINDER.

Results: One hundred and seventeen features, out of 3613, were statistically different among groups. We estimated that they correspond to about 80 metabolites, of which we were able to putatively annotate about half. The identification of the mercapturic acids of acrolein, 1,3-butadiene, and crotonaldeide, chemicals known to be present in tobacco smoke, supports the validity of the proposed approach. With a lower level of confidence, we annotated the glucuronide conjugated of 3-hydroxycotinine and the sulfate conjugate of methoxyphenol; finally, with the lowest degree of confidence, several other sulfate conjugates of small molecules were annotated.

Short discussion/conclusions: The proposed approach seems to be useful for the investigation of exposure to toxicants in humans.