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Title: Do volatile organic compounds discriminate between eosinophils and neutrophils in vitro?

Dr. Florence 4443 Schleich fschleich@chu.ulg.ac.be MD ¹, Jan 4444 Dallinga j.dallinga@maastrichtuniversity.nl ², Monique 4445 Henket moniquehenket@hotmail.com ¹, Agnes 4447 Boots a.boots@maastrichtuniversity.nl ², Prof. Dr Emiel 4448 Wouters e.wouters@mumc.nl MD ³, Prof. Dr Renaud 4449 Louis r.louis@chu.ulg.ac.be MD ¹ and Prof. Frederik-J. 4453 van Schooten f.vanschooten@maastrichtuniversity.nl ². ¹ Respiratory Medicine - GIGA I³ Lab, CHU Sart-Tilman, Liege, Belgium, 4000 ; ² Health Risk Analysis and Toxicology, Maastricht University, Maastricht, Netherlands, 6200 and ³ Respiratory Medicine, University Hospital, Maastricht, Netherlands, 6200 .

Body: Inflammation associated oxidative stress leads to peroxidation of polyunsaturated fatty acids thereby generating volatile organic compounds (VOCs) excreted in exhaled air. The purpose of the present study is to examine whether specific VOCs are associated with eosinophilic and neutrophilic inflammation, and thus offers the possibility of noninvasive monitoring of both asthma inflammatory phenotypes. Methods Eosinophils and neutrophils were isolated from 27ml blood of 16 healthy non-smokers by gradient centrifugation using lymphoprep. Eosinophils were isolated from neutrophils by immunomagnetic cell separation (MACS) using anti-CD16. The average absolute number of eosinophils and neutrophils upon isolation was 3.5 x 106 and 19.4 x 106 respectively. Cells were incubated in RPMI at 37°C and activated with phorbol 12-myristate 13-acetate (100ng/ml). Headspace air was sampled at time 0', 30', 60' and 90' by introduction of ultra-pure nitrogen in closed flasks at a flow rate of 200 ml/min during 10 min. The air was pushed out onto a carbon tube and the total amount of trapped VOCs (volatome) was analysed by time-of-flight GC-MS. Results From the 2005 compounds present in the volatome, those present in at least 8% of the samples (1123 compounds) were used for further analysis. Discriminant analysis (SPSS statistics19) showed that 5 VOCs were able to distinguish between both culturing types with 100% and 96% correct classification in original and cross-validated set respectively. Chemical identification of the compounds is ongoing and these are potential candidates to check in asthmatic patients for their possible diagnostic value in asthma phenotyping.