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Title: Influence of inflammatory phenotype in severe refractory asthma on metabolomic profile of oxidative stress in exhaled breath condensate (EBC)

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Body: Severe refractory asthma (SRA) is still a disputable medical condition. It is supposed that intensity of inflammation and oxidative stress (ROS) exceeds the level in controlled asthma.Aims: Our hypothesis was that oxidative stress metabolomics in exhaled breath condensate (EBC) differs in SRA in distinct phenotypes. We harvested EBC of 40 SRA patients from center for SRA and markers of ROS were compared between subgroups of eosinophilic (EA, n=13) and non-eosinophilic (NEA, n=26) phenotype defined by peripheral blood eosinophilia (PBE) >4%, results were compared to 21 healthy controls. Methods: Metabolomic analysis of EBC using liquid chromatography and mass spectrometry was used to detect concentrations of 22 markers of ROS (malondialdehyde, leukotriens, 4-hydroxy-2-noneal (4-HNE). EBC was collected by standardized protocol (EcoScreen). Measured results were analyzed together with FEV1, FeNO50, PBE and subgroups statistically evaluated. Results: EA and NEA did not differ in gender, age (48.2 vs 51.2), presence of atopy, asthma control test score (13.0 vs 14.5), FEV1 (1.5 vs 1.6 L/s) (all $p>0.05$). In EA were higher FeNO levels (18 vs 46 ppb, $p=0.01$), hyperinflation (RV 3.84 vs 3.02 l, $p=0.049$), asthma exacerbations (6.3 vs 2.9 per year, $p<0.01$), rhinosinusitis (57.7 vs 92.3%). 4HNE was higher in NEA group (52.1 vs 40.1 pg/ml, $p<0.03$). All other measured markers in EBC did not differ between EA and NEA, but were higher in comparison to control group (all $p<0.001$). Conclusion: Our data shows increased lipoperoxidation of ω-3 and 6 fatty acids by ROS only in NEA. We conclude that NEA phenotype of SRA would benefit from antioxidative therapy.