Induced sputum (IS) allows to measure mediators of asthmatic inflammation in bronchial secretions. Aspirin-exacerbated respiratory disease (AERD) is recognized as a distinct asthma phenotype, usually with a severe course, eosinophilic airway inflammation and increased production of pro-inflammatory biomarkers such as endothelial alarmin-like cytokines (IL-33, IL-25, and TSLP), all of which could activate innate lymphoid cells type 2 (ILC2s). ILC2 activation is dependent on local respiratory epithelium damage and results in the generation of pro-inflammatory cytokines. It was shown that ILC2s are recruited to nasal mucosa of AERD patients after COX-1 inhibitor administration, correlating with enhanced production of prostaglandin D2 (PGD2) and cysteinyl leukotrienes (cys-LTs). On the one hand, ILC2s activate and recruit mast cells and eosinophils by production of cytokines such as IL-5 and IL-9 and on the other ILC2s are activated in response to released PGD2 and cysLTs.

**The aim of the study will be to assess for the first time the possible changes of** (1) the number or percentage, (2) phenotype, and (3) functional characteristics of both blood and sputum ILC2s at baseline before aspirin challenge as compared to those at the time of developing aspirin bronchospasm in AERD patients. We are going to use cytometry for a broad range of surface (CD127+, CD161+, CRTH2+ and EP2+, EP4+) and intracellular markers (IL-5+, IL-13+) expression to accurately identify and phenotype ILC2s.

**The research is to be carried out at baseline before aspirin challenge and during acute aspirin-induced bronchospasm in AERD patients:**

**(1)** measure the percentage of circulating ILC2s and the number of sputum ILC2s (CD161+, CRTH2+, EP2+, EP4+) using flow cytometry analyses, **(2)** perform phenotypic analyses of blood and sputum ILC2s expressing $CRTH2^+$ and $EP2^+$, $EP4^+$ receptors at base and during acute aspirin-induced respiratory reaction. CRTH2 is a receptor for PGD2 and EP2, EP4 are receptors for PGE2, **(3)** perform functional analyses of blood and sputum ILC2s expressing intracellular levels of $IL\text{-}5^+$ and $IL\text{-}13^+$ , **(4)** evaluate the influence of aspirin on epithelial production of IL-33, TSLP and IL-25 directly affecting ILC2s. Serum and induced sputum supernatant (ISS) IL-33, TSLP, and IL-25 levels will be assessed using ELISA kit in cell culture serum and supernatant, **(5)** measure the level of type 2 cytokines (IL-4, IL-5, IL-6, IL-9, and IL-13) using ELISA in serum and ISS, **(6)** measure the level of some eicosanoids (leukotrienes C4, D4, E4, PGD2 and PGE2) in ISS using mass spectrometry and urinary PGD2 metabolite and LTE4 using ELISA, **(7)** evaluate sputum cells analyses (cell phenotypes of asthma base on induced sputum), **(8)** measure both serum and sputum neutrophilia, and ISS level of IL6, IL8, IL17, **(9)** measure FeNO in exhaled breath with standardized methods.

**Reasons for choosing the research topic:** This research is primarily designed to expand and gain new knowledge about the effect of aspirin on the number, phenotype and functional characteristics of both serum and sputum ILC2s in AERD patients. Even more important is the fact that the results of this study will provide evidence supporting an effector role for ILC2s in patients with AERD and in particular consider the potential of anti-ILC2 (anti-CRTH+, anti-IL-13) therapeutic implications.