

SUPPLEMENTARY MATERIAL

Supplementary E1. THE MEGA STUDY: Methodology and main objectives

THE MEGA Study is a multicentre prospective cohort study including consecutive patients with objectively confirmed asthma from eight university hospitals in Spain. MEGA is carried out within the framework of the CIBER of Respiratory Diseases.

This cohort includes 532 subjects. Standard data collection methods were used in all participating centres. An electronic database and case report forms were designed to collect study data. We included patients with asthma and seen during the study period in each hospital, aged 18–75 years, with intermittent, mild, moderate, or severe asthma, who have been diagnosed based on the Global Initiative for Asthma (GINA) criteria [3], at least 1 year before inclusion. Control was assessed following criteria from GINA [3], and patients were subsequently classified as controlled, partially controlled and not controlled. Patients were excluded only if they have other acute or chronic active lung disorders, or significant psychiatric disorders. All patients signed an informed consent. Individuals were contacted via the outpatient clinic of the participating institutions.

A standardised clinical history was completed for each patient, including variables that have been agreed by a multidisciplinary team of project investigators. Validated Spanish versions of the following questionnaires were administered: Asthma Control Test (ACT) in which patients with 20 points or more were classified as controlled asthma and scores up to 19 points were considered uncontrolled asthma), Mini-Asthma Quality of Life Questionnaire (Mini-AQLQ), the Sino-Nasal Outcome Test 22 (SNOT-22), and the Hospital Anxiety and Depression Scale (HADS). Adherence was evaluated using the 4-item Morisky-Green questionnaire on adherence, which includes 4 questions with yes/no response options and is scored from 0 to 4. The tool reflects 3 levels of adherence on the basis of this score: high (0 points), intermediate (1 to 2 points), and low (3 to 4 points) [Supl 1].

All study subjects underwent a detailed clinical examination, including body mass index (BMI) and respiratory function tests (baseline spirometry, bronchodilator test, lung volume measurement by plethysmography, fraction of exhaled nitric oxide [FeNO], and CO transfer test [DLCO] using the single-breath method), following the recommendations of the European Respiratory Society. Methacholine challenge (PC_{20}) was performed at baseline. Chest x-ray and skin prick tests (SPT) with common aeroallergens were performed at the beginning of the study. The panel of aeroallergens comprised the following: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*, *Cupressus arizonica*, *Platanus acerifolia*, *Olea europaea*, *Phleum pratense*, *Artemisia vulgaris*, *Parietaria judaica*, *Salsola kali*, *Blatella orientalis*, and danders (cat and dog). SPTs will be considered positive with wheal diameters of at least 3 mm compared to the negative control (saline). Histamine (10 mg/ml) was used as a positive control. Atopy is defined as the presence of at least one positive SPT or aeroallergen-specific immunoglobulin E (IgE) in serum. The number of eosinophils were determined in peripheral blood samples. The number of eosinophils in induced sputum (IS) was determined by Wright-Giemsa staining for a differential cell count after mucolysis of the sputum samples with dithiothreitol, as described [Supl 2]. Eosinophilic sputum was considered when $\geq 3\%$ eosinophils in sputum were observed while neutrophilic asthma was considered if the percentage of neutrophils was at least 61% [Supl 3].

Supplementary References

Supl 1. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. *Med Care*. 1986;24:67-74.

Supl 2. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace, D, et al. Indices of Airway Inflammation in Induced Sputum: Reproducibility and Validity of Cell and Fluid-Phase Measurements. *Am J Respir Crit Care Med*. 1996;154:308-17.

Supl 3. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*. 2006;11:54-61.