Seasonal Variations in the Concentration of Particulate Matter in the Air of Cracow Affect the Magnitude of CD4+ T cell Subsets Cytokine Production in Patients with Inflammatory and Autoimmune Disorders

Adrianna Gałuszka-Bulaga [1], Kazimierz Węglarczyk [1], Paweł Latacz [2], Katarzyna Jodłowska-Cicio [3,4], Mariusz Korkosz [3], Joanna Pera [2], Agnieszka Słowik [2], Maciej Siedlar [1] and Jarek Baran [1,*]

[1] Department of Clinical Immunology, Institute of Pediatrics, Jagiellonian University Medical College, 30-663 Cracow, Poland; adrianna.galuszka@doctoral.uj.edu.pl (A.G.-B.); kazimierz.weglarczyk@uj.edu.pl (K.W.); misiedla@cyf-kr.edu.pl (M.S.)

[2] Department of Clinical Neurology, Jagiellonian University Medical College, 31-008 Cracow, Poland; pawel.latacz@uj.edu.pl (P.L.); joanna.pera@uj.edu.pl (J.P.); agnieszka.slowik@uj.edu.pl (A.S.)

[3] Department of Rheumatology and Immunology, Jagiellonian University Medical College, 30-688 Cracow, Poland; katarzyna.jodlowska@gmail.com (K.J.-C.); mariusz.korkosz@uj.edu.pl (M.K.)

[4] Division of Rheumatology and Immunology, University Hospital, 30-688 Cracow, Poland

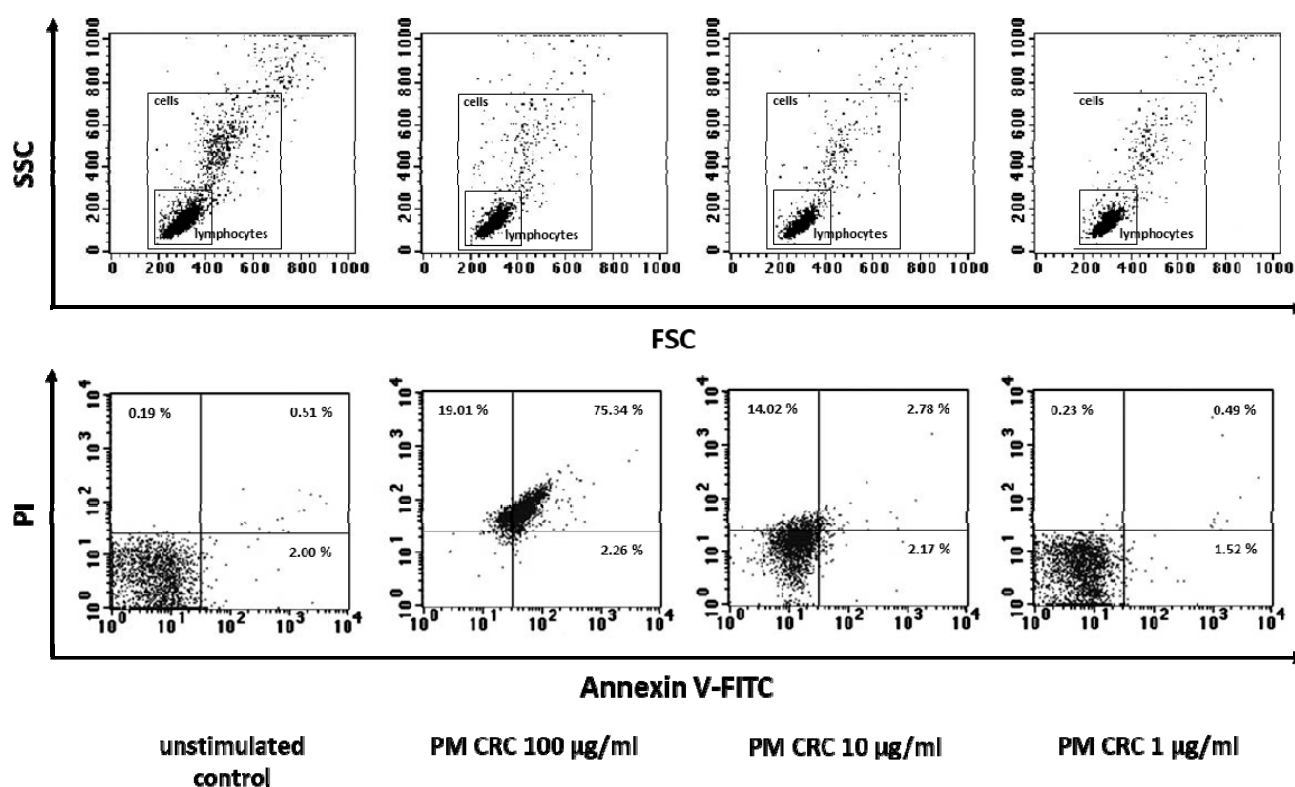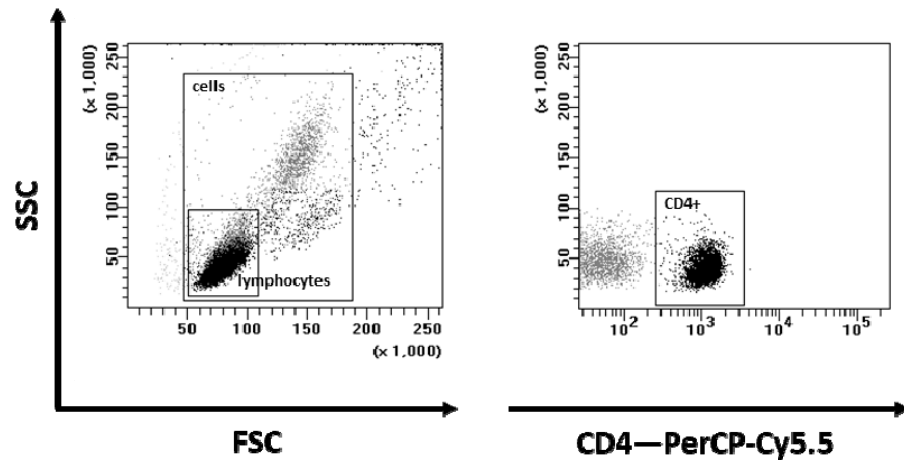* Correspondence: mibaran@cyf-kr.edu.pl

Figure S1. Flow cytometry analysis of viability of cells after exposure of PBMCs to Cracow air pollutants (PM CRC). Gating strategy for lymphocytes identification. Lymphocytes were defined according to FSC vs. SSC parameters from PBMCs. Dot plots Annexin V-FITC vs. Propidium iodide (PI) show lymphocytes positive for Annexin V and PI in unstimulated control or cells stimulated with PM CRC in three different concentration (100, 10 and 1 μg/mL) to establish the non-toxic dose of air pollution for investigated cells. Shown are the data from one representative experiment using PBMCs from healthy donor.
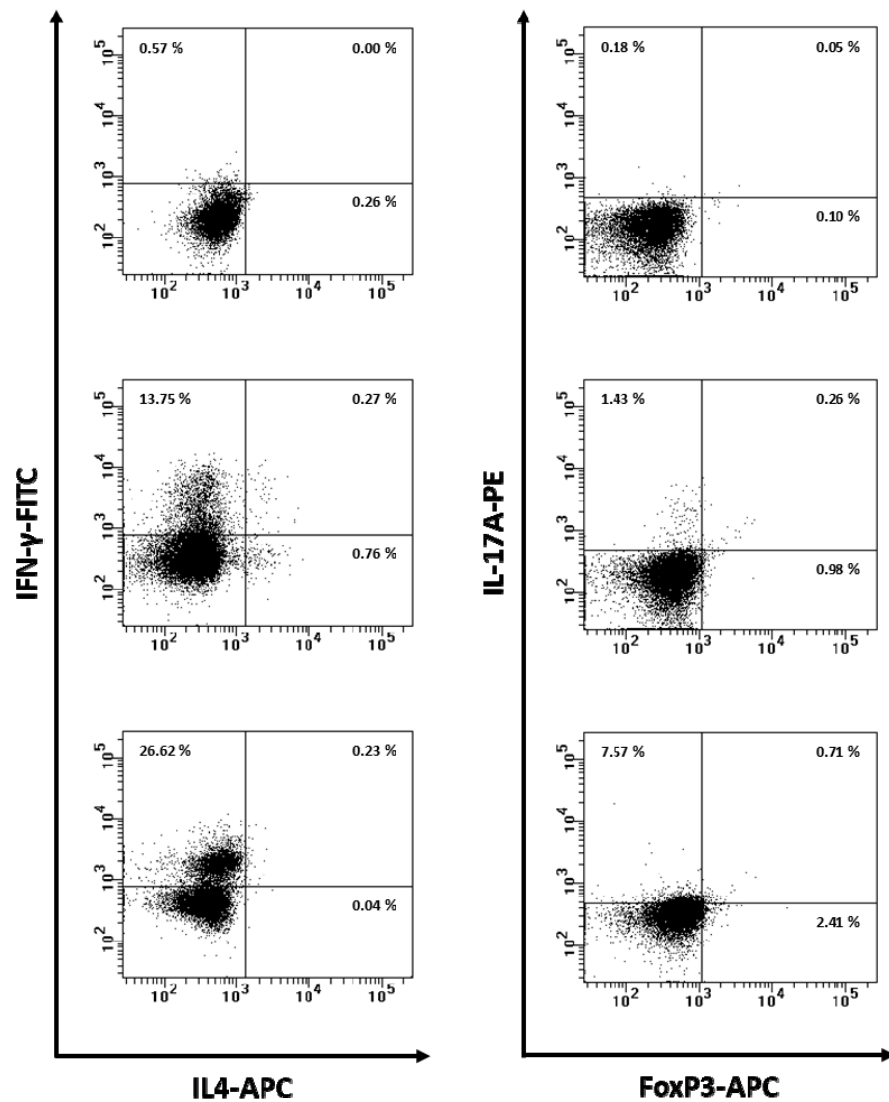
A

B

Figure S2. Flow cytometry analysis of intracellular proteins characteristic for specific Th subsets after exposure of PBMC to Cracow air pollutants (PM CRC). (A) Gating strategy for CD4+ T cell identification. Lymphocytes were defined according to FSC vs. SSC parameters from PBMC and then Th cells were identified according to CD4 expression. (B) Dot plots (IFN-γ-FITC vs. IL-4-APC; IL-17A-PE vs. FoxP3-APC) show CD4+ T cells positive for IFN-γ, IL-4, IL-17A and FoxP3 in unstimulated control, cells stimulated with PMA (50 ng/mL) + ionomycin (100 ng/mL) (positive control) or stimulated with PM CRC (10 μg/mL). Shown are the data from one representative experi-

ment using PBMCs from healthy donor. The expression of intracellular proteins IFN-γ, IL-4, IL-17A and FoxP3 was analyzed by flow cytometry after the cells were stained with cocktail of fluorescently conjugated monoclonal antibodies, specific for IFN-γ (Th1); IL-4 (Th2); IL-17A (Th17); FoxP3 (Treg).
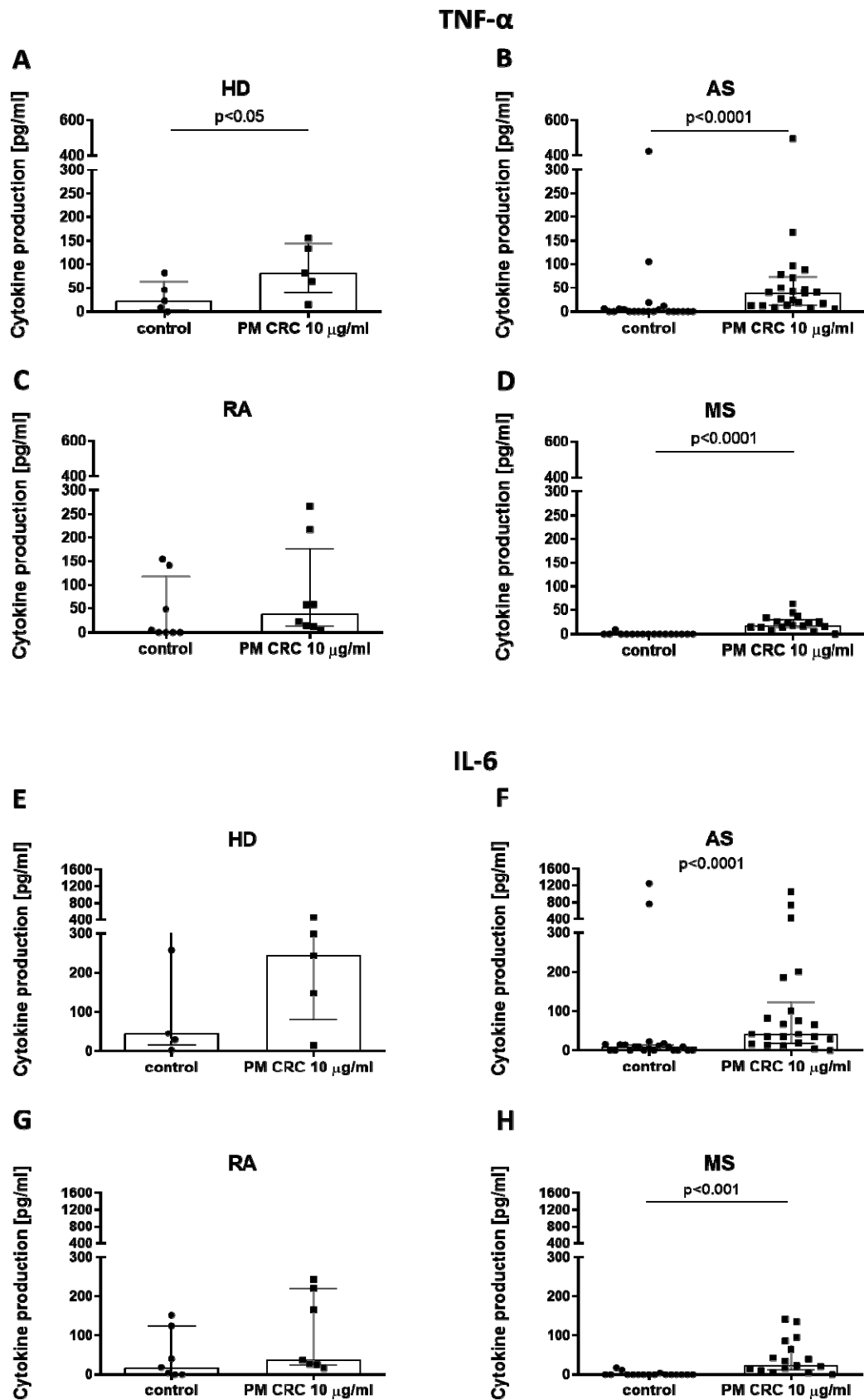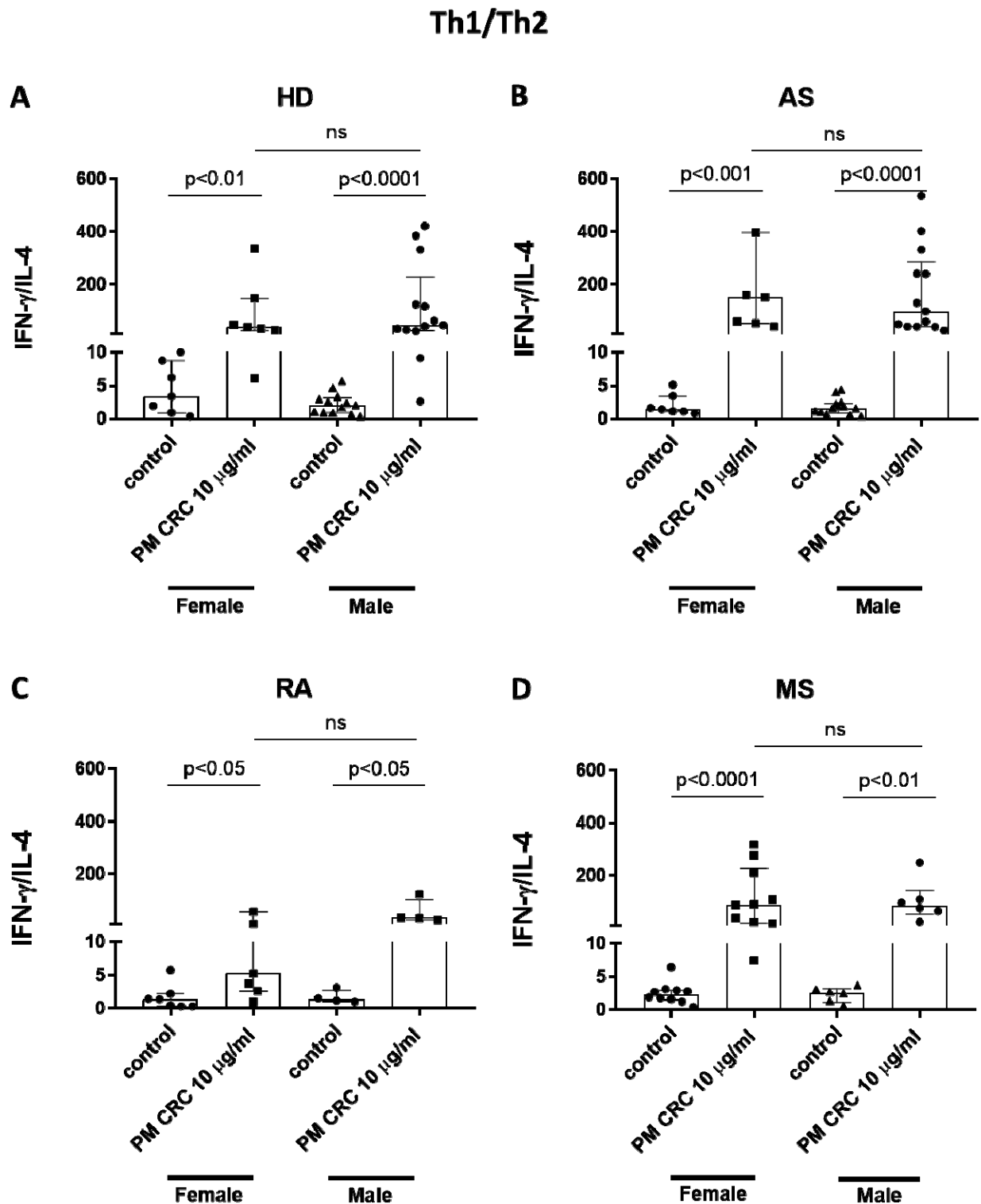
TNF-α

IL-6

Th1/Th2

Figure S4. Effect of the exposure of PBMCs to PM CRC on the ratio of Th1/Th2 cells by gender in patients and HD. After PBMCs stimulation, the frequency of Th1 and Th2 cells was quantified as the percentage of cells positive for IFN-γ and IL-4, respectively. The ratio of IFN-γ positive (%) to

IL-4 positive (%) CD4+ T cells (Th1/Th2) was calculated in HD (A) and patients with AS (B), RA (C) and MS (D Data are presented as median ± interquartile range from 7 independent experiments for females and 13 for males in HD (A) and AS (B) group, 7 for females and 4 for males in RA (C) group, for 10 females and for 6 males in MS (D) group. Differences between the groups were considered statistically significant at the following p values: p < 0.05; p < 0.01; p < 0.001; p < 0.0001.

Th17/Treg

Figure S5. Effect of the exposure of PBMCs to PM CRC on the ratio of Th17/Treg cells by gender in patients and HD. After PBMCs stimulation, the frequency of Th17 and Treg cells was quantified as the percentage of cells positive for IL-17A and FoxP3, respectively. The ratio of IL-17A positive (%) to FoxP3 positive (%) CD4+ T cells (Th17/Treg) was calculated in HD (A) and patients with AS (B), RA (C) and MS (D). Data are presented as median ± interquartile range from 7 independent experiments for females and 13 for males in HD (A) and AS (B) group, 7 for females and 4 for males in RA (C) group, for 10 females and for 6 males in MS (D) group. Differences between the groups

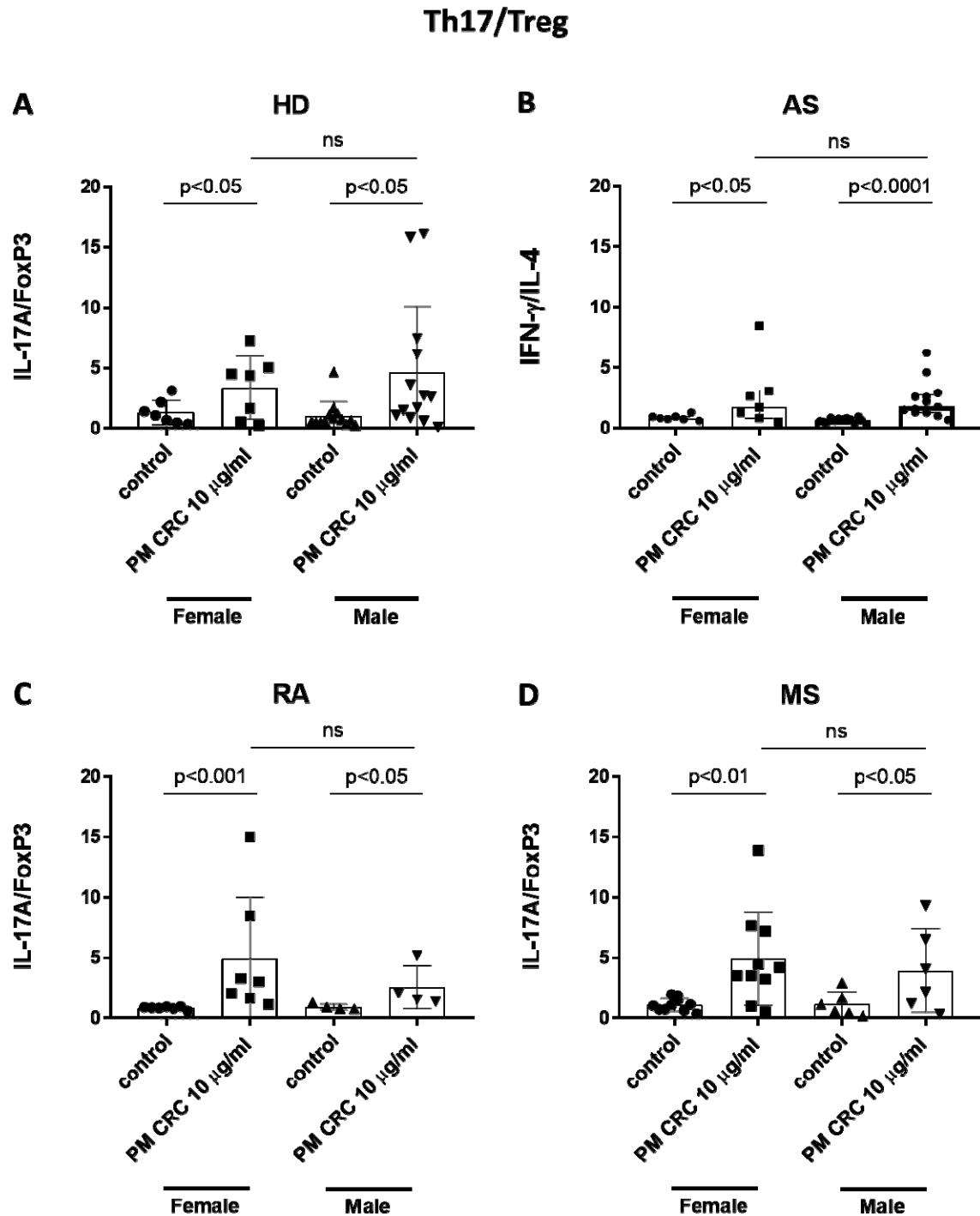were considered statistically significant at the following p values: $p < 0.05$; $p < 0.01$; $p < 0.001$; $p < 0.0001$.