Appendix S1.

MATERIALS AND METHODS

Subjects

The study was approved by the local Bioethical Committee according to the principles of the Declaration Helsinki (No. 283/2008 and No. 672/2012). Twenty-four subjects with PsA and 26 controls with no history of psoriasis or any other skin disease, matched by age and sex, were recruited to the study. The control group was selected for the study from among hospital staff and volunteers. Exclusion criteria were: history of CVD, chronic renal or liver disease, diabetes mellitus, skin disease other than psoriasis, malignancies or any significant abnormalities in blood count. The patients with PsA did not receive any topical or systemic therapy at least 4 weeks prior to the initiation of the study, and had never been treated with a TNF-α antagonist. Subjects underwent clinical evaluation at the time of recruitment. Demographic data as well as information regarding presence of conventional cardiovascular risk factors (hypertension, diabetes, obesity, smoking habit) and medication use were documented.

Patients underwent clinical evaluation, performed by trained health professionals, at the time of recruitment. The diagnosis of PsA was confirmed by the Classification Criteria for Psoriatic Arthritis (CASPAR) (23). The baseline evaluation included disease activity score (DAS) 28 and assessment of peripheral joint (68 joints for tenderness and 66 joints for swelling). All patients had peripheral type of PsA; either asymmetrical oligoarthritis or symmetrical polyarthritis, according to the Moll & Wright classification (24). In the majority of cases the wrist and metacarpophalangeal and interphalangeal joints of the hands were affected (94.2%). Four (17%) patients reported inflammatory back pain. In addition, all patients displayed current psoriatic skin lesions and nail involvement. The severity of skin involvement was assessed with the PASI. There were no statistically significant differences regarding incidence of assessed cardiovascular risk factors, such as obesity, hypertension, smoking habit and medication use, between patients with PsA and controls. Fifteen ml peripheral blood samples were taken on ethylenediametetraacetic acid (EDTA) from all studied subjects in order to evaluate the number of CEPCs. Seventeen patients with PsA agreed to donate an additional 10 ml of peripheral blood, at the same time point, in order to assess the levels of CRP and VEGF.

Assessment of CEPCs

Peripheral blood samples, taken on EDTA, were incubated for 10 min at 4°C with 10 µl FcR-blocking reagent (Human FcR-Blocking Reagent, Miltenyi Biotec GmbH (Bergisch Gladbach, Germany), cat. 1200-000-44) and for 30 min at 4°C with selected antibodies at the concentrations suggested by the manufacturer. To determine numbers of CEPCs (CD133$^+$, VEGFR-2/KDR$^+$ cells), samples were labelled with APC-conjugated anti-CD133 antibodies (anti-human CD133 APC, eBiosciences, cat. 17-1338-42) and PE-conjugated anti-VEGFR-2/KDR antibodies (anti-human VEGFR-2(KDR) – Phycoerythrin Conjugated Mouse IgG1, R&D Systems, cat. FAB 357P). Isotype matched, labelled immunoglobulins (Mouse γ1 APC, BD Biosciences (San Jose, CA, USA), cat. 345818; Mouse IgG1 Isotype Control Phycoerythrin Conjugated, R&D Systems, cat. CN IC002P) were used for each sample as a negative control. After incubation, cells were treated for 10 min at room temperature with Lysing Solution (Lysing Solution, BD Biosciences, cat. 349202) to eliminate erythrocytes. After careful washing, cells were resuspended in 300 µl of PBS and 50 µl of fluorescent CytoCount beads (DakoCytomation, Glostrup, Denmark) were added for precise cell number evaluation. The samples were then subjected to flow cytometry analysis using a FACSCalibur cytometer (Becton Dickinson). At least 150,000 events were collected. FACS analysis was performed using CellQuest software (Becton Dickinson) and appropriate cell gating to eliminate platelets and dead cells (debris gate). Cell viability was determined using propidium iodide (PI). CEPCs were evaluated using a lymphocyte gate and data were expressed as cell counts per ml of blood.

VEGF evaluation

VEGF was measured by ELISA using a commercially available kit (Quantikine Human VEGF Immunoassay, R&D Systems, Minneapolis, MN, USA, cat. DVE00), according to manufacturer's instructions. Extinction was measured at a wavelength of 450 nm and correction wavelength of 540 nm using a Wallac Victor spectrophotometer (Perkin Elmer, Waltham, MA, USA). Results were obtained from the standard curve and expressed as pg/ml.

Statistical analysis

Statistical analysis was performed using the software Statistica version 9.0 (Windows XP) and GraphPad Prism version 5.0. The mean, median, maximal, minimal and standard deviation for continuous variables, such as age, mean duration of disease, PASI, DAS28, and CRP, were calculated. To compare continuous variables, such as age and CEPC number, the *t*-test (for normally distributed data) or Mann-Whitney *U* test (for non-normally distributed data) was used. The significance of differences between categorical variables, such as sex, obesity, hypertension, and smoking habit, was determined using the χ^2 Pearson test, χ^2 with Yates correction test or Fisher's exact test. Relations between continuous variables of interest were assessed by Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$.