Letters to the Editors

Changes in atherosclerosis markers during tocilizumab treatment in rheumatoid arthritis: preliminary results

Sirs,

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that is associated with increased mortality due to accelerated atherosclerosis and characterised by increased cardiovascular (CV) mortality in comparison with the general population (1). Inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF), acute phase proteins, lipids, and endothelial and coagulation markers play an important role in accelerated atherosclerosis (2). IL-6 is a pivotal cytokine that is involved in the pathogenesis of RA, and also in many aspects of CV disease. It is important in the first phase of atherosclerosis as it leads to endothelial damage and stimulates acute phase proteins such as C-reactive protein (CRP), which is involved in the pathogenesis of plaque (3).

Tocilizumab (TCZ), a humanised monoclonal antibody that binds to membrane-bound and soluble forms of IL-6R, blocks IL-6 binding to its receptor. Many published studies indicate that a blockade of IL-6R intracellular signalling may be beneficial for patients with RA (4). The aim of our study was to compare different markers of accelerated atherosclerosis in patients with RA at baseline and after 6 months of treatment with TCZ (dose 8 mg/kg every 4 weeks).

This prospective study involved 16 female RA outpatients with a mean age of 56±9.3 years, 5.2±1.2 years of disease activity, and active disease (mean DAS-28 5.2±0.8), who started treatment with TCZ. All of the patients received a stable dose of prednisone 6.2±1.2 mg/day and methotrexate (MTX) 12.3±1.4 mg/week. Rheumatoid factor (RF) positivity was detected in 81.2% of the patients, and anti-cyclic citrullinated peptide (anti-CCP) antibodies in 87%.

Clinical data (tender joint count, swollen joint count, global health) and the following laboratory data were collected at baseline and after six months: inflammatory cytokines TNF-α (Human TNF-alpha Quantikine Immunoassay RD System INC, Minneapolis, USA), IL-6 (Human IL6 Instant Enzyme-linked immunosorbent assay (ELISA), Bioscience, Bender MedSystems, GmbH, Vienna, Austria), IL-10 (Human IL10 Instant Enzyme-linked immunosorbent assay (ELISA), Bioscience, Bender MedSystems, GmbH, Vienna, Austria), and IL-8 (Human IL8 Instant Enzyme-linked immunosorbent assay (ELISA), Bioscience, Bender MedSystems, GmbH, Vienna, Austria), acute phase proteins ESR, CRP (Beckman Unicel Coulter DxC 800 Synchron Central System, Fullerton, CA, USA), serum amyloid A (N Latex SAA BNII nephelometric analyser, Siemens, Marburg, Germany); lipid profile: total cholesterol (TC), triglycerides (TG), HDL and LDL cholesterol (Beckman Unicel Coulter DxC 800 Synchron Central System, Fullerton, CA, USA) and lipoprotein a (Lpa) (N Latex Lpa BNII nephelometric analyser, Siemens, Marburg, Germany); endothelial and coagulation markers such as plasminogen activator inhibitor-1 (PAI-1) (Berichrom PAI-1 System, Siemens, Marburg, Germany), von Willebrand factor (Biomerieux Immunological Test; wWF: RCO Siemens, Marburg, Germany), β2GPI antibodies (anti-beta 2 glycoprotein, IgG and IgM Technogenetics) and F1+F2 fibrin fragments (Enzygnost F1 + F2 monoclonal. Siemens, Marburg, Germany), and CD4+/CD28-T lymphocyte cells (flow cytometric system Cytomics FC 500, Beckman Coulter, Fullerton, CA, USA).

The patients were classified as being in remission if their DAS28 was <2.6 and as having low disease activity if their DAS28 was between 2.6 and 3.2.

Student's *t*-test was used to evaluate the changes in disease activity. The statistical analysis was performed using ANOVA for parametric variables and the Kruskall-Wallis test for non-parametric analysis.

After 6 months, 10 patients were considered to be in remission (group A), and six as having low disease activity (group B).

There were significant differences between these two groups in terms of the following parameters: ESR (12.6±3.2 *vs*. 28.5±4.8 mm/h; $p<0.05$); CRP (0.8±0.4 *vs*. 1.8±0.4 mg/dL, $p<0.05$); SAA (6.2±2.4 *vs*. 13.3±3.4 mg/dL; $p<0.05$); CD4+/CD28- cells (0.4±0.2 *vs*. 0.8±0.1 cells/mL; $p<0.01$); IL-6 (26.3±11.4 *vs*. 9.6±3.5 pg/mL, $p<0.05$); TNF-α (13.1±2.4 *vs*. 26.2±3.5 pg/mL, $p<0.01$). There were no differences in lipid profiles or coagulation markers (Table I).

Table I. Patient characteristics at baseline and after six months of TCZ treatment by DAS28 values.

Parameters	Baseline DAS28: 5.2± 0.8 16 patients	After 6 months of TCZ treatment DAS28: <2.6 10 patients	After 6 months of TCZ treatment DAS28: 2.6-3.2 6 patients
ESR, mm/h	42.6 ± 7.2	12.6 ± 3.2*	28.5 ± 4.8
CRP, mg/dL	3.8 ± 0.8	0.8 ± 0.4*	1.8 ± 0.4
SAA, mg/dL	15.2 ± 2.4	6.2 ± 2.4*	13.3 ± 3.4
Total cholesterol,. mg/dL	196 ± 16	213 ± 16	206 ± 18
Triglycerides, mg/dL	141 ± 13	151 ± 14	158 ± 15
HDL cholesterol, mg/dL	68 ± 12	65 ± 11	69 ± 13
LDL cholesterol, mg/dL	126 ± 14	132 ± 13	129 ± 14
Lpa, mg/dL	28 ± 3	27 ± 3	26 ± 3
PAI-1, U/mL	3.3 ± 0.2	3.6 ± 0.2	3.7 ± 0.4
von Willebrand factor, %	137 ± 12	135 ± 11	143 ± 9
β2GPI U/ml	9.2 ± 2.2	9.4 ± 3.2	9.9 ± 4.5
F1+F2, pM/L	209 ± 11	212 ± 13	231 ± 11
CD4+/CD28-, %	1.3 ± 0.4	0.4 ± 0.2**	0.8 ± 0.1
TNF-α, pg/mL	31.1 ± 3.4	13.1 ± 2.4*	26.2 ± 3.5
IL-6, pg/mL	33.5 ± 2.4	9.6 ± 3.5*	26.3 ± 11.4

*$p<0.05$; **$p<0.01$

ESR: erythrocyte sedimentation rate (ESR); CRP: C-reactive protein; SAA: serum amyloid A; IL-6: interleukin 6; TNF: tumour necrosis factor; Lpa: lipoprotein; β2GPI: anti-beta 2 glycoprotein; PAI-1: plasminogen activator inhibitor-1; HDL cholesterol: high-density lipoprotein; LDL cholesterol: low-density lipoprotein.

These preliminary results show that six months' treatment with TCZ not only decreases inflammation and induces remission, which is an important endpoint for decreasing CV risk, but also reduces the levels of circulating CD4+/CD28- T cells, which are known to play an important role in accelerated atherosclerosis.

One study found increased levels of CD4+/CD28- T cells in 87 patients with RA in comparison with 30 controls (5), and it is known that CD4+/CD28- T cells are expanded in the blood of patients with unstable angina and found in extracts of coronary arteries containing unstable plaques (6). It has also been demonstrated that peripheral blood CD4+/CD28- T cell expansion is associated with higher carotid intima media thickness (IMT) in RA patients, and may be a stronger marker of sub-clinical atherosclerosis than circulating anti-cyclic citrullinated peptide (anti-CCP) antibodies (7). A recent study has shown that decreased levels of circulating CD28- T cells in RA patients treated with abatacept correlate with clinical response (8).

An open-label, randomised controlled trial has shown that TCZ can reduce arterial stiffness in patients with RA (9). TCZ increases flow-mediated dilatation (FMD), but decreases pulse wave velocity (PWV), whether these beneficial arterial changes are direct effects of the IL-6/IL-6 receptor pathway inhibition warrants further studies (10).

In conclusion, although further large-scale studies are required, these preliminary data indicate that six months' treatment with TCZ not only decreased inflammation, but also more significantly reduced the levels of the circulating CD4+/CD28- cells involved in accelerated atherosclerosis in the patients who achieved a DAS28 of <2.6 than in those who did not.

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