Concise report

Calprotectin more accurately discriminates the disease status of rheumatoid arthritis patients receiving tocilizumab than acute phase reactants

José Inciarte-Mundo, Virginia Ruiz-Esquide, Maria Victoria Hernández, Juan D. Cañete, Sonia Raquel Cabrera-Villalba, Julio Ramírez, Jordi Yagüe and Raimon Sanmartí

Abstract

Objective. To compare the accuracy of serum calprotectin levels, CRP and ESR in stratifying disease activity in RA patients receiving tocilizumab (TCZ).

Methods. Cross-sectional study of 33 RA patients receiving TCZ. DAS28, Simplified Disease Activity Index, Clinical Disease Activity Index, joint counts and serum levels of CRP, ESR, calprotectin and TCZ were measured. Associations between calprotectin, ESR and CRP and articular indices were analysed by correlation and linear regression. The accuracy and discriminatory capacity of calprotectin was assessed by receiver operating characteristic curves (area under the curve).

Results. Calprotectin levels, but not CRP or ESR, were strongly correlated with all composite indices (all r coefficients over 0.50). Calprotectin, but not CRP or ESR, was significantly lower in patients in remission compared with those with low disease activity [1.57 μg/ml (s.d. 1) vs 3.35 μg/ml (s.d. 1), P = 0.001]. In a fully adjusted model (R² = 0.82), DAS28-ESR increased 0.48 units per μg/ml calprotectin increase (P < 0.001). Using a DAS28 >3.2 as the reference variable, calprotectin showed an area under the curve of 0.922, and the best cut-off was 5.19 μg/ml (odds ratio 11.5). CRP levels, but not calprotectin, were dependent on detectable TCZ trough serum levels.

Conclusion. Calprotectin serum levels seem to be an accurate biomarker for assessing disease activity in RA patients receiving TCZ.

Key words: calprotectin, rheumatoid arthritis, tocilizumab, disease activity.

Introduction

Tocilizumab (TCZ) is a humanized anti–IL-6 receptor mAb approved for use in RA. TCZ has demonstrated efficacy in controlling the signs and symptoms of RA, beneficial effects on radiographic progression and a significant improvement in patient quality of life [1–3]. TCZ inhibits stimuli to hepatocytes via IL-6 [4], dramatically improving the acute phase response (APR), including CRP serum levels and ESR, which form part of composite disease activity indices and response criteria in RA [5], leading to a possible overestimation of the response rate, especially when the DAS28 is used [6]. Therefore, CRP and ESR may not be accurate biomarkers for assessing disease activity in patients receiving TCZ.
Recently, calprotectin (S100A8 and S100A9), a member of the S100 protein family, has been identified as an important endogenous damage-associated molecular pattern molecule that is released by activated phagocytes and recognized by toll-like receptor 4 on multiple inflammatory cell types, such as monocytes and granulocytes [7]. Calprotectin has strong pro-inflammatory effects, and its levels reflect local ongoing inflammation rather than a systemic inflammatory response, due to its release at local inflammation sites [8]. High calprotectin levels have been found in SF and serum from RA patients [9], and correlate with disease activity [10]. The objective of this study was to analyse the accuracy of calprotectin (compared with APR) in discriminating the clinical disease status of RA patients receiving TCZ.

Methods

A cross-sectional study including all RA patients (ACR 1987 criteria) from our arthritis unit receiving TCZ was carried out. Demographic data, disease duration, autoantibody status (ACPA/RF), radiological data (erosive disease), previous biologic therapy, TCZ treatment duration and dosage (a TCZ dose of 4–6 kg every 4 weeks) was considered a reduced dosage) and concomitant synthetic DMARD therapy, were collected.

Before TCZ administration, all patients underwent a clinical assessment, including 28 swollen and tender joint counts (28-SJC and 28-TJC) and physician and patient global assessment with visual analogue scales (0–100 mm). Disease activity indices were subsequently calculated [DAS28-ESR, Simplified Disease Activity Index, (SDAI) and Clinical Disease Activity Index (CDAI)].

In addition, blood samples were obtained just before TCZ administration. ESR was measured using the Westergren method (NV < 20 mm/h), and CRP by nephelometry (lowest detection limit of 0.01 mg/dL; NV < 0.8 mg/dL). Calprotectin serum levels were determined using an ELISA Test Kit [CALPROLAB Calprotectin ELISA (ALP) CALPRO AS, Norway] in accordance with the manufacturer’s protocol. Briefly, 100 μL of each standard, control and diluted 1:20 sample in duplicate wells were incubated at room temperature for 40 min; three washings were made, 100 μL of the conjugated enzyme were added and then plates were incubated at room temperature for 40 min. After three washes and addition of the enzyme substrate, the optical density values at 405 nm were determined using an ELISA reader. To reduce variation in calprotectin determination, the whole procedure was performed in a Triturus autoanalyzer; the coefficients of variation were 5% within and 13% between assays. Serum calprotectin levels were also measured in 40 healthy blood donors. Serum TCZ trough levels were detected by bridging ELISA (LISA-TRACKER Duo Tocilizumab, LTT005 (Theradiag, Croissy-Beaubourg, France)), according to the product insert, with 1 μg/ml the lower level of quantification.

Continuous data were presented as mean (s.d.), and categorical variables as absolute frequency with percentage. Normality was tested with skewness tests and bin-graphs, and the data for most variables were normal or very close to a normal distribution. Groups were compared using the Student’s t-test or Mann–Whitney test when appropriate. Correlations were assessed using Pearson’s correlation coefficient. Linear regression models were used to assess the association between calprotectin and DAS28-ESR. Multivariate models were constructed to analyse the effect of covariates, and to fully adjust the association between calprotectin and DAS28. The discriminatory capacity of calprotectin was analysed using receiver operating characteristic curves with activity yes/no (no: DAS28 ≤ 3.2) as the gold standard, and the best cut-off in terms of sensitivity, specificity and likelihood ratios was identified. The area under the curve was calculated using Hanley’s corrected CIs. The odds ratio for the best cut-off of calprotectin was calculated. The analysis was made using STATA version 11 (STATA Corp, College Station, TX, USA). The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the Hospital Clinic of Barcelona [Clinical Research Ethics Committee of the Hospital Clinic de Barcelona (CEIC Hospital Clinic)]. Informed consent was obtained from all patients before study enrolment.

Results

A total of 33 patients were included, of whom 91% were female, with a mean age of 53 years. Mean disease duration was 15 years, 67% were RF or ACPA positive, 79% had erosive disease, 70% had previously received biologic therapy, 79% had received concomitant treatment with synthetic DMARDs (mostly MTX) and 57% low doses of glucocorticoids (mean prednisone dose 2.3 mg/day). The mean time of TCZ therapy was 26 months (s.d. 15), and 48.5% of patients had received a low dosage. Remission (DAS28 ≤ 2.6) and low disease activity (DAS28 2.6–3.2) were observed in 11 (33%) and 13 (39%) patients, respectively (supplementary Table S1 available at Rheumatology Online).

Serum calprotectin levels were higher in RA patients than in healthy controls [3.20 μg/ml (s.d. 2) vs 1.5 μg/ml (s.d. 0.8), P = 0.001]. Calprotectin levels did not differ according to age or sex and correlated strongly with all composite disease activity indices (Fig. 1A–C) and the 28 SJC/TJC (Fig. 1J and K). Adjusted analysis showed a significant association between serum calprotectin levels and DAS28 according to a number of covariates (combined therapy, reduced dose, use of glucocorticoids, disease duration and presence of autoantibodies or erosive disease). Backward selection of variables did not substantially modify the association between calprotectin and DAS28 (supplementary Table S2 available at Rheumatology Online). A weak correlation was found between calprotectin and CRP, but not with ESR. In a fully adjusted model (R2 = 0.82), DAS28-ESR increased 0.48 units per μg/ml calprotectin increase (P < 0.001). The accuracy analysis with activity by DAS28 as the reference variable showed an area under the curve of 0.922 (95% CI...
The scatterplots (A–O) illustrate the distribution of calprotectin, CRP and ESR levels with the corresponding DAS-28 ESR. SDAI: Simplified Disease Activity Index; CDAI: Clinical Disease Activity Index; SJC: swelling joint count; TJC: tender joint count. R: Pearson’s correlation coefficient.
Discussion

Our results show that calprotectin seems to be a good biomarker for the assessment of disease activity in RA patients receiving TCZ, and is apparently more accurate than APR. The dramatic effect of TCZ on APR, markedly decreasing CRP levels, is established [11]. The pronounced effects of TCZ on APR might lead to an overestimation of the response rate when disease activity indices that include APR, such as the DAS/DAS28 or SDAI, are used. In RA patients receiving TCZ, higher rates of remission were obtained using the DAS28 than using the CDAI or SDAI, due to the high weighting of ESR in the DAS28 score [6]. The DAS28 overestimates the rates of remission in RA patients treated with TCZ in comparison with the CDAI, which does not include APR [12].

We not uncommonly observe patients with a pronounced reduction in CRP or ESR values after TCZ therapy, accompanied by persistent disease activity as shown by a high swollen joint count. Ten of our 24 patients (42%) had very low CRP serum levels (<0.2 mg/dl) accompanied by a swollen joint count of >2. CRP and ESR were only weakly correlated with the DAS28; no correlation between APR and SDAI/ CDAI or SJC/TJC was found. Neither ESR nor CRP discriminates between clinical disease activity states, such as remission or low disease activity. Therefore, APRs may not be sensitive biomarkers for the accurate assessment of disease activity in RA patients receiving TCZ.

This is the first study to analyse serum calprotectin levels in patients receiving TCZ. Studies have shown that serum calprotectin levels correlate with disease activity in RA [13], and other inflammatory conditions [14–16] and that decreases in serum calprotectin levels after TNF inhibition are sensitive to change [17], and predict radiographic progression [18]. Our results show that calprotectin correlates better with all disease articular indices and joint counts, independently of covariates, than APR. Calprotectin seems to accurately discriminate between disease activity states, including remission and low disease activity.

Pharmacokinetic studies of TCZ show that a significant proportion of RA patients have undetectable serum trough TCZ levels [19], as confirmed by our results. Nishimoto et al. [20] found that TCZ normalized CRP values in RA patients as long as free TCZ remained above 1 μg/ml in serum [20]; in contrast; we found that serum calprotectin levels were independent of detectable serum trough TCZ levels.

Our study has some limitations, including the small sample size and the cross-sectional design, which make it difficult to ascertain the exact effects of TCZ on serum calprotectin levels and their sensitivity to change, as previously reported after TNF antagonist therapy [17]. Moreover, although we tried to find a cut-off point to discriminate active from low disease activity/remission, this
would require prospective studies with a large sample size.

In summary, serum calprotectin levels may accurately help stratify disease activity in RA patients receiving TCZ. Calprotectin strongly correlated with composite joint indices, whereas there was a weak or no correlation with APR. Calprotectin levels seem to distinguish between patients in remission or with low disease activity, despite the recognized reduction in APR observed after TCZ treatment, independently of serum trough TCZ levels. Calprotectin levels could be considered an accurate biomarker for the assessment of disease activity in RA patients receiving TCZ.

Acknowledgements

The authors acknowledge the statistical assistance provided by Dr Loreto Carmona and technical advice from David Buss.

Funding: This study was financially supported by grants from the Hospital Clinic of Barcelona ‘Emili Letang 2013’ and the Catalan Society of Rheumatology.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

References


15. Moncrieffe H, Ursu S, Holzinger D et al. A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRPs/14 protein. Rheumatology 2013;52:1467-76.


