Serum tocilizumab trough concentration can be used to monitor systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study

EL Kneepkens1, IAM van den Oever1, CH Plasencia2, D Pascual-Salcedo3, A de Vries4, M Hart5, MT Nurmohamed1,6, A Balsa2, T Rispens5, G Wolbink1,5

1Department of Rheumatology, Amsterdam Rheumatology and Immunology Centre, location Reade, Amsterdam, The Netherlands, 2Department of Rheumatology, La Paz hospital Madrid, Spain, 3Immunology Unit, La Paz hospital Madrid, Spain, 4Sanquin Diagnostic Services Amsterdam, The Netherlands, 5Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Centre Amsterdam, and 6Department of Rheumatology, Amsterdam Rheumatology and Immunology Centre, VU Medical Centre, Amsterdam, The Netherlands

Objectives: To investigate the pharmacokinetics (PK) and dynamics of tocilizumab (TCZ) in daily practice.

Method: An observational study of 66 consecutive RA patients treated with TCZ 8 mg/kg once every 4 weeks intravenously, monitored for 24 weeks. Spearman’s rank test was used to investigate the correlation between TCZ concentration and C-reactive protein (CRP). Clinical improvement was assessed at week 24 using the Disease Activity Score in 28 joints (DAS28) compared to baseline, and its relationship with TCZ concentration was investigated using linear regression analyses. TCZ trough concentrations and anti-drug antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) and antigen binding test, respectively.

Results: At baseline, 26 patients (39.4%) had a CRP level above 10 mg/L with a median (interquartile range, IQR) of 37.7 (21.9–49.7) mg/L. A TCZ concentration above 1 mg/L was sufficient to normalize CRP levels. Spearman’s rank test showed a correlation coefficient of −0.460 (p < 0.0001). The TCZ concentration varied widely, with concentrations < 1 mg/L in 17–31% of patients, depending on the time point of measurement. Anti-TCZ antibodies were detected in one sample. Linear regression analyses showed a coefficient of 0.080 with a 95% confidence interval (CI) of 0.039–0.113 (p < 0.001) for the association between TCZ concentration and DAS28. No confounders were identified.

Conclusions: The TCZ standard regimen results in a wide variety of serum TCZ trough concentrations; this is mostly due to target binding and to a lesser extent to immunogenicity. The majority of patients obtained TCZ concentrations > 1 mg/L, which is sufficient for CRP normalization. Therefore, dose taper strategies might be possible in a substantial proportion of patients.

The pathogenesis of rheumatoid arthritis (RA) involves the release of pro-inflammatory mediators such as interleukin (IL)-6. IL-6 is a multifunctional cytokine and is associated with inflammation, chronic synovitis (1, 2), bone destruction of joints (3), and the pathogenesis of RA (4, 5). Moreover, IL-6 is the most important cytokine-stimulating hepatocyte to produce C-reactive protein (CRP) (1, 6, 7). IL-6 can activate target cells, such as hepatocytes, through the IL-6 receptor (IL-6R), which occurs in the body as a membrane-bound (mIL-6R) and a soluble form (sIL-6R); the activation takes place through classic- and trans-signalling pathways, respectively. The sIL-6R/IL-6 complex can only activate cells that express cell-surface glycoprotein-130 (8).

Tocilizumab (TCZ) is a humanized antibody that competitively inhibits both sIL-6R and mIL-6R and is an effective treatment for RA (9, 10). Currently, TCZ can be administered intravenously (iv) or subcutaneously (11), with or without concomitant methotrexate (10). Randomized controlled trials (RCTs) have shown that TCZ treatment substantially reduces biomarkers of inflammation, such as CRP, and influences serum levels of IL-6 and sIL-6R (10–14). With regard to CRP, Nishimoto et al have shown that a TCZ concentration above 1 mg/L is sufficient for CRP normalization (12). Although clinical response rates are promising, not all patients seem to respond adequately to TCZ treatment, as has also been reported for anti-tumour necrosis factor (TNF) treatment (15).

Clinical inefficacy with regard to biological treatment is multifactorial, but in anti-TNF treatment immunogenicity
may be a major factor influencing the pharmacokinetics (PK). The presence of high titres of anti-drug antibodies (ADA) reduces the amount of free drug available to bind the target, resulting in a reduced clinical response in the majority of patients with detectable ADA (16). Evolving evidence shows that the PK of TCZ is influenced by target binding (amount of IL-6R) and to a lesser extent by immunogenicity (10–14, 17).

The identification of factors that can predict the clinical response to a biological agent is important because this knowledge can be used to optimize treatment in individual patients. Currently, all data on serum TCZ concentrations, immunogenicity, and clinical response are obtained from RCTs. The aim of this study was to investigate variation in serum TCZ trough concentrations and the relationship with clinical measurements in RA during 24 weeks of follow-up.

**Method**

**Patient population and study design**

This prospective observational cohort consisted of 66 consecutively included adult RA patients diagnosed according to the American College of Rheumatology (ACR) 1987 revised criteria (18). All patients started TCZ between April 2009 and June 2014. Two cohorts were combined (The Netherlands, n = 34; Spain, n = 32). All patients had active disease, that is a Disease Activity Score in 28 joint counts using the erythrocyte sedimentation rate (DAS28-ESR) > 3.2, despite prior treatment with disease-modifying anti-rheumatic drugs (DMARDs) and/or biologics. All patients started with the TCZ standard regimen (8 mg/kg every 4 weeks iv) and concomitant DMARDs with or without prednisone, only with concomitant prednisone or TCZ as monotherapy. Adaptations in the TCZ regimen could be made, based on the expert opinion of the rheumatologist, in the case of: clinical inefficacy, adverse events, sustained low disease activity, or remission. Patients were eligible for inclusion in the final analyses if a serum sample (trough concentration) from at least one follow-up visit from week 12 onwards was available, taken after the TCZ standard regimen and in combination with the availability of corresponding measurements of DAS28 and/or CRP. The study was approved by the Medical Ethics Committee of Slotervaart Hospital and the Jan van Breemen Research Institute/Reade, Amsterdam, The Netherlands, and by the Medical Ethics Committee of La Paz Hospital, Madrid, Spain. All patients gave written informed consent in accordance with the Helsinki Declaration of 1975, as revised in 1983.

**Outcome measures**

Disease activity was measured with the DAS28-ESR (19). In the Dutch cohort, DAS28, parameters of inflammation (CRP and ESR), and serum trough samples were collected at baseline and 4, 12, and 24 weeks thereafter. In the Spanish cohort, serum samples and parameters of inflammation were collected before every TCZ infusion whereas DAS28 and its separate components were measured at baseline and at 24 weeks. The duration of follow-up in this study was 24 weeks.

To investigate the relationship between serum TCZ trough concentration (described in the following as TCZ concentration) and clinical response at week 24, defined as an improvement compared to baseline in DAS28 (ΔDAS28) and swollen joint count in 28 joints (ΔSJC28). To obtain the concentration–effect curve at week 24, the last observation carried forward (LOCF) method was used for patients in whom follow-up data from week 12 were available but not yet those from week 24. This seemed appropriate as the steady state of the TCZ standard regimen is seen, on average, from week 8 onwards (20). The relationship between TCZ concentration and serum CRP (described in the following as CRP) was investigated separately as CRP can be used as a surrogate marker for systemic IL-6R blockade (1, 6, 7).

**TCZ concentration measurement**

To measure TCZ concentrations an immunoassay was developed using rabbit anti-TCZ antibodies to capture TCZ, and rabbit anti-TCZ F(ab’)2 fragments for detection. Maxisorp enzyme-linked immunosorbent assay (ELISA) plates were coated overnight at room temperature with 0.125 μg/mL rabbit anti-TCZ in phosphate-buffered saline (PBS). The specific rabbit anti-idiotype antibodies were produced analogously as described for natalizumab (21). Plates were washed five times with PBS/0.02% Tween (PT), then washed and incubated for 1 h with patient serum that had been serially diluted in high-performance ELISA (HPE) buffer. After washing five times with PT, plates were incubated for 1 h with biotinylated anti-TCZ F (ab’)2 fragments (125 ng/mL in HPE buffer). After washing, streptavidin–poly-horseradish peroxidase (poly-HRP; Sanquin, Amsterdam, The Netherlands) (1:10 000, in HPE buffer) was added for 1 h at 37°C. After washing, the ELISA was developed with 100 μg/mL tetramethylbenzidine in 0.11 M sodium acetate (pH 5.5) containing 0.003% (v/v) H2O2. The reaction was stopped with 2 M H2SO4. Absorption was measured at 450 nm relative to a titration curve of TCZ in each plate. The lower limit of quantification (LLOQ) in serum was 200 ng/mL; the overall precision and accuracy were 8% and 93%, respectively. Serum samples were collected at the trough concentration, that is just before the next infusion.

**Anti-TCZ antibody measurement**

Measurement of anti-TCZ antibodies was essentially carried out as described previously (22, 23). One
microlitre of serum diluted in buffer containing intravenous immunoglobulin (IVIg) F(ab′)2 to prevent anti-hinge reactivity (23) was incubated overnight with 1 mg Protein A Sepharose (GE Healthcare, Chalfont St Giles, UK) and 2.5 ng biotinylated F(ab′)2 TCZ in a final volume of 800 μL. Subsequently, samples were washed with 0.005% PT and about 1 ng 125I-labelled streptavidin was added in a 800-μL final volume of PBS albumin Tween [PBS/0.01 M ethylenediaminetetraacetic acid (EDTA)/0.3% bovine serum albumin/0.004% Tween-20/0.05% Na3I] and incubated overnight. Unbound label was removed by washing and Sepharose-bound radioactivity was measured. Antibody levels were compared to a standard serum sample of an immunized rabbit containing ADA and expressed in arbitrary units (AU). A lower limit of detection was based on mean +3 standard deviations (sd) measured in a panel of 50 sera from healthy donors and 15 sera containing anti-cyclic citrullinated peptide (anti-CCP) antibodies, antinuclear antibodies (ANA), and/or rheumatoid factor (RF).

Statistical analyses

For statistical analyses, SPSS version 21.0 and Graph Pad Prism 6.0 for Windows were used. The results are displayed as number and percentage or mean ± sd when normally distributed, or as median and interquartile range (IQR) when non-normally distributed. For differences in baseline characteristics between patients of the Spanish cohort vs. the Dutch, an independent-sample t-test, the Mann–Whitney U test, or the χ2 test was used, as appropriate. The threshold for significance was set at p < 0.05. Spearman’s rank test was used to investigate the correlation between TCZ concentration and CRP. A linear regression analysis was used to investigate the relationship between TCZ concentration and ΔDAS28 at week 24. Potential confounders of this relationship were investigated using the baseline characteristics (Table 1), excluding the separate components of the DAS28, as the baseline DAS28 was already included. A variable was considered to be a confounder if it changed the regression coefficient by ≥ 10%. For this analysis, the LOCF method was used as described earlier. Sensitivity analyses showed no significant difference in baseline characteristics or outcome data (ΔDAS28 and TCZ concentration) at week 12 between patients with and without outcome data at week 24.

Results

The baseline characteristics of the 66 RA patients included in this study are shown in Table 1. Patients in the Spanish cohort had a significantly lower median ESR (mm/h) level of 23.5 (IQR 14.3–35.8) compared to 44 (IQR 22.5–63.0) for the Dutch patients (p = 0.009). The Dutch patients more often used ≥ 1 prior biological [34 (100%)] compared with the Spanish patients [19 (59.4%); p < 0.001].

Discontinuation and follow-up

A total of eight patients (12.1%) discontinued TCZ treatment between weeks 12 and 24 due to inefficacy.

Table 1. Baseline demographics and clinical characteristics of the total patient population (n = 66).

| Demographics | Value |
|--------------|-------|
| Age (years)  | 56.0 ± 12.9 |
| Female       | 54 (81.8) |
| BMI (kg/m²)  | 26.4 ± 5.4 |
| Spanish      | 32 (48.5) |
| Disease status |       |
| Disease duration (years) | 11.0 (5–17) |
| RF positive  | 47 (71.2) |
| Anti-CCP antibody positive | 47 (71.2) |
| CRP (mg/L)   | 6.8 (2.1–31.9) |
| ESR (mm/h)   | 34 (16.5–47.5) |
| DAS28        | 5.4 ± 1.4 |
| Tender joint count | 9.5 (4.0–14.5) |
| Swollen joint count | 6.0 (2.8–10.3) |
| VAS GDA patient (0–100 mm) | 60.0 ± 25.8 |

| DMARD therapy | Value |
|---------------|-------|
| Prior biologicals | 53 (80.3) |
| Methotrexate use at baseline | 42 (63.6) |
| Methotrexate dose (mg/week) | 15.5 ± 7.3 |
| Prednisone use at baseline | 46 (69.7) |
| Prednisone dose (mg/day) | 5.8 ± 4.4 |
| Other DMARD use (with or without methotrexate) | 29 (43.9) |

BMI, Body mass index; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, Disease Activity Score in 28 joints; VAS, visual analogue scale in mm (scale 0–100); GDA, general disease activity; DMARD, disease-modifying anti-rheumatic drug. Values given as number (percentage), mean ± standard deviation, or median (interquartile range).
(n = 5) or adverse events (n = 3). For three patients, clinical data and serum from week 12 were available but they had not yet had 24 weeks of follow-up at the time of data extraction.

**Serum TCZ concentration and anti-TCZ antibodies**

The available samples and median TCZ concentrations at each time point are shown in Table 2, with the lowest and highest concentrations and the number of patients with TCZ concentrations below 1 mg/L.

In total, nine patients had TCZ concentrations below 1 mg/L at ≥ 2 subsequent visits, of which there were three patients at every time point. This means that some patients had TCZ concentrations below 1 mg/L repeatedly, despite receiving the TCZ standard regimen. However, this could not be established in all patients because of some missing samples, and therefore this number might be an underestimation. Although, TCZ concentrations below 1 mg/L were found (repeatedly) in several patients, an anti-TCZ antibody signal was seen in only two patients with the assay used. In one of these patients, a weak anti-TCZ antibody signal was detected consistently, including in the pretreatment samples, independently of TCZ concentration. This patient was therefore not considered anti-TCZ antibody positive. A sample from the other patient was found to be positive at week 4, which was confirmed by subsequent inhibition experiments using unlabelled TCZ F(ab')2. This patient had TCZ concentrations below 1 mg/L at week 4; these had increased to ≥ 1 mg/L at week 24 with subsequent normalization of serum CRP levels.

**Serum TCZ concentration, inflammation parameters, and disease activity**

At baseline, 26 patients (39.4%) had a CRP level above 10 mg/L with a median of 37.7 mg/L (IQR 21.9–49.7). To investigate the relationship between TCZ concentration and CRP levels, all samples were stratified from low to high according to TCZ concentration with correlating CRP levels, as shown in Figure 1. This figure includes all samples from week 4 onwards, thus one dot represents one sample and not one patient. Based on this figure, a TCZ concentration above 1 mg/L is sufficient to normalize serum CRP levels (≤ 10 mg/L). Spearman’s rank correlation coefficient was calculated (based on the sample included in Figure 1) and showed a significant but moderately strong negative correlation coefficient of –0.460 (p < 0.0001).

To provide an insight into the course of CRP normalization over time with the corresponding TCZ concentration, Figure 1 was also divided at each time point (i.e. weeks 4, 12, and 24), see Supplementary Material. This supplementary figure shows that only one patient had an elevated CRP level at the time point of drop-out (week 12) and in almost all other patients CRP normalized over time. None of the patients had increased CRP levels during follow-up in combination with TCZ > 1 mg/L at any time point.

A similar course of improvement was seen for ESR; nevertheless, the cut-off of TCZ of 1 mg/L was less marked compared to CRP. With TCZ concentrations above 6 mg/L, no increased ESR levels (> 20 mm/h) were found (data not shown).

**Concentration–effect curve of TCZ at week 24**

In Figure 2, the relationship between TCZ concentration and ΔDAS28 (Figure 2A) and ΔSJC28 (Figure 2B) for

---

Table 2. Median serum tocilizumab (TCZ) trough concentrations during the 24 weeks of follow-up.

|                    | Week 4 | Week 12 | Week 24 |
|--------------------|--------|---------|---------|
| Number (%) of patients on TCZ | 66 (100) | 66 (100) | 55 (83.3) |
| Number (%) of available samples | 49 (74.2)* | 62 (93.9)* | 53 (96.4)† |
| Median TCZ (mg/L) | 3.4    | 9.1     | 10.6    |
| Minimum TCZ (mg/L) | 0      | 0       | 0       |
| Maximum TCZ (mg/L) | 18.2   | 35.5    | 35.4    |
| Number (%) of patients with TCZ < 1 mg/L | 15 (30.6)† | 15 (24.2)† | 9 (17.0)† |

* Percentage is based on the number of patients on TCZ.
† Percentage is based on the number of available samples at each time point.
TCZ and CRP. CRP is mainly produced by hepatocytes through IL-6 activation and can therefore be used as a surrogate marker for systemic IL-6R binding (1, 6, 7). Target binding that influence the PK of TCZ has been suggested previously in several studies. Inhibition assays have shown that the binding between IL-6 and sIL-6R was suppressed, in a dose-dependent manner, by adding TCZ at concentrations between 0.002 and 4 mg/L (31). Nishimoto et al show that TCZ concentrations above 1 mg/L resulted in more than 95% inhibition of CRP production (12). Another clinical trial showed that these TCZ concentrations were obtained in the majority of patients from week 8 onwards (11). In addition, the TCZ concentration

as was seen previously in anti-TNF treatment. Moreover, the majority of patients obtained TCZ concentrations that were sufficient to normalize serum CRP. A statically significant ΔDAS28 improvement with increasing TCZ concentrations was seen, but for clinical implications this change was small.

For anti-TNF treatment it has been shown that immunogenicity can have a profound effect on the PK (16). However, the PK of TCZ appears to be different because anti-TCZ antibodies were detected only in one patient, although TCZ concentrations below 1 mg/L were found (repeatedly) in several patients. Moreover, these low concentrations were especially evident during the early treatment phase, which is not in accordance with reported PK variations due to immunogenicity in anti-TNF treatment. The influence of immunogenicity might have been underestimated in this study as the assay used to detect anti-TCZ antibodies is drug sensitive, meaning that only ADA exceeding TCZ concentration will be detected (24, 25). Previously reported data from RCTs suggest that immunogenicity is not a major factor in the PK of TCZ (10, 11, 13), but these trials included a different patient population from that encountered in daily clinical practice. Moreover, comparing ADA results is difficult because ADA production and detection can be influenced by several factors, such as time point of sampling, assay format, concomitant immunomodulation therapy (e.g. methotrexate), and dosing (26). Thus, it remains open to question whether TCZ has a less immunogenic structure, or detectability is more complex due to drug interference, or immunological tolerance is induced by high dosing (27, 28). Therefore, it would be of interest to investigate the immunogenicity of TCZ with a drug tolerant assay similar to that performed previously for adalimumab (29, 30).

Another explanation for the variation in TCZ concentration between patients is target binding. In patients with more target, (i.e. IL-6R), clearance of TCZ is increased, and thus lower serum TCZ trough concentrations will be detected by the assay. Moreover, a TCZ concentration above 1 mg/L was sufficient to normalize CRP levels, and Spearman’s rank test showed a statistically significant moderately strong negative correlation between TCZ and CRP. CRP is mainly produced by hepatocytes through IL-6 activation and can therefore be used as a surrogate marker for systemic IL-6R binding (1, 6, 7). Target binding that influence the PK of TCZ has been suggested previously in several studies. Inhibition assays have shown that the binding between IL-6 and sIL-6R was suppressed, in a dose-dependent manner, by adding TCZ at concentrations between 0.002 and 4 mg/L (31). Nishimoto et al show that TCZ concentrations above 1 mg/L resulted in more than 95% binding of sIL-6R in an sIL-6R/TCZ immune complex with subsequent inhibition of CRP production (12). Another clinical trial showed that these TCZ concentrations were obtained in the majority of patients from week 8 onwards (11). In addition, the TCZ concentration

the individual patients at week 24 is presented. Twelve patients (18%) did not achieve a ΔDAS28 of ≥ 1.2, which is considered to be a clinically significant change. Eight of these 12 patients had a TCZ concentration < 1 mg/L. In addition, three of these eight patients had a CRP level above 10 mg/L. Linear regression analysis showed a regression coefficient of 0.080 with a 95% confidence interval (CI) of 0.039–0.113 (p < 0.001) for the association between TCZ concentration and ΔDAS28 at week 24 of treatment. No confounders were identified.

Eight patients had more swollen joints at week 24 of treatment compared to baseline, of whom five had a TCZ concentration below 1 mg/L. Two of these five patients had increased CRP levels, two other patients had increased CRP levels at previous time points, and one had normal CRP levels. In general, the SJC had improved or stabilized in the majority of patients with TCZ concentrations of approximately 4 mg/L.

Discussion

The aim of this study was to investigate, in a prospective observational cohort, the variation in serum TCZ trough concentrations and the relationship with clinical measurements in RA during 24 weeks of follow-up. This study shows that serum TCZ trough concentrations vary widely between patients on the TCZ standard regimen,
at which 50% of its maximal effect was observed was lower in patients with high IL-6 levels at baseline, which may be the result of IL-6 overproduction or lower expression of sIL-6R, and thus slower clearance (14). Different amounts of target, in the normal or inflammatory state, might be explained by sIL-6R polymorphisms (32, 33); however, to our knowledge, this was not studied in combination with serum TCZ concentrations.

Although CRP can be used as a surrogate marker for systemic IL-6R binding, clinical response is more complex. Clinical response is multifactorial and clinical outcome measurements frequently used in RA [such as DAS28, the Clinical Disease Activity Index (CDAI) (34), or the Simplified Disease Activity Index (SDAI) (35)] are composite measurements that reflect total disease activity but do not discriminate between the role of a particular cytokine and other factors (e.g. other cytokines, established bone damage, psychological and social factors). Linear regression analysis showed a statistically significant ΔDAS28 improvement with increasing TCZ concentrations; however, for clinical purposes this change was small. Moreover, normalized CRP did not result in a good clinical outcome, measured with DAS28 or SJC28, in all patients. In addition, the predictive value of IL-6, sIL-6R, or CRP on clinical outcome is contradictory (12, 13, 17, 36, 37). Nevertheless, TCZ, like all biologics given in RA, is a molecular targeting therapy and thus the highest obtainable result is complete target blockade; however, this does not necessarily translate to an appropriate clinical response in all patients. The assay used for TCZ concentrations measures the surplus of unbound TCZ, and a detectable serum trough concentration means that all systemic, and potentially all local (38, 39), IL-6R is blocked. Therefore, TCZ concentration measurements can be used as a surrogate marker for systemic target blockade. Considering the number of non-responders to biological treatment and the high costs associated with these therapies, a dose based on target levels seems more rational. To apply therapeutic drug monitoring (TDM) of biologicals in daily clinical practice for treatment optimization, an optimal therapeutic concentration range for an effective target blockade must be identified (40, 41). Because of the direct relationship between IL-6 and CRP, TCZ is a suitable biological agent for investigating the optimal therapeutic concentration range for complete target blockade vs. clinical response. To investigate the additional value of TDM, a prospective TCZ dose taper trial is necessary, including clinical measurements, TCZ concentrations and other potential markers, for target blockade (IL-6, sIL-6R, CRP, and calprotectin) (12, 13, 17, 35, 36, 42–44), as well as biomarkers for progression of bone damage because IL-6 plays an important role in bone metabolism (3, 45).

Some limitations of the current study need to be addressed. Two cohorts were combined to increase patient numbers, resulting in slight differences in measurements per time point. Differences in patient characteristics were limited and both cohorts consisted of mainly Caucasians. However, possible bias due to non-compliance, change in TCZ dose, or interval or wrong timing of sampling was excluded since TCZ was given intravenously.

In conclusion, the TCZ standard regimen results in a wide variety of serum TCZ trough concentrations between patients, and target binding seems to provide a better explanation for this variation than immunogenicity. Moreover, the majority of patients obtained TCZ concentrations above 1 mg/L, which is sufficient to normalize serum CRP. Therefore, the TCZ standard regimen is an overtreatment with regard to systemic IL-6R blockade in the majority of RA patients; however, clinical response is multifactorial and might require more than just sufficient blockade of a single cytokine pathway.

Acknowledgements

We are grateful to the research nurses and medical doctors for performing the clinical assessments and the technicians of Sanquin Diagnostics Services for performing the assays. We also thank Professors R. Mathot and L. Aarden for reviewing the manuscript and for their valuable comments.

References

1. Swaak AJ, van Rooyen A, Nieuwenhuis E, Aarden L. Interleukin-6 (IL-6) in synovial fluid and serum of patients with rheumatic diseases. Scand J Rheumatol 1988;17:469–74.
2. Usón J, Balsa A, Pascual-Salcedo D, Cabezas JA, Gonzalez-Tarrio JM, Martín-Mola E, et al. Soluble interleukin 6 (IL-6) receptor and IL-6 levels in serum and synovial fluid of patients with different arthropathies. J Rheumatol 1997;24:2069–75.
3. Kotake S, Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, et al. Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 1996;11:88–95.
4. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol 2007;7:429–42.
5. Lipsky PE. Interleukin-6 and rheumatic diseases. Arthritis Res Ther 2006;8(Suppl 2):S4.
6. Castell JV, Geiger T, Gross V, Andus T, Walter E, Hirano T, et al. Plasma clearance, organ distribution and target cells of interleukin-6 hepatocyte-stimulating factor in the rat. Eur J Biochem 1988;177:357–61.
7. Kato A, Watanabe T, Yamazaki M, Deki T, Suzuki M. IL-6R distribution in normal human and cynomolgus monkey tissues. Regul Toxicol Pharmacol 2009;53:46–51.
8. Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci 2012;8:1237–47.
9. Smolen JS, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E, et al. Effect of interleukin-6-receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. Lancet 2008;371:987–97.
10. Maini RN, Taylor PC, Szechinski J, Pavelka K, Broll J, Balint G, et al. Double-blind randomized controlled clinical trial of the interleukin-6-receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. Arthritis Rheum 2006;54:2817–29. Erratum in Arthritis Rheum 2008;59:887.
11. Ogata A, Tanimura K, Sugimoto T, Inoue H, Urata Y, Matsubara T, et al. Phase III study of the efficacy and safety of subcutaneous versus intravenous tocilizumab monotherapy in patients with rheumatoid arthritis. Arthritis Care Res (Hoboken) 2014;66:344–54.
12. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significance in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood 2008;112:3959–64.

13. Levi M, Grange S, Frey N. Exposure-response relationship of tocilizumab, an anti-IL-6 receptor monoclonal antibody, in a large population of patients with rheumatoid arthritis. J Clin Pharmacol 2013;53:151–9.

14. Frey N, Grange S, Woodworth T. Population pharmacokinetic analysis of tocilizumab in patients with rheumatoid arthritis. J Clin Pharmacol 2010;50:754–66.

15. Arora A, Mahajan A, Spurden D, Boyd H, Porter D. Long-term drug survival of TNF inhibitor therapy in RA patients: a systematic review of European National Drug Registers. Int J Rheumatol 2013;2013:764518.

16. Maneiro JR, Salgado E, Gomez-Reino JJ. Immunogenicity of monoclonal antibodies against tumor necrosis factor used in chronic immune-mediated inflammatory conditions: systematic review and meta-analysis. JAMA Intern Med 2013;173:1416–28.

17. Nishina N, Kikuchi J, Hashizume M, Yoshimoto K, Kameda H, Takeuchi T. Baseline levels of soluble interleukin-6 receptor predict clinical remission in patients with rheumatoid arthritis treated with tocilizumab: implications for molecular targeted therapy. Ann Rheum Dis 2014;73:945–7.

18. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.

19. Prevoo ML, van’t Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.

20. Food and Drug Administration. 2013 (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125276s064lbl.pdf). Accessed 22 June 2016.

21. Rispens T, Leeuwen Av, Vennegoor A, Killestein J, Aalberse RC, Aarden L, Rispens T, et al. Antibodies to constant domains of therapeutic monoclonal antibodies. J Clin Pharmacol 1996;36:82–5.

22. Wolbink GJ, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA 2011;305:1460–8.

23. Hart MH, de Vrieze H, Wouters D, Wolbink GJ, Killestein J, de Groot ER, et al. Differential effect of drug interference in immunogenicity assays. J Immunol Methods 2011;372:196–203.

24. Wolbink GJ, Aarden LA, Dijkmans BA. Dealing with immunogenicity of biologicals: assessment and clinical relevance. Curr Opin Rheumatol 2009;21:211–15.

25. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, et al. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998;41:1552–63.

26. Chainge B, Watier H. Monoclonal antibodies in excess: A simple way to avoid immunogenicity in patients? J Allergy Clin Immunol 2015;136:814–16.

27. van Schouwenburg PA, Bartelds GM, Hart MH, Aarden L, Wolbink GJ, Wouters D. A novel method for the detection of antibodies to adalimumab in the presence of drug reveals “hidden” immunogenicity in rheumatoid arthritis patients. J Immunol Methods 2010;362:82–8.
Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary figure S1

Please note that the editors are not responsible for the content or functionality of any supplementary material supplied by the authors. Any queries should be directed to the corresponding author.