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Original article

# Effect of tocilizumab on neutrophils in adult patients with rheumatoid arthritis: pooled analysis of data from phase 3 and 4 clinical trials

**Robert J. Moots[1], Anthony Sebba[2], William Rigby[3], Andrew Ostor[4], Benjamin Porter-Brown[5], Francis Donaldson[5], Sophie Dimonaco[5], Andrea Rubbert-Roth[6], Ronald van Vollenhoven[7] and Mark C. Genovese[8]**

## Abstract

**Objectives.** To investigate changes in neutrophil count and occurrences of infection in RA patients treated with the IL-6 receptor-α inhibitor tocilizumab (TCZ).

**Methods.** Data were pooled from patients who received i.v. TCZ (4 mg/kg + MTX, 8 mg/kg ± DMARDs, 10 mg/kg) or placebo + DMARDs in phase 3/4 clinical trials, long-term extensions or a pharmacology study. Neutrophil counts were measured routinely according to the Common Toxicity Criteria for Adverse Events grades; TCZ dosing was adjusted if necessary. Covariates associated with decreased neutrophil counts were assessed with multivariate regression analysis. Infection rates within 30 days of neutrophil count changes were calculated per 100 patient-years of TCZ exposure.

**Results.** In placebo-controlled parts of trials, more TCZ-treated than placebo-treated patients had grade 1/2 or 3/4 neutrophil counts (TCZ: 28.2%/3.1%; placebo: 8.9%/0.2%). In placebo-controlled trials + long-term extensions, 4171 patients provided 16204.8 patient-years of TCZ exposure. Neutrophil counts decreased through week 6 from baseline [mean (S.D.) change, −2.17 (2.16) $\times$ $10^9$/l) and remained stable thereafter. Rates (95% CI) of serious infections within 30 days of normal [4.66 (4.31, 5.03)], grade 1/2 [2.48 (1.79, 3.34)] and 3/4 [2.77 (0.34, 10.01)] neutrophil counts were similar. Baseline neutrophil count $<2 \times 10^9$/l and female gender were associated with grade 3/4 neutrophil counts [odds ratio (OR) (95% CI): 19.02 (6.76, 53.52), 2.55 (1.40, 4.66)]. Patients who stopped TCZ in response to decreased neutrophil count returned more quickly to normal levels than patients who reduced or continued their dose.

**Conclusion.** Decreases in neutrophil counts in patients taking TCZ do not appear to be associated with serious infections and are normalized by current risk mitigation guidelines.

**Key words:** infections and arthritis, neutrophils, inflammation, rheumatoid arthritis, biological therapies

CLINICAL SCIENCE

**Rheumatology key messages**

- A small proportion of tocilizumab-treated RA patients in clinical trials experienced grade 3 or 4 neutrophil counts.
- Decreased neutrophil counts during tocilizumab treatment in RA do not appear to be associated with serious infections.
- A risk mitigation strategy of dose interruption effectively addresses neutrophil decreases in RA.

[1]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK, [2]Department of Rheumatology, University of South Florida, Tampa, FL, [3]Medicine/Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Lebanon, NH, USA, [4]Department of Rheumatology, Addenbrookes Hospital, Cambridge, [5]Roche Products Limited, Welwyn Garden City, UK, [6]Department of Internal Medicine, University of Cologne, Cologne, Germany, [7]Department of Medicine, Karolinska Institute, Stockholm, Sweden and [8]Division of Rheumatology, Stanford University Medical Center, Palo Alto, CA, USA

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Correspondence to: Robert J. Moots, Institute of Ageing and Chronic Disease, University of Liverpool, Clinical Sciences Centre, University Hospital Aintree, Longmoor Lane, Liverpool L9 7AL, UK.
E-mail: rjmoots@liv.ac.uk

## Introduction

In RA, neutrophils are involved in inflammation through the release of cytokines and chemokines that regulate the function of other immune cells, the upregulation of expression of plasma membrane receptors (e.g. MHC class II antigens), cell–cell interactions (e.g. NK cell activation) and the release of proteases that activate or deactivate cytokines and chemokines [1]. Activated neutrophils are a major source of soluble IL-6Rs, which initiate trans-signalling in other immune cells that do not constitutively express IL-6R; this is a central process in chronic inflammation [1–4].

It remains unclear whether IL-6 has an effect on neutrophil kinetics; preclinical and *ex vivo* studies have provided conflicting results [5–7]. IL-6 induces neutrophil demargination and release from the bone marrow marginal pool *in vivo* [8]. Preclinical studies suggest that a reduction in neutrophil count with the IL-6R-$\alpha$ inhibitor tocilizumab (TCZ) may result from increased margination of circulating neutrophils into the bone marrow rather than from the drug-induced neutropenia observed with myelotoxic drugs [8–10].

TCZ is a recombinant, humanized, anti-human IL-6R mAb that binds to soluble and membrane-bound IL-6R-$\alpha$ and inhibits IL-6 signalling pathways [11, 12]. Decreased neutrophil counts have been reported in trials of TCZ in RA patients [13, 14]. Blocking IL-6 signalling with TCZ is associated with transient neutropenia, but *ex vivo* findings show this does not directly affect neutrophil functions associated with host defence [7].

An analysis of long-term safety data from TCZ phases 3 and 4 trials was performed to assess changes in neutrophil count in RA patients treated with TCZ, to determine whether decreased neutrophil count was associated with increased risk for infection, to investigate baseline covariates that may be associated with reduction in neutrophil count and to review responses to the current risk mitigation guidance for decreased neutrophil count in patients receiving TCZ.

## Patients and methods

### Patient populations

Patients were included from the i.v. TCZ long-term extension (LTE) all-exposure and pooled placebo-controlled populations. The LTE all-exposure population included all patients who received $\geqslant 1$ dose of TCZ 4 or 8 mg/kg and had at least one post-randomization safety assessment in five phase 3 studies [15–19] and their LTEs, patients who received 8 mg/kg TCZ in a phase 4 study [14] and 23 patients who received a single dose of TCZ 10 mg/kg in a clinical pharmacology study (and had the option to enrol in an LTE) [20]. Data were analysed from initial TCZ infusion (baseline) through 2 May 2012. For some analyses, patients from the LTE all-exposure population were grouped as having anti-TNF inadequate response (aTNF-IR) [17] or DMARD inadequate response (DMARD-IR) [15, 16, 19] or as MTX naïve (subset of patients who had never received MTX) [18]. Patients in the phase 3/4 studies were randomly assigned to TCZ 8 mg/kg ± DMARD, TCZ 4 mg/kg + DMARD or placebo + DMARD every 4 weeks. All patients in the LTEs received open-label TCZ 8 mg/kg ± DMARD, which could be temporarily interrupted and reduced to 4 mg/kg, if needed, for risk mitigation. The pooled placebo-controlled population included patients who received placebo or TCZ during the double-blind, placebo-controlled phase of each phase 3 study [15–19]. All studies from which data were taken were approved by the ethics committees for each participating site and complied with the principles of Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent.

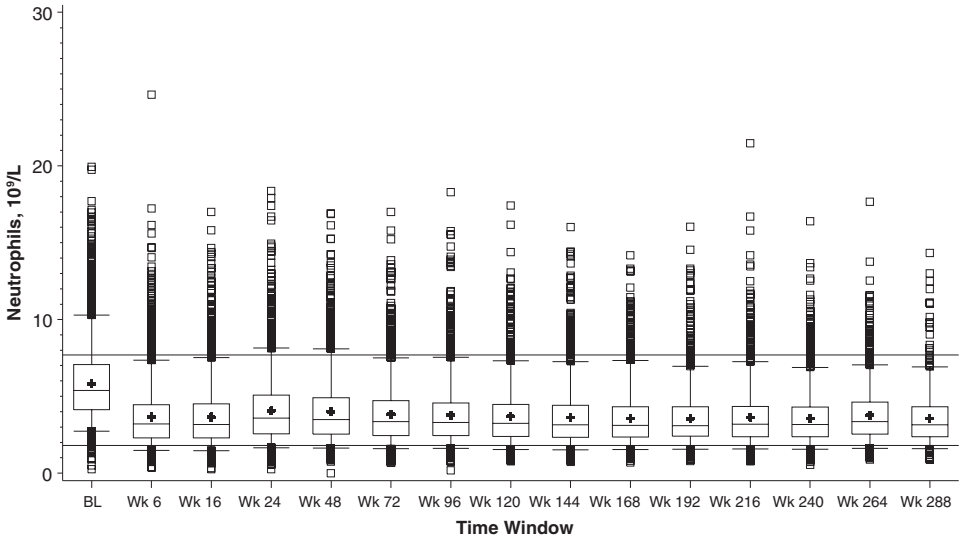
### Assessments

Grades of neutrophil decreases were assessed according to the Common Toxicity Criteria for Adverse Events, version 3.0 [20]. Adverse events (AEs) and serious AEs (SAEs) were reported using the Medical Dictionary for Regulatory Activities, version 15.0. Rates of AEs and SAEs around events of neutrophil count decreases were examined. These were defined as AEs or SAEs that occurred within 30 days before or after the worst laboratory result indicative of grade 1/2 or 3/4 neutropenia. Neutrophil counts were performed every 4 weeks in the placebo-controlled studies or every 8 or 12 weeks in the LTEs. Protocol-defined TCZ dose modifications within the study protocols were reflective of risk mitigation recommendations in the USA and European labels [21, 22] such that TCZ was interrupted if absolute neutrophil count (ANC) was $<1.0 \times 10^9$/l and discontinued if ANC was $<0.5 \times 10^9$/l.

### Statistical analyses

Baseline was defined as the day of first TCZ infusion. AE rates were expressed per 100 patient-years (PY) of exposure with 95% CIs. Multiple occurrences of AEs were counted. Individual PY duration was calculated as date of the last safety observation minus date of the first TCZ dose plus 1. Results are provided in the form of summary statistics unless otherwise stated and were calculated based on all available non-missing data. Percentages are based on total number of patients assessed within a category. Summary of the time to last neutrophil depression to return to normal was based on a patient's last depression. Patients with a worst neutrophil count of grade 1/2 or 3/4 were analysed separately. Dose modification was considered to be a change in TCZ dose following any neutrophil depression of the relevant grade during the study. Multivariate logistic regression analysis using a stepwise backwards elimination method was performed to investigate the relationship between decreased neutrophil counts (grade 3/4) and baseline variables [see supplementary Table S1, available at *Rheumatology* Online, for baseline demographics by Common Toxicity Criteria (CTC) neutrophil grade] in the LTE all-exposure population. A significance level of 0.05 was used for inclusion in the model. Covariates included were age at screening, gender and the following baseline parameters:

**Fig. 1** Mean neutrophil counts over time (long-term extension all-exposure population)




Worst values per visit are used. Data are shown only if the number of assessments at a time point was $\geqslant$5% of *N* (*N* = 4171).

weight, disease duration, DAS28, oral CS dose, MTX dose, number of previous aTNFs, concomitant DMARD use (other than MTX), IL-6, soluble IL-6R, CRP and neutrophil count (supplementary Table S2, available at *Rheumatology* Online). Patients with missing data for one or more analysis covariates were not included in the regression model. No imputation was performed for any missing data. Regression analysis was based on approximately two-thirds ($n = 2732$) of patients participating in the LTE studies.

## Results

### Patients

The LTE all-exposure population included 4171 patients, providing a total duration of 16 204.8 PY of TCZ exposure. The pooled placebo-controlled population included 4098 patients; 1454 received placebo + DMARDs and 2644 received TCZ ± DMARDs. Baseline characteristics in the LTE all-exposure population were similar in the three patient groups, with exceptions as expected for each patient population; they were also similar between placebo- and TCZ-treated patients in the placebo-controlled pooled population (supplementary Table S3, available at *Rheumatology* Online). Mean (S.D.) baseline neutrophil counts ranged from 5.4 (2.25) $\times 10^9$/l in MTX-naïve patients to 6.3 (2.66) $\times 10^9$/l in aTNF-IR patients.

### Neutrophil counts and CTC grades

In the LTE all-exposure population, mean neutrophil counts decreased from baseline to week 6 within the normal range [mean (S.D.) change, −2.17 (2.16) $\times$ $10^9$/l) and remained stable thereafter (Fig. 1). In the LTE all-exposure population, similar proportions of DMARD-IR, aTNF-IR and MTX-naïve patients experienced all grades of neutrophil count, except for grade 2, which was reported in 12.1% of aTNF-IR patients, 19.1% of DMARD-IR patients and 20.1% of MTX-naïve patients (Table 1). Grade 3/4 neutrophil counts were experienced by 6% of DMARD-IR and aTNF-IR patients and by 7% of MTX-naïve patients; most (4.7–6.7%) were grade 3. In the placebo-controlled pooled population, greater proportions of patients treated with TCZ than with placebo + DMARDs experienced all grades of neutrophil count (Table 1).

Most patients who experienced grade 3/4 neutrophil counts did so on only a single visit, two consecutive visits or non-consecutive visits (90.0% of TCZ-treated patients in the placebo-controlled pooled population and 93.9, 88.9 and 83.3% for DMARD-IR, aTNF-IR and MTX-naïve LTE patients, respectively; Table 2).

Logistic regression analysis of grade 3/4 neutrophil count *vs* baseline characteristics demonstrated that baseline neutrophil count and gender were significantly associated with the occurrence of grade 3/4 neutrophil count at any time in the LTE all-exposure population ($P < 0.0001$ and $P = 0.0022$, respectively). Patients with baseline neutrophil counts $<2 \times 10^9$/l were 19 times more likely to experience grade 3/4 neutrophil counts than patients with baseline neutrophil counts $\geqslant 2 \times 10^9$/l [odds ratio (OR) = 19.02 (95% CI: 6.76, 53.52)]. Female patients were more than twice as likely as male patients to experience grade 3/4 counts [OR = 2.55 (95% CI: 1.40, 4.66)]. Results of a sensitivity analysis including baseline TCZ dose did not affect the outcome of the model (supplementary Table S4, available at *Rheumatology* Online).

In the LTE all-exposure population, recovery to normal neutrophil levels was shorter for patients who stopped TCZ than for those who did not. Time to recovery was longer after grade 3/4 neutrophil count than after grade

**Table 1** Worst neutrophil common toxicity criteria grades

| Neutrophils | Placebo-controlled pooled population | | LTE all-exposure population | | | |
|---|---|---|---|---|---|---|
| | Placebo+DMARD[a] *n* = 1454 | All TCZ[b] *n* = 2644 | DMARD-IR *n* = 2904 | aTNF-IR *n* = 464 | MTX-naïve *n* = 417 | All TCZ[c] *N* = 4171 *n* = 4163[d] |
| Normal | 1320 (90.8) | 1814 (68.6) | 1514 (52.1) | 280 (60.3) | 220 (52.8) | 2256 (54.2) |
| Grade 1 | 88 (6.1) | 461 (17.4) | 655 (22.6) | 101 (21.8) | 83 (19.9) | 900 (21.6) |
| Grade 2 | 41 (2.8) | 284 (10.7) | 554 (19.1) | 56 (12.1) | 84 (20.1) | 757 (18.2) |
| Grade 3 | 3 (0.2) | 73 (2.8) | 164 (5.6) | 22 (4.7) | 28 (6.7) | 223 (5.4) |
| Grade 4 | 0 | 8 (0.3) | 17 (0.6) | 5 (1.1) | 2 (0.5) | 27 (0.6) |

Percentages are based on overall *N* unless *n* is shown. Data are *n* (%), where *n* = number of patients who experienced the CTC grade for neutrophil count as their worst event. Grade 1 neutrophil count is defined as ANC < lower limit of normal to $1.5 \times 10^9$/l; grade 2, ANC <1.5 to $1.0 \times 10^9$/l; grade 3, ANC <1.0 to $0.5 \times 10^9$/l; grade 4, ANC $<0.5 \times 10^9$/l. [a]CTC neutrophil grade data were missing for two patients. [b]CTC neutrophil grade data were missing for four patients. [c]Includes 201 MTX non-naïve patients from Jones *et al.* [18], 162 patients from Gabay *et al.* [14] and 23 patients from the clinical pharmacology study. [d]Number of patients who had neutrophil measurements. ANC: absolute neutrophil count; aTNF: anti-TNF; IR: inadequate response; LTE: long-term extension.

**Table 2** Patterns of common toxicity criteria grades 1 or 2 and 3 or 4 neutrophil counts

| | Placebo-controlled pooled population | | LTE all-exposure population | | |
|---|---|---|---|---|---|
| | Placebo+DMARD *n* = 1454 | All TCZ *n* = 2644 | DMARD-IR *n* = 2904 | aTNF-IR *n* = 464 | MTX-naïve *n* = 417 |
| Grade 1 or 2, *n*[a] | 127 | 818 | 1382 | 180 | 196 |
| Single visit, *n* (%) | 73 (57.5) | 282 (34.5) | 333 (24.1) | 47 (26.1) | 43 (21.9) |
| 2 consecutive visits, *n* (%) | 23 (18.1) | 155 (18.9) | 252 (18.2) | 34 (18.9) | 38 (19.4) |
| 3 to ⩾ 6 consecutive visits, *n* (%) | 12 (9.4) | 183 (22.4) | 477 (34.5) | 48 (26.7) | 70 (35.7) |
| Non-consecutive visits, *n* (%) | 19 (15.0) | 198 (24.2) | 320 (23.2) | 51 (28.3) | 45 (23.0) |
| Grade 3 or 4, *n*[a] | 3 | 80 | 180 | 27 | 30 |
| Single visit, *n* (%) | 3 (100) | 55 (68.8) | 110 (61.1) | 14 (51.9) | 13 (43.3) |
| 2 consecutive visits, *n* (%) | 0 | 8 (10.0) | 25 (13.9) | 5 (18.5) | 4 (13.3) |
| 3 to ⩾ 6 consecutive visits, *n* (%) | 0 | 8 (10.0) | 11 (6.1) | 3 (11.1) | 5 (16.7) |
| Non-consecutive visits, *n* (%) | 0 | 9 (11.3) | 34 (18.9) | 5 (18.5) | 8 (26.7) |

[a]Number of patients within neutrophil grade after baseline. Consecutive indicates laboratory samples that follow from each other. Non-consecutive indicates patients who have grade of neutrophil counts on ⩾1 non-consecutive visit. Categories are mutually exclusive—patients were included in their worst post-baseline category, with worst category considered highest number of consecutive visits, followed by non-consecutive visits, then single visit. Grade 1 neutrophil count is defined as ANC < lower limit of normal to $1.5 \times 10^9$/l; grade 2, ANC <1.5 to $1.0 \times 10^9$/l; grade 3, ANC <1.0 to $0.5 \times 10^9$/l; grade 4, ANC $<0.5 \times 10^9$/l. ANC: absolute neutrophil count; aTNF: anti-TNF; IR: inadequate responder; LTE: long-term extension; TCZ: tocilizumab.

1/2 neutrophil count (Table 3). Mean (minimum–maximum) time from last neutrophil depression to return to normal levels was shorter for patients who stopped TCZ [30.5 (4–135) days after grade 1/2 count and 42.2 (3–372) days after grade 3/4 count] or who reduced and then stopped TCZ [31.3 (26–64) days and 40.5 (22–59) days, respectively] compared with those who reduced only TCZ [62.4 (8–166) days and 112.6 (3, 1316) days, respectively] or who did not modify their dose [62.9 (3–316) days and 119.7 (1–1019) days, respectively]. Grade 1/2 neutrophil counts of 172 patients were recorded as not having returned to normal because no neutrophil counts were taken after the last grade 1/2 episode. Of the 26 patients whose grade 3/4 counts did not return to normal, 16 patients had no further neutrophil data because they were lost to follow-up or reached the end of the study, and 10 patients' counts improved to grade 1/2 at the last measurement.

### Infections and serious infections

In the pooled placebo-controlled population, there were no serious infections around grade 3/4 neutrophil counts for placebo-treated or TCZ-treated patients and no

**Table 3** Normalization of neutrophil counts according to tocilizumab dose modifications

| Time from last depression to return from normal[a], days | Grade 1 or 2 | Grade 3 or 4 |
|---|---|---|
| Patients who reduced TCZ | 62.39 [63 (8, 166)] $n = 80$ | 112.60 [57 (3, 1316)] $n = 52$ |
| Patients who stopped TCZ | 30.50 [28 (4, 135)] $n = 237$ | 42.18 [27.5 (3, 372)] $n = 28$ |
| Patients who reduced then stopped TCZ | 31.32 [28 (26, 64)] $n = 22$ | 40.50 [40.5 (22, 59)] $n = 2$ |
| Patients who did not amend their TCZ dose | 62.93 [78 (3, 316)] $n = 1145$ | 119.73 [70.0 (1, 1019)] $n = 142$ |

Data are mean [median (minimum, maximum)]. Grade 1 neutrophil count is defined as ANC < lower limit of normal –$1.5 \times 10^9$/l; grade 2, ANC <1.5–$1.0 \times 10^9$/l; grade 3, ANC <1.0–$0.5 \times 10^9$/l; grade 4, ANC <$0.5 \times 10^9$/l. [a]A patient's neutrophil level was considered to have 'normalized' if it returned to normal after the last neutrophil depression. ANC: absolute neutrophil count; LTE: long-term extension; TCZ: tocilizumab.

infections around grade 3/4 neutrophil counts in placebo-treated patients. In TCZ-treated patients, rates of infection and serious infection were similar around normal neutrophil count, grade 1/2 neutrophil count and grade 3/4 neutrophil count.

In the LTE all-exposure population, the overall rate of serious infection was low across all populations (Table 4). Rates of infection and serious infection were highest in aTNF-IR patients, reported at 144.07/100 PY (95% CI: 138.3, 150.0) and 6.26/100 PY (95% CI: 5.1, 7.6), respectively. The most common serious infections in the LTE all-exposure population were pneumonia (0.68/100 PY, 95% CI: 0.56, 0.82) and cellulitis (0.52/100 PY, 95% CI: 0.41, 0.64). In all three groups in the LTE all-exposure population, rates of infection and serious infection around normal, grade 1/2 and grade 3/4 neutrophil counts were generally similar, with no consistent patterns across treatment groups. The rate of all infections was generally highest in aTNF-IR patients across all grades of neutrophil count, consistent with the overall higher infection rate in these patients. Rates of infection and serious infection occurring around grade 3/4 neutrophil count were low and did not show any particular pattern around treatment groups; however, the duration of TCZ exposure was also low. Two events of serious infection occurring around grade 3/4 neutrophil count were reported: empyema in a DMARD-IR patient (occurring within 30 days after the grade 3/4 neutrophil count) and pneumonia in an MTX-naïve patient (occurring before the grade 3/4 neutrophil count). No serious infections around grade 3/4 neutrophil count were reported in aTNF-IR patients. Analysis of serious infections in patients who had grade 3/4 neutrophil counts at any time indicated no serious infections occurred in the LTE all-exposure population other than in the two patients identified, who had grade 3/4 neutrophil counts within 30 days of serious infection.

## Discussion

As reflected in the USA and European labels [21, 22], the proportion of patients with reduced neutrophil counts (all grades) in the pooled placebo-controlled population was greater in TCZ-treated patients than in placebo-treated patients. Most patients who experienced reduced neutrophil counts did so during a single visit, two consecutive visits or non-consecutive visits. The fact that a minority had grade 3/4 neutrophil counts on $\geqslant 3$ consecutive visits indicates that risk minimisation measures required in the clinical development programme were effective.

Overall, patients treated with TCZ in the LTE all-exposure population had initial decreases in mean neutrophil levels that remained within the normal range thereafter, and the rate of neutrophil levels over time remained unchanged. There was some evidence of greater incidence of grade 2 or 3 neutrophil counts in the LTE all-exposure population compared with TCZ-treated patients in the pooled placebo-controlled population, likely because of the relative proportions of patients in each population who received TCZ for the first time and because some patients switched from 4 mg/kg in the placebo-controlled studies to 8 mg/kg in the LTE studies. Approximately 40% of patients evaluated received TCZ for the first time or switched from 4 to 8 mg/kg in the LTE studies. Data from subcutaneous administration of TCZ have also demonstrated decreases in neutrophil levels to an equal or lesser extent than observed with intravenous administration [23, 24].

The serious infection rate was low across all groups in the LTE all-exposure population, but was higher in aTNF-IR patients than in DMARD-IR and MTX-naïve patients. This pattern was also observed for all infections and likely reflects the greater disease burden and cumulative use of immunosuppressive treatments in aTNF-IR patients. The rate of serious infection was stable over time in the LTE all-exposure population, as previously reported [13, 25]. Rates of serious infection observed in the LTE all-exposure population were similar to published rates in LTEs and registries for RA patients treated with aTNFs [26–29].

The occurrence of serious infection in patients treated with TCZ does not appear to be associated with reduced neutrophil counts. In the pooled placebo-controlled population, no serious infections occurred around grade 3/4 neutrophil counts. However, the duration of exposure to TCZ (16.86 PY) was low around grade 3/4 events. Furthermore, the all-infection rate in the TCZ-treated patients around grade 3/4 neutrophil counts was generally similar to that around grade 1/2 counts. In the LTE all-exposure population, rates of serious infection were similar around normal neutrophil counts, grade 1/2 counts and grade 3/4 counts, suggesting no association of serious infection with reduction in neutrophils, because the rates

**Table 4** Rates of infection and serious infection adverse events

| Infection adverse events | Placebo-controlled pooled population | | LTE all-exposure population | | | |
|---|---|---|---|---|---|---|
| | Placebo+DMARD[a] *n* = 1454 | All TCZ *n* = 2644 | DMARD-IR *n* = 2904 | aTNF-IR *n* = 464 | MTX-naïve *n* = 417 | All TCZ[b] *N* = 4171 *n* = 4163[c] |
| Overall duration, PY | 742.69 | 1560.28 | 11 815.11 | 1629.80 | 1748.96 | 16 204.77 |
| Overall infections | 99.77 [92.7, 107.2] (741) | 109.92 [104.8, 115.2] (1715) | 87.07 [85.4, 88.8] (10 287) | 144.07 [138.3, 150.0] (2348) | 79.76 [75.6, 84.1] (1395) | 92.73 [91.25, 94.22] (15 026)[d] |
| Overall serious infections | 3.37 [2.2, 5.0] (25) | 4.74 [3.7, 6.0] (74) | 4.10 [3.7, 4.5] (484) | 6.26 [5.1, 7.6] (102) | 4.69 [3.7, 5.8] (82) | 4.42 [4.11, 4.76] (717)[d] |
| Duration around normal neutrophil count, PY | 855.36 | 1406.7 | 10 457.0 | 1486.7 | 1557.7 | 14 396.0 |
| Infections around normal neutrophil count | 96.80 [90.32, 103.63] (828) | 104.64 [99.36, 110.13] (1472) | 82.69 [80.96, 84.45] (8647) | 138.16 [132.25, 144.27] (2054) | 74.40 [70.18, 78.81] (1159) | 88.07 [86.54, 89.61] (12 678) |
| Serious infections around normal neutrophil count | 3.16 [2.08, 4.59] (27) | 5.05 [3.94, 6.37] (71) | 4.34 [3.95, 4.76] (454) | 6.52 [5.29, 7.96] (97) | 4.81 [3.79, 6.04] (75) | 4.66 [4.31, 5.03] (671) |
| Duration around grade 1/2 neutrophil count, PY | 28.64 | 250.22 | 1307.5 | 135.89 | 180.93 | 1736.6 |
| Infections around grade 1/2 neutrophil count | 111.75 [76.44, 157.76] (32) | 112.30 [99.55, 126.23] (281) | 84.21 [79.31, 89.33] (1101) | 130.99 [112.45, 151.71] (178) | 83.46 [70.68, 97.88] (151) | 88.97 [84.58, 93.52] (1545) |
| Serious infections around grade 1/2 neutrophil count | 10.48 [2.16, 30.62] (3) | 2.40 [0.88, 5.22] (6) | 2.22 [1.49, 3.19] (29) | 3.68 [1.19, 8.59] (5) | 3.32 [1.22, 7.22] (6) | 2.48 [1.79, 3.34] (43) |
| Duration around grade 3/4 neutrophil count, PY | 0.34 | 16.86 | 50.70 | 7.24 | 10.31 | 72.15 |
| Infections around grade 3/4 neutrophil count | 0 | 136.44 [86.49, 204.73] (23) | 108.48 [81.72, 141.20] (55) | 193.33 [105.69, 324.37] (14) | 29.10 [6.00, 85.05] (3) | 109.49 [86.69, 136.46] (79) |
| Serious infections around grade 3/4 neutrophil count | 0 | 0 | 1.97 [0.05, 10.99] (1) | 0 | 9.70 [0.25, 54.05] (1) | 2.77 [0.34, 10.01] (2) |
| Duration for patients with serious infection and grade 3/4 neutrophil count, PY | NA | NA | NA | NA | NA | 8.13 |
| Serious infections in patients with grade 3/4 neutrophil count at any time | NA | NA | NA | NA | NA | 24.60 [2.98, 88.88] (2) |

[a]Includes patients receiving MTX monotherapy. [b]Includes 201 MTX non-naïve patients from Jones *et al.* [18], 162 patients from Gabay *et al.* [14] and 23 patients from the clinical pharmacology study. [c]Number of patients with neutrophil assessment. [d]Calculated based on $n = 4171$. Percentages are based on overall *N* unless *n* is shown. Data are rates per 100 PY [95% CI] (number of events), unless stated otherwise. Multiple occurrences of the same adverse event in one individual are counted each time. PY refers to duration in study, calculated from first dose of tocilizumab to last available safety assessment plus 1. Around grade 3/4 neutrophil count is defined as the last time period of ±30 days of the neutrophil laboratory result. Around grade 1/2 is defined as ±30 days of the neutrophil laboratory result that is not around grade 3/4. Neutrophil normal is defined as the remaining period. Grade 1 neutrophil count is defined as ANC <lower limit of normal –$1.5 \times 10^9$/l; grade 2, ANC <1.5–1.0 $\times 10^9$/l; grade 3, ANC <1.0–0.5 $\times 10^9$/l; grade 4, ANC <0.5 $\times 10^9$/l. ANC: absolute neutrophil count; aTNF: anti-TNF; IR: inadequate responder; LTE: long-term extension; NA: not applicable; PY: patient-years; TCZ: tocilizumab.

around grade 3/4 were driven by only two serious infection events. The rate of all infections around all neutrophil count categories was generally lower in the LTE all-exposure population than in the pooled placebo-controlled population, perhaps because patients with acceptable tolerability were more likely to continue TCZ treatment in the LTE or because of less frequently scheduled neutrophil count testing in the LTE studies than in the placebo-controlled studies. These analyses were limited by the frequency of laboratory assessments in the studies (i.e. neutropenia might have occurred at any time within 30 days before the actual assessment, but the value included came only from samples collected at patient visits). To account for this, a ±30-day window around each visit was used.

The mechanism of the decreased neutrophil counts with TCZ is unknown. In a primate model of arthritis managed with TCZ, the absence of bone marrow myeloid hyperplasia or hypoplasia when ANC was reduced and the lack of neutrophil morphological abnormalities strongly suggested that neither peripheral sequestration nor incomplete granulopoiesis was the underlying

mechanism of the reduced circulating neutrophils [30]. A recent study [7] of neutrophil function and survival, which included RA patients treated with TCZ in an open-label, phase 4 trial, demonstrated that TCZ treatment did not have a direct effect on neutrophil functions, including apoptosis, phagocytosis, respiratory burst, chemotaxis or expression of adhesion molecules. *In vitro* evidence suggests that IL-6 increases circulating neutrophils by releasing them from marginated pools in bone marrow [8]. Thus, TCZ may potentially reverse this effect. Neutrophil counts were elevated in a monkey model of arthritis and were rapidly increased by the administration of IL-6, a kinetic effect suggestive of migration of neutrophils from the marginal pool to the circulation. The increase in neutrophils was inhibited by TCZ [9]. Furthermore, IL-6 administration resulted in reduced neutrophil expression of the adhesion molecule CD162 (involved in margination of neutrophils) by IL-8 and granulocyte macrophage–colony-stimulating factor; TCZ decreased IL-8 and GM-CSF levels and could reverse the IL-6–mediated inhibition of CD162 expression, further supporting the hypothesis of TCZ-induced margination of neutrophils [9]. A study to determine the effects of TCZ on neutrophil kinetics was recently completed, and results are expected soon (ClinicalTrials.gov, NCT01991990).

A population pharmacokinetics/pharmacodynamics model of neutrophil time course suggested that with higher TCZ exposures, neutrophil counts reach a trough, with no further decreases (supplementary Fig. S1A and S1B, available at *Rheumatology* Online). The exposure-dependent effect on neutrophil decreases did not result in an increased incidence of grade 4 neutrophil counts, consistent with findings from TCZ clinical trials (supplementary Table S5, available at *Rheumatology* Online).

Multivariate logistic regression analysis indicated that female gender and baseline neutrophil count $<2 \times 10^9$/l were significantly associated with the occurrence of grade 3/4 neutrophil count, whereas the association of baseline oral CS use approached significance ($P = 0.0517$). In the current prescribing information, TCZ initiation is not recommended if the baseline neutrophil count is $<2 \times 10^9$/l [21, 22], based on previous observations in the TCZ clinical trial programme. Results of the multivariate regression analysis are consistent with the retrospective cohort analysis of aTNFs, which showed that baseline neutrophil count was a significant independent predictor of neutropenia after aTNF therapy [31]. Although observational data demonstrate that female RA patients have more severe disease than male RA patients and are more likely to be treated with DMARDs and aTNFs [32], this may not explain the current results in which female gender was associated with grade 3/4 neutrophil count independently of disease status or co-medication parameters included in the analysis. In addition, previous pharmacokinetic analyses found no clinically relevant difference in TCZ exposure between male and female RA patients (unpublished data, Roche Products Ltd, 2007). It may be that a covariate not included in the current analysis or the heavy gender imbalance in RA (~80% female in the current analysis) influenced the results.

Most decreased neutrophil counts returned to normal during study participation. Moreover, the time to normalization was shorter for patients who stopped TCZ than for those whose doses were not modified. These data highlight the importance of neutrophil monitoring and risk mitigation in patients treated with TCZ. Consistent with the data presented here and the risk mitigation strategies outlined in current US [21] and European [22] labels, TCZ should not be initiated in patients with ANC $<2.0 \times 10^9$/l, and neutrophils should be monitored during TCZ treatment in accordance with label recommendations, with dose interruption recommended for ANC $0.5–1.0 \times 10^9$/l and discontinuation of TCZ for ANC $<0.5 \times 10^9$/l.

### Conclusion

Results from this pooled analysis of TCZ clinical trial and LTE data demonstrate that a small proportion of patients had grade 3/4 neutrophil counts with TCZ treatment and that, consistent with label recommendations, low baseline neutrophil count is the strongest predictor of grade 3/4 neutrophil count. Neutrophil decreases typically occur on one visit or two consecutive visits. Although infections may develop in patients during TCZ treatment, no temporal relationship between decreased neutrophil count and serious infection has been observed to date. Overall, patients treated with TCZ who experience decreased neutrophil counts do not appear to be at increased risk for infection compared with those who do not experience such decreases. The risk mitigation strategy of dose interruption is effective in addressing neutrophil decreases. Investigation of the mechanisms of TCZ-induced decreases in neutrophils is ongoing.

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## Supplementary data

Supplementary data are available at *Rheumatology* Online.

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