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Original article

Risk factors of serious infections in patients with rheumatoid arthritis treated with tocilizumab in the French Registry REGATE

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Abstract

Objectives. Observational studies have already reported the risk of serious infections in RA treated with tocilizumab, but in limited samples. The aim of this study was to investigate the predictive risk factors for serious infections in the largest European registry of patients treated with tocilizumab for RA.

Methods. A total of 1491 RA patients included in the French REGistry–RoAcTEmra were analysed to calculate the incidence rate of first serious infections rate after initiation of tocilizumab. To identify independent factors associated with serious infections, a Cox model was performed.

Results. Among the 1491 patients, average age 56.6 (13.6) years, 125 serious infections occurred in 122 patients (incidence rate of serious infection: 4.7/100 patient-years). Univariate analysis identified initial ACPA positivity as the only factor associated with a lower risk of serious infection [hazard ratio (HR) = 0.56, 95% CI: 0.36, 0.88]. Other factors significantly associated with a higher risk of serious infections were DAS28, concomitant Leflunomide (LEF) treatment, and absolute neutrophil count (ANC) at baseline. Initial ANC above 5.0×10^9/l (HR = 1.94, 95% CI: 1.32, 2.85; $P < 0.001$), negative ACPA (HR = 1.79, 95% CI: 1.15, 2.78; $P = 0.012$) at baseline and concomitant LEF treatment (LEF alone *vs* no treatment, HR = 2.18, 95% CI: 1.22, 3.88; $P = 0.009$) remained significantly associated with first serious infections in multivariate analysis after imputation for missing data.

Conclusion. The rate of first serious infections in current practice is similar to that reported in clinical trials. High ANC (above 5.0×10^9 at baseline), negative ACPA and concomitant therapy with LEF are predictive factors of serious infection, requiring in this case a tighter surveillance.

Key words: rheumatoid arthritis, observational study, registry, tolerance, safety, serious infection, risk factors, tocilizumab, DMARDs, steroids

CLINICAL SCIENCE

Rheumatology key messages

- For RA patients treated with tocilizumab in routine, the incidence of serious infections is 4.7/100 patient-years.
- High neutrophil count, negative ACPA and leflunomide association at baseline are predictive of serious infections with tocilizumab in RA.

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Introduction

The prognosis of RA has considerably improved over the last two decades, especially with access to biological agents such as monoclonal antibodies, and fusion protein. Over the years, excluding biosimilars, five different TNF antagonists and four non–TNF-targeted biologic drugs have obtained an indication for RA treatment [1]. The most frequently used non–TNF-targeted biologics are abatacept, an inhibitor of T cell co-stimulation; rituximab, a B cell depleting agent and tocilizumab (TCZ), an inhibitor of IL-6 receptor. These new therapeutic drugs have, however, some side effects, in particular serious infections, which need to be considered in the risk–benefit balance. The French registries Orencia and Rheumatoid Arthritis (ORA), and Autoimmunity and Rituximab (AIR) have already reported that the rate of serious infections was, respectively, 4.1 and 5/100 patient-years (PYs) for abatacept and rituximab [2, 3]. For anti-TNF biodrugs, the serious infections rate ranged between 3 and 6/100 PYs [4–6]. TCZ is the most recent biologic drug that has obtained the label for RA, with a lower i.v. dose proposed in USA compared with in Europe (4 mg *vs* 8 mg/kg/month). From pivotal studies, adverse events were mainly serious infections, infusion reaction, neutropenia, cholesterol and liver enzymes increase [7]. Some observational studies reported safety of TCZ in real life, but with a restricted number of patients. In the present study, we analysed the rates of serious infections, and the associated risk factors in 1491 RA patients treated with TCZ and included in the REGistry–RoAcTEmra (REGATE) registry, which was set up by the French Society of Rheumatology in 2011.

Methods

The REGATE registry is an ongoing nationwide prospective cohort study investigating the long-term safety and efficacy of TCZ for treating RA. The primary end point of this observational study is safety. All side effects are collected for patients while under TCZ, and also if TCZ is stopped during the 5 years of follow-up. The REGATE study was conducted in full concordance with the principles of the Declaration of Helsinki and with the laws, and regulations of France. This study was therefore approved by the French authorities (Comité Consultatif sur le Traitement de l'information en matière de Recherche dans le domaine de la Santé, and Commission Nationale de L'Informatique et des Libertés) and registered as No. 910346. All the rheumatology units were invited to take part in the registry by regular mail, and electronic mail, using the mailing list of the French Society of Rheumatology. Seventy-eight centres accepted the invitation to participate in patient inclusion, and one declined. No centre that agreed to participate dropped out after the centre was opened. The current 78 centres involved in the REGATE registry are mainly departments of rheumatology, and there are a few departments of internal medicine. In each participating centre, all consecutive eligible patients were included and informed patient consent was obtained. With information from the hospital pharmacies on the total amount of TCZ prescribed for RA, it has been estimated that the 78 centres participating in the REGATE registry were representing over 85% of the TCZ prescriptions throughout the country, since the majority of rheumatology departments (especially those from university hospitals) participated in the study. Thus, the patients included were representative of those for whom TCZ was prescribed for RA in real life. Recruitments started on 13 January 2011, and the last patient was recruited on 5 May 2013. Data were collected at baseline (at the time of the patient's first exposure to the drug), at 3- and 6-month visits, then every 6 months, using an electronic case report form (eCRF). If a patient had no follow-up visits for >10 months, either the primary care physician, or the private rheumatologist was contacted. Seven research study nurses were specifically trained in RA, TCZ and the use of eCRFs by the coordinator of the study (J.M.) and the contract research organization RCTs. The database was closed in March 2015 in order to analyse the serious infections in the registry (defined as an infection that developed within the 3 months following the last TCZ infusion, and requiring hospitalization, i.v. antibiotics or resulting in death). The charts of all patients with serious infections were reviewed by two of the authors (J.M. and A.C.). For each patient with a serious infection according to the eCRF, it was verified in the charts that patients were hospitalized due to their infection, and/or received i.v. antibiotics, or died. Serious infections that occurred under TCZ or in the next 3 months following drug discontinuation were analysed. Roche Chugaï sponsored the study, but did not participate in the study design, analysis, writing of the report or the decision to submit the manuscript.

Statistical analysis

For patient-time of follow-up (expressed in PYs), the first infusion date and the last follow-up date were used for calculations. For calculation of the incidence rate of the first serious infection, the time of the first serious infection was used as the right censoring rule. Patients who developed more than one infection were censored at the time of their first infection. For patients without any report of serious infection, a minimum of 3 months after the last TCZ injection date, and the last follow-up date was used for the calculation of PYs (if the last injection date was equal to the last follow-up date; data were censored at the last injection date). The incidence of first serious infections is presented as event per 100 PYs with the corresponding 95% CI. The Kaplan–Meier method was used to assess the cumulative probability of remaining serious infection–free after 3 years (with corresponding 95% CI). We investigated the relationships between serious infections occurring during TCZ exposure, and potential predictors, that is, age, sex, disease severity (baseline DAS in 28 joints, and disease duration), the number of previous DMARDs and TNF antagonists, RF positivity, ACPA positivity, neutrophil count (dichotomized at the median), comorbidities, concomitant use of steroids, DMARDs and biological criteria. Neutrophil count was included in the analysis since neutropenia is

associated with infections occurrence, and TCZ is known to induce neutropenia. Both univariate, and multivariate analyses were performed, using as the outcome of interest the time to first serious infection during the follow-up period. Single and multivariate Cox models were performed to identify independent factors associated with serious infections. Variables showing univariate association with the dependent variable with a $P < 0.20$ were candidates for the multivariate model, and were also used for imputation of missing data. Multiple imputations by chained equations were used to compensate for missing data, and 20 complete datasets were created. After completion, a forward selection algorithm was applied to each dataset to select variables for the final model. Variables that appear in 12 of 20 models (60%) were entered into a final multivariate model. (Age and disease duration were forced in all multivariate models.) Assumptions of Cox models were checked (particularly log linearity and proportional hazards). Results are expressed as crude hazard ratios (HRs) (univariate analysis), or adjusted HR (multivariate analysis) with 95% CIs. A $P < 0.05$ was considered statistically significant. Statistical analyses were computed with SAS v9.4 software (SAS Institute Inc, Cary, NY, USA).

Results

Characteristics of RA patients and concomitant medication at TCZ start

A total of 1496 RA patients, from 78 centres, all enrolled at the time of their first exposure to the drug, were included in the REGATE registry. The analysis was then carried out on 1491 patients, since 5 patients had no follow-up without any explanation. The mean (S.D.) follow-up period was 27.6 (13.1) months (3429 PYs). For this analysis, all the patients received TCZ only by i.v. injection. The characteristics of the patients are presented in Table 1. The mean (S.D.) age was 56.6 (13.6), and the mean (S.D.) disease duration was 12.2 (9.9) years. Patients had active disease at inclusion, with a mean DAS28 of 5.1 (1.4). Before TCZ initiation, 15.8% had not received any biologic agent. The median (range) time between the last dose of the previous biologic and the first infusion of TCZ was 1.58 (0–120) months. TCZ was administrated as monotherapy in 598/1486 patients (40.2%), and with a concomitant DMARD in the remaining 888 (59.8%) patients, mainly MTX ($n = 705/888$; 79.4%) and LEF (119/888; 13.4%). Most of the patients ($n = 1017/1486$; 68.4%) were receiving CSs at initiation of TCZ, with a mean (S.D.) dose of 10.9 (23.6) mg/day. Of the 1491 patients, RF and ACPA data were available in 1252 and 1179 patients, respectively, and positive in 78.5% ($n = 983$) and 81.6% ($n = 962$) of them, respectively. Of the 1491 patients, CRP and ESR data were available at baseline for 1316 and 1362 patients, with, respectively, a median (range) value of 12 (4–30) mg/l and 26 mm (12–45). The mean absolute number of neutrophils at baseline was 5.260 G/l (2.429). At baseline, 475/1484 (32.0%) patients had a history of chronic lung diseases, and 317/1491 (21.3%) patients were ever smokers. It was noted that 188/1484 patients (12.7%) had a history of serious infection before TCZ start.

Rate of serious infections

A total of 125 serious infections, which required hospitalization and/or i.v. antibiotics, and, or resulted in death, occurred in 122 patients (119 patients with one infection, and three patients with two infections) during or in the next 3 months following TCZ withdrawal. The total exposure to treatment, or to first infection was 2553 PYs, and the incidence rate of the first serious infections was 4.7/100 PYs [95% CI (3.8/100 PYs; 5.5/100 PYs)]. (This was similar to the event rate of all serious infections, which was 4.6/100 PYs [95% CI (3.8/100 PYs; 5.4/100 PYs)].) The mean duration between TCZ initiation and first serious infection occurrence was 12.8 months (S.D 10.6). The most frequent infections were lung and respiratory tract and skin/soft tissue in 35 (28%) and 32 (26%) cases, respectively (Fig. 1A). The remaining serious infections were urogenital, gastrointestinal tract and articular sites. The three deaths were related to septic shock secondary to pyelonephritis (one patient) and to lung infections (one patient had a lung infection related to pneumocystosis and the other patient had one related to *Haemophilus influenzae* pneumonia). All three were >65 years old and were treated with a MTX combination. All three had serious comorbidities. Pyelonephritis developed in a woman with obesity who had a history of bedsore grafts, osteoporosis and joint replacement. Pneumocystosis developed in a woman with a history of complicated diverticulosis, joint replacement and treated arrhythmia. The patient with *Haemophilus influenzae* pneumonia was a man with lung fibrosis. In most other cases, a favourable outcome was observed. For the patients who received antibiotics orally, or systemically, the penicillin family was the antibiotic prescribed as first line in almost half of the cases ($n = 55$; 44%). Surgery was needed for 16 patients, mainly for articular infections. A pathogen was identified in 41 cases. *Staphylococcus and Streptococcus* bacteria were the main germs identified, in 14 and 5 cases, respectively. Gram-negative bacilli were the second in frequency, with 13 cases (4 *Pseudomonas aeruginosa*, 3 *Escherichia coli*, 2 *Pasteurella multocida*, 1 *Salmonella typhi*, 1 *Bacteroides fragilis*, 1 *Klebsiella pneumoniae*, 1 *H. influenzae*). According to the definition of opportunistic non-viral germs [8], two opportunistic infections were reported: one *Pneumocystosis carinii* and one *Mycobacterium tuberculosis*. Seven serious infections related to a virus were documented: two varicella-zoster virus, two parvovirus B19, one respiratory syncytial virus, one Herpes simplex virus and one influenza virus (Fig. 1B). TCZ was definitively stopped for 35 patients. Infections occurred with a regular frequency during follow-up (Fig. 2). The incidence of serious infection was stable over the first 3 years of follow-up in the registry. The probability of remaining serious infection–free during follow-up in REGATE was 87.3% 95% CI (84.9, 89.7) at 36 months.

Table 1 Baseline characteristics of 1491 patients with RA treated with tocilizumab in the REGATE registry

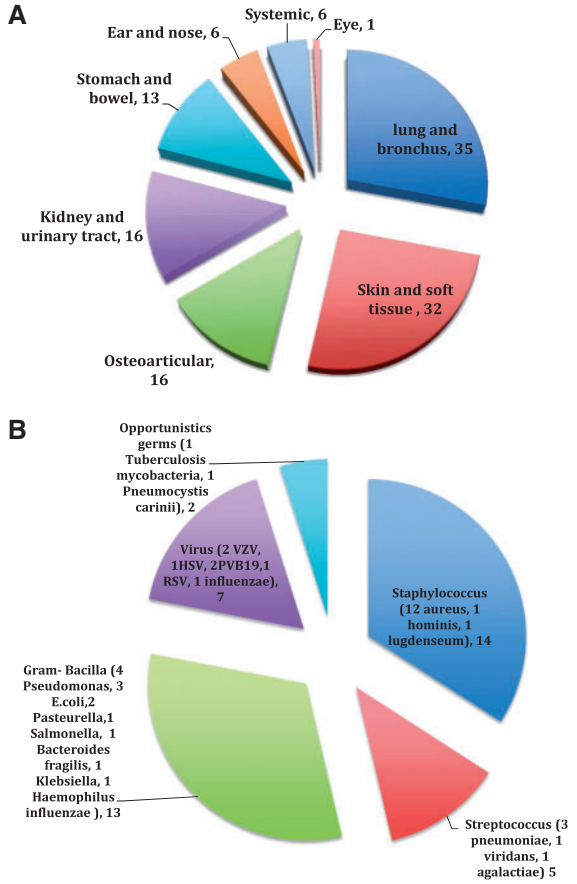
Characteristics	Value	Number of available data per outcome, (*n*) %
Age, mean (S.D.), years	56.6 (13.6)	1491 (100)
Sex, *n* (%)		1491 (100)
Female	1191 (79.9)	
Male	300 (20.1)	
Disease duration, mean (S.D.), years	12.2 (9.9)	1412 (94.7)
Smoking, *n* (%)		1491 (100)
Never	1174 (78.7)	
Ever	317 (21.3)	
History of cancer, *n* (%)	80 (5.4)	1482 (99.4)
Chronic lung disease, *n* (%)	475 (32)	1484 (99.5)
Cardiovascular disease, *n* (%)	286 (19.1)	1492 (99.4)
Renal insufficiency, *n* (%)	93 (6.3)	1484 (99.5)
Diabetes, *n* (%)	154 (10.3)	1491 (100)
Hypercholesterolemia, *n* (%)	124 (8.3)	1484 (99.5)
Previous or recurrent severe infection, *n* (%)	188 (12.7%)	1484 (99.5)
Orthopaedic surgery for RA, *n* (%)	382 (26.7)	1432 (96.0)
Previous DMARD, mean (S.D.)	2.3 (1.4)	1491 (100)
Previous biologic DMARD, mean (S.D.)	2.0 (1.5)	1491 (100)
Last biologic treatment, *n* (%)		1482 (99.4)
Anti-TNF	770 (52.0)	
Rituximab	202 (13.6)	
Abatacept	276 (18.6)	
None	234 (15.8)	
Previous anti-TNF treatment, *n* (%)		1426 (95.6)
0	234 (16.4)	
1	511 (35.8)	
2	517 (36.3)	
3	156 (10.9)	
4	8 (0.6)	
RF-positive, *n* (%)	983 (78.5)	1252 (84.0)
ACPA positive, *n* (%)	962 (81.6)	1179 (79.1)
DAS28-ESR, mean (S.D.)	5.1 (1.4)	1203 (80.7)
ESR, mean (S.D.), mm/h	32.7 (26.5)	1362 (91.3)
ANC (S.D.; G/l)	5.26 (2.43)	1336 (89.6)
DMARDs[a] combination, *n* (%)	888 (59.8)	1486 (99.7)
MTX	705	
LEF	119	
Other	64	
CSs, *n* (%)	1017 (68.4)	1486 (99.7)
CSs[b], mean (S.D.), mg/day	10.9 (23.6)	999/1017[b] (98.2)
CS > 15 mg/day , mean n (%)	123 (8.4)	1468 (98.4)

[a]Defined as the day of TCZ first infusion. [b]For patients who have taken CSs. TCZ: tocilizumab; ANC: absolute neutrophil count; *n*: absolute number; %: percentage; h: first hour; G: Giga.

Predictive factors of serious infections

To identify predictive factors of serious infection, bivariate analysis was performed between RA patients with and without serious infections during the period of follow-up. ACPA positivity at baseline was the only factor significantly associated with a lower risk of serious infection (HR = 0.56, 95% CI: 0.36, 0.88). Factors significantly (<0.05) associated with a higher risk of serious infections were DAS28-ESR, and initial number of neutrophils above 5.0×10^9 at baseline. Other selected factors with a higher risk of serious infections ($P < 0.20$) were age, disease duration, history of orthopaedic surgery, number of previous DMARDs, CRP at baseline, concomitant DMARDs treatment and CSs >15 mg/day at initiation of TCZ (Table 2). All these factors were included in multivariate analysis. After imputation (the amount of missing data varies between 0% for age and 21% for ACPA) and adjustment on age and disease duration, initial neutrophils above 5.0×10^9 (HR = 1.94, 95% CI: 1.32, 2.85; $P < 0.001$), negative ACPA (HR = 0.56, 95% CI: 0.36, 0.88; $P = 0.012$) at baseline and concomitant LEF treatment (LEF alone *vs* no treatment, HR = 2.18, 95% CI: 1.22, 3.88; $P = 0.009$) were still significantly associated with a higher risk of serious infections (Table 3).

FIG. 1 Sites of serious infections ($n = 125$) and type of germs identified ($n = 41$) in REGATE registry



(**A**) Sites of infection. (**B**) Germs identified. VZV: Varicella and Zona Virus; HSV: Herpes Simplex Virus; PVB19: Parvovirus B19; RSV: respiratory syncytial virus; *E. coli*: *Escherichia coli*.

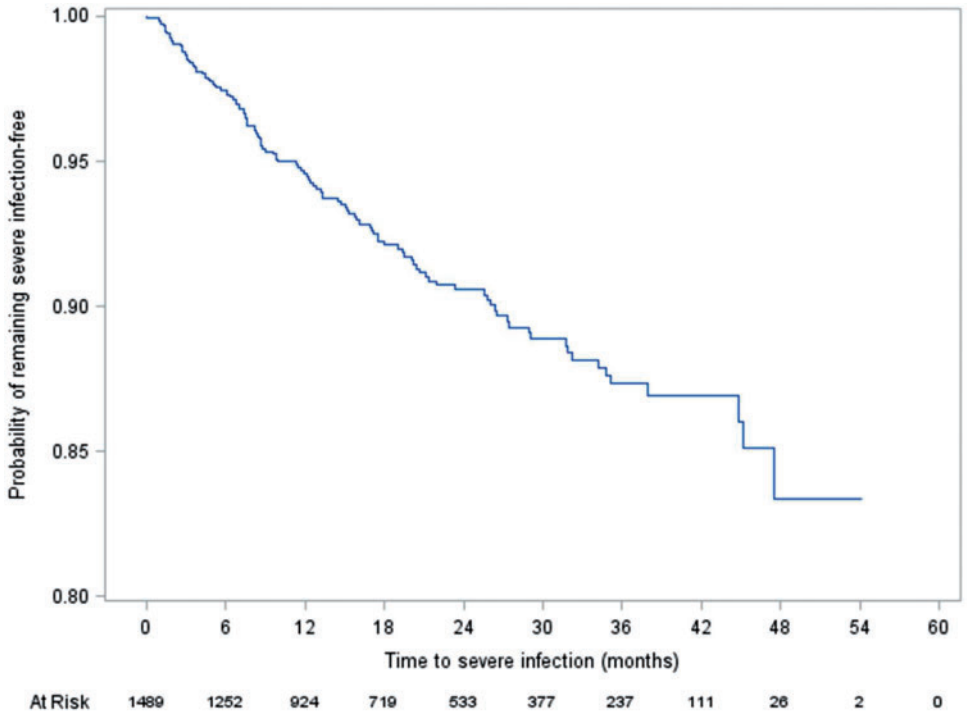
Discussion

In the REGATE registry, the incidence rate of serious infections was 4.7/100 PYs with a regular frequency during the 27.6 months of follow-up. Lung/respiratory tract and skin/soft tissue are the two more frequent sites of serious infections. Predictive factors of serious infections are: initial absolute neutrophil count above 5.0 × 109/l, negative ACPA at baseline, and concomitant LEF treatment.

TCZ was first developed in Japan, and safety of this biodrug was first reported in this country. Thus, in Japanese RA patients, a meta-analysis of six randomized clinical trials, and five long-term extension studies was performed. This meta-analysis included 601 patients with moderate to serious RA, with a total of 2188 PYs of exposure. The median treatment duration was 3.8 years, and the rate of serious infections was 6.22/100 PYs [9]. Another post-marketing surveillance programme in Japan reported the safety of TCZ in 3881 RA patients [total exposure 1793.5 PYs; mean observation period (S.D.) 24.1 (7.4) weeks]. The risk of serious infections was higher, with an incidence rate of 9.2/100 PYs, but the definition of serious infections was not very well detailed [10]. In Europe and North America, TCZ was available 1 year later. Long-term extension data from five randomized controlled TCZ trials ($n = 4211$), their open-label extension phases ($n = 3512$) and a drug interaction study ($n = 23$) reported the safety of TCZ in these international trials [11]. No new safety signals were identified for the total duration of observation (12 293 PYs). The rate of serious infections was 5.0/100 PYs, which is very close to the 4.7/100 PYs observed in the non-trial population included in the REGATE registry. In the German observational study ROUTINE, which included 850 RA patients, the rate of serious infection was higher, with a rate of 9.8/100 PYs [12]. Using a definition of serious infections derived from the regular adverse event log page (primary System Organ Class: infections and infestations), the rate was 4.4/100 PYs. Nevertheless, in that observational study, the sample size was lower than in our cohort, with 850 RA patients included, and only two-thirds of them remaining in the study at 52 weeks. In a smaller cohort of 112 German RA patients, the rate was much higher, with 17.9/100 PYs [13]. Exposure was not reported in this study, and that may explain the difference. In the French registries ORA and AIR, rates of serious infections were 4.1/100 PYs for abatacept and 5/100 PYs for rituximab [2, 3]. In a large American observational study including 3152 RA patients with slightly more than 1 year of follow-up, the incidence rate for bacterial infections requiring hospitalization was 2.8/100 PYs for abatacept, 4.4/100 PYs for rituximab and 3.0/100 PYs for anti-TNF [5]. Data for TCZ were not available in this study. In our study, the incidence of all serious infection was stable over the first 3 years of follow-up in the registry, and it was quite similar, whatever the site of infection was (supplementary Fig. S1, available at *Rheumatology* Online). This is in contrast with TNF inhibitors, which have a higher incidence of serious infections in the first 3 months of follow-up, as observed in the British Society for Rheumatology Biologics Register, and in the Swedish Biologics Register Anti-Rheumatic Treatment in Sweden (ARTIS) [4, 14].

In the present study, most of the infections resolved with antibiotics for bacterial infections. Two of the three deaths observed in our study were related to a pulmonary opportunistic infection. Like the other biodrugs, the preferential sites infected were, first, lung and upper respiratory tract, second, cellulitis and soft tissue, and third, kidney and urogenital tract [5]. In the REGATE registry, the serious osteoarticular infections frequency was 12.8% (16 of 125), which is quite similar to frequencies observed with rituximab and abatacept in the two other French registries, with 12.2 and 14%, respectively [2, 3]. Predictive factors of serious infections were explored in a German study, but in 112 RA patients [13]. Serious infections, defined by infections requiring hospitalization, were related to longer disease duration, exposure to more than three previous DMARDs and concomitant therapy with proton-pump inhibitors. Moderate infections, defined by infections requiring a visit to a physician, or the use of

FIG. 2 Probability of remaining serious infection–free during follow-up in REGATE (Kaplan–Meier)



The curve represents cumulative probability of remaining serious infection–free against months of follow-up with a zoom on the 0.8 and 1 scale. The cumulative probability of remaining serious infection–free was 87.3%, 95% CI (84.9, 89.7) at 36 months.

antibiotics, were related with concomitant therapy with prednisone, concomitant therapy with LEF, previous exposure to rituximab and high disease activity as measured by the DAS-28 score as significant predictors. In our cohort of RA patients, in univariate analysis, variables corresponding to disease activity at baseline such as DAS28 (ESR or CRP), CRP, absolute neutrophil count and concomitant LEF were associated with a higher risk of developing serious infections, with the two last factors confirmed by multivariate analysis. The combination of DMARDs and TCZ has been associated with increased risk of infections in the TCZ in Combination With Traditional DMARD Therapy (TOWARD) study [15]. We confirmed that LEF co-medication with TCZ should require more attention for safety, especially in patients with a higher risk of infection.

The association of neutrophils at baseline above 5.0×10^9/l with increased risk of serious infections might reflect disease activity. Indeed, CRP, DAS28 and high Polynuclear neutrophils (PNN) are associated significantly or almost significantly with serious infections, and the percentage of patients treated with steroids is higher in the group with severe infections (70.5% *vs* 68.3%), suggesting the need for steroids because of higher disease activity at inclusion in the REGATE registry. RA patients with high disease activity may require intensification of their therapies, which may consequently enhance the risk of developing infections. This is the first time that a high neutrophils level before initiation of TCZ has been identified as a risk factor for serious infection. Implication of IL-6 and neutrophils in immunity is well known, and the close relation between these two factors is highlighted by neutropenia being observed in ~3% of the patients treated with TCZ. Despite evidence that neutropenia can be associated with an increase risk of infection, it is less evident at first look how to explain this association with a high neutrophils level. This increase in neutrophils at baseline might correspond to disease activity, since in our study DAS28 and CRP were also associated with this risk of serious infection in univariate analysis. In a cohort of RA patients treated with DMARD (RADIUS 1), it has been shown that disease activity independently correlates with serious infection event risk. In this study, a 5 U Clinical Disease Activity Index increase corresponded with a 7.7% increased serious infection event risk (adjusted HR = 1.077, 95% CI: 1.044, 1.112; $P < 0.0001$) [16]. More recently, Emery *et al*. [17] observed that in patients treated with etanercept, or synthetic DMARD, there was a linear association between the serious infection rate, and DAS28. Overall, a DAS28 change of 1 unit during follow-up predicted a 27% increase in serious infection rates [17]. Finally, we also observed that the risk of serious infections increased even more for ACPA-positive RA patients, even in a multivariate model after imputation

Table 2 Risk factors for serious infections (univariate analysis)

Characteristics at baseline	Patient with severe infection (*n* = 122)	Patient without severe infection (*n* = 1369)	Hazard ratio (95% CI)	*P*-value
Age, mean (S.D.), years	58.7 (15.0)	56.4 (13.4)	1.15 (per 10) (0.99, 1.32)	0.0547
Female, *n*/*N* (%)	92/122 (75.4)	1099/1369 (80.3)	0.77 (0.51, 1.16)	0.20
Disease duration, mean (S.D.), months	13.5 (10.2)	12.1 (9.9)	1.09 (per 6) (0.98, 1.21)	0.099
Smoking, *n*/*N* (%)	22/122 (18.0)	295/1369 (21.6)	0.82 (0.52, 1.30)	0.40
History of cancer, *n*/*N* (%)	7/122 (5.7)	73/1360 (5.4)	1.26 (0.59, 2.71)	0.55
Chronic lung disease, *n*/*N* (%)	41/122 (33.6)	434/1362 (31.9)	1.10 (0.76, 1.60)	0.61
History of renal insufficiency, *n* (%)	10/122 (8.2)	83/1362 (6.1)	1.35 (0.71, 2.58)	0.36
Diabetes I or II, *n*/*N* (%)	16/122 (13.1)	138/1369 (10.1)	1.36 (0.81, 2.31)	0.25
Hypercholesterolaemia, *n*/*N* (%)	12/122 (9.8)	112/1362 (8.2)	1.26 (0.69, 2.28)	0.45
Previous or recurrent serious infection, *n*/*N* (%)	13/122 (10.7)	175/1362 (12.9)	0.80 (0.45, 1.42)	0.45
History of orthopaedic surgery, *n*/*N* (%)	36/110 (32.7)	346/1322 (26.2)	1.36 (0.91, 2.02)	0.136
Previous DMARDs, mean (S.D.)	2.5 (1.7)	2.3 (1.4)	1.09 (0.96, 1.23)	0.169
Previous biologic treatment, *n*/*N* (%)	103/121 (85.1)	1145/1361 (84.1)	1.12 (0.68, 1.85)	0.65
Previous anti-TNF treatment, *n*/*N* (%)	97/115 (84.4)	1095/1311 (83.5)	1.10 (0.66, 1.82)	0.72
RF positive, *n*/*N* (%)	72/98 (73.5)	911/1154 (78.9)	0.75 (0.48, 1.17)	0.21
ACPA positive, *n*/*N* (%)	65/92 (70.7)	897/1087 (82.5)	0.55 (0.35, 0.86)	0.0093
Initial ESR, mean (S.D.), mm/h	35.6 (27.2)	32.5 (26.5)	1.04 (per 10) (0.97, 1.11)	0.26
Initial CRP, mean (S.D.), mg/l	29.6 (40.5)	23.0 (31.7)	1.04 (per 10) (0.99, 1.09)	0.078
Initial DAS28-ESR, mean (S.D.)	5.4 (1.3)	5.0 (1.4)	1.20 (1.03, 1.40)	0.0197
Initial ANC >5 G/l, *n*/*N* (%)	76/110 (69.1)	685/1226 (55.9)	1.75 (1.17, 2.63)	0.0067
DMARDS combination, *n*/*N* (%)				0.090
None	43/122 (35.3)	555/1364 (40.7)	1	
MTX alone	58/122 (47.5)	647/1364 (47.4)	1.02 (0.69, 1.51)	
LEF alone	16/122 (13.1)	103/1364 (7.8)	1.99 (1.12, 3.54)	
Other	5/122 (4.1)	59/1364 (4.3)	0.93 (0.37, 2.34)	
CSs >15 mg/day, *n*/*N* (%)	5/120 (4.2)	118/1348 (8.8)	0.55 (0.22, 1.34)	0.18

Serious infections occurred during treatment with tocilizumab or within 3 months of its discontinuation. ANC: Absolute Neutrophil count; Initial: corresponds to the value just before initiation of tocilizumab; *n*: absolute number; h: first hour; G: Giga.

Table 3 Risk factors for serious infections (multivariate analysis)

Risk factors at baseline	Hazard ratio (95% CI)	*P*-value
Age, per 10 years	1.14 (0.99, 1.32)	0.064
Disease duration, per 6 months	1.07 (0.96, 1.19)	0.21
APCA positive	0.56 (0.36, 0.88)	0.012
Initial ANC >5.0 G/l	1.94 (1.32, 2.85)	0.001
DMARDS combination, *n* (%)		
None	1	
MTX alone	1.14 (0.76, 1.71)	0.53
LEF alone	2.18 (1.22, 3.88)	0.009
Other	0.84 (0.33, 2.14)	0.72

Multivariate Cox model of risk factors of serious infections that occurred during treatment with tocilizumab or within 3 months of its discontinuation. ANC: absolute neutrophil count.

of missing data. If ACPA has been proposed as a predictive factor for response to treatment with e.g. rituximab and abatacept, it is the first time that ACPA has been described as a potential protective factor against developing serious infections. ACPA-negative and ACPA-positive RA have different outcomes. Absence of ACPA could delay introduction of the first DMARD beyond the window of opportunity. Several studies have shown that patients treated early, e.g. within 3 months of the onset of symptoms, have favourable outcomes compared with patients treated later after symptom onset, even in patients with ACPA-positive [18, 19]. The management of these ACPA-negative RA patients could then require a different treatment strategy compared with that of patients with ACPA-positive RA, which could expose them to a higher risk of infections. There was no difference between the characteristics (such as disease duration) for ACPA-positive and -negative patients. However, to confirm that the lost of window of opportunity could explain why being ACPA negative is a risk factor, it would have been relevant to know the delay between date of diagnosis and initiation

of first DMARD. Unfortunately, we did not collect that information in the eCRFs for the REGATE registry. Physical function has been associated with infection in patients with RA [20]. In the REGATE registry, the mean HAQ is 1.4 (0.7) in the group with serious infections ($n = 32$) *vs* 1.2 (0.8) for the group without serious infections ($n = 235$). Because missing data was very high (82%), we could not include this criteria in the statistical analysis and confirm that physical function was indeed associated with serious infections in RA patients treated with TCZ.

The main strength of our study was the number of RA patients followed up, even after TCZ was stopped, allowing an imputation to TCZ based on the time between serious infections and last infusion. Our study has several limitations, especially the absence of a control group treated with synthetic DMARDs to estimate the risk of serious infections imputed to TCZ itself. Nonetheless, the risk of serious infections observed is very similar to that of other European studies using a similar definition of serious infections [11, 12]. A selection bias might explain the association of LEF and the risk of infections, since those patients who were not treated with MTX might have had some comorbidities such as lung disease. However, this repartition of synthetic DMARDs is quite usual, and the association between LEF and serious infections was observed in another study [15].

Conclusion

This is the largest observational study reporting serious infections in RA patients treated with TCZ. The rate of serious infections is in the range observed with other biologics, and is very close to rates observed in pivotal studies. Predictive factors of serious infections are high neutrophils above 5.0 $\times$ 10^9/l, negative ACPA and LEF association at baseline; therefore, increased attention should be especially given to these RA patients treated with TCZ.

Authors contribution: J.M. and J.S. coordinated the REGATE registry. J.M. validated serious infections, analysed the data and wrote the manuscript. A.C. validated serious infections, and participated in data interpretation. G.B. performed statistical analysis. J.M., E.D., R.M.F., S.R., B.C., J.E.G., T.S., M.S., O.V., A.S., M.D., X.M., P.R. and J.S. contributed to the design of the REGATE registry and data interpretation. P.R. wrote the statistical analysis plan.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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