Antiarrhythmic potential of anti-cytokine therapy in rheumatoid arthritis: Tocilizumab reduces QTc interval by controlling systemic inflammation.

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Antiarrhythmic potential of anti-cytokine therapy in rheumatoid arthritis: tocilizumab reduces QTc interval by controlling systemic inflammation.

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Contributorship statement

Pietro Enea LAZZERINI: Conception and design of the work; analysis and interpretation of data; drafting the manuscript; final approval of the version to be published.

Maurizio ACAMPA: acquisition, analysis and interpretation of data; revising the manuscript critically for important intellectual content; final approval of the version to be published.

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ABSTRACT

Objectives. Rheumatoid arthritis (RA) patients were twice as likely to experience sudden cardiac death compared with non-RA subjects. Although the underlying mechanisms have not been clarified, evidence points at the effects of systemic inflammation on ventricular repolarisation. Accordingly, corrected QT interval (QTc) prolongation is more frequent in RA than in non-RA subjects, associating with C-reactive protein (CRP), and predicting all-cause mortality. Tocilizumab (TCZ) is an anti-interleukin 6-receptor antibody that potently inhibits inflammatory activation in RA with a rapid normalization of acute phase reactants, including CRP. Therefore, we here put forward the hypothesis that TCZ may normalize QTc by dampening systemic inflammation thus reducing the arrhythmic risk in RA patients.

Methods. Seventeen consecutive patients with active RA who were scheduled to receive TCZ once every 4 weeks, underwent clinical visit, ECG recordings and blood sampling just before the first injection with TCZ, and then after 3 and 6 months of treatment.

Results. At baseline, patients showed a high prevalence of QTc prolongation (76%; mean QTc: 452.3±35.8 ms). TCZ treatment was associated with a rapid and significant reduction of QTc to mean values lower than 440 ms (up to 428.1±34.4 ms). QTc shortening correlated throughout the study time with the decrease in both CRP, and, more strongly, circulating TNFα levels.

Conclusion. These data provide further evidence of the strict link existing between the degree of systemic inflammation and QTc duration in RA, also suggesting an anti-arrhythmic potential for TCZ treatment that may beneficially impact the mortality of these patients.

SIGNIFICANCE & INNOVATION

Innovation

- In RA patients with active disease, TCZ treatment is associated with a rapid and significant reduction in QTc duration; such an effect seems to be mainly due to the potent inhibitory effect of the drug on systemic inflammation.

Significance

- Providing further evidence of the strict link existing between the degree of systemic inflammation and QTc duration in RA.
- Suggesting an anti-arrhythmic potential for TCZ treatment that may beneficially impact the mortality of RA patients.

INTRODUCTION

Cardiovascular disease (CVD) is responsible for about 50% of premature deaths in patients affected with rheumatoid arthritis (RA) [1]. In particular, RA patients were twice as likely to experience sudden cardiac death (SCD) compared with non-RA subjects (adjusted estimated cumulative incidence at 30 years of follow-up, 6.7% vs. 3.8%) [2], a hazard ratio comparable with that observed in patients with diabetes mellitus, a well recognized risk factor for CVD in the general population [3].

The pathophysiological basis of this phenomenon is not fully understood, but the fact that in RA patients inflammatory indicators are significantly associated with increased cardiovascular mortality after adjusting for other cardiovascular risk factors [1,4] strongly suggests that systemic inflammation may *per se* play a key role. The promotion of accelerated atherosclerosis is the most investigated mechanism possibly linking inflammatory activation and cardiovascular death in RA [1]. However, the finding that the doubling of the risk of SCD observed in RA patients persisted after adjustment for the history of hospitalized or unrecognized myocardial infarction and revascularization procedures [2] implies the factors other than accelerated coronary artery disease are significantly involved in the phenomenon.

Indeed, evidence suggests that RA patients may display a higher risk of developing life-threatening arrhythmias also as a result of non-structural cardiac abnormalities. In particular, enduring systemic inflammation may cause a delay in ventricular repolarization by directly affecting the electrophysiological properties of cardiomyocytes. Such a phenomenon emerges in the clinical setting as a corrected QT interval (QTc) prolongation, an established electrocardiographic predictor of arrhythmic risk and sudden death in the general population [5]. As indicated by several studies [6-11], a direct modulating activity of pro-inflammatory cytokines on cardiac ion channels regulating action potential duration (APD) seems to be one of the main pathophysiological mechanisms involved.

Accordingly, in a cohort of 101 patients with chronic inflammatory arthritis, in which a significant positive correlation between CRP values and QTc duration was demonstrated, we found that RA patients have a longer QTc when compared with both spondyloarthritis patients and healthy controls [12]. These findings were very recently confirmed by Chauhan and coll. [13] in a larger population based inception cohort of 650 RA patients along with an age- and sex-matched comparison cohort of 650 non-RA patients. Moreover, in a prospective study carried out on 357 RA patients, the group of Kitas [14] found that prolonged QTc is a strong predictor of death as a 50 ms increase in QTc interval associated with a doubling of the hazard for all-cause mortality. The evidence that in this population QTc prolongation was independently associated with CRP levels, and the fact that the significance of the association between QTc and all-cause mortality was lost after the adjustment for CRP, once more and robustly supported the hypothesis that systemic inflammation plays a key mechanistic role in the phenomenon.

Tocilizumab (TCZ) is a novel humanized anti-interleukin 6 (IL-6) receptor antibody licensed for the treatment of RA. By blocking IL-6 signal transduction, this drug potently inhibits systemic inflammation characterizing the disease with a rapid normalization of acute phase reactant levels, including CRP [15]. Therefore, we here put forward the hypothesis that by dampening systemic inflammation TCZ may normalize QTc duration thus reducing the arrhythmic risk in patients with RA.

MATERIALS AND METHODS

Study population. We studied 17 consecutive patients (2 males) affected with RA. All the patients had active disease, as indicated by a disease activity score in 28 joints based on erythrocyte sedimentation rate level (DAS28-ESR) of more than 3.2. Demography, clinical history, treatment, and laboratory data of patients are reported in table 1 (more clinical details are provided as supplementary material).

All patients were treated with TCZ (8 mg/Kg body weight) intravenously once every 4 weeks. They underwent clinical visit, ECG recordings and blood sampling before the first administration with TCZ (T0), and then after 3 (T1) and 6 months (T2) of treatment, just before drug infusion.

Local Ethical Committee approved the study, and patients gave their written informed consent in accordance with the Principles of the Declaration of Helsinki.

ECG recordings. QTc interval was manually measured on a 12-lead electrocardiogram (ECG), according to standard criteria [16,17]. Detailed technical information is provided as supplementary material. The upper limit for QTc interval in normal healthy subjects is 440 ms [18]. Moreover, large studies demonstrated that a progressively increasing risk of SCD was observed starting at a cut-off point of 440 ms [18-20]. Accordingly, in this study a QTc > 440 ms was considered as prolonged.

In order to assess the reliability of the manual QTc measurement, in the same visit in all the patients we also evaluated the QTc with a computerized method based on an ambulatory twelve-channel ECG recording system (Prima-Holter, Cardioline, Remco, Vignate-Milano, Italy) in a 15-minute period. Detailed technical information is provided as supplementary material.

Clinical assessment of disease activity

Clinical response to treatment was primarily assessed using changes in DAS28-ESR. Moreover, changes in the following variables were also evaluated: Disease Activity Score in 28 joints based on

C-reactive protein level (DAS28-CRP), swollen joint count, tender joint count, patient's assessment of pain on a 100-mm visual analogue scale (VAS), patient's assessment of general health (GH), and Health Assessment Questionnaire–Disability Index (HAQ–DI). Finally, the clinical disease activity index (CDAI) was assessed (more information is provided as supplementary material).

Laboratory investigation. Blood samples were taken to measure high sensitivity C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and tumor necrosis factor α (TNFα) levels. Moreover, in consideration of the potential impact of concomitant electrolyte imbalances on QTc duration [21], the serum levels of potassium, calcium and magnesium were also measured throughout the study time. Detailed technical information is provided as supplementary material.

Statistical analysis. Descriptive statistics were performed by including mean and standard deviation. Normal distribution of quantitative variables was preliminary tested using the Kolmogorov-Smirnov test to select parametric (normal distribution) or non parametric (non normal distribution) inferential statistical methods. Accordingly, the following parametric or non parametric statistical analyses were respectively carried out: repeated measures analysis of variance (RM-ANOVA) and Tukey-Kramer post-hoc test or Friedman test (nonparametric RM-ANOVA), and Dunn post-hoc test to evaluate differences in quantitative variables throughout the time; Pearson or Spearman correlation analysis to verify possible statistical association between quantitative variables. The chi-squared test for trend (Mantel-Haenszel linear-by-linear association chi-squared test) was performed to evaluate differences between categorical variables throughout the time.

In any case, a p value <0.05 was considered as significant. All statistical analyses were computed using GraphPad-InStat package (GraphPad, La Jolla, CA 92037 USA).

Statistical power estimation was performed and results are provided as supplementary material.

RESULTS

Changes in clinical parameters of disease activity. At baseline, patients showed clinical data suggestive of active disease, in most cases of severe degree (DAS28-ESR >5.1 in 65% of the patients). Treatment with TCZ was associated with an evident decrease in the disease activity, as demonstrated by the significant improvement in DAS28-ESR, DAS28-CRP, VAS, GH, HAQ-DI and CDAI scores, as well as in the swollen and tender joints counts. Such an effect was rapid, since a significant reduction in all these parameters, except for HAQ, was already observed after a follow-up period of 3 months (T1) (Table 2).

Changes in laboratory findings. Systemic inflammation parameters, i.e. ESR and CRP, were elevated at baseline and showed a corresponding reduction under TCZ treatment. Also in this case, a 3 month-period of therapy was sufficient to induce a significant decrease in both the parameters below the normal reference values (Table 2).

On the contrary, no changes in electrolyte serum concentrations were observed throughout the study time (Table 2).

Changes in QTc duration. At standard ECG, patients showed baseline mean QTc values above the normal healthy subject upper limit of 440 ms. Accordingly, 13 out of 17 patients (76%) displayed a prolonged QTc in basal conditions. TCZ treatment was associated with a significant reduction in QTc duration, until mean values lower than 440 ms. More in details, at T1 the mean QTc decreased of about 24 ms in comparison to baseline, and this reduction stably persisted also at T2 (Table 3 and Figure 1). Concomitantly, the number of patients with QTc prolongation progressively decreased to 7 (41%), and 5 (29%) at T1 and T2, respectively (Table 3). A similar trend was observed when using established gender-specific cut-offs for prolonged QTc, as defined in the Rotterdam Study [20] and, more recently, employed in the Oregon Sudden Unexpected Death Study [22] (men: normal, ≤430 ms; borderline-prolonged, 431-450 ms; and abnormally-prolonged, >450 ms; women: normal, ≤450 ms; borderline-prolonged, 451-470 ms; and abnormally-prolonged, >470 ms).

Notably, in the general population a significant increase in the risk of SCD was observed in subjects with both borderline-prolonged, and abnormally-prolonged QTc interval [20]. Also using these cut-offs, the number of RA patients displaying a prolonged QTc significantly decreased throughout the study time (T0:10/17,59%; T1:6/17,35%; T2:4/17,24%; chi-squared test for trend, p=0.03).

The reliability of these findings was confirmed by the strong direct correlation between QTc values manually measured at the standard ECG, and QTc parameters automatically recorded with the ambulatory ECG monitoring for each patient (QTc max, r=0.68, p<0.0001; QTc mean, r=0.71, p<0.0001; Pearson correlation test). It should be underlined that since QTc intervals measured using ambulatory ECG monitoring do not correspond quantitatively to those for standard ECG, it is recognized that data obtained from the two methodologies are not suitable for direct comparison [16]. Nevertheless, in accordance with standard ECG findings, the ambulatory monitoring QTc parameters showed a progressive and significant reduction during TCZ treatment (Table 3).

Association between QTc and systemic inflammation. Values of QTc were significantly and directly correlated with CRP levels in the whole population throughout the study time (Figures 2-3). On the contrary, there was no significant association of QTc with ESR, DAS28-ESR, DAS28-CRP, VAS, GH, HAQ-DI, swollen joint count, tender joint count, and CDAI, although for some of these parameters a trend was observed (data not shown; CDAI: Figures 2-3).

Among the parameters evaluated, CRP reflects most accurately the degree of systemic inflammation, in turn strictly dependent on the circulating levels of pro-inflammatory cytokines. On the basis of these considerations, in 10 randomly selected patients of the population under study we also measured TNFα serum concentration at the different time points. In these patients, in which QTc decreased over time with mean values nearly overlapping those observed in the whole population (T0:456.1±41.8 ms; T1:433.1±34.6 ms; T2:426.1±34.0 ms), TCZ treatment was associated with a significant reduction in TNFα levels (T0:84.3±26.6 pg/ml; T1:63.5±30.3* pg/ml; T2:59.9±37.7** pg/ml; RM-ANOVA, p=0.0058; *p<0.05,**p<0.01 versus T0: post-hoc Tukey-

Kramer multiple comparison test). Notably, a strong direct correlation between QTc and circulating TNFα levels was found throughout the study time (Figures 2-3).

DISCUSSION

In the present study, we provide evidence for the first time that in RA patients with active disease TCZ treatment is associated with a rapid and significant reduction in QTc duration. Such an effect seems to be mainly due to a potent inhibitory effect on systemic inflammation, as suggested by the evidence that QTc shortening correlates with the decrease in both CRP and, more strongly, circulating TNFα levels. Since accumulating evidence indicates that in RA prolonged QTc is driven by high inflammatory burden and predicts all-cause of death, our findings suggest that TCZ treatment may have a beneficial impact on the mortality of patients affected with RA. Intriguingly, such mechanisms may be more generally working in all the diseases characterized by chronic systemic inflammation, thereby significantly emphasizing the relevance of our findings.

It is well recognized that patients with RA have an increased mortality when compared to age- and sex-matched subjects of the general population, with an overall standard mortality ratio of approximately two [4]. Such an excess of deaths is mainly due to CVD, as the result of both increased CVD morbidity and case fatality [1]. Accordingly, SCD occurs two-times more frequently in RA-patients than in non-RA subjects (hazard ratio 1.94, 95% CI 1.06-3.55) [2]. Although the underlying mechanisms of the high risk of SCD in RA have not completely clarified, the leading hypothesis is that chronic systemic inflammation, by accelerating the development of coronary artery disease, may induce myocardial latent ischemia and/or fibrosis, in turn responsible for myocardial electric instability [1,2]. The recent evidence that QTc prolongation has a higher incidence in RA than in non-RA subjects [12,13], also correlating with inflammatory markers [12-14] and predicting all-cause mortality [14], supports the hypothesis that such a inflammation-driven pro-arrhythmic substrate may be expressed in terms of changes in ventricular repolarisation. In fact, it is well established that the more QTc prolongs, the higher is the risk that abnormal premature depolarisations prior to completion of repolarisation (early afterdepolarizations, EADs) occur and generate malignant ventricular arrhythmias such as torsade de pointes, which can progress to ventricular fibrillation and SCD [5].

In accordance with these premises, in our study population, largely constituted by RA patients with high-activity disease, an elevated incidence of QTc prolongation was observed at baseline. In these subjects, TCZ treatment significantly reduced the prevalence of QTc prolongation, leading to mean QTc values below the upper normal limit of 440 ms. This finding may have very relevant clinical implications. In fact, large studies in the general population demonstrated that a progressively increasing risk of SCD was observed starting at a cut-off point of 440 ms [18-20]. Moreover, Panoulas and coll. [14] reported how a 50 ms increase in QTc interval was associated with a 2.17 (95% CI 1.21-3.90) increase in the odds for all-cause mortality in patients with RA, a risk already arising for values included in the "normal range" for the general population. In fact, in this study, patients with a QTc ≥ 425 ms exhibited a significantly higher risk of death compared with those having a shorter QTc (1.91, 95% CI 1.01-3.22). Finally, it should be noted that mean QTc values measured in our RA patients before and after TCZ treatment (about 452 and 428 ms, respectively) are comparable to those found in the Oregon Sudden Unexpected Study [22] when the mean QTc duration of coronary artery disease patients with and without SCD was compared (about 450 and 433 ms, respectively). Keeping all these considerations in mind, the evidence here provided that TCZ treatment reduced QTc to mean values even below 430 ms (with a large part of patients displaying a QTc < 425 ms) suggests that this drug may lower mortality in RA patients, presumably by decreasing the arrhythmic risk.

The other main result of the present study consists on the fact that during TCZ treatment a significant correlation between QTc and inflammatory markers was observed throughout the time. The existence of a harmful interplay connecting inflammatory burden and ventricular repolarisation is recently arising in RA [23], as indicated by the significant association between acute phase reactant levels and QTc duration [12-14]. Moreover, the evidence that the predictive value of QTc prolongation for all-cause mortality is lost after the adjustment for CRP levels [14] further and strongly confirms this view. Accordingly, we found that in TCZ-treated patients QTc shortening associated with CRP decrease, thus making likely the hypothesis that the drug affects ventricular

repolarisation indirectly by controlling the disease and, more particularly, the degree of systemic inflammation. In fact, in accordance with previous studies [13,14] we did not find a significant correlation between QTc and clinical parameters of disease activity (DAS28-ESR, DAS28-CRP, VAS, GH, HAQ-DI) which, differently from CRP, are also dependent on disease-related factors other than systemic inflammation. This suggests the systemic effects of the inflammatory process underlying RA are specifically and critically involved in the genesis of the phenomenon.

Although the exact mechanism linking systemic inflammation and QTc prolongation in RA is not well known as yet, the finding that TCZ rapidly (within 3 months) normalizes QTc duration virtually rules out the possibility that it is driven by an underlying, subclinical coronary atherosclerosis, as it is a structural process requiring a longer period of time to both take place and (if possible) regress. Thus, it seems much more conceivable that functional mechanisms may be implicated, possibly involving direct (and reversible) effects of pro-inflammatory cytokines on cardiomyocyte electrophysiology. A number of basic studies support this view, in particular suggesting a cytokine-dependent activity on specific potassium and calcium channels critically involved in ventricular repolarisation and action potential duration (APD). Perfused hearts from transgenic mice overexpressing TNFα exhibited a prolonged APD and re-entrant ventricular arrhythmias [24]; left ventricular myocytes isolated from these animals revealed a robust decrease in the transient outward potassium current (Ito) associated with a reduced expression of the corresponding potassium channel protein [8]. Several authors reported consistent findings when rat ventricular myocytes were cultured with TNFα, also demonstrating the involvement of a molecular cascade including iNOS overexpression, oxidant species generation, NFκB activation, and potassium-channel-interacting protein 2 (KChIP-2) inhibition [9,11,25]. Moreover, Wang and coll. [7] showed that TNFα down-regulates in-vitro the rapid delayed-rectifier potassium current (IKr) by impairing the function of the HERG potassium channel via the stimulation of reactive oxygen species. Although it is far probable that similar effects on potassium channels are also exerted by the other main pro-inflammatory cytokines IL-6 and IL-1, no specific studies evaluated this topic as

yet. Nevertheless, experiments on pig and mouse ventricular cells clearly demonstrated the ability of both these cytokines to prolong APD, possibly by enhancing L-type calcium current (ICaL) [6,10]. In accordance with these premises, we demonstrated a strong direct association between changes in QTc duration and TNFα levels during TCZ treatment. Notably, we chose to monitor just such a cytokine, not only because it has a key role in the pathogenesis of RA and it is the most studied in terms of effects on cardiac electrophysiology, but also because it is well known that TCZ administration does not reduce circulating IL-6 levels (which, on the contrary, increase as a result of an inhibition of the IL-6 receptor-mediated consumption of IL-6) [26] thus rendering this cytokine not suitable for a correlation study. At the same time, since TNFα and IL-6 are strictly linked each other (TNFα is highly inducible in response to IL-6, and vice versa [27]) and both drive inflammatory activation in RA, it is conceivable that TNFα levels may reflect the degree of IL-6 receptor-dependent inhibition of systemic inflammation actually obtained in our patients as a result of TCZ treatment.

This work has some limitations, including the small sample-size, the short follow-up period, the absence of control groups, as well as the lack of information on indirect mechanisms possibly linking systemic inflammation and ventricular repolarisation (in particular, changes in the autonomic nervous system activity [12]). Thus, further ad-hoc prospective studies on larger groups of patients are warranted, also to evaluate the actual impact of TCZ therapy on the prevention of SCD in RA patients.

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Table 1

Characteristics of study patients

Patients, n	17
Age, years	52.3±11.3
Sex, F/M	15/2
Disease duration, years	13.6±11.1
Erosions on radiographs, n	12
RF positive, n	9
ACPA positive, n	7
Family history of CVD, n	6
Concomitant diseases, n	10
Autoimmune thyroiditis	3
Hypertension	2
Diabetes	2
Chronic viral hepatitis	2
Dyslipidemia	1
Ongoing treatment, n	
Steroids*(mean dose)	10 (8.1 mg daily)
Methotrexate (mean dose)	3 (12.5 mg weekly)
Leflunomide (mean dose)	1 (20 mg daily)
Hydroxychloroquine (mean dose)	1 (400 mg daily)
Cyclosporine A(mean dose)	1 (50 mg daily)
NSAIDs	8
ACE/Angiotensin II receptor inhibitors	2
Beta-blockers	2
Diuretics	1
Insulin	1
Thyroxine	3
Previous biologic treatments, n	
Etanercept	9
Adalimumab	8
Infliximab	4
Rituximab	3
Golimumab	1

CVD,cardiovascular disease; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibody; NSAIDs: non-steroidal anti-inflammatory drugs; ACE: angiotensin converting enzyme.
*Prednisone-equivalent dose.

Table 2

Changes in clinical and laboratory parameters during TCZ treatment

	T0	T1	T2	*p*
DAS28-ESR	5.5±1.2	3.4±1.6***	3.0±1.1***	**<0.001**
DAS28-CRP	4.9±1.0	3.0±1.2***	2.8±0.8***	**<0.001**
Swollen joint count	6.1±4.2	3.1±3.2**	2.4±2.4***	**<0.001**
Tender joint count	7.0±4.1	3.3±3.8*	2.4±2.1*	**0.003**
VAS	67.6±23.6	45.9±24.8*	43.2±21.4**	**<0.001**
GH	72.1±16.3	47.6±27.5*	46.2±22.1**	**<0.001**
HAQ-DI	1.5±0.6	1.3±0.6	1.1±0.6**	**<0.001**
CDAI	25.6±10.8	14.7±10.9***	10.8±7.0***	**<0.001**
ESR, mm/h (n.v.<35)	54.2±50.0	16.1±17.6**	10.8±11.2***	**<0.001**
CRP, mg/dl (n.v.<0.5)	1.50±1.6	0.21±0.3**	0.09±0.1***	**<0.001**
Potassium, mEq/L (n.v.3.5-5.5)	4.2±0.3	4.1±0.3	4.1±0.4	n.s.
Calcium, mEq/L (n.v.8-11)	9.2±0.5	9.2±0.3	9.3±0.4	n.s.
Magnesium, mEq/L (n.v.1.5-2.5)	1.9±0.2	2.0±0.1	1.9±0.2	n.s.

DAS28-ESR: disease activity score in 28 joints based on erythrocyte sedimentation rate levels; DAS28-CRP: disease activity score in 28 joints based on C-reactive protein levels; VAS: visual analogue scale; GH: patient's assessment of general health; HAQ-DI: Health Assessment Questionnaire–Disability Index; CDAI: clinical disease activity index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Differences were evaluated by the repeated measures analysis of variance (RM-ANOVA) and Tukey-Kramer post-hoc test, or Friedman test (nonparametric RM-ANOVA) and Dunn post-hoc test; *p<0.05, **p<0.01, ***p<0.001 vs. T0 (Tukey-Kramer or Dunn post-hoc test).

Table 3

Changes in QTc parameters during TCZ treatment

	T0	T1	T2	*p*
Standard ECG				
QTc, ms	452.3±35.8	428.8±34.3**	428.1±34.4**	**0.001**
Patients with QTc>440 ms, n	13 (76%)	7 (41%)	5 (29%)	**0.006**
15 minute-ambulatory ECG monitoring				
QTc MAX, ms	471.5±18.8	455.5±24.0*	447.4±25.8**	**0.002**
QTc MEAN, ms	432.2±21.0	425.0±26.5	413.3±21.2**	**0.009**

ECG: electrocardiogram; QTc: corrected QTc interval; QTc MAX: longest corrected QTc interval; QTc MEAN: mean corrected QTc interval.

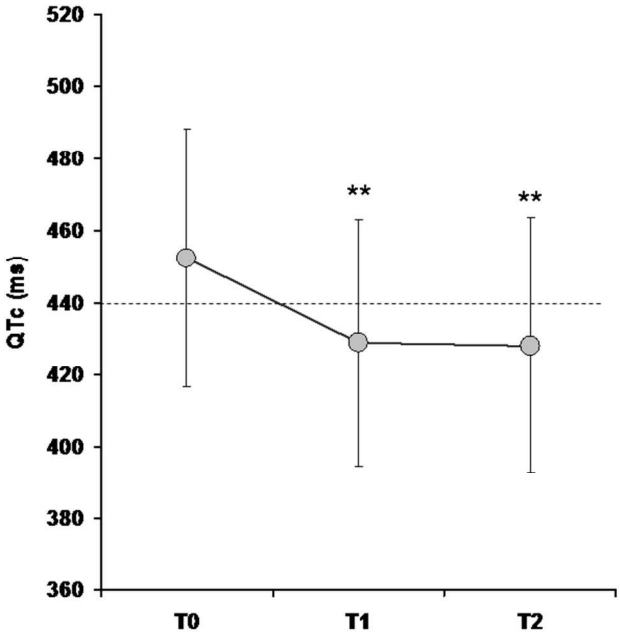
Differences in quantitative variables were evaluated by the repeated measures analysis of variance (RM-ANOVA) and Tukey-Kramer post-hoc test; *p<0.05, **p<0.01 vs. T0. Difference in categorical variables were evaluated by the chi-squared test for trend.

LEGEND TO FIGURES

Figure 1. Changes in mean QTc duration during TCZ treatment. T0: baseline, T1: 3 months, T2: 6 months. Repeated measures analysis of variance (RM-ANOVA), p=0.001; **p<0.01 versus T0: post-hoc Tukey-Kramer multiple comparison test.

Figure 2. Relation between QTc duration and circulating CRP levels (A), TNFα levels (B), or clinical disease activity index, CDAI (C) in the whole study population throughout the study time. Spearman's rank correlation test.

Figure 3. Relation between QTc duration and changes in circulating TNFα levels (A), CRP levels (B), or clinical disease activity index, CDAI (C) in the whole study population throughout the study time. Pearson or Spearman's rank correlation test.



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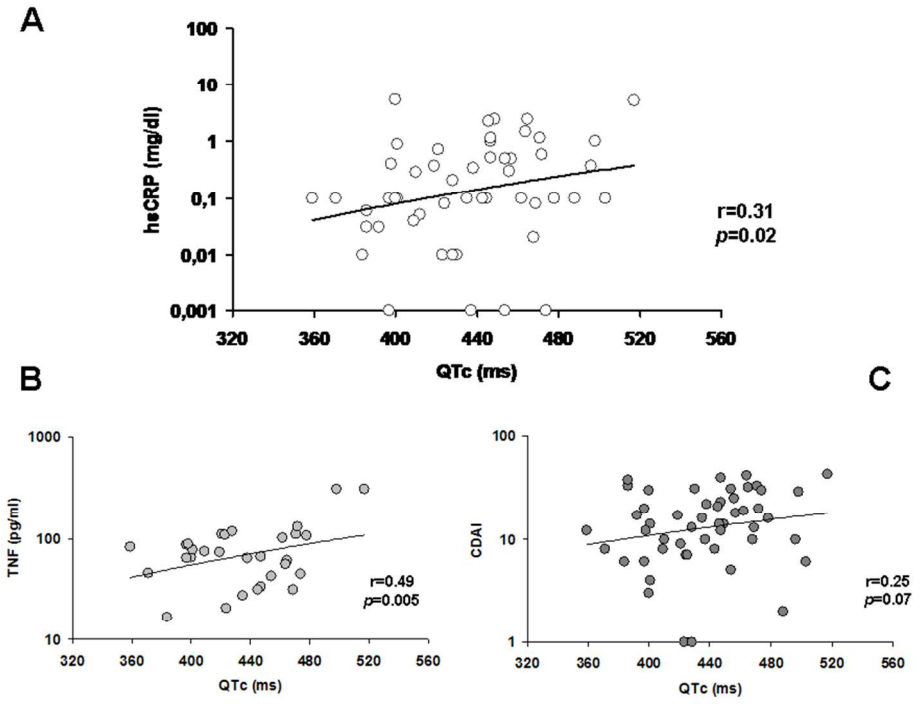

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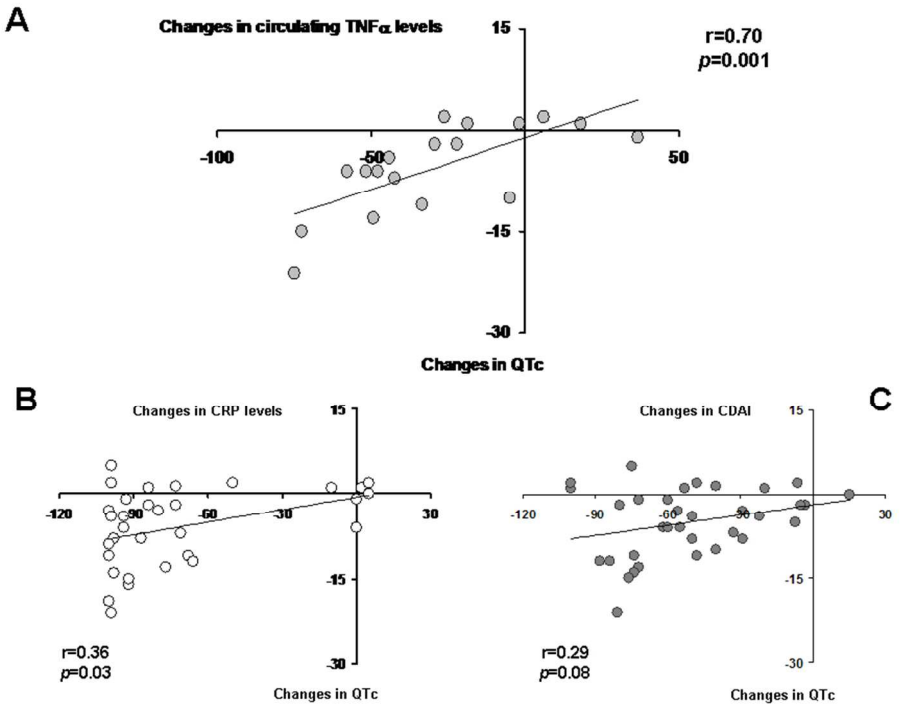

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