Association Between Corrected QT Interval and Inflammatory Cytokines in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Corrected QT (QTc) interval predicts all-cause and cardiovascular mortality and may contribute to the increased mortality risk in rheumatoid arthritis (RA). Animal experiments have shown that proinflammatory cytokines [tumor necrosis factor (TNF)-α and interleukin 1 (IL-1)] can prolong cardiomyocyte action potential. We sought to determine whether elevations in circulating inflammatory cytokines were independently associated with QTc prolongation in patients with RA.

Methods. One hundred twelve patients [median age 62 (interquartile range 17) yrs; 80 women (71%)] from a well-characterized RA cohort underwent baseline 12-lead electrocardiograms for QT interval measurement and contemporary blood sampling to assess concentrations of inflammatory markers including C-reactive protein (CRP), TNF-α, and interleukins (IL-1α, IL-1β, IL-6, IL-10). QTc was calculated using the Bazett ($QT_{BAZ} = QT \div \sqrt{RR}$) and Framingham Heart Study ($QT_{FHS} = QT + 0.154 \times [1 - RR]$) heart rate correction formulas.

Results. Inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10) were positively correlated with QT_{BAZ} (Spearman rank correlation coefficient rho = 0.199, 0.210, 0.222, 0.333; all $p < 0.05$). In multivariable regression analysis, these associations were all confounded by age except IL-10, where higher tertile groups were independently and positively associated with QT_{BAZ} ($\beta = 0.202$, $p = 0.023$) and QT_{FHS} ($\beta = 0.223$, $p = 0.009$) when compared to the lower tertile. CRP (per unit increase) was independently associated with QT_{BAZ} ($\beta = 0.278$, $p = 0.001$), but not QT_{FHS}.

Conclusion. To our knowledge, ours is the first study demonstrating a contemporary link between inflammatory cytokines and QT interval in humans. Our results suggest that a lower inflammatory burden may protect against QTc prolongation in patients with RA. However, further studies are required to confirm the effects of pro- and antiinflammatory cytokines on QTc interval. (First Release Jan 15 2015; J Rheumatol 2015;42:421–8; doi:10.3899/jrheum.140861)

Key Indexing Terms:

RHEUMATOID ARTHRITIS | CARDIOVASCULAR | CYTOKINES
INFLAMMATION | ARRHYTHMIA | QTc INTERVAL

The QT interval represents the time from onset of ventricular depolarization (beginning of the Q wave) to completion of repolarization (end of the T wave). Corrected QT (QTc) prolongation is a weak independent predictor of all-cause and cardiovascular mortality in the general population[1], but a more significant one in patients with rheumatoid arthritis (RA)[2]. Compared to the general population, the risk of sudden cardiac death is almost doubled in RA[3] and the case fatality rate (death rate following an acute cardiovascular event) is also increased[4,5,6]. This increased risk may in part be explained by prolonged QTc interval, a likely surrogate of sudden arrhythmic death[2,7].

Given the variability of the QT interval in relation to heart rate, several formulae are used to yield a corrected measure (QTc). The Bazett formula ($QT_{BAZ} = QT \div \sqrt{RR}$), one of the earliest and most commonly used heart rate correction formulae, has been criticized because of an overcorrection and undercorrection of QT interval at high and low heart rates, respectively[8]. To overcome this problem, a linear regression model using data from the Framingham Heart Study ($QT_{FHS} = QT + 0.154 \times [1 - RR]$) was developed[9].

Prior studies (including 1 in patients with RA) have suggested that QTc prolongation (QT_{BAZ}) may be driven by increased inflammatory burden[2,10,11,12,13,14]. However, few

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studies have assessed the direct effects of inflammatory cytokines on QT interval. In 1 animal experiment, tumor necrosis factor (TNF)-α prolonged the cardiomyocyte action potential by inhibiting the hERG (the human *ether-à-go-go*–related) gene (KCNH2) that codes for a protein known as $K_v11.1$, the α subunit of a potassium ion channel[11]. In another animal study, interleukin 1 (IL-1) was found to prolong cardiomyocyte action potential through its effects on calcium channels[15]. In addition, systemic inflammation may lead to QTc prolongation by accelerating the development of structural heart disease[7], a major risk factor for QTc prolongation that is more prevalent in patients with RA[16]. Acceleration of atherosclerosis[17], as well as induction of myocardial injury and remodeling[18,19,20], have been suggested as potential mechanisms by which systemic inflammation may directly promote the development of structural heart disease[7]. Alterations in the autonomic nervous system (ANS) function may also result in QTc prolongation[7,13]. Reduced cardiac parasympathetic activity[21] and increased sympathetic activity[22], a pattern of ANS dysfunction prevalent in patients with RA[23,24,25,26,27], have been shown to contribute to QTc prolongation. The relationship between inflammation and ANS remains to be fully elucidated and is a focus of research[28,29,30,31]. Evidence from animal experiments demonstrates that IL-1β[32], IL-6[33], and TNF-α[34] cause sympathoexcitation, which potentially may prolong QT interval. Collectively, these studies in animal models suggest several potential mechanisms by which proinflammatory cytokines may lead to prolongation of the QT interval, but it remains unclear whether such pathways are involved in humans.

Our study aimed to test the hypothesis that serum proinflammatory and antiinflammatory cytokine concentrations were correlated with contemporary QTc interval in patients with RA. We also sought to determine whether these associations were affected by the QT correction method used (QT_{BAZ} vs QT_{FHS}).

MATERIALS AND METHODS

One hundred forty-six subjects from a well-characterized cohort of patients with RA[35,36] were assessed with 12 lead electrocardiograms (ECG) for QTc interval and contemporary blood samples that were analyzed for biochemical markers of inflammation. Disease Activity Score at 28 joints (DAS28)[37] was used to assess their current disease activity. RA diagnosis was based on the revised 1987 American College of Rheumatology classification criteria[38]. Patients were recruited consecutively at the outpatient clinics of the Dudley Group National Health Service Foundation Trust, UK, from July 2004 to October 2006. Our study was approved by the local ethics committee and all subjects gave written informed consent.

QT interval (time between the beginning of a Q wave and the end of a T wave) was manually measured by 2 experts and corrected for heart rate using the Bazett formula (methods described in detail elsewhere[2]). Additionally, QT was corrected using the Framingham Heart Study formula ($QT_{FHS} = QT + 0.154 \times [1 - RR]$)[9]. Serum was collected on the same day and frozen immediately at –70°C until analyzed. Concentrations of IL-6, TNF-α, IL-1β, and IL-10 were analyzed by flow cytometry using FlowCytomix kits (Bender MedSystems GmbH). Briefly, FlowCytomix kits allowed the analyses of multiple targets in 1 sample. Uniquely color-coded polystyrene beads were coated with monoclonal antibodies that targeted the required cytokine, following which they were allowed to react with the sample. A biotinylated detection antibody specific for a different epitope on the cytokine was then added. Finally, after the addition of a streptavidin-phycoerythrin complex, the emitted fluorescent signals were detected using flow cytometry. C-reactive protein (CRP; Vitros 5.1-FS, Ortho Clinical Diagnostics) and erythrocyte sedimentation rate (StaRRsed Compact, Vitech Scientific Ltd.) were also analyzed. History of ischemic heart disease was defined as having any of the following: angina diagnosed by a physician or elicited by the use of the Rose questionnaire[39], myocardial infarction, angioplasty, or coronary artery bypass grafting or/and ischemic heart failure. The Minnesota Code Classification System was used to code for the presence of ST abnormality on the ECG[40].

Statistical analysis was performed using SPSS software, version 19 (IBM SPSS Inc.). Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Correlations between QTc (QT_{BAZ}, QT_{FHS}) interval and continuous variables were assessed using Pearson correlation for parametric and Spearman correlation coefficient (rho) for nonparametric data. Associations between QTc (QT_{BAZ}, QT_{FHS}) interval and dichotomous categorical variables were assessed using a 2-tailed, independent Student t test. In addition, cytokines and CRP were divided into tertiles. Differences in QTc interval between tertiles was assessed using a 1-way ANOVA with Tukey posthoc test. Stepwise multivariable linear regression analysis was performed to determine independent associations between QTc (QT_{BAZ} and QT_{FHS} separately) interval and other pertinent measured variables (variables considered clinically significant or those with a univariate association with QTc demonstrating a p value < 0.1 were included in the model). Cytokine tertile groups were entered into the regression models as continuous variables (calculating a β per tertile increase). To minimize the risk of collinearity, variables that are known to interact were entered into separate models (e.g., β-blocker, ischemic heart disease, cytokines). Values were expressed as mean ± SD for parametric data, median [interquartile range (IQR)] for nonparametric data, and frequency (percentages) for categorical variables. A p value of less than 0.05 was considered statistically significant throughout.

RESULTS

Of the 146 patients studied, 34 (23%) were excluded because of the presence of bundle branch block precluding QT interval assessment. Analysis was performed on the remaining 112 patients. Median age (IQR) was 62.4 years (51.5–68.6) with 80 females (71%; Table 1). Other baseline patient characteristics are summarized in Table 1.

Heart rate correction. Mean QTc interval was 423.2 ± 22.5 ms (QT_{BAZ}) and 412.5 ± 18.0 ms (QT_{FHS}). QT_{BAZ} was positively correlated with heart rate while QT_{FHS} was not (Table 2).

Demographic and disease-related variables. QTc interval (QT_{BAZ} and QT_{FHS}) was weakly positively correlated with age and was higher in women compared to men (QT_{BAZ} = 12.5 ms and QT_{FHS} = 7.8 ms higher; Table 3). Patients with a history of ischemic heart disease had a higher QTc interval (QT_{BAZ} = 16.8 ms and QT_{FHS} = 20.4 ms higher). Hydroxychloroquine (HCQ) use was associated with a higher QTc interval (QT_{BAZ} = 12.9 ms, QT_{FHS} = 9.8 ms higher). There was a trend for β blockade to lower QT_{BAZ}, but not QT_{FHS}. Aside from HCQ, no other medications, including biologic agents (i.e., TNF-α inhibitors) and those with a known association with risk of Torsades de Pointes

Table 1. Baseline characteristics for patients with RA. Values are mean ± SD for parametric data, median (IQR) for nonparametric data, or n (%) for categorical data.

Characteristic	n = 112
Demographic	
Age, yrs	62.4 (51.5–68.6)
Female sex	80 (71)
BMI[a], kg/m^2	27.9 ± 5.2
Comorbidities and medications	
Osteoarthritis	44 (39)
Hypertension[b]	88 (79.5)
Ischemic heart disease	12 (10.7)
Hypercholesterolemia	16 (14)
Antihypertensive agent	47 (42)
ACE inhibitor	29 (25.9)
B blocker	22 (19.6)
Diuretic	18 (16.1)
RA-related	
Disease duration, yrs	9 (3–15)
RF-positive	87 (78)
Anti-CCP–positive[c]	77 (69)
DAS28[d]	4.2 ± 1.3
Methotrexate	98 (88)
Sulfasalazine	80 (71)
Hydroxychloroquine	22 (20)
Gold	20 (18)
NSAID	26 (23)
Biological agent	10 (9)
Electrocardiographic features	
QT interval, ms	393.6 ± 31.0
Corrected QTc interval, ms	
QT_{BAZ}	423.2 ± 22.5
QT_{FHS}	412.5 ± 18.0
Heart rate, beats/min	71.0 ± 13.1
Left ventricular hypertrophy, Sokolow-Lyon	7 (6)
Q wave abnormality	6 (5)
ST abnormality[e]	6 (5)
Inflammatory markers	
CRP, mg/l	9 (5–19.8)
ESR, mm/h	21.5 (10–38.8)
IL-6, pg/ml	16.8 (4–52.7)
TNF-α, pg/ml	8.3 (5–35.8)
IL-1α, pg/ml	2.7 (0–24.1)
IL-1β, pg/ml	1.1 (0–7.4)
IL-10, pg/ml	1.0 (0–5.9)

[a] n = 109. [b] Blood pressure ≥ 140 or 90 or taking antihypertensives. [c] n = 105. [d] n = 111. [e] Coded using the Minnesota Code Classification System. RA: rheumatoid arthritis; IQR: interquartile range; BMI: body mass index; ACE: angiotensin-converting enzyme; RF: rheumatoid factor; anti-CCP: anticyclic citrullinated peptide; DAS28: Disease Activity Score at 28 joints; NSAID: nonsteroidal antiinflammatory drug; QTc: corrected QT; QT_{BAZ}: QT corrected using the Bazett formula; QT_{FHS}: QT corrected using the Framingham Heart Study formula; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL: interleukin; TNF-α: tumor necrosis factor-α.

(thiazide diuretics, amitriptyline, and other antidepressants; www.crediblemeds.org), were associated with QTc interval.

Patients with hypercholesterolemia had a higher QT_{FHS}, while there were trends for higher QT_{FHS} in patients with coexistent osteoarthritis, those receiving proton pump inhibition, and in seropositive patients (anticyclic citrullinated peptide, rheumatoid factor). There were no significant associations between QTc interval and ECG abnormalities (including left ventricular hypertrophy), other comorbidities (including hypertension), medications, and RA-related characteristics (DAS28; Table 3).

Inflammatory markers. Correlation analysis showed that CRP, TNF-α, IL-6, IL-1β, and IL-10 were all significantly associated with QT_{BAZ} (weakly positive) while IL-1α showed only a trend (Table 2). However, when QT_{FHS} was used, only IL-1β and IL-10 were significantly associated (weakly positive) while there was a trend with TNF-α and IL-1α. There was no significant association between QT_{FHS} and CRP or IL-6.

QT_{BAZ} and QT_{FHS} were significantly lower in the first tertile for IL-10 compared to the second and third; this was attenuated after multivariable adjustment for age, sex, presence of ischemic heart disease or hypercholesterolemia, HCQ, and β-blocker use (Table 4). QT_{FHS} was significantly lower in the first tertile for TNF-α compared to the third tertile; however, this was insignificant after adjustment for age and other variables (Table 4). QT_{BAZ} tended to be lower in the first tertile for CRP compared to the third tertile. There was no significant association between IL-6, IL-1α, and IL-1β tertile groups and QTc interval.

Multivariable linear regression. Stepwise linear regression analysis showed that age, sex, and CRP were independently associated with QT_{BAZ} interval, accounting for 22% of the variation (Table 5). The associations (and trends) found between the cytokines and QT_{BAZ}/QT_{FHS} in univariate analysis were all confounded by age with the exception of IL-10 (Table 5). IL-10 tertile group (when entered into the model as a continuous variable) was independently associated with QT_{BAZ} and QT_{FHS} intervals independent of other factors (including age, sex, β-blocker or HCQ use, and presence of ischemic heart disease).

DISCUSSION

To our knowledge, our study is the first to report a correlation between QTc interval and contemporary serum concentrations of proinflammatory and antiinflammatory cytokines in humans. CRP and IL-10 demonstrated a positive correlation with QTc, suggesting that a higher inflammatory burden associates independently with longer duration of QTc interval. Given that QTc prolongation increases the risk of potentially life-threatening arrhythmias (e.g., Torsades de Pointes), our results are in keeping with a possible mechanistic link between increased inflammatory burden in RA and cardiovascular mortality. Cardiovascular case fatality in RA is reported to decline in parallel with better control of disease activity[41], which supports the hypothesis that a lower inflammatory burden may protect against QTc prolongation and hence reduce the risk of sudden cardiac death.

Table 2. Correlations between QT, QTc (QT_{BAZ}, QT_{FHS}) heart rate, age, CRP, ESR, and cytokines. Values are correlation coefficient (Spearman rank rho or Pearson product r[a]), p, and n.

Variable	Heart Rate[a]	Age	CRP	ESR	IL-6	TNF-α	IL-1α	IL-1β	IL-10
QT	–0.807** < 0.001 112	0.038 0.694 112	–0.126 0.185 112	–0.205* 0.030 112	–0.057 0.552 112	0.099 0.299 112	0.113 0.235 112	0.132 0.165 112	0.108 0.257 112
QT_{BAZ}	0.517** < 0.001 112	0.293** 0.002 112	0.202* 0.033 112	0.134 0.158 112	0.222* 0.016 112	0.199* 0.036 112	0.158 0.097 112	0.210* 0.026 112	0.333** < 0.001 112
QT_{FHS}	–0.041 0.668 112	0.244** 0.009 112	0.088 0.356 112	–0.010 0.917 112	0.129 0.175 112	0.180 0.057 112	0.180 0.058 112	0.220* 0.020 112	0.294** 0.002 112
Heart rate			0.251** 0.008 112	0.248** 0.008 112	0.175 0.064 112	0.034 0.724 112	–0.031 0.744 112	0.004 0.744 112	0.109 0.251 112
CRP				0.568** < 0.001 112	0.185** 0.0051 112	0.021 0.830 112	–0.004 0.965 112	0.088 0.356 112	0.0083 0.387 112
ESR					0.323** 0.001 112	0.153 0.106 112	0.144 0.129 112	0.196* 0.038 112	0.121 0.203 112
IL-6						0.300** 0.001 112	0.497** < 0.001 112	0.511** < 0.001 112	0.205* 0.030 112
TNF-α							0.784** < 0.001 112	0.767** < 0.001 112	0.775** < 0.001 112
IL-1α								0.917** < 0.001 112	0.853** < 0.001 112
IL-1β									0.902** < 0.001 112

* $p < 0.05$. ** $p < 0.01$. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL: interleukin; TNF: tumor necrosis factor; QT_{BAZ}: QT corrected using the Bazett formula; QT_{FHS}: QT corrected using the Framingham Heart Study formula.

Of the cytokines analyzed, IL-10 was independently associated with QTc interval prolongation. IL-10, the major antiinflammatory cytokine, is released by monocytes, macrophages, and B cells in response to inflammatory stimuli, and is known to inhibit the production and release of proinflammatory cytokines (including TNF-α, IL-1β, and IL-6)[42,43,44,45]. In addition, IL-10 reduces the surface expression of TNF receptors and promotes the release of TNF receptors into the circulation[46,47]. There are a number of possible explanations for the positive correlation between IL-10 concentration and QTc prolongation found in our study. The surge of IL-10 production is known to follow that of proinflammatory cytokines[45] and hence, increased levels of IL-10, as seen in RA[48], may represent increased inflammation. Conversely, undetectable levels of IL-10 may indicate lower circulating concentrations of TNF-α and IL-1, and may result in a lower QTc interval; given that both TNF-α[11] and IL-1[15] were shown to prolong cardiomyocyte action potential duration in animal studies. This is supported by our finding that IL-10 was strongly correlated with other proinflammatory cytokines, including TNF-α (rho = 0.775, $p < 0.001$), IL-1α (rho = 0.853, $p < 0.001$), and IL-1β (rho = 0.902, $p < 0.001$).

Additionally, given that the release of IL-10 is stimulated by adrenaline[45] and that sympathoexcitation may be associated with QTc prolongation[22], lower levels of IL-10 may reflect lower resting sympathetic nerve activity. Finally, the direct effects of IL-10 on cardiomyocyte action potential are currently unknown and further studies are warranted to explore the mechanisms by which IL-10 and other inflammatory cytokines may affect QTc interval.

In our study, we found a significant and independent association between CRP and QT_{BAZ}, but no association between CRP and QT_{FHS}. We are the first to report such a discrepancy between QT_{BAZ} and QT_{FHS}; to our knowledge, prior studies showing associations between QTc interval and CRP all used the Bazett heart rate correction formula. In 1 study of 101 patients with inflammatory arthritis, elevated concentrations of high-sensitivity CRP were associated with higher QT_{BAZ}, but also a higher resting heart rate[13]. One possible explanation is that heart rate may be confounding the relationship between QTc interval and CRP; once heart

Table 3. Significant associations between QTc and selected variables. Associations between QTc interval and dichotomous categorical variables were tested using 2-tailed, unpaired Student t test. Multiple factors (found in Table 1) were assessed; however, only those with $p < 0.1$ were included in this table. Values are mean ± SD unless otherwise specified.

Variable	n	QTc Interval, ms	t	p
QT_{BAZ}				
Sex				
Women	80	426.7 ± 21.0	2.733	0.007**
Men	32	414.2 ± 23.9		
Ischemic heart disease				
Yes	12	438.2 ± 22.3	2.503	0.014*
No	100	421.4 ± 22.0		
β blocker				
Yes	22	415.6 ± 19.5	–1.775	0.079
No	90	425.0 ± 22.9		
HCQ				
Yes	22	433.5 ± 22.1	2.448	0.016*
No	90	420.6 ± 21.8		
QT_{FHS}				
Sex				
Women	80	414.8 ± 17.3	2.103	0.038*
Men	32	407.0 ± 18.8		
Ischemic heart disease				
Yes	12	430.8 ± 17.3	3.948	< 0.001**
No	100	410.4 ± 16.9		
Hypercholesterolemia				
Yes	16	424.0 ± 23.9	2.840	0.005**
No	96	410.6 ± 16.2		
HTN				
Yes	24	412.5 ± 13.6	–0. 011	0.071
No	88	412.6 ± 19.1		
OA				
Yes	44	416.1 ± 18.0	1.719	0.088
No	68	410.2 ± 17.7		
Proton pump inhibitor				
Yes	22	418.8 ± 18.9	1.847	0.067
No	90	411.0 ± 17.5		
HCQ				
Yes	22	420.4 ± 16.6	2.336	0.021*
No	90	410.6 ± 17.9		
RF-positive				
Yes	87	414.1 ± 18.7	1.749	0.083
No	25	407.0 ± 14.2		
Anti-CCP–positive				
Yes	77	413.7 ± 18.5	2.016	0.053
No	28	406.1 ± 18.5		

* $p < 0.05$. ** $p < 0.01$. QTc: corrected QT; QT_{BAZ}: QT corrected using the Bazett formula; QT_{FHS}: QT corrected using the Framingham Heart Study formula; RF: rheumatoid factor; anti-CCP: anticyclic citrullinated peptide; HCQ: hydroxycholorquine; HTN: hypertension; OA: osteoarthritis.

rate is adequately corrected with the stricter formula (QT_{FHS}), this relationship disappears. This is further supported by our finding that CRP was correlated with heart rate. Interactions between the immune and ANS could also explain the associations we found between QTc interval and inflammation. Both the sympathetic and parasympathetic nervous system have been shown to contribute to QTc interval[21,22]. Left cardiac sympathetic denervation shortens QTc interval in patients with the congenital long QT syndrome[22], while cholinergic stimulation with pyridostigmine shortens QTc interval in patients with coronary artery disease[21]. In patients with RA, increased sympathetic and reduced parasympathetic activities have been identified[24,25,26,27] and could contribute to QTc interval prolongation. Evidence suggests a bidirectional relationship between the immune system and ANS. For example, IL-6

Table 4. Associations between QTc and inflammatory markers according to tertiles before and after multivariable adjustments.

Inflammatory Marker	Measurement	Tertile 1	Tertile 2	Tertile 3	Univariate[1] F	Univariate[1] p	Multivariable[2] Age F	Multivariable[2] Age p	Multivariable[2] A F	Multivariable[2] A p	Multivariable[2] B or C[3] F	Multivariable[2] B or C[3] p
CRP	mg/ml	**≤ 7.0**	**8–13**	**> 14**								
n		44	31	37								
QT_{BAZ}	ms	417.4 ± 22.9	425.5 ± 18.4	428.0 ± 24.2	2.552	0.083	2.675	0.073	4.078	0.020*†	2.497	0.087
QT_{FHS}	ms	410.9 ± 19.2	414.5 ± 17.0	412.8 ± 17.6	0.362	0.697	0.375	0.688	1.760	1.155	0.177	0.319
IL-6	pg/ml	**≤ 6.3**	**6.31–37.23**	**> 37.24**								
n		38	37	37								
QT_{BAZ}	ms	417.0 ± 20.7	426.3 ± 21.4	426.3 ± 24.6	2.214	0.114	0.954	0.388	0.972	0.382	0.615	0.543
QT_{FHS}	ms	409.5 ± 16.0	415.1 ± 17.5	413.1 ± 20.2	0.942	0.393	0.892	0.413	0.505	0.605	0.245	0.783
TNF-α	pg/ml	**≤ 6.14**	**6.15–23.99**	**> 24.0**								
n		38	37	37								
QT_{BAZ}	ms	419.1 ± 23.5	421.4 ± 20.8	429.2 ± 22.5	2.109	0.126	1.174	0.313	0.883	0.417	1.320	0.271
QT_{FHS}	ms	408.6 ± 15.7	410.8 ± 17.3	418.4 ± 19.7	3.153	0.047†	1.823	0.167	1.381	0.256	1.570	0.213
IL-1α	pg/ml	**0.0**	**0.01–9.45**	**> 9.46**								
n		41	34	37								
QT_{BAZ}	ms	422.9 ± 27.4	419.1 ± 18.1	427.1 ± 19.9	1.123	0.329	1.036	0.358	1.214	0.301	0.860	0.426
QT_{FHS}	ms	410.8 ± 17.9	410.1 ± 16.7	416.8 ± 18.9	1.549	0.217	0.248	0.781	1.141	0.323	0.907	0.407
IL-1β	pg/ml	**≤ 0.45**	**0.46–3.51**	**> 3.52**								
n		38	37	37								
QT_{BAZ}	ms	418.4 ± 26.2	423.9 ± 21.2	427.2 ± 19.2	1.460	0.237	0.860	0.426	0.507	0.604	0.838	0.435
QT_{FHS}	ms	408.2 ± 17.1	411.7 ± 18.0	412.5 ± 18.0	2.806	0.065	2.004	0.140	1.584	0.210	1.800	0.170
IL-10	pg/ml	**0.0**	**0.01–2.0**	**> 2.01**								
n		40	35	37								
QT_{BAZ}	ms	413.4 ± 20.8	429.8 ± 23.7	427.4 ± 19.9	6.506	0.002*†	5.555	0.005*†	3.255	0.042*†	4.212	0.017*†
QT_{FHS}	ms	405.6 ± 14.9	415.6 ± 18.2	417.1 ± 19.0	4.989	0.008*†	3.976	0.022*†	2.498	0.087	3.344	0.039†

Values in bold face are the cytokine concentration in each tertile. [1] Univariate analysis performed using 1-way ANOVA with Tukey posthoc test. [2] Multivariable analysis performed with adjustments for age alone: model A (age, sex, presence of ischemic heart disease, and hydroxychloroquine use), and [3] either model B (age, sex, β blocker use, and hydroxychloroquine use for QT_{BAZ}) or model C (age, sex, presence of hypercholesterolemia, and hydroxychloroquine use for QT_{FHS}), with least significant difference posthoc test. Posthoc significance $p < 0.05$. * tertile 1 vs 2, † tertile 1 vs 3. QTc: corrected QT; CRP: C-reactive protein; QT_{BAZ}: QT corrected using the Bazett formula; QT_{FHS}: QT corrected using the Framingham Heart Study formula; IL: interleukin; TNF-α: tumor necrosis factor-α.

has been shown to increase sympathetic activity in rats[33], while the ANS has been implicated in regulation of the immune response through cholinergic nerve fibers, known as the cholinergic inflammatory reflex[31]. Indeed, the principal vagal neurotransmitter acetylcholine inhibits the release of cytokines TNF-α, IL-1β, and IL-6, but not IL-10[49], and direct electrical stimulation of the vagus nerve in rats during endotoxemia inhibited synthesis of TNF-α[49].

The weak correlation between CRP and IL-6 found in our study and reported in prior studies[50,51] is interesting given that IL-6 stimulates hepatocytes to produce CRP. This finding may be because of hysteresis (i.e., the delayed effect of IL-6 on CRP production may not be reflected in concurrent blood sampling), or perhaps the pleiotropic effects of IL-6 (i.e., antiinflammatory), or altered activity in RA. One of the major limitations of our present study is the cross-sectional nature of the associations between inflammatory cytokines and QTc interval. With a lack of followup data, these results cannot prove causality and therefore should be viewed as hypothesis-generating. Another limitation is the one-off blood sampling that does not allow for cytokine fluctuations over time to be measured. Finally, the variables included in our regression models explained about 21%–24% of the variation in QTc interval, suggesting the presence of other confounders not being explained. These may include autonomic dysfunction, electrolyte abnormalities, structural heart disease, and the presence of genetic polymorphisms causing congenital long QT syndrome. Studies are needed to further explore the relationship between inflammatory cytokines and QTc prolongation. It is likely that concurrent assessments of ANS function and QTc interval with multiple methods of heart rate correction can help provide greater understanding of the precise mechanisms by which inflammatory cytokines contribute to QTc prolongation in RA.

Our study, to our knowledge, is the first to report a contemporary association between higher serum proinflammatory and antiinflammatory cytokine concentrations and QTc prolongation in patients with RA. Undetectable concentrations of IL-10 were independently associated with shorter QTc interval while proinflammatory cytokines (TNF-α, IL-1β) were positively associated with prolonged QTc interval — although this association was attenuated when adjusting for age. Further studies are needed to

Table 5. Stepwise multivariable linear regression with QTc interval as dependent variable. To minimize risk of collinearity, these were entered into separate models: β blocker, heart disease, and hypercholesterolemia, as well as anti-CCP positivity and RF positivity. Standardized β and unstandardized (B) coefficients are shown.

Variable	β	B	95% CI	p
QT_{BAZ} as dependent variable, stepwise linear regression				
Model 1, $R^2 = 0.220$				
Age	0.263	0.487	0.175–0.800	0.003‡
Female sex	0.236	11.719	3.354–20.083	0.006‡
CRP	0.278	0.300	0.118–0.483	0.001‡
Model 2, $R^2 = 0.211$				
Age	0.264	0.489	0.169–0.808	0.003‡
Female sex	0.210	10.441	1.894–18.987	0.017†
β blocker	–0.177	–9.973	–19.647 – –0.298	0.043†
IL-10, tertiles, per unit increase	0.202	5.457	0.754–10.160	0.023†
QT_{FHS} as dependent variable, stepwise linear regression				
Model 3, $R^2 = 0.238$				
Heart disease	0.351	20.321	10.562–30.080	< 0.001‡
HCQ	0.248	11.197	3.650–18.744	0.004‡
IL-10, tertiles, per unit increase	0.223	4.830	1.205–8.455	0.009‡

The following variables were entered into the stepwise multivariable regression analysis: models 1 and 2 (age, female sex, CRP, IL-6, TNF-α, IL-1α, IL-1β, IL-10, heart disease, β blocker, and hydroxychloroquine), and Model 3 [age, female sex, TNF-α (tertiles), IL-1β (tertiles), IL-10 (tertiles), heart disease, hypercholesterolemia, hydroxychloroquine, RF positivity, and anti-CCP positivity]. † $p < 0.05$. ‡ $p < 0.01$. QTc: corrected QT; anti-CCP: anticyclic citrullinated peptide antibodies; RF: rheumatoid factor; QT_{BAZ}: QT corrected using the Bazett formula; CRP: C-reactive protein; IL: interleukin; QT_{FHS}: QT corrected using the Framingham Heart Study formula; HCQ: hydroxychloroquine.

confirm the effect of circulating inflammatory cytokine levels on QTc interval and to assess whether this effect is contemporary or cumulative.

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