

**ORIGINAL RESEARCH**

# Risk of QTc Interval Prolongation Associated With Circulating Anti-Ro/SSA Antibodies Among US Veterans: An Observational Cohort Study

Pietro Enea Lazzerini <sup>id</sup>, MD\*; Gabriele Cevenini, BEng\*; Yongxia Sarah Qu, MD, PhD\*; Frank Fabris, MS; Nabil El-Sherif, MD; Maurizio Acampa <sup>id</sup>, MD; Alessandra Cartocci <sup>id</sup>, MSc; Franco Laghi-Pasini, MD; Pier Leopoldo Capecci, MD; Mohamed Boutjdir, PhD; Deana Lazaro, MD

**BACKGROUND:** Anti-Sjögren's syndrome-related antigen A-antibodies (anti-Ro/SSA-antibodies) are responsible for a novel form of acquired long-QT syndrome, owing to autoimmune-mediated inhibition of cardiac human ether-a-go-go-related gene-potassium channels. However, current evidence derives only from basic mechanistic studies and relatively small sample-size clinical investigations. Hence, the aim of our study is to estimate the risk of QTc prolongation associated with the presence of anti-Ro/SSA-antibodies in a large population of unselected subjects.

**METHODS AND RESULTS:** This is a retrospective observational cohort study using the Veterans Affairs Informatics and Computing Infrastructure. Participants were veterans who were tested for anti-Ro/SSA status and had an ECG. Descriptive statistics and univariate and multivariate logistic regression analyses were performed to identify risk factors for heart rate-corrected QT interval (QTc) prolongation. The study population consisted of 7339 subjects (61.4±12.2 years), 612 of whom were anti-Ro/SSA-positive (8.3%). Subjects who were anti-Ro/SSA-positive showed an increased prevalence of QTc prolongation, in the presence of other concomitant risk factors (crude odds ratios [OR], 1.67 [1.26–2.21] for QTc >470/480 ms; 2.32 [1.54–3.49] for QTc >490 ms; 2.77 [1.66–4.60] for QTc >500 ms), independent of a connective tissue disease history. Adjustments for age, sex, electrolytes, cardiovascular risk factors/diseases, and medications gradually attenuated QTc prolongation estimates, particularly when QT-prolonging drugs were added to the model. Nevertheless, stepwise-fully adjusted OR for the higher cutoffs remained significantly increased in anti-Ro/SSA-positive subjects, particularly for QTc >500 ms (2.27 [1.34–3.87]).

**CONCLUSIONS:** Anti-Ro/SSA-antibody positivity was independently associated with an increased risk of marked QTc prolongation in a large cohort of US veterans. Our data suggest that within the general population individuals who are anti-Ro/SSA-positive may represent a subgroup of patients particularly predisposed to ventricular arrhythmias/sudden cardiac death.

**Key Words:** anti-Ro/SSA ■ connective tissue diseases ■ general population ■ QTc prolongation ■ sudden death risk

**T**he QT-interval on the ECG reflects the duration of the ventricular action potential, in turn generated by transmembrane flow of ions, mainly inward depolarizing currents through sodium and

calcium channels, and outward repolarizing currents through potassium channels.<sup>1</sup> Heart rate-corrected QT-interval (QTc) prolongation, currently defined as QTc >470 ms for men and 480 ms for women,<sup>2</sup> is a

Correspondence to: Pietro Enea Lazzerini, MD, Department of Medical Sciences, Surgery and Neurosciences, University of Siena, Siena, Italy. E-mail: lazzerini7@unisi.it and Deana Lazaro, MD, Medical Service (III), VA New York Harbor Healthcare System, 800 Poly Place, Brooklyn, NY 11209. E-mail: deana.lazaro@va.gov

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\*Dr Lazzerini, Prof Cevenini, Dr Boutjdir, and Dr Lazaro contributed equally to this work.

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## CLINICAL PERSPECTIVE

### What Is New?

- In a large cohort of US veterans, subjects who were anti-Sjögren's syndrome-related antigen A-antibodies positive (anti-Ro/SSA), independent of a history of connective tissue disease, showed an increased prevalence of heart rate-corrected QT interval prolongation, in the presence of other concomitant risk factors.
- Circulating anti-Ro/SSA-antibodies were independently associated with a  $\approx 2$ -times higher risk of marked QTc prolongation.
- Anti-Ro/SSA positivity was a significant contributor to marked heart rate-corrected QT interval prolongation occurrence, even representing one of the most important risk factors involved; a synergy between anti-Ro/SSA-antibodies and most of the traditional risk factors was demonstrated.

### What Are the Clinical Implications?

- Our data suggest that within the general population subjects who are anti-Ro/SSA-positive may represent a subgroup with an increased predisposition to life-threatening ventricular arrhythmias, particularly when other QT-prolonging risk factors are concomitantly present.
- It is proposed that subjects who are anti-Ro/SSA-positive should receive counseling about drugs and management of other risk factors that may increase the risk for malignant arrhythmias; at the same time, specific anti-Ro/SSA testing should be considered in patients with "idiopathic" rhythm disturbances, as it could intriguingly open novel avenues in antiarrhythmic treatment, primarily immunomodulatory therapies.

## Nonstandard Abbreviations and Acronyms

<b>Anti-Ro/SSA-antibodies</b>	anti-Sjögren's syndrome-related antigen A-antibodies
<b>CTD</b>	connective tissue disease
<b>hERG</b>	human ether-a-go-go-related gene
<b>IHD</b>	ischemic heart disease
<b>LQTS</b>	long QT-syndrome
<b>QTc</b>	heart rate-corrected QT interval
<b>SCD</b>	sudden cardiac death
<b>TdP</b>	torsades de pointes
<b>VA</b>	ventricular arrhythmia

well-recognized risk factor for arrhythmic events and sudden cardiac death (SCD) in the general population.<sup>3</sup> Specifically, the term long-QT syndrome (LQTS) designates a clinical condition characterized by the presence of a prolonged QTc, and an increased predisposition to develop life-threatening ventricular arrhythmias (VAs), particularly torsades de pointes (TdP).<sup>2,4</sup> Notably, the more the QTc prolongs, the greater the TdP risk, becoming significantly high when QTc >500 ms.<sup>2</sup>

Although LQTS may be congenital or acquired, inherited forms are relatively rare ( $\approx 1:2000$  apparently healthy newborns).<sup>5</sup> Conversely, acquired LQTS is rather common, most frequently associated with electrolyte imbalances, medications, and structural heart diseases.<sup>6</sup> Other well-recognized risk factors for acquired LQTS/TdP include female sex, older age, and bradycardia.<sup>2</sup> It should be noted that in most cases multiple simultaneous risk factors need to be present for TdP occurrence or to develop QTc prolongation.<sup>2,7</sup> Because numerous often-redundant ion channel mechanisms are implicated in preserving the normal action potential, multiple QT-prolonging factors are required to significantly disrupt ventricular repolarization (*multihit theory*).<sup>7</sup>

Immune-inflammatory activation is increasingly recognized as an emerging risk factor for QTc prolongation and TdP.<sup>7-10</sup> In particular, recent evidence demonstrates that anti-Ro/SSA-antibodies are responsible for a novel form of acquired LQTS because of an autoimmune-mediated inhibition of cardiac human-ether-a-go-go-related gene (hERG)-potassium channels.<sup>8,11,12</sup>

Anti-Ro/SSA-antibodies, including anti-Ro/SSA-52 kDa and anti-Ro/SSA-60 kDa subtypes, are frequently detected in patients with connective tissue diseases (CTD).<sup>13</sup> Several studies provided evidence that in these patients, and their children, anti-Ro/SSA-antibodies (specifically anti-Ro/SSA-52 kDa) can promote LQTS<sup>14-18</sup> and VAs<sup>19-21</sup> by blocking the hERG-potassium current.<sup>11</sup> Such a block is caused by the anti-Ro/SSA-antibody direct binding to the hERG-potassium pore region, acutely interfering with gating properties and chronically resulting in reduced expression of the channel.<sup>11,12,22</sup>

Notably, anti-Ro/SSA-antibodies are also detected in a significant percentage of apparently healthy subjects (0.5–2.7%)<sup>23-26</sup> and are associated with TdP occurrence regardless of the presence or absence of a manifest CTD.<sup>22,27</sup> In fact, in a cohort of 25 consecutive patients with TdP prospectively collected from the general population, anti-Ro/SSA-52 kDa positivity was found in 60% of cases, always in the absence of a concomitant CTD.<sup>22</sup> These findings intriguingly suggest that anti-Ro/SSA-antibodies may be also silently involved in promoting VAs and SCD in a percentage of subjects of the general population.

Although these data point to anti-Ro/SSA-antibodies as a novel cause of LQTS/TdP, current evidence derives only from basic experimental studies and clinical investigations involving relatively small samples of patients. Moreover, no population data are presently available on the percentage of carriers of anti-Ro/SSA who actually manifest the LQTS and thereby are at increased risk of TdP and SCD when other QT-prolonging risk factors are concomitantly present.

In order to address this gap in knowledge, we undertook an observational cohort study using US Veterans Affairs Informatics and Computing Infrastructure to estimate the risk of QTc prolongation associated with the presence of anti-Ro/SSA-antibodies in a large population of unselected subjects.

## METHODS

The authors declare that all supporting data are available within the article (and its supplementary files).

### Study Design and Setting

This is a retrospective cross-sectional study using Veterans Affairs Informatics and Computing Infrastructure, which provides a secure, central analytic platform for performing research using data collected from veterans at 1400 points of care. Veterans Affairs Informatics and Computing Infrastructure Clinical Data Warehouse, which is a relational database, was interrogated using structured query language. Logical Observation Identifiers Names and Codes were used to search for all patients who have been tested for anti-Ro/SSA-antibodies and who had at least 1 ECG from October 1999 until December 2016. Using a unique patient identifier, we obtained the following data: demographic data, laboratory data including anti-Ro/SSA status, electrolytes, thyrotropin, C-reactive protein, medications, diagnoses, and ECG. QTc measurement was automated using the Bazett's formula ( $QT/RR$  interval<sup>1/2</sup>) and confirmed by a cardiologist who was independent of the study and blinded to anti-Ro/SSA status. Patient-related QTc and laboratory values were computed by averaging multiple available measurements (Table S1).

### Participants

We included all subjects who were tested for anti-Ro/SSA-antibodies and underwent ECG. Exclusion criteria were complete bundle branch block (QRS interval >120 ms), ventricular paced rhythm, atrial fibrillation or flutter, and profound brady- and tachycardia (<50 bpm and >120 bpm, respectively).

## Anti-Ro/SSA-Antibody Status

The presence of total circulating anti-Ro/SSA-antibodies was assessed by an immune-enzymatic method or by immunoblotting. In the large majority of the cases, the results were provided qualitatively (positive/negative), whereas a quantitative result was available in only few subjects (4%). Moreover, the cutoff level used was not always the same, depending on the single point of care. For this reason, the subjects were classified in a categorical manner as anti-Ro/SSA-positive or -negative based on the specific reference cutoff used for each anti-Ro/SSA determination.

## Study Objectives

The primary study objective was to estimate the risk of QTc prolongation associated with circulating anti-Ro/SSA-antibodies. The secondary study objective was to investigate the synergy between anti-Ro/SSA-antibodies and other concomitant risk factors in prolonging QTc.

## Data Analyses

The definition of QTc prolongation was based on current recommendations by the American Heart Association/American College of Cardiology. In particular, we employed 3 different, progressively more stringent cutoffs: (1) >470 ms in men or >480 in women, representing ≈99th percentile QTc values for otherwise healthy postpubertal individuals (American Heart Association/American College of Cardiology: *abnormal QTc prolongation*); (2) >490 ms; and (3) >500 ms, representing a marker of significant arrhythmic risk for both sexes (American Heart Association/American College of Cardiology: *marked or highly abnormal QTc prolongation*).<sup>2</sup>

## Statistical Analysis

Descriptive statistics were computed, including mean and SD for quantitative variables, frequency count and percentage for qualitative variables. Kolmogorov-Smirnov test was applied to verify normality distribution of the quantitative variables, that is, age, electrolytes (potassium, calcium, magnesium), thyrotropin, and C-reactive protein. When normality was accepted/refused, Student *t* or Mann-Whitney tests were respectively used to compare groups.

Fisher exact test was applied to 2×2 contingency tables to evaluate the association between frequencies of qualitative variables; Mantel-Hantzel chi-square test for trend was used for the association of dichotomous and ordinal variables (2×*n* tables). When the association was between a binary risk factor and a long QTc condition (identified by a QTc

cutoff value), the odds ratio (OR) and its 95% CI were also evaluated.

Multivariate stepwise logistic regression analysis was carried out to identify, among all risk factors included in the model (independent variables), a statistically significant minimum subset of factors with the highest possible accuracy to predict QTc interval. In the stepwise process, 1 independent variable was added to or removed from the discriminant model at each step based on the criterion of maximum likelihood ratio. The process stops when no more statistical significant variables can be entered or removed. OR was calculated for significant variables. Statistical significance of OR was assessed by evaluating the 95% CI of sample estimates. To minimize the impact of confounding factors on the relationship between anti-Ro/SSA-positivity and QTc prolongation, a subgroup of the overall cohort was selected by the nearest neighbor matching procedure based on propensity score. Specifically, the variables used for propensity score calculation were age, sex, potassium, calcium, history of tobacco use, diabetes mellitus, ischemic heart disease (IHD), anesthetics, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, H<sub>2</sub>-histamine receptor antagonists, inotropes, proton pump inhibitors, and vasoconstrictors. As a result, for each subject who was anti-Ro/SSA-positive, 3 subjects who were anti-Ro/SSA-negative were selected, in order to obtain a significant sample size and at the same time maintain satisfactory patient matching. A balance check of the matched cohorts was performed by statistically evaluating for each variable the significance of the difference, as well as the changes in the standardized mean difference before and after matching. A statistical significance level of 95% ( $P < 0.05$ ) was always considered. SPSS software, release 10, was used for all statistical computations.

## Ethics

The data were de-identified and the study was approved by the Veterans Affairs New York Harbor Healthcare System Institutional Review Board under ID#01443.

## RESULTS

### Characteristics of the Overall Cohort

We found 8522 subjects in the database who were tested for anti-Ro/SSA status and underwent ECG. However, 1183 were excluded for the presence of complete bundle branch block, ventricular paced rhythm, profound brady- or tachycardia, or atrial fibrillation/flutter patterns. As a result, 7339 subjects were eventually included in the study, with a mean age of

61.4±12.2 years. Most of them were male (79.1%), and White (69.3%). In the whole population, 612 individuals were anti-Ro/SSA-positive (8.3%), and 6727 tested negative (Table 1). Depending on the specific cutoff used, QTc prolongation was found in 6.5% (QTc >470 [men]/480 [women] ms; n=479), 2.3% (QTc >490 ms; n=170), and 1.3% (QTc >500 ms; n=96) of subjects, respectively (Table 1).

In the univariate analysis, a number of variables were found to be consistently associated throughout the different cutoffs with an increased risk of QTc prolongation in the whole population, including demographic factors (age, sex), laboratory data (anti-Ro/SSA positivity, potassium, calcium, thyrotropin, C-reactive protein, cardiovascular risk factors/diseases (history of tobacco use, diabetes mellitus, IHD, and drugs [anesthetics, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, H<sub>2</sub>-histamine receptor antagonists, inotropes, proton pump inhibitors, and vasoconstrictors]) (data not shown). Notably, although mean QTc values were higher in the presence versus absence of each risk factor, in all cases they were within the normal range (ie, <470 ms men/480 ms women) (Table S2), thereby in some way supporting the view that no one factor alone is able to prolong QTc interval over limits (multihit theory).<sup>7</sup>

### Association Between Anti-Ro/SSA Positivity and QTc: Univariate Analysis

Empirical distribution of QTc values by the presence or absence of anti-Ro/SSA-antibodies is presented in the Figure 1. Subjects who were anti-Ro/SSA-positive showed a significantly longer mean QTc than individuals who were anti-Ro/SSA-negative (436.8±28.2 versus 430.8±25.6 ms;  $P < 0.001$ ), a difference persisting when the subjects were analyzed by race (Table 2). Although within the range of normal, mean values of QTc in subjects who were anti-Ro/SSA-positive were comparable or higher than those observed in the presence of most of the other QT-prolonging risk factors (including several well-recognized factors, such as older age, female sex, hypokalemia, hypocalcemia, diabetes mellitus, IHD, use of antibiotics, antidepressants, antihistaminics, antipsychotics, diuretics, opiates, diuretics, antiemetics) (Table S2). Subjects who were anti-Ro/SSA-positive were at higher risk of presenting with QTc prolongation, which gradually increased as the cutoff used was progressively more stringent. Specifically, in the group who were anti-Ro/SSA-positive the absolute prevalence of QTc prolongation ranged from 10% (versus 6.2% in the group who were negative;  $P = 0.001$ ) for abnormal QTc prolongation (>470 [men]/480 [women] ms), to 3.1%



**Table 1. Demographic and Health Characteristics of the Overall Cohort**

Characteristic	
Total, n	7339
Demography	
Age, y	61.4±12.2
Men, n	5803 (79.1%)
White, n	5084 (69.3%)
Black, n	1277 (17.4%)
Others/unknown, n	978 (13.3%)
Body mass index	29.5±6.2
ECG findings	
QTc, ms	431.3±25.9
QTc >470 men/480 women	479 (6.5%)
QTc >490 ms, n	170 (2.3%)
QTc >500 ms, n	96 (1.3%)
Laboratory data	
Anti-Sjögren's syndrome-related antigen A-antibodies positive	612 (8.3%)
Potassium, mEq/L	4.2±0.3
Calcium, mg/dL	9.3±0.4
Magnesium, mg/dL	2.0±0.2
Thyrotropin, µUI/L	2.4±4.3
C-reactive protein, mg/L	13.0±32.3
Cardiovascular risk factors/diseases	
History of tobacco use, n	518 (7.1%)
Hypertension, n	405 (5.5%)
Diabetes mellitus, n	2100 (28.6%)
Ischemic heart disease, n	1218 (16.6%)
Drugs	
Anesthetics, n	85 (1.2%)
Antiarrhythmics, n	82 (1.1%)
Antibiotics, n	3893 (53.0%)
Antidepressants, n	3870 (52.7%)
Antiemetics, n	1449 (19.7%)
Antifungals, n	1362 (18.6%)
Antihistaminics, n	2160 (29.4%)
Antimalarials, n	718 (9.8%)
Antimaniacs, n	110 (1.5%)
Antipsychotics, n	1630 (22.2%)
Bronchodilators, n	2617 (35.7%)
Cholinesterase inhibitors, n	182 (2.5%)
Diuretics, n	2918 (39.8%)
H <sub>2</sub> -histamine receptor antagonists, n	102 (1.4%)
Immunosuppressants, n	145 (2.0%)
Inotropes, n	32 (0.4%)
Opiates, n	358 (4.9%)
Proton pump inhibitors, n	1322 (18.0%)
Phosphodiesterase inhibitors, n	1248 (17.0%)
Vasoconstrictors, n	183 (2.5%)

For continuous variables, the data are expressed as mean±SD. For categorical variables, the data are expressed as n (%). QTc indicates heart rate-corrected QT interval.

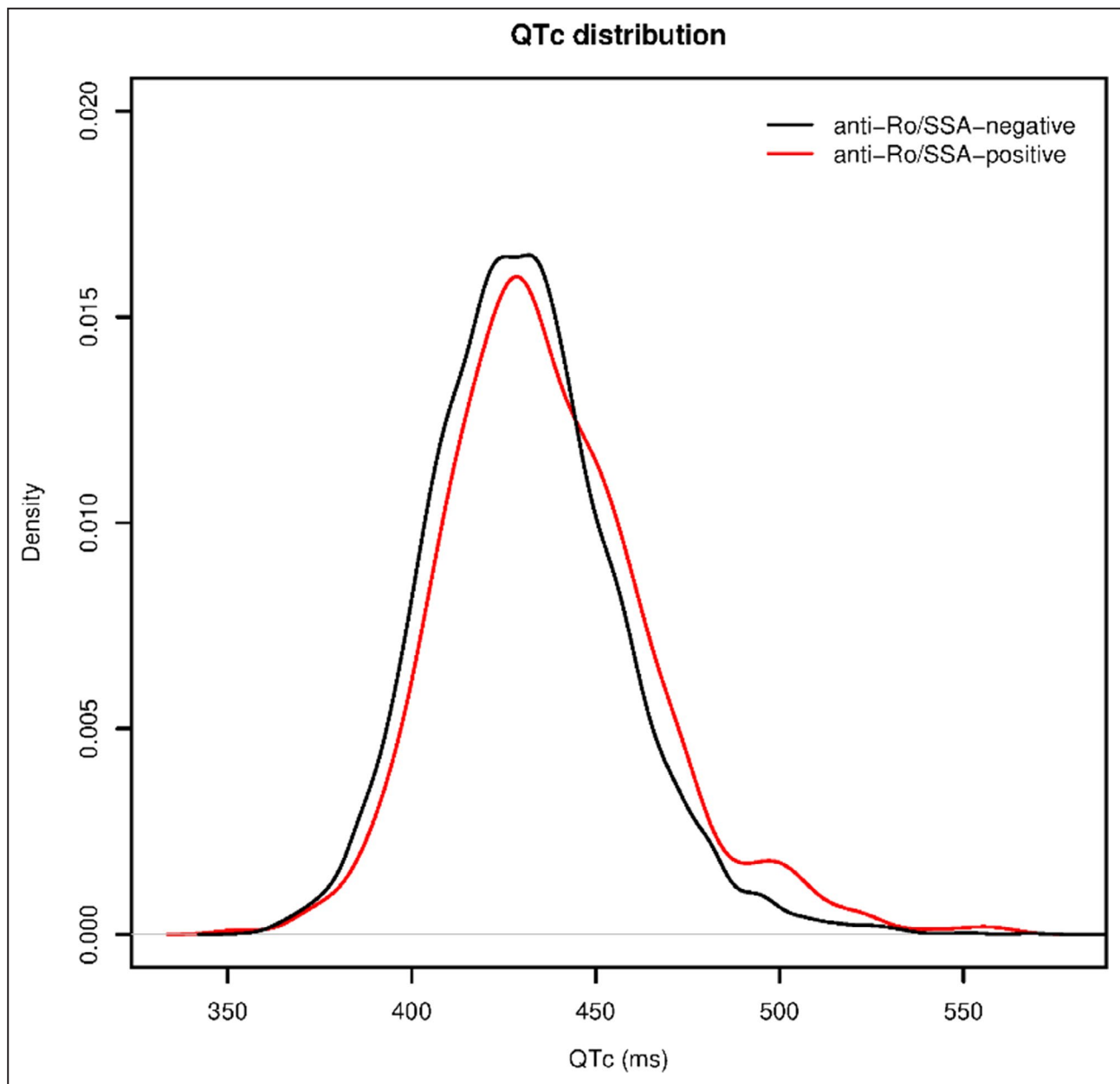
(versus 1.1%;  $P<0.001$ ), for highly abnormal QTc prolongation (>500 ms). The crude ORs in subjects who were anti-Ro/SSA-positive versus -negative were 1.67 (1.26–2.21) for QTc >470/480 ms, 2.32 (1.54–3.49) for QTc >490 ms, and 2.77 (1.66–4.60) for QTc >500 ms, respectively (Table 2). Notably, a significant increase in the OR was already present for lower cut-offs (440–460 ms); however, the OR value remained substantially the same ( $\approx 1.5$ ) until the 470/480 ms cutoff, then showing a sharp rise from 490 ms onwards (Figure S1). Accordingly, a parallel increase in the percentage of individuals who were anti-Ro/SSA-positive was observed in the groups with prolonged QTc, until reaching a value  $\approx 20\%$  among subjects with highly abnormal QTc prolongation (whole population: 8.3%; >470/480 ms: 12.7%; >490 ms: 17.1%; >500 ms: 19.8%;  $P<0.001$ , chi-square test for trend).

### QT-Prolonging Risk Factors in Subjects Who Were Anti-Ro/SSA-Positive Versus -Negative

In subjects who were anti-Ro/SSA-positive, the global burden of QT-prolonging risk factors was significantly increased when compared with those who were anti-Ro/SSA-negative. Subjects who were anti-Ro/SSA-positive were older and more frequently females; had lower potassium and calcium levels; were more frequently affected with IHD and treated with drugs with QT-prolonging potential (specifically, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, and vasoconstrictors), when compared with subjects who were negative (Table 3).

### Relationship Between Anti-Ro/SSA Positivity, History of Connective Tissue Disease, and QTc

In our veteran population, a history of CTD, including systemic lupus erythematosus, discoid lupus, Sjögren syndrome, systemic sclerosis, idiopathic inflammatory myositis, mixed CTD, undifferentiated CTD, and rheumatoid arthritis, was present in 993 subjects (13.5%). As expected, it was more frequently found in subjects who were anti-Ro/SSA-positive (288/612; 47.1%) than anti-Ro/SSA-negative (705/6727: 10.5%), with an OR >7 (Table S3). However, a positive history for CTD was not a necessary condition for the occurrence of anti-Ro/SSA-associated QTc prolongation. In fact, when individuals who were anti-Ro/SSA-positive without history of CTD (ie,  $\approx 50\%$  of cases) were selectively considered, the mean QTc and frequencies of QTc prolongation persisted substantially unmodified and



**Figure.** Empirical distribution of QTc values in the overall cohort.

Anti-Ro/SSA-antibodies indicates anti-Sjögren's syndrome-related antigen A-antibodies; and QTc, heart rate-corrected QT interval.

significantly higher with respect to subjects who were anti-Ro/SSA-negative (Table S4). Accordingly, the percentage of subjects who were anti-Ro/SSA-positive without CTD remained stable around 50% throughout the different cutoffs (whole population: 52.9%; >470/480 ms: 52.5%; >490 ms: 51.7%; >500 ms: 47.4%;  $P=0.97$ , chi-square test for trend).

There was a trend, although not statistically significant, for an increase in QTc values in subjects who were anti-Ro/SSA-positive with CTD when compared with those who were anti-Ro/SSA-positive but without CTD (Table S3). In this regard, it is important to note that subjects who were anti-Ro/SSA-positive with CTD

showed a higher burden of QT-prolonging risk factors than anti-Ro/SSA-positive without CTD, specifically an increased prevalence of female sex and a more frequent use of some medications (ie, antibiotics, antidepressants, antihistaminics, antimalarials, diuretics, proton pump inhibitors) (Table S5).

To further improve the confidence that anti-Ro/SSA-antibodies are the cause of QTc prolongation, rather than merely serving as a surrogate marker of an autoimmune state, we evaluated the impact of 7 other autoantibodies (all well recognized to be associated with CTDs, ie, antibodies anti-cyclic citrullinated peptide, anti-centromere, anti-double stranded DNA,

**Table 2. Mean QTc Values and QTc Prolongation Frequencies in Anti-Ro/SSA-Positive (n=612) Versus Anti-Ro/SSA-Negative (n=6727) Subjects of the Study Population**

QTc	n	Positive	n	Negative	Odds Ratio (95% CI)	P Value
Mean values, ms						
All subjects	612	436.8±28.2	6727	430.8±25.6	...	<0.001*
White	390	437.1±28.4	4694	430.5±25.4	...	<0.001*
Black	41	437.3±28.6	1236	431.0±26.0	...	0.010*
QTc prolongation frequencies, %						
QTc >470 ms Men/480 ms women	612	10.0	6727	6.2	1.67 (1.26–2.21)	0.001*
QTc >490 ms	612	4.7	6727	2.1	2.32 (1.54–3.49)	<0.001*
QTc >500 ms	612	3.1	6727	1.1	2.77 (1.66–4.60)	<0.001*

For continuous variables, the data are expressed as mean±SD. For categorical variables, the data are expressed as n (%). Differences in quantitative variables were evaluated by the 2-tail Mann-Whitney test or unpaired *t* test. Differences in categorical variables were evaluated by the 2-sided Fisher's exact test. Anti-Ro/SSA indicates anti-Sjögren's syndrome-related antigen A-antibodies; Negative, anti-Ro/SSA-negative subjects; Positive, anti-Ro/SSA-positive subjects; and QTc, heart rate-corrected QT interval.

\**P*<0.05.

anti-Sjögren syndrome-related antigen B, anti-ribonucleoprotein, anti-topoisomerase-I, and anti-Smith)<sup>28</sup> on the prevalence of QTc prolongation in the population who were anti-Ro/SSA-negative. As shown in Table S6, none of these autoantibodies showed any significant association with the risk of QTc prolongation, regardless of the specific cutoff used.

### Association Between Anti-Ro/SSA Positivity and QTc Prolongation: Multivariate Analysis

The role of anti-Ro/SSA as a risk factor for long QTc was evaluated using a multivariate logistic regression analysis by gradually adding potential adjustment or confounding risk factors. Such factors were selected by means of the aforementioned preliminary analyses, sequentially performed to identify (1) which variables were crudely associated in the whole population with a significant risk of QTc prolongation, throughout different cutoffs, and then (2) which among these selected variables were also significantly different when populations who were anti-Ro/SSA-positive versus -negative were specifically compared. The variables selected by the first step were age, sex, potassium, calcium, thyrotropin, C-reactive protein, history of tobacco use, diabetes mellitus, IHD anesthetics, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, H<sub>2</sub>-histamine receptor antagonists, inotropes, proton pump inhibitors, and vasoconstrictors. Among these, the variables selected by the second step were age, sex, potassium, calcium, IHD antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors.

Based on this final set of selected variables, the following multivariate models were performed:

1. Model 1: anti-Ro/SSA, age (as continuous variable), and sex;
2. Model 2: model 1 plus electrolytes (potassium and calcium levels, as continuous variables);
3. Model 3: model 2 plus cardiovascular risk factors/diseases (IHD);
4. Model 4: model 3 plus use of any drug with QT-prolonging potential (including antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, and vasoconstrictors).

The association between anti-Ro/SSA and QTc prolongation was progressively attenuated by the 4 levels of adjustment, particularly when QT-prolonging drugs were added to the model (Tables 4 through 6). Nevertheless, fully adjusted OR for the highest cutoff remained significantly increased in subjects who were anti-Ro/SSA-positive. Particularly, OR for QTc >500 ms was 1.92 (1.02–3.62) (Table 6), indicating a ≈2-times higher risk of developing a highly abnormal QTc prolongation in the presence of circulating anti-Ro/SSA antibodies. The role of anti-Ro/SSA-positivity as an independent predictor of QTc prolongation was further supported and better characterized by additionally applying a stepwise method to the regression logistic analysis (in which all the variables selected by the aforementioned 2-step selection process were entered to identify a minimum subset of factors with the highest possible prediction accuracy for QTc prolongation). In fact, with this approach, we additionally demonstrated that the presence of QTc prolongation >490 and >500 ms was explained by only a few variables (ie, age, calcium, potassium, anti-Ro/

**Table 3. QTc Prolonging Risk Factors in Anti-Ro/SSA-Positive (n=612) Versus Anti-Ro/SSA-Negative (n=6727) Subjects of the Study Population**

Risk factor	n	Positive	n	Negative	Odds Ratio (95% CI)	P Value
Quantitative variables						
Age	606	63.5±11.8 y	6654	61.2±12.2 y	1.016 (1.009–1.023)	<0.001*
Potassium	611	4.1±0.3 mEq/L	6701	4.1±0.3 mEq/L	0.681 (0.510–0.909)	0.023*
Calcium	602	9.2±0.4 mg/dL	6438	9.3±0.4 mg/dL	0.435 (0.352–0.536)	<0.001*
Thyrotropin	550	2.6±3.4 µIU/mL	5783	2.4±4.4 µIU/mL	1.009 (0.994–1.024)	0.22
C-reactive protein	154	12.5±29.1 mg/L	1176	13.0±32.7 mg/L	0.999 (0.994–1.005)	0.83
Categorical variables						
Demographic factors						
Female sex	612	26.3%	6727	20.4%	1.39 (1.15–1.68)	0.001*
Cardiovascular risk factors/diseases						
Smoking	612	8.8%	6727	7.7%	1.16 (0.87–1.56)	0.31
Diabetes mellitus	612	29.1%	6727	26.8%	1.12 (0.93–1.34)	0.24
Ischemic heart disease	612	24.7%	6727	15.9%	1.74 (0.43–2.11)	<0.001*
Drugs						
Anesthetics	612	1.6%	6727	1.1%	1.47 (0.76–2.86)	0.23
Antiarrhythmics	612	5.1%	6727	0.8%	6.98 (4.43–11.0)	<0.001*
Antibiotics	612	62.9%	6727	52.1%	1.56 (1.31–1.85)	<0.001*
Antidepressants	612	62.4%	6727	51.9%	1.54 (1.30–1.83)	<0.001*
Antiemetics	612	29.1%	6727	18.9%	1.76 (1.46–2.12)	<0.001*
Antihistaminics	612	40.8%	6727	28.4%	1.74 (1.47–2.06)	<0.001*
Antimalarials	612	48.9%	6727	6.2%	14.38 (11.93–17.33)	<0.001*
Antipsychotics	612	32.0%	6727	21.3%	1.74 (1.45–2.08)	<0.001*
Bronchodilators	612	48.7%	6727	34.5%	1.80 (1.53–2.13)	<0.001*
Cholinesterase inhibitors	612	4.2%	6727	2.3%	1.87 (1.22–2.85)	0.006*
Diuretics	612	44.4%	6727	39.3%	1.23 (1.04–1.45)	0.014*
H <sub>2</sub> -histamine receptor antagonists	612	1.1%	6727	1.4%	0.81 (0.37–1.75)	0.71
Inotropes	612	1.0%	6727	0.4%	2.55 (1.05–6.22)	0.046*
Opiates	612	4.9%	6727	4.9%	1.01 (0.68–1.47)	0.92
Proton pump inhibitors	612	22.4%	6727	17.6%	1.35 (1.10–1.65)	0.004*
Vasoconstrictors	612	1.8%	6727	0.7%	2.66 (1.37–5.16)	0.007*

For continuous variables, the data are expressed as mean±SD. For categorical variables, the data are expressed as n (%). Differences in quantitative variables were evaluated by the 2-tail Mann-Whitney test or unpaired *t* test. Difference in categorical variables were evaluated by the 2-sided Fisher's exact test. Anti-Ro/SSA indicates anti-Sjögren's syndrome-related antigen A-antibodies; Negative, anti-Ro/SSA-negative subjects; Positive, anti-Ro/SSA-positive subjects; and QTc, heart rate-corrected QT interval.

\**P*<0.05.

SSA, diuretic use) among which anti-Ro/SSA positivity was in both cases significantly included (>500 ms; OR 2.27 [1.34–3.87]) (Table 7; Figure S2), thereby pointing to circulating anti-Ro/SSA-antibodies as one of the most important risk factors for marked QTc prolongation in this population.

To further support these findings, several additional sensitivity analyses were conducted. A first sensitivity analysis was performed on a subgroup of 2384 subjects selected by nearest neighbor matching procedure, in which the impact of confounding factors was minimized. As shown in Table S7, this population included 596 subjects who were anti-Ro/

SSA-positive and 1788 anti-Ro/SSA-negative, strictly matched for all QT-prolonging risk factors, except for antiarrhythmic and antimalarial use (because of the small sample size of treated subjects and clinical considerations<sup>29</sup>; more details are provided in the Online-Data S1). Also in this case, stepwise multivariate logistic regression analysis (taking into account all variables, including antiarrhythmic and antimalarial use) confirmed anti-Ro/SSA-positivity as an independent predictor of QTc prolongation >500 (OR 2.18 [1.18–4.02]), along with age and calcium levels (Table S8). Notably, the evidence that both antimalarials and antiarrhythmics were removed during the



**Table 4. Multivariate Logistic Regression Analysis Showing the Odds Ratio of Anti-Ro/SSA-Positivity for QTc Interval Prolongation >470 ms Men/480 ms Women, After Adjustment for Confounders**

Adjustment Factors	n	Odds Ratio	95% CI	P Value
None	7339	1.67	1.26–2.21	0.001*
Age, sex	7260	1.58	1.18–2.12	0.002*
Age, sex, electrolytes <sup>†</sup>	6949	1.40	1.04–1.88	0.025*
Age, sex, electrolytes <sup>†</sup> , cardiovascular risk factors/diseases <sup>‡</sup>	6949	1.35	1.00–1.81	0.049*
Age, sex, electrolytes <sup>†</sup> , cardiovascular risk factors/diseases <sup>‡</sup> , Drugs <sup>§</sup>	6949	1.06	0.75–1.51	0.73

Anti-Ro/SSA indicates anti-Sjögren's syndrome-related antigen A-antibodies; and QTc, heart rate-corrected QT interval.

\* $P < 0.05$ .

<sup>†</sup>Including potassium, calcium.

<sup>‡</sup>Including ischemic heart disease.

<sup>§</sup>Including antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors.

stepwise analysis process ruled out a significant influence of these variables on the relationship between anti-Ro/SSA-antibodies and QTc prolongation. As an additional confirmation, by stratifying these subjects according the antiarrhythmics and antimalarials use, we found that even in the absence of these drugs subjects who were anti-Ro/SSA-positive showed a prevalence of QTc >500 ms significantly higher than in those who were anti-Ro/SSA-negative (antiarrhythmic untreated subjects,  $n=2308$ : OR 2.04 [1.03–3.94],  $P < 0.05$ ; antimalarial untreated subjects,  $n=1953$ : OR 2.26 [1.00–4.79],  $P < 0.05$ ).

**Table 5. Multivariate Logistic Regression Analysis Showing the Odds Ratio of Anti-Ro/SSA-Positivity for QTc Interval Prolongation >490 ms, After Adjustment for Confounders**

Adjustment Factors	n	Odds Ratio	95% CI	P Value
None	7339	2.32	1.54–3.49	<0.001*
Age, sex	7260	2.09	1.37–3.17	0.001*
Age, sex, electrolytes <sup>†</sup>	6949	1.85	1.20–2.83	0.005*
Age, sex, electrolytes <sup>†</sup> , cardiovascular risk factors/diseases <sup>‡</sup>	6949	1.77	1.15–2.72	0.009*
Age, sex, electrolytes <sup>†</sup> , cardiovascular risk factors/diseases <sup>‡</sup> , drugs <sup>§</sup>	6949	1.63	0.98–2.70	0.060 <sup>  </sup>

Anti-Ro/SSA indicates anti-Sjögren's syndrome-related antigen A-antibodies; and QTc, heart rate-corrected QT interval.

\* $P < 0.05$ .

<sup>†</sup>Including potassium, calcium.

<sup>‡</sup>Including ischemic heart disease.

<sup>§</sup>Including antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors.

<sup>||</sup> $P < 0.07$

Moreover, a further sensitivity analysis was conducted to evaluate the impact of confounding factors on anti-Ro/SSA-associated QTc prolongation by comparing the mean QTc and the prevalence of QTc prolongation with different cutoffs in patients who were anti-Ro/SSA-positive with or without a specific confounding factors versus subjects who were anti-Ro/SSA negative. Again, an increase in QTc parameters with respect to subjects who were anti-Ro/SSA-negative persisted in all cases when subjects who were anti-Ro/SSA-positive without the specific risk factor were considered, in most cases the differences remained statistically significant (16/19, 84%, in 1 or more parameters) (Table S9).

Furthermore, 2 additional sensitivity analyses were performed to exclude that temporality, that is, the fact that ECG acquisition, anti-Ro/SSA testing, and biochemical parameters measured had not been always performed at the same time point may have biased our results. First, by analyzing the whole cohort of the 612 patients who were anti-Ro/SSA-positive, we found that in most cases anti-Ro/SSA test preceded ECG ( $n=494$ , Ro-PRE group, ie, the group in which ECG was taken after anti-Ro/SSA-antibodies were discovered); whereas in 72 subjects the 2 examinations were concomitant (Ro-CC group, ie, the group in which ECG and anti-Ro/SSA testing were performed at the same time), and in only 46 the anti-Ro/SSA testing followed the ECG (Ro-POST group, that is, the group in which ECG was taken before anti-Ro/SSA-antibodies were discovered). Interestingly, the mean QTc found in the Ro-PRE and Ro-CC groups were very similar ( $437.5 \pm 28.6$  and  $436.1 \pm 23.8$ , respectively) and overlapping that found in the whole population who were anti-Ro/SSA-positive ( $436.8 \pm 28.2$ ; Table 2). Conversely, in the Ro-POST group (in which there was an increased risk of including subjects underwent ECG when circulating anti-Ro/SSA antibodies were still not present) the mean QTc was significantly shorter ( $430.7 \pm 29.9$ ), and overlapping that observed in the whole population who were anti-Ro/SSA-negative ( $430.8 \pm 25.6$ ; Table 2). Based on these findings, stepwise multivariate logistic regression analysis was repeated after excluding the Ro-POST group subjects, without any substantial change in the ORs for anti-Ro/SSA (>490: 1.93 [1.23–2.93]; >500: 2.27 [1.28–3.82]).

Moreover, the impact of temporality of electrolyte measurements on ECG findings was also evaluated. Given that, by nature, their influence on QTc is transient, we focused on the subjects in whom ECG and electrolytes were evaluated concomitantly. In this group, including the majority of subjects (potassium:  $n=5432$ ; calcium:  $n=4873$ ), the mean QTc in the presence or absence of the risk factor (ie, hypokalemia or hypocalcemia, respectively) (Table S10) was similar to that found in the whole population, thereby providing

**Table 6. Multivariate Logistic Regression Analysis Showing the Odds Ratio of Anti-Ro/SSA Positivity for QTc Interval Prolongation >500 ms, After Adjustment for Confounders**

Adjustment Factors	n	Odds Ratio	95% CI	P Value
None	7339	2.77	1.66–4.60	<0.001*
Age, sex	7260	2.48	1.46–4.20	0.001*
Age, sex, electrolytes <sup>†</sup>	6949	2.19	1.28–3.73	0.004*
Age, sex, electrolytes <sup>†</sup> , cardiovascular risk factors/diseases <sup>‡</sup>	6949	2.09	1.22–3.57	0.007*
Age, sex, electrolytes <sup>†</sup> , cardiovascular risk factors/diseases <sup>‡</sup> , drugs <sup>§</sup>	6949	1.92	1.02–3.62	0.045*

Anti-Ro/SSA indicates anti-Sjögren's syndrome-related antigen A-antibodies; and QTc, heart rate-corrected QT interval.

\* $P < 0.05$ .

<sup>†</sup>Including potassium, calcium.

<sup>‡</sup>Including ischemic heart disease.

<sup>§</sup>Including antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors.

evidence that temporality of electrolyte measurements did not significantly bias the results of our study.

### Synergy Between Anti-Ro/SSA Positivity and Other Concomitant QT-Prolonging Risk Factors

A single QT-prolonging risk factor per se is frequently not able to induce significant QTc prolongation. To evaluate whether circulating anti-Ro/SSA antibodies can synergistically act with other concomitant risk factors to prolong QTc, we compared mean QTc values in subjects who were anti-Ro/SSA-positive versus -negative in the presence of various recognized QT-prolonging risk factors. We found that for any of the 15 risk factors analysed with a sample size of at least 50 subjects for each category, subjects who were

anti-Ro/SSA-positive showed an increased QTc value and/or QTc prolongation prevalence when compared with the subjects who were anti-Ro/SSA-negative, in most cases such a difference being statistically significant (10/15) (Table S9).

## DISCUSSION

The key findings of the present study are the following: (1) in a large cohort of US veterans, subjects who were anti-Ro/SSA-positive, independent of a history of CTD, showed an increased prevalence of QTc prolongation, in the presence of other concomitant risk factors; (2) circulating anti-Ro/SSA-antibodies were independently associated with a  $\approx 2$ -times higher risk of marked QTc prolongation; (3) anti-Ro/SSA positivity was a significant contributor to marked QTc prolongation occurrence, even representing one of the most important risk factors involved; and (4) a synergy between anti-Ro/SSA-antibodies and most of the traditional risk factors was demonstrated. Altogether, our study provides evidence that anti-Ro/SSA positivity is a strong and independent risk factor for marked QTc prolongation in a large cohort of US veterans. These data suggest that within the general population subjects who were anti-Ro/SSA-positive may represent a subgroup with an increased predisposition to life-threatening VAs, particularly when other QT-prolonging risk factors are concomitantly present.

Autoimmune cardiac channelopathies represent a recently recognized novel mechanism for cardiac arrhythmias.<sup>8</sup> In fact, the existence of a number of arrhythmogenic autoantibodies cross-reacting with specific cardiac ion channels involved in action potential genesis has been demonstrated. Specifically, by targeting calcium, potassium, or sodium channels, these autoantibodies lead to significant electrophysiological changes promoting conduction disturbances

**Table 7. Demographic, Clinical, Laboratory, and Therapeutic Variables Independently Associated With QTc Interval Prolongation >490 and >500 ms in the Study Population: A Stepwise Multivariate Logistic Regression Analysis**

Dependent Variable	Independent Variables	Model Coefficients ( $\beta$ )	Odds Ratio (95% CI)	P Value
QTc >490 ms	Age	0.052	1.05 (1.04–1.07)	<0.001*
	Calcium	–0.804	0.45 (0.31–0.66)	<0.001*
	Diuretics	0.623	1.86 (1.34–2.66)	<0.001*
	Anti-Ro/SSA	0.639	1.90 (1.24–2.90)	0.003*
	Potassium	–0.755	0.47 (0.27–0.83)	0.009*
QTc >500 ms	Age	0.044	1.05 (1.03–1.07)	<0.001*
	Calcium	–0.903	0.41 (0.25–0.67)	<0.001*
	Anti-Ro/SSA	0.821	2.27 (1.34–3.87)	0.002*
	Diuretics	0.539	1.71 (1.11–2.64)	0.015*

Variables entered in the multivariate analysis include age, sex, potassium, calcium, ischemic heart disease, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors. Anti-Ro/SSA indicates anti-Sjögren's syndrome-related antigen A-antibodies; and QTc, heart rate-corrected QT interval.

\* $P < 0.05$ .

and/or life-threatening tachyarrhythmias, both in patients with manifest autoimmune diseases and in apparently healthy individuals.<sup>7,8,10</sup> Anti-Ro/SSA is the most investigated anti-ion channel antibody and its role in the pathogenesis of the congenital heart block via a molecular mimicry-driven interaction with calcium channels of the fetal conduction system is well established.<sup>8,30–32</sup> More recently, a growing body of evidence demonstrated that anti-Ro/SSA-antibodies can also target the hERG-potassium channel leading to a novel form of acquired LQTS of autoimmune origin.<sup>8,11</sup> Several clinical studies in adults and newborns provided evidence that subjects who were anti-Ro/SSA-positive frequently show a prolonged QTc,<sup>14–19</sup> whose length correlates with circulating autoantibody levels<sup>16,17,33</sup> and incidence of complex VAs,<sup>19,20</sup> including TdP.<sup>22,27</sup> From a molecular point of view, we established that purified IgG/anti-Ro/SSA-52 kDa from patients who were antibody positive with LQTS/TdP acutely inhibited the rapid component of the delayed rectifier potassium current in human embryonic kidney-293 cells and guinea-pig ventricular myocytes.<sup>11,22</sup> Such effects, found in both subjects with LQTS with CTD and in patients with TdP without any sign of autoimmune disease, were a result of a specific interaction of the autoantibody with the extracellular loop of the hERG-potassium channel, between segments S5 and S6 of the pore-forming subunit, where a significant homology with the Ro/SSA-52 kDa antigen is present.<sup>11,22</sup> Other authors demonstrated that this region is also critical for the occurrence of the chronic effects of anti-Ro/SSA-52 kDa on the channel, that is, reduced expression by triggering channel internalization.<sup>12,27</sup> Finally, we provided evidence that immunization of adult guinea-pigs with the Ro/SSA-52 kDa antigens reproduced QTc prolongation in these animals, because of the autoantibody-mediated prolonging effect on ventricular action potential duration via the rapid component of the delayed rectifier potassium current inhibition.<sup>11</sup> Although these data strongly support the role of anti-Ro/SSA as a novel, nontraditional, risk factor for LQTS/TdP in patients with CTD, information regarding its actual clinical impact on the general population is substantially missing as only studies on relatively small groups of selected patients are currently available.<sup>14–19,22,27,33</sup>

In the present study, we provide evidence for the first time that in a large population of over 7000 US veterans, including 612 with circulating anti-Ro/SSA, subjects who were autoantibody positive showed a longer mean QTc duration (+6.0 ms) along with a higher prevalence of QTc prolongation when compared with subjects who were anti-Ro/SSA-negative. In the univariate analysis, the greater the QTc cutoff used, the greater was the estimated OR for QTc prolongation, with an OR close to 3 for QTc >500 ms. By progressively setting a higher QTc cutoff, a parallel rise in the percentage of

subjects who were anti-Ro/SSA-positive among those with QTc prolongation was observed. In the highest cutoff of QTc >500 ms,  $\approx 1$  in every 5 subjects with marked QTc prolongation (19.8%) showed circulating anti-Ro/SSA. Although these data are remarkable, it should be considered that in the group who were anti-Ro/SSA-positive a significantly higher burden of QT-prolonging risk factors was found. It is well known that anti-Ro/SSA-antibodies are strongly associated with the presence of CTDs,<sup>13</sup> frequently characterized by a multisystemic involvement, cardiovascular (particularly IHD) and noncardiovascular,<sup>34</sup> leading to an increased probability of needing medications, including QT-prolonging drugs. Accordingly, in our cohort, the prevalence of CTD in the group who were anti-Ro/SSA-positive was over 7-times higher than in those who were anti-Ro/SSA-negative. Moreover, among subjects who were anti-Ro/SSA-positive, those with a history of CTD were more frequently treated with antibiotics, antidepressants, antihistaminics, antimalarials, diuretics, and proton pump inhibitors when compared with those without. In addition, large population studies have demonstrated that the likelihood of presenting circulating anti-Ro/SSA is higher in women and in advanced age,<sup>23,25</sup> both also representing a recognized risk factor for QTc prolongation. As mentioned earlier, subjects who were anti-Ro/SSA-positive in our population were older and more frequently female when compared with those who were negative. The concomitant factors partly responsible for QTc prolongation in these subjects are likely synergistic with anti-Ro/SSA in promoting QTc prolongation. Indeed, according to the multihit theory,<sup>7</sup> more than 1 factor/hit is necessary in most cases to significantly disturb ventricular repolarization and induce a clinically measurable lengthening of QTc.<sup>7,35</sup> In this view, anti-Ro/SSA may intriguingly represent a risk factor for QTc prolongation both directly by inhibiting the hERG-potassium channel and indirectly by affecting the probability that other QT-prolonging risk factors are at the same time present in the individual subject.

The results obtained from the multivariable analysis robustly supported this hypothesis. In fact, although adjustments for concomitant risk factors progressively reduced the magnitude of the OR associated with the presence of circulating anti-Ro/SSA, a  $\approx 2$ -times increased risk of marked QTc prolongation was still observed in subjects who were anti-Ro/SSA-positive even after full adjustment. Moreover, quantitative assessment by stepwise analysis demonstrated that anti-Ro/SSA positivity was included in the small subset of critical variables explaining the occurrence of QTc prolongation >490 and >500 ms in our cohort. These findings provide evidence for a strong and independent role of anti-Ro/SSA in increasing the risk for having a prolonged QTc, thereby, confirming that the direct

electrophysiologic effects of these autoantibodies have a clinically relevant impact also in a large population of unselected individuals. The multivariate analysis also demonstrated how the anti-Ro/SSA-associated QT-prolonging potential was significantly enhanced when other traditional risk factors are present. By introducing age, sex, electrolytes, IHD, and medications step by step in the model, a parallel reduction in ORs for QTc prolongation was observed (even resulting in no significant independent effect for the 470/480 ms cut-off). Moreover, by specifically focusing on the bivariate interaction existing between anti-Ro/SSA and each of the other risk factors present in the US veteran population, we found that in most cases the anti-Ro/SSA positivity copresence with other risk factors was associated with additional and significant QT-prolonging effects.

Another important finding of the present study is that anti-Ro/SSA represents a risk factor for QTc prolongation in the US veteran population regardless of the presence or absence of a manifest CTD. In fact, a significant increase of both mean QTc duration and QTc prolongation prevalence was also observed in subjects who were anti-Ro/SSA-positive without a history of CTD when compared with those who were anti-Ro/SSA-negative. In addition, none of the other CTD-associated autoantibodies evaluated (as surrogate markers of an autoimmune disease state) showed any significant association with the QTc prolongation risk. This evidence further supports the view that anti-Ro/SSA-antibodies are per se able to promote LQTS by exerting direct electrophysiological effects on the heart, thereby suggesting that these autoantibodies are a risk factor for QTc prolongation in the whole population. Thus, the clinical implications extend not only to patients with clinical symptoms of CTD but more widely in all subjects showing anti-Ro/SSA positivity, even though asymptomatic. Notably, in accordance with a previous large population study,<sup>23</sup> the fraction of subjects who were anti-Ro/SSA-positive without CTD in our cohort were elevated,  $\approx 50\%$  of cases. The key implication of these results is that in the general population, a number of subjects who carry a concealed anti-Ro/SSA may be predisposed to QT-related malignant arrhythmias, and in whom only specific testing can reveal the presence of the risk factor. Concordant with this view is a study cohort of patients with TdP consecutively enrolled from the general population, more than a half showed circulating anti-Ro/SSA, and in all cases without any history of CTD.<sup>22</sup> Importantly, the number of subjects potentially exposed to this risk is not negligible. In fact, previous studies in the general population demonstrated that circulating anti-Ro/SSA are found in up to  $\approx 2.5\%$  of the cases,<sup>23,25</sup> a percentage who rise to  $\approx 4\%$  when people older than 50 years are specifically considered.<sup>23,25</sup> In our population, such

a proportion further increases to  $\approx 8\%$ , at least in part owing to the fact that US veterans are older, with a mean age  $>60$  years. Thus, it can be estimated that 1 individual of every  $\approx 40$  in the general population ( $\approx 15$  in elderly) may be anti-Ro/SSA-positive. In this scenario, our data suggest that 1 in every 5 older US veterans with marked QTc prolongation have circulating anti-Ro/SSA. This evidence provides strong support to the hypothesis that these autoantibodies might be silently involved in a number of unexplained arrhythmias or SCDs in the general population. To this end, specific autoantibody testing in patients with "idiopathic" rhythm disturbances could lead to novel treatment and prophylactic opportunities.<sup>8</sup>

The study has several limitations and strengths. First, our population includes US veterans who are predominantly male, White, and older, a fact that might in some way limit the generalizability of the results to a wider population. On the other hand, the use of national large-scale data, collected during routine medical care from a network of integrated health-care systems, significantly minimized the selection bias. Moreover, previous clinical and experimental data strongly suggest that the hERG-blocking activity of anti-Ro/SSA-antibodies is restricted to the anti-52 kDa subtype<sup>11,12,22</sup> and that relatively high antibody levels are necessary to promote QTc prolongation.<sup>33</sup> However, no adequate information is available in our cohort regarding the subtype specificities or plasma concentrations of anti-Ro/SSA-antibodies in subjects who were positive, and this may represent another limitation. Still, such limitation may conversely represent a strength of the study. In fact, based on the general population data demonstrating that among subjects who were anti-Ro/SSA-positive more than 40% tested negative for anti-Ro/SSA-52 kDa-antibodies,<sup>25</sup> it is expected that a significant number of subjects in our cohort labelled as anti-Ro/SSA-positive actually did not present the anti-52 kDa subtype. In addition, among those anti-Ro/SSA-52 kDa-positive, it is plausible that only a fraction had circulating antibody levels as high as necessary to exert a clinically relevant effect. Thus, the testing method used in our cohort probably underestimated the potential impact of anti-Ro/SSA-antibodies on QTc. The evidence that an independent association was found, further supports the hypothesis that the anti-Ro/SSA-associated QTc prolongation has a relevant impact in the clinical setting. Further population studies are warranted to expressly evaluate the impact of subtype specificity and concentration on QTc prolongation risk. Patients' selection modality and measurement temporality represent other potential limitations to highlight. In fact, the retrospective enrolment of patients in whom an anti-Ro/SSA test had been done (possibly because of a pre-existing clinical suspicion of CTD) may have increased



the probability that the test was positive, and that a CTD was present, when compared with that expected in the general population. However, as discussed previously, the percentage of subjects who were anti-Ro/SSA-positive reported by large studies in the elderly general population<sup>23,25</sup> is similar to what we found, suggesting a relatively small bias impact. Moreover, the influence of a possible higher CTD prevalence in our population has been minimized by the sensitivity analysis we performed demonstrating no significant differences in QTc parameters by comparing subjects who were anti-Ro/SSA-positive with or without a history of CTD. Regarding the temporal bias, it was attenuated by the fact that for each variable, for a specific patient, we used the mean value resulting from multiple measurements performed throughout the time points. Nevertheless, the sensitivity analyses focused on the temporal relationship between ECG execution and anti-Ro/SSA-antibody or electrolyte testing provided evidence that temporality of measurements did not significantly bias the results of our study.

In conclusion, anti-Ro/SSA positivity was independently associated with an increased risk of marked QTc prolongation in a large cohort of US veterans, regardless of the presence or absence of a manifest CTD. Our findings suggest that within the general population subjects with circulating anti-Ro/SSA may be predisposed to VAs/SCD. It is proposed that subjects who were anti-Ro/SSA-positive should receive counseling about drugs and management of other risk factors that may increase the risk for malignant arrhythmias. At the same time, specific anti-Ro/SSA testing should be considered in patients with “idiopathic” rhythm disturbances, as it could intriguingly open novel avenues in antiarrhythmic treatment, primarily immunomodulatory therapies.

## ARTICLE INFORMATION

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### Affiliations

From the Department of Medical Sciences, Surgery and Neurosciences (P.E.L., F.L.-P., P.L.C.) and Department of Medical Biotechnologies (G.C., A.C.), University of Siena, Italy; VA New York Harbor Healthcare System, SUNY Downstate Medical Center, New York, NY (Y.S.Q., F.F., N.E.-S., M.B., D.L.); Department of Cardiology, New York Presbyterian Brooklyn Methodist Hospital, Brooklyn, NY (Y.S.Q.); Stroke Unit, University Hospital of Siena, Italy (M.A.); and NYU School of Medicine, New York, NY (M.B.).

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### Supplementary Material

Data S1

Tables S1–S10

Figures S1–S2

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## **SUPPLEMENTAL MATERIAL**

## **Data S1.**

### **Supplemental Results**

**Subjects selection by nearest neighbor matching procedure.** Many attempts were performed to obtain the best matching. In particular, we initially entered all the variables (i.e. age, sex, potassium, calcium, history of tobacco use, diabetes mellitus, ischemic heart disease, anesthetics, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, H<sub>2</sub>-histamine receptor antagonists, inotropes, proton pump inhibitors, vasoconstrictors, and antimalarials), also considering different matching ratios (1:3, 1:2, 1:1). However, when antimalarials were added to the model, the quality of the matching worsened, even with the 1:1 ratio, with several variables resulting in a significant difference between the two groups. Clinical considerations can explain these findings. In fact, antimalarials are very frequently used for the treatment of autoimmune diseases, particularly those associated with anti-Ro/SSA-antibodies.<sup>29</sup>

Regarding antiarrhythmics, a statistically significant difference between the matched cohorts specifically arose for the 1:3 ratio only (no significant differences for 1:2 and 1:1 matching ratios). While these data are more difficult to explain in clinical terms, the above findings suggest that they may be due to the fact that the total number of antiarrhythmic-treated patients (only 82 in the whole cohort of 7339 subjects, see Table 1) is not enough to allow a 1:3 matching ratio.

In addition, even if we decided to analyse the 1:2 or the 1:1 cohort, in both cases, the sample size would not have been sufficient for such a very complex regression model (20 regressors entered).

**Table S1. Descriptive statistics of QTc and laboratory measurements in the overall cohort (n=7339).**

<b>Variable</b>	<b>Number of patients evaluated</b>	<b>Total number of measurements</b>	<b>Median (interquartile range) number of measurements per patient</b>
QTc	7339	33969	2 (1-6)
Potassium	7327	206902	19 (8-38)
Calcium	7072	153022	14 (5-30)
Magnesium	5109	48400	4 (2-12)
TSH	6575	58345	6 (3-12)
CRP	1561	1561	1 (1-1)

QTc: heart rate-corrected QT interval; TSH: thyroid-stimulating hormone; CRP: C-reactive protein.

**Table S2. Mean QTc values in the presence or absence of specific QT-prolonging risk factors in the overall population.**

QTc (ms)	n	Risk factor present	n	Risk factor absent	ΔQTc (ms)	<i>P</i>
Anti-Ro/SSA	612	436.8±28.2	6727	430.8±25.6	+6.0	<b>&lt;0.001</b>
<b>Demographic factors</b>						
Age>65 years	2940	434.3±26.8	4320	429.0±24.8	+5.3	<b>&lt;0.001</b>
Female sex	1536	434.2±23.4	5803	430.5±26.4	+3.7	<b>&lt;0.001</b>
<b>Laboratory data</b>						
Calcium<9.5 mg/dl	4873	433.3±26.2	2168	428.0±24.8	+5.3	<b>&lt;0.001</b>
Potassium<4.0 mEq/l	2074	435.0±25.4	5238	429.8±25.9	+5.2	<b>&lt;0.001</b>
TSH>3.5 μUI/ml	763	436.7±27.1	5570	430.7±25.1	+6.0	<b>&lt;0.001</b>
CRP>20 mg/L	163	443.0±23.2	1167	432.6±25.7	+10.4	<b>&lt;0.001</b>
<b>CV risk factors/diseases</b>						
Smoking	518	435.4±25.0	6781	431.0±25.9	+4.3	<b>&lt;0.001</b>
Diabetes mellitus	2100	436.7±26.0	5239	429.3±25.5	+7.4	<b>&lt;0.001</b>
IHD	1218	437.9±27.4	6121	430.0±25.3	+7.9	<b>&lt;0.001</b>
<b>Drugs</b>						
Anesthetics use	85	442.6±23.9	7254	431.2±25.9	+11.4	<b>&lt;0.001</b>
Antiarrhythmic use	82	447.9±31.5	7257	431.1±25.8	+16.8	<b>&lt;0.001</b>
Antibiotic use	3893	434.1±25.4	3446	428.1±26.1	+6.0	<b>&lt;0.001</b>
Antidepressant use	3870	433.6±25.2	3469	428.8±26.3	+4.8	<b>&lt;0.001</b>
Antiemetic use	1449	437.9±25.8	5890	429.7±25.6	+8.2	<b>&lt;0.001</b>
Antihistaminic use	2560	435.6±25.3	5179	429.6±25.9	+6.0	<b>&lt;0.001</b>
Antimalarial use	718	439.5±26.3	6621	430.4±25.7	+9.1	<b>&lt;0.001</b>
Antipsychotic use	1630	435.5±25.4	5709	430.1±25.9	+5.4	<b>&lt;0.001</b>
Bronchodilator use	2617	436.3±25.5	4722	428.5±25.7	+7.8	<b>&lt;0.001</b>
Cholinesterase inhibitor use	182	437.2±26.8	7157	431.2±25.8	+6.0	<b>&lt;0.001</b>
Diuretic use	2918	436.8±26.6	4421	427.6±24.7	+9.2	<b>&lt;0.001</b>
H <sub>2</sub> -histamine receptor antagonist use	102	443.6±29.1	7237	431.1±25.8	+12.5	<b>&lt;0.001</b>
Inotrope use	32	448.7±24.3	7307	431.2±25.9	+17.5	<b>&lt;0.001</b>
Opiate use	358	436.5±25.6	6981	431.0±25.9	+5.5	<b>&lt;0.001</b>
Proton pump inhibitor use	1322	436.0±25.6	6017	430.3±25.8	+5.7	<b>&lt;0.001</b>
Vasoconstrictor use	183	438.9±25.5	7156	431.1±25.9	+7.8	<b>&lt;0.001</b>

Data are expressed as mean±standard deviation.

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

P values <0.05 are presented in bold.



**Table S3. History of connective tissue disease in anti-Ro/SSA-positive (n=612) vs anti-Ro/SSA-negative (n=6727) subjects of the study population.**

	<b>n</b>	<b>Anti-Ro/SSA-Positive</b>	<b>n</b>	<b>Anti-Ro/SSA-Negative</b>	<b>OR (95% CI)</b>	<b><i>P</i></b>
CTD (total)	288	47.1%	705	10.5%	7.59 (6.36-9.06)	<b>&lt;0.001</b>
SLE	125	20.4%	194	2.9%	8.64 (6.78-11.02)	<b>&lt;0.001</b>
SS*	122	19.9%	114	1.7%	14.44 (11.01-18.94)	<b>&lt;0.001</b>
RA	75	12.3%	324	4.8%	2.76 (2.11-3.60)	<b>&lt;0.001</b>

CTD: connective tissue disease (including systemic lupus erythematosus, discoid lupus, Sjogren’s syndrome, rheumatoid arthritis, systemic sclerosis, idiopathic inflammatory myopathies, mixed CTD, and undifferentiated CTD); SLE: systemic lupus erythematosus; SS: Sjogren’s syndrome; RA: rheumatoid arthritis; OR: odds ratio; CI: confidence interval.

\*Including both primary and secondary [associated to another CTD] Sjogren’s syndrome.

Data are expressed as n (%).

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

Difference in categorical variables were evaluated by the two-sided Fisher’s exact test.

P values <0.05 are presented in bold.

**Table S4. Mean QTc values and QTc prolongation frequencies in anti-Ro/SSA-negative vs anti-Ro/SSA-positive subjects, with and without history of connective tissue disease.**

QTc	Anti-Ro/SSA-negative	Anti-Ro/SSA-positive	<i>Anti-Ro/SSA-positive without CTD</i>	<i>Anti-Ro/SSA-positive with CTD</i>
n	6727	612	324	288
Mean QTc (ms)	430.8±25.6	436.8±28.2***	435.0±28.7**	438.8±27.5***
QTc>470 ms ♂/ 480 ms ♀	6.2%	10.0%**	9.9%*	10.1%*
QTc>490 ms	2.1%	4.7%***	4.6%**	4.9%**
QTc>500 ms	1.1%	3.1%***	2.8%*	3.5%**

For continuous variables, the data are expressed as mean ±standard deviation. For categorical variables, the data are expressed as n (%).

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

Difference in categorical variables were evaluated by the two-sided Fisher's exact test.

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs anti-Ro/SSA-negative subjects.

p>0.05 for all comparisons between anti-Ro/SSA-positive subjects, with CTD vs without CTD,.

**Table S5. QTc prolonging risk factors in anti-Ro/SSA-positive subjects with (n=288) or without (n=324) history of connective tissue disease.**

Risk factor	n	Anti-Ro/SSA-positive with CTD	n	Anti-Ro/SSA-positive without CTD	OR (95% CI)	p
<i>Quantitative variables</i>						
Age	287	63.5±12.5 years	319	63.4±11.4 years	-	0.89
Potassium	288	4.1±0.3 mEq/L	323	4.1±0.3 mEq/L	-	0.54
Calcium	284	9.2±0.4 mg/dl	318	9.2±0.4 mg/dl	-	0.90
TSH	278	2.4±2.3 µUI/ml	272	2.8±4.2 µUI/ml	-	0.19
CRP	104	12.8±31.9 mg/L	50	11.7±22.6 mg/L	-	0.82
<i>Categorical variables</i>						
<b>Demographic factors</b>						
Female sex	288	32.6%	324	20.7%	1.86 (1.29-2.68)	<b>&lt;0.001</b>
<b>Cardiovascular risk factors/diseases</b>						
Smoking	288	9.0%	324	8.6%	1.05 (0.60-1.83)	0.88
Diabetes	288	27.8%	324	30.2%	0.89 (0.62-1.25)	0.53
IHD	288	24.7%	324	24.7%	1.00 (0.69-1.44)	1.00
<b>Drugs</b>						
Anesthetics	288	2.1%	324	1.2%	1.70 (0.48-6.09)	0.53
Antiarrhythmics	288	6.3%	324	4.0%	1.59 (0.77-3.31)	0.27
Antibiotics	288	67.7%	324	58.6%	1.48 (1.06-2.06)	<b>0.024</b>
Antidepressants	288	66.7%	324	58.6%	1.41 (1.14-4.13)	<b>0.045</b>
Antiemetics	288	27.1%	324	30.9%	0.83 (0.58-1.18)	0.33
Antihistaminics	288	70.5%	324	14.5%	14.07(9.43-20.99)	<b>&lt;0.001</b>
Antimalarials	288	71.9%	324	28.4%	6.44 (4.53-9.17)	<b>&lt;0.001</b>
Antipsychotics	288	32.3%	324	31.8%	1.02 (0.72-1.43)	0.93
Bronchodilators	288	50.3%	324	47.2%	1.13 (0.82-1.55)	0.47
Cholinesterase inhibitors	288	3.1%	324	5.2%	0.58 (0.26-1.33)	0.23
Diuretics	288	50.0%	324	39.5%	1.53 (1.11-2.11)	<b>0.011</b>
H <sub>2</sub> -histamine receptor antagonists	288	1.4%	324	0.9%	1.51 (0.33-6.79)	0.71
Inotropes	288	0.7%	324	1.2%	0.56 (0.10-3.08)	0.69
Opiates	288	5.9%	324	4.0%	1.50 (0.72-3.15)	0.35
Proton pump inhibitors	288	26.4%	324	18.8%	1.55 (1.05-2.27)	<b>0.026</b>
Vasoconstrictors	288	2.1%	324	2.5%	0.84 (0.29-2.45)	0.79

OR: odds ratio; CI: confidence interval; TSH: thyroid-stimulating hormone; CRP: C-reactive protein; IHD: ischemic heart disease.

For continuous variables, the data are expressed as mean ±standard deviation. For categorical variables, the data are expressed as n (%).

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

Difference in categorical variables were evaluated by the two-sided Fisher's exact test.

P values <0.05 are presented in bold.

**Table S6. QTc prolongation frequencies in anti-Ro/SSA-negative subjects stratified on the basis of the presence/absence of other autoantibodies associated with connective tissue diseases.**

Autoantibody	Status	n	QTc> 470 ms ♂/480 ms ♀	QTc>490 ms	QTc>500 ms
<i>Anti-CCP</i>	-	1086	51 (4.7%)	20 (1.8%)	10 (0.9%)
	+	201	9 (4.5%)	4 (2.0%)	2 (1.0%)
	<b>OR (95%CI)</b>		0.95 (0.40-1.99)	1.08 (0.27-3.28)	1.08 (0.11-5.13)
<i>Anti-Centromere</i>	-	2714	138 (5.1%)	37 (1.4%)	17 (0.6%)
	+	44	3 (6.8%)	1 (2.3%)	0 (0.0%)
	<b>OR (95%CI)</b>		1.37 (0.27-4.37)	1.68 (0.04-10.48)	0 (0.00-15.41)
<i>Anti-dsDNA</i>	-	4132	246 (6.0%)	74 (1.8%)	37 (0.9%)
	+	506	41 (8.1%)	11 (2.2%)	8 (1.6%)
	<b>OR (95%CI)</b>		1.39 (0.96-1.98)	1.22 (0.58-2.33)	1.78 (0.71-3.91)
<i>Anti-La/SSB</i>	-	6595	406 (6.2%)	136 (2.1%)	76 (1.2%)
	+	104	10 (9.6%)	5 (4.8%)	1 (1.0%)
	<b>OR (95%CI)</b>		1.62 (0.75-3.15)	2.40 (0.75-5.93)	0.83 (0.02-4.89)
<i>Anti-RNP</i>	-	4633	288 (6.2%)	92 (2.0%)	46 (1.0%)
	+	300	18 (6.0%)	4 (1.3%)	3 (1.0%)
	<b>OR (95%CI)</b>		0.96 (0.55-1.58)	0.67 (0.18-1.79)	1.01 (0.20-3.16)
<i>Anti-Scl70</i>	-	4416	260 (5.9%)	83 (1.9%)	43 (1.0%)
	+	90	6 (6.7%)	2 (2.2%)	2 (2.2%)
	<b>OR (95%CI)</b>		1.14 (0.40-2.63)	1.19 (0.14-4.55)	2.31 (0.27-9.12)
<i>Anti-SM</i>	-	4928	302 (6.1%)	94 (1.9%)	47 (1.0%)
	+	98	10 (10.2%)	2 (2.0%)	1 (1.0%)
	<b>OR (95%CI)</b>		1.74 (0.80-3.40)	1.07 (0.13-4.09)	1.07 (0.03-6.40)

Anti-CCP: anti-cyclic citrullinated peptide antibodies; anti-dsDNA: anti-double stranded DNA antibodies; anti-La/SSB: anti-Sjogren Syndrome-related antigen B antibodies; anti-RNP: anti-ribonucleoprotein antibodies; anti-Scl70: anti-topoisomerase I antibodies; anti-Sm: anti-Smith antibodies; OR: odds ratio; CI: confidence interval. The data are expressed as n (%).

Difference in categorical variables were evaluated by the two-sided Fisher's exact test. P>0.05 for all comparisons.

**Table S7. QTc prolonging risk factors in anti-Ro/SSA-positive (n=596) vs anti-Ro/SSA-negative (n=1788) subjects of the propensity score-selected population.**

Risk factor	n	Positive	n	Negative	SMD <i>pre</i>	SMD <i>post</i>	<i>p</i>
<b>Quantitative variables</b>							
Age	596	63.5±11.9 years	1788	63.6±11.7 years	0.191	0.003	0.82
Potassium	596	4.1±0.3 mEq/L	1788	4.1±0.3 mEq/L	0.110	0.021	0.18
Calcium	596	9.2±0.4 mg/dl	1788	9.2±0.4 mg/dl	0.327	0.015	0.57
<b>Categorical variables</b>							
<b>Demographic factors</b>							
Female sex	596	26.2%	1788	26.0%	0.139	0.004	0.96
<b>Cardiovascular risk factors/diseases</b>							
Smoking	596	8.7%	1788	9.1%	0.041	0.014	0.81
Diabetes	596	29.5%	1788	29.5%	0.051	0.001	1.00
IHD	596	25.0%	1788	23.7%	0.221	0.030	0.54
<b>Drugs</b>							
Anesthetics	596	1.7%	1788	1.9%	0.045	0.017	0.86
Antiarrhythmics	596	5.0%	1788	2.6%	0.258	<b>0.129</b>	<b>0.004</b>
Antibiotics	596	63.3%	1788	61.7%	0.219	0.031	0.52
Antidepressants	596	62.8%	1788	62.8%	0.215	<0.001	1.00
Antiemetics	596	29.4%	1788	29.4%	0.240	0.001	1.00
Antihistaminics	596	41.1%	1788	42.2%	0.264	0.023	0.67
Antimalarials	596	49.0%	1788	7.8%	1.086	<b>1.028</b>	<b>&lt;0.001</b>
Antipsychotics	596	32.6%	1788	32.4%	0.244	0.002	0.96
Bronchodilators	596	49.0%	1788	47.8%	0.292	0.024	0.64
Cholinesterase inhibitors	596	4.0%	1788	4.4%	0.108	0.019	0.73
Diuretics	596	44.6%	1788	43.3%	0.104	0.027	0.57
H <sub>2</sub> -histamine receptor antagonists	596	1.2%	1788	1.1%	0.024	0.011	0.82
Inotropes	596	1.0%	1788	0.9%	0.072	0.012	0.81
Opiates	596	5.0%	1788	5.5%	0.001	0.020	0.75
Proton pump inhibitors	596	22.8%	1788	22.4%	0.119	0.011	0.82
Vasoconstrictors	596	2.3%	1788	2.2%	0.015	0.011	0.87

Positive: anti-Ro/SSA-positive subjects; Negative: anti-Ro/SSA-negative subjects; IHD: ischemic heart disease. SMD pre/post: standardized mean differences, before/after matching.

For continuous variables, the data are expressed as mean ±standard deviation. For categorical variables, the data are expressed as n (%).

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

Difference in categorical variables were evaluated by the two-sided Fisher's exact test.

SMD values >0.1 and p values <0.05 are presented in bold.



**Table S8. Demographic, clinical, laboratory, and therapeutic variables independently associated with QTc interval prolongation >500 ms in the propensity score-selected population (n=2384): a stepwise multivariate logistic regression analysis.**

Dependent variable	Independent variables	Model coefficients ( $\beta$ )	OR (95%CI)	<i>p</i>
QTc>500 ms	Age	0.045	1.05 (1.02-1.08)	<b>0.001</b>
	Calcium	-1.089	0.34 (0.18-0.64)	<b>0.001</b>
	Anti-Ro/SSA	0.777	2.18 (1.18-4.02)	<b>0.013</b>

P values <0.05 are presented in bold.

Variables entered in the multivariate analysis include: age, sex, potassium, calcium, ischemic heart disease, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors.

**Table S9. Mean QTc values and QTc prolongation frequencies in anti-Ro/SSA-negative vs anti-Ro/SSA-positive subjects, with and without specific QT-prolonging risk factors.**

Risk factor	Anti-Ro/SSA	n	Mean QTc (ms)	QTc> 470 ms ♂ / 480 ms ♀	QTc>490 ms	QTc>500 ms
Whole cohort	-	6727	430.8±25.6	418 (6.2%)	141 (2.1%)	77 (1.1%)
	+	612	436.8±28.2***	61 (10.0%)***	29 (4.7%)***	19 (3.1%)***
<65 years	-	3999	428.5±24.6 <sup>ooo</sup>	171 (4.3%) <sup>o</sup>	46 (1.2%) <sup>ooo</sup>	24 (0.6%)
	+	321	434.6±26.7*	22 (6.9%)*	13 (4.1%)*	9 (2.8%)*
>65 years	-	2655	433.8±26.5 <sup>oo</sup>	232 (8.7%) <sup>o</sup>	90 (3.4%)	49 (1.9%)
	+	285	438.8±29.5**	37 (13.0%)***	15 (5.3%)**	9 (3.2%)**
Calcium >9.5 mg/dl	-	2040	427.6±24.6 <sup>oo</sup>	84 (4.1%) <sup>o</sup>	24 (1.2%)	12 (0.6%)
	+	127	435.6±26.6	12 (9.5%)	3 (2.4%)	2 (1.6%)
Calcium <9.5 mg/dl	-	4398	432.8±25.9 <sup>oo</sup>	327 (7.4%) <sup>o</sup>	115 (2.6%) <sup>oo</sup>	64 (1.5%) <sup>oo</sup>
	+	475	437.5±28.7***	49 (10.3%)***	26 (5.5%)***	17 (3.6%)***
Potassium >4 mEq/l	-	4835	429.3±25.6 <sup>ooo</sup>	281 (5.8%) <sup>oo</sup>	95 (2.0%) <sup>oo</sup>	53 (1.1%) <sup>oo</sup>
	+	403	435.8±28.8**	40 (9.9%)**	18 (4.5%)**	12 (3.0%)**
Potassium < 4 mEq/l	-	1866	434.5±25.2 <sup>o</sup>	134 (7.2%)	44 (2.4%) <sup>o</sup>	23 (1.2%) <sup>o</sup>
	+	208	438.9±26.8***	21 (10.1%)*	11 (5.3%)**	7 (3.4%)*
Ischemic heart disease-	-	5660	429.6±25.2 <sup>ooo</sup>	303 (5.4%)	97 (1.7%) <sup>oo</sup>	51 (0.9%) <sup>oo</sup>
	+	461	435.0±27.0**	34 (7.4%)	18 (3.9%)*	11 (2.4%)*
Ischemic heart disease+	-	1067	437.3±26.9	115 (10.8%) <sup>o</sup>	44 (4.1%)	26 (2.4%)
	+	151	442.3±30.9***	27 (17.9%)***	11 (7.3%)***	8 (5.3%)***
History of tobacco-	-	6209	430.4±25.6 <sup>ooo</sup>	375 (6.0%) <sup>ooo</sup>	126 (2.0%) <sup>ooo</sup>	71 (1.1%) <sup>ooo</sup>
	+	558	436.6±28.5***	55 (6.9%)**	27 (4.8%)***	17 (3.1%)***
History of tobacco+	-	518	435.0±25.0	43 (8.3%)	15 (2.9%)	6 (1.2%)
	+	54	439.2±25.3*	6 (11.1%)	2 (3.7%)	2 (3.7%)
Diabetes mellitus-	-	4923	428.7±25.2 <sup>ooo</sup>	251 (5.1%) <sup>ooo</sup>	83 (1.7%) <sup>ooo</sup>	42 (0.9%) <sup>ooo</sup>
	+	434	436.5±27.9***	40 (9.2%)*	19 (4.4%)**	15 (3.5%)***
Diabetes mellitus+	-	1804	436.7±25.7	167 (9.3%)	58 (3.2%)	35 (1.9%)
	+	178	437.5±28.8**	21 (11.8%)**	10 (5.6%)**	4 (2.3%)
Antiarrhythmics-	-	6676	430.7±25.5 <sup>ooo</sup>	405 (6.1%) <sup>oo</sup>	138 (2.1%) <sup>ooo</sup>	76 (1.1%) <sup>oo</sup>
	+	581	436.2±27.9***	56 (9.6%)**	26 (4.5%)***	17 (2.9%)**
Antiarrhythmics+	-	51	447.8±31.5	13 (25.5%)	3 (5.9%)	1 (2.0%)
	+	31	448.2±31.9**	5 (16.1%)*	3 (9.7%)*	2 (6.5%)
Antibiotics-	-	3219	427.7±25.8 <sup>oo</sup>	171 (5.3%)	60 (1.9%)	37 (1.1%)
	+	227	432.8±28.2	19 (8.4%)	8 (3.5%)	6 (2.6%)
Antibiotics+	-	3508	433.6±25.1 <sup>ooo</sup>	247 (7.0%) <sup>o</sup>	81 (2.3%) <sup>oo</sup>	40 (1.1%) <sup>o</sup>
	+	385	439.1±28.0***	42 (10.9%)***	21 (5.5%)***	13 (3.4%)**
Antidepressants-	-	3239	428.6±26.1	189 (5.8%)	67 (2.1%)	42 (1.3%)
	+	230	431.8±28.3	16 (7.0%)	8 (3.5%)	6 (2.6%)
Antidepressants+	-	3488	432.9±24.9 <sup>ooo</sup>	229 (6.6%) <sup>ooo</sup>	74 (2.1%) <sup>ooo</sup>	35 (1.0%) <sup>ooo</sup>
	+	382	439.8±27.7***	45 (11.8%)***	21 (5.5%)***	13 (3.4%)***
Antiemetics-	-	5456	429.3±25.5 <sup>ooo</sup>	304 (5.6%)	104 (1.9%)	58 (1.1%)
	+	434	434.1±26.6*	31 (7.1%)	13 (3.0%)	8 (1.8%)
Antiemetics+	-	1271	437.2±25.0 <sup>o</sup>	114 (9.0%) <sup>oo</sup>	37 (2.9%) <sup>ooo</sup>	19 (1.5%)
	+	178	443.3±30.8***	30 (16.9%)***	16 (9.0%)***	11 (6.2%)***
Antihistaminics-	-	4817	429.1±25.6 <sup>ooo</sup>	269 (5.6%) <sup>ooo</sup>	91 (1.9%) <sup>oo</sup>	55 (1.1%) <sup>o</sup>
	+	362	434.9±28.9*	37 (10.2%)**	17 (4.7%)**	10 (2.8%)*
Antihistaminics+	-	1910	435.1±25.1 <sup>o</sup>	149 (7.8%)	50 (2.6%)	22 (1.2%) <sup>oo</sup>
	+	250	439.5±27.0***	24 (9.6%)*	12 (4.8%)*	9 (3.6%)**
Antimalarials-	-	6308	430.3±25.5	379 (6.0%)	128 (2.0%) <sup>oo</sup>	68 (1.1%) <sup>oo</sup>
	+	313	432.0±29.2	27 (8.6%)	14 (4.5%)**	10 (3.2%)**
Antimalarials+	-	419	437.8±26.3	39 (9.3%)	13 (3.1%)	9 (2.1%)
	+	299	441.8±26.1***	34 (11.4%)**	15 (5.0%)**	9 (3.0%)*

<i>Antipsychotics-</i>	-	5293	429.6±25.7 <sup>°°°</sup>	302 (5.7%) <sup>°°</sup>	107 (2.0%) <sup>°°°</sup>	60 (1.1%) <sup>°°</sup>
	+	416	435.8±28.1**	39 (9.4%)*	20 (4.8%)**	13 (3.1%)**
<b><i>Antipsychotics+</i></b>	-	1434	435.1±25.0	116 (8.1%)	34 (2.4%)	17 (1.2%) <sup>°</sup>
	+	196	439.0±28.3***	22 (11.2%)*	9 (4.6%)*	6 (3.1%)*
<i>Bronchodilators-</i>	-	4408	428.2±25.4 <sup>°°</sup>	224 (5.1%) <sup>°°</sup>	77 (1.7%) <sup>°°</sup>	45 (1.0%) <sup>°°</sup>
	+	314	433.9±29.2	28 (8.9%)	14 (4.5%)**	10 (3.2%)**
<b><i>Bronchodilators+</i></b>	-	2319	435.8±25.3 <sup>°</sup>	194 (8.4%)	64 (2.8%) <sup>°</sup>	32 (1.4%) <sup>°</sup>
	+	298	439.9±26.8***	33 (11.1%)**	15 (5.0%)**	9 (3.0%)*
<i>Cholinesterase inhibitors-</i>	-	6571	430.7±25.6 <sup>°°°</sup>	401 (6.1%) <sup>°°°</sup>	134 (2.0%) <sup>°°°</sup>	74 (1.1%) <sup>°°°</sup>
	+	586	437.7±28.3***	59 (10.1%)*	28 (4.8%)*	18 (3.1%)*
<b><i>Cholinesterase inhibitors+</i></b>	-	156	436.9±27.1	17 (10.9%)	7 (4.5%)	3 (1.9%)
	+	26	439.4±25.6	2 (7.7%)	1 (3.9%)	1 (3.9%)
<i>Diuretics-</i>	-	4081	427.0±24.2 <sup>°°°</sup>	151 (3.7%) <sup>°°°</sup>	46 (1.1%) <sup>°°°</sup>	26 (0.6%) <sup>°°°</sup>
	+	340	435.3±29.8*	38 (11.2%)*	19 (5.6%)*	12 (3.5%)*
<b><i>Diuretics+</i></b>	-	2646	436.7±26.7	267 (10.1%)	95 (3.6%)	51 (1.9%)
	+	272	438.7±25.9***	23 (8.5%)	10 (3.7%)	7 (2.6%)*
<i>Inotropes-</i>	-	6701	430.7±25.6 <sup>°°°</sup>	413 (6.2%) <sup>°°°</sup>	139 (2.1%) <sup>°°°</sup>	76 (1.1%) <sup>°°°</sup>
	+	606	436.8±28.3***	61 (10.1%)*	29 (4.8%)*	19 (3.1%)*
<b><i>Inotropes+</i></b>	-	26	451.1±25.6	5 (19.2%)	2 (7.7%)	1 (3.8%)
	+	6	438.4±15.3	0 (0.0%)	0 (0.0%)	0 (0.0%)
<i>Proton pump inhibitors-</i>	-	5542	429.8±25.5 <sup>°°°</sup>	312 (5.6%) <sup>°°°</sup>	105 (1.9%) <sup>°°°</sup>	58 (1.0%) <sup>°°</sup>
	+	475	436.1±28.7***	49 (10.3%)*	23 (4.8%)*	14 (3.0%)*
<b><i>Proton pump inhibitors+</i></b>	-	1185	435.6±25.5	106 (8.9%)	36 (3.0%)	19 (1.6%)
	+	137	439.1±26.5***	12 (8.8%)	6 (4.4%)	5 (3.7%)*
<i>Vasoconstrictors-</i>	-	6558	430.6±25.6 <sup>°°°</sup>	400 (6.1%) <sup>°°°</sup>	134 (2.0%) <sup>°°°</sup>	74 (1.1%) <sup>°°°</sup>
	+	598	436.8±28.3***	61 (10.2%)*	29 (4.9%)*	19 (3.2%)*
<b><i>Vasoconstrictors+</i></b>	-	169	439.1±25.8	18 (10.7%)	7 (4.1%)	3 (1.8%)
	+	14	435.8±21.4	0 (0.0%)	0 (0.0%)	0 (0.0%)

For continuous variables, the data are expressed as mean ±standard deviation, For categorical variables, the data are expressed as n (%).

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

Differences in categorical variables were evaluated by the two-sided Fisher's exact test.

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 for comparisons (in grey) between anti-Ro/SSA-positive subjects with or without a specific risk factor vs all anti-Ro/SSA-negative subjects in the whole cohort (n=6727, in bold).

<sup>°</sup>p<0.05, <sup>°°</sup>p<0.01, <sup>°°°</sup>p<0.001 for comparison between anti-Ro/SSA-negative and positive within the same risk factor.

**Table S10. Mean QTc values in the presence/absence of hypocalcemia or hypokalemia in the subjects in whom electrocardiogram and electrolyte measurements were performed at the same time point.**

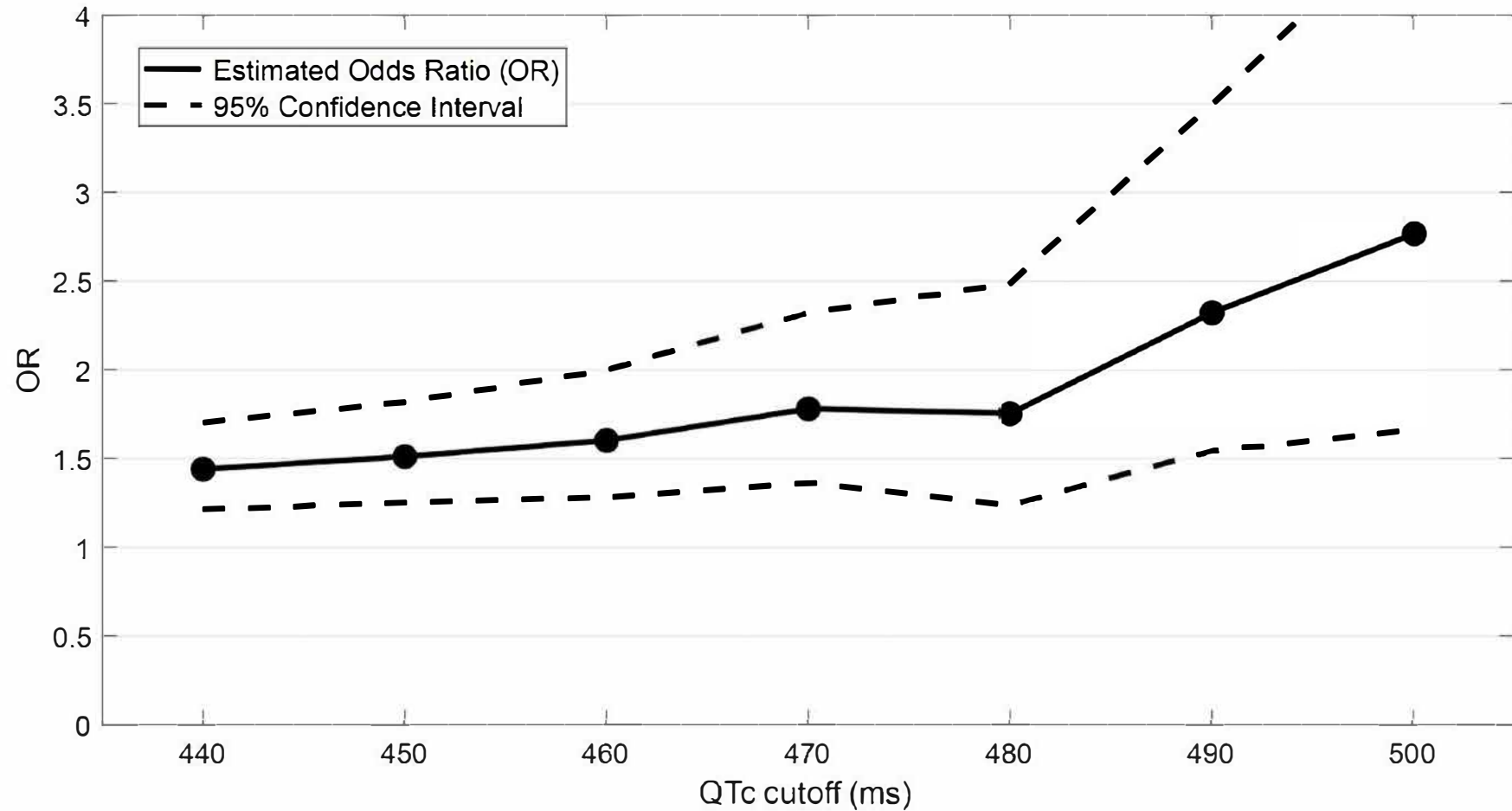
QTc (ms)	n	Risk factor present	n	Risk factor absent	ΔQTc (ms)	<i>p</i>
Calcium<9.5 mg/dl	3505	434.1±25.8	1368	428.6±24.7	+5.5	<b>&lt;0.001</b>
Potassium<4.0 mEq/l	1582	435.8±25.7	3850	430.7±25.4	+5.1	<b>&lt;0.001</b>

Data are expressed as mean±standard deviation.

Differences in quantitative variables were evaluated by the two-tail Mann-Whitney test or unpaired T-test.

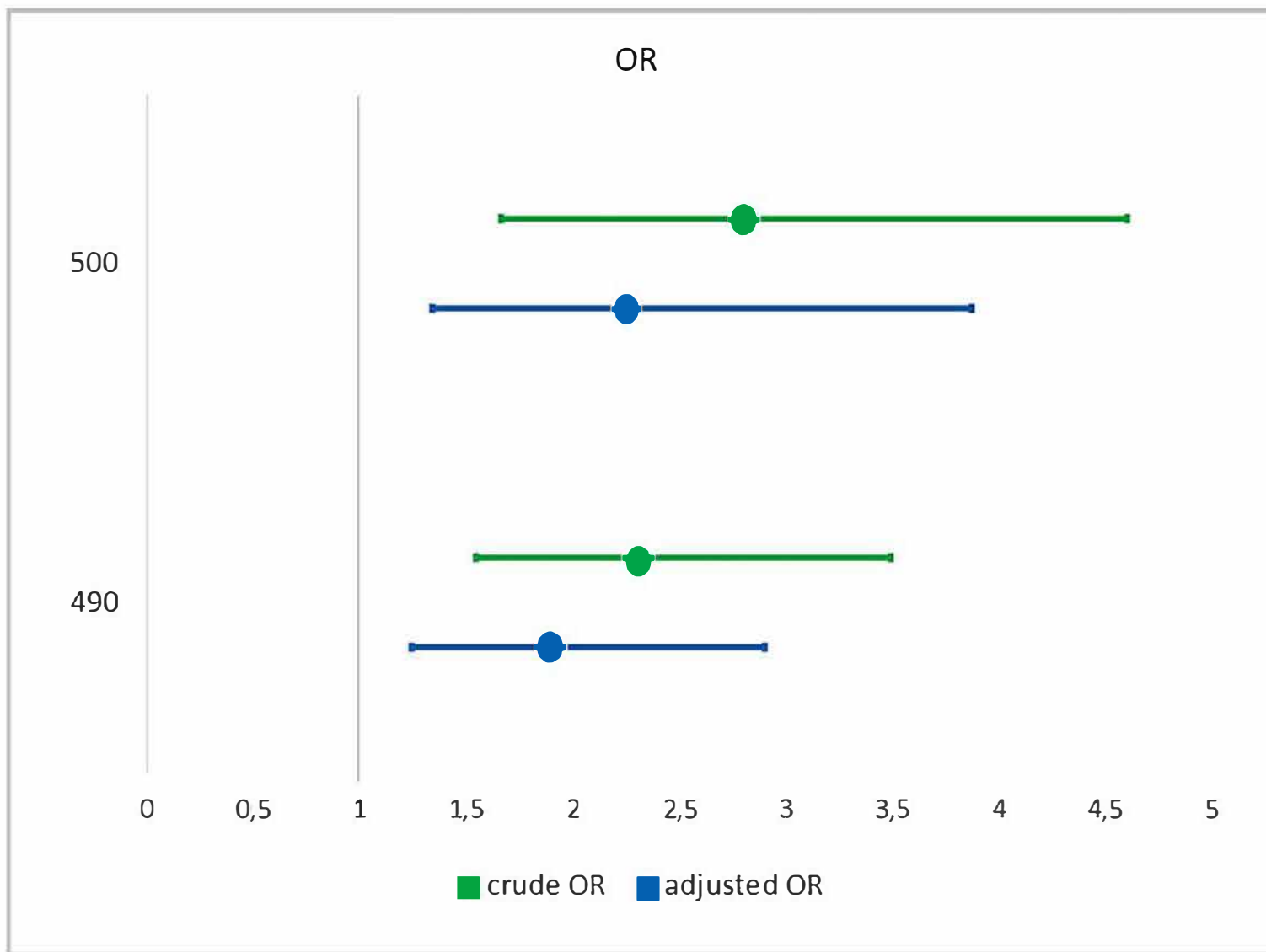
P values <0.05 are presented in bold.

**Figure S1. Behavior of the odds ratio (OR) associated with the positivity of circulating anti-Ro/SSA-antibodies, as the cut-off value of QTc increases.**





**Figure S2. Crude and adjusted odds ratios (OR) for QTc prolongation >490 ms, and >500 ms associated with the positivity of circulating anti-Ro/SSA-antibodies.**



Variables entered in the stepwise logistic regression analysis include age, sex, potassium, calcium, ischemic heart disease, antiarrhythmics, antibiotics, antidepressants, antiemetics, antihistaminics, antimalarials, antipsychotics, bronchodilators, cholinesterase inhibitors, diuretics, inotropes, proton pump inhibitors, vasoconstrictors.