Elevated IL-1β levels in anti-Ro/SSA connective tissue diseases patients with prolonged corrected QTc interval

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Abstract

Objective

Patients with systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) have increased IL-1β levels. IL-1β and other pro-inflammatory cytokines have a modulating activity on cardiac ion channels and have been associated with increased arrhythmic risk in rheumatoid arthritis patients. Likewise, adult patients with connective tissue diseases (CTDs) may have prolonged QTc intervals associated with the presence of anti-Ro/SSA antibodies.

Our objective was to evaluate the presence of serum IL-1β in subjects with CTDs, in relation to the presence of anti-Ro/SSA antibodies and QTc interval duration.

Methods

12-lead electrocardiograms (ECG) were performed and blood was withdrawn, measuring electrolytes, IL-1β anti-Ro/SSA antibodies by ELISA in 73 patients with CTDs.

Results

55 patients were anti-Ro/SSA positive and 18 were anti-Ro/SSA negative. Patients with anti-Ro/SSA positive antibodies had a significantly greater median IL-1β serum level: 7.29 (range: 0.17–17.3 pg/ml) compared to patients with anti-Ro/SSA negative antibodies whose median was: 1.67 (range 0.55–4.12 pg/ml) p<0.001. The mean QTc interval values obtained in both groups were not significantly different (417.7±23.1 vs. 414.7±21.2, p=0.63). The QTc interval was prolonged in 11 (20%) patients, who were all anti-Ro/SSA positive versus 0 (0 %) in anti-Ro/SSA negative patients p=0.05.

Median IL-1β levels were: 8.7 (range: 2.69–15.1 pg/ml) in patients with prolonged QTc interval versus median: 5.0 (range: 0.17–17.3 pg/ml) in those with normal QTc interval values (<440ms) p=0.006.

Conclusion

IL-1β is elevated in patients with CTDs that have both anti-Ro/SSA antibodies and prolonged QTc intervals.

Key words

connective tissue diseases, QTc interval, IL-1β

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Introduction

IL-1β is a 17.5 kDa, 153 amino-acid peptide, initially produced as a 35 kDa precursor with no biological activity. Maturation of the precursor form involves cleavage by Caspase-1, originally termed interleukin-1β-converting-enzyme, releasing the soluble and mature form of IL-1β (1). IL-1β stimulates the expression of genes associated with inflammation and immune response and plays a key role in the pathogenesis of autoimmune diseases. To date, the discovered immunologic effects of IL-1β are: CD4 T cell activation, Th17 differentiation, dendritic cell maturation, NK and B-cell activation (2).

Systemic lupus erythematosus (SLE) is characterised by the production of autoantibodies, B cell hyperactivation and defective clearance of immune complexes. Increased plasma levels of inflammatory cytokines, such as IL-1β and interleukin-6 (IL-6), type I and type II interferons were described in SLE patients (3, 4)) .There is evidence suggesting that IL-1β is crucial for the synthesis of IgG autoantibodies (5). Furthermore, low serum concentration of IL-1 receptor antagonist (IL-1Ra) has been detected in SLE patients with kidney involvement, adding further evidence to the role of IL-1β in SLE (6).

Primary Sjögren's syndrome is another autoimmune systemic disease, characterised by chronic inflammation of exocrine glands. Peripheral blood mononuclear cells secreting IL-1β and tumour necrosis factor-α (TNF-α) are increased in pSS patients' plasma (7). Elevated levels of IL-1β and other pro-inflammatory cytokines were also detected in salivary glands (8) and tears (9) of patients with pSS, and these cytokines have shown to stimulate collagenases that might promote acinar disruption (10).

A study involving human cardiac tissue showed that myocardial trabeculae treated with IL-1β presented a significant decrease in contractility (11)), suggesting that IL-1β might also have negative inotropic and chronotropic effects, most likely due to changes in calcium homeostasis (11).

It has been demonstrated that IL-1β modifies the electrical properties of guinea pig cardiac cells via lipid second messengers that alter calcium channel conductance (12). In rheumatoid arthritis (RA) patients, elevated CRP levels were independently associated with QTc prolongation supporting the hypothesis that systemic inflammation plays a role in arrhythmic risk (13). Furthermore, Lazzerini *et al*. provided evidence that inhibition of systemic inflammation with tocilizumab was associated with QTc shortening and CRP reduction (14). Thus, these above mentioned observations suggest a causative link between cytokines, ion channel modulation and arrhythmia predisposition (15).

In adult patients with connective tissue diseases (CTDs) a prolonged corrected QT (QTc) interval was described as the most frequent abnormality found associated to the presence of anti-Ro/SSA antibodies (16, 17). These antibodies were primarily linked to cardiac conduction disturbances in fetus from anti-Ro/SSA positive mothers (18-20). They were originally identified in patients with pSS and SLE, but can also be found in patients with other CTDs or asymptomatic women. In SLE patients, the anti-Ro/SSA antibodies are frequently detected in those presenting dermatologic manifestations (21). Serum containing autoantibodies directed against the Ro/SSA antigens may recognise one or both cellular proteins with molecular weights of approximately 52 kD and 60 kD. To date, the way by which anti-Ro/SSA antibodies affect the ventricular repolarisation in patients with SLE and pSS remains unknown. A possible mechanism extrapolated from CHB hypothesis is cross-reactivity of anti-Ro/SSA antibodies with 190-kDaα1D Ca channel protein in the myocyte sinus node (22). Other authors, proposed that inhibition of rapid and slow cardiac delayed rectifier potassium current (IKr) by anti-Ro/SSA antibodies might explain QTc prolongation in adult patients with CTD (16). The aim of the present study was to evaluate the presence of serum IL-1β in subjects with CTDs and relate it to the presence of anti-Ro/SSA (positive or negative) antibodies and prolonged QTc intervals.

Patients and methods

Study population

Seventy-three patients with CTDs were included in the present work, 55 were anti-Ro/SSA positive and 18 were anti-Ro/SSA negative. Patients were considered anti-Ro/SSA positive if anti-Ro/SSA was greater than 25 U/ml measured by enzyme-linked immunosorbent assay (ELISA). The CTDs included were: SLE, pSS, myositis, scleroderma, RA, mixed connective tissue disease (MCTD) and undifferentiated connective tissue disease (UCTD) diagnosed following the standard classification criteria for each disease (23-27).

Demographic, clinical characteristics and ongoing treatment of study patients are detailed in Table I.

All enrolled patients signed an informed consent. The study was conducted according to the tenets of the Helsinki Declaration 1975, revised in 2000.

Patients presenting other systemic diseases such as diabetes, cardiac disease, as well as those taking medications which could potentially affect the QTc interval (antiarrhythmics, antibiotics, antipsychotics, tricyclics and tetracyclics antidepressants, selective serotonin reuptake inhibitors, domperidone, human immunodeficiency virus protease inhibitors, methadone) were excluded from the present study. Patients with electrolyte disturbances (Na^+, K^+, Ca^{++}, Mg^{++}) were excluded as well. Patients were allowed to take hydroxychloroquine (HCQ).

On enrolment day, 12-lead electrocardiograms (ECG) were performed and blood was withdrawn measuring antibodies, electrolytes and IL-1β in all patients.

Serologic studies

ELISA tests for total anti-Ro/SSA and anti-La/SSB were performed with a commercial kit based on purified antigens (Orgentec; Germany). The test used a mixture of 60 Kd and 52 Kd Ro-proteins. The assays were carried out according to the manufacturer's protocols on an automated ELISA instrument (Brio, from Radim) and values greater than 25 UI/ml were considered positive. Serum IL-1β was measured by ELISA, carried out according to the manufacturer's protocols (Cayman, MI, USA), expressing the results as pg/ml. The normal values ranges between 0.2-5.8 pg/ml.

ECG procedure

A single cardiologist, blinded to the patients' clinical and laboratory findings measured heart rate, QRS, PR, and QTc intervals.

QTc intervals were measured with Bazett formula in lead II of a 12-lead ECG. QT interval was measured from the onset of QRS complex to the point where the T wave ends. QT interval varies inversely with the heart rate; therefore, a correction for the heart rate (or the duration of the RR interval) was performed. A corrected QT interval was calculated from Bazett's formula: QTc = QT interval ÷ square root of the RR interval (in seconds). Prolonged ECG intervals were defined as the following: PR as longer than 200 msec, QRS as longer than 100 msec, and QTc as greater than or equal to 440 msec.

Statistical analysis

Differences between the 2 groups were evaluated performing 2-tailed Student's *t*-test for normally distributed unpaired continuous variables and the 2-tailed Mann-Whitney test for not normally distributed continuous variables. The 2-sided Fisher's exact test evaluated categorical variables. Pearson's correlation test was employed to detect correlations amongst QTc intervals and laboratory parameters. A multivariate logistic regression analysis was performed to evaluate the parameters associated to prolonged QTc intervals. In any case, *p*-values less than 0.05 were considered significant. All statistical analyses were performed using Epi Info™ 7 (Centers for Disease Control and Prevention-CDC).

Table I. Demographic and clinical characteristics in anti-Ro/SSA positive vs. negative patients.

	Anti-Ro/SSA positive (n=55)	Anti-Ro/SSA negative (n=18)	*p*
Age, media SD	47.2 ± 12	40 ± 11.1	0.04
Female sex, n (%)	52 (92.9)	14 (82.4)	0.20
Diagnosis, n			
SLE	16	14	
pSS	20	1	
UCTD	11	1	
SSc	3	0	
MCTD	1	0	
RA	3	1	
Myositis	2	0	
Disease duration years, media SD	7.5 ± 9.1	4.2 ± 3.9	0.17
Hydroxychloroquine, n (%)	31 (56.4)	12 (70.6)	0.44

Systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS), undifferentiated connective tissue disease (UCTD), scleroderma (SSc), mixed connective tissue disease (MCTD), rheumatoid arthritis (RA).

Results

ELISA Anti-Ro/SSA findings

Fifty-five patients were anti-Ro/SSA positive and 18 were negative. Anti-Ro/SSA levels measured by ELISA were 125±85.4 U/ml in the total population, 163.8±60.1 U/ml in anti-Ro/SSA positive *versus* 7.7±5.7 U/ml in anti-Ro/SSA negative. Twenty-three (31.5%) patients were anti-La/SSB positive. Anti-Ro/SSA positive patients were significantly older 47.2±12 than anti-Ro/SSA negative patients 40±11.1 p=0.04.

IL-1β findings

Median IL-1 β serum levels in all patients was 6.12 (range: 0.17–17.3 pg/ml). IL-1β serum levels in patients with anti-Ro/SSA positive antibodies were significantly higher: median: 7.29 (range: 0.17–17.3 pg/ml) than those with anti-Ro/SSA negative antibodies median: 1.67 (range 0.55–4.12 pg/ml) p<0.001 (Fig. 1). A positive correlation between IL-1β levels and anti-Ro/SSA titers was found: r 0.480 p<0.00.

QTc interval findings

The Figure 2 scattergram shows the QTc interval values expressed in msec

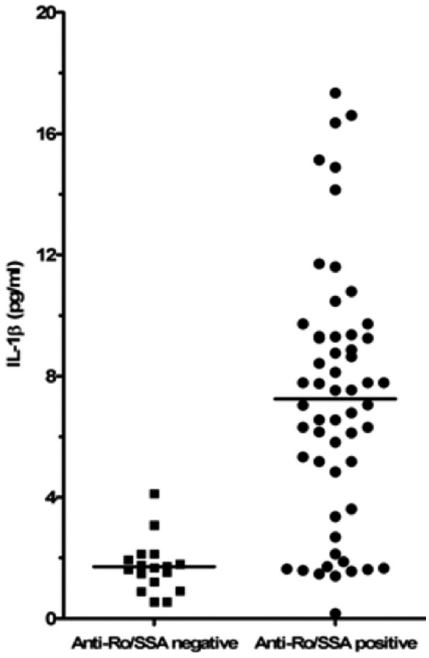

Fig. 1. IL-1 β levels in anti-Ro/SSA positive and negative patients.

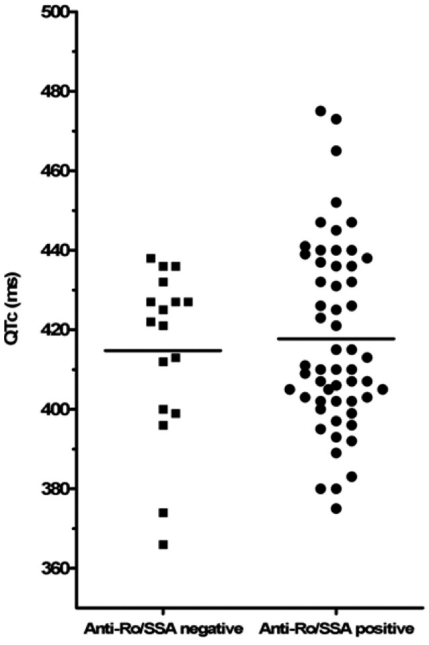

Fig. 2. Corrected QT values in anti-Ro/SSA positive and negative patients.

for each of the 55 anti-Ro/SSA positive patients and 18 anti-Ro/SSA negative patients. The mean QTc interval values obtained in both groups were not significantly different 417.7±23.1 *versus* 414.7±21.2, p=0.63. No correlation was found between QTc and anti-Ro/SSA measured by ELISA r - 0.06 p=0.57.

IL-1β levels in patients with prolonged QTc intervals were: median 8.7 (range: 2.69–15.1 pg/ml) *versus* median: 5.0 (range: 0.17–17.3 pg/ml) in those with normal QTc value (<440ms) p=0.006 (Fig. 3). A correlation analysis between IL-1 β and QTc showed r -0.08 p=0.48. Univariable analyses: QTc interval was prolonged in 11 (20%) patients, who were all anti-Ro/SSA positive *versus* 0 (0%) in anti-Ro/SSA negative patients p=0.05. Table II summarises clinical and laboratory findings in patients with normal and prolonged QTc intervals. Eleven patients in our study presented prolonged QTC: 4 patients had UCTD, 3 pSS, 2 SLE and 2 had scleroderma.

The Multivariable analysis: IL-1β was the only variable predictive of prolonged QTc intervals when adjusted by age, sex, and anti-Ro/SSA OR 1.86 (95% CI 1.01–1.39).

Fig. 3. IL-1 β levels in patients with prolonged and normal QTc intervals

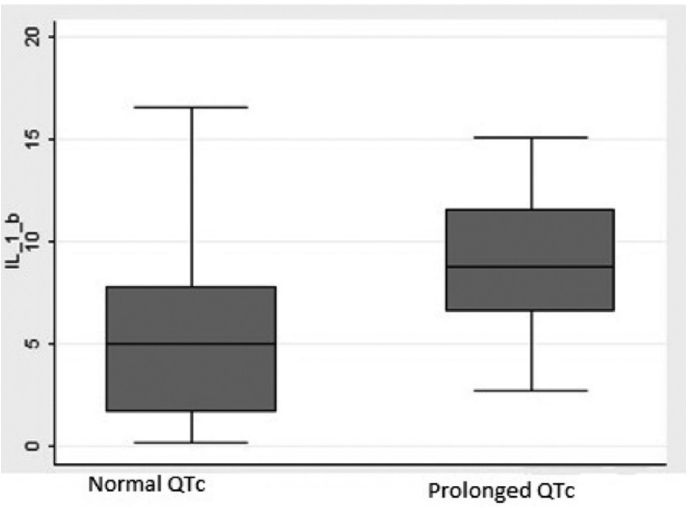

Table II. Clinical and laboratory findings in patients with normal and prolonged QTc intervals.

	QTc <440 n=62	QTc ≥440 n=11	*p*
Age, mean (SD)	44.5 (12.9)	51 (11.1)	0.12
Female sex n (%)	55 (88.7)	100	0.58
Diagnosis, n (%)			
SLE	28 (45.16)	2 (18.1)	
pSS	18 (29.3)	3 (27.2)	
RA	4 (6.4)	0	
UCTD	8 (12.9)	4 (36.3)	
MCTD	1 (1.61)	0	
Scleroderma	1 (1.61)	2 (18.1)	
Myositis	2 (3.23)	0	
HCQ use, %	36 (59.2)	7 (63.4)	1
IL-1 β levels, median pg/ml (range)	5.0 (0.17-17.3)	8.7 (2.69-15.1)	0.006
Anti-Ro/SSA levels, mean UI/ml (SD)	120.3 (89)	153.3 (56.3)	0.24
Anti-Ro/SSA positive, n (%)	11 (20)	0	0.05

Systemic lupus erythematosus (SLE), primary Sjogren syndrome (pSS), rheumatoid arthritis (RA), undifferentiated connective tissue disease (UCTD) and mixed connective tissue disease (MCTD).

Discussion

The present study examined IL-1β serum levels in subjects with CTDs and related them to the presence of anti-Ro/SSA antibodies. We found that anti-Ro/SSA positive patients had higher IL-1β levels compared to anti-Ro/SSA negative patients. Although overall QTc intervals were not different between the two groups, there were 11 anti-Ro/SSA positive patients with prolonged QTc intervals and their IL-1β levels were higher than in patients with normal QTc intervals.

Some studies have suggested that there is a link between anti-Ro/SSA antibodies and prolonged QTc intervals (16, 17, 28). It was initially reported by Lazzerini *et al*. who studied 57 patients with CTDs and found that 58% of the

anti-Ro/SSA patients had QTc intervals greater than 440 msec; in contrast to none of the control patients (16). A recent study from the same author found a direct correlation between anti-Ro/SSA 52 kd levels and QTc interval duration in 47 patients with CTDs (28). Data from Bourre-Tessier *et al*. (17) which studied a large group of SLE patients found a 5.1 to 12.6 increased risk of QTc interval prolongation in anti-Ro/SSA positive patients. However, association studies of anti-Ro/SSA and QTc interval prolongation are controversial, as others failed to confirm this association in patients with CTDs (29, 30). To date, the specific mechanisms linking the presence of anti-Ro/SSA with prolonged QTc intervals have not been elucidated, nevertheless some hypothesis had been proposed. Nakamura *et al*. provided evidence that serum IgG from a patient with acquired long QT syndrome provoked HERG potassium channel reduction. The authors hypothesise that anti-Ro/SSA antibodies can directly cross-react with the KCNH2 channel and provoke reduced expression by facilitating channel endocytosis (31). IL-1 β may synergistically act with the effects directly exerted by anti-Ro/SSA antibodies, thereby enhancing the impact of these antibodies on action potential duration up to produce a clinically measurable effect on ventricular repolarisation.

The existence of a relationship between ventricular repolarisation and systemic inflammation is arising in RA, chronic inflammatory arthritis and ankylosing spondylitis patients (13, 32, 33). Recent studies demonstrated significant association between QTc duration and CRP not only in rheumatic conditions also in chronic hypertensive patients (34).

To further emphasise the effect of inflammation, pro inflammatory cytokines on cardiomyocyte electrophysiology a recent study from Lazzerini *et al*. provided evidence that treatment with Tocilizumab in active RA patients was associated with rapid reduction in QTc duration, such an effect was correlated to decreased CRP and TNF-α levels. IL-1 β levels were not measured in this study (14) .

Results obtained in the present study indicating that IL-1β is increased in patients with anti-Ro/SSA antibodies as well as in patients with prolonged QTc intervals, may help clarify the mechanisms of QTc interval prolongation (functional mechanisms like the effect of proinflammatory cytokines on cardiomyocyte electrophysiology) in anti-Ro/SSA positive patients, which to date have remained unknown.

A prolonged action potential duration was induced on guinea pig ventricular myocytes with recombinant human IL 1-β, this effects were mediated at least in part by changes in the conductance of L-type calcium channels (12). A number of other studies investigated the effects of TNF on HERG potassium channel demonstrating channel down regulation via stimulation of reactive oxygen species (35).

A large number of investigators have explored the potential association of cytokines as biological markers in the activity and organ involvement in the course of SLE, yet only few specific studies of IL-1 β in SLE are available. In one study IL-1 Ra and IL-1β levels were evaluated in 20 patients with SLE. IL-1 β levels were slightly increased in only 3 patients and IL-1Ra correlated with IL-1β serum levels (6). Another study presented evidence that IL-1β is elevated in a subgroup of patients with CTDs, irrespective of the disease entity, but associated to anti nRNP antibodies (36).

Several studies have focused on the role of local IL-1β in pSS, however fewer studies focus on the systemic expression of the molecules. A recent study from Willeke *et al*. (7) showed a systemic increase of IL-1β secreting peripheral blood mononuclear cells and their levels correlated with disease duration, recurrent parotid swelling and rheumatoid factor titers. None of the publications to date in either SLE or pSS have studied the relationship between IL-1β levels, anti-Ro/SSA antibodies and prolonged QTc intervals or cardiac disease.

Our study had several limitations:

- Patients in the anti-Ro/SSA positive group were older than patients in the anti-Ro/SSA negative group. This finding could be explained by a predominance of SLE patients in the anti-Ro/SSA negative group, compared to a predominance of pSS patients in the anti-Ro/SSA positive group. Age is a variable associated to prolonged QTc intervals, yet QTc interval prolongation was adjusted by age using multivariate logistic regression analysis, and the results only showed an association with IL-1β levels. it can be argued that SLE predominance in one group and pSS in the other might be a bias if the prolonged QTc interval was associated with only one of these diseases.
- Anti-Ro/SSA antibodies were measured in the subjects with an ELISA kit using as antigen a mixture of 60kD and 52kD Ro-proteins, thereby not analysing the putative different impact of the two autoantibody subtypes, *i.e*. anti-Ro/SSA-52kD and anti-Ro/SSA-60kD on QTc interval. Recent work demonstrated how in anti-Ro/SSA-positive adult patients with CTD the occurrence of QTc prolongation seems strictly and selectively dependent on the anti-Ro/SSA-52kD level, without no significant role of the anti-Ro/SSA-60kD subtype (28). These findings may explain why considering anti-Ro/SSA antibodies as a whole, no significant correlation was find between prolonged QTc and anti-Ro/SSA levels despite all patients with QTc prolongation are anti-Ro/SSA-positive.

In conclusion, our results demonstrate that IL-1β is elevated in CTDs patients with anti-Ro/SSA antibodies. Further studies are needed to evaluate whether this cytokine might be the chemical mediator contributing to the prolonged QTc intervals found in anti-Ro/SSA positive patients.

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References

1. DINARELLO CA: Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. *Ann N Y Acad Sci* 1998; 856: 1-11.
2. DINARELLO CA: A clinical perspective of IL-1beta as the gatekeeper of inflammation. *Eur J Immunol* 2011; 41: 1203-17.
3. WILLIS R, SEIF AM, MCGWIN G, JR., *et al*.:

Effect of hydroxychloroquine treatment on pro-inflammatory cytokines and disease activity in SLE patients: data from LUMINA (LXXV), a multiethnic US cohort. *Lupus* 2012; 21: 830-5.
4. LEE HM, SUGINO H, NISHIMOTO N: Cytokine networks in systemic lupus erythematosus. *J Biomed Biotechnol* 2010: 676284.
5. JANDL RC, GEORGE JL, DINARELLO CA, SCHUR PH: The effect of interleukin 1 on IgG synthesis in systemic lupus erythematosus. *Clin Immunol Immunopathol* 1987; 45: 384-94.
6. STURFELT G, ROUX-LOMBARD P, WOLLHEIM FA, DAYER JM: Low levels of interleukin-1 receptor antagonist coincide with kidney involvement in systemic lupus erythematosus. *Br J Rheumatol* 1997; 36: 1283-9.
7. WILLEKE P, SCHOTTE H, SCHLUTER B *et al*.: Interleukin 1beta and tumour necrosis factor alpha secreting cells are increased in the peripheral blood of patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2003; 62: 359-62.
8. FOX RI, KANG HI, ANDO D, ABRAMS J, PISA E: Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. *J Immunol* 1994; 152: 5532-9.
9. SOLOMON A, DURSUN D, LIU Z, XIE Y, MACRI A, PFLUGFELDER SC: Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. *Invest Ophthalmol Vis Sci* 2001; 42: 2283-92.
10. AZUMA M, MOTEGI K, AOTA K, HAYASHI Y, SATO M: Role of cytokines in the destruction of acinar structure in Sjögren's syndrome salivary glands. *Lab Invest* 1997; 77: 269-80.
11. CAIN BS, MELDRUM DR, DINARELLO CA *et al*.: Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. *Crit Care Med* 1999; 27: 1309-18.
12. LI YH, ROZANSKI GJ: Effects of human recombinant interleukin-1 on electrical properties of guinea pig ventricular cells. *Cardiovasc Res* 1993; 27: 525-30.
13. PANOULAS VF, TOMS TE, DOUGLAS KM *et al*.: Prolonged QTc interval predicts all-cause mortality in patients with rheumatoid arthritis: an association driven by high inflammatory burden. *Rheumatology* (Oxford) 2014; 53: 131-7.
14. LAZZERINI PE, ACAMPA M, CAPECCHI PL *et al*.: Antiarrhythmic Potential of Anticytokine Therapy in Rheumatoid Arthritis: Tocilizumab Reduces Corrected QT Interval by Controlling Systemic Inflammation. *Arthritis Care Res* (Hoboken) 2015; 67: 332-9.
15. DUNCAN DJ, YANG Z, HOPKINS PM, STEELE DS, HARRISON SM: TNF-alpha and IL-1beta increase Ca2+ leak from the sarcoplasmic reticulum and susceptibility to arrhythmia in rat ventricular myocytes. *Cell Calcium* 2010; 47: 378-86.
16. LAZZERINI PE, ACAMPA M, GUIDERI F *et al*.: Prolongation of the corrected QT interval in adult patients with anti-Ro/SSA-positive connective tissue diseases. *Arthritis Rheum* 2004; 50: 1248-52.
17. BOURRE-TESSIER J, CLARKE AE, HUYNH T *et al*.: Prolonged corrected QT interval in anti-Ro/SSA-positive adults with systemic lupus erythematosus. *Arthritis Care Res* (Hoboken) 2011; 63: 1031-7.
18. BRUCATO A, FRASSI M, FRANCESCHINI F *et al*.: Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: a prospective study of 100 women. *Arthritis Rheum* 2001; 44: 1832-5.
19. COSTEDOAT-CHALUMEAU N, AMOURA Z, LUPOGLAZOFF JM *et al*.: Outcome of pregnancies in patients with anti-SSA/Ro antibodies: a study of 165 pregnancies, with special focus on electrocardiographic variations in the children and comparison with a control group. *Arthritis Rheum* 2004; 50: 3187-94.
20. BRUCATO A, CIMAZ R, CATELLI L, MERONI P: Anti-Ro-associated sinus bradycardia in newborns. *Circulation* 2000; 102: E88-9.
21. BIAZAR C, SIGGES J, PATSINAKIDIS N *et al*.: Cutaneous lupus erythematosus: first multicenter database analysis of 1002 patients from the European Society of Cutaneous Lupus Erythematosus (EUSCLE). *Autoimmun Rev* 2013; 12: 444-54.
22. KARNABI E, BOUTJDIR M: Role of calcium channels in congenital heart block. *Scand J Immunol* 2010; 72: 226-34.
23. BOHAN A, PETER JB: Polymyositis and dermatomyositis (second of two parts). *N Engl J Med* 1975; 292: 403-7.
24. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23: 581-90.
25. ALARCON-SEGOVIA D, CARDIEL MH: Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. *J Rheumatol* 1989; 16: 328-34.
26. MOSCA M, TAVONI A, NERI R, BENCIVELLI W, BOMBARDIERI S: Undifferentiated connective tissue diseases: the clinical and serological profiles of 91 patients followed for at least 1 year. *Lupus* 1998; 7: 95-100.
27. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
28. LAZZERINI PE, CAPECCHI PL, ACAMPA M *et al*.: Anti-Ro/SSA-associated corrected QT interval prolongation in adults: the role of antibody level and specificity. *Arthritis Care Res* (Hoboken) 2011; 63: 1463-70.
29. GORDON PA, ROSENTHAL E, KHAMASHTA MA, HUGHES GR: Absence of conduction defects in the electrocardiograms [correction of echocardiograms] of mothers with children with congenital complete heart block. *J Rheumatol* 2001; 28: 366-9.
30. COSTEDOAT-CHALUMEAU N, AMOURA Z, HULOT JS *et al*.: Corrected QT interval in anti-SSA-positive adults with connective tissue disease: comment on the article by Lazzerini *et al*. *Arthritis Rheum* 2005; 52: 676-7; author reply 677-8.
31. NAKAMURA K, KATAYAMA Y, KUSANO KF *et al*.: Anti-KCNH2 antibody-induced long QT syndrome: novel acquired form of long QT syndrome. *J Am Coll Cardiol* 2007; 50: 1808-9.
32. LAZZERINI PE, ACAMPA M, CAPECCHI PL *et al*.: Association between high sensitivity C-reactive protein, heart rate variability and corrected QT interval in patients with chronic inflammatory arthritis. *Eur J Intern Med* 2013; 24: 368-74.
33. SENEL S, COBANKARA V, TASKOYLU O, GUCLU A, EVRENGUL H, KAYA MG: Effect of infliximab treatment on QT intervals in patients with ankylosing spondylitis. *J Investig Med* 2011; 59: 1273-5.
34. CHANG KT, SHU HS, CHU CY *et al*.: Association between C-reactive protein, corrected QT interval and presence of QT prolongation in hypertensive patients. *Kaohsiung J Med Sci* 2014; 30: 310-5.
35. WANG J, WANG H, ZHANG Y, GAO H, NATTEL S, WANG Z: Impairment of HERG K(+) channel function by tumor necrosis factor-alpha: role of reactive oxygen species as a mediator. *J Biol Chem* 2004; 279: 13289-92.
36. AOTSUKA S, NAKAMURA K, NAKANO T *et al*.: Production of intracellular and extracellular interleukin-1 alpha and interleukin-1 beta by peripheral blood monocytes from patients with connective tissue diseases. *Ann Rheum Dis* 1991; 50: 27-31.