

Systemic inflammation as a novel QT-prolonging risk factor in patients with torsades de pointes

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Heart

Systemic inflammation as a novel QT-prolonging risk factor in patients with Torsades de Pointes

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Since classical risk factors cannot fully explain TdP events in a number of patients, we hypothesized that systemic inflammation may represent in the general population. Methods:Forty consecutive patients who experienced TdP(TdP-cohort) were prospectively enrolled independent of ongoing therapies and concomitant diseases, in order to determine C-reactive proteinc(CRP) and circulating levels of pro-inflammatory cytokines(Li-6, TMFc,IL-1). An additional 43 patients with different inflammatory conditions and elevated CRP (Linflammatory-cohort, 30% of patients showed elevated CRP levels(median-v3 mg/dl), with a definite inflammatory (CRP cohort, 30% of patients), and a concentration of the evels were available in 154/c0 case. Li-6, but not TMFc and Li-1, levels are cassicated with a decrease in L-6 levels and a rapid and significant QTc shortening(-20.5 ms). Conclusion. The data are first to show that systemic inflammation via elevated Li-6 levels and a rapid and significant QTc shortening(-20.5 ms). Conclusion. The data are first to show that systemic inflammation via elevated Li-6 levels and a rapid and significant QTc shortening(-20.5 ms). Conclusion. The data are first to show that systemic inflammation via elevated Li-6 levels may represent a novel QT-prolonging risk factor controlity in the presence of other classical via divers, thus opening new avenues in anti-arrhythmic therapy.	Since classical risk factors cannot fully explain TdP events in a number of patients, we hypothesized that systemic Inflammation may represent a fusion of the systemic Inflammation may represent in the general population. We can be approximately explained to a nogoing therapies and concombanding to the determine of reactive protein(CRP) and circulating levels of pro-inflammatory colotions, were prospectively enrolled, and general explained to a nogoing therapies and concombanding patients with different inflammatory conditions and elevated CP (reference). Results in the 17-boord, 80% of patients who experienced TdP(TdP-cohort) were prospectively enrolled, and general explained to a nogoing therapies and concombanding patients with different inflammatory conditions and elevated CP (reference). Results in the 17-boord, 80% of patients who experience and elevated CP in the determine of concombanding and the systemic inflammatory controls. Results in the 18-boord, 80% of patients and a repid and significant of the determine of concombanding and the systemic inflammation with the determine of the determine of concombanding and the systemic inflammation with the determine of the determine of concombanding and the systemic inflammation with the determine of the determine	
SCHOLARONE Manuscripts	Example 2	Since classical risk factors cannot fully explain TdP events in a number of patients, we hypothesized that systemic inflammation may represent a currently overlooked risk factor contributing to TdP development in the general population. Methods.Forty consecutive patients who experienced TdP(TdP-cohort) were prospectively enrolled independent of ongoing therapies and concomitant diseases, in order to determine C-reactive protein(CRP) and circulating levels of pro-inflammatory cytokines(IL-6,TNF α ,IL-1). An additional 43 patients with different inflammatory conditions and elevated CRP(Inflammatory-cohort), were prospectively enrolled, and QTc and cytokine levels were measured during active disease and after a CRP decrease by >75% subsequent to therapy. Results.In the TdP-cohort, 80% of patients showed elevated CRP levels(median:~3 mg/dl), with a definite inflammatory disease identifiable in 18/40 cases. IL-6, but not TNF α and IL-1, levels were ~20-times higher in TdP-cohort compared to healthy controls and to patients with active rheumatoid arthritis. In the inflammatory-cohort, QTc prolongation was common(mean values:454.3±30.4 ms). In these patients, CRP reduction was associated with a decrease in IL-6 levels and a rapid and significant QTc shortening(-20.5 ms). Conclusion.The data are first to show that systemic inflammation via elevated IL-6 levels may represent a novel QT-prolonging risk factor contributing to TdP occurrence in the presence of other classical risk factors, thus opening new avenues in anti-arrhythmic therapy.
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Systemic inflammation as a novel QT-prolonging risk factor in patients with Torsades de Pointes.

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ABSTRACT

Objective.Increasing evidence indicates systemic inflammation as a new potential cause of acquired long QT-syndrome(LQTS), via cytokine-mediated changes in cardiomyocyte ion channels. Torsades de pointes(TdP) is a life-threatening polymorphic ventricular tachycardia occurring in LQTS patients, usually when multiple QT-prolonging factors are simultaneously present.

Since classical risk factors cannot fully explain TdP events in a number of patients, we hypothesized that systemic inflammation may represent a currently overlooked risk factor contributing to TdP development in the general population.

Methods. Forty consecutive patients who experienced TdP(TdP-cohort) were prospectively enrolled independent of ongoing therapies and concomitant diseases, in order to determine C-reactive protein(CRP) and circulating levels of pro-inflammatory cytokines(IL-6,TNF α ,IL-1). An additional 43 patients with different inflammatory conditions and elevated CRP(Inflammatory-cohort), were prospectively enrolled, and QTc and cytokine levels were measured during active disease and after a CRP decrease by >75% subsequent to therapy.

Results. In the TdP-cohort, 80% of patients showed elevated CRP levels(median:~3 mg/dl), with a definite inflammatory disease identifiable in 18/40 cases. IL-6, but not TNF α and IL-1, levels were ~20-times higher in TdP-cohort compared to healthy controls and to patients with active rheumatoid arthritis. In the inflammatory-cohort, QTc prolongation was common(mean values:454.3±30.4 ms). In these patients, CRP reduction was associated with a decrease in IL-6 levels and a rapid and significant QTc shortening(-20.5 ms).

Conclusion.The data are first to show that systemic inflammation via elevated IL-6 levels may represent a novel QT-prolonging risk factor contributing to TdP occurrence in the presence of other classical risk factors, thus opening new avenues in anti-arrhythmic therapy.

Key words: Torsades de pointes; long-QT syndrome; sudden death; systemic inflammation; interleukin-6.

INTRODUCTION

Torsades de pointes(TdP) is a peculiar polymorphic ventricular tachycardia characterized by a pattern of twisting points occurring in patients with long QT syndrome(LQTS), both acquired and congenital. It is life-threatening as it can degenerate into ventricular fibrillation(VF) and cause sudden cardiac death(SCD)¹. TdP develops in patients with a markedly prolonged QTc(usually >500 msec), a condition in most cases requiring the simultaneous presence of multiple QTc-prolonging factors, congenital and/or acquired, synergistically operating in impairing ion channels responsible for the ventricular repolarization process. In fact, normal cardiac repolarization depends critically on the interplay of multiple ion currents, and these provide some redundancy(*repolarization reserve*) to protect against excessive QTc prolongation in critical settings¹.

Clinically recognizable congenital LQTS, mainly resulting from mutations affecting genes encoding for potassium or sodium channels, is a well-established risk factor for TdP. Among acquired risk factors, concurrent use of more than one QT-prolonging drug, and electrolyte imbalances are those most frequently implicated in TdP development. Other currently known causes of acquired LQTS and TdP include structural heart diseases, bradyarrhythmias, endocrine disorders, liver diseases, nervous system injuries, HIV infection, starvation, hypothermia and toxins¹. However, since the above "classical" risk factors cannot fully explain the development and recurrence of TdP events in a number of patients in which QTc remains prolonged despite elimination of the classical triggers, identification of previously unrecognized risk factors represents a field of increasing interest. Indeed, in the recent years both genetic (occult "latent" congenital LQTS, genetic polymorphisms reducing repolarization reserve)^{1,2} and acquired (autoimmune-mediated)^{3,4} conditions are emerging as novel and/or clinically silent risk factors significantly impacting the likelihood of TdP in the general population.

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In this scenario, mounting evidence indicates inflammatory activation as a new cause of acquired LQTS⁵. In fact, both cardiac and systemic inflammation are associated with QTc prolongation and higher propensity to develop TdP, as demonstrated by accumulating data obtained in patients with myo/endocarditis⁵, and systemic autoimmune diseases, particularly rheumatoid arthritis (RA)^{6,7} and connective tissue diseases^{5,6}, as well as in apparently healthy subjects from the general population^{5,8,9}. The putative underlying mechanisms are complex but essentially cytokine-mediated including direct actions on cardiomyocyte ion channels expression and function^{5,6}. As such, it seems conceivable that systemic inflammation, regardless of its origin, may represent a currently overlooked risk factor for QTc prolongation and TdP development in the general population. To test this hypothesis, we prospectively collected: (i)patients who experienced TdP associated with QTc prolongation to assess the incidence and the degree of systemic inflammation, as well as (ii)patients with systemic inflammatory diseases of different origin to evaluate the relationship between inflammatory markers and QTc during active disease and remission.

PATIENTS AND METHODS

Study populations.

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

We prospectively enrolled (2008-2016) 40 consecutive patients who presented with TdP, independent of ongoing therapies and concomitant diseases(TdP-cohort). Demographic, clinical and laboratory characteristics of study patients, as well as ongoing treatment with QTc-prolonging medications are provided in Table 1 and in the Supplementary-Table 1. Shortly after the first TdP episode (no later than 48h), patients underwent a venous withdrawal to determine circulating levels of C-reactive protein(CRP) and pro-inflammatory cytokines (IL-6, TNF α , IL-1). Since no established reference values for cytokine levels are available, two reference control groups were employed: (i)a positive control group, comparable for CRP levels of 10 patients with RA, a well-recognized immuno-mediated disease characterized by chronic high-grade systemic inflammation. All these RA patients had active disease as indicated by a disease activity score in 28 joints (DAS28) of more than 3.2 (in most cases [60%] of severe degree, i.e. DAS28 >5.1¹⁰); (ii)a negative control group of 10 healthy subjects(HC) age- and sex-matched with RA patients. Demographic, clinical and laboratory characteristics of control and RA groups are reported in the Supplementary-Table 2.

Finally, to assess the clinical impact of systemic inflammation on ventricular repolarization, we prospectively enrolled 43 patients with elevated CRP levels as the result of different inflammatory conditions, including acute inflammatory processes, septic or aseptic, or chronic immuno-mediated diseases during flares, comparable to those observed in the TdP cohort(Table 2). In these patients (Inflammatory-cohort), whose demographic, clinical and laboratory characteristics are detailed in Table 3, QTc and cytokine levels were measured during active disease and after different therapeutic interventions resulting in a CRP decrease >75% compared to the baseline.

ECG recordings.

Diagnosis of TdP was based on the presence of at least one episode of polymorphic ventricular arrhythmia and a rate ranging from 160 to 240 beats/minute, associated with QTc prolongation¹(Supplementary Figure 1). The QTc measurement is detailed in supplemental Methods.

Laboratory analysis.

CRP and cytokine measurement is detailed in supplemental Methods.

Statistical analysis.

The following parametric or non-parametric statistical analyses were respectively carried out: the Kruskal-Wallis test (non-parametric analysis of variance, ANOVA), and Dunn multiple comparison post-hoc, or the two-tail Student's paired "t" test, or the two-tail Wilcoxon matched-pairs test to evaluate differences in quantitative variables; the Spearman rank correlation-test to verify possible statistical association between quantitative variables. The two-sided Fisher's exact test was performed to evaluate statistical correlation between categorical variables. p values <0.05 were considered significant (GraphPad-InStat, version 3.06 for Windows 2000, GraphPad, San Diego,CA, USA;Microsoft Corp.,Redmond, WA).

RESULTS

TdP patients' characteristics. As expected¹,most patients in TdP-cohort were females(~70%) and older than 65 years(mean age:~75 years). Moreover, a high prevalence of recognized QTc-prolonging risk factors of acquired origin were present, the most recurrent condition being the presence of an underlying cardiac disease(82%), followed by electrolyte imbalances(70%) and QTc-prolonging medications(57%). Regarding specific risk factors, hypokalemia was the most common(60%). Anti-Ro/SSA-52kD antibodies were detected in 52% of the cases and in only two patients there was a history of autoimmune disease (1 RA, 1 celiac disease). Among drugs, amiodarone was the most frequently used (27%). Notably, in almost all cases more than one known QTc-prolonging factor was simultaneously identifiable; on average >4 (Table 1;Supplementary-Table 1). In addition, a significant proportion of patients (8/40, 20%) showed TdP rapidly degenerating to VF.

Inflammatory markers in TdP patients. A definite inflammatory disease was present in 18/40 patients(45%), most frequently an acute infection(n=12), but also chronic immune-mediated diseases (n=5), or acute aseptic inflammatory processes (n=1)(Table 2;Supplementary-Table 1). Notably, besides these subjects, the majority of TdP patients(80%) showed elevated CRP levels (median value 2.92 mg/dl) over 5-times higher than the upper normal limit (Table 2;Supplementary-Table 1; Figure 1).

TdP patients had elevated IL-6 levels, comparable with those observed in patients with active RA and about 20-times higher when compared to HC(Table 2;Supplementary-table 2;Figure 2A), which strongly correlated with CRP concentration(Figure 2B). Conversely, TNF α and IL-1 β levels in the TdP-cohort were not different from HC. Specifically, TNF α levels were significantly higher in RA patients than both TdP subjects and HC(Table 2; Supplementary-table 2; Figure 2C-D).

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Relationship between inflammatory markers and QTc in patients with inflammatory diseases. Patients with active inflammatory diseases (Inflammatory-cohort) displayed high prevalence of QTc prolongation (37%; QTc>440 ms: 63%), with a mean QTc>450 ms (Table 4). Therapeutic interventions, including antibiotics, anti-inflammatory drugs or protease inhibitors depending on the specific inflammatory disease present, were associated with a rapid (mean follow-up time 20.7 ± 25.4 days) and significant reduction in both CRP levels(mean decrease 88.7%) and QTc duration (Δ QTc=-20.5 ms, from 454.3±30.4 to 433.8±25.8 ms)(Table 4;Figure 3A-B). Accordingly, the prevalence of QTc prolongation significantly decreased (from 37% to 12%, p=0.010;table 4). Values of QTc significantly correlated with CRP levels (rho=0.30, p=0.0057) throughout the study time (Figure 4A). Conversely, no significant changes were observed in other laboratory parameters, including electrolyte levels, or echocardiography findings(Table 4).

Notably, in our patients a concomitant and significant reduction in mean heart rate(HR) values (-9.2 bpm, from 81.7 ± 14.0 to 72.5 ± 11.7 bpm;Table 4) was observed, correlating with CRP levels (rho=0.38, p=0.0003;Spearman rank correlation-test). Since the Bazett formula may over- or underestimate QTc at higher and lower HRs,¹¹ respectively, we additionally evaluated QTc using alternative correction formulas, i.e. Fridericia, Framingham and Hodges, the latter being recognized as the formula showing the least heart-rate dependence.¹¹ Although less marked, a significant QTc reduction was found in all cases (Fridericia: -11.6 ms, p=0.0008; Framingham: -9.3 ms, p=0.0047; Hodges: -11.4 ms, p=0.0005). Notably, QTc values obtained with Bazett formula strongly correlated with QTc values obtained with Friedericia, Framingham, and Hodges formulae(Supplementary-table 3).

Determination of circulating inflammatory cytokines revealed high IL-6 levels during active disease, >20-times higher than HC, which almost normalized after therapeutic interventions(Table 4;Figure 4B). IL-6 levels throughout the study time, significantly correlated with HR(rho=0.33, p=0.010, Spearman rank correlation-test) and, more strongly, with QTc (rho=0.50, p<0.0001;Figure

4C).

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DISCUSSION

The main findings of the present study are the following: (i) in unselected patients with TdP (TdPcohort), elevated CRP levels were present in the large majority of cases (80%; median value:~3 mg/dl), and a definite inflammatory disease identifiable in almost 50% of subjects; (ii) in this cohort, IL-6 levels were elevated and comparable with those observed in patients with severe active RA, and ~20-times higher than in HC. (iii) In patients with elevated CRP levels from different inflammatory conditions (Inflammatory-cohort), QTc prolongation was common; in these subjects, CRP reduction was associated with significant QTc shortening, which correlated with the decrease in IL-6 levels. Altogether, these data suggest that systemic inflammation, via elevation of IL-6 levels, may represent a novel risk factor for QTc prolongation contributing in the presence of other classical risk factors for TdP occurrence.

A large body of evidence indicates that systemic inflammation is associated with an increased risk of malignant ventricular arrhythmias and SCD, both in patients with overt cardiac diseases and in apparently healthy subjects. Indeed, large prospective studies have demonstrated that inflammatory markers, particularly CRP and IL-6, are strong and independent predictors of SCD in the general population¹²⁻¹⁴. Moreover, in patients with cardiomyopathy and implantable cardioverter-defibrillator(ICD) CRP and IL-6 levels are associated with the risk of appropriate ICD shock for ventricular tachycardia/fibrillation and electrical storm^{15,16}. Furthermore, large population-based studies demonstrated that in patients with chronic immune-mediated inflammatory diseases, particularly chronic inflammatory arthritis, the incidence of ventricular arrhythmias, cardiac arrest and SCD is higher than in the general population, also correlating with disease activity^{5,6,17,18}. Although the most likely underlying mechanism linking inflammatory markers and life-threatening ventricular arrhythmias/SCD is the promotion of coronary atherosclerosis, accumulating evidence supports the hypothesis that systemic inflammation may also *per se* be arrhythmogenic by inducing cytokine-mediated electric myocardial remodelling^{5,6}. In particular, many experimental studies

demonstrated that inflammatory cytokines (IL-6, $TNF\alpha$, IL-1) induce profound changes in potassium and calcium channels resulting in a prolongation of cardiomyocyte action potential duration(APD) and thus QT interval prolongation on the surface ECG. Transgenic mice overexpressing $TNF\alpha$ showed decrease of the cardiac transient outward current(I_{to}) and the corresponding potassiumchannel protein, as well as prolonged APD and increased susceptibility to re-entrant ventricular arrhythmias^{19,20}. In-vitro incubation of rat ventricular myocytes with TNF α resulted in a significant I_{to} inhibition²¹⁻²³. Wang et al.²⁴ demonstrated that TNF α down-regulates in-vitro the rapid component of the delayed rectifier potassium current(IKr) by impairing the hERG potassiumchannel function expected to also prolong APD. Finally, experiments on ventriculocytes demonstrated that IL-6 and IL-1 also prolong APD by enhancing L-type calcium current $(I_{CaL})^{25,26}$. In particular, evidence indicates that IL-6 induces phosphorylation of the serine residue at the position 1829 of the Cav1.2 calcium channel subunit via the action of extracellular signalregulated kinase, and that this phosphorylation increases I_{CaL}²⁶.In accordance with these data, a significant relationship between QTc duration and systemic inflammatory activation, as assessed by CRP and cytokine levels, has been demonstrated in large populations of apparently healthy subjects^{8,9}, as well in chronic inflammatory diseases, such as RA and connective tissue diseases, which frequently were associated with QTc prolongation²⁷⁻²⁹. In patients with RA, anti-cytokine therapy with tocilizumab(TCZ), an anti-interleukin 6-receptor antibody, was associated with a rapid QTc shortening which correlated with the decrease in CRP levels²⁸.Collectively, these data suggest that the link between inflammatory markers and SCD may be at least in part explained by a higher propensity to develop long QT-associated malignant arrhythmias, particularly TdP that rapidly degenerate into VF.

The results from the present study provide support to this point of view. In our cohort of unselected consecutive TdP patients, systemic inflammatory activation as reflected by elevated CRP levels was

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a very common finding, occurring in 80% of subjects. CRP values were in many cases markedly increased (up to ~30 mg/dl;median:~3 mg/dl) indicating the presence of high-grade systemic inflammation, more frequently as a result of acute infections (with different organ localization, including 6 sepsis), but also active chronic inflammatory diseases (chronic inflammatory arthritis, polymyalgia rheumatica) or acute aseptic inflammatory processes (acute pancreatitis). In this cohort, the incidence of chronic inflammatory arthritis (2 RA and 1 undifferentiated arthritis) was 7.5%, a value 5 to 15-times higher than expected for this condition in the general population (0.5-1.5%⁶. In addition, in a significant proportion of patients (35%) with no clearly defined inflammatory processes were diagnosed despite increases in CRP levels comparable to those observed in the entire cohort (median: 2.9 mg/dl, range: 0.67-11.6). These findings point to the concept that regardless of the specific aetiology, pathogenesis and targeted organs, systemic inflammation may per se represent the true risk factor for TdP development, via the action of common basic mediators involved in the development of the different inflammatory processes. The results of the present study together with data from previously cited electrophysiological studies¹⁹⁻ ²⁶strongly suggest that a key role is played by inflammatory cytokines, specifically IL- 6^{26} . In fact, unlike TNF α and IL-1, in our TdP-cohort circulating IL-6 was markedly (and selectively) elevated, ~20-times higher than HC and overlapping with those observed in a control group of RA patients with severe active disease. Furthermore, in TdP-cohort IL-6 levels robustly correlated with CRP concentration(rho=0.65). This latter finding, although expected (CRP is mainly produced by hepatocytes in response to $IL-6^{30}$), nevertheless provides further support to the hypothesis that IL-6 is the mediator linking systemic inflammation and arrhythmic risk in patients with TdP.

From a pathophysiological point of view, the most likely mechanism by which systemic inflammation could increase TdP risk is a prolonging effect on the QT interval, possibly via IL-6-mediated electrophysiological changes in cardiomyocyte APD. Accordingly, in the second part of the study, we demonstrated that in patients with elevated CRP levels resulting from different

inflammatory conditions (Inflammatory-cohort), QTc was frequently prolonged, but it was rapidly shortned a soon as inflammation was controlled. These patients showed elevated baseline IL-6 levels which almost normalized after treatement. Also, QTc shortening correlated with the decrease in CRP and IL-6 levels. The fact that QTc changes occurred rapidly (on average~20 days) suggests functional mechanisms independent of any structural heart modification. This hypothesis is consistent with in-vitro experiments demonstrating the ability of IL-6 in causing I_{CaL}-mediated APD-prolongation in cardiomyocytes²⁶.Additional support is provided by previous data obtained in RA patients where selective IL-6 blockade was associated with significant QTc reduction occurring after 3 months of treatment²⁸.

Besides exerting direct activities on the myocardium, IL-6 could also prolong QTc via indirect effects mediated by the autonomic nervous system. In fact, by targeting autonomic centres of the brain, inflammatory cytokines can increase the sympathetic outflow, in turn controlling cytokine production via inhibitory β 2-adrenergic receptors on circulating lympho-monocytes(Inflammatory reflex).⁵ Indeed, central sympathetic over-activity affects the whole body, including the heart where marked and complex changes in myocardial electrophysiology result in a net effect of APD prolongation^{5,6}. Accordingly, we found that suppression of inflammation was associated with a significant reduction of the sympathetic drive on the heart, as reflected by the decrease in the mean HR, which correlated with CRP and IL-6. Nevertheless, the fact that IL-6 levels more strongly correlated with QTc(rho=0.50, p<0.0001) than HR(rho=0.33, p=0.010) suggests that direct myocardial effects probably play a predominant role in IL-6-mediated OTc prolongation.

It is noteworthy that inflammation alone cannot explain marked QTc prolongation observed in the TdP patients. In fact, based on the results of the present (Inflammatory-cohort), and the above RA study²⁸, inflammatory mechanisms are *per se* probably not able to induce a QTc prolongation as critical to induce TdP(actually this is true for all recognized causes of LQTS when present alone¹). Rather, by reducing the ventricular repolarisation reserve, inflammation may represent a

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contributing factor synergistically operating with the other QT-prolonging factors concomitantly present. Indeed, as expected, in the TdP-cohort the majority of patients exhibit more than 1 risk factor (on average~4), more frequently structural heart disease, electrolyte imbalances, drugs, and anti-Ro/SSA antibodies. However, while most of these factors are well recognized and thus carefully taken in account for TdP treatment, the potential arrhythmogenic role of systemic inflammation in these patients is to date largely overlooked.

The potential limitations of this study include the relative small sample size and the lack of a genetic testing for concomitant LQTS-associated mutations. However, it should be underlined how TdP is an uncommon event, maybe also because not easily documentable for the high propensity of this arrhythmia to induce SCD. Accordingly, in several patients, TdP rapidly degenerated to VF and cardiac arrest. This suggests the hypothesis that the actual TdP incidence in the general population may be largely underestimated, thus supporting the view that this arrhythmia may contribute more than expected to explain SCD occurring in subjects with elevated inflammatory markers.

Although the genetic characterization would have been useful for a more accurate definition of the total load of risk factors in the single patient (case series identified subclinical congenital LQTS in 5-20% of cases of drug-induced TdP¹), we anticipate that the presence of systemic inflammation and genetic mutations along with other classical acquired risk factors will together predispose to TdP.

In conclusion, our data, *for the first time*, provide evidence that systemic inflammation may represent a novel risk factor contributing to TdP development in the general population. The data also support the recommendation to translate into the clinical practice that in patients with a systemic inflammatory state, regardless of its origin, the potential impact of this condition on ventricular repolarisation should be carefully kept in mind, particularly when one or more QTprolonging drugs are required. Inflammatory mediators, particularly IL-6, may represent an

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Table 1.Demographic, clinical and laboratory characteristics of patients with Torsades de pointes.

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Table 2. Ongoing inflammatory diseases, and circulating levels of CRP and inflammatory cytokines in patients with Torsades de pointes.

Definite inflammatory diseases, n	18(45%)
Acute infections	12(30%)
Sepsis	6(15%)
Pneumonia	4(10%)
Acute infective endocarditis	2(5%)
Urinary tract infection	1(2%)
Acute cholecystitis	1(2%)
Chronic immune-mediated diseases	5(12%)
Rheumatoid arthritis	2(5%)
Polymyalgia rheumatica	2(5%)
Undifferentiated chronic arthritis	1(2%)
Other	1(2%)
Acute pancreatitis	1(2%)
CRP, mg/dl	2.92(0.1-29.65)
Patients with CRP > 0.5 mg/dl, n	32(80%)
IL-6, pg/ml*	10.0(0.51-497.3)
TNFalpha, pg/ml*	0.61(0.13-81.35)
IL-1beta, pg/ml*	0.16(0.1-5.95)

CRP: C-reactive protein; IL-6: interleukin-6; TNFalpha: Tumor necrosis factor alpha; IL-1beta: interleukin-1beta. Except where indicated otherwise, values are expressed as median (range). *Available in 30 out of 40 patients.

Heart

Table 3. Demographic and clinical characteristics inflammatory di	, and ongoing therapies of patients with seases
Patients, n	43
Age.vears(range)	$72.2\pm21.3(24-98)$
Females, n	27
Definite inflammatory diseases, n	43
Acute infections	22
Pneumonia	13
Sepsis	5
Urinary tract infection	4
Cholecystitis /cholangitis	3
Acute bronchitis	3
Skin infection	1
Immune-mediated diseases	11
Rheumatoid arthritis	10
Cryoglobulinemic vasculitis	1
Other	3
Acute microcrystalline arthritis	2
Acute pancreatitis	1
Imipenem Clarithromycin Metronidazole Levofloxacin Vancomycin Cefotaxime Rifampicin Teicoplanin Oxacillin Colistin Anti-inflammatory drugs Corticosteroids	2 3 3 2 2 1 1 1 1 1 1 4 9
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Age is expressed as mean ±standard deviation (range).

Table 4. Changes in clinical, electrocardiographic, laboratory and echocardiography parameters in patients with inflammatory disorders (n=24), during active disease (PRE) and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST).

	PRE	POST	р
CRP,mg/dl	14.2±9.4	1.6±1.4	<0.0001
IL-6 [†] , pg/ml	13.9 (0.2-64.2)	3.4 (0.2-9.6)	<0.0001
TNFalpha†, pg/ml	0.75 (0.60-3.95)	0.75 (0.60-8.60)	n.s.
IL-1†, pg/ml	0.39 (0.18-1.16)	0.20 (0.17-1.36)	n.s.
QT,ms	391.1±36.9	396.3±39.7	n.s.
RR,ms	753.3±119.2	844.8±127.4	<0.0001
Heart rate, bpm	81.7±14.0	72.5±11.7	<0.0001
QTc,ms	454.3±30.4	433.8±25.8	<0.0001
Patients with prolonged QTc, n	16(37%)	5(12%)	0.010
Potassium, mEq/L	4.1±0.5	4.1±0.6	n.s.
Calcium, mEq/L	8.7±0.6	8.7±0.5	n.s.
Magnesium, mEq/L	1.9±0.3	1.8 ± 0.4	n.s.
Creatinine mg/dl	1 1±0 6	0 9±0 4	ns
pO2. mmHg	70.1±11.7	73.9±9.8	n.s.
рН	7.44±0.05	7.44 ± 0.04	n.s.
Fiection fraction %	56 4+5 0	57 3+4 8	ns
Left ventricular internal dimension mm	30.4 ± 5.0 17.5 ± 1.7	37.3 ± 4.0 17.4 ± 5.1	n.s.
Estimated pulmonary artery pressure, mmHg	32.8±7.2	29.6±5.9	n.s.
QT-prolonging drugs, n	0.67±0.8	0.79±0.9	n.s.

CRP: C-reactive protein; IL-6: interleukin-6; TNFalpha: Tumor necrosis factor alpha; IL-1: interleukin-1.

Values are expressed as mean ±standard deviation, or median (range). †Data available in 30 out of 43 patients.

Differences were evaluated by the two-tail Student's paired "t" test, or the two-tail Wilcoxon matched pairs test. Difference in categorical variables were evaluated by the two-sided Fisher's exact test.

Heart

Figure Legends

Figure 1. CRP levels in patients with Torsades de pointes (TdP), patients with rheumatoid arthritis (RA) and healthy controls (HC). TdP patients, n=40; RA patients, n=10; HC, n=10. Kruskal-Wallis test (p<0.01), with Dunn test,**p<0.01. Horizontal dotted line indicates the upper limit of reference values, i.e. 0.5 mg/dl.

Figure 2.Serum cytokine levels in patients with Torsades de pointes (TdP), patients with rheumatoid arthritis (RA) and healthy controls (HC). TdP patients, n=40; RA patients, n=10; HC, n=10. (A) IL-6 levels. Kruskal-Wallis test (p<0.01), with Dunn test,**p<0.01. (B) Relationship between CRP and IL-6 levels in TdP patients. Spearman test. (C) TNF α levels. Kruskal-Wallis test (p=0.01), with Dunn test,*p<0.05. (D) IL-1 levels. Kruskal-Wallis test (p>0.05.).

Figure 3. Changes in the corrected QT (QTc) interval duration in patients with active inflammatory diseases during, during active disease (PRE) and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST). (A) Representative ECG strips of a patient with acute pancreatitis, during active disease (PRE; CRP 27.0 mg/dl) and after a 13-day treatment with gabesate mesilate (POST; CRP 1.64 mg/dl). Red vertical lines in lead V5 show QT interval. (B)QTc changes observed before (PRE) and after (POST) treatment in the entire study population. Patients, n=43. Two-tail Student's paired "t" test,***p<0.0001.

Figure 4. Changes in inflammatory markers and their relationship with the corrected QT (QTc) interval duration in patients with inflammatory diseases. (A) Relationship between QTc and CRP levels throughout the time. Patients, n=43. Spearman test. (B) IL-6 levels during active disease (PRE), and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST); n=30. Two-tail Wilcoxon matched pairs test, ***p<0.0001.

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Figure 1

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Figure 3

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Heart

SUPPLEMENTAL MATERIAL

Supplemental Methods

ECG recordings.

In patients with TdP, the QTc interval was manually measured on a standard 12-lead ECG, from the onset of the Q wave or the onset of the QRS complex to the end of the T wave, defined as the return to the T-P baseline. When prominent U waves (>1 mm) merging into T waves were present, they were included in QT measurement [1]. QT interval, determined as the longest hand-measured QT interval in any lead [2] was corrected for heart rate by the Bazett formula (dividing the QT interval by the square root of the R-R interval) to yield the QTc value. Since the Bazett formula may over-or underestimate QTc at higher and lower HRs [3], respectively, we additionally evaluated QTc using alternative correction formulas, i.e. Fridericia, Framingham and Hodges, the latter being recognized as the correction formula showing the least heart-rate dependence [3]. QTc was measured from 3 non-consecutive beats (mean value) by a single investigator.

In patients without TdP, measurement of the heart rate, RR, QT, and QTc intervals was automated. These parameters were obtained from three ECGs consecutively recorded and the mean values were used. A single investigator blinded to the clinical and laboratory findings of the patients reviewed all ECGs to validate the measured intervals. All patients showed sinus rhythm and an RR interval longer than 521 ms and shorter than 1,111 ms (Bazett's formula considers values outside this range to be unreliable).

According to the American Heart Association/American College of Cardiology (AHA/ACC) guidelines, QTc was considered prolonged if \geq 450 ms in males, or \geq 460 ms in females [2].

Laboratory analysis.

Blood samples were centrifuged at 1000 rpm and serum samples were stored at -80°C.

CRP was assayed by a particle-enhanced turbidimetric method (COBAS-6000 platform, Roche Diagnostics GmbH; Mannheim, Germany) and the values were expressed as mg/dl (normal values <0.5).Circulating levels of IL-6, TNF α and IL-1 were evaluated by multiplex assay for cytokine quantification (Bioplex, Bio-Rad, Hercules, CA). Cytokine concentrations were calculated using a standard curve established from serial dilutions of each cytokine standard as described in the manufacturer's protocol and expressed as pg/ml.

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Supplementary Table 1. Patients' characteristics by case

Patient	Age	Gender	QTc (msec)	Ongoing	CRP	Concomitant QTc-prolonging fa	ctors
				inflammatory diseases	(mg/dl)	Non-pharmacologic	Drugs
1	78	Ŷ	690	Sepsis (Enterococcus faecalis)	29.65	Left ventricular hyperthrophy,chronic kidney disease hypokaliemia	Levofloxacin,Fluconazole
2	74	0	620	Acute infective endocarditis (Staphilococcus Hominis)	20.15	Subarachnoid haemorrhage,chronic coronary artery disease,sinus bradycardia,diabetes mellitus(type II),chronic kidney disease,anti-Ro/SSA	-
3	46	Ŷ	570	Sepsis (Candida Albicans)	18.39	Chronic coronary artery disease ,hypokaliemia	Amiodarone,Ciprofloxacin
4	81	Ŷ	640	Sepsis (Staphilococcus Hominis) in HIV infection	11.69	Acute coronary syndrome, hypokaliemia, HIV infection, chronic kidney disease	Protease inhibitors
5	84	Ŷ	530	NI	11.6	Acute coronary syndrome, left ventricular hyperthrophy, anti-Ro/SSA	Sertraline
6	85	Ŷ	675	Pneumonia and sepsis	11.6	Left ventricular hyperthrophy, diabetes mellitus(type II), hypokaliemia, hypomagnesemia	Amiodarone, Sertraline
7	75	Ŷ	530	Undifferentiated chronic arthritis	10.0	Left ventricular hyperthrophy, chronic coronary artery disease, complete AVB, diabetes mellitus(type II), hypokaliemia, hypocalcemia, hypomagnesemia	-
8	74	S	500	Pneumonia (Enterobacter Cloacae)	8.76	Sinus bradycardia	Fluconazole
9	91	8	520	NI	7.87	High-grade AVB, chronic kidney disease, hypocalcemia,	-
10	80	Ŷ	530	Prosthetic mitral valve endocarditis (Staphilococcus Epidermidis)	7.53	Sinus bradycardia,hypokaliemia, hypocalcemia	2/1
11	84	Ŷ	680	Polymyalgia rheumatica	7.32	Acute complete AVB,left ventricular hyperthrophy	Amiodarone
12	85	8	660	Pneumoniae and Sepsis	7.15	Acute coronary syndrome, heart failure, left ventricular hyperthrophy	Amiodarone

				(Escherichia Coli)			
13	60	2	510	Pneumonia	5.77	Acute coronary syndrome, hypokaliemia, anti-Ro/SSA	-
14	30	9	600	NI	5.55	Anorexia nervosa, hypokaliemia, anti-Ro/SSA	Fluoxetine,Clozapine, Clomipramine
15	53	0	520	Rheumatoid arthritis	5.5	Acute coronary syndrome, heart failure, diabetes mellitus(type II), hypocalcemia, anti-Ro/SSA	-
16	88	Ŷ	640	NI	4.21	Acute coronary syndrome, left ventricular hyperthrophy, hypokaliemia	Amiodarone, Chlarythromici
17	68	8	520	Sepsis (Staphilococcus Haemoliticus)	3.81	Hypokaliemia, hypocalcemia, chronic kidney disease, diabetes mellitus(type II), chronic coronary artery disease	Promazine,Prometazine, Dexmedetomidine
18	79	2	610	NI	3.22	Acute coronary syndrome, hypokaliemia, anti-Ro/SSA	Amiodarone
19	77	Ŷ	620	NI	3.16	Acute complete AVB	Donezepil
20	81	Ŷ	660	NI	3.12	Dilated cardiomyopathy	Amiodarone
21	95	Ý	570	NI	2.73	Hypothiroidism, left ventricular hyperthrophy, anti-Ro/SSA	Sertraline
22	65	Ŷ	560	NI	2.66	Hypokaliemia, hypocalcemia	Quetiapine, Nortriptyline, Trimipramine
23	78	8	660	Acute pancreatitis	2.1	Hypocalcemia,hypokaliemia,anti-Ro/SSA	Amiodarone
24	82	Ŷ	600	Urinary tract infection	1.7	Hypokaliemia, hypomagnesemia, anti-Ro/SSA	Citalopram,Promazine, Chlarythromicine
25	85	Ŷ	495	Polymyalgia rheumatica	1.59	Acute II° degree 2:1 AVB, dilated cardiomyopathy, hypokaliemia	Citalopram
26	87	Ŷ	550	Rheumatoid arthritis	1.21	Heart failure,complete AVB,hypothyroidism,chronic kidney disease	-
27	82	Ŷ	660	NI	1.14	Chronic coronary artery disease,hypokaliemia,hypocalcemia,anti-Ro/SSA	Amiodarone
28	81	8	580	Acute cholecystitis	1.1	Sinus bradycardia,hypokaliemia, chronic kidney disease,diabetes mellitus(type II)	Escitalopram,Memantine
29	68	8	490	NI	0.91	Hypokaliemia,left ventricular hyperthrophy,liver cirrhosis,anti-Ro/SSA	-
30	71	Ŷ	530	NI	0.74	Dilated cardiomyopathy, hypocalcemia, anti-Ro/SSA	Amiodarone
31	84	Ŷ	660	NI	0.68	Left ventricular hyperthrophy, diabetes mellitus(type II), hypokaliemia, hypocalcemia	-
32	81	Ŷ	630	NI	0.67	left ventricular hyperthrophy,acute complete AVB,anti- Ro/SSA	27
33	91	Ŷ	650	-	0.44	II° degree 2:1 AVB left ventricular hyperthrophy, hypokaliemia, hypocalcemia, amti-Ro/SSA	-
34	85	Ŷ	730	-	0.36	Hypocalcemia, hypokaliemia, sinus bradycardia	-
35	72	Ŷ	910	-	0.3	Hypocalcemia, hypomagnesemia,	Levofloxacin, Fluconazole.

Heart

						anti-Ro/SSA	Citalopram
36	67	3	620	-	0.23	Sinus bradycardia, left ventricular	Leuprolide
						hyperthrophy, hypokaliemia,	
37	85	9	600	-	0.19	Sinus bradycardia and AVBs(II-III° degree), hypokaliemia	Amiodarone
38	77	Ŷ	570	-	0.1	Diabetes mellitus(type II),anti-Ro/SSA	Sotalol
39	45	6	520	-	0.1	Acute coronary syndrome, left ventricular hyperthrophy	-
						,hypokaliemia,hypocalcemia,anti-Ro/SSA	
40	87	4	580	-	0.1	Chronic coronary artery disease, heart failure, left	Sertraline, haloperidol
						ventricular hyperthrophy diabetes mellitus(type II),	_

rio-ventricular block; HIV:human immunodetneseuxy virus, w.... CRP: C-reactive protein; AVB:atrio-ventricular block; HIV:human immunodeficiency virus; anti-Ro/SSA: anti-Ro/SSA antibodies; NI: not identified.

Supplementary Table 2. Demographic, clinical and laboratory characteristics of rheumatoid arthritis patients and healthy controls.

	RA	НС
Patients,n	10	10
Age, years	56.8±10.2	55.5±4.3
Females,n	8(80%)	8(80%)
Disease duration, years DAS28* Patients with DAS28 $>$ 5.1* n	14.4±11.4 5.8±1.3 6(60%)	-
Tatients with DA528 > 5.1°, it	0(0078)	-
CRP,mg/dl	1.89(0.73-5.46)	0.09(0.09-0.28)
Patients with CRP > 0.5 mg/dl,n	10(100%)	0
IL-6,pg/ml	11.52(0.49-75.75)	0.49(0.49-1.25)
TNFalpha,pg/ml	1.38(0.61-33.63)	0.60 (0.60-3.24)
IL-1,pg/ml	0.18(0.11-5.73)	0.15(0.08-0.29)

RA: rheumatoid arthritis; HC: healthy controls; DAS28: disease activity score in 28 joints; CRP: C-reactive protein; IL-6: interleukin-6; TNFalpha: Tumor necrosis factor alpha; IL-1beta: interleukin-1beta. Values are expressed as mean ±standard deviation, or median (range).

*DAS28 <2.6: disease remission; 2.6-3.2: mild active disease; 3.2-5.1: moderate active disease; <5.1: severe active disease[18].

Heart

Supplementary Table 3. QTc interval changes in the Inflammatory cohort [during active disease (PRE) and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST)], as assessed by using three alternative correction formulae, and their correlation with the QTc calculated with the Bazett's formula.

	QTc Fridericia	QTc Framingham	QTc Hodges
PRE (ms)	430.9±29.1	429.1±27.4	429.4±26.9
POST (ms)	419.3±27.9	419.8±27.5	418.0±28.2
ΔQTc (ms)	- 11.6	- 9.3	-11.4
р	0.0008	0.0047	0.0005
Correlation with Q	Tc Bazett		
rho	0.88	0.86	0.83
p	<0.0001	<0.0001	<0.0001

QTc: corrected QTc interval; rho=Spearman's rank correlation coefficient.

Differences in QTc values (PRE/POST) were evaluated by the two-tail Wilcoxon matched pairs test. Correlations with the QTc calculated with Bazett's formula were evaluated by the Spearman rank correlation test.





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Heart

LEGEND TO SUPPLEMENTARY FIGURE

<section-header><section-header><section-header><section-header><section-header><section-header><section-header> Supplementary Figure 1. Electrocardiographic findings of a patient with Torsades de pointes (TdP) and high-grade systemic inflammation. ECG strip in sinus rhythm (A) and during TdP (B,C) from patient #4 who presented sepsis (*Staphylococcus Hominis*), and has elevated CRP levels (11.69 mg/dl) and a QTc of 640 ms. Vertical lines in lead V1 show QT interval.