









ORIGINAL RESEARCH

Transient Hypogonadism Is Associated With Heart Rate–Corrected QT Prolongation and Torsades de Pointes Risk During Active Systemic Inflammation in Men

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BACKGROUND: Systemic inflammation and male hypogonadism are 2 increasingly recognized “nonconventional” risk factors for long-QT syndrome and torsades de pointes (TdP). Specifically, inflammatory cytokines prolong, while testosterone shortens the heart rate–corrected QT interval (QTc) via direct electrophysiological effects on cardiomyocytes. Moreover, several studies demonstrated important interplays between inflammation and reduced gonad function in men. We hypothesized that, during inflammatory activation in men, testosterone levels decrease and that this enhances TdP risk by contributing to the overall prolonging effect of inflammation on QTc.

METHODS AND RESULTS: We investigated (1) the levels of sex hormones and their relationship with inflammatory markers and QTc in male patients with different types of inflammatory diseases, during active phase and recovery; and (2) the association between inflammatory markers and sex hormones in a cohort of male patients who developed extreme QTc prolongation and TdP, consecutively collected over 10 years. In men with active inflammatory diseases, testosterone levels were significantly reduced, but promptly normalized in association with the decrease in C-reactive protein and interleukin-6 levels. Reduction of testosterone levels, which also inversely correlated with 17- β estradiol over time, significantly contributed to inflammation-induced QTc prolongation. In men with TdP, both active systemic inflammation and hypogonadism were frequently present, with significant correlations between C-reactive protein, testosterone, and 17- β estradiol levels; in these patients, increased C-reactive protein and reduced testosterone were associated with a worse short-term outcome of the arrhythmia.

CONCLUSIONS: During systemic inflammatory activation, interleukin-6 elevation is associated with reduced testosterone levels in males, possibly deriving from an enhanced androgen-to-estrogen conversion. While transient, inflammatory hypotestosteronemia is significantly associated with an increased long-QT syndrome/TdP risk in men.

Key Words: interleukin-6 ■ QTc interval ■ sudden cardiac arrest ■ systemic inflammation ■ testosterone ■ torsades de pointes

The heart rate–corrected QT interval (QTc), a surrogate of the average action potential duration in ventricular myocytes, is widely used in the clinical practice as an important marker of arrhythmic risk.^{1,2}

In fact, whenever QTc prolongs over 470 ms in men or 480 ms in women, a condition designated as long-QT syndrome (LQTS), the vulnerability to malignant ventricular arrhythmias, particularly torsades de pointes

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CLINICAL PERSPECTIVE

What Is New?

- In male patients with active inflammatory diseases, testosterone levels were significantly reduced, but normalized within days in association with C-reactive protein and interleukin-6 decrease. Reduction of testosterone levels, which also inversely correlated with 17- β estradiol over time, significantly contributed to mediate inflammation-associated heart-rate corrected QT prolongation.
- In men with torsades de pointes, both active systemic inflammation and hypogonadism were frequently present, with significant correlations between C-reactive protein, testosterone and 17- β estradiol levels. In these patients, increased C-reactive protein and reduced testosterone tended to be associated with worse arrhythmia outcomes.

What Are the Clinical Implications?

- During systemic inflammatory activation, interleukin-6 elevation correlates with reduced testosterone levels in males, possibly deriving from an enhanced androgen-to-estrogen conversion. While transient, inflammatory hypotestosteronemia significantly associates with heart rate-corrected QT interval prolongation and torsades de pointes risk.
- In patients with inflammatory conditions, hypotestosteronemia and heart rate-corrected QT interval prolongation, a prompt and effective control of the underlying inflammatory disease might be a crucial measure to lower arrhythmic risk.
- Administration of interleukin-6 blocking drugs and testosterone replacement therapy could represent additional antiarrhythmic interventions in the short term, particularly in patients with torsades de pointes refractory to conventional treatments.

Nonstandard Abbreviations and Acronyms

| | |
|-------------|----------------------------------|
| LQTS | long-QT syndrome |
| QTc | heart rate-corrected QT interval |
| SHBG | sex hormone-binding globulin |
| TdP | torsades de pointes |

(TdP), progressively increases (QTc >500 ms, high risk; QTc >600 ms, very high risk).² TdP is a polymorphic ventricular tachycardia characterized by a pattern of twisting points, which specifically develops in patients with LQTS, and that can degenerate in ventricular

fibrillation (VF) and sudden cardiac death.³ LQTS is traditionally classified as congenital, attributable to inherited mutations in genes encoding for cardiac ion channels and regulatory proteins, or acquired.^{4,5} While congenital LQTS is relatively rare,⁶ acquired LQTS is common⁷ and recognizes many possible causes, primarily medications blocking the human ether-à-go-go potassium channel, and electrolyte imbalances (hypokalemia, hypocalcemia, and hypomagnesemia),^{2,5} but also structural heart diseases, bradyarrhythmias, endocrine and liver diseases, nervous system injuries, starvation, hypothermia, and toxins.^{5,8} All these factors have in common the aptitude to induce the dysfunction of ≥ 1 of the channels regulating the action potential duration, leading to an inward shift in the balance of the total currents. However, all of the above “conventional” risk factors cannot explain all cases of LQTS/TdP development and recurrence observed in clinical practice.²

In this scenario, an increasing number of “non-conventional” risk factors for LQTS/TdP are recently emerging,^{9–12} among which systemic inflammation¹³ and male hypogonadism¹⁴ represent 2 of the most recognized. A large body of clinical and experimental evidence has demonstrated that inflammatory activation can prolong action potential duration/QTc^{15–17} and increase TdP risk^{15,17} via direct modulatory effects of interleukin-6 and other cytokines on several cardiac ion currents, principally the rapid and the slow components of the delayed rectifier potassium current, the transient outward current, and the L-type calcium current (inflammatory cardiac channelopathies).^{13,16,18–21} Accumulating evidence also supports an important role for male hypogonadism in promoting LQTS/TdP,^{22–25} mainly explained by the removal of the physiological action potential duration shortening effect of testosterone on the ventricular cardiomyocyte.²⁶ In fact, it is well demonstrated that testosterone can decrease QTc by both increasing the rapid and slow components of the repolarizing delayed rectifier potassium current, and decreasing the depolarizing L-type calcium current.¹⁴ Accordingly, it has long been known that women normally have a longer QTc than men and are at a greater risk of developing TdP.²

In addition, several studies provided evidence for a direct interplay between inflammation and depressed gonad function in males.²⁷ Testosterone levels are frequently reduced in men with chronic inflammatory diseases,²⁸ and already in 1989 Rivier and Vale²⁹ had demonstrated how hypogonadism could be induced by injecting proinflammatory cytokines in rats. Consistent findings have been also recently reported in men with severe COVID-19,³⁰ an acute inflammatory disease characterized by high-level circulating cytokines along with an increased prevalence of QTc prolongation and life-threatening ventricular arrhythmias.^{31,32}

Several mechanisms may account for these changes, both central on gonadotropin secretion and peripheral on androgen-to-oestrogen conversion.²⁸ In fact, inflammatory cytokines, such as interleukin-6, interleukin-1, and tumor necrosis factor- α , can directly inhibit gonadotropin-releasing hormone secretion in the hypothalamus, and luteinizing hormone (LH) release from anterior pituitary,³³ as well as stimulate in adipose stromal cells the enzyme aromatase, which catalyzes the biotransformation of testosterone to estradiol.^{34,35}

Based on this background, it is likely that, during inflammatory activation in men, testosterone levels decrease and that this contributes to the overall prolonging effect of inflammation on QTc. Thus, the aim of the present study was to analyze the acute impact of systemic inflammation on circulating levels of testosterone and other sex hormones in men, also defining whether these alterations play a role in increasing the risk of LQTS/TdP. To address this objective, we investigated (1) the levels of sex hormones and their relationship with inflammatory markers and QTc duration in male patients with different types of acute inflammatory diseases, during the active phase and recovery; and (2) the association between inflammatory markers and sex hormones in a cohort of male patients who developed extreme QTc prolongation and TdP, consecutively collected from the general population over 10 years.

METHODS

The authors declare that all supporting data are available within the article and its online supplemental files.

A local ethics committee approved the study, and patients from all groups gave their oral and written informed consent in accordance with the Principles of the Declaration of Helsinki.

Study Populations

To evaluate the influence of systemic inflammatory activation on sex hormones in men and their relationship with QTc, we prospectively collected 22 men with elevated CRP (C-reactive protein) levels attributable to different inflammatory diseases, including acute infections, chronic immune-mediated diseases during flares, or acute microcrystalline arthritis (inflammatory cohort). These subjects underwent blood sample withdrawals and ECG recordings during the active phase (PRE), and after different pharmacological treatments resulting in a CRP decrease >75% when compared with baseline conditions (POST). None of the patients in this cohort was under treatment with drugs potentially influencing circulating levels of sex hormones, specifically androgen-deprivation therapy (including gonadotropin-releasing hormone receptor

agonists/antagonists, cytochrome-17 inhibitors, non-steroidal androgen-receptor antagonists) or opioids.³⁶ Moreover, we excluded subjects presenting with or developing electrolyte abnormalities (hypokalemia, hypocalcemia, hypomagnesemia) or atrial fibrillation/flutter (needing more complicate methods for QT interval correction). Patients taking QT interval-prolonging medications with conditional, possible, or known risk of TdP (www.crediblemeds.org) were excluded only if they were treated with these drugs in PRE, but not in POST conditions, that is, those withdrawing liable drugs between PRE and POST (but keeping patients maintaining the same liable drug between PRE and POST). Detailed demography and clinical features of the inflammatory cohort are reported in Table S1.

A second cohort of 10 men, comparable in terms of age and comorbidities (comorbidity controls) but without signs of systemic inflammation, was enrolled as a confirmatory group to further compare laboratory and ECG findings of the inflammatory cohort, in both PRE and POST conditions (demography, clinical, and laboratory details depicted in Table S1).

Finally, male subjects belonging to a cohort of 75 consecutive TdP patients, prospectively collected in our institution in the period 2008 to 2020 independent of ongoing therapies and concomitant diseases, were also studied to analyze the putative relationship existing between sex hormones and inflammatory activation. During this 12-year period, a total of 30 men with TdP came to our observation. However, 7 were excluded for ongoing treatments with drugs potentially affecting sex hormones (androgen-deprivation therapy for prostatic cancer, n=5; methadone maintenance therapy, n=2). Moreover, in 4 additional subjects, a blood sample obtained within 24 hours from TdP to perform laboratory investigations was not available. As a result, 19 male patients with TdP were included in the study (TdP cohort), whose demographic, clinical, and laboratory characteristics are reported in Table S2. Notably, inflammatory cohort patients and comorbidity controls were selected to be comparable in terms of age with these 19 patients with TdP (TdP cohort: 76 [45–91] years; inflammatory cohort: 79 [36–93] years; comorbidity controls: 78.5 [67–85] years).

Laboratory Analysis

All subjects under study underwent a blood withdrawal in the morning (before 11:00 AM) to measure circulating levels of inflammatory markers and sex hormones. Specifically, in patients with TdP, the blood sample was obtained within 24 hours of arrhythmia occurrence. In the other subjects (inflammatory cohort and controls), blood draws and ECG recordings were simultaneous. Measurements of CRP and cytokines (including interleukin-6, interleukin-1, interleukin-10, and tumor necrosis

factor- α) are detailed in Data S1. The levels of the following sex hormones and related proteins were assessed: testosterone (total, free, and bioavailable), sex hormone-binding globulin (SHBG), 17 β -estradiol, progesterone, follicle-stimulating hormone (FSH), and LH. All measurements were performed by an automatic chemiluminescent immunoassay system. Testosterone (total), LH, and FSH were measured by UniCel Dxl 800 (Beckman Coulter), while SHBG, dehydroepiandrosterone sulfate, androstenedione, progesterone, and 17- β estradiol levels by Immulite 2000 (Siemens). Following the current guidelines of the European Society of Endocrinology/European Academy of Andrology,³⁷ free and bioavailable testosterone were calculated according to a standard formula (available at <http://www.issam.ch/freetesto.htm>) based on SHBG and total testosterone concentration, considering albumin as a constant (average albumin concentration of 4.3 g/dL). This parameter more accurately reflects the level of bioactive testosterone than does the sole measurement of total serum testosterone. Testosterone circulates in plasma unbound (free, ~2%–3%), bound to specific plasma proteins (SHBG), and weakly bound to nonspecific proteins such as albumin. The SHBG-bound fraction is biologically inactive, because of the high binding affinity of SHBG for testosterone. Free testosterone measures the free fraction; bioavailable testosterone includes free plus weakly bound to albumin.

ECG Recordings

Measurements of heart rate, RR interval, QT interval, and QTc (Bazett's formula) in patients with inflammatory diseases and controls was automated and obtained from 3 ECG strips consecutively recorded. A single investigator (cardiologist) blinded to the clinical and laboratory findings of the patients reviewed all ECGs to validate the measured intervals. Since the Bazett's formula may over- or underestimate QTc at higher and lower heart rates, respectively, we additionally evaluated QTc using alternative correction formulas, ie Fridericia [QT/RR interval^{1/3}], and Hodges [QT+1.75 (heart rate–60)], the latter being recognized as the correction formula showing the least heart rate dependence.³⁸ Since all the subjects in the study were men, QTc was considered prolonged if >470 ms, in accordance with the American Heart Association/American College of Cardiology recommendations.²

Diagnosis of TdP was based on the presence of at least 1 episode of polymorphic ventricular arrhythmia with a rate ranging from 160 to 240 beats/min, associated with QTc prolongation.⁹ In patients with TdP, the QT interval was manually measured on a standard 12-lead ECG.³⁹

The details of QTc measurement in different groups are provided in Data S1.

Statistical Analysis

Descriptive statistics is reported as frequency count and percentage for qualitative data, and mean \pm standard deviation (SD) or median and interquartile range for quantitative data.

We first performed a sample size and power analysis (more details are provided in Data S1) to define the number of subjects to include in the inflammatory cohort for comparison in PRE versus POST conditions, as well as in the control group. Specifically, by using a nonparametric approach based on the 2-sided Wilcoxon test, and considering $\alpha=0.05$, $1-\beta=0.85$, and an effect size of 0.7, a sample size of 22 patients was obtained. Based on this first sample, a further power analysis was then performed to select the size of the control group. In this case, by considering the use of a 2-sided Mann-Whitney test, with $\alpha=0.05$, $1-\beta=0.80$, effect size=1.1, and a ratio between the size of groups of about 2:1, a control group size of 10 patients was calculated.

Because of sample size in each group and data distribution, the following nonparametric statistical analyses were carried out: the 2-tailed Wilcoxon test or the 2-tailed Mann-Whitney test to evaluate differences in quantitative variables between 2 groups of data paired (inflammatory markers, sex hormones, and ECG parameters in patients with inflammatory disease, PRE versus POST) or unpaired (comparisons of sex hormones in inflammatory disease or patients with TdP versus controls; testosterone and 17- β estradiol in patients with TdP with CRP >2 mg/dL versus CRP <2 mg/dL), respectively; the Spearman rank correlation to verify possible statistical association between quantitative variables in patients with inflammatory disease (sex hormones versus CRP, interleukin -6, or QTc; testosterone versus 17- β estradiol; interleukin -6 versus QTc) or TdP (sex hormones versus CRP; testosterone versus 17- β estradiol); and the 2-sided McNemar test to evaluate statistical association between categorical variables in patients with inflammatory diseases (QTc prolongation, PRE versus POST). The Bonferroni correction for multiple tests was applied when patients with inflammatory diseases in PRE/POST conditions were compared with controls. Moreover, the Kruskal-Wallis test, and Dunn multiple comparison post hoc test, were performed to evaluate differences in quantitative variables (age, total testosterone) among 3 groups (patients with inflammatory disease, patients with TdP, controls; patients with inflammatory disease, PRE, patients with TdP with CRP >2 mg/dL, controls; patients with inflammatory disease, POST, patients with TdP with CRP <2 mg/dL, controls). 17- β estradiol assay had a detection limit of 20 pg/mL. Statistical handling of values <20 pg/mL is detailed in Data S1.

Finally, mediation analysis (R, version 4.0.1; R Foundation for Statistical Computing) was performed to evaluate the role of testosterone as mediator of part

of the effects of interleukin-6 on QTc. Three linear regressions were performed: one with QTc as outcome and interleukin-6 as the independent variable to estimate the effect a ; one with the addition of testosterone to estimate a' and c ; and one with testosterone as outcome and IL-6 as independent variable (b). The resultant mediating effect of testosterone on the relationship between interleukin-6 and QTc (a'') was tested using a mediation model by means of a bootstrap method.

$P \leq 0.05$ were considered significant (InStat, version 3.06 for Windows 2000; GraphPad Software).

RESULTS

Levels of Sex Hormones in Male Patients With Inflammatory Diseases and Relationship With Inflammatory Markers

In male patients with active inflammatory diseases, total testosterone levels were significantly lower than in noninflamed comorbidity controls (Table 1; Table S2; Figure S1A), with a significant proportion of inflamed patients (11/22; 50%) showing levels ≤ 1.1 ng/mL, that is, falling into the female range according to our internal laboratory reference values. Free and bioavailable testosterone levels also showed a significant reduction in these subjects when compared with controls (Table 1; Table S1; Figure S1B and S1C). Circulating 17- β estradiol was conversely increased, and in almost a quarter of cases (5/22; 23%) they reached values ≥ 100 pg/mL, as normally observed in premenopausal women only⁴⁰ (Table 1; Table S1; Figure S1D). The other sex hormones evaluated, including progesterone, FSH, and LH, as well as SHBG, did not show any significant difference when inflamed patients were compared with controls (all $P > 0.05$; 2-tailed Mann-Whitney test) (Table 1; Table S1).

Depending on the specific inflammatory disease present, treatment with antibiotics or anti-inflammatory drugs was associated with a prompt (mean follow-up time 14.7 \pm 18.1 days, median 10.0 [2–90] days) and marked decrease of CRP (median decrease, 85.3%), despite no complete return to control levels. In POST conditions, testosterone levels increased (total, free, bioavailable), while 17- β estradiol decreased, in all cases reaching values not significantly different from controls (Figure 1A through 1D; Table 1; Table S1; Figure S1). In POST conditions, only 18% (4/22) and 5% (1/22) of the patients showed total testosterone levels ≤ 1.1 ng/mL, and 17- β estradiol levels ≥ 100 pg/mL, respectively. A strong inverse correlation between testosterone and CRP levels was observed over time (Figure 2A through 2C). Moreover, total testosterone also inversely correlated with 17- β estradiol concentration (Figure S2A). No significant variations of SHBG, progesterone, FSH,

and LH levels were observed when PRE and POST conditions were compared (Table 1). In particular, it is important to highlight the absence of significant modifications in gonadotropin concentrations despite significant variations in testosterone levels.

Circulating inflammatory cytokine concentration, specifically interleukin-6, was elevated during active disease, and significantly decreased after treatment to near normal values (no complete return to control levels) (Table 1). Levels of interleukin-6 inversely correlated over time with those of testosterone, even more strongly than observed for CRP (Figure 2D through 2F), as well as directly with those of 17- β estradiol (Figure S2B). Interleukin-1, tumor necrosis factor- α , and interleukin-10 were all within normal reference values in the PRE condition, and did not show any noticeable modification after therapeutic interventions (Table 1).

QTc in Male Patients With Inflammatory Diseases and Relationship With Testosterone and Interleukin-6 Levels

In patients with active inflammatory diseases, median values of QTc were 472.0 (416–544), that is, over the currently recognized limit for QTc prolongation in men (470 ms). Accordingly, in PRE conditions, QTc prolongation was a common finding, with almost a quarter of the subjects even showing marked QTc prolongation (>500 ms) (Table 1).

Systemic inflammation recovery attributable to therapeutic interventions was associated with a significant reduction of median QTc duration, and QTc >470 ms prevalence (Table 1), inversely correlating with testosterone levels over time (Figure 3A through 3C). Other laboratory parameters, including electrolytes, renal function, or echocardiography findings, were normal at baseline and did not show significant changes throughout the study period (Table S3). Because heart rate was higher in our patients in PRE versus POST conditions because of inflammation-induced sympathetic activation,^{18,41,42} we repeated the same analysis with 2 alternative QT-correction formulas (ie, Fridericia, and Hodges) to exclude that a QTc overestimation by Bazett's formula at higher heart rates could have biased the results. Again, QTc-Fridericia and QTc-Hodges significantly reduced after treatment and maintained a significant inverse association with testosterone levels (Table 1; Figure S3A through S3F). No association was present between QTc and other sex hormones, including gonadotropins, regardless of the specific formula used (Table S4).

At the same time, we found a robust direct correlation over time between QTc duration and interleukin-6 levels (Figure 4A), again persisting also when QTc-Fridericia or QTc-Hodges were alternatively considered (Figure S4). The strength of the association existing

Table 1. Laboratory, Electrocardiographic, and Echocardiography Parameters in Male Patients With and Without Inflammation

| Parameter | Male patients with inflammatory diseases (n=22) | | | Male patients without inflammation (controls) (n=10) |
|--|---|---------------------------------------|----------|--|
| | Before therapeutic intervention (PRE) | After therapeutic intervention (POST) | P value | |
| CRP, mg/dL (r.v. <0.5) | 10.9 (7.9–23.2) | 1.6 (0.4–2.5) | <0.0001* | 0.18 (0.1–0.4) |
| Interleukin-6, pg/mL (r.v. 0.49–1.25) | 14.0 (10.1–53.2) | 2.8 (0.8–12.4) | <0.0001* | 0.3 (0.1–7.2) |
| Interleukin-1, pg/mL (r.v. 0.08–0.29) | 0.27 (0.2–0.4) | 0.26 (0.2–0.4) | 0.30 | 0.05 (0.0–0.1) |
| Tumor necrosis factor- α , pg/mL (r.v. 0.60–3.24) | 0.75 (0.6–0.8) | 0.75 (0.6–0.9) | 0.68 | 0.42 (0.4–0.8) |
| Interleukin-10, pg/mL (r.v. 0–3.60) | 0.56 (0.5–0.8) | 0.55 (0.4–1.6) | 0.59 | 0.46 (0.4–0.6) |
| Total testosterone, ng/mL | 1.10 (0.7–1.5) | 1.90 (1.5–2.6) | <0.001* | 2.22 (1.4–3.3) |
| Free testosterone, ng/mL | 0.021 (0.009–0.027) | 0.031 (0.019–0.043) | <0.001* | 0.044 (0.024–0.05) |
| Bioavailable testosterone, ng/mL | 0.50 (0.2–0.6) | 0.76 (0.5–1.0) | <0.001* | 1.02 (0.6–1.2) |
| SHBG, nmol/L | 56.8 (23.1–48.3) | 40.2 (30.0–56.2) | 0.46 | 39.8 (34.4–53.5) |
| 17- β estradiol, pg/mL | 37.9 (10.8–103.4) | 15.5 (10.0–39.8) | 0.0052* | 16.7 (10.0–27.2) |
| Progesterone, ng/mL | 0.10 (0.1–0.1) | 0.10 (0.1–0.3) | 0.46 | 0.10 (0.1–0.2) |
| FSH, mIU/mL | 11.0 (4.8–19.0) | 10.2 (5.1–22.5) | 0.23 | 6.8 (4.9–14.6) |
| LH, mIU/mL | 6.9 (0.5–28.0) | 6.9 (1.9–26.7) | 0.54 | 4.0 (1.0–21.0) |
| QT, ms | 394 (361–423) | 398 (370–420) | 0.78 | 406 (368–421) |
| RR, ms | 758 (655–840) | 833 (731–992) | <0.001* | 995 (868–1074) |
| Heart rate, bpm | 79.0 (71.8–91.8) | 72.5 (60.0–82.0) | <0.001* | 60.6 (56.3–71.1) |
| QTc-Bazett, ms | 472.0 (436–499) | 444.5 (426–452) | <0.001* | 417.0 (397–423) |
| Patients with prolonged QTc [†] , n (%) | 12 (55) | 2 (9) | 0.004* | 0 (0) |
| Patients with QTc >500 ms, n (%) | 5 (23) | 1 (5) | 0.13 | 0 (0) |
| QTc-Fridericia, ms | 436.5 (417–474) | 423.5 (407–443) | 0.0033* | 408.0 (386–422) |
| Patients with prolonged QTc [†] , n (%) | 6 (27) | 1 (5) | 0.09 | 0 (0) |
| Patients with QTc >500 ms, n (%) | 2 (9) | 1 (5) | 1.0 | 0 (0) |
| QTc-Hodges, ms | 436.5 (413–466) | 420.5 (406–439) | 0.0037* | 411.0 (384–422) |
| Patients with prolonged QTc [†] , n (%) | 5 (23) | 1 (5) | 0.13 | 0 (0) |
| Patients with QTc >500 ms, n (%) | 1 (5) | 1 (5) | 1.0 | 0 (0) |

Cytokine level ranges measured in an internal reference group of healthy controls. Therapeutic interventions resulted in a >75% CRP decrease when compared with the baseline. Data are expressed as median (interquartile range) or frequency (percentage). Differences in continue variables were evaluated by the 2-tailed Wilcoxon matched pairs test. Difference in categorical variables were evaluated by McNemar test. CRP indicates C-reactive protein; FSH, follicle-stimulating hormone; LH, luteinizing hormone; QTc, heart rate-corrected QT interval; r.v., reference values; and SHBG, sex hormone-binding globulin.

*Statistically-significant p-values (<0.05).

[†]QTc >470 ms.

between QTc and interleukin-6 was even stronger than that observed between QTc and testosterone levels (QTc-Bazett/interleukin-6: $\rho=0.72$ versus QTc-Bazett/total testosterone: $\rho=-0.49$), thereby suggesting that testosterone lowering may represent, as anticipated, only 1 of the mechanisms by which inflammation, via interleukin-6 increase, can promote QTc prolongation. To provide support to this premise, a mediation analysis was performed. The total effect of interleukin-6 on QTc ($a=0.66$; $P<0.001$) was composed of a direct effect $a'=0.55$ ($P<0.001$), and an indirect effect $a''=0.11$ ($P=0.039$) mediated by the reduction of testosterone levels (Figure 4B). In practice, it means that for every 1 pg/mL increase of interleukin-6, a mean QTc prolongation of 0.66 ms is observed, $\approx 20\%$ of which is attributable to the testosterone-lowering potential

of this cytokine (on average, 0.11 ms every 1 ng/mL decrease of testosterone). Thus, although the direct effect explains most of the QT-prolonging activity of interleukin-6 ($\approx 80\%$), concomitant testosterone reduction also plays a significant role in the determinism of interleukin-6-associated QTc prolongation.

Inflammation and Sex Hormones Levels in Male Patients With Torsades de Pointes

In our cohort of consecutive, unselected male patients with TdP, signs of systemic inflammatory activation, as demonstrated by elevated CRP levels (>0.5 mg/dL), were present in the large majority of cases. Specifically, systemic inflammation was significantly active (CRP >2 mg/dL) in approximately one-half of subjects and

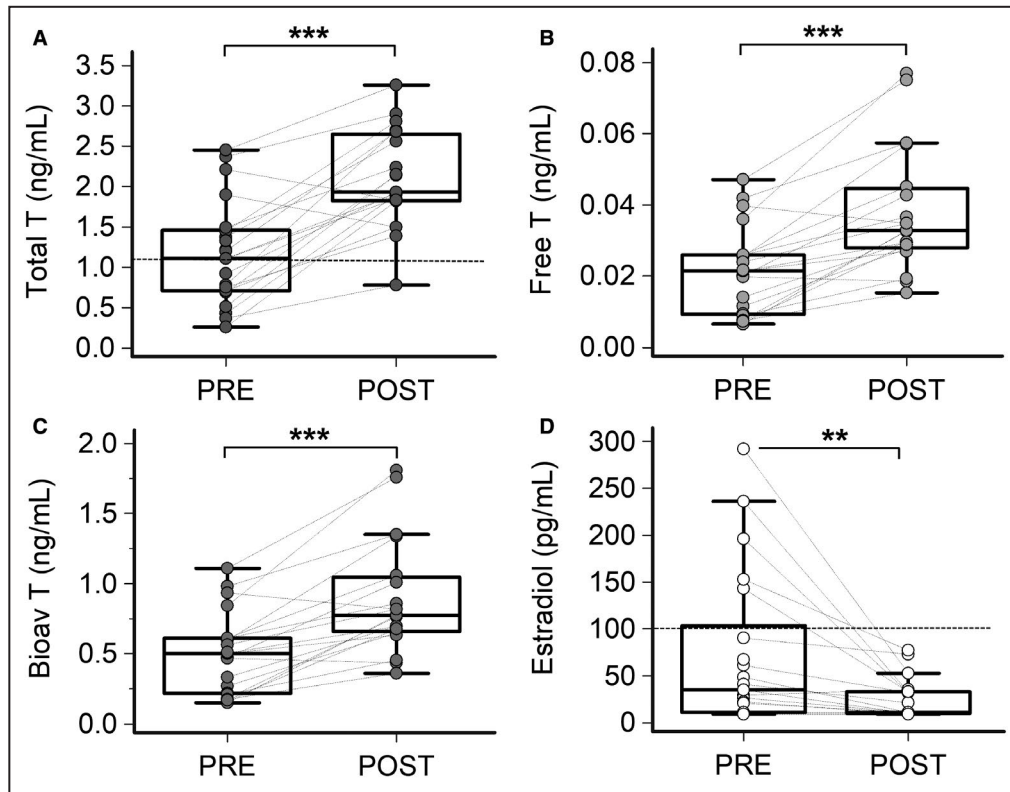


Figure 1. Testosterone and 17- β estradiol levels in male patients with inflammatory diseases, during active disease (PRE) and after therapeutic interventions resulting in a C-reactive protein decrease >75% (POST) when compared with the baseline.

A, Total testosterone; 2-tailed Wilcoxon matched-pairs test, *** P <0.001. Values below the horizontal dotted line indicate a female range (upper limit of reference values in premenopausal women, ie, 1.1 ng/mL). **B**, Free testosterone; 2-tailed Wilcoxon matched-pairs test, *** P <0.001. **C**, Bioavailable testosterone; 2-tailed Wilcoxon matched-pairs test, *** P <0.001. **D**, 17- β estradiol; 2-tailed Wilcoxon matched-pairs test, ** P <0.01. Values above the horizontal dotted line indicate a female range (lower limit of reference values in premenopausal women, ie, 100 pg/mL). Patients, n =22. Bioav indicates bioavailable; Estradiol, 17- β estradiol; and T, testosterone.

highly active (CRP >5 mg/dL) in approximately one-third (Table 2; Figure 5A). A definite inflammatory disease was identified in 10 of 19 patients (53%), most frequently acute infections, but also immune-mediated diseases during flares or acute pancreatitis (Table 2).

In these subjects, significantly lower testosterone levels were observed when compared with controls (1.10 [0.14–3.89] versus 2.22 [0.66–4.29] ng/mL; P =0.035; 2-tailed Mann-Whitney test). Hypogonadism (testosterone levels <2.5 ng/mL) was present in most patients (14/19; 74%), frequently a profound hypogonadism reaching very low values as per the female range (\leq 1.1 ng/mL in 9/19; 47%) (Table 2; Table S2). Free and bioavailable testosterone levels were consistently reduced (both P =0.031, 2-tailed Mann-Whitney test), while circulating 17- β estradiol was higher than in controls (39.6 [10.0–214.0] versus 16.7 [10.0–44.0] pg/mL; P =0.034, two-tailed Mann-Whitney test), with 5 of 19 patients with TdP (26%) showing values \geq 100 pg/mL, as usually observed

in premenopausal women⁴⁰ (Table 2; Table S2). Progesterone, FSH, LH, and SHBG levels were not significantly different when patients with TdP were compared with controls (all P >0.05; 2-tailed Mann-Whitney test) (Table 2; Table S2).

Circulating levels of testosterone (total, free, and bioavailable) showed a robust inverse correlation with CRP, in turn directly associated with 17- β estradiol concentration (Figure 5B through 5E). Moreover, an inverse association between total testosterone and 17- β estradiol levels was also found (ρ =−0.46; P =0.045; Spearman test). To further analyze the relationship between sex hormones and the degree of inflammatory activation in men with TdP, we divided the subjects in 2 groups based on the presence or not of significantly active systemic inflammation (cutoff: CRP, 2 mg/dL). Despite no significant age difference between the 2 groups (78 [53–91] versus 74 [45–89] years; P =0.97; 2-tailed Mann-Whitney test), patients with TdP with CRP>2 mg/dL displayed total, free, and

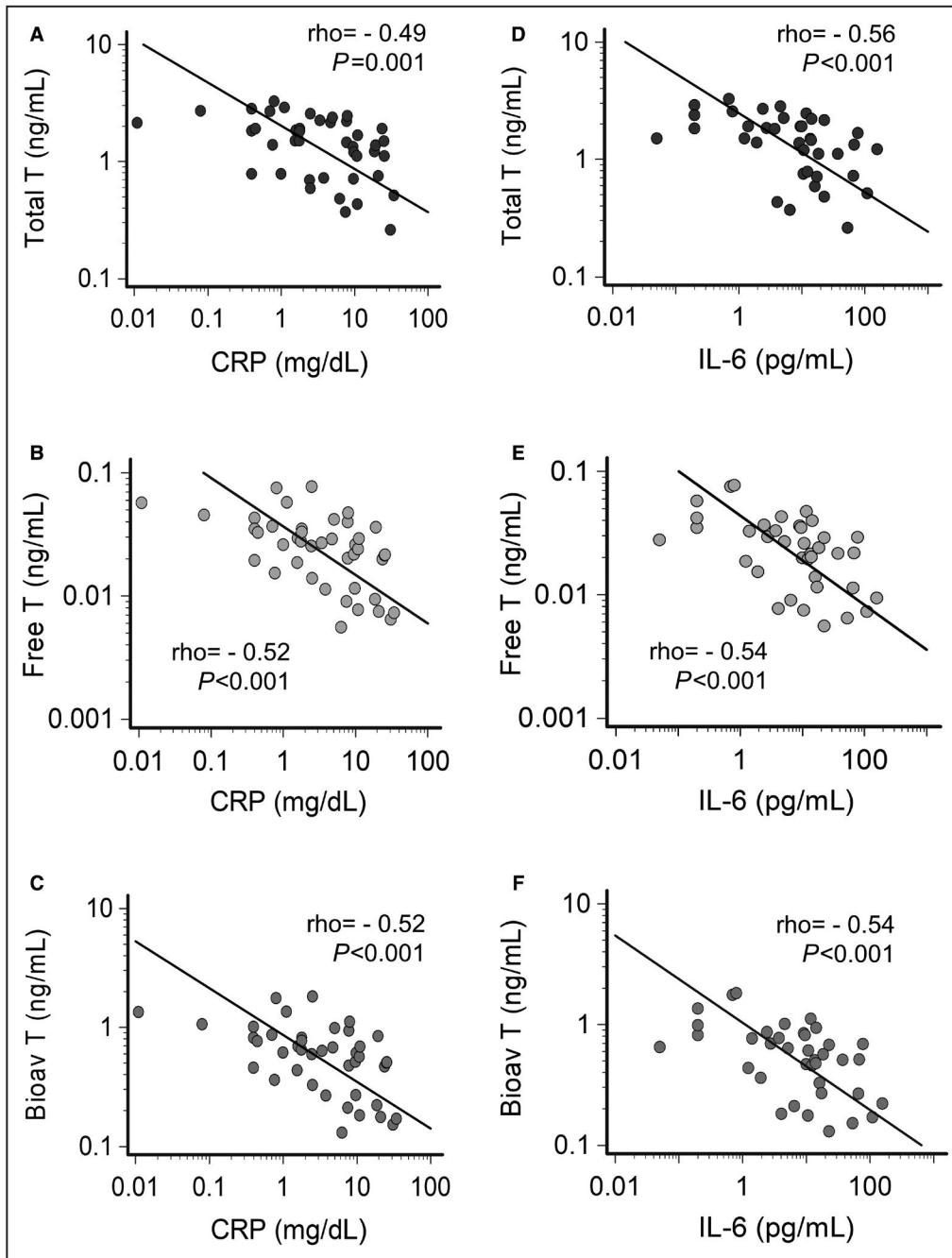


Figure 2. Correlation between testosterone levels and inflammatory markers in male patients with inflammatory diseases.

A, Relationship between total testosterone and CRP levels. **B**, Relationship between free testosterone and CRP levels. **C**, Relationship between bioavailable testosterone and CRP levels. **D**, Relationship between total testosterone and IL-6 levels. **E**, Relationship between free testosterone and IL-6 levels. **F**, Relationship between bioavailable testosterone and IL-6 levels. Spearman test. Patients, $n=22$. Bioav indicates bioavailable; CRP, C-reactive protein; IL-6, interleukin-6; and T, testosterone.

bioavailable testosterone levels significantly lower (>2 times) and $17\text{-}\beta$ estradiol levels significantly higher (>4 times), respectively, when compared with those with $\text{CRP} < 2$ mg/dL (Figure 6A through 6D). Testosterone concentrations in patients with TdP with $\text{CRP} > 2$ mg/dL were comparable with those observed in males

with active inflammatory diseases (PRE), both being significantly lower when compared with controls (Figure S5A); conversely, no difference was found between TdP subjects with $\text{CRP} < 2$ mg/dL, patients with inflammatory diseases in the recovery phase (POST), and controls (Figure S5B).

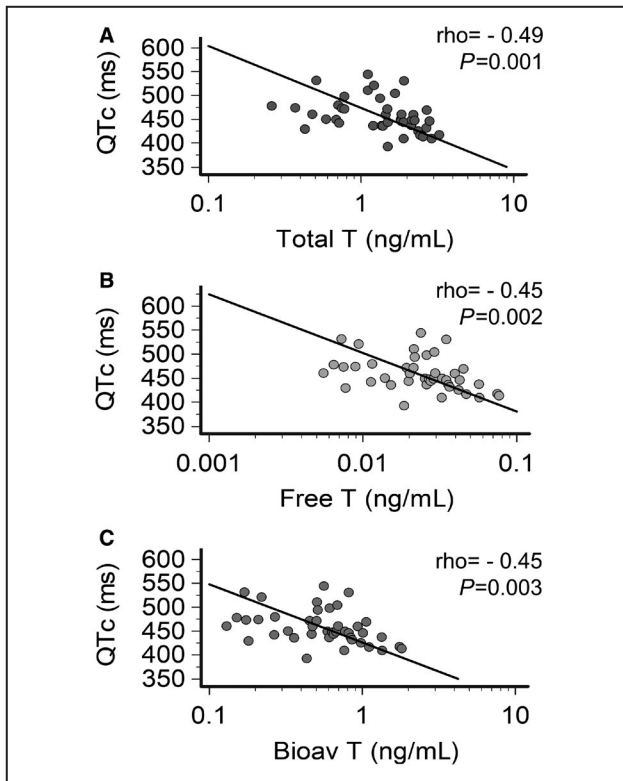


Figure 3. Correlation between QTc interval and testosterone levels in male patients with inflammatory diseases.

A, Relationship between QTc and total testosterone levels. **B**, Relationship between QTc and free testosterone levels. **C**, Relationship between QTc and bioavailable testosterone levels. Spearman test. Patients, $n=22$. Bioav indicates bioavailable; QTc, heart rate–corrected QT interval based on Bazett's formula; and T, testosterone.

In a significant proportion of cases (8/19; 42%), patients with TdP developed VF/sudden cardiac arrest and/or underwent electric shock (TdP rapidly degenerated to VF/sudden cardiac arrest [$n=2$]; out-of-hospital VF/sudden cardiac arrest followed by direct current shock, only later revealing to be a manifestation of TdP episodes [$n=2$]; direct current shock for sustained TdP not responsive to medical therapy [$n=4$]). Most cases of complicated TdP occurred in the group with significantly active systemic inflammation (CRP >2 mg/dL: 6/9, 67% versus CRP <2 mg/dL: 2/10, 20%; 2-sided Fisher's exact test, $P=0.069$). Moreover, patients with complicated TdP, considered as a whole, showed significantly lower testosterone levels (≈ 2.5 times) when compared with those with uncomplicated TdP (0.76 [0.14–2.62] versus 1.94 [0.79–3.89] ng/mL; 2-tailed Mann-Whitney test, $P=0.033$).

Finally, as anticipated, most patients with TdP were burdened by many concomitant traditional QT-prolonging risk factors (>5 on average), more commonly cardiac and extracardiac diseases, electrolyte abnormalities, anti-Ro/SSA antibodies, and drugs

(Table S2). Indeed, based on the multihit theory, >1 factor/hit usually needs to be present in a specific patient to disturb ventricular repolarization so critically to provoke the extreme QTc prolongation required for TdP occurrence.¹⁹ In this regard, the prevalence of severe hypogonadism (total testosterone ≤ 1.1 ng/mL, 47% of cases) was unpredictably high in our TdP cohort, as it was similar to that found for other well-recognized TdP risk factors, such as electrolyte imbalances (53%) or use of QT-prolonging medications (47%) (Table S2).

DISCUSSION

The most important findings of the present study are the following: (1) In male patients with active inflammatory diseases, regardless of specific etiology and organ localization, testosterone levels were significantly reduced, but promptly normalized within days in association with CRP and interleukin-6 level decrease; reduction of testosterone levels, which also inversely correlated with 17- β estradiol levels over time, significantly contributed to mediate the observed inflammation-induced QTc prolongation; and (2) in consecutive men with TdP, both active systemic inflammation and hypogonadism were frequently present, with significant correlations between CRP, testosterone, and 17- β estradiol levels; in these patients, increased CRP and reduced testosterone tended to be associated with a worse outcome for the arrhythmia, that is, degeneration to VF/sudden cardiac arrest and/or requirement of electric shock.

In recent years, there was a growing interest in understanding the impact of acute inflammation on the risk of arrhythmic events in general^{13,19,43,44} and of LQTS/TdP in particular,^{15,18} an awareness further emphasized by the current COVID-19 outbreak.^{31,45,46} Several underlying mechanisms have been identified to date, mostly mediated by the pleiotropic effects of inflammatory cytokines able to enhance myocardial electric instability both directly, modulating cardiac ion channels function, and indirectly, increasing the sympathetic drive on the heart.^{13,19,20,41} However, given the wide spectrum of cytokine-induced multisystem changes during inflammatory activation, which deeply affects, among others, the endocrine system,^{35,47,48} it is plausible that additional mechanisms of hormonal nature may also contribute to explain the strong link between inflammation and LQTS/TdP risk. Indeed, a key role of testosterone in regulating ventricular repolarization is increasingly recognized,^{14,23} and male hypogonadism was recently demonstrated to be a reversible cause of TdP in men.^{22,24,49} Moreover, several studies have demonstrated that gonadic function is significantly suppressed in men affected with inflammatory diseases.^{27,28} Thus, it could be anticipated that

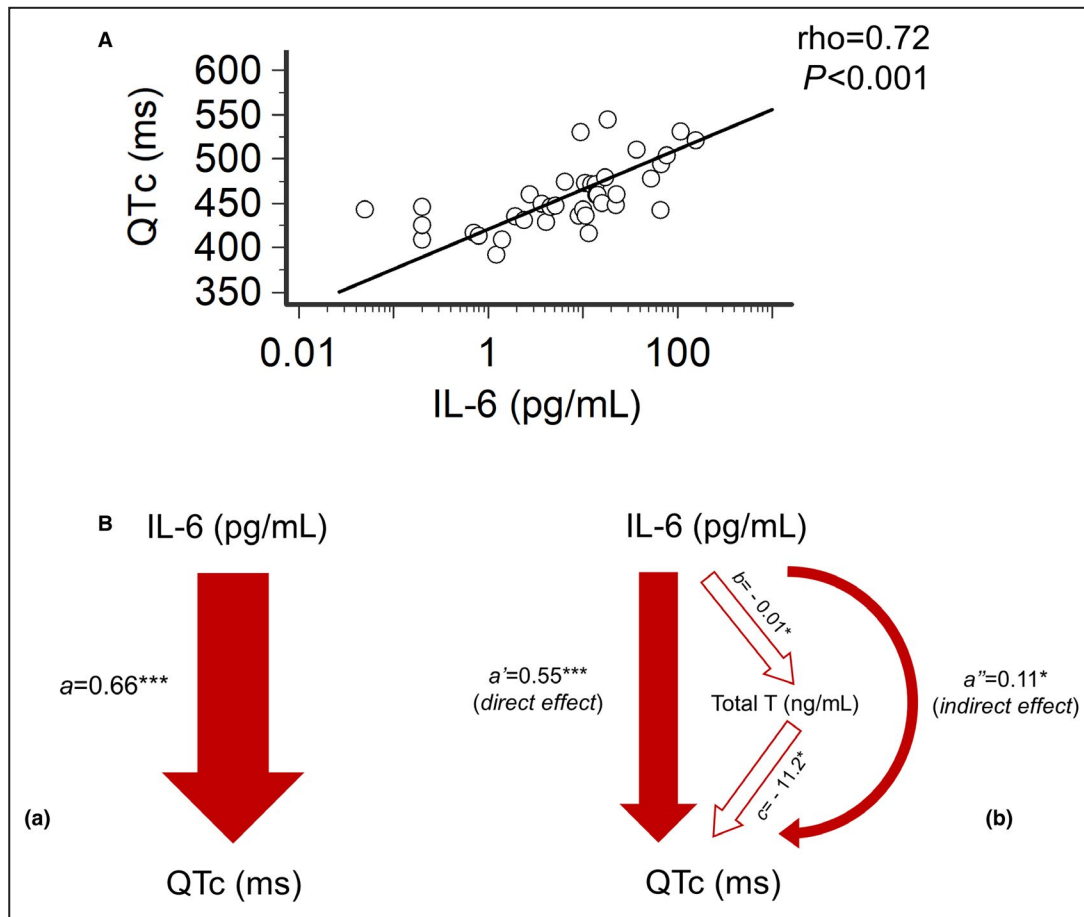


Figure 4. Correlation between IL-6 levels and QTc interval in male patients with inflammatory diseases and relative contribution of direct and indirect effects.

A, Relationship between heart rate-corrected QT interval (QTc) and IL-6 levels. Spearman test. **B**, Mediation analysis of the relationship between QTc and IL-6. **(a)** Total effect of IL-6 on QTc; ($a=0.66$). **(b)** Decomposition of IL-6 effect on QTc: the direct effect of IL-6 on QTc is expressed by the coefficient a' ($=0.55$); the indirect effect ($a''=0.11$), mediated by testosterone reduction, is the product of path coefficients b ($=-0.01$) and c ($=-11.2$). Linear regression analysis, *** $P<0.001$, * $P<0.05$. Patients, $n=22$. IL-6 indicates interleukin-6; QTc, heart rate-corrected QT interval based on Bazett's formula; and T, testosterone.

inflammation-associated hypogonadism in males is a significant contributor of the increased LQTS/TdP risk observed during the inflammatory response. The present study provides support to this hypothesis, along with important details regarding time-scale and size of the arrhythmogenic effects mediated by testosterone changes in the course of inflammation.

First, we confirmed that inflammation-driven perturbations of sex hormones in men are relevant, and for the first time demonstrated that they appear rapidly but just as quickly reverse. In fact, in our cohort of men with active inflammatory diseases of different origin and etiology, testosterone levels were significantly reduced, in one-half of the cases even reaching very low concentrations as physiologically found in premenopausal women. In these patients, disease-specific treatments leading to marked CRP lowering resulted in a prompt normalization of testosterone levels, in the course of a

few days/weeks only. Overall, circulating testosterone showed a strict inverse correlation with CRP and interleukin-6 over time. While transient, these modifications were relevant, given that during the active phase median testosterone concentration was nearly half, and the rate of marked hypogonadism 3 times more frequent when compared with the recovery period. An increased androgen-to-estrogen conversion seems to be the main underlying mechanism, likely attributable to cytokine-dependent enhancement of aromatase activity in the adipose tissue. This is supported by the evidence here provided that $17\text{-}\beta$ estradiol levels markedly increased in PRE conditions and then normalized in the POST phase, with a significant correlation with both testosterone (inverse) and interleukin-6 (direct) levels over time. Nevertheless, the fact that, despite signs of peripheral hypogonadism, no compensatory increase in gonadotropins was observed (normal

Table 2. Inflammatory Diseases, C-Reactive Protein, and Sex Hormone Levels in Male Patients With Torsades de Pointes

| | |
|----------------------------------|---------------------|
| Patients, n | 19 |
| Mean CRP*, mg/dL | 4.49±6.23 |
| Median CRP*, mg/dL | 1.72 (0.91–5.77) |
| Patients with CRP >0.5 mg/dL | 16/19 (84) |
| Patients with CRP >2 mg/dL | 9/19 (47) |
| Patients with CRP >5 mg/dL | 6/19 (32) |
| Definite inflammatory diseases | 10/19 (53) |
| Acute infections | 7/10 (70) |
| Pneumonia | 4/7 (58) |
| Sepsis | 1/7 (14) |
| Endocarditis | 1/7 (14) |
| Acute bronchitis | 1/7 (14) |
| Immune-mediated diseases | 2/10 (20) |
| Rheumatoid arthritis | 1/2 (50) |
| Undifferentiated arthritis | 1/2 (50) |
| Other | 1/10 (10) |
| Acute pancreatitis | 1/1 (100) |
| Total testosterone, ng/mL | 1.10 (0.8–2.6) |
| Free testosterone, ng/mL | 0.017 (0.009–0.043) |
| Bioavailable testosterone, ng/mL | 0.40 (0.2–1.0) |
| SHBG, nmol/L | 47.5 (28.8–74.3) |
| 17-β estradiol, pg/mL | 39.6 (10.0–108.0) |
| Progesterone, ng/mL | 0.10 (0.1–0.2) |
| FSH, mIU/mL | 5.8 (3.7–11.8) |
| LH, mIU/mL | 4.5 (2.8–10.3) |

Data are expressed as frequency (percentage), mean±SD, or median (interquartile range). CRP indicates C-reactive protein; FSH, follicle-stimulating hormone; LH, luteinizing hormone; and SHBG, sex hormone-binding globulin.

*Reference values <0.5 mg/dL.

levels in absolute, but inappropriately low for circulating testosterone) suggests that an additional central component may also contribute to the phenomenon. Accordingly, the inhibitory effects of cytokines on gonadoliberein and gonadotropin secretion are well recognized,³³ as well as that of the 17-β estradiol increase on LH release in men.⁵⁰

While these hormonal alterations, in a teleological perspective, sound understandable since reproduction is not convenient during active inflammatory illnesses, it is also conceivable that such a transient “feminization” may increase the predisposition of men to LQTS/TdP, to a similar extent to what is usually observed in women. The validity of his hypothesis is demonstrated for the first time by our results, representing the other major finding of the present study. In fact, during active inflammation, men showed a transient but significant prolongation of the QTc, which over time correlated directly with circulating interleukin-6 and inversely with testosterone levels, regardless of the specific QT-correction

formula used. Mediation analysis provided evidence that interleukin-6 is a primary factor involved in inflammation-driven QTc prolongation, both directly and indirectly, by reducing testosterone. While direct effects, probable expression of the impact of interleukin-6 on the cardiomyocyte electrophysiology,^{13,16,19} seems to be predominant, testosterone-mediated indirect effects may also add a significant contribution accounting for ≈20% of the overall changes.

A support to the clinical and epidemiological relevance of these mechanisms in promoting arrhythmogenesis is provided by the second part of the study, in which we demonstrated how in a consecutive cohort of men with TdP both inflammatory activation and hypogonadism are common and interconnected findings. In fact, at the moment of arrhythmia occurrence, active inflammatory diseases and/or significant CRP elevation along with female-range testosterone levels were found in ≈50% of subjects, with a robust inverse association between CRP and testosterone serum concentrations. In men with TdP with significantly active systemic inflammation, testosterone levels overlapped with those found in male patients of the inflammatory cohort during the active phase, thereby pointing to shared mechanisms. This view is further strengthened by the evidence that circulating 17-β estradiol significantly correlated with CRP (directly) and testosterone (inversely) levels also in patients with TdP, a finding suggestive of an increased inflammation-driven androgen-to-estrogen conversion in peripheral tissues.

Our results not only confirm in a larger cohort the datum first reported by Salem et al²² that hypogonadism is very common in men who develop TdP, but also provide a new insight in the underlying mechanisms and prognostic significance. Specifically, the present study suggests that a concomitant inflammatory activation may be the main cause of apparently unexplained testosterone deficiency in most male patients with TdP. Indeed, by reviewing the data reported by Salem et al,^{22,51} a concomitant inflammatory disease was present in 6 of 7 men with TdP with hypogonadism, that is, sepsis (n=3), endocarditis (n=2), or lung infection (n=1). In addition, the present finding that inflammation-induced male hypogonadism tends to be associated with a worse prognosis of arrhythmia could reinforce the potential importance of a prompt diagnosis and treatment of this condition. Finally, it is intriguing to speculate how such mechanisms might contribute to explain the higher COVID-19 mortality observed in men,⁵² given that in this disease high levels of circulating cytokines³¹ coexist with low testosterone concentrations,³⁰ and increased prevalence of QTc prolongation and life-threatening ventricular arrhythmias.^{31,32}

In conclusion, the present findings provide evidence that during systemic inflammatory activation,

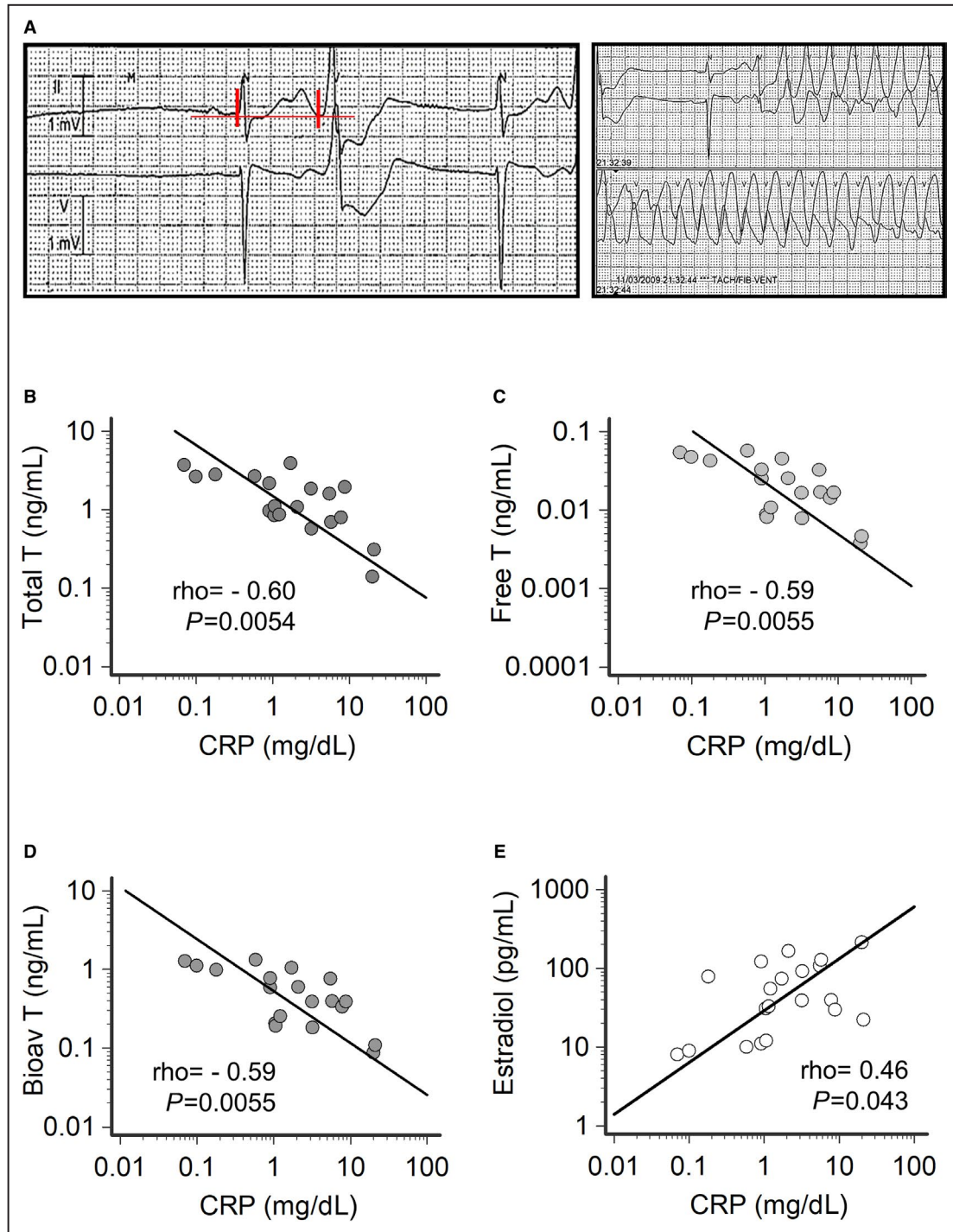


Figure 5. Correlation between testosterone, 17- β estradiol and C-reactive protein levels in male patients with Torsades de Pointes.

A, ECG strips during Torsades de Pointes from a patient with acute infective endocarditis (CRP, 20.2 mg/dL), low testosterone levels (total, 0.14 ng/mL; free, 0.0037 ng/mL; bioavailable, 0.09 ng/mL) and high 17- β estradiol levels (214 pg/mL). In this patient, torsades de pointes degenerated in ventricular fibrillation and required electric shock. Vertical red lines show QT interval (620 ms). **B**, Relationship between total testosterone and CRP levels. **C**, Relationship between free testosterone and CRP levels. **D**, Relationship between bioavailable testosterone and CRP levels. **E**, Relationship between 17- β estradiol and CRP levels. Spearman test. Patients, $n=19$. Bioav indicates bioavailable; CRP, C-reactive protein; Estradiol, 17- β estradiol; and T, testosterone.

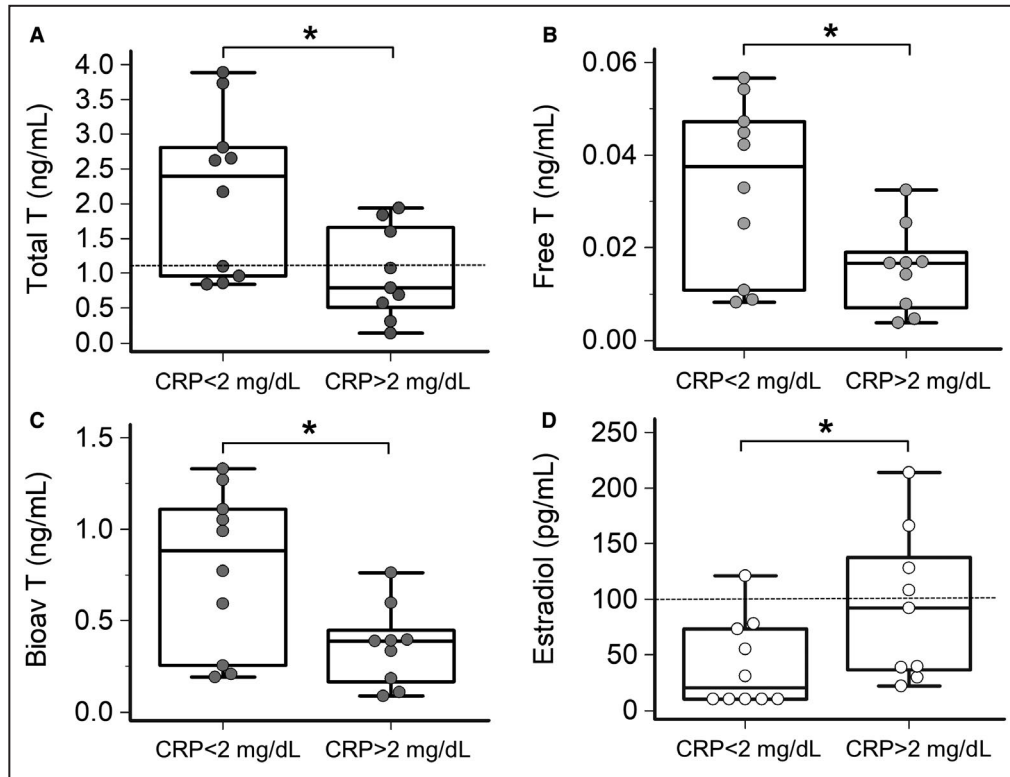


Figure 6. Comparison of testosterone and 17- β estradiol levels in male torsades de pointes patients with medium-high vs absent-low degree systemic inflammation (C-reactive protein >2 mg/dL vs <2 mg/dL).

A, Total testosterone; 2-tailed Mann-Whitney test, * $P < 0.05$. Values below the horizontal dotted line indicate a female range (upper limit of reference values in premenopausal women, ie, 1.1 ng/mL). **B**, Free testosterone; 2-tailed Mann-Whitney test, * $P < 0.05$. **C**, Bioavailable testosterone; 2-tailed Mann-Whitney test, * $P < 0.05$. **D**, 17- β estradiol; 2-tailed Mann-Whitney test, * $P < 0.05$. Values above the horizontal dotted line indicate a female range (lower limit of reference values in premenopausal women, ie, 100 pg/mL). Patients with CRP <2 mg/dL, n=10; patients with CRP >2 mg/dL, n=9. Bioav indicates bioavailable; Estradiol, 17- β estradiol; and T, testosterone.

interleukin-6 elevation is associated with reduced testosterone levels in men, possibly deriving from an enhanced androgen-to-estrogen conversion. While transient, inflammatory hypotestosteronemia may significantly contribute to acutely increase the risk of LQTS and TdP in men. The epidemiological and prognostic impact of these arrhythmogenic mechanisms seems to be relevant, as suggested by the fact that they are actively involved in a high portion of men with TdP consecutively enrolled from the general population, particularly in those with a poorer immediate outcome.

From a therapeutic perspective, our data indicate that a specific treatment of the underlying inflammatory disease might be the crucial step to interrupt the entire pathogenic cascade from the beginning. Nevertheless, they also intriguingly suggest that administration of interleukin-6–blocking drugs (tocilizumab, sarilumab)^{45,53} and testosterone replacement therapy could represent additional important antiarrhythmic interventions in the short term, particularly in patients with TdP complicated by or refractory to conventional treatments.

ARTICLE INFORMATION

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Disclosures

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Supplemental Material

Data S1
Tables S1–S4
Figures S1–S5

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SUPPLEMENTAL MATERIAL

Data S1.

SUPPLEMENTAL METHODS

Laboratory analysis.

Blood samples were centrifuged at 4000 rpm and serum samples were stored at -80°C. CRP was measured by a particle-enhanced turbidimetric method (COBAS-6000 platform, Roche Diagnostics GmbH; Mannheim, Germany) and values were reported as mg/dl (normal values <0.5). Serum concentration of inflammatory cytokines IL-6, TNF α and IL-1, and the anti-inflammatory cytokine IL-10 were evaluated by multiplex assay for cytokine quantification (Bioplex, Bio-Rad, Hercules, CA, USA). Cytokine levels 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. Since no established reference values for cytokine levels are currently available, an internal reference control group of 10 healthy subjects (mean age 55.5 \pm 4.3 years) without clinical signs of ongoing acute infections was used.

ECG recordings.

The QT interval was measured between 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. QT interval was measured in patients and controls using standard 12-lead ECG (25 mm/s and 10 mV/cm) of a commercially available recording system (Cardioline ECT WS 2000, Remco Italia, Vignate-Milano, Italy).

In patients with TdP, the QT interval was manually measured on a standard 12-lead ECG.

When prominent U waves (>1 mm) merging into T waves were present, they were included in the QT measurement. QT interval, determined as the longest hand-measured QT interval in any lead, was corrected for heart rate by the Bazett's formula to yield the QTc value. QTc was measured from 3 non-consecutive beats (mean value) by a single investigator.

Statistical analysis

Effect-size estimation. To define the number of subjects to include in the Inflammatory-cohort for comparison in PRE vs POST condition, a sample size and power analysis was performed. Given that no publications with a similar study design were found in the literature, we used a two-step approach to define the effect-size to use. In the first step, we carried on a preliminary analysis on the first 10 patients enrolled, in order to evaluate means and standard deviations of the different sex hormones in PRE and POST conditions. Since the resulting effect size varied depending on the specific hormone considered (specifically: testosterone 0.9, SHBG: 0.1, free testosterone 1.3, bioavailable testosterone 1.05, estradiol 0.7, progesterone 0.2, LH 0.45, FSH 0.7), we averaged these values, thereby obtaining an effect size of 0.7 (mean: 0.68; median 0.70). Based on this value, we then calculated a sample size of 22 patients, and then completed in the second step the enrollment of the remaining 12 patients.

Handling of 17- β estradiol values below the detection limit. The lower detection limit for the 17- β estradiol assay was 20 pg/ml. We handled this issue in the analysis by assuming that in all cases in which the result was <20 pg/ml, the value was 10 pg/ml, i.e. the middle value between 0 and 20.

Table S1. Demographic and clinical characteristics of male patients with inflammatory diseases and comorbidity controls.

| | PATIENTS | CONTROLS |
|---|-----------------|------------------|
| N | 22 | 10 |
| Age, years | 79 (73.0-86.3) | 78.5 (74.0-83.3) |
| Comorbidities | | |
| Cardiovascular disease, n | 11/22 (50%) | 7/10 (70%) |
| CAD/vasculopathy, n | 8/11 (72%) | 5/7 (71%) |
| DCM/HF, n | 3/11 (27%) | 1/7 (14%) |
| LVH, n | 2/11 (18%) | 3/7 (43%) |
| Diabetes, n | 6/22 (27%) | 1/10 (10%) |
| COPD, n | 4/22 (18%) | 2/10 (20%) |
| Chronic kidney disease, n | 1/22 (5%) | 1/10 (10%) |
| Mean CRP*, mg/dl | 14.7±9.4 | 0.24±0.2 |
| Median CRP*, mg/dl | 10.9 (7.9-23.2) | 0.18 (0.1-0.4) |
| Patients with CRP>0.5 mg/dl, n | 22/22 (100%) | 0/10 |
| Patients with CRP>2 mg/dl, n | 21/22 (95%) | - |
| Patients with CRP>5 mg/dl, n | 20/22 (91%) | - |
| Inflammatory diseases | | |
| <i>Acute infections</i> | 16/22 (73%) | - |
| Pneumonia | 8/16 (50%) | - |
| Sepsis | 2/16 (12%) | - |
| Biliary tract infection | 2/16 (12%) | - |
| Acute bronchitis | 2/16 (12%) | - |
| Urinary tract infection | 1/16 (6%) | - |
| Spondylodiscitis | 1/16 (6%) | - |
| <i>Immune-mediated diseases</i> | 4/22 (18%) | - |
| Rheumatoid arthritis | 2/4 (50%) | - |
| Inflammatory bowel disease | 1/4 (25%) | - |
| Polymyalgia rheumatica | 1/4 (25%) | - |
| <i>Other</i> | 2/22 (9%) | - |
| Acute microcrystalline arthritis | 2/2 (100%) | - |
| Therapeutic interventions for inflammatory disease | | |
| <i>Antibiotics</i> | 16/22 (73%) | - |
| Piperacillin/Tazobactam | 7/16 (44%) | - |
| Ceftriaxone | 4/16 (25%) | - |
| Levofloxacin | 2/16 (12%) | - |
| Vancomycin | 2/16 (12%) | - |
| Clarithromycin | 2/16 (12%) | - |
| Amoxicillin/Clavulanate | 1/16 (6%) | - |
| Oxacillin | 1/16 (6%) | - |
| Ceftazidime | 1/16 (6%) | - |
| Teicoplanin | 1/16 (6%) | - |
| Imipenem | 1/16 (6%) | - |
| Meropenem | 1/16 (6%) | - |
| Metronidazole | 1/16 (6%) | - |
| <i>Anti-inflammatory drugs</i> | 6/22 (27%) | - |
| Tocilizumab | 2/6 (33%) | - |
| Corticosteroids | 2/6 (33%) | - |
| Colchicine | 2/6 (33%) | - |

CRP: C-reactive protein; CAD: coronary artery disease; DCM/HF: dilated cardiomyopathy/heart failure; LVH: left ventricular hypertrophy; COPD: chronic obstructive pulmonary disease; - : not applicable.

*Reference values <0.5 mg/dl.

Data are expressed as frequency (percentage), median (interquartile range) or mean±standard deviation.

Table S2. Demographic, clinical and laboratory characteristics of male patients with Torsades de Pointes.

| | |
|---|----------------------|
| Patients,n | 19 |
| Age, years | 76 (68.0-81.0) |
| Mean QTc,ms (range) | 553.4±59.2 (480-700) |
| Mean QTc-prolonging risk factor number per patient* | 5.1±1.8 |
| Electrolyte imbalances,n | 10/19 (53%) |
| Hypokaliemia | 7/19 (37%) |
| Hypocalcemia | 5/14 (36%) |
| Hypomagnesemia | 0/12 |
| Concomitant diseases†,n | 19/19 (100%) |
| <i>Cardiac diseases</i> | 18/19 (95%) |
| Dilated cardiomyopathy/heart failure | 10/18 (56%) |
| Acute coronary syndrome | 6/18 (33%) |
| Chronic coronary artery disease | 4/18 (22%) |
| Left ventricular hypertrophy | 4/19 (22%) |
| II-III degree atrioventricular block | 4/18 (22%) |
| Bradycardia | 2/18 (11%) |
| <i>Extra-cardiac diseases</i> | 12/19 (63%) |
| Diabetes mellitus type II | 9/12 (75%) |
| Chronic kidney disease | 6/12 (50%) |
| Subarachnoid haemorrhage | 1/12 (8%) |
| Cirrhosis | 1/12 (8%) |
| Starvation | 1/12 (8%) |
| Anti-Ro/SSA positivity,n | 9/18(50%) |
| Systemic inflammation,n‡ | 16/19 (84%) |
| QTc prolonging-medications,n | 9/19 (47%) |
| Amiodarone | 4/9 (44%) |
| Trazodone | 2/9 (22%) |
| Azytromycin | 1/9 (11%) |
| Clarithromycin | 1/9 (11%) |
| Fluconazole | 1/9 (11%) |
| Sotalole | 1/9 (11%) |
| Sertraline | 1/9 (11%) |
| Bortezomib | 1/9 (11%) |
| Mean medication number per patient | 0.7±0.8 |

*Including electrolyte imbalances, diseases, anti-Ro/SSA positivity, systemic inflammation, and QTc-prolonging medications.

†Diseases recognized to be a risk factor for QTc prolongation.

‡Increased C-reactive protein level (>0.5 mg/dl) with or without a definite inflammatory disease.

Serum calcium or magnesium measurements available before replacement therapy in 14 and 12 out of 19 patients, respectively; anti-Ro/SSA antibodies tested in 18 out of 19 patients.

Data are expressed as frequency (percentage), median (interquartile range) or mean±standard deviation.

Table S3. Laboratory and echocardiography parameters, and QT-prolonging medications given in male patients with inflammatory diseases (n=22), during active disease (PRE) and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST).

| | PRE | POST | <i>p</i> |
|---|-----------|-----------|----------|
| Potassium, mEq/L (r.v.3.5-5.5) | 4.0±0.5 | 4.3±0.6 | 0.09 |
| Calcium, mg/dl (r.v.8-11) | 8.6±0.4 | 8.6±0.5 | 0.68 |
| Magnesium, mg/dl (r.v.1.5-2.5) | 2.0±0.2 | 1.9±0.3 | 0.06 |
| Creatinine, mg/dl (r.v.0.7-1.2) | 1.2±0.5 | 1.1±0.4 | 0.07 |
| pO ₂ , mmHg (r.v.70-100) | 66.3±9.7 | 73.9±9.0 | 0.19 |
| pH (r.v.7.35-7.45) | 7.46±0.03 | 7.44±0.04 | 0.13 |
| Ejection fraction, % (r.v.>50) | 55.9±5.6 | 56.8±5.2 | 0.09 |
| Left ventricular internal dimension, mm (r.v.<56) | 48.9±5.3 | 48.8±6.0 | 1.00 |
| Estimated pulmonary artery pressure, mmHg (r.v.<30) | 35.3±6.9 | 32.0±6.4 | 0.10 |
| QT-prolonging medications, n/patient | 0.6±0.7 | 0.7±0.8 | 0.25 |

r.v.: reference values.

Values are expressed as mean±standard deviation.

Differences were evaluated by the two-tail Wilcoxon matched pairs test.

Table S4. Correlations between 17-β estradiol, progesterone, gonadotropins, and QTc interval based on different correction formulas in male patients with inflammatory diseases (n=22).

| | QTc | QTc-F | QTc-H |
|--------------------------|------------------------------|------------------------------|------------------------------|
| 17-beta Estradiol | rho= -0.15 <i>p</i> =0.34 | rho=0.05 <i>p</i> =0.72 | rho=0.09 <i>p</i> =0.56 |
| Progesterone | rho=0.13 <i>p</i> =0.39 | rho=0.29 <i>p</i> =0.06 | rho=0.24 <i>p</i> =0.11 |
| FSH | rho= -0.21 <i>p</i> =0.18 | rho=0.05 <i>p</i> =0.72 | rho=0.02 <i>p</i> =0.092 |
| LH | rho=0.25 <i>p</i> =0.10 | rho= -0.10 <i>p</i> =0.51 | rho= -0.13 <i>p</i> =0.32 |

QTc: heart rate-corrected QT interval based on the Bazett's formula; QTc-F: heart rate-corrected QT interval based on the Fridericia's formula; QTc-H: heart rate-corrected QT interval based on the Hodges's formula; FSH: follicle stimulating hormone; LH: luteinizing hormone.

Correlations were evaluated by the Spearman test.

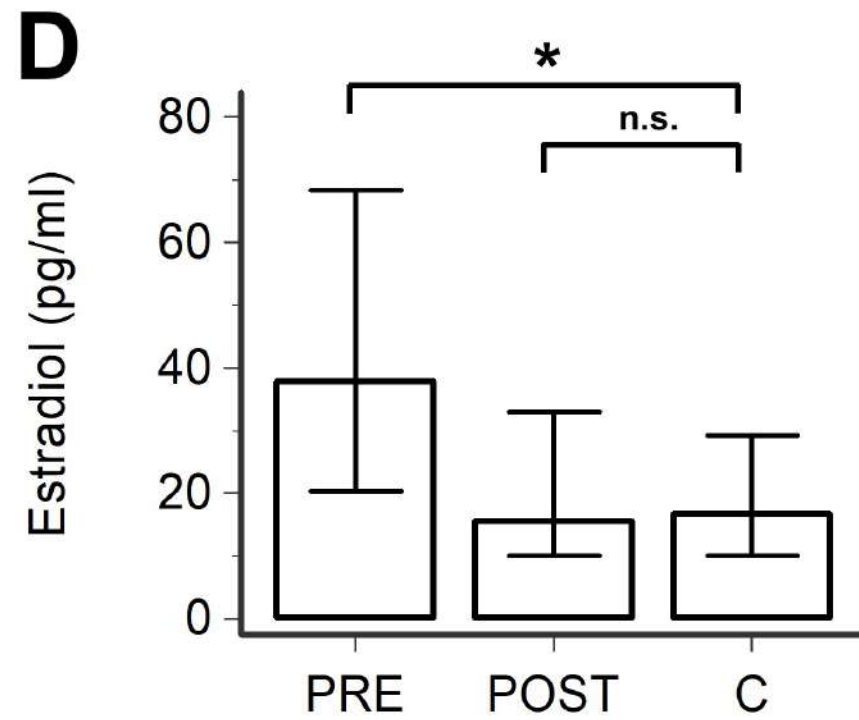
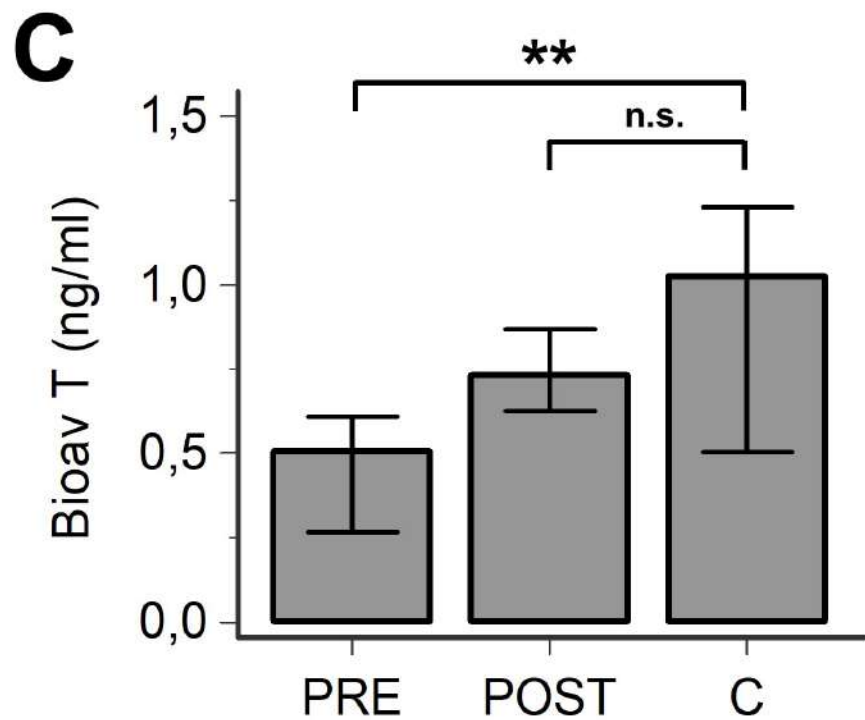
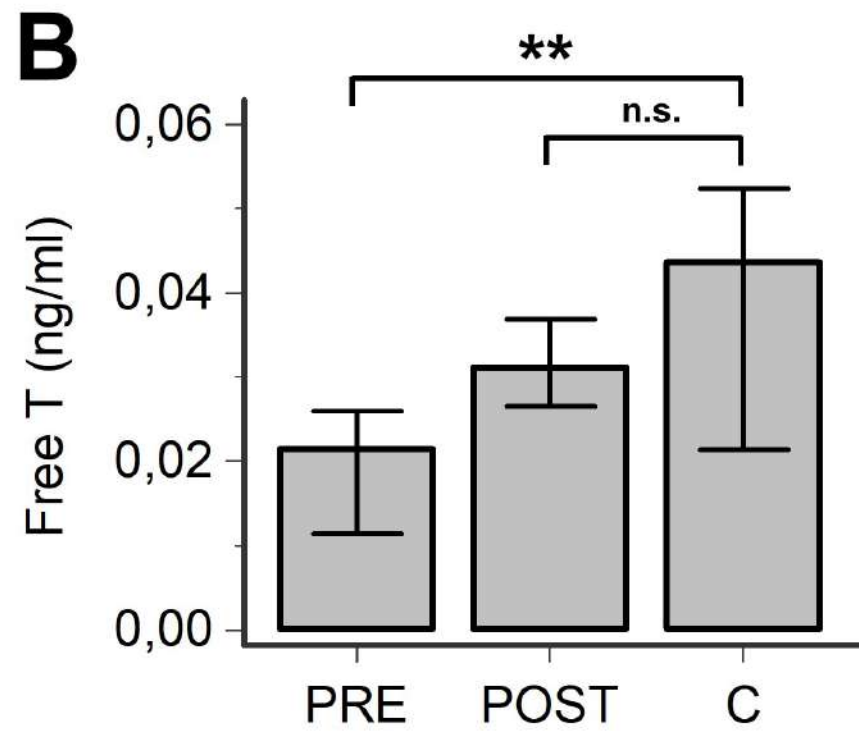
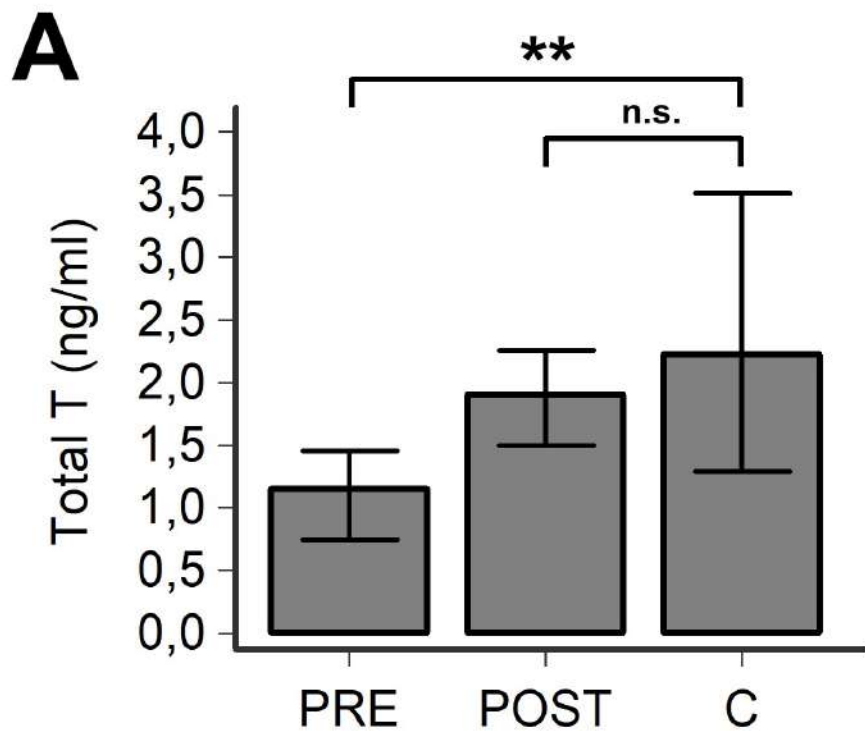


Figure S1

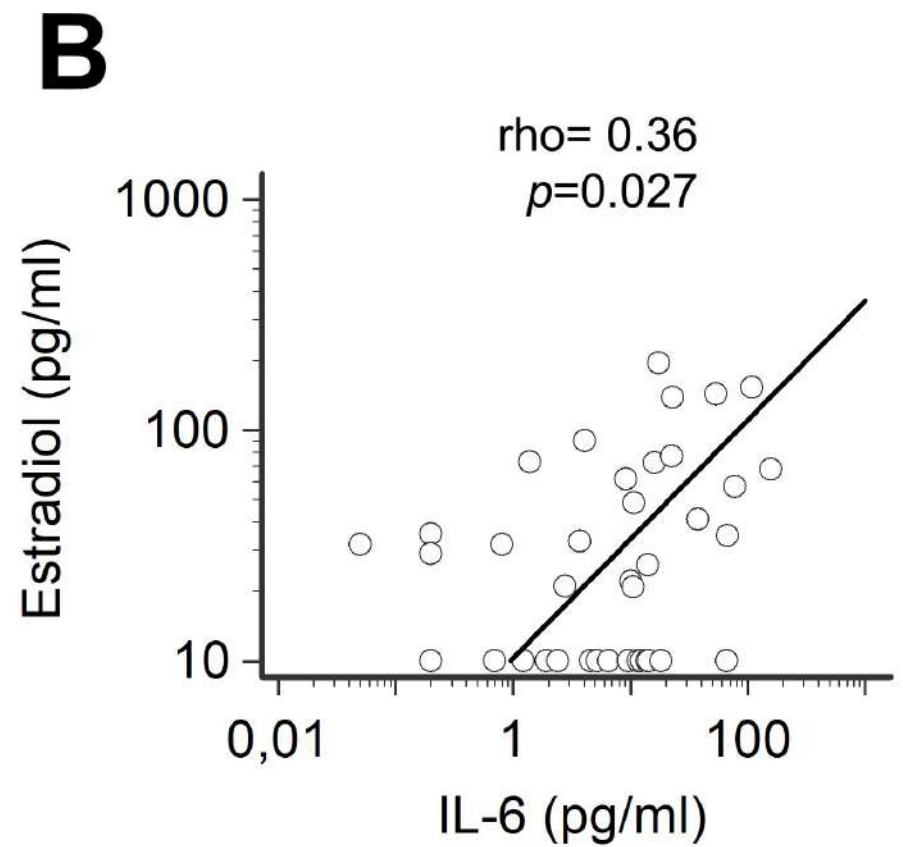
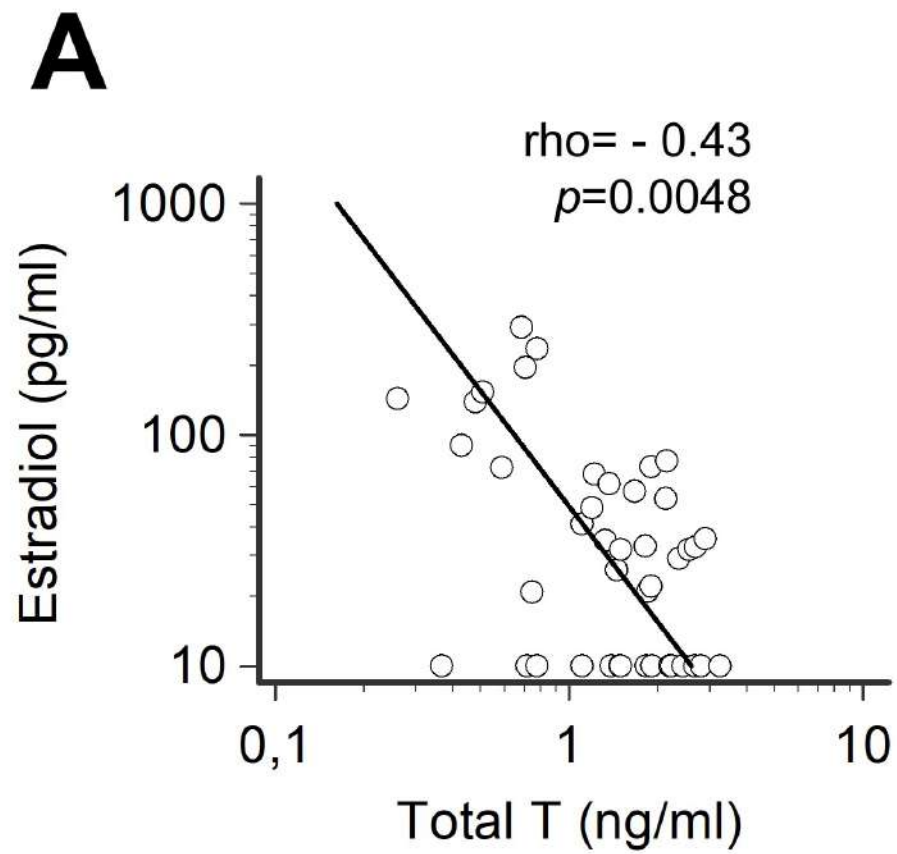


Figure S2

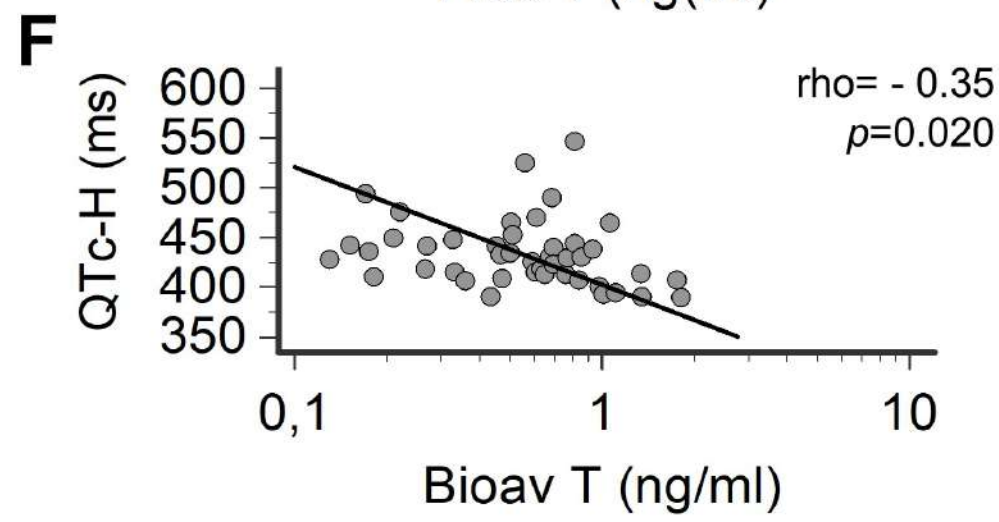
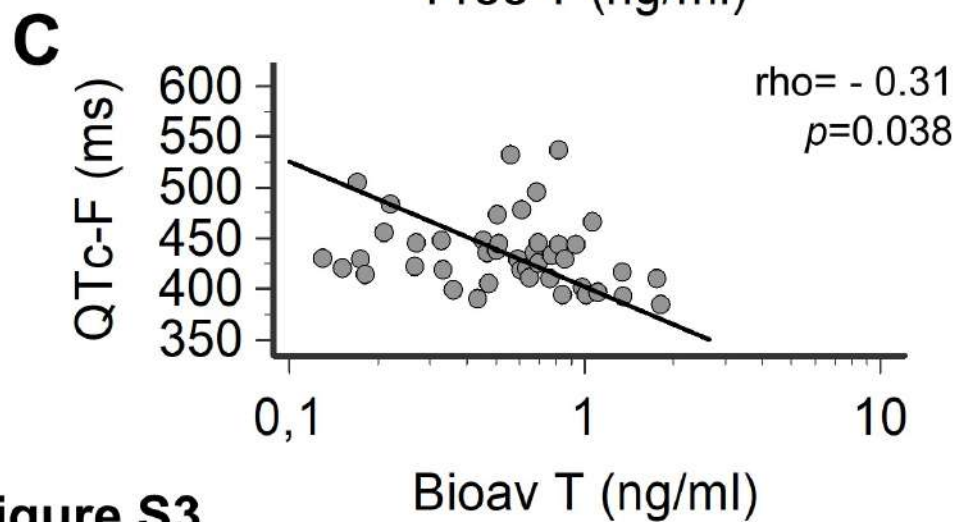
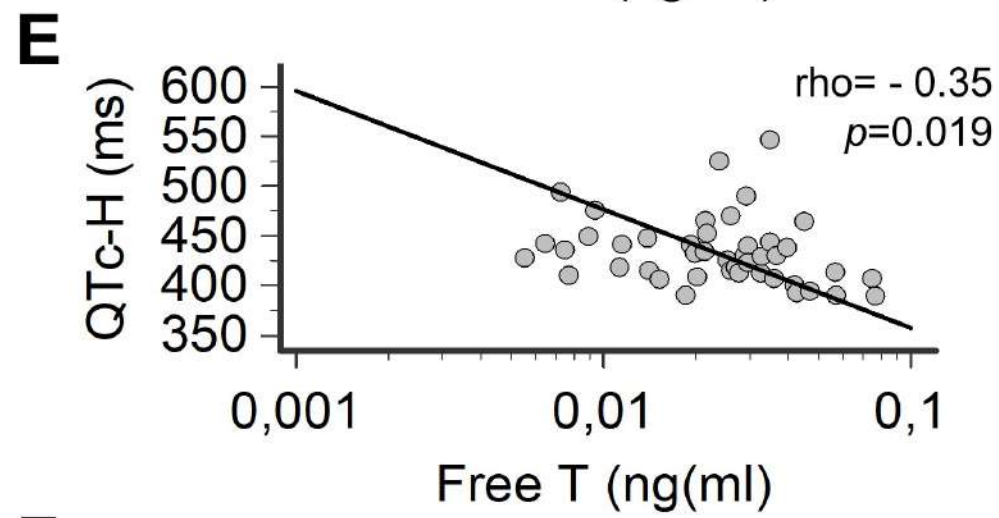
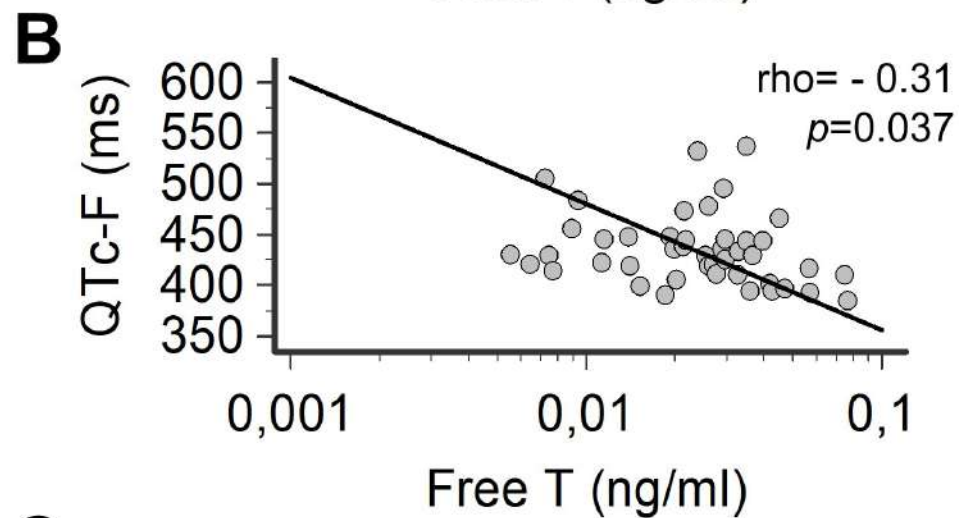
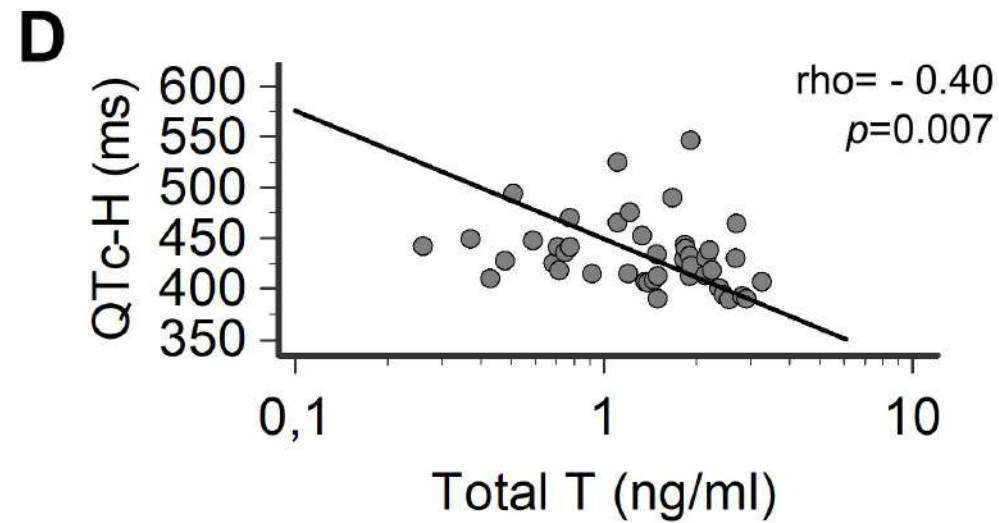
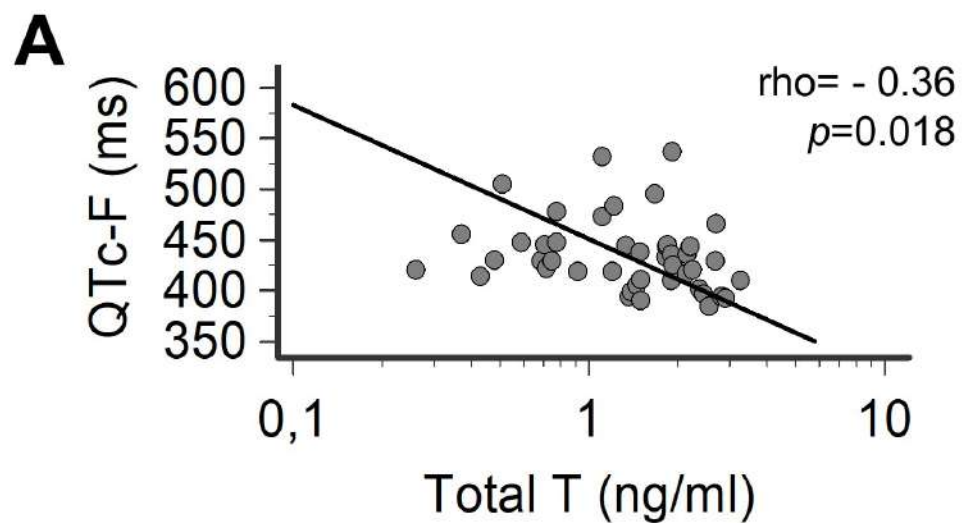


Figure S3

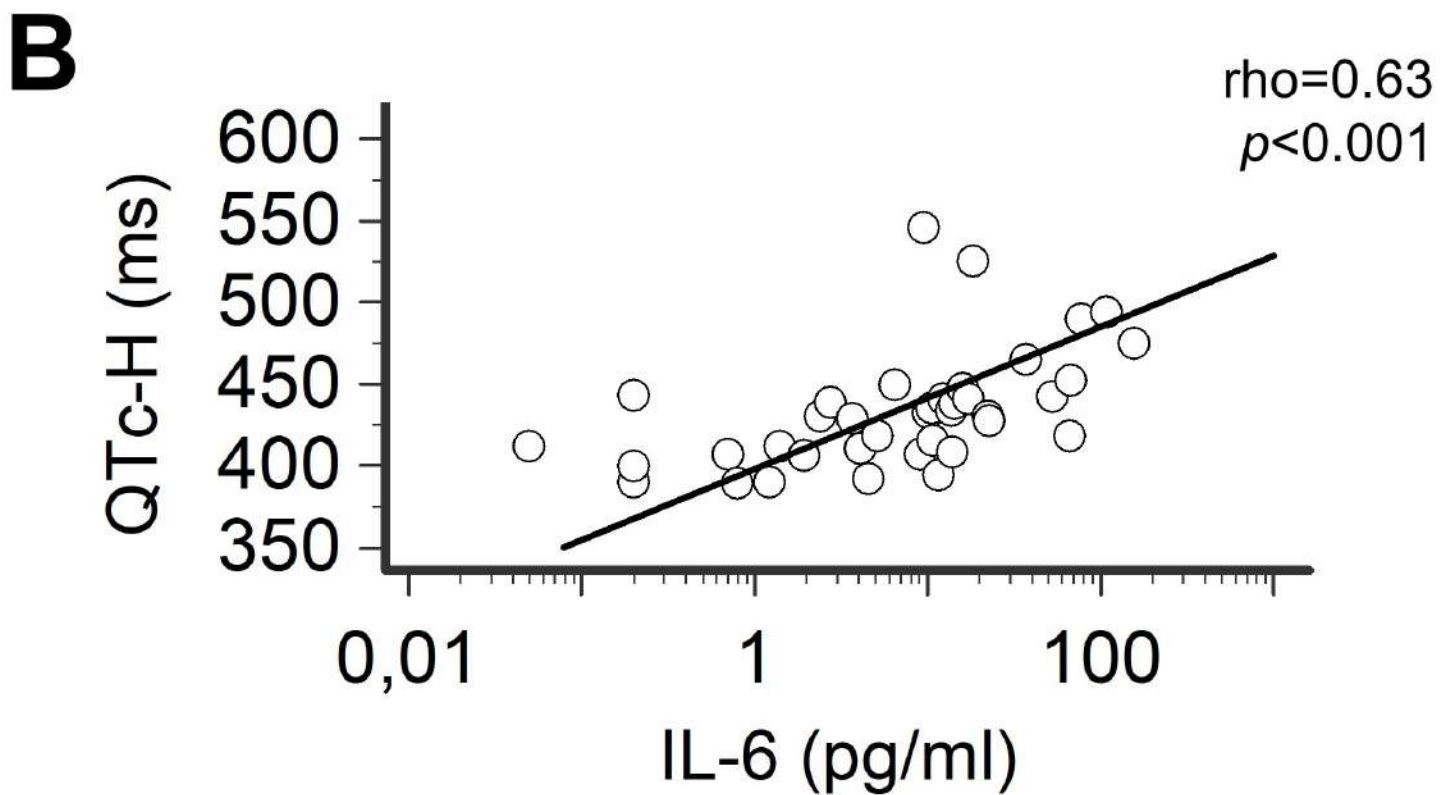
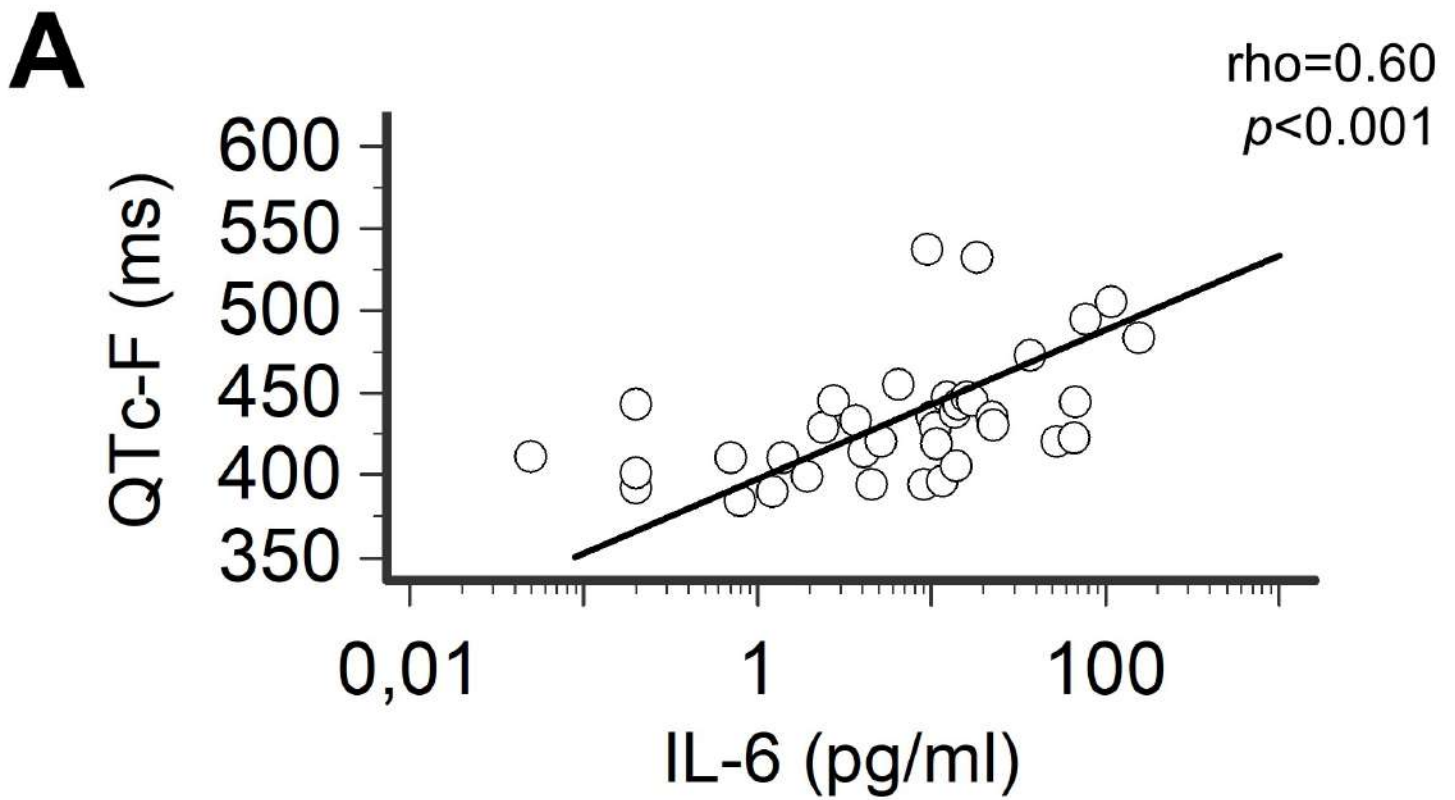


Figure S4

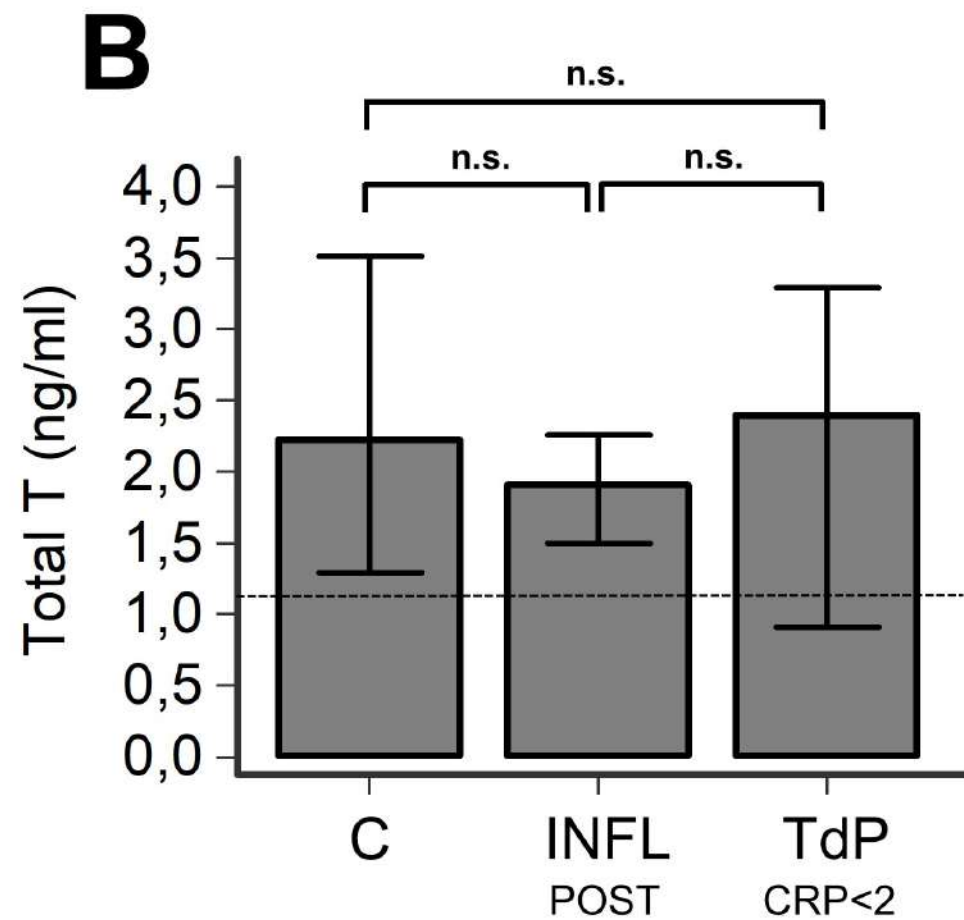
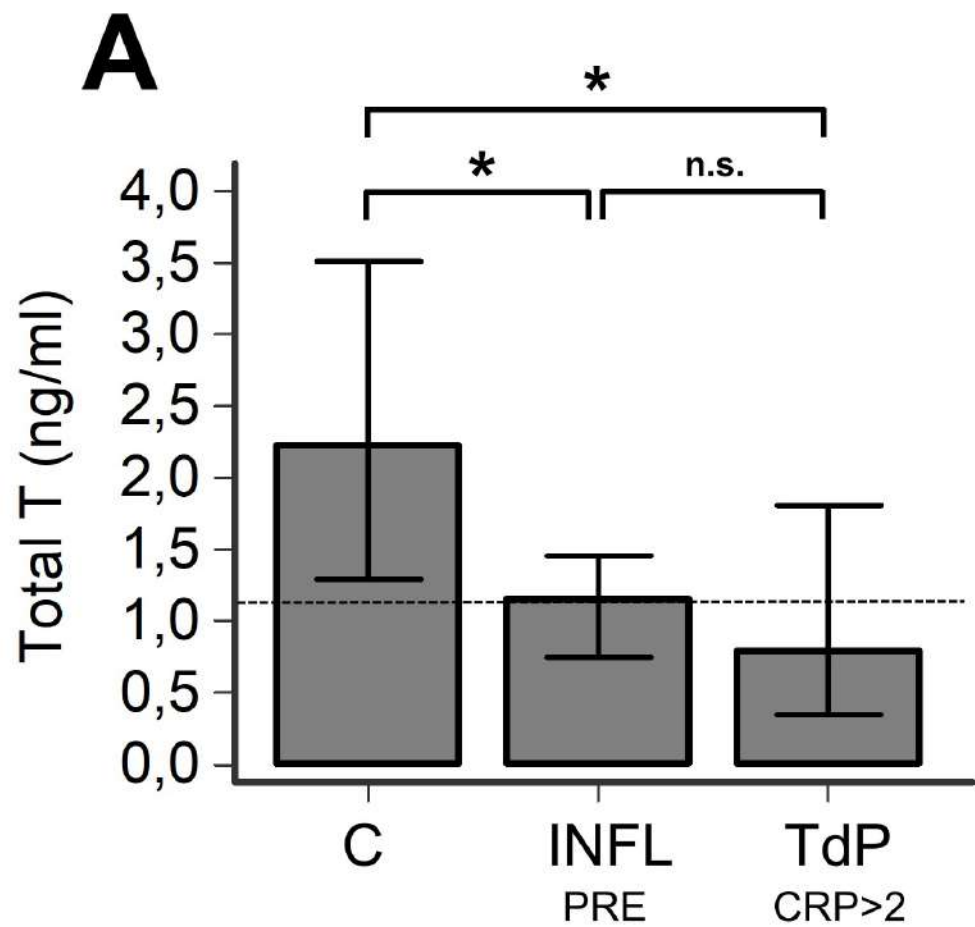


Figure S5

Supplemental Figure Legends:

Figure S1. Comparison of testosterone and 17- β estradiol levels in male patients with inflammatory diseases, during active disease (PRE) and after therapeutic interventions resulting in a CRP decrease >75% when compared to the baseline (POST), and controls.

(A) Total testosterone; two-tail Mann-Whitney test, ** $p < 0.01$, n.s. not significant. (B) Free testosterone; two-tail Mann-Whitney test, ** $p < 0.01$, n.s. not significant. (C) Bioavailable testosterone; two-tail Mann-Whitney test, ** $p < 0.01$, n.s. not significant. (D) 17- β estradiol; two-tail Mann-Whitney test, * $p < 0.025$, n.s. not significant.

Patients, $n=22$; controls, $n=10$. T: testosterone; Estradiol; 17- β estradiol; Bioav: bioavailable; C: controls.

Figure S2. Correlation between 17- β estradiol, total testosterone and IL-6 levels in male patients with inflammatory diseases. (A) Relationship between 17- β estradiol and total testosterone levels. (B) Relationship between 17- β estradiol and IL-6 levels.

Spearman test. Patients, $n=22$. T: testosterone; Estradiol; 17- β estradiol; IL-6: interleukin-6.

Figure S3. Correlation between QTc interval based on the Fridericia's and Hodges's formulas and testosterone levels in male patients with inflammatory diseases. (A) Relationship between heart rate-corrected QT interval based on the Fridericia's formula (QTc-F) and total testosterone levels. (B) Relationship between QTc-F and free testosterone levels. (C) Relationship between QTc-F and bioavailable testosterone levels. (D) Relationship between heart rate-corrected QT interval based on the Hodges's formula (QTc-H) and total testosterone levels. (E) Relationship between QTc-H and free testosterone levels. (F) Relationship between QTc-H and bioavailable testosterone levels.

Spearman test. Patients, $n=22$. T: testosterone; Bioav: bioavailable.

Figure S4. Correlation between QTc interval based on the Fridericia's and Hodges's formulas and IL-6 levels in male patients with inflammatory diseases. (A) Relationship between heart rate-corrected QT interval based on the Bazett's formula (QTc) and IL-6 levels. (A) Relationship between heart rate-corrected QT interval based on the Fridericia's formula (QTc-F) and IL-6 levels. (B) Relationship between heart rate-corrected QT interval based on the Hodges's formula (QTc-H) and IL-6 levels.

Spearman test. Patients, n=22. IL-6: interleukin-6.

Figure S5. Comparison of testosterone levels in male patients with inflammatory diseases, Torsades de Pointes, and controls. (A) Comparison of total testosterone levels in patients with active inflammatory diseases, Torsades de Pointes subjects with medium-high degree systemic inflammation, and controls. Kruskal-Wallis test, $p < 0.001$ $p = 0.0082$; Dunn post-hoc multiple comparison test, $*p < 0.05$, n.s. not significant. (B) Comparison of total testosterone levels in patients with non-active inflammatory diseases, Torsades de Pointes subjects with absent-low degree systemic inflammation, and controls. Kruskal-Wallis test, $p = 0.42$, n.s. not significant.

Inflammatory disease patients, n=22; TdP patients, n=19; controls, n=10. T: testosterone; C: controls; INFL-PRE: inflammatory diseases patients during active disease; INFL-POST: inflammatory diseases patients after therapeutic interventions resulting in a C-reactive protein level decrease $>75\%$ when compared to the baseline; TdP-CRP >2 : Torsades de pointes patients with medium-high degree systemic inflammation, i.e. C-reactive protein levels >2 mg/dl, n=9; TdP-CRP <2 : Torsades de pointes patients with absent-low degree systemic inflammation, i.e. C-reactive protein levels <2 mg/dl, n=10.