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, TNFα 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±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		
Acute infections	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%)	-
Immune-mediated diseases	4/22 (18%)	-
Rheumatoid arthritis	2/4 (50%)	-
Inflammatory bowel disease	1/4 (25%)	-
Polymyalgia rheumatica	1/4 (25%)	-
Other	2/22 (9%)	-
Acute microcrystalline arthritis	2/2 (100%)	-
Therapeutic interventions for inflammatory disease		
Antibiotics	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%)	-
Anti-inflammatory drugs	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%)
Cardiac diseases	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%)
Extra-cardiac diseases	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	*p*
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 *p*=0.34	rho=0.05 *p*=0.72	rho=0.09 *p*=0.56
Progesterone	rho=0.13 *p*=0.39	rho=0.29 *p*=0.06	rho=0.24 *p*=0.11
FSH	rho= -0.21 *p*=0.18	rho=0.05 *p*=0.72	rho=0.02 *p*=0.092
LH	rho=0.25 *p*=0.10	rho= -0.10 *p*=0.51	rho= -0.13 *p*=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.

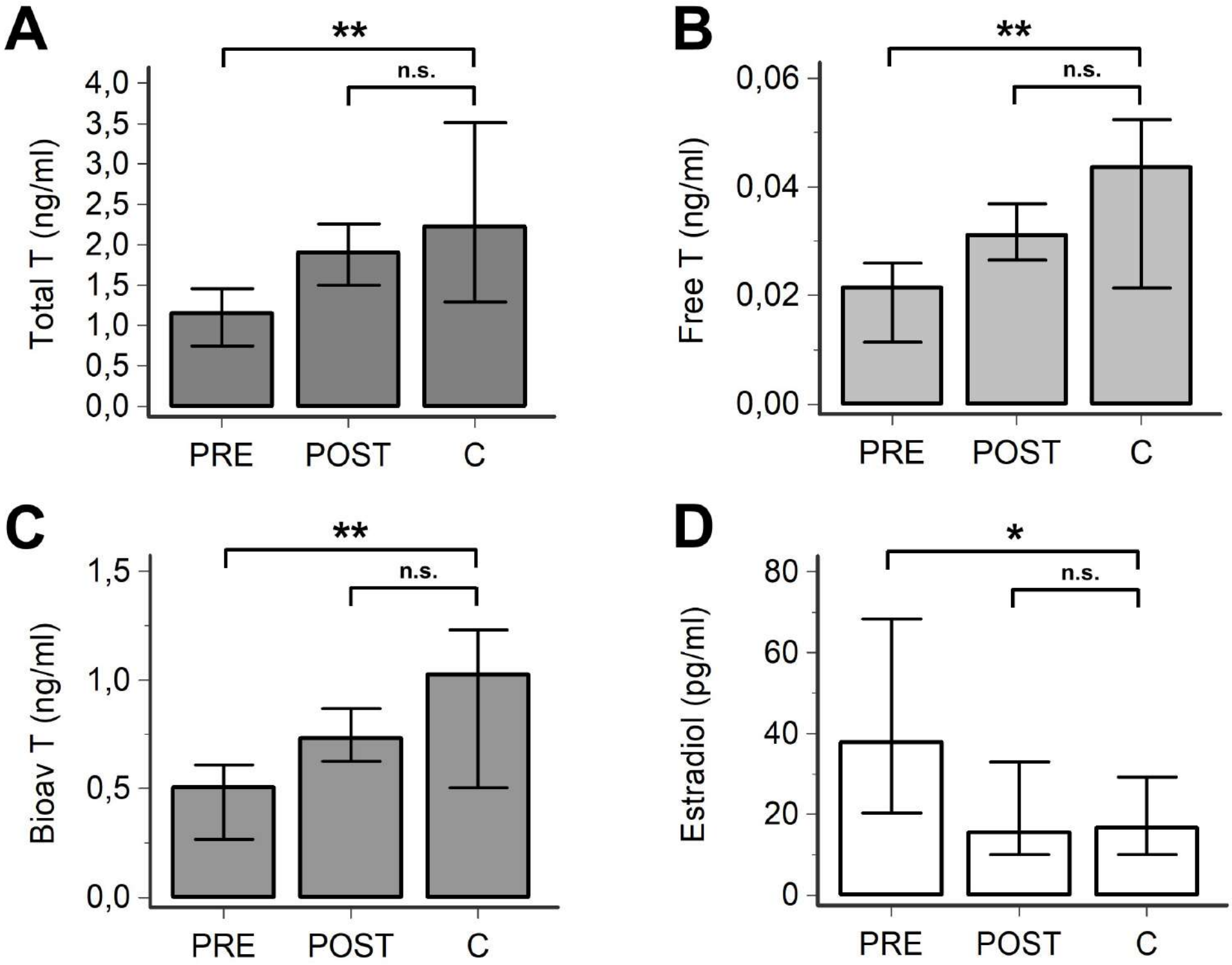
A
**
n.s.
Total T (ng/ml)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
PRE
POST
C
B
**
n.s.
Free T (ng/ml)
0,06
0,04
0,02
0,00
PRE
POST
C
C
**
n.s.
Bioav T (ng/ml)
1,5
1,0
0,5
0,0
PRE
POST
C
D
*
n.s.
Estradiol (pg/ml)
80
60
40
20
0
PRE
POST
C

Figure S1

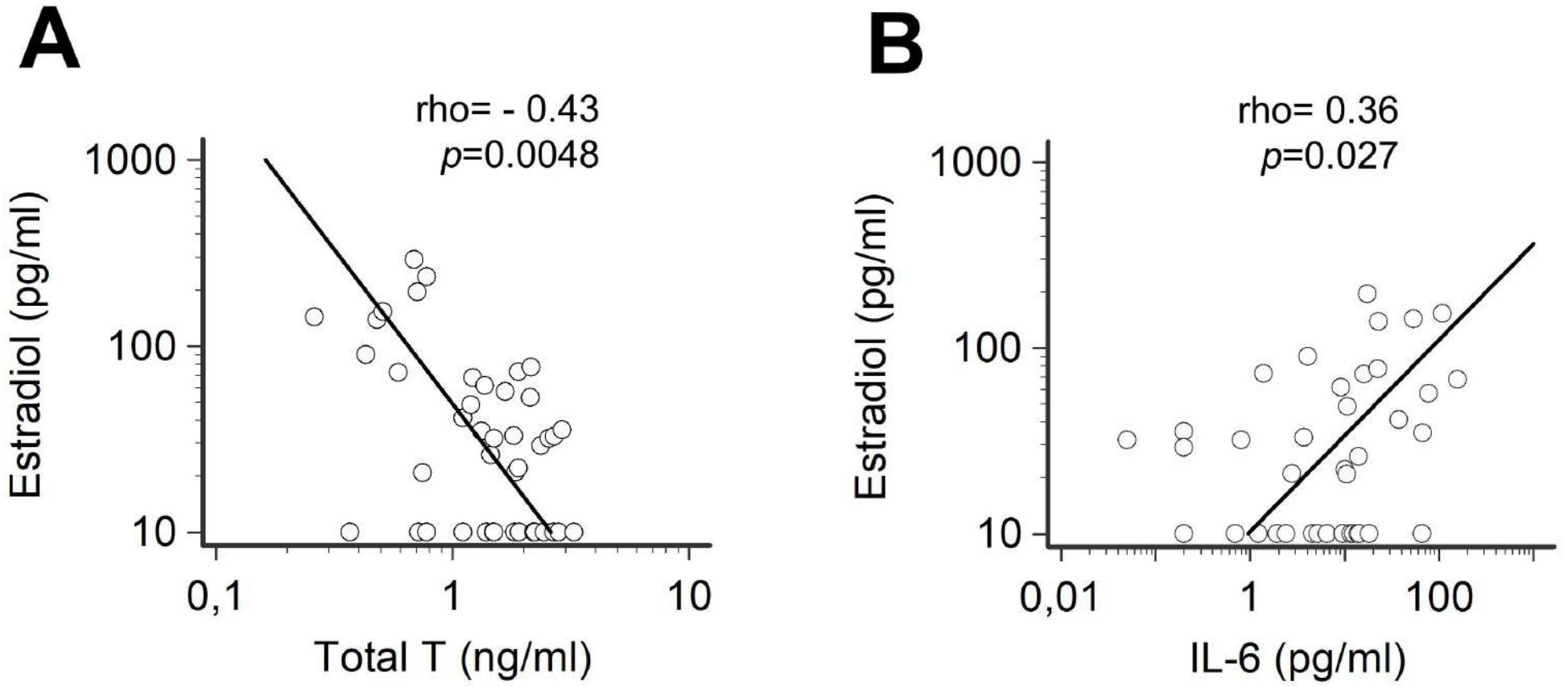
A
rho= - 0.43
p=0.0048
1000
100
10
Estradiol (pg/ml)
0,1
1
10
Total T (ng/ml)
B
rho= 0.36
p=0.027
1000
100
10
Estradiol (pg/ml)
0,01
1
100
IL-6 (pg/ml)

Figure S2

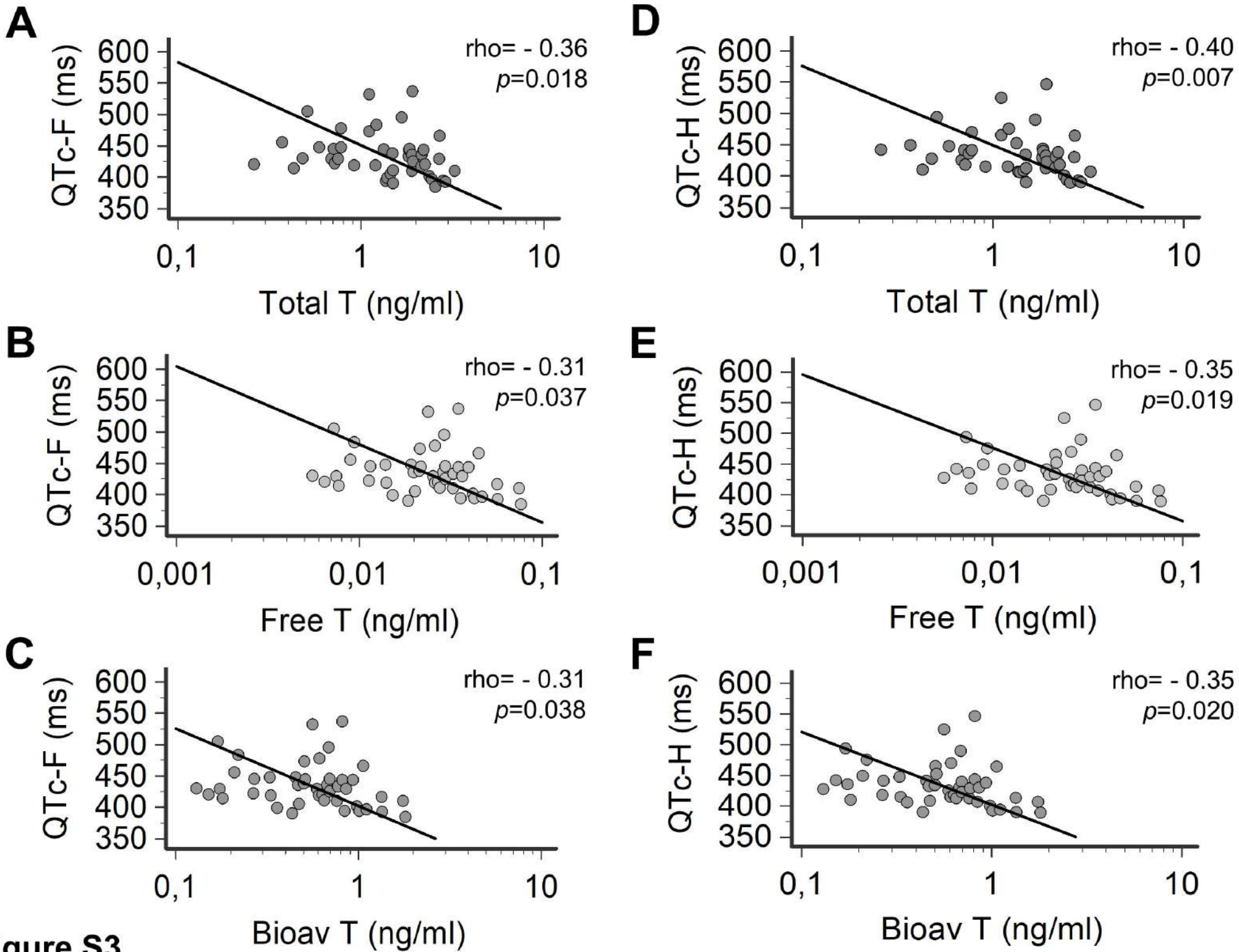
A
QTc-F (ms)
600
550
500
450
400
350
rho= - 0.36
p=0.018
0,1
1
10
Total T (ng/ml)
B
QTc-F (ms)
rho= - 0.31
p=0.037
0,001
0,01
0,1
Free T (ng/ml)
C
QTc-F (ms)
rho= - 0.31
p=0.038
0,1
1
10
Bioav T (ng/ml)
D
QTc-H (ms)
rho= - 0.40
p=0.007
Total T (ng/ml)
E
QTc-H (ms)
rho= - 0.35
p=0.019
Free T (ng(ml)
F
QTc-H (ms)
rho= - 0.35
p=0.020
Bioav T (ng/ml)

Figure S3

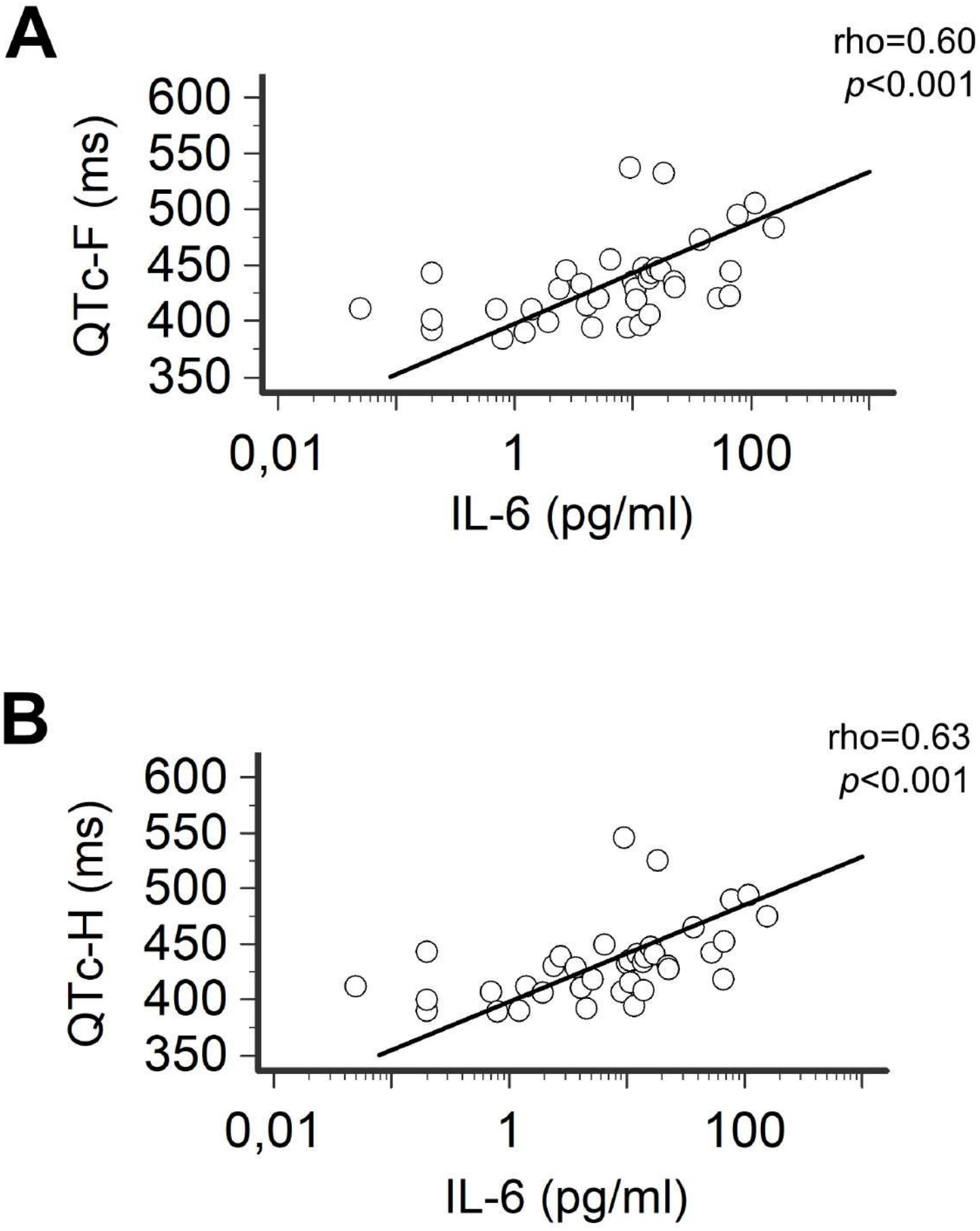
A
rho=0.60
p<0.001
600
550
500
450
400
350
QTc-F (ms)
0,01
1
100
IL-6 (pg/ml)
B
rho=0.63
p<0.001
600
550
500
450
400
350
QTc-H (ms)
0,01
1
100
IL-6 (pg/ml)

Figure S4

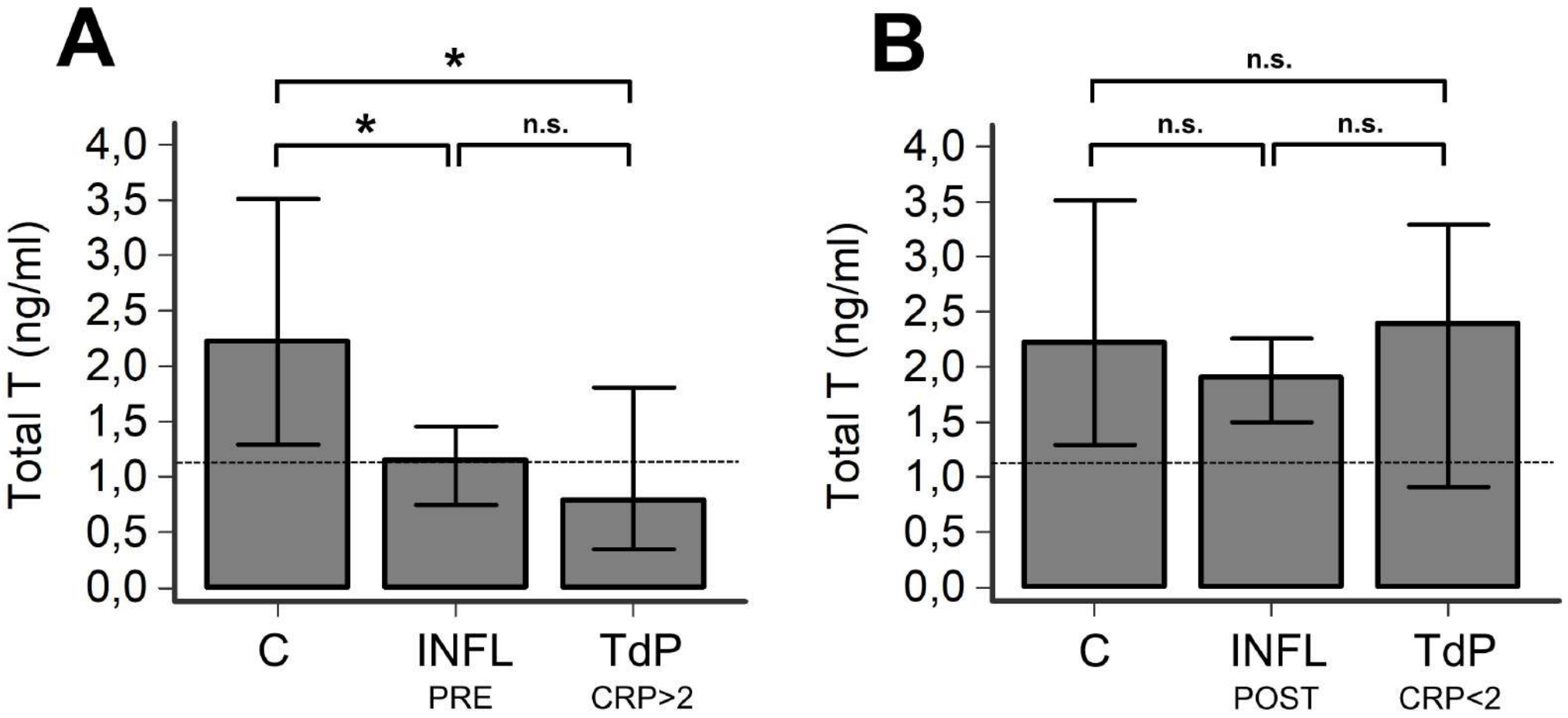
A
*
*
n.s.
Total T (ng/ml)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
C
INFL
PRE
TdP
CRP>2
B
n.s.
n.s.
n.s.
Total T (ng/ml)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
C
INFL
POST
TdP
CRP<2

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. (**B**) Relationship between QTc-H and free testosterone levels. (**C**) 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.