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ORIGINAL RESEARCH ARTICLE

Impact of residual inflammation on myocardial recovery and cardiovascular outcome in Takotsubo patients

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Abstract

Aims Recent insights have emphasized the importance of myocardial and systemic inflammation in Takotsubo syndrome (TTS). In a large registry of unselected patients, we sought to evaluate whether residual high inflammatory response (RHIR) could impact cardiovascular outcome after TTS.

Methods and results Patients with TTS were retrospectively included between 2008 and 2018 in three general hospitals. Three hundred eighty-five patients with TTS were split into three subgroups, according to tertiles of C-reactive protein (CRP) levels at discharge (CRP <5.2 mg/L, CRP range 5.2 to 19 mg/L, and CRP >19 mg/L). The primary endpoint was the impact of RHIR, defined as CRP >19 mg/L at discharge, on cardiac death or hospitalization for heart failure.

Follow up was obtained in 382 patients (99%) after a median of 747 days. RHIR patients were more likely to have a history of cancer or a physical trigger. Left ventricular ejection fraction (LVEF) at admission and at discharge were comparable between groups. By contrast, RHIR was associated with lower LVEF at follow up (61.7% vs. 60.7% vs. 57.9%; $P = 0.004$) and increased cardiac late mortality (0% vs. 0% vs. 10%; $P = 0.001$). By multivariate Cox regression analysis, RHIR was an independent predictor of cardiac death or hospitalization for heart failure (hazard ratio: 1.87; 95% confidence interval: 1.08 to 3.25; $P = 0.025$).

Conclusions Residual high inflammatory response was associated with impaired LVEF at follow up and was evidenced as an independent factor of cardiovascular events. All together, these findings underline RHIR patients as a high-risk subgroup, to target in future clinical trials with specific therapies to attenuate RHIR.

Keywords Takotsubo syndrome; Residual high inflammatory response; Systemic inflammatory response syndrome; Predictive factor; Late cardiac death; Late cardiovascular outcome

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Introduction

Recent advances on the pathophysiology of Takotsubo syndrome (TTS) have underlined the key role for catecholamine's surge, that trigger a switch in the beta-2 adrenergic signalling from Gs to Gi-protein leading to further cardiodepression. Although the initial cardiodepression during TTS is likely to be explained by this pathway, the pathophysiological mechanisms of long-term adverse prognosis in TTS remain to be fully characterized.[1] Challenging the initial paradigm of a benign condition associated with complete recovery, recent insights have emphasized the existence of persistent long-term structural functional and metabolic changes after TTS, possibly associated with ongoing inflammation. TTS is associated with a

marked increase in markers of myocardial inflammation, sustained myocardial oedema on magnetic resonance imaging (MRI) and with as substantial concomitant reduction in myocardial phosphocreatine ratio (PCr/ATP) as an indicator of energetic impairment.[2] As well as a direct toxicity of catecholamines, other key mechanisms on late TTS outcome may rely on the activation of inflammatory components. Intramyocardial inflammatory activation at the acute phase of TTS have been previously reported, either directly by endomyocardial biopsy or indirectly by MRI. More recently, macrophage-mediated cellular inflammatory response in the myocardium was evidenced by ultrasmall superparamagnetic particles of iron oxide (USPIO)-enhanced MRI, together with systemic and sustained inflammatory response.[3] Besides the detrimental effect of inflammation at the acute phase, challenging insights have emphasized the view that inflammation might set the basis of sustained damage and the development of a long-term heart failure phenotype including energetic impairment and therefore might be considered as a promising therapeutic target.[4]

In the present study, we sought to investigate the long-term impact of residual high inflammatory response (RHIR) on cardiovascular outcomes in a large cohort of unselected TTS patients. In addition, determinants of RHIR during TTS were investigated.

Methods

Study design and population

We conducted a multicentre, retrospective study from September 2008 to September 2018 in three general hospitals (Colmar, Haguenau, and Strasbourg, France). Patients with suspected TTS were identified out of 62 214 coronary angiograms recorded in the cardiac catheterization laboratory database, using the key words 'stress', 'takotsubo', or 'catecholamine'. The diagnosis of TTS was made according to the European Society of Cardiology Heart Failure Association criteria.[5] Exclusion criteria included a diagnosis of acute coronary occlusion, percutaneous coronary intervention, myocarditis, and cardiac arrest at first medical contact. Two cardiologists reviewed all the cases, and the diagnosis of TTS was based on a consensus agreement. Cases were recorded in the Alsace Takotsubo registry. The study protocol was approved by the institutional review board of the University.

Clinical and biological assessment

All baseline clinical data and follow-up variables were recorded and entered into a secure, ethics-approved database, after careful reviewing of patients' medical electronic records. Baseline characteristics included medical history, cardiovascular risk factors, medications, electrocardiogram at the time of admission, in-hospital dynamic electrocardiogram changes, coronary angiograms, and left ventricular ejection fraction (LVEF). LVEF was assessed using two-dimensional transthoracic echocardiography (TTE) and the biplane Simpson method. Serial biological parameters, including C-reactive protein (CRP), white blood cells count, BNP, and troponin were measured at admission, peak (highest), and discharge. Previous data have underlined the relationship between CRP levels, LVEF impairment, and neurohormonal activation.[6]

Residual high inflammatory response

After exclusion of in-hospital deaths [n = 33 (7%)], 385 patients with TTS were split into three subgroups, according to tertiles of CRP levels at discharge: $<$33 percentile (CRP $<$5.2 mg/L, n = 138), 33–66 percentile (CRP range 5.2 to 19 mg/L, n = 131) and $>$66 percentile (CRP $>$19 mg/L, n = 116). RHIR was defined as hospital discharge CRP $>$19 mg/L.

Outcomes

In-hospital complications including arrhythmias, cardiogenic shock, and death were collected by careful reviewing of the patient electronic medical records. Patients' follow up was obtained through telephone interviews, using a standardized questionnaire about health status and symptoms from the cardiologist, the family physician, or by hospital records. Follow-up LVEF was assessed using either MRI or TTE if not available.

The primary endpoint of the study was a composite endpoint of cardiovascular mortality (defined as death resulting from myocardial infarction, sudden cardiac death, heart failure, stroke, TTS recurrence, or resulting from other cardiovascular causes) and hospitalization for heart failure. The secondary endpoints included overall mortality, neoplastic mortality, and TTS recurrence.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. Continuous variables between all groups were compared using ANOVA or Kruskal–Wallis test, as appropriate. Pearson's χ^2 test was used to compare categorical variables. Continuous variables were analysed for normal distribution using Shapiro–Wilk test or graphically. Predictive factors of RHIR were investigated using logistic regression analysis. Associations between RHIR and occurrence of clinical meaningful events (cardiac death, death from non-cardiac

causes, death from neoplasia, rehospitalization for heart failure, and TTS recurrence) were assessed by Kaplan–Meier analysis and log-rank test. Time to event was defined as the time from TTS diagnosis to the date of event, with patients censored at when events occurred or at the end of the study. Multivariate analysis of survival rates was done using Cox models. All tests were two sided. A P value <0.05 was considered significant. Calculations were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographics

A total of 450 TTS patients were identified and enrolled in the Alsace Takotsubo registry. After exclusion of in-hospital deaths [n = 33 (7%)] and patients for whom CRP at discharge was not available, 385 patients were included in our study. Baseline characteristics, TTE, and biological parameters are reported in *Table 1*. Age and gender were equally distributed among groups. History of cancer (14.5% vs. 23.7% vs. 31.9%; P = 0.004) together with physical trigger (30.4% vs. 46.6% vs. 60.3%; $P < 0.001$) were more frequently observed in RHIR patients. Higher heart rate (81 bpm vs. 87 bpm vs. 96 bpm.; $P < 0.001$) on admission was shown in RHIR patients, as well as increased QT prolongation (456 ms vs. 474 ms vs. 479 ms; P = 0.007). Higher biological inflammatory parameters (including CRP and white blood cells count) and BNP levels on admission at peak and discharge were more frequently recorded among RHIR patients ($P < 0.001$). By contrast, troponin levels as a marker of myocardial damage were equivalent between groups.

In-hospital outcomes

Supraventricular arrhythmia occurred more frequently in the RHIR group (12.4% vs. 20.0% vs. 30.2%; P = 0.002). By contrast, no significant differences in life-threatening arrhythmias were evidenced during hospitalization between the three subsets of patients. Stay in critical care unit (CCU) and concomitant infection was more frequently observed in RHIR patients. In-hospital complications are listed in *Table 2*. At discharge, use of betablockers was equivalent among groups (70.1% vs. 65.6% vs. 58.3%; P = 0.144).

Mid-term and long-term outcomes

Mid-term and long-term outcomes were available for 382 (99%) patients with a median follow up of 747 days (interquartile range: 240 to 1518 days). At follow up, lower LVEF could be evidenced in RHIR patients (61.7% vs. 60.7% vs. 57.9%; P = 0.004). Cardiac death was higher in RHIR patients (0% vs. 0% vs. 10%; P = 0.001) (*Table 3*). In addition, the proportion of patients with impaired LVEF ($<$50%) at follow up was higher in the RHIR subgroup (*Figure 1*). Conversely, non-cardiac death, neoplasia-related death, and TTS recurrence were not significantly different among groups (*Table 3*). Kaplan–Meier curves are given in *Figure 1* and show a higher survival free from cardiac death or heart failure in non-RHIR patients, with a log-rank P of 0.007.

Early, mid-term, and long-term outcomes in patients without concomitant in-hospital infection

To circumvent a possible interaction between concomitant in-hospital infection and RHIR, outcomes were also analysed after the exclusion of patients with ongoing infectious process. In the infection-free cohort (n = 238) supraventricular arrhythmia, cardiovascular death, or heart failure and cardiovascular death were more frequently observed in RHIR patients. Likewise, the proportion of patients with impaired LVEF at follow up was higher in RHIR patients (*Table 4*).

Predictors of residual high inflammatory response

By univariate regression analysis, a history of cancer, diabetes mellitus, high BNP level on admission ($>$400 ng/L), CCU stay, and concomitant infection were significant predictors of RHIR occurrence. By multivariate logistic regression analysis, history of cancer, high BNP levels on admission, CCU stay, and concomitant infection were still evidenced as independent predictors of RHIR (*Table 5*).

Predictors of cardiac mortality and heart failure

By univariate Cox analysis, age, history of cancer, history of vascular disease, history of atrial fibrillation, de novo atrial fibrillation, renal dysfunction, LVEF impairment at follow up, lower systolic, and diastolic pressure, RHIR were associated with higher rates of cardiac mortality and heart failure at maximal follow up. By multivariate Cox regression analysis, age [hazard ratio (HR): 1.09; 95% confidence interval (CI): 1.03 to 1.15; P = 0.001], history of cancer (HR: 9.29; 95% CI: 3.24 to 26.61; $P < 0.001$), history of vascular disease (HR: 3.03; 95% CI: 1.25 to 7.35; P = 0.014), LVEF at follow up (HR 0.93 95% CI: 0.89 to 0.98; P = 0.005), and RHIR (HR: 1.87; 95% CI: 1.08 to 3.25; P = 0.02), remained independent factors of cardiac mortality and heart failure (*Table 6*).

Table 1 Characteristics of Takotsubo patients at baseline, according to tertiles of CRP at discharge

CRP level at discharge	<5.2 mg/L (*n* = 138)	5.2–19 mg/L (*n* = 131)	>19 mg/L (RHIR patients, *n* = 116)	*P* value
Demographics				
Age (years)—*mean ± SD*	69.6 ± 12.7	72.3 ± 13.0	71.2 ± 12.6	0.218
Female sex—*n/N* (%)	125/138 (90.6%)	115/131 (97.8%)	95/116 (81.9%)	0.116
Comorbidities—*n/N* (%)				
Cancer	20/138 (14.5%)	31/131 (23.7%)	37/116 (31.9%)	0.004
COPD or asthma	25/118 (21.2%)	22/103 (21.4%)	22/96 (22.9%)	0.947
Psychiatric disorders	42/138 (30.4%)	48/131 (36.6%)	40/116 (34.5%)	0.550
Neurologic disorders	5/118 (4.2%)	8/104 (7.7%)	5/99 (5.1%)	0.526
Dementia	10/138 (7.2%)	11/130 (8.5%)	9/115 (7.8%)	0.934
Chronic inflammatory disease	11/138 (8.0%)	8/130 (6.2%)	13/116 (11.2%)	0.352
Cardiovascular risk factor—*n/N* (%)				
Hypertension	85/138 (61.6%)	77/131 (58.8%)	68/116 (58.6%)	0.857
Diabetes mellitus	23/138 (16.7%)	20/131 (15.3%)	29/116 (25.0%)	0.110
Dyslipidaemia	61/138 (44.2%)	55/131 (42.0%)	51/116 (44.0%)	0.725
Current smoking	25/138 (18.1%)	22/131 (16.8%)	20/116 (17.2%)	0.959
Prior smoking	26/138 (18.8%)	22/131 (16.8%)	28/116 (24.1%)	0.332
Cardiovascular history—*n/N* (%)				
Peripheric or coronary artery disease	23/136 (16.9%)	25/131 (19.1%)	24/116 (20.7%)	0.742
Paroxysmal or persistent AF	15/138 (10.9%)	30/131 (22.9%)	24/116 (20.7%)	0.024
Permanent AF	4/138 (2.9%)	9/131 (6.9%)	9/116 (7.8%)	0.167
Stroke	16/138 (11.6%)	17/131 (13.0%)	13/116 (11.2%)	0.901
Prior TTS	5/138 (3.6%)	5/131 (3.8%)	2/116 (1.7%)	0.550
Beta-blockers prior to admission—*n/N* (%)	29/132 (22.0%)	33/125 (26.4%)	30/110 (27.3%)	0.584
Trigger—*n/N* (%)				
Physical	42/138 (30.4%)	61/131 (46.6%)	70/116 (60.3%)	<0.001
Emotional	53/138 (38.4%)	42/131 (32.1%)	24/116 (20.7%)	<0.001
Unknown	43/138 (31.2%)	27/131 (20.6%)	22/116 (19.0%)	<0.001
Symptom on admission—*n/N* (%)				
Chest pain	98/138 (71.0%)	61/131(46.6%)	52/116 (44.8%)	<0.001
Dyspnoea	48/138 (34.8%)	56/131 (42.7%)	56/116 (48.3%)	0.089
Syncope	5/138 (3.6%)	6/131 (4.6%)	6/116 (5.2%)	0.831
Hemodynamics on admission—*mean ± SD*				
Heart rate (bpm)	81 ± 19	87 ± 18	96 ± 19	<0.001
Blood pressure (mmHg)				
Systolic	126 ± 29	129 ± 28	132 ± 123	0.868
Diastolic	91 ± 70	73 ± 15	67 ± 13	0.076
Wall motion abnormalities—*n/N* (%)				
Apical	90/138 (65.2%)	100/131 (76.3%)	90/116 (77.6%)	0.046
Mid-ventricular	41/138 (29.7%)	26/131 (19.8%)	23/116 (19.8%)	0.090
Basal	4/138 (2.90%)	4/131 (3.10%)	0/116 (0.00%)	0.055
LVEF (%)—*mean ± SD*				
On admission	40.5 ± 10.4	38.7 ± 11.2	37.7 ± 12.1	0.136
At discharge	52.8 ± 10.7	51.9 ± 11.5	51.3 ± 12.2	0.593
Corrected QT (ms) on admission—*mean ± SD*	456 ± 41	474 ± 51	479 ± 53	0.007
Concomitant CAD	37/129 (28.7%)	48/122 (39.3%)	45/104 (43.3%)	0.053
Creatinine kinase on admission (μmol/L)—*mean ± SD*	65.6 ± 23.2	82.2 ± 68.7	89.2 ± 80.4	0.007
Inflammation markers—*mean ± SD* WBC (10^9/L)				

(*Continues*)

Table 1 (continued)

CRP level at discharge	<5.2 mg/L (n = 138)	5.2–19 mg/L (n = 131)	>19 mg/L (RHIR patients, n = 116)	P value
On admission	9.72 ± 4.43	11.4 ± 4.84	12.8 ± 5.68	<0.001
At peak	10.7 ± 5.04	13.1 ± 5.88	14.7 ± 5.86	<0.001
At discharge	7.25 ± 2.75	8.15 ± 3.03	8.71 ± 3.60	0.001
CRP (mg/L)				
On admission	7.58 ± 17.3	28.6 ± 41.1	81.0 ± 86.6	<0.001
At peak	18.1 ± 40.5	53.1 ± 60.9	125 ± 95.2	<0.001
Cardiac biomarkers—*mean* ± *SD*				
BNP (ng/L)				
On admission	415 ± 656	813 ± 956	1171 ± 1247	<0.001
At peak	595 ± 792	1096 ± 1,157	1446 ± 1398	<0.001
At discharge	332 ± 339	474 ± 488	688 ± 916	0.001
Troponin I (μg/L)				
On admission	3.33 ± 7.37	2.81 ± 3.95	2.89 ± 5.54	0.760
At peak	5.17 ± 8.30	5.53 ± 11.4	4.05 ± 6.81	0.425
At discharge	2.51 ± 7.86	1.45 ± 2.81	1.38 ± 2.54	0.188
Beta-blockers between Days 0 and 5	94/134 (70.1%)	86/131 (65.6%)	67/115 (58.3%)	0.144

Data are expressed as mean ± standard deviation (*SD*) or as number (*N*)/total number (*N*) (%).
AF, atrial fibrillation; BNP, brain natriuretic peptide; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; RHIR, residual high inflammatory response; TTS, Takotsubo syndrome; WBC, white blood cell.

Discussion

The current report drawn from a cohort of 385 TTS patients is the first study to specifically evaluate the impact of RHIR on late cardiovascular outcomes including heart failure. The salient results of the present study are as follows: (i) at the acute phase, RHIR is associated with enhanced neurohormonal activation contrasting with similar LVEF impairment. (ii) Predictive factors of RHIR are a history of cancer, high BNP levels, critical care unit stay, and concomitant infection. (iii) Whilst LVEF at hospital discharge was equivalent between groups, lower LVEF could be evidenced at follow up in RHIR patients. (iv) RHIR has a dramatic impact on late outcomes, including a higher cardiac mortality.

Altogether, our findings suggest that residual inflammation at hospital discharge could contribute to impaired recovery and identify a subset of vulnerable patients after TTS onset.

Acute inflammation in Takotsubo syndrome

Inflammation is one of the earliest events in cardiac stress situations. Numerous studies based on animal models, human endomyocardial biopsies, or USPIO-enhanced MRI to target macrophage infiltration have depicted the time course of inflammation within the myocardium during TTS, consisting in a mononuclear cells infiltrate, contraction band necrosis, and myocardial inflammation-mediated oedema.[3,7–9] Interestingly, similar histological patterns could be evidenced during septic shock and correlation between epinephrine dose and monocyte infiltration have been reported in this setting.[10]

Mechanisms of inflammation during Takotsubo syndrome

Recent insights have emphasized the view that activation of adrenergic signalling pathways contributes to enhanced cytoadhesins expression by bone marrow cells, but also by cardiac endothelial cells (ICAM-1) which may favour diapedesis and development of sterile inflammation and remodelling of the failing heart.[11] Other authors have stressed the importance of the shedding of endothelial glycocalyx components, such as Syndecan-1, that occurs at the acute phase of TTS to the same extent that was observed in acute myocardial infarction. Impairment of the glycocalyx distorts vascular rheology, alters normal laminar flow patterns, and may contribute to endothelial dysfunction and oxidative stress, paving the way to increased coronary vascular permeability, myocardial oedema, and sustained vasomotor dysfunction.[12] The importance of systemic inflammatory burden in TTS is emphasized by the re-

Table 2 In-hospital complications according to tertiles of CRP at discharge

CRP level at discharge	Whole cohort (n = 385)	<5.2 mg/L (n = 138)	5.2–19 mg/L (n = 131)	>19 mg/L (RHIR patients, n = 116)	P value
Cardiogenic shock—*n/N* (%)	36/385 (9.4%)	7/138 (5.1%)	13/131 (9.9%)	16/116 (13.8%)	0.057
Concomitant infection—*n/N* (%)	146/384 (38%)	31/138 (22.5%)	51/131 (38.9%)	64/115 (55.7%)	<0.001
Cardiac arrest—*n/N* (%)	5/384 (1.3%)	2/138 (1.4%)	3/130 (2.3%)	0/116 (0.0%)	0.143
IABP—*n/N* (%)	8/384 (2.1%)	1/138 (0.7%)	4/130 (3.1%)	3/116 (2.6%)	0.309
ECLS—*n/N* (%)	1/385 (0.3%)	0/138 (0.0%)	1/131 (0.8%)	0/116 (0.0%)	0.339
De novo supraventricular arrhythmia—*n/N* (%)	78/383 (20.4%)	17/137 (12.4%)	26/130 (20.0%)	35/116 (30.2%)	0.002
Life-threatening arrhythmia—*n/N* (%)					
Ventricular tachycardia	5/385 (1.3%)	3/138 (2.2%)	2/131 (1.5%)	0/116 (0.0%)	0.152
Torsade de pointe	4/385 (1.0%)	2/138 (1.4%)	1/131 (0.8%)	1/116 (0.9%)	0.841
Sinus dysfunction	8/385 (2.1%)	4/138 (2.9%)	1/131 (0.8%)	3/116 (2.6%)	0.366
Third degree block	3/385 (0.8%)	1/138 (0.7%)	1/131 (0.8%)	1/116 (0.9%)	0.293

Data are expressed as number (n)/total number (N) (%).
CRP, C-reactive protein; ECLS, extra-corporeal life support; IABP, intra-aortic balloon pump; RHIR, residual high inflammatory response; TTS, Takotsubo syndrome.

lease of specific patterns of cytokines whilst controversies are still ongoing concerning the respective importance of anti-inflammatory[13] or pro-inflammatory cytokines.[2,14] Evidences that low-grade inflammation persists at 5 months were recently provided with the demonstration of elevated IL-6 levels in TTS patients.[3]

Among the various mechanisms involved in the induction of cytokines release and cytoadhesins expression by endothelial cells, a key role of p53 has been established. In heart failure, several animal models have highlighted the importance of the catecholamine/beta-2 adrenergic/reactive oxygen species (ROS) p53 signalling pathway in the induction of cardiac dysfunction. The primordial importance of this pathway is highlighted by the demonstration that catecholamine/beta-2 stimulation regulates p53 in endothelial cells and macrophages and induces cardiac inflammation whilst monocyte infiltration catecholamine/beta-2 cardiac dysfunction could be blunted in p53 endothelial cells KO mice.[11] Cytokines and ROS released by activated inflammatory cells 'neutrophils burst' could contribute directly to myocardial damage.[15] Recent data have underlined that increased release of nitric oxide (NO) may occur in TTS and that NO, in the presence of superoxide anion (O_2^-), potentially induces the formation of peroxynitrite anions ($ONOO^-$) prompting oxidative stress, activation of poly (ADP) ribose polymerase-1 (PARP-1) that induces 'energy sink'. Moreover, in animal TTS models, it was established that oxidative stress promotes a 2.5-fold up-regulation of the pro-inflammatory arrest in thioredoxin-interacting protein (TXNIP) with a significant apex to base gradient. The importance of this pathway was substantiated by the fact that PARP-1 inhibitors were demonstrated to limit the severity of systolic functional impairment (apical strain rate and apical fractional shortening area) simultaneously to the reduction of TXNIP expression within the myocardium.[16]

Interplay between brain natriuretic peptide and inflammation

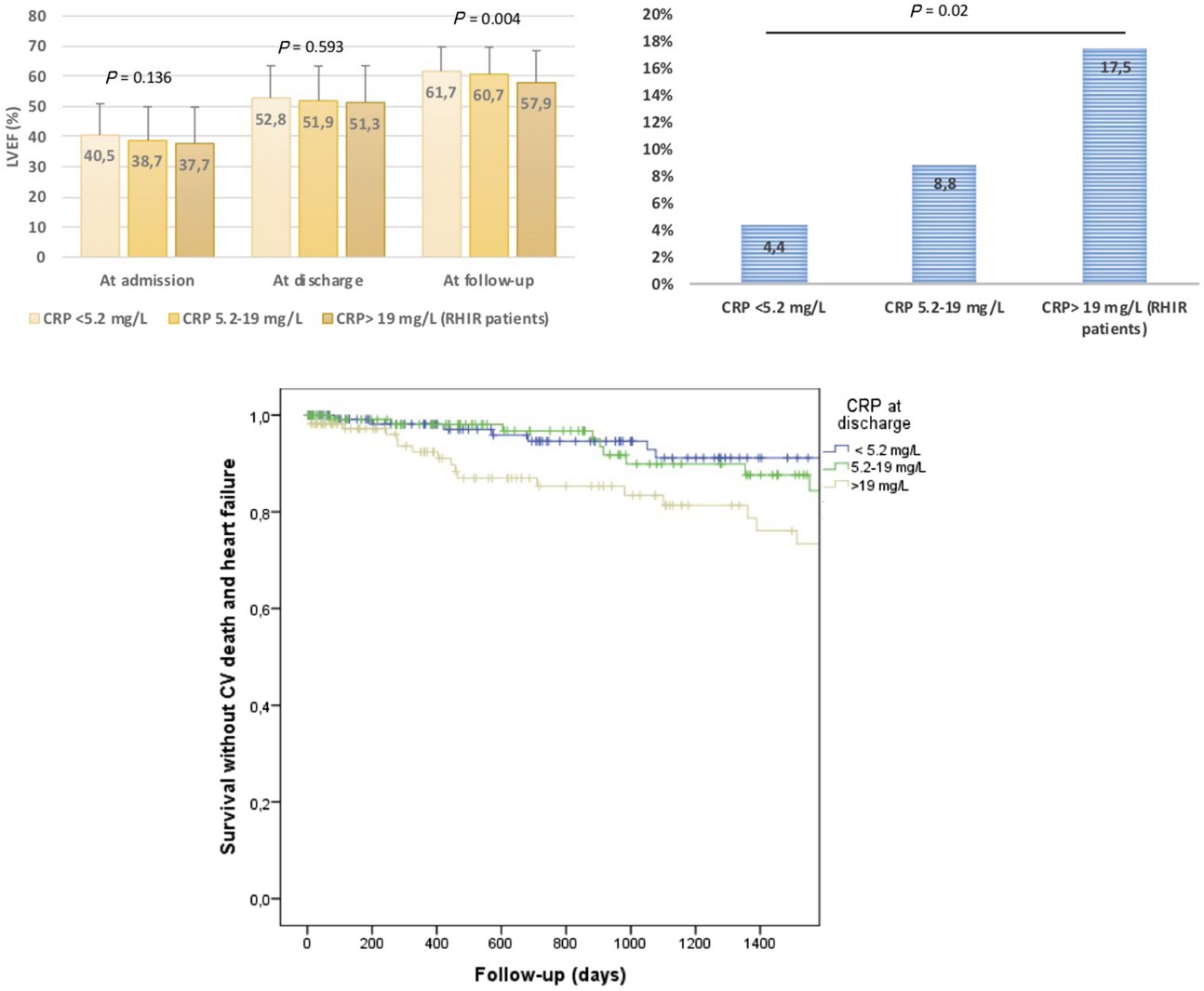
Although stretch and left ventricular wall tension are likely to be the main contributors of production and secretion of BNP, other evidences point to a direct link between inflammatory

Table 3 Follow-up outcomes of TTS patients according to tertiles of CRP at discharge

CRP level at discharge	Whole cohort (n = 385)	<5.2 mg/L (n = 138)	5.2–19 mg/L (n = 131)	>19 mg/L (RHIR patients, n = 116)	P value
LVEF (%)—*mean ± SD*	60.1 ± 9.3	61.7 ± 8.05	60.7 ± 8.98	57.9 ± 10.6	0.004
LVEF <50%—*n/N* (%)	37/375 (9.9%)	6/136 (4.4%)	11/125 (8.8%)	20/114 (17.5%)	0.002
Death—*n/N* (%)					
Cardiovascular death	11/362 (3%)	0/131 (0.0%)	0/121 (0.0%)	11/110 (10.0%)	<0.001
Neoplasia related death	14/362 (3.9%)	3/131 (2.3%)	3/121 (2.5%)	8/110 (7.3%)	0.106
Other causes of death	24/362 (6.6%)	5/131 (3.8%)	10/121 (8.3%)	9/110 (8.2%)	0.269
Rehospitalization for heart failure—*n/N* (%)	39/382 (10.2%)	9/138 (6.5%)	15/129 (11.6%)	15/115 (13.0%)	0.188
Rehospitalization for other cardiovascular cause—*n/N* (%)	55/384 (14.3%)	16/138 (11.6%)	18/130 (13.8%)	21/116 (18.1%)	0.331
Composite endpoint (death or rehospitalization for heart failure)—*n/N* (%)	45/382 (11.8%)	9/138 (6.50%)	15/129 (11.6%)	21/115 (18.3%)	0.016
TTS recurrence—*n/N* (%)	12/384 (3.1%)	7/138 (5.1%)	3/130 (2.3%)	2/116 (1.7%)	0.263

Data are expressed as mean ± standard deviation (*SD*) or as number (n)/total number (N) (%).
CRP, C-reactive protein; LVEF, left ventricular ejection fraction; RHIR, residual high inflammatory response; TTS, Takotsubo syndrome.

Figure 1 Central illustration (left): LVEF (%) at admission, discharge and follow up according to tertiles of CRP at discharge in Takotsubo patients. (Right): Takotsubo patients (%) with LVEF <50% at follow up according to tertiles of CRP at discharge. (Bottom): Kaplan–Meier curve for survival free from cardiovascular death or heart failure, stratified according to tertiles of CRP level at discharge, in Takotsubo patients. CRP, C-reactive protein; LVEF, left ventricular ejection fraction; RHIR, residual high inflammatory response.



burden and BNP. In rats, immobilization stress induces the production of heat shock protein 70 by the myocardium, a potent activator of the inflammatory response[17] and enhances atrial and B-type natriuretic peptide expression.[18] In the setting of TTS, other authors have emphasized that increased release of BNP may occur in the presence of myocardial inflammation despite minimal changes in cardiac distension.[19] The binding of BNP to natriuretic peptide A (equivalent to particulate guanylate cyclase A) results in the generation of the second messenger cGMP that controls numerous functions such as myocardial relaxation, decreased oxidative stress, and reduction of vascular permeability. Accordingly, the concept that BNP displays at least in part anti-inflammatory effects enabling the reduction of ROS formation associated to the 'neutrophil burst' and could contribute to the limitation of the tissular injury was recently raised.[19,20,21] In the present study, enhanced release of BNP could be observed on admission, at peak, and at discharge in patients with an important inflammatory response whilst LVEF were comparable among groups on admission and at discharge. Although we could not differentiate whether BNP is induced by the inflammatory response or represents mostly an anti-inflammatory agent involved in the limitation of myocardial damage, the present data clearly highlight the importance of the interplay between inflammation response and BNP secretion during TTS.

Table 4 Follow-up outcomes of infection-free TTS patients according to tertiles of CRP at discharge

	Whole infection-free cohort (n = 238)	CRP <5.2 mg/L (n = 107)	CRP 5.2–19 mg/L (n = 80)	CRP >19 mg/L (n = 51)	P value
Supraventricular arrhythmia—*n/N* (%)	31/237 (13.1%)	9 (8.5%)	10 (12.5%)	12 (25.3%)	0.032
Cardiovascular death or heart failure —*n/N* (%)	26/236 (11.0%)	5 (4.7%)	9 (11.4%)	12 (24%)	0.002
Cardiovascular death—*n/N* (%)	5/223 (2.2%)	0 (0%)	0 (0%)	5 (10%)	<0.001
Rehospitalization for heart failure—*n/N* (%)	22/236 (9.3%)	5 (4.7%)	9 (11.4%)	8 (16%)	0.056
Neoplasic death—*n/N* (%)	9/22 3 (4%)	3 (2.9%)	2 (2.8%)	4 (8%)	0.270
Takotsubo recurrence—*n/N* (%)	8/238 (3.4%)	6 (5.6%)	1 (1.2%)	1 (2.0%)	0.216
LVEF at admission—*mean ± SD*	40 ± 10	40 ± 10	41 ± 10	38 ± 11	0.288
LVEF at discharge—*mean ± SD*	51 ± 11	52 ± 10	50 ± 11	49 ± 12	0.167
LVEF at follow up—*mean ± SD*	59 ± 9	61 ± 8	60 ± 9	56 ± 11	0.009
LVEF <50% at follow up—*n/N* (%)	27/232 (11.6%)	5 (4.8%)	9 (11.7%)	13 (26%)	0.001

Data are expressed as mean ± standard deviation (*SD*) or as number (*n*)/total number (*N*) (%).
CRP, C-reactive protein; LVEF, left ventricular ejection fraction; RHIR, residual high inflammatory response; TTS, Takotsubo syndrome.

Table 5 Univariate and multivariate analyses for prediction of RHIR in Takotsubo patients

	Univariate			Multivariate		
	OR	95% CI	P value	OR	95% CI	P value
Age	1.00	0.98–1.02	0.796			
Female sex	0.54	0.29–1.00	0.052			
Comorbidities						
Cancer	2.00	1.22–3.28	<0.001	2.17	1.09–4.31	0.026
COPD or asthma	1.10	0.61–1.95	0.744			
Dementia	0.99	0.44–2.25	0.997			
Chronic inflammatory disease	1.65	0.78–3.47	0.165			
Cardiovascular risk factor						
Hypertension	0.93	0.60–1.45	0.769			
Diabetes mellitus	1.75	1.02–2.98	0.039	1.62	0.77–3.41	0.201
Dyslipidaemia	1.00	0.65–1.55	0.986			
Current smoking	0.98	0.55–1.75	0.956			
Prior smoking	1.46	0.86–2.48	0.156			
Cardiovascular history						
PVD or coronary artery disease	1.19	0.68–2.05	0.533			
Paroxysmal or persistent AF	1.29	0.74–2.25	0.353			
Permanent AF	1.65	0.68–3.99	0.261			
Stroke	0.90	0.45–1.78	0.769			
Prior TTS	0.45	0.09–2.10	0.314			
Emotional trigger	0.88	0.66–1.18	0.409			
Concomitant infection	2.86	1.82–4.48	<0.001	1.87	1.00–3.52	0.050
CCU stay	2.62	1.49–4.60	0.001	2.19	1.09–4.40	0.026
Wall motion abnormalities						
Apical	1.43	0.86–2.39	0.161			
Mid-ventricular	0.74	0.43–1.27	0.281			
Cardiogenic shock	1.99	0.99–4.00	0.053			
BNP >400 ng/L on admission	1.00	1.00–1.00	<0.001	3.30	1.77–6.16	0.001
Troponin I on admission	0.99	0.95–1.03	0.784			

AF, atrial fibrillation; BNP, brain natriuretic peptide; COPD, chronic obstructive pulmonary disease; CCU, critical care unit; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; OR, odds ratio; PVD, peripheral vascular disease; RHIR, residual high inflammatory response; TTS, Takotsubo syndrome; 95% CI, 95% confidence interval.

Evidences for chronic low-grade inflammation in Takotsubo syndrome

Other authors have stressed the importance of and an adaptive immune response triggered by cardiomyocyte necrosis in the time course of TTS. Representing an essential mechanism of wound healing, immune cells infiltration into the damaged myocardium could also trigger a process named sterile inflammation, as the immune system is activated despite the lack of any discernible infectious insult. This mechanism could lead to ongoing inflammation[1] as reflected by elevated IL-6 levels still evidenced 5 months after TTS onset. In the present report, residual inflammation is still evidenced more than 1 week after TTS onset in a substantial part of the

Table 6 Univariate and multivariate analyses for prediction of cardiac death and/or rehospitalization for heart failure in Takotsubo patients

	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age	1.04	1.02–1.07	0.002	1.09	1.03–1.15	0.001
Female sex	0.600	0.28–1.25	0.170			
Comorbidities						
Cancer	2.61	1.45–4.73	0.001	9.29	3.24–26.61	<0.001
COPD or asthma	1.39	0.774–2.49	0.272			
Psychiatric disorders	0.35	0.04–2.59	0.306			
Neurologic disorders	1.87	0.78–4.43	0.155			
Dementia	1.94	0.93–4.02	0.075			
Chronic inflammatory disease	0.87	0.27–2.81	0.816			
Cardiovascular risk factor						
Hypertension	1.84	0.96–3.50	0.062			
Diabetes mellitus	1.25	0.63–2.47	0.516			
Dyslipidaemia	1.03	0.58–1.84	0.910			
Current smoking	0.66	0.28–1.57	0.354			
Prior smoking	1.34	0.68–2.64	0.398			
Cardiovascular history						
PVD or coronary artery disease	2.36	1.30–4.27	0.005	3.03	1.25–7.35	0.014
Paroxysmal or persistent AF	3.68	2.05–6.63	<0.001	1.38	0.36–5.24	0.634
Permanent AF	4.01	1.96–8.47	<0.001	0.53	0.09–3.11	0.484
Stroke	1.93	0.92–4.01	0.077			
Prior TTS	1.98	0.47–8.17	0.347			
Beta-blockers prior to admission	2.09	1.14–3.85	0.017	0.50	0.15–1.69	0.270
Beta-blockers between Days 0 and 5	0.82	0.44–1.55	0.553			
Trigger	1.17	0.79–1.73	0.426			
Hemodynamics on admission						
Heart rate (bpm)	1.00	0.98–1.02	0.998			
Blood pressure (mmHg)						
Systolic	0.98	0.97–1.00	0.046	0.98	0.96–1.01	0.373
Diastolic	0.96	0.94–0.99	0.004	0.96	0.92–1.00	0.006
QT on admission	1.00	0.99–1.01	0.980			
LVEF						
On admission	1.01	0.98–1.04	0.503			
At discharge	1.00	0.97–1.02	0.923			
At follow up	0.96	0.93–0.990	0.009	0.93	0.89–0.98	0.005
Wall motion abnormalities						
Apical	1.35	0.67–2.73	0.400			
Mid-ventricular	0.73	0.35–1.53	0.412			
Concomitant CAD	1.80	0.96–3.36	0.064			
Creatinine kinase on admission	1.00	1.00–1.01	0.021	1.00	0.99–1.01	0.361
Concomitant infection	1.13	0.62–2.03	0.694			
CCU stay	1.76	0.83–3.75	0.140			
CRP tertile (RHIR)	1.79	1.24–2.59	0.002	1.87	1.08–3.25	0.025
WBC at discharge	1.07	0.98–1.158	0.093			
BNP at discharge	1.00	1.00–1.00	0.208			
Troponin I at discharge	1.01	0.94–1.09	0.724			

AF, atrial fibrillation; BNP, brain natriuretic peptide; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; PVD, peripheral vascular disease; HR, hazard ratio; CCU, critical care unit; LVEF, left ventricular ejection fraction; RHIR, residual high inflammatory response; TTS, Takotsubo syndrome; WBC, white blood cell; 95% CI, 95% confidence interval.

cohort. The clinical relevance of this paradigm was recently demonstrated in TTS. Using USPIO-MRI to monitor inflammatory macrophages infiltration within the myocardium, Scally and co-workers have demonstrated that TTS is characterized by a myocardial macrophage inflammatory infiltrate together with an increase in systemic pro-inflammatory cytokines that persist at least 5 months, suggesting a low-chronic inflammatory state.[2] In line with this observation, other recent cardiac MRI data show that TTS is characterized by a state of intra-myocardial oedema secondary to a global left ventricular inflammatory response, which is detectable early after the index event and persists well beyond the resolution of segmental left ventricular contractile dysfunction.[9]

To evidence a possible noxious role of RHIR on the cardiovascular compartment, late follow up was focused on cardiac events. Despite similar LVEF at discharge, RHIR patients were characterized by enhanced neurohormonal activation possibly witnessing a persistent myocardial infiltration by leukocytes.

The invariable recovery of LVEF has misled the medical community into believing that TTS is benign and transient. Despite a well-recognized early mortality, few data have described the physiological and clinical status of these patients in the long term. In a small case control study, Scally and co-workers have demonstrated that lower exercise capacity and metabolic performance were still evidenced 20 months after TTS onset. Apical T1 prolongation, as a possible marker of

microscopic fibrosis, together with subtle alteration of the left apical strain and the wringing motion 'twist' were evidenced in TTS patients whilst no difference of LVEF could be observed. In our larger cohort, lower LVEF could be evidenced in RHIR patients at follow up consistent with a noxious impact of on-going chronic low-grade inflammation. This finding points to the fact that in case of ongoing inflammation noxious alteration of the myocardium may occur, leading to LVEF impairment. These data are in keeping with the observation that higher rates of cardiovascular mortality and recurrent heart failure could be evidenced in RHIR patients. By multivariable analysis, advanced age, history of cancer or vascular diseases, low diastolic pressure, low LVEF, and RHIR were evidenced as independent predictors of the combined primary endpoint. Importantly, the association between RHIR and adverse outcome was still evidence even after the exclusion of patients with concomitant infection (*Table 4*). Other reports have suggested that systemic inflammation could be associated with adverse events in TTS.[14] Besides the noxious role of inflammation in athero-thrombotic burden, systemic inflammation could also pave the way to arrhythmias as observed in the early phase possibly causing fatal events.[22–24] Accordingly, we could evidence prolonged QT duration in RHIR patients. In experimental studies, various pro-inflammatory cytokines such as TNF-alpha and IL-6 are demonstrated to induce action potential duration and QT prolongation by decreasing transient outward current It_o or by enhancing L type calcium current.[25] Altogether, the present data identify RHIR patients as a vulnerable high-risk subgroup requiring a close follow up. Along these lines, therapies to attenuate RHIR in TTS represent an appealing subject for future research.

Study limitation

Owing to the retrospective nature of our study, there are inherent limitations related to cofounding known or unknown factors. The time points of measurements of CRP at discharge varied. However, this is a large study, and to our knowledge, the only to specifically focus on RHIR in TTS and to evaluate its impact on cardiovascular late outcome. Inflammation evaluation was restricted to CRP. Other parameters reflecting inflammation may have been assessed such as cytokines measurements or monocytes count. Evaluation of LVEF at discharge was not standardized and was not assessed by a central Echo Lab. Finally, the lack of systematic evaluation of heart failure medical therapy including ACEi/ARBs or aldosterone receptor antagonists constitutes another limitation when interpreting the data.

Conclusions

Residual high inflammatory response was associated with enhanced neurohormonal activation at the acute phase and impaired LVEF at follow up. RHIR was evidenced as an independent factor of cardiac death or heart failure. All together, these findings underline RHIR patients as a high-risk subgroup, to target in future clinical trials with specific therapies to attenuate RHIR.

Conflict of interest

None declared.

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