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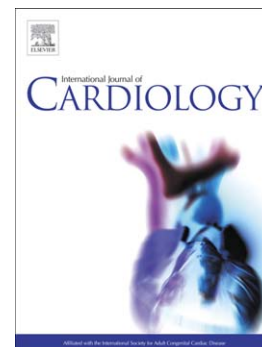
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TITLE PAGE:

(a) Title: Glycocalyx shedding is markedly increased during the acute phase of Takotsubo cardiomyopathy

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ABSTRACT

Background: Acute myocardial infarction (AMI) and other forms of myocardial acute oxidative stress are associated with variable “shedding” of the endothelial glycocalyx (GCS) which can be quantitated ex vivo by release into plasma of glycocalyx components such as Syndecan-1 (SD-1). Previous studies have implicated release of both catecholamines and BNP as potential accentuating factors in GCS: since these are prominent aspects of the pathogenesis of Takotsubo cardiomyopathy (TTC), we hypothesised that TTC is associated with increased GCS and the extent of GCS is predictable on the basis of NT-proBNP and catecholamine releases.

Methods: SD-1 concentrations were measured in 48 TTC patients acutely and after 3 months, and compared with those in 12 healthy controls, and 17 patients with AMI. Correlations were sought between SD-1 levels markers of severity of TTC episodes in individual patients.

Results: Acute SD-1 concentrations in TTC patients were elevated significantly ($p < 0.0001$, 1-way ANOVA) compared to control values. There were no significant correlations between SD-1 concentrations and any markers of severity of acute TTC episodes, such as NT-proBNP or catecholamine release. Over 3 months, SD-1 concentrations fell significantly ($p = 0.0002$) to approximately the same values as in control subjects.

Conclusions: TTC is associated acutely with a marked increase in GCS. Potentially, GCS might contribute to increased coronary vascular permeability in TTC, thus dissociating development of myocardial oedema from severity of associated inflammation. Prevention of GCS represents a potential therapeutic option in TTC.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Key words: Takotsubo cardiomyopathy, glycocalyx shedding, syndecan-1, myocardial inflammation.

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MAIN TEXT

1. Introduction

Takotsubo cardiomyopathy (TTC) or “broken heart syndrome” is a form of potentially reversible acute left ventricular systolic dysfunction which affects mainly women aged over 50 years, usually after physical or emotional stress and often resembles acute myocardial infarction (AMI) clinically [1]. It is interesting to reflect that although TTC was first characterised only 24 years ago [2], it is anything but rare, accounting for approximately 10% of “heart attacks” in women [3]. It has been recently reported in a large cohort study that TTC is associated with a substantial early mortality rate due to lethal arrhythmias and cardiogenic shock [4]. In-hospital mortality rates are approximately 2-6%, with high levels of residual disability for at least 3 months [3], and high risk of recurrence [5]. There are currently no proven strategies for the treatment of acute TTC or for the prevention of recurrence and these are urgently required.

TTC is known to be precipitated by surges of catecholamine release/exposure, in ageing (especially female) hearts. Recent studies have suggested that TTC is associated with a marked increase in markers of myocardial inflammation [such as brain natriuretic peptide (BNP)/NT-proBNP release [6] and prolonged myocardial oedema on cardiovascular magnetic resonance (CMR) [7] and with a substantial associated reduction in myocardial phosphocreatine to ATP ratio (PCr : ATP) [8], indicative of energetic impairment.

AMI and other forms of myocardial acute oxidative stress are associated with variable “shedding” of vascular and other glycocalyx [9], which controls both permeability and shear sensing in the macro-and micro-vasculature. This glycocalyx shedding (GCS) can be quantitated by release into plasma of glycocalyx components such as

Syndecan-1 (SD-1), which is the most prevalent proteoglycan. Previous *in vitro* studies have implicated release both of catecholamines [10] and BNP [11] as potential accentuating factors in GCS: since these are prominent aspects of the pathogenesis of TTC, we hypothesised that TTC (i) would be associated with increased GCS both acutely and during subsequent 3 months of continued myocardial inflammation and (ii) extent of GCS would be predictable on the basis of NT-proBNP and catecholamine release.

2. Methods: The study was designed as a comparison between cases of TTC and healthy postmenopausal female controls; a small group of patients with AMI, which is known to be associated with SD-1 release [9], served as a comparator group.

2.1 Patient population

(a) TTC group.

Consecutive TTC patients were prospectively identified on the basis of the following criteria: (1) chest pain and/or dyspnoea \geq 30min, (2) ST/T wave changes and/or biomarker elevation, (3) periapical or midventricular akinesis/hypokinesis, and (4) no evidence for a diagnosis of myocardial infarction on coronary angiography or cardiovascular magnetic resonance (CMR).

(b) Control group.

The control group of 12 females were recruited via newspaper advertisements. Subjects with current or previous symptomatic myocardial ischaemia were excluded.

(c) AMI group.

Patients who had been diagnosed with an AMI (23 ± 6 hours post onset of symptoms) were selected (n = 17).

For both TTC and AMI patients' blood, sampling for SD-1 levels occurred 1-3 days post onset of symptoms. In 36 of 48 TTC patients, the blood samples were taken within 24 hours post onset of symptoms.

This investigation complies with the principles outlined in the Declaration of Helsinki and was approved by the Institutional Ethics of Human Research Committee (Central Northern Adelaide Health Service: the Queen Elizabeth Hospital and Lyell McEwin Hospital; protocol number: 009014). Written informed consent was obtained from all participants before study entry.

2.2 Quantitation of glycocalyx shedding

Venous blood specimens were collected into EDTA-coated tubes, centrifuged at 4 °C at 1000g for 10 mins, and plasma was stored at -80 °C until assay. GCS was quantitated via measurement of syndecan-1 concentrations in plasma (sCD138 ELISA Kit, Diaclone Research, France). In the TTC patients, these evaluations occurred at the time of diagnosis and 3 months thereafter.

2.3 Relationships between glycocalyx shedding and severity of TTC

(a) Biochemical correlates

Plasma concentrations of normetanephrine and metanephrine, non-acidic derivatives of norepinephrine and epinephrine, were measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Serial routine biochemical investigations including assays of plasma NT-proBNP using immunoassay (Elecsys E 170, Roche Diagnostics, Mannheim, Germany), high sensitivity plasma C-reactive protein (hs-CRP) concentrations utilizing a latex-enhanced immunoturbidometric assay (Olympus au5400, Dallas, Texas, USA), Creatine kinase (CK), and troponin T were performed.

(b) Pathophysiological correlates

The lowest blood pressure during the first 24 hours of admission was obtained from patients' clinical records. Global indices of LV deficit and tissue inflammation were derived from echocardiographic and cardiovascular magnetic resonance imaging studies. T2-weighted CMR was performed as previously described utilizing a Philips Achieva 1.5 Tesla machine (Best, Netherlands)[7]. T2-weighted signal intensity (T2w-SI) was utilized as an index of myocardial oedema/inflammation.

2.4 Statistics

Data were analyzed using the SPSS software (version 15, Chicago, Illinois, USA) and presented as mean and SD or median and interquartile range depending on data distributions.

Comparisons between SD-1 levels in TTC patients at admission, AMI patients and control subjects were performed utilizing 1-way ANOVA. Unpaired t-tests were utilized further to compare the differences in SD-1 levels between individual groups (controls vs. acute TTC and acute TTC vs. AMI). P values were corrected for multiple testing using Bonferroni correction. A separate comparison between acute vs. 3 month TTC values was analyzed utilizing paired t-tests.

In order to test the hypothesis that the extent of GCS is correlated with markers of TTC severity, syndecan-1 concentrations were correlated with (i) acute LV function, (ii) lowest blood pressure (BP) on admission, (iii) peak plasma catecholamine levels, and (iv) hs-CRP, NT-proBNP concentrations, and T2 signal intensity (T2-SI) via linear regression or Spearman's correlation as appropriate. To evaluate potential determinants of acute GCS, backwards stepwise multiple logistic regression was performed. Parameters for inclusion in multivariate analysis were chosen on the bases of (i) a priori hypothetical rational for association (age, lowest BP within 24 hours of

admission, acute LVEF, Troponin concentrations), and/or (ii) association ($p < 0.2$) on univariate analysis (NT-proBNP, metanephrine concentrations).

A value of $p < 0.05$ was considered significant.

3 Results

3.1 Patients' characteristics

The clinical characteristics of the study population (TTC, control and AMI reference groups) are described in the Table. 48 female patients diagnosed with TTC were studied. There were no differences in age, gender and cardiovascular risk factors between the TTC, control and AMI subjects.

3.2 Glycocalyx shedding in TTC

(a) SD-1 elevations in TTC

As shown in Figure 1, the SD-1 concentrations were on average $56 \mu\text{g/L}$ higher for patients in the acute phase of TTC than for healthy subjects [mean 97 ± 65 (SD) vs. $41 \pm 10 \mu\text{g/L}$, $p = 0.005$, unpaired t-test, corrected for multiple testing using Bonferroni test].

(b) SD-1 elevations in AMI

SD-1 levels were lower in acute TTC compared to acute MI patients (mean 97 ± 65 vs. $256 \pm 208 \mu\text{g/L}$, $p < 0.0001$, unpaired t-test, corrected for multiple testing using Bonferroni test).

(c) Despite incomplete resolution of inflammation, SD-1 concentrations at 3 months post TTC attacks fell significantly in comparison with the acute levels ($p = 0.0002$, paired t-test) and did not differ significantly from values as in control subjects (Figure 2).

3.3 Associations between SD-1 concentrations and extent of severity of acute episodes

There were no correlations between SD-1 concentrations and extent of severity of acute episodes, as measured by lowest BP within 24 hours of admission, acute LV EF, Troponin, NT-proBNP, normetanephrine and metanephrine concentrations.

On multivariate analyses, the extent of SD-1 elevation in TTC patients was not related to any of the chosen markers of attack severity or to patients' age.

4. Discussion

TTC is often associated with hypotension during its acute stages, and with the development of myocardial oedema [7]. The extent of hypotension is not well correlated with left ventricular systolic dysfunction [12]. While the oedema has been ascribed to myocardial inflammation, this association has never been shown to be causative.

In the current study, we have shown that SD-1 release into plasma is markedly increased during the acute stages of TTC, and was approximately the same in magnitude as in AMI. Furthermore, this release returned to normal levels 3 months post onset of TTC.

Several factors already implicated in TTC are known to activate the process of GCS. Notably, both catecholamines and BNP are known stimuli for the process [10, 11]. Furthermore, there is considerable evidence that activation of matrix metalloproteinases represents a major stimulus for glycocalyx disruption [13], but no studies to date have evaluated this potential issue in TTC.

One residual issue is the precise sequence of biochemical/physiological events in TTC. For example, SD-1 release might be a "secondary" event, stimulated via

inflammation-mediated BNP release [11]. Alternatively, it could be a “primary” mechanistic change, whereby catecholamine effects translate to neutrophil permeation of damaged coronary vessels and thus to myocardial inflammation.

These findings should also focus attention on vascular function in TTC. GCS results not only in endothelial dysfunction [14] but also in abnormal responses to shear stress [15]. A study by Cecchi [16] showed that microvascular coronary reactivity was indeed abnormal in TTC, but the cause of this was not evaluated. GCS also facilitates monocyte adhesion to the venular endothelium and subsequent transmigration into the myocardium [17].

These findings also have substantial potential clinical applicability. For example, it may be that myocardial oedema accounts for impaired ventricular torsion and resultant decreases in cardiac output. Oedema within the pulmonary circulation could occur without substantial elevation of wedge pressures, via transudation of pulmonary capillary fluid. Importantly, ACE inhibitors, which appear to prevent recurrences of TTC, may limit matrix metalloproteinase (MMP) activation [18, 19]. Furthermore, low dose doxycycline represents a further potential therapeutic avenue via MMP inhibition [13].

The study has some limitations. The precise time course of release SD-1 was not investigated and it is also uncertain whether the process has completely resolved after 3 months. If indeed this is the case, the reason for hysteresis between inflammation and syndecan-1 release is uncertain. Given that the precipitant for TTC episodes was in theory a “pulse” release of catecholamines, it would be desirable to compare the time course of such a “pulse” with that of onset of glycocalyx shedding. This is in practice very difficult, because the time of onset of TTC cannot always be precisely estimated, and because only levels of the catecholamine derivatives normetanephrine

and metanephrine were measured (and even so, at a single time point). There was also an inevitable and variable delay between patient presentation and the diagnosis of TTC, so that initial sampling occurred 1-3 days post onset of symptoms: again this may have obscured the “time” peak of SD-1 release.

In conclusion, TTC is associated with acute release into plasma of SD-1. Investigation of the possible role of this process in the pathophysiology and potential therapeutics of TTC is appropriate.

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Figure legends:

Figure 1: Plasma SD-1 levels in control subjects, acute TTC, and AMI patients. $p < 0.0001$ for heterogeneity (1-way ANOVA); (** = 0.005 for acute TTC vs. controls, * < 0.0001 for acute TTC vs. AMI, unpaired t-test, corrected for multiple testing using Bonferroni test).

Figure 2: Changes in plasma syndecan-1 levels in individual TTC patients over 3 months' recovery ($p = 0.0002$, paired t-test).

Table: Patients' characteristics. *There were no statistically significant differences between groups in any parameter.*

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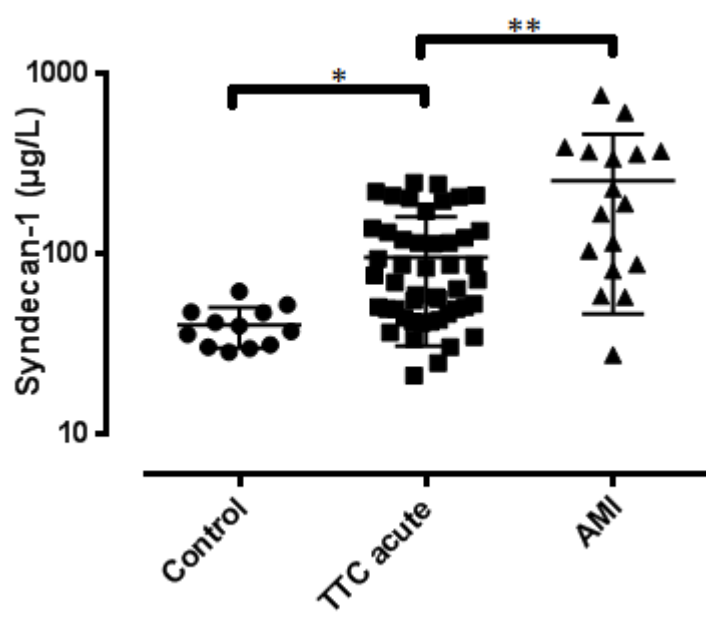


Figure 1

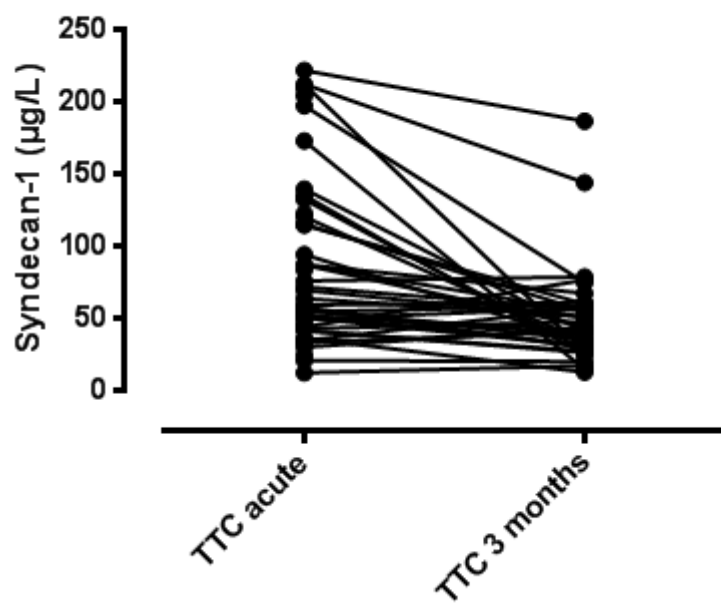


Figure 2

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Table: Patients' characteristics. *There were no statistically significant differences between groups in any parameter.*

Parameters	TTC (n = 48)	Controls (n = 12)	AMI (n = 17)
Age (mean \pm SD)	67 \pm 14	64 \pm 7	70 \pm 14
Current/past smoking (%)	28	11	25
Hypertension (%)	63	32	63
Diabetes mellitus (%)	28	21	13
Dyslipidemia (%)	40	32	31

Highlights:

- TTC is associated acutely with marked increase in GCS.
- GCS might contribute to increased coronary vascular permeability in TTC, thus dissociating development of myocardial oedema from severity of associated inflammation.
- Prevention of GCS represents a potential therapeutic option in TTC.