Biomedicine & Pharmacotherapy 136 (2021) 111259

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Original article

Persistent long-term platelet activation and endothelial perturbation in women with Takotsubo syndrome

Patrizia Amadio [a,1], Benedetta Porro [a,1,*], Viviana Cavalca [a], Silvia Stella Barbieri [a], Sonia Eligini [a], Susanna Fiorelli [a], Alessandro Di Minno [a,2], Alessandra Gorini [a,b], Mattia Giuliani [a], Josè Pablo Werba [a], Nicola Cosentino [a], Paolo Olivares [a], Simone Barbieri [a], Fabrizio Veglia [a], Elena Tremoli [a], Daniela Trabattoni [a]

[a] Centro Cardiologico Monzino I.R.C.C.S., Milan, Italy
[b] Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

ARTICLE INFO

Keywords:
Takotsubo syndrome
Endothelial dysfunction
Platelet activation
Aspirin
Long-term follow-up

ABSTRACT

Background: Takotsubo (TTS) syndrome is an acute cardiac condition characterized by transient and reversible left ventricle dysfunction that mainly affects postmenopausal women. Catecholamine burst is the most accredited mechanism underpinning TTS onset and leading to endothelial dysfunction and platelet activation. Even if the use of low dose acetylsalycilic acid (ASA) in this clinical setting is based on both clinical presentation and unfavorable long-term prognosis, its efficacy has been recently challenged.

Aim: This study was designed to assess endothelial function, residual thromboxane formation and platelet aggregation in TTS women on low-dose ASA treatment at long-term follow-up.

Methods: Twenty-eight females with previously diagnosis of TTS syndrome were enrolled. Data were compared to those obtained from 23 coronary artery disease (CAD) women with a history of acute myocardial infarction, and 26 control subjects with no TTS or clinically evident CAD. Psychological and clinical profile were assessed in all study groups at the enrollment. Main metabolites involved in L-arginine/nitric oxide pathway, urinary prostacyclin, serum and urine thromboxane metabolites were measured by LC—MS/MS methods. Thrombomodulin levels were quantified using an ELISA kit, and platelet aggregation, carried out on platelet rich-plasma, was induced by ADP or by epinephrine (EPI), norepinephrine (NORE) and TRAP-6, alone or in association with ADP and evaluated by Born's method.

Results: In TTS women an endothelial derangement, characterized by reduced citrulline production and increased thrombomodulin concentration, with no perturbation in prostacyclin levels, was evidenced. In addition, despite ASA treatment, TTS displayed a higher residual thromboxane formation, in parallel with an enhanced platelet response to compared to CAD.

Conclusions: Our study highlighted the presence of endothelial perturbation in TTS patients even at long-term from the index event. The residual thromboxane production and platelet aggregation still leave open the question about the use of low dose ASA in this clinical setting.

1. Introduction

Takotsubo syndrome (TTS) is an acute cardiac condition characterized by a transient and reversible left ventricular dysfunction that mainly affects postmenopausal women [1]. Its clinical presentation resembles an acute coronary syndrome, with severe chest pain, dyspnea, electrocardiographic and echocardiographic abnormalities, but without any haemodinamically significant stenosis [2].

The acute TTS event is often preceded by a sudden stressful emotional or physical experience occurring few hours or days before

* Corresponding author at: Unit of Metabolomics and Cellular Biochemistry of Atherothrombosis, Centro Cardiologico Monzino, IRCCS, Via Parea 4, 20138, Milan, Italy.
E-mail address: benedetta.porro@ccfm.it (B. Porro).
[1] Equally contributed.
[2] Present address: Department of Pharmacy Federico II University Naples, Italy.

https://doi.org/10.1016/j.biopha.2021.111259
Received 6 October 2020; Received in revised form 4 January 2021; Accepted 7 January 2021
Available online 12 January 2021

[3–7], able to induce substantial rise of plasma catecholamine levels [8], that, in turn, may elicit platelet activation [9]. Previous studies have indeed shown that patients presenting the highest levels of plasma adrenaline during the acute phase of TTS display increased platelet reactivity during the 3-months follow-up [10].

In parallel to an increased platelet activation, mental stress is also able to induce endothelial dysfunction, characterized by an imbalance between vasoconstricting and vasodilating factors [11,12]. Indeed, patients with a history of TTS have a reduced flow-mediated vasodilatation, even years after the event [13].

From a clinical point of view, even if the prognosis of TTS was formerly thought to be benign, both the short- and the long-term mortality are higher than previously recognized [2]. In addition, while there is uncertainty regarding the effective TTS recurrence rate, it has been shown that long-term Major Adverse Cardiovascular and Cerebrovascular Events (MACCE) rate is about 9.9 % per-patients per year [14,15]. Based on clinical presentation and on the presumed platelet activation low acetylsalicylic acid (ASA) treatment has been initially recommended and it is generally maintained also at long-term. Its potential efficacy has been recently investigated in a large TTS patients' Registry [16]. Specifically, the study failed to show a reduced risk of MACCE at short and long term, raising some doubts about the use of this treatment schedule in TTS. In addition, there is no evidence about a direct effect of ASA on catecholamine production, considered the main process involved in TTS acute event [17].

Based on these premises, aim of this work was to evaluate the usefulness of ASA treatment in TTS patients at long term follow up through the assessment of endothelial function, thromboxane formation and platelet aggregation.

2. Materials and methods

2.1. Study population

We carried out an open, single center study at Centro Cardiologico Monzino (CCM). We enrolled 28 post-menopausal females attending our Women Center with history of TTS syndrome (occurred 3.1 [0.8, 6.3] years, median [interquartile range], before enrollment) and 23 age-matched women with a history of acute myocardial infarction, either ST-elevated (STEMI) or not ST-elevated (NSTEMI), (occurred 3.7 [1.6, 7.4] years, median [interquartile range], before enrollment). At the index event, both TTS and CAD patients underwent clinical evaluation with cardiac biomarkers assessment (CK-MB, high-sensitivity Troponin I (hs-TnI), electrocardiogram, transthoracic echocardiogram (TTE), and coronary angiogram. Based on the results of their clinical evaluation and on ASA treatment, patients were selected and enrolled in this study.

For comparison, we enrolled 26 control women with no TTS or clinically evident CAD, with no ASA treatment, to define normal reference ranges for the laboratory variables investigated.

Demographics, cardiovascular risk factors, medical history and pharmacological treatment were collected on admission and at the follow-up for all patients and control subjects recruited.

The study was carried out in accordance with the Declaration of Helsinki and approved by the local Ethic research committee of CCM (R584/17-CCM 586). Written informed consent to participate was obtained from all subjects.

2.2. Psychological assessment

Considering the possible role of psychological determinants in the pathogenesis of TTS, a psychological assessment, consisting of an open question investigating the occurrence of one or more stressful events in the week before the acute clinical episode and of 7 self-report validated questionnaires, was conducted. Six out of seven of these questionnaires assessed the patients' psychological characteristics at the time of the evaluation, including depressive symptoms (Beck Depression Inventory – II; BDI-II) [18], state and trait anxiety (State-Trait Anxiety Inventory –Y; STAI-Y) [19], perceived stress (Perceived Stress Scale; PSS) [20], health related anxiety (Health Anxiety Questionnaire; HAQ) [21], and Type-D personality traits (Type-D Scale; DS-14) [22]. Finally, the Impact of Event Scale-Revised (IES-R) [23] was administered to evaluate the impact of stressful event (if any) that preceded the acute cardiac episode. Differently from the other questionnaires used to evaluate the actual patients' psychological conditions, the IES-R may be used to assess post-traumatic symptoms related to events occurred up to 10 years before the evaluation [24].

2.3. Blood sampling and biochemical measurements

2.3.1. Plasma

EDTA and citrated anticoagulated blood was centrifuged at 3000xg for 10 min at 4 °C or at room temperature, respectively, within 30 min after being drawn. Plasma was separated and aliquots were stored at −80 °C until analysis.

2.3.2. Serum

whole blood was collected in serum separator tubes and placed in a water bath at 37 °C for 2 h. Serum was then separated by a centrifugation at 1700xg for 10 min at room temperature and aliquots were stored at −80 °C until analysis.

2.3.3. Platelet rich plasma (PRP)

PRP was obtained from blood collected with sodium citrate (0.105 M), and immediately separated after blood withdrawal by a centrifugation at 200xg for 10 min. After PRP removal, platelet poor plasma (PPP) was obtained by an additional centrifugation at 1400xg at room temperature for 15 min and used as a reference sample for aggregation experiments.

2.3.4. Urine

an overnight urine collection was carried out and samples stored at −80 °C until analysis. The analytes estimated values were corrected for the urinary creatinine levels and expressed as pg/mg creatinine.

2.3.5. Biosynthetic pathway of NO representing endothelial function

arginine, the NO synthesis substrate, and the catabolic products, ornithine and citrulline, together with synthesis inhibitors asymmetric and symmetric dimethylargines (ADMA and SDMA) were simultaneously determined by liquid chromatography – tandem mass spectrometry (LC–MS/MS) in plasma EDTA, as previously described [25].

In addition, to investigate the systemic production of vascular prostacyclin in vivo, its major urinary metabolite, i.e., 2,3-dinor-6-keto-$PGF_{1\alpha}$, was measured by LC–MS/MS method [26].

2.3.6. Thromboxane (TXB_2) formation

Serum levels of TXB_2 was measured using a LC–MS/MS method previously developed and validated [27]. The systemic biosynthesis of TXA_2 was assessed by measuring 11-dehydro-TXB_2 by a previously developed and validated LC–MS/MS method [28].

2.3.7. Thrombomodulin release

Thrombomodulin levels were measured in citrated plasma using the Quantikine® Human Thrombomodulin/BDCA-3 Immunoassay (R&D Systems Europe), following the manufacturer's instructions.

Blood count, levels of catecholamines, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone and cortisol were carried out at the central laboratory of CCM.

2.4. Platelet aggregation

Platelet aggregation was performed in PRP samples, without adjusting platelet count, on CHRONO-LOG® Aggregometer 490−2D

(Chrono-log Corporation, Havertown, PA) [29] in the presence of stimuli as indicated: ADP (0.25–2 μM), Epinephrine (EPI) (1 μM), Norepinephrine (NORE) (4 mM) or TRAP-6 (8 μM). EPI, NORE and TRAP were also used in association with ADP 0.25 μM. The rate of aggregation was expressed as maximal Area Under the Curve (AUC expressed as percent*time) recorded after 5 min from the addition of the aggregating agent.

2.5. Statistical analysis

Continuous variables are presented as mean ± SD when normally distributed, or as median and interquartile ranges (IQR) otherwise. Categorical variables are presented as absolute numbers and percentages.

Comparisons between groups (TTS *vs* CAD and TTS *vs* Control) were performed by general linear models (GLM) adjusted for age, after log-transformation of variables with right-skewed distribution.

All tests were two-sided, and a P value of less than 0.05 was required for statistical significance. For tests regarding the primary endpoint (assessment of endothelial function, thromboxane formation and platelet aggregation in TTS compared to CAD patients), a Bonferroni correction for 12 independent comparisons was also applied, setting the threshold to $p = 0.0042$.

All calculations were computed with the aid of the SAS software package (Version 9.4 SAS Institute Inc., Cary, NC).

3. Results

3.1. Population characteristics

The main demographic and clinical characteristics of the subjects enrolled in this study are listed in Table 1 and Suppl Table 1.

TTS and CAD patients were comparable for hematological parameters, main cardiovascular risk factors and ASA therapy. Both groups shared similar baseline characteristics and an available clinical follow-up after the index event (data not shown). At the index event, mild specific cardiac enzyme elevation (peak hs Tn-I = 4307 [2380, 7810] ng/mL; CK—MB = 63 [32, 91] n/L), in association with a significant left ventricular systolic function depression (mean LVEF 41 ± 8.3 %) was evidenced in TTS patients, fully recovered to normality during the subsequent follow-up period (LVEF 67 ± 5.5 %). The mean time from TTS symptoms onset to emergency room presentation was 7 ± 17.9 h. No difference was observed in the levels of cortisol among the two study groups, whereas adrenaline was higher in TTS compared to CAD (Table 1), even if values were in the normal range. TTS or CAD were older than control women, thus data were adjusted for age.

As regard to the psychological investigation, in the majority of patients, TTS occurred after an emotional stress (89 %) in the week before their acute event, including the death of a relative, a friend or a significant person, a serious quarrel with a relative or a friend, an assault suffered or witnessed (e.g. snatch or domestic violence), and the personal involvement in a car accident or in a building collapse. According to a recent TTS consensus paper [14,15], all the reported events can be considered as emotional triggers. On the contrary, no CAD patients explicitly reported the occurrence of stressful experiences before their acute cardiac event.

No difference was found in the psychological questionnaires, except for the HAQ where TTS patients reported higher scores related to the need of reassurance, whereas CAD patients scored higher in the illness-related interference subscale (Table 2).

3.2. Endothelial markers

The components of the NO pathway were measured in plasma of TTS and CAD patients and in control subjects. Comparable levels of arginine were found in the three groups (Fig. 1A and Suppl. Fig. 1A). On the other hand, Citrulline concentrations and the related Citrulline/Arginine ratio were lower in TTS compared to CAD (Fig. 1B and C). When TTS were compared to controls, only the reduction in Citrulline levels was statistically significant (Suppl Fig. 1B). Ornithine levels were lower in TTS compared to CAD, but similar to controls (Fig. 1D and Suppl. Fig. 1D). All together these data are strongly suggestive of a potential lower production of NO in TTS patients compared to the other two groups. This lower NO production may be attributable to a reduced activation of NO synthase as shown by diminished Citrulline levels, with no difference in the concentrations of enzyme and arginine transport inhibitors ADMA or SDMA, respectively (data not shown).

In addition, TTS patients showed augmented circulating levels of the

Table 1
Demographic and clinical characteristics, hormone levels and pharmacological treatments of TTS and CAD patients.

Variable	TTS (n = 28)	CAD (n = 23)	p value
Demographic and clinical characteristics			
Age - years	67.9 ± 8.9	69.5 ± 8.8	0.51
Hypercolesterolemia - no. (%)	15(53.6 %)	16(69.6 %)	0.24
Hypertension - no. (%)	22(78.6 %)	19(82.6 %)	0.27
RBC ($x10^6$/μL)	4.6 ± 0.4	4.8 ± 0.5	0.06
WBC ($x10^3$/μL)	6.3 ± 1.5	7.0 ± 1.6	0.08
PLT ($x10^3$/μL)	236.6 ± 44.3	207.0 ± 54.3	**0.03**
IPF (%)	5.5 ± 2.8	4.1 ± 1.7	0.22
MPV (fL)	10.4 ± 0.9	10.6 ± 0.9	0.24
Hb (g/dL)	13.2 ± 1.2	14.2 ± 1.4	**0.008**
Hct (%)	40.0 ± 2.8	42.5 ± 3.9	**0.01**
MCV (fL)	90.5 ± 4.2	90.0 ± 4.4	0.73
MCH (pg)	30.3 ± 1.3	30.0 ± 1.7	0.55
MCHC (%)	33.2 ± 0.7	33.3 ± 0.7	0.70
RDW-SD (fL)	42.3 ± 2.9	44.0 ± 4.1	0.11
RDW-CV (%)	13.2 ± 0.9	13.5 ± 1	0.30
Hormone levels			
Cortisol (ng/mL)	117.9 ± 38.2	117.1 ± 41.8	0.52
Adrenaline (ng/L)	41.6 ± 19.6	33.9 ± 23.5	**0.05**
Noradrenaline (ng/L)	469.8 ± 145.5	614.7 ± 320.1	0.38
Pharmacological treatments			
Statins - no. (%)	10(35.7 %)	16(69.5 %)	**0.02**
β-blockers - no. (%)	20(71.4 %)	18(78.2 %)	0.75
ACE inhibitors - no. (%)	12(42.8 %)	11(47.8 %)	0.78
Calcium channel blockers - no. (%)	0 (0%)	0 (0%)	
Diuretics - no. (%)	0(0%)	7(30.4 %)	**0.002**
Aspirin - no. (%)	17(60.7 %)	15(65.2 %)	0.78
Angiotensin receptor blockers - no. (%)	0(0%)	4(17.4 %)	**0.03**

Table 2
Psychological characteristics of TTS and CAD patients. **BDI-II**: Beck Depression Inventory – II; **DS-14**: Type-D personality traits; **STAI-Y**: State-Trait Anxiety Inventory –Y; **HAQ**: Health Anxiety Questionnaire; **PSS**: Perceived Stress Scale; **IES**: Impact of Event Scale.

Psychological assessment		TTS (n = 28)	CAD (n = 23)	p value
BDI-II		7.96 ± 5.6	9.71 ± 6.1	0.37
DS-14	*Negative Affectivity*	8(4;14)	10(5;15)	0.57
	Social Inhibition	6(1.5;9.5)	5.5(2;12)	0.71
STAI-Y	*State*	37.57 ± 9.9	38.36 ± 9.1	0.86
	Trait	38.75 ± 11.1	40.73 ± 7.4	0.50
PSS		16.71 ± 8	18.05 ± 5.9	0.55
HAQ	*Interference*	0(0;1)	2(1;3)	**<0.001**
	Fear of death and diseases	4(2;5)	5(4;7)	0.07
	Worry about health	4(2.5;7)	5.5(4;8)	0.60
	Reassurance	2(1.5;4)	1(0;2)	**0.02**
	Total	10(7;12)	13.5(10;19)	**0.01**
IES	*Avoidance*	14.5(2;21)	16(7.5;21.5)	0.50
	Intrusiveness	14(2.5;18.5)	11.5(6;18)	0.98
	Total	28(7.5;40.5)	24(16;38)	0.96

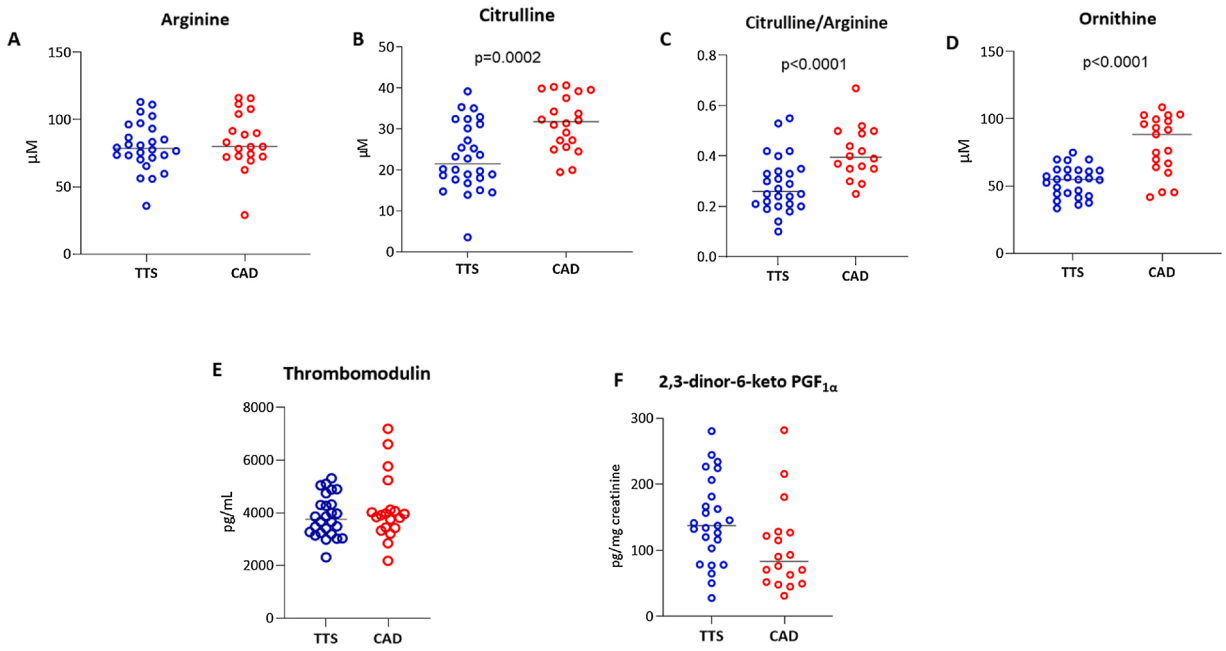

Fig. 1. Endothelial markers in TTS (blue circles) and CAD (red circles) patients. Plasma levels of A) Arginine, B) Citrulline and D) Ornithine were measured in TTS and CAD patients to evaluate NO pathway components. C) Citrulline/Arginine ratio was additionally assessed. Endothelial damage was also evaluated measuring E) thrombomodulin levels in plasma of TTS and CAD patients. F) 2,3-dinor-6-keto $PGF_{1\alpha}$, the main metabolite of prostacyclin, was measured in urine of TTS and CAD patients. Data are represented as individual values. Black line indicates median value.

soluble form of Thrombomodulin compared to control subjects (even this difference did not reach statistical significance after Bonferroni correction) and similar to those observed in CAD group (Fig. 1E and Suppl. Fig. 1E). This finding provides new evidence of the presence of vascular endothelial cell damage in this clinical setting.

As regard to prostacyclin, its main urinary metabolite, 2,3-dinor-6-keto-$PGF_{1\alpha}$, showed comparable levels in the three groups (Fig. 1F and Suppl. Fig. 1F).

3.3. *Platelet serum thromboxane formation and activation in response to aggregating agents*

As previously mentioned, TTS and CAD patients were under continuous treatment with low dose ASA (100 mg/d), which specifically is expected to inhibit TXB_2 biosynthesis by platelets. The serum levels of TXB_2 in TTS patients were significantly higher than those found in CAD (Fig. 2A). Residual TXB_2 levels in TTS serum, however, were, as expected, significantly lower than those measured in controls, who were ASA naïve (Fig. 2A). In addition, TTS patients had greater levels of 11-dehydro-TXB_2 in urine compared to CAD, even this difference did not reach statistical significance after Bonferroni correction. No difference was observed between TTS and controls (Fig. 2B).

We then analysed the response of platelets to different aggregating agents. PRP aggregation in response to low dose ADP (0.25−1µM) was comparable among TTS and CAD patients and controls (Suppl. Fig. 2). In contrast, PRP aggregation of TTS patients induced by 2 µM ADP, in spite of ASA treatment, was greater than that of CAD, but remained still similar to controls, as shown by representative tracings and AUC of PRP aggregation (Fig. 3). PRP of TTS patients showed enhanced aggregation in response to EPI or NORE alone or in combination with 0.25 µM ADP with respect to CAD patients (Fig. 4). PRP from TTS, however, in response to these latter agonists displayed a slightly lower aggregation compared to that of controls ASA naïve (Fig. 4). After Bonferroni correction, only the aggregation induced by NORE, alone or in combination with ADP, remains statistically higher in TTS compared to CAD.

Interestingly no difference was observed in terms of PRP aggregation in response to TRAP among the three groups (Suppl. Fig. 3).

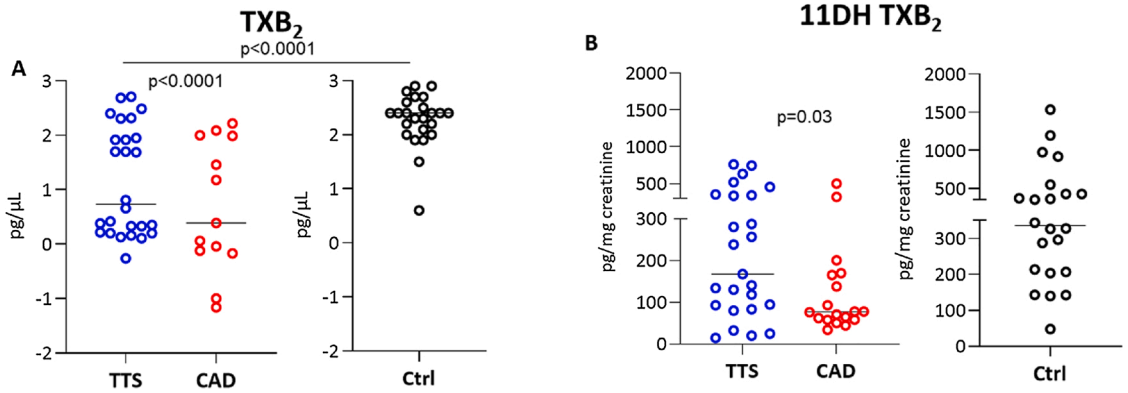

Fig. 2. Thromboxane formation. Metabolites of thromboxane A_2 measured in A) serum and B) urine of TTS (blue circles), CAD (red circles) patients and in control subjects (black circles). Data are represented as individual values. Black line indicates median value.

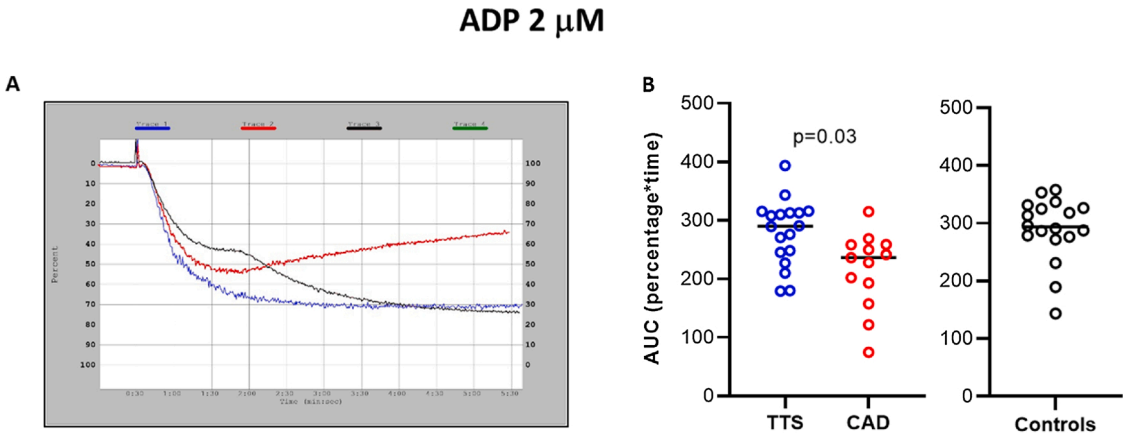

Fig. 3. Platelet aggregation in response to the highest dose of ADP. Platelet rich plasma (PRP) isolated from TTS, CAD patients and control subjects was stimulated with ADP 2 μM and platelet aggregation was recorded for 5 min. A) Representative platelets aggregation in TTS (blue line), CAD (red line) patients and control (black line) subjects and B) maximal AUC of platelets aggregation in TTS (blue circles), CAD (red circles) patients and control (black circles) subjects. Data are represented as individual values. Black line indicates median value.

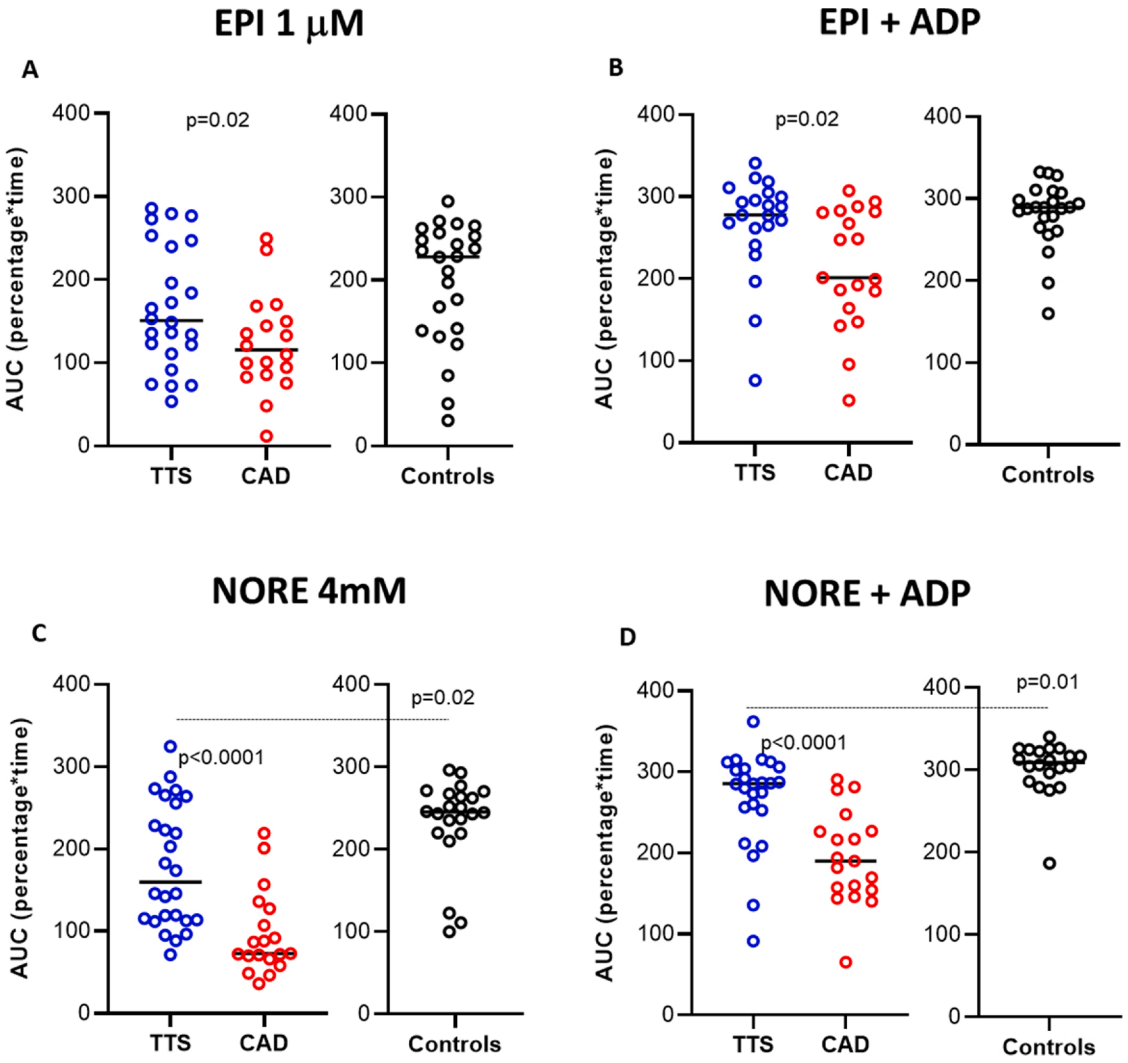

Fig. 4. Platelets aggregation in response to Epinephrine (EPI) and Norepinephrine (NORE) alone or in combination with ADP. Platelet rich plasma (PRP) isolated from TTS, CAD and control subjects was stimulated with A) EPI 1 μM or C) NORE 4 mM alone and combined with ADP (B, D); platelet aggregation was recorded for 5 min. A-D) Maximal AUC of platelets aggregation in TTS (in blue), CAD (in red) patients and control (in black) subjects are reported. Data are represented as individual values. Black line indicates median value.

4. Discussion

This study shows that women previously diagnosed as TTS, several years after the index event, display alterations in the production of endothelial markers involved in vascular tone and in thrombosis, as well as residual platelet activation, in spite of low dose ASA treatment.

TTS is still a poorly diagnosed disease that has been recognized within years to be potentially associated with severe clinical complications. The disease includes a transient left ventricular dysfunction without obstructive coronary disease, often associated to a rise in catecholamine levels.

Treatment with ASA is currently used in this pathological condition, even in the absence of recognised coronary clear-cut atherosclerotic involvement. Indeed, previous studies show that at 3 months after the acute event, TTS patients show an activated platelet response, which apparently is more marked in those with the highest levels of plasma catecholamines [10]. On the other hand, when the clinical presentation of TTS is associated with CAD, administration of low dose ASA is mandatory, on the basis of guidelines [14,15].

The efficacy of low dose ASA in TTS has been recently challenged by the analysis of the Inter-TAK registry, since no difference in MACCE was found after 30 days and 5 years clinical follow-up between TTS patients receiving or not low dose ASA at discharge after the acute event [16].

Our study, performed in a relatively small number of women with an episode of TTS that occurred 3.1 [0.8, 6.3] years earlier, provides evidence that, even on the long-term, the residual platelet thromboxane biosynthesis, measured in serum [an endogenous stimulus that fully activates the capacity of platelets to produce this arachidonic acid metabolite] is significantly greater than that of women with CAD, thus suggesting the occurrence in TTS women of a reduced capacity of ASA to fully inhibit platelet thromboxane formation.

Urinary thromboxane metabolite excretion is considered a non-invasive biomarker of platelet activation in vivo [30] and increased excretion of this oxidation product of arachidonic acid has been reported in a variety of patients with cardiovascular atherogenic risk factors [31–35]. Excretion of 11-dehydro-TXB_2 was greater in TTS than in CAD, but similar to that of control subjects ASA naïve. Altogether, these findings indicate a reduced effect of ASA on thromboxane formation in TTS patients even on a long-term follow-up after the index event.

The residual thromboxane formation is considered a marker of potential increased thrombotic risk in several clinical settings and may be linked to suboptimal inhibition of platelet aggregation. We deeply investigated the behaviour of platelets in terms of aggregation in response to a variety of agonists.

Using ADP as aggregating agent, we found a trend to a reduced inhibition of PRP aggregation in TTS patients treated with low dose ASA compared with CAD only when we stimulated platelets with the highest ADP concentration (2 μM), paralleling data observed in the two clinical settings concerning the levels of thromboxane in serum. Of note is the fact that no difference in PRP aggregation was found among the three groups when lower doses of ADP were used as stimulus (0.25–1 μM).

As previously mentioned, the onset of TTS is mostly associated with a burst of catecholamines that are responsible of a variety of effects at different levels [15]. Of note, epinephrine levels were higher in TTS compared to CAD, highlighting that the alteration in its production may persist for long time after the index event. As consequence, we hypothesize that such TTS patients, whose acute TTS event was, in the most cases, preceded by an acute psychological stress, have an altered neurohormonal response both at resting conditions and as a response to stress that make them more vulnerable to stressful events than others. Nevertheless, such hypothesis need to be deepen with further research.

On the other hand, catecholamines are known to influence platelet aggregation [9]. In our TTS group, the aggregation of PRP in response to epinephrine was higher than in CAD and similar to that obtained in the control group. Interestingly, the aggregation in response to norepinephrine alone or in combination with ADP was significantly greater in TTS compared with CAD, but slightly lower than that of controls. These findings go in parallel with the plasma levels of cathecolamines, suggesting a more pronounced involvement of norepinephrine in TTS clinical setting.

Several factors, beside catecholamines, may be responsible for the limited effect of ASA in TTS compared to CAD. They include activation of inflammatory patterns, differences in ASA absorption or metabolism, platelet turnover with increments in circulating reticulocytes [36]. A reduced capacity of antithrombotic properties of endothelium may also be considered. Of note is the fact that in plasma of TTS greater levels of thrombomodulin, a marker of endothelial damage, were found. In addition, thrombomodulin levels in TTS were similar to those of CAD, who presumably display alterations in endothelial function due to the presence of coronary disease and/or specific cardiovascular risk factors. In addition, changes were found both in the synthesis of products of the NO pathway and indirectly in NOS activity in TTS, compared to CAD and controls. Citrulline and the citrulline/arginine ratio were found lower in TTS compared to CAD, indicating a potential impairment of NO production after long-term follow-up in TTS, which may support alterations in the microcirculation, that have been reported in this pathological condition [37]. Finally, about the psychological variables, the only difference observed, refers to one aspect of the patients' health anxiety. In particular, CAD patients showed a higher level of illness interference compared to TTS. This observation may be explained considering that CAD women usually experience a long-lasting threatening event, with severe prolonged consequences on their everyday life.

5. Conclusions

Our study highlights the presence of endothelial perturbation in TTS patients even at long-term follow-up after the index event. In addition, the residual thromboxane production and platelet aggregation still leaves open the question about the use of low dose ASA in this clinical setting. However, since our study was conducted on a relative small number of subjects, further investigations are needed in order to validate our findings in a large cohort of patients.

Funding

This work was supported by funding from the Italian Ministry of Health, Rome, Italy (Ricerca Corrente RC 2018 CI 1.5 ID 2634621, 2019 MPP 2B ID 2755316).

CRediT authorship contribution statement

Patrizia Amadio: Formal analysis, Data curation, Investigation. **Benedetta Porro:** Formal analysis, Data curation, Investigation. **Viviana Cavalca:** Formal analysis, Data curation, Conceptualization, Supervision, Funding acquisition. **Silvia Stella Barbieri:** Formal analysis, Data curation, Funding acquisition. **Sonia Eligini:** Conceptualization, Supervision. **Susanna Fiorelli:** Investigation. **Alessandro Di Minno:** Investigation. **Alessandra Gorini:** . **Mattia Giuliani:** . **Josè Pablo Werba:** . **Nicola Cosentino:** . **Paolo Olivares:** . **Simone Barbieri:** . **Fabrizio Veglia:** . **Elena Tremoli:** Conceptualization, Supervision. **Daniela Trabattoni:** Conceptualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

References

[1] A.R. Lyon, E. Bossone, B. Schneider, U. Sechtem, R. Citro, S.R. Underwood, M. N. Sheppard, G.A. Figtree, G. Parodi, Y.J. Akashi, F. Ruschitzka, G. Filippatos, A. Mebazaa, E. Omerovic, Current state of knowledge on Takotsubo syndrome: a position statement from the taskforce on Takotsubo syndrome of the heart failure

association of the European Society of Cardiology, Eur. J. Heart Fail. 18 (1) (2016) 8–27.
[2] F. Pelliccia, J.C. Kaski, F. Crea, P.G. Camici, Pathophysiology of Takotsubo syndrome, Circulation 135 (24) (2017) 2426–2441.
[3] A. Compare, R. Bigi, P.S. Orrego, R. Proietti, E. Grossi, A. Steptoe, Type D personality is associated with the development of stress cardiomyopathy following emotional triggers, Ann. Behav. Med. 45 (3) (2013) 299–307.
[4] A. Prasad, Apical ballooning syndrome: an important differential diagnosis of acute myocardial infarction, Circulation 115 (5) (2007) e56–9.
[5] V. Vidi, V. Rajesh, P.P. Singh, J.T. Mukherjee, R.M. Lago, D.M. Venesy, S. Waxman, C.T. Pyne, T.C. Piemonte, D.E. Gossman, R.W. Nesto, Clinical characteristics of tako-tsubo cardiomyopathy, Am. J. Cardiol. 104 (4) (2009) 578–582.
[6] J.R. Ghadri, V.L. Cammann, C. Templin, The international Takotsubo registry: rationale, design, objectives, and first results, Heart Fail. Clin. 12 (4) (2016) 597–603.
[7] F. Galli, F. Bursi, S. Carugo, Traumatic events, personality and psychopathology in Takotsubo syndrome: a systematic review, Front. Psychol. 10 (2019) 2742.
[8] Y.J. Akashi, H.M. Nef, A.R. Lyon, Epidemiology and pathophysiology of Takotsubo syndrome, Nat. Rev. Cardiol. 12 (7) (2015) 387–397.
[9] G. Anfossi, M. Trovati, Role of catecholamines in platelet function: pathophysiological and clinical significance, Eur. J. Clin. Invest. 26 (5) (1996) 353–370.
[10] I.J. Nunez-Gil, E. Bernardo, G. Feltes, J. Escaned, H.D. Mejia-Renteria, J.A. De Agustin, D. Vivas, L. Nombela-Franco, P. Jimenez-Quevedo, C. Macaya, A. Fernandez-Ortiz, Platelet function in Takotsubo cardiomyopathy, J. Thromb. Thrombolysis 39 (4) (2015) 452–458.
[11] L.E. Spieker, D. Hurlimann, F. Ruschitzka, R. Corti, F. Enseleit, S. Shaw, D. Hayoz, J.E. Deanfield, T.F. Luscher, G. Noll, Mental stress induces prolonged endothelial dysfunction via endothelin-a receptors, Circulation 105 (24) (2002) 2817–2820.
[12] L. Sandrini, A. Ieraci, P. Amadio, M. Zara, S.S. Barbieri, Impact of acute and chronic stress on thrombosis in healthy individuals and cardiovascular disease patients, Int. J. Mol. Sci. 21 (21) (2020).
[13] M. Naegele, A.J. Flammer, F. Enseleit, S. Roas, M. Frank, A. Hirt, P. Kaiser, S. Cantatore, C. Templin, G. Frohlich, M. Romanens, T.F. Luscher, F. Ruschitzka, G. Noll, I. Sudano, Endothelial function and sympathetic nervous system activity in patients with Takotsubo syndrome, Int. J. Cardiol. 224 (2016) 226–230.
[14] J.R. Ghadri, I.S. Wittstein, A. Prasad, S. Sharkey, K. Dote, Y.J. Akashi, V. L. Cammann, F. Crea, L. Galiuto, W. Desmet, T. Yoshida, R. Manfredini, I. Eitel, M. Kosuge, H.M. Nef, A. Deshmukh, A. Lerman, E. Bossone, R. Citro, T. Ueyama, D. Corrado, S. Kurisu, F. Ruschitzka, D. Winchester, A.R. Lyon, E. Omerovic, J. J. Bax, P. Meimoun, G. Tarantini, C. Rihal, Y.H. S, F. Migliore, J.D. Horowitz, H. Shimokawa, T.F. Luscher, C. Templin, International expert consensus document on Takotsubo syndrome (Part II): diagnostic workup, outcome, and management, Eur. Heart J. 39 (22) (2018) 2047–2062.
[15] J.R. Ghadri, I.S. Wittstein, A. Prasad, S. Sharkey, K. Dote, Y.J. Akashi, V. L. Cammann, F. Crea, L. Galiuto, W. Desmet, T. Yoshida, R. Manfredini, I. Eitel, M. Kosuge, H.M. Nef, A. Deshmukh, A. Lerman, E. Bossone, R. Citro, T. Ueyama, D. Corrado, S. Kurisu, F. Ruschitzka, D. Winchester, A.R. Lyon, E. Omerovic, J. J. Bax, P. Meimoun, G. Tarantini, C. Rihal, Y.H. S, F. Migliore, J.D. Horowitz, H. Shimokawa, T.F. Luscher, C. Templin, International expert consensus document on Takotsubo syndrome (Part I): clinical characteristics, diagnostic criteria, and pathophysiology, Eur. Heart J. 39 (22) (2018) 2032–2046.
[16] F. D'Ascenzo, S. Gili, M. Bertaina, M. Iannaccone, V.L. Cammann, D. Di Vece, K. Kato, A. Saglietto, K.A. Szawan, A.H. Frangieh, B. Boffini, M. Annaratone, A. Sarcon, R.A. Levinson, J. Franke, L.C. Napp, M. Jaguszewski, M. Noutsias, T. Munzel, M. Knorr, S. Heiner, H.A. Katus, C. Burgdorf, H. Schunkert, H. Thiele, J. Bauersachs, C. Tschope, B.M. Pieske, L. Rajan, G. Michels, R. Pfister, A. Cuneo, C. Jacobshagen, G. Hasenfuss, M. Karakas, W. Koenig, W. Rottbauer, S.M. Said, R. C. Braun-Dullaeus, A. Banning, F. Cuculi, R. Kobza, T.A. Fischer, T. Vasankari, K.E. J. Airaksinen, G. Opolski, R. Dworakowski, P. MacCarthy, C. Kaiser, S. Osswald, L. Galiuto, F. Crea, W. Dichtl, W.M. Franz, K. Empen, S.B. Felix, C. Delmas, O. Lairez, I. El-Battrawy, I. Akin, M. Borggrefe, J.D. Horowitz, M. Kozel, P. Tousek, P. Widimsky, E. Gilyarova, A. Shilova, M. Gilyarov, G. Biondi-Zoccai, D. E. Winchester, C. Ukena, M. Neuhaus, J.J. Bax, A. Prasad, C. Di Mario, M. Bohm, M. Gasparini, F. Ruschitzka, E. Bossone, R. Citro, M. Rinaldi, G.M. De Ferrari, T. Luscher, J.R. Ghadri, C. Templin, Impact of aspirin on Takotsubo syndrome: a propensity score-based analysis of the InterTAK Registry, Eur. J. Heart Fail. 22 (2) (2020) 330–337.
[17] C.C. Smith, A.P. Wilson, D.J. Betteridge, The influence of aspirin on plasma and platelet catecholamine levels, and platelet function in normal man, Clin. Auton. Res. 3 (1) (1993) 49–53.
[18] A.T. Beck, R.A. Steer, R. Ball, W. Ranieri, Comparison of beck depression inventories -IA and -II in psychiatric outpatients, J. Pers. Assess. 67 (3) (1996) 588–597.
[19] C.D. Spielberger, State-Trait Anxiety Inventory: Bibliography, 2nd ed., 1989.
[20] S. Cohen, T. Kamarck, R. Mermelstein, A global measure of perceived stress, J. Health Soc. Behav. 24 (4) (1983) 385–396.
[21] M.P. Lucock, S. Morley, The health anxiety questionnaire, Br. J. Health Psychol. 1 (Part 2) (1996) 137–150.
[22] J. Denollet, DS14: standard assessment of negative affectivity, social inhibition, and Type D personality, Psychosom. Med. 67 (1) (2005) 89–97.
[23] M. Horowitz, N. Wilner, W. Alvarez, Impact of Event Scale: a measure of subjective stress, Psychosom. Med. 41 (3) (1979) 209–218.
[24] N. Morina, T. Ehring, S. Priebe, Diagnostic utility of the impact of event scale-revised in two samples of survivors of war, PLoS One 8 (12) (2013) e83916.
[25] I. Squellerio, E. Tremoli, V. Cavalca, Quantification of arginine and its metabolites in human erythrocytes using liquid chromatography-tandem mass spectrometry, Anal. Biochem. 412 (1) (2011) 108–110.
[26] V. Cavalca, B. Rocca, I. Squellerio, A. Dragani, F. Veglia, F. Pagliaccia, B. Porro, S. S. Barbieri, E. Tremoli, C. Patrono, In vivo prostacyclin biosynthesis and effects of different aspirin regimens in patients with essential thrombocythaemia, Thromb. Haemost. 112 (1) (2014) 118–127.
[27] I. Squellerio, B. Porro, P. Songia, F. Veglia, D. Caruso, E. Tremoli, V. Cavalca, Liquid chromatography-tandem mass spectrometry for simultaneous measurement of thromboxane B2 and 12(S)-hydroxyeicosatetraenoic acid in serum, J. Pharm. Biomed. Anal. 96 (2014) 256–262.
[28] V. Cavalca, F. Minardi, S. Scurati, F. Guidugli, I. Squellerio, F. Veglia, L. Dainese, A. Guarino, E. Tremoli, D. Caruso, Simultaneous quantification of 8-iso-prostaglandin-F(2alpha) and 11-dehydro thromboxane B(2) in human urine by liquid chromatography-tandem mass spectrometry, Anal. Biochem. 397 (2) (2010) 168–174.
[29] D. Baldassarre, M. Amato, S. Eligini, S.S. Barbieri, L. Mussoni, B. Frigerio, M. Kozakova, E. Tremoli, C.R. Sirtori, S. Colli, Effect of n-3 fatty acids on carotid atherosclerosis and haemostasis in patients with combined hyperlipoproteinemia: a double-blind pilot study in primary prevention, Ann. Med. 38 (5) (2006) 367–375.
[30] C. Patrono, B. Rocca, Measurement of thromboxane biosynthesis in health and disease, Front. Pharmacol. 10 (2019) 1244.
[31] B. Rocca, G. Ciabattoni, R. Tartaglione, S. Cortelazzo, T. Barbui, C. Patrono, R. Landolfi, Increased thromboxane biosynthesis in essential thrombocythemia, Thromb. Haemost. 74 (5) (1995) 1225–1230.
[32] R. Landolfi, G. Ciabattoni, P. Patrignani, M.A. Castellana, E. Pogliani, B. Bizzi, C. Patrono, Increased thromboxane biosynthesis in patients with polycythemia vera: evidence for aspirin-suppressible platelet activation in vivo, Blood 80 (8) (1992) 1965–1971.
[33] F. Santilli, G. Davi, A. Consoli, F. Cipollone, A. Mezzetti, A. Falco, T. Taraborelli, E. Devangelio, G. Ciabattoni, S. Basili, C. Patrono, Thromboxane-dependent CD40 ligand release in type 2 diabetes mellitus, J. Am. Coll. Cardiol. 47 (2) (2006) 391–397.
[34] G. Davi, M.T. Guagnano, G. Ciabattoni, S. Basili, A. Falco, M. Marinopiccoli, M. Nutini, S. Sensi, C. Patrono, Platelet activation in obese women: role of inflammation and oxidant stress, JAMA 288 (16) (2002) 2008–2014.
[35] G. Di Minno, G. Davi, M. Margaglione, F. Cirillo, E. Grandone, G. Ciabattoni, I. Catalano, P. Strisciuglio, G. Andria, C. Patrono, et al., Abnormally high thromboxane biosynthesis in homozygous homocystinuria. Evidence for platelet involvement and probucol-sensitive mechanism, J. Clin. Invest. 92 (3) (1993) 1400–1406.
[36] V. Cavalca, B. Rocca, F. Veglia, G. Petrucci, B. Porro, V. Myasoedova, R. De Cristofaro, L. Turnu, A. Bonomi, P. Songia, L. Cavallotti, M. Zanobini, M. Camera, F. Alamanni, A. Parolari, C. Patrono, E. Tremoli, On-pump cardiac surgery enhances platelet renewal and impairs aspirin pharmacodynamics: effects of improved dosing regimens, Clin. Pharmacol. Ther. 102 (5) (2017) 849–858.
[37] B. Porro, S. Eligini, F. Veglia, A. Lualdi, I. Squellerio, S. Fiorelli, M. Giovannardi, E. Chiorino, A. Dalla Cia, M. Crisci, J.P. Werba, E. Tremoli, V. Cavalca, Nitric oxide synthetic pathway in patients with microvascular angina and its relations with oxidative stress, Oxid. Med. Cell. Longev. 2014 (2014), 726539.