Platelet function in Takotsubo cardiomyopathy

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Abstract Takotsubo cardiomyopathy (TK) includes a transient left ventricular dysfunction without obstructive coronary disease, sometimes after stressful situations with elevated catecholamines. Since catecholamines activate platelets we aimed to study the platelet influence in a TK setting. We included 32 patients with a TK diagnosis, 13 with an acute coronary syndrome (ACS) and 18 healthy volunteers. Once consent informed was obtained, blood samples were extracted and processed (at admission and after 3 months follow-up). Clinical, ecg, echocardiographic and angiographic features were thoroughly recorded. Previous treatment before admission was similar between groups. No differences were observed in clinical features or any of the acute markers studied regarding platelet reactivity between TK compared to ACS. After follow-up, aggregation levels and platelet reactivity showed differences, mainly due to the antithrombotic therapy prescribed at discharge, but similar to volunteers. Circulating epinephrine during the acute phase was significantly higher in TK (p < 0.001). Patients with higher levels of epinephrine had elevated platelet activation and aggregation after 3 months. No differences were observed in Takotsubo acute platelet aggregation compared to patients with ACS, in spite of higher blood levels of adrenaline. Takotsubo patients had elevated platelet aggregation and activation compared with ACS patients at 3 months follow-up because they were less frequently on chronic clopidogrel and ASA. However, they had similar platelet aggregation and activation levels to healthy volunteers despite treatment with low-dose ASA. Takotsubo patients who had higher levels of adrenaline in the acute phase displayed increased platelet reactivity during follow-up.

Keywords Acute coronary syndrome · Platelet aggregation · Platelets · Takotsubo syndrome

Introduction

Takotsubo cardiomyopathy (TK) usually includes a transient left ventricular dysfunction without responsible obstructive coronary artery disease [1–4]. Its diagnosis is growing around the world mainly due to the increasing knowledge in the medical community after its description in Japan in the early 90s [1, 3]. Stressful situations have been reported as a trigger of TK by several authors [5–7]. In addition, high levels of catecholamines (mainly epinephrine) and a specific B-adrenergic receptor distribution in the myocardium of patients with TK support a causative link between stress and this entity [8–11]. However, a definitive pathophysiological explanation is still lacking [4, 12, 13]. Despite TK has been classified as a cardiomyopathy, it shares many clinical, analytical and electrocardiographic characteristics with acute coronary syndromes [14]. Since platelet activation plays a key
role in the genesis of acute coronary syndromes, it is justified
the long-term double antithrombotic therapy recommendation

In addition, we know catecholamines cause platelet
activation [16–21], thus, we hypothesized cathecolamine-
induced platelet activation might be partly responsible for
the clinical presentation of TK cardiomyopathy.

Methods

Study patients

We designed a prospective registry on Takotsubo syndrome
(march 2008–march 2012). To be eligible, patients had to
fulfill the modified Mayo criteria as previously published
(march 2008–march 2012). To be eligible, patients had to
be identified during coronary angiography on the grounds of absence of significant
obstructive coronary lesions and left ventriculography sug-
gestive of TK. Once consent informed was obtained from the
patient, a blood sample was extracted and processed. A control
cohort of patients with ACS admitted at our institution, mat-
ched by date of admission, gender, age ±5 years, hyperten-
sion, diabetes, creatinine clearance, acute coronary syndrome
type—with or without ST segment elevation, acute coronary
syndrome—infection type I-patients, was included. Patients
presenting with ACS over the weekend or holiday periods
were excluded from the beginning, due to the impossibility to
process blood samples ad hoc. In addition, serial blood
extractions, EKG tracings and repeated echocardiograms were
performed to track changes, including recovery of regional left
ventricular wall motion abnormalities. All patients were
managed by their attending cardiologist according to current
clinical guidelines at that time. After discharge, a 3-month
follow-up visit was scheduled, including a new blood sam-
ing and an echocardiogram. Finally, a cohort of healthy
volunteers facilitated some blood samples to compare with the
follow up patients samples. Exclusion criteria: any antiplate-
let, anticoagulants or anti-inflammatory drug taken within 2
previous weeks, abnormal platelet or leukocyte count, any
history of abnormal bleeding, thrombosis, active inflammatory
disease or coronary artery disease. All of them provided a
written consent to participate in the procedures of this study.

Platelet laboratory assessment

Platelet aggregation

Platelet aggregation was assessed using light transmittance
aggregometry (LTA). In brief, LTA was performed in platelet-
rich plasma (PRP) by the turbidimetric method in a four-channel
aggregometer (Chrono-Log 490 Model, Chrono-Log Corp.,
Havertown, Pennsylvania) according to standard protocols. The
PRP was obtained as a supernatant after centrifugation of cit-
rated blood at 800 rpm for 10 min and platelet-poor plasma
(PPP) was obtained after a second centrifugation of samples at
2,500 rpm for 10 min. Platelet count in PRP was adjusted to a
range of 250,000 platelet/μL by dilution with autologous PPP
when the platelet count was out of range. Light transmission was
adjusted to 0 % with PRP and to 100 % with PPP for each
measurement. Curves were recorded during 5 min, and platelet
aggregation was determined as the maximal percent change in
light transmittance from baseline using PPP as a reference.
Adenosine diphosphate (ADP) 5 μM was used to assess
P2Y_{12}-dependent pathway aggregation. Epinephrine 1, 5 and
20 μM and norepinephrine 15 mM, and dopamine 30 mM
were used to assess P2Y_{12}-independent pathway aggregation.

Flow cytometry analyses

Platelet surface expression of activated GP IIb/IIIa was
assessed using PAC-1 (PAC1-FITC conjugated, Becton–
Dickinson, Rutherford, New Jersey) antibodies and.
P-selectin surface expression was assessed using a phycoc-
erythrin-conjugated anti-CD62P (Becton–Dickinson, San
Jose, California) antibody. Both, GP IIb/IIIa and P-selectin
expression were assessed before and after addition of ADP
0.5 μM and epinephrine 5 μM Samples were analyzed
within 2 h by flow cytometry using a Beckman Coulter
Gallios flow cytometer (Coulter, Miami, Florida) and a
total of 10,000 CD61-positive (Coulter, Miami, Florida)
events were collected with all light scatter and fluorescence
parameters in a logarithmic mode. Platelets were gated on
the basis of light scatter and CD61 expression. Activated
platelets were defined as the percentage of CD61-positive
events expressing the activated confirmation of PAC-1
binding and P-selectin (CD62P). Data were expressed as
the percentage of platelets positive for antibody binding.

Serum epinephrine levels

Blood from serum tubes were centrifuged at 2,500 rpm for
15 min. Serum samples were frozen at −70 °C until lab-
oratory determinations. Total levels of epinephrine were
assessed by ELISA kit (IBL International GMBH, Ham-
burg, Germany) at baseline and 3 months follow-up sam-
pies following the manufacturer’s instructions.

Statistical analysis

Baseline characteristics are expressed as mean ± standard
development or median (inter-quartile range) for continuous
variables and absolute number for categorical variables.
Comparisons between groups were made with Pearson’s
Chi square-test for categorical variables and the t test or
Results

A total of 32 patients fulfilling the TK Mayo criteria were included in the study, along with 13 patients with ACS, and 18 healthy volunteers. Table 1 shows demographic data of the study population.

Potential physical or emotional triggers were found more frequently in the TK cohort (Table 2). No statistically significant differences between TK and ACS controls were found for epidemiological features, Table 3. Moreover, there were no differences regarding the time of onset of symptoms \((p = 0.70)\), type of pain, palpitations, syncope or shock on admission. Time to cardiac cath was short in all cases (for TK: median 0, interquartile range 0–1 days; for ACS median 0, interquartile range: 0–1 day; \(p = \text{ns}\)).

During hospital stay, TK patients displayed less leucocytes and platelet count, less troponin I and CK peak, and similar EF (Table 4). Mitral regurgitation (MR) was an issue with better outcomes in the TK group, with lower MR grade after follow up. On the contrary, ACS patients displayed MR more frequently at follow-up. Previous medical treatment before admission was similar between groups, Table 5. During hospitalization, the logical differences were associated with greater proportion of heart failure in the TK group and the higher thrombotic burden in ACS-patients. At discharge, treatment regimens differed in the same way (Table 5). Median in-stay was 7 days for both groups. Follow-up after discharge was carried out in all cases approximately 3 months after index...
Follow-up events were more frequent in the ACS cohort (Table 6). After three months, a normal LVEF was displayed in all TK patients (inclusion criteria). Then, mean LVEF was slightly higher in TK patients at that point (Table 4).

### Platelet study

Stimulation with ADP epinephrine (1, 5 and 20 μM), nor-epinephrine and 15 mM 30 mM dopamine revealed that patients with TK presented similar levels of platelet aggregation than those with ACS during the acute phase (Fig. 1a, of note, all patients were on full antithrombotic therapy, at the time of the cardiac cath). The ADP increase in platelet aggregation after platelet previously stimulated with epinephrine (1, 5 and 20 μM), nor-epinephrine and dopamine 30 mM 15 mM was similar in patients with STK group compared with the group of patients ACS (Fig. 1b), as well.

Regarding platelet reactivity, the acute activation of integrin GPIIb/IIIa by binding the antibody PAC-1 and P-selectin expression on the platelet surface was also measured. Both determinations were performed by flow cytometry before (rest) and during platelet stimulation with 0.5 μM ADP and 5 μM epinephrine. No significant differences were observed either in none of those markers studied between TK patients compared to patients with ACS (Fig. 1c).
After follow up, aggregation levels and platelet reactivity showed differences (Fig. 2a, b, c), mainly due to the antithrombotic therapy at discharge (double antiplatelet therapy for ACS Vs only aspirin—21 over 32 in TK patients, Table 5). Platelet function was also assessed in healthy volunteers ($n=18$, age $36 \pm 6$ years) and compared with stable TK patients (in the 3-month visit). We observed that TK patients had levels of aggregation and platelet activation similar to healthy volunteers, in spite no healthy volunteer was on any drug (Fig. 2c).

Interestingly, circulating levels of epinephrine during the acute phase of the disease were significantly higher in TK patients compared with ACS patients due a differential release of catecholamines produced in the acute phase, Fig. 3.

In order to identify those patients with higher basal catecholamine release, we divided the population of patients with TK in three groups according to epinephrine level (25, 50 and 75 percentile). We defined patients with high baseline levels of epinephrine as those belonging to the 75th percentile and those with low levels when below this 75th percentile. We observed that the patients with higher acute levels of epinephrine had elevated platelet aggregation and activation at 3 months follow-up (Fig. 4). Perhaps, this result was not observed in the baseline sample as a result of the intensive antiplatelet therapy used during the acute phase.

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**Fig. 1 a** Platelet aggregation levels in patients with Takotsubo syndrome and acute coronary syndrome (controls) in the acute phase, together with the results displayed in healthy volunteers (no treatment). **b** Levels of platelet activation in the acute phase of TK, ACS patients, and volunteers (group without treatment)

**Fig. 2 a** Platelet aggregation levels in patients with Takotsubo syndrome and acute coronary syndrome (controls) in the stable phase, after 3 months follow up. **b** Platelet aggregation levels after ADP $5 \mu$M co-stimulation 3 months after admission. **c** Levels of platelet activation in patients with TK and ACS at 3 months follow-up, compared with healthy volunteers
Discussion

This work represents one of the first studies on platelets in the context of TK [22]. Although TK syndrome still does not have a definitive pathophysiological explanation, it has been suggested the key influence of catecholamines in what it seems a transient condition similar to myocardial stunning [8, 11]. Moreover, as demonstrated in previous works, plasma catecholamine levels are conclusively much higher in the TK setting than in ACS, including patients in Killip class III after a myocardial infarction [10]. Our results agree(s) with these previous data. This fact, together with a different beta receptor distribution in the left ventricle could have a key influence in this unclear condition [8]. Also, on the one hand, platelet activity plays a key role in the genesis and management of myocardial infarction [15] and, on the other hand, catecholamines stimulate platelet activation [17, 19, 20, 23], as is clearly seen in some laboratory tests. Thus, it seemed reasonable to explore the influence of platelets in a condition with high catecholamine levels as TK [10]. It is noteworthy that despite a growing number of publications on TK [24] there are no data in this regard. This issue gives us an idea of the logistical difficulties for the study in this rare disease [25]. Additionally, in the acute setting, the comparison between ACS and TK regarding platelet activation is hampered by the intense antithrombotic treatment that both clinical situations require [15, 26].

Despite these pharmacological differences on platelet aggregation, the clinical course is better in the TK [21, 26], even in spite of the short follow-up and the limited number of patients, as previously published elsewhere [21]. This point suggests that platelets maybe are not as important in TK as in ACS patients. After LV recovery, three months later, it is not easy to establish actual differences between TK and ACS platelet activity, mostly because ACS patients are in the vast majority on double antiplatelet therapy [15], while TK are only on aspirin, if any. TK patients displayed in our study, for this reason, platelet aggregation levels much higher that ACS patients. However, at 3 months, Epinephrine levels remained higher in TK arm, although the differences did not reach statistical significance in this occasion. These results, limited by lack of statistical power, could point higher basal levels of catecholamines in asymptomatic TK patients, which may have pathophysiological significance. Besides, as previously published as well, patients with Takotsubo syndrome have a better clinical outcome than those with ACS during follow-up despite worst Killip class at presentation [26].

Interestingly, in other matters, when we compared platelet activity in TK patients with some healthy volunteers we found no significant differences, despite being some TK patients on aspirin. This issue, along with the previously mentioned data, seriously challenges the overall indication of aspirin therapy in TK, arising the question how much dose and for how long?. Also, from our point of view, the use of dual antiplatelet therapy is not usually justified for TK patients. Of course, one must consider thoroughly the clinical profile of the patient, when making this decision, because they often are elderly women with multiple cardiovascular risk factors, requiring aspirin for other reasons.

During follow up, stratifying by levels of epinephrine, we observed that TK patients with higher levels of epinephrine had elevated platelet activation and aggregation. So, as final clinical implication for our findings, this could be a way to determine which patients might theoretically benefit from receiving antiplatelet treatment since globally the prognosis is good whether or not receiving aspirin.

Limitations

First, the small number of patients included in the study. On top of that, we had to exclude some TK patients presenting on weekend and vacation days because of technical
inability to process blood samples. Nevertheless, all TK patients were collected prospectively and continuously included in a register, participating or not in this study, and are published elsewhere, with a long term clinical follow up [21]. The need for antithrombotic therapy in all patients during the acute phase, for ethical reasons, decreases the possibility of obtaining differences at that moment. However, it is an approximation to the actual practice.

**Conclusion**

No differences were found in Takotsubo acute platelet aggregation compared to patients with ACS, probably due to the intensive antiplatelet therapy, although they presented higher blood levels of adrenaline. Takotsubo patients had elevated platelet aggregation and activation compared with ACS patients at 3 months follow-up possibly because they were less frequently on chronic clopidogrel and ASA. However, they had similar platelet aggregation and activation levels to healthy volunteers dogrel and ASA. However, they had similar platelet aggregation and activation levels to healthy volunteers.

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**Conflict of interest** None.

**References**