Peer Review File

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Reviewer A

The authors present a nice updated review on Takotsubo syndrome.
The manuscript is well written and carefully presented.
I would only propose a few minor remarks.

General reply: Many thanks for the review and the interesting and positive comments.

Pg 7.
Although the metanalysis you mentioned did not confirm the presence of high catecholamine levels, some other papers pointed out again higher levels than in patients with regular type I infarctions in the acute phase (https://pubmed.ncbi.nlm.nih.gov/25052832/).

Reply: Thank you for pointing out this important issue. Following the reviewer's suggestion we have add the following sentence (page 6, lines 23-24; and page 7 1-2): "*Some studies demonstrated significantly elevated levels of plasma catecholamines and stress-related circulating neuropeptides during the acute phase of TTS compared to patients with ST-elevation myocardial infarction (STEMI) (22, 29).*"

Pg 8.
…Overall an increased sympathetic stimulation appears to play a critical role in TTS….
Here, Lyon et al proposed an interesting theory on the change of Prot G stimul to Prot G inh in ventricular cardiomyocytes, explaining the segmental motion abnormality. https://pubmed.ncbi.nlm.nih.gov/18094670/

Reply: We appreciate the reviewer's insightful comment. We have partially reformulated the sentence and included the previously mentioned article information as requested (page 7, line 25-page9 line 10): "*Overall, an increased sympathetic stimulation and increased tissue catecholamine levels due to impaired reuptake appear to play a critical role in TTS. In this regard, some authors proposed that elevated levels of circulating epinephrine prompt a shift in the internal pathway of signal transmission within ventricular cardiomyocytes, transitioning from G(s) protein to G(i) protein signalling, via the beta(2)-adrenoceptor. While this transition safeguards against the proapoptotic consequences of intense beta(1)-adrenoceptor activation, it also has a detrimental impact on contractility. This impact seems most pronounced in the apical myocardium, which possesses the highest density of beta-adrenoceptors (39,40). However, the precise underlying mechanism of TTS remains elusive, leading to the open discussion of other pathophysiological theories.*"

Pg 13
A study demonstrated that takotsubo syndrome is metabolically different than AMI, showing limited myocardial energy production capacity during the acute phase. https://pubmed.ncbi.nlm.nih.gov/34357333/. In the future, this could have diagnostic importance.

Reply: Thank you again for this important contribution. In this regard, we have included the following sentence (page 14, lines 8-10): "*Indeed, one study demonstrated that TTS is metabolically different from AMI, showing limited myocardial energy production capacity during the acute phase (96).*"

Reviewer B

The review is well conceived. However, some aspects need to be taken into account.

General reply: Thank you very much for your review, and the interesting and constructive

comments.

1. I suggest to summarize the "Pathogenesis and pathophysiology" that are too long and dispersive.

Reply: In response to this suggestion, we have consolidated certain elements within the 'Pathogenesis and Pathophysiology' section. Moreover, we respectfully ask for the reviewer's consideration concerning the incorporation of particular facets into this same section, as proposed by multiple other reviewers. Recognizing the intrinsic uncertainty surrounding TTS's pathophysiology and the existence of various theories, we consider essential to provide an encompassing exploration of this important aspect.

2. Authors should expand the discussion about secondary TTS.

Reply: Thank you for this comment, following your suggestion we have expanded the discussion about secondary TTS (page 17, lines 1-12): *"In this regard, we must highlight that TTS triggered by physical factors showed higher mortality in the short and long term, but not all physical triggers influence the prognosis of patients in the same way. Within this group, patients whose physical trigger was hypoxia and TTS secondary to neurological disorders were clearly associated with increased rates of adverse events and in-hospital mortality (104,105). Probably, differences in the short- and long-term prognosis of TTS patients are the result of adding pathophysiological differences that may exist among each of the triggers, as well as the individual prognosis of each of the comorbidities. These data provide additional evidence to support the idea that under the TTS label, there could be as yet undiscovered very different clinical profiles, and therefore reinforce the concept that TTS is not a benign entity."*

We also refer to the response to Reviewer C, which is also related to this issue.

3. Authors can add some consideration about pheochromocytoma-induced TTS (De Angelis et al. Pheochromocytoma-induced cardiogenic shock: A multicentre analysis of clinical profiles, management and outcomes. Int J Cardiol. 2023 Jul 15;383:82-88)

Reply: Many thanks, this is a truly interesting article, and we have included now some considerations about it (page 16, lines 22-25; page 17 line 1): *"Recently one study showed that Pheochromocytoma-induced cardiogenic shock should be suspected in case of TTS progressing to cardiogenic shock (CS), particularly if an atypical TTS echocardiographic pattern is observed, or in case of a CS with severe cyclic blood pressure fluctuation and rapid hemodynamic deterioration, associated with increased inflammatory markers (103)."*

4. I would also suggest reviewing the " Management and medical therapy" paragraph which is a little bit chaotic, and superficial. I suggest to summarize and outlining this paragraph differentiating the different clinical scenarios (TTS complicated by cardiogenic shock, TTS complicated by apical thrombus, LVOTO...)

Reply: *Thank you for your observation and advice. Following your suggestions, we have summarized the section and differentiated some relevant clinical scenarios in the way the reviewer suggested.*

-Page 24, line 15: *TTS complicated by acute heart failure*

-Page 25, line 3: *TTS complicated by cardiogenic shock and left ventricular outflow tract obstruction*

-Page 26, line 22: *TTS complicated by left ventricular thrombus*

-Page 27, line 8: *Long-term pharmacological treatment of TTS*

5. It could be also interesting to mention some evidence about the use of mechanical circulatory support in each of these scenarios.

Reply: Thank you again for this suggestion. We have included a new paragraph pointing out to this relevant issue (page 25, lines 16-22): *"Interestingly, mechanical circulatory support for TTS-related shock is increasingly reported, with a growing use of veno-arterial extracorporeal membrane oxygenation and Impella, while those using intra-aortic balloon pump declined (153, 154). Currently, available clinical data support this approach with an excellent overall survival rate (153, 154). However, prospective studies are needed to evaluate the safety and efficacy of different devices as well as the timing of mechanical circulatory support in this special patient population."*

Reviewer C

No reference is made about the classification of Takotsubo Syndrome (TTS) depending on the cause that triggers it. As much as TTS is commonly triggered by intense emotional stress, it can be secondarily triggered by drugs, substances of abuse, metabolic disorders, and neurological syndromes. The latter forms are increasingly being studied by the scientific community; in my opinion, it is therefore necessary to reference them.

General reply: Many thanks for your review and this interesting comment.

Reply: We totally agree with this point. It is crucial to differentiate between TTS depending on emotional stress or "primary TTS" and the "physically triggered" or secondary TTS. In fact, those related to secondary forms and especially related to neurological syndromes seem to carry a worse prognosis. This issue was also raised by other reviewers. Following this important suggestion, we have highlighted the TTS classification depending on the trigger nature (primary vs secondary forms) that we previously included, modified one paragraph headline, and included the following sentences plus some important references:

- Page 16, line 3, paragraphs heading: *"Classifications of Takotsubo syndrome"*
- Page 16, lines 5-6: ***"We accept so far two different categories of TTS patients: primary and secondary forms."***
- Page 15, line 12-13: "*…for another (medical, surgical, anaesthetic, obstetric, neurological, or psychiatric) condition."*
- Page 17, lines 1-12: "In this regard, we must highlight that TTS triggered by physical factors showed higher mortality in the short and long term, but not all physical triggers influence the prognosis of patients in the same way. Within this group, patients whose physical trigger was hypoxia and TTS secondary to neurological disorders were clearly associated with increased rates of adverse events and in-hospital mortality (104,105). Probably, differences in the short- and long-term prognosis of TTS patients are the result of adding pathophysiological differences that may exist among each of the triggers, as well as the individual prognosis of each of the comorbidities. These data provide additional evidence to support the idea that under the TTS label, there could be as yet undiscovered very different clinical profiles, and therefore reinforce the concept that TTS is not a benign entity."

-Ref 104: Uribarri A, et al. Short- and Long-Term Prognosis of Patients With Takotsubo Syndrome Based on Different Triggers: Importance of the Physical Nature. J Am Heart Assoc. 2019;8(24):e013701

-Ref 105: Cammann VL, Scheitz JF, von Rennenberg R, Jäncke L, Nolte CH, Szawan KA, et al. Clinical correlates and prognostic impact of neurologic disorders in Takotsubo syndrome. Sci Rep.

2021;11(1):23555.

Reviewer D

Salamanca and Alfonso's general review on Takotsubo syndrome (TTS) relies mostly on publications over 5 years' old and it is not comprehensive. As oppose to the title, it does not take a view. The enigma of TTS remains what it is and the review does not point the way forward.

General reply: We sincerely thank the reviewer for their comments and critiques, which we are confident have greatly contributed to improving our work. Considering the limitations of text and length, we have tried to create a comprehensive review that allows the reader to examine in the most comprehensive possible way a complex pathology that the TTS entails. From the title, we have attempted to capture the reader's attention; its interrogative format, we believe, encourages the reader to read the review and increase their understanding of the entity, but undoubtedly capturing the path ahead to unravel the mystery of TTS.

1. There is a plethora of reviews on TTS. How is this review different and how were the referenced papers selected?

Reply: We genuinely appreciate your thoughtful question. While it is true that there is a wealth of reviews available on TTS, our review stands out in a few key aspects. Firstly, our approach involved an exhaustive search for articles published in recent years trying to cover a wide and updated spectrum of topics including epidemiology, physiopathology, diagnosis, clinical manifestations, treatment, and prognosis, and even incorporating assessments of numerous recent reviews. We tried to meticulously select papers that not only covered the core aspects of TTS but also incorporated diverse perspectives, cutting-edge research, and emerging insights. We believe that this allowed us to construct a holistic view that considers the multifaceted complexity of TTS.

Furthermore, we believe thanks to the changes made based on your comments and those of the rest of the reviewers, we could eventually address some gaps and discrepancies present in the existing literature. By critically evaluating the referenced papers, we've identified areas where consensus is lacking and where further investigation is needed. This adds an extra layer of value to our review as it not only summarizes existing knowledge but also highlights directions for future research. Thanks again.

2. There is a blatant contradiction between multivessel vasospasm and then disregarding vasospasm as a trigger for TTS since apical ballooning encompasses more than one coronary territory. The Texas group's work should be included (P Angellini). All the non-vasospasm theories do not explain the TTS variants – mid LV and basal LV TTS. Acute TTS is clearly ischaemic in nature with ST elevation which settles into deep T inversion. Further, the notion that vasospasm could not be elicited following convalescence does not preclude the vasospasm aetiology. Vasoreactivity is not a permanent state. For example, patients presenting with thyrotoxicosis and vasospasm. Vasoreactivity settles with its treatment. The authors can review the characteristics of patients with recurrent TTS and those with persistent anginal symptoms.

Reply: Thank you for bringing this issue to our attention. Following your recommendation, we have included this sentence and referenced the cited Texas group's work (page 8, lines 19-25): "*In this regard, Angellini et al. proposed that TTS onset is likely driven by vasospasm and preexisting endothelial dysfunction as all the non-vasospasm theories do not explain the TTS non-apical variants (45). Nevertheless, using intracoronary acetylcholine testing, one study found a predisposition to coronary vasospasm in only 21% of TTS patients (46). Some concern persists that acetylcholine testing is not reliable; however, this objection could be attributable to improper timing of testing (45).*"

3. The fact that TTS is overwhelmingly common in oestrogen deficient older women does suggest a central role for this hormone in preventing TTS. People on oestrogen replacement therapy (ORT) are protected from TTS. This section should be expanded. Although younger women with early menopause are not thought to be at risk of TTS perhaps many are on ORT but this can be reviewed more in depth.

Reply: Thank you again for pointing out this relevant issue. We agree with the reviewer that hormonal factors, and most likely estrogen deprivation, play a role in the development of TTS and could also be involved in its treatment. However, we cannot ignore conflicting aspects that some studies have highlighted. Following this insightful recommendation from the reviewer, we have replaced the old subsection with this new, more developed one. (page 11, lines 18-25; and page 12, lines 1-14):

"*Estrogen deprivation, hormonal and reproductive factors*

The high prevalence of TTS in postmenopausal females strongly suggests a potential hormonal influence. It has been observed that a reduction in estrogen levels following menopause increases the vulnerability to TTS (72). Estrogens play a role in regulating endothelial nitric oxide synthase activity, which influences vasomotor tone (73). Additionally, in perimenopausal women they attenuate the sympathetic response to mental stress and reduce catecholamine-induced vasoconstriction (74,75). The demonstration of estrogen supplementation in animal studies reducing the occurrence of TTS when faced with emotional stress (76,77) has prompted researchers to posit that reproductive factors might be involved in the initiation of TTS. To date, however, there is limited information in humans, and few small clinical studies examining the role of hormonal and reproductive factors in the development of TTS. One interesting study compared estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone levels in women with TTS, AMI, and healthy ones and found higher concentrations of estradiol in TTS compared to both controls (78). Another small study compared reproductive characteristics of TTS female cases, AMI controls, and healthy women and showed that TTS women more frequently reported a history of irregular menses and menopausal symptoms, higher parity, and using hormone replacement therapy compared to AMI and healthy female controls (79). These hormonal and reproductive factors undoubtedly play a role in the pathophysiology of TTS and could have therapeutic implications yet to be clarified."

4. Identify gaps in knowledge and the way forward to solve the mystery of TTS.

Reply: Thank you for this suggestion. Indeed, throughout the manuscript, we highlight in the various sections on pathophysiology (thoroughly discussing each of the suggested and potentially involved mechanisms) and treatment, those gaps in evidence that the reviewer astutely calls for. All of these should undoubtedly serve as a source of inspiration for future research in this exciting field. In this regard, we have also included a concluding paragraph encouraging such exploration and investigation. Thank you again (page 30, lines 3-22):

" Gaps in knowledge and future directions

Currently, several key gaps persist in the understanding of this intriguing condition, underscoring the need for continued and deeper research. The complete elucidation of its pathophysiology remains elusive, and there is also an absence of comprehensive knowledge about the various phenotypes and their associated prognoses. The treatment approaches for both the acute phase and follow-up are still not fully established, and the specificity of acute and chronic biomarkers for diagnosing and prognosticating TTS remains uncertain. These critical aspects warrant dedicated focus through robust multicenter research initiatives. For instance, to the best of our knowledge, nowadays there are two ongoing randomized clinical trials, NACRAM (N-Acetylcysteine and Ramipril Takotsubo Syndrome Trial, ACTRN12616000781448) and BROKEN-SWEDE-HEART (Optimized Pharmacological Treatment for Broken Heart

[Takotsubo] Syndrome, NCT04666454), currently investigating different treatment strategies for patients with TTS (176,177). This, therefore, underscores the need for immediate action, encompassing not only fresh research initiatives addressing these aspects but also the implementation of randomized clinical trials. These trials would assess the effectiveness of existing treatment strategies (i.e. BB) while also delving into innovative approaches that draw from historical insights and emerging pathophysiological understandings of this captivating condition."

Reviewer E

Thank you for allowing me the opportunity to review this comprehensive review on Takotsubo syndrome.

The review is generally well written and provides a detailed review of this interesting condition but this could be improved further:-

General Reply: We greatly appreciate the reviewer for their thorough review and their insightful and positive comments. All of these have been valuable in significantly improving our new version of the work.

1. Where is the Introduction? Your paper starts with an abstract and immediately on the section of Definition and Epidemiology. There should always be an Introduction in all papers describing the aim of this review article.

Reply: Thank you, the reviewer is right, and we have added now an introduction to start the paper.

"*Introduction*

Takotsubo syndrome (TTS), also known as stress-induced cardiomyopathy or broken heart syndrome, is a unique and intriguing condition that has gained recognition in recent years. TTS mimics the symptoms of acute coronary syndrome (ACS) but is characterized by distinct cardiac abnormalities, often triggered by emotional or physical stressors (1). This syndrome is marked by transient left ventricular dysfunction. However, there are numerous aspects within its pathophysiology that remain incompletely understood, and its treatment primarily relies on international consensus and expert recommendations without the support of randomized clinical trials. In this review, we delve into the epidemiology, pathophysiology, clinical presentation, diagnostic challenges, evolving management strategies, and the gaps in knowledge for future directions of TTS."

2. "Takotsubo syndrome (TTS) is an acute cardiac condition characterized by a form of transient regional wall motion abnormalities (RWMA) in the absence of culprit epicardial coronary artery disease on angiography." This statement is not entirely true as coronary artery disease can exist in TTS as per the InterTAK Diagnostic Criteria. It is also important to mention that TTS is an acute condition characterised by reversible left ventricular dysfunction.

Reply: We absolutely agree with the reviewer in the fact, as he/she wisely pointed out, that coronary artery disease can exist in TTS. In this regard we already acknowledge this important point in our review (page 19, lines 3-6): *"All of them share a common essence, but it is worth noting that the InterTAK criteria recognize the possibility of significant coronary artery disease coexisting with TTS and also acknowledge that pheochromocytoma may act as a trigger for TTS."* Moreover, we have changed the mentioned sentence (page 4, lines 15-17): *"Takotsubo syndrome (TTS) is an acute cardiac condition characterized by a form of transient regional wall motion abnormalities (RWMA) and left ventricular dysfunction."*.

3. "Recurrence of TTS is estimated to be 1.8% per-patient year." This statement should not appear in the section of Definition and epidemiology. There should be another section discussing about Recurrence TTS.

Reply: This is an important suggestion. Thank you again. We have elaborated a short section regarding TTS recurrence (page 29, lines 15-24; page 30, lines 1-2):

"*Takotsubo syndrome recurrence*

Patients with TTS are at risk of TTS recurrence. The recurrence rate for TTS is estimated to be 1.8% per patient-year (8). Interestingly, a variable TTS pattern upon recurrence is observed in up to 20% of cases (8). However, there are conflicting results regarding potential predictors of TTS recurrence (173,174) Unfortunately, we lack evidence about the optimal treatment to prevent such recurrences. It's important to note that the triggering factors can also vary in cases of recurrence. Moreover, some authors advocate that neurological, and psychiatric disorders might be considered as predisposing factors for recurrence development (175). Notably, a significant number of recurrences occurred several years after the initial event. Consequently, these findings highlight the importance of extended follow-up for TTS patients and the identification of effective preventive strategies."

4. " In terms of ECG manifestations, QT prolongation and T-wave inversion are more often reported in African-American women with TTS." This statement should not appear in the section of Definition and epidemiology. This should be discussed in the section of ECG manifestation of TS.

Reply: We have now presented the sentence in the ECG manifestations section. Thank you.

5. I think you need to quote and discuss about this recent large-scale head-to-head comparative study which provides important insight into ethnic disparities between Japanese and European TS patients from 9 countries in the section of epidemiology. Imori Y, Kato K, Cammann VL, et al. Ethnic comparison in takotsubo syndrome: novel insights from the International Takotsubo Registry. Clin Res Cardiol. 2021.

Reply: Thank you for pointing out this interesting paper. We have added to the text the following (page 5, lines 10-15): **"***Differences in clinical characteristics and in-hospital outcomes between Japanese and European TTS patients exist as Japanese patients were older, more likely to be male, and the presence of physical triggering factors was more common. Nevertheless, ethnicity does not impact the outcome in TTS patients and the worse in-hospital outcome in Japanese patients is mainly driven by the higher prevalence of physical triggers (15)."*

6. You should separate Definition and epidemiology into 2 sections.

Reply: Thank you, following this recommendation we separate each other.

7. In the epidemiology section - you should discuss about seasonal variation of Takotsubo syndrome. Two prior Northern Hemisphere studies reported a reverse circadian pattern for TS and ST-segment elevation MI. Summers MR, Dib C, Prasad A. Chronobiology of Tako-tsubo cardiomyopathy (apical ballooning syndrome). J Am Geriatr Soc 2010; 58: 805–6. Song BG, Oh JH, Kim HJ, Kim SH, Chung SM, Lee M et al. Chronobiological variation in the occurrence of Tako-tsubo cardiomyopathy: experiences of two tertiary cardiovascular centers. Heart Lung 2013; 42: 40–7.
There is also one study to date investigating the seasonal variation of TS in comparison to patients who presented with MI in Southern Hemisphere. Looi JL, Lee M, Grey C, Webster M, To A, Kerr AJ. Seasonal variation in Takotsubo syndrome compared with myocardial infarction: ANZACS-QI 16. N Z Med J 2018; 131: 21–9.
You should also mention about high incidence of psychiatric disorders in patients with TTS.

Reply: Thank you for bringing this interesting issue to our attention. We have included and discussed the mentioned papers as follows (page 5, lines 21-24): "*There are some conflicting*

results regarding possible seasonal variations in TTS. However, several studies from northern and southern hemispheres have shown a pattern of seasonal variation in TTS that is reversed compared with AMI, with peaks during summer (19-22)."

8. "As in other pathologies, it is crucial to comprehend the fundamental causes and mechanisms of the disease in order to develop suitable treatments to prevent acute complications and long-term adverse events or recurrences." I don't think you need this statement.

Reply: Following your suggestion we have removed the sentence. Thank you.

9. Quote this study in the section of Coronary spasm/Myocardial bridging and long left anterior descending coronary artery- To AC, Kay P, Khan AA, Kerr AJ. Coronary artery anatomy and apical sparing in apical ballooning syndrome: implications for diagnosis and aetiology. Heart Lung Circ 2010; 19:219–24. This study reported evidence that the coronary artery distribution and the frequent finding of apical sparing make the coronary spam hypothesis less likely.

Reply: Thank you for the contribution. Following the recommendation, we have included the reference as the reviewer suggested in Myocardial bridging and long left anterior descending coronary artery.

10. What about the role of LVOT obstruction in the pathophysiology of TS? You need to discuss about this as studies have suggested that LVOT obstruction, by markedly increasing apical intraventricular pressure and wall stress, could predispose to LV apical stunning.
References - To AC, Khan AA, Kay P, Kerr AJ. Resting systolic anterior motion of mitral valve apparatus: association with apical ballooning syndrome. Circ Heart Fail 2008; 1: 84–5. Merli E, Sutcliffe S, Gori M, Sutherland GG. Tako-Tsubo cardiomyopathy: new insights into the possible underlying pathophysiology. Eur J Echocardiogr 2006; 7: 53–61. Akashi YJ, Goldstein DS, Barbaro G, Ueyama T. Takotsubo cardiomyopathy: a new form of acute, reversible heart failure. Circulation 2008; 118: 2754–62. Looi JL, Gabriel R, Khan A, To A, Lee M, Stewart R et al. Left ventricular morphology and response to betaadrenergic stimulation in apical ballooning syndrome. Eur Heart J Cardiovasc Imaging 2012; 13: 510–6.

Reply: Thank you very much for this interesting point. Indeed, LVOTO has been one of the mechanisms proposed in TTS, and therefore, it is fair to discuss it. However, we believe (and surely the reviewer will agree with us) that it is generally a theory also questioned due to its variable prevalence that in many series accounts for less than 25% of LVOTO in TTS patients, along with the existing conflicting data. Following the reviewer's recommendation, we have added the following paragraph (page 9, lines 8-15):

"Left ventricular outflow tract obstruction

Several studies have hinted at the potential significance of left ventricular outflow tract obstruction (LVOTO) in contributing to the development of TTS. LVOT obstruction can result in a marked increase in apical intraventricular pressure and wall stress, potentially predisposing the left ventricular apex to stunning. However, there are conflicting data, and even the variable but by no means majority prevalence of LVOTO in TTS patients can raise doubts about its consistency (52-55)."

11. You have discussed too in depth and given a lot of information in the section of Pathogenesis and pathophysiology. Can you summarise? This section alone can be a paper itself.

Reply: Thank you for this suggestion. We refer you to the new version with changes where we have shortened the section on Pathogenesis and pathophysiology in some aspects, although other reviewers have requested expanding some subsections inside this. Please understand the effort to condense without omitting relevant information or disregarding valuable suggestions from other

reviewers including you.

12. "During admission, the primary group showed more intensive antithrombotic and anxiolytic medical management, while secondary forms showed higher biomarker elevation, longer in-hospital stay and evolutive complications (82). " This shouldn't be discussed in the section of Clinical Presentation.

Reply: Thank you. According to this suggestion we have removed the sentence.

13. Can you split Clinical Presentation and anatomical characteristics into 2 different sections? Combining these 2 together is confusing to the readers.

Reply: Thank you for this suggestion. As the reviewer may see we have split both of them into two different sections.

14. As you mentioned, four different variants of TS have been described including apical, mid-ventricular, basal and focal types. A fifth variant of TS has also been described recently where the mid-LV is hyperdynamic but the apex and base are akinetic or hypokinetic (a reverse mid-ventricular Takotsubo). Bridgman PG, Chan CW. The fifth takotsubo variant. Echocardiography 2017; 34: 122–3.

Reply: Thank you very much for this suggestion. We included the following sentence (page 17, lines 19-21): "A fifth variant of TS has also been proposed recently where the mid-LV is hyperdynamic, but the apex and base are akinetic or hypokinetic (a reverse mid-ventricular Takotsubo) (106)."

15. Can you show the other 2 variants – basal and focal types?

Reply: We appreciate the recommendation, but we wanted to emphasize the most common forms to maintain a highly illustrative and practical spirit. In addition, cases with rare variants are much more difficult to illustrate in a static figure. We kindly request the understanding of the reviewer in this regard.

16. The Clinical Presentation should focus on how TTS patients present. The Anatomical characteristics should be discussed in the section of Cardiac imaging. The entire section of Clinical presentation and anatomic characteristics is too confusing for readers. Need to rewrite.

Reply: We kindly refer the reviewer to response number 13. Thank you again.

17. This section Clinical, electrocardiographic, and imaging diagnosis should also be separated into Clinical Criteria for TTS, ECG patterns in TTS, Cardiac biomarkers in TTS Imaging in TTS.

Reply: Thank you for this suggestion that has permitted us improved the clarity of our presentation. Following the reviewer's advice, we have separated the former "Clinical, electrocardiographic, and imaging diagnosis" in the suggested subsections.

18. ECG patterns in TTS – are you aware of this paper by Looi JL, Wong CW, Lee M, Khan A, Webster M, Kerr AJ. Usefulness of ECG to differentiate Takotsubo cardiomyopathy from acute coronary syndrome. Int J Cardiol 2015; 199: 132–40? This study is the largest cohort published to date in Australasia and one of the largest cohorts internationally, evaluating ECG differences between TTS and MI patients.

Reply: Thank you for this suggestion. We have included the article (Page 20, lines 13-14): *"Interestingly, even ECG changes seen in TTS within two days of presentation are distinctive and*

important clues for clinicians to suspect the diagnosis (120)"

19. You mention about The Mayo Clinic Diagnostic Criteria and the InterTAK Diagnostic Criteria for the diagnosing TTS. What about the InterTAK diagnostic score? You need to discuss about The InterTak Diagnostic score.
Recently Looi et al has developed a score using demographic, clinical and ECG data to distinguish women with NSTEMI from those with NSTE-TS. Looi JL, Poppe K, Lee M, Gilmore J, Webster M, To A et al. A score to differentiate Takotsubo syndrome from non-ST-elevation myocardial infarction in women at the bedside. Open Heart 2020; 7: e001197. And this Clinical score has been externally validated. External Validation of a Clinical Score to Differentiate Takotsubo Syndrome From Non-ST-Elevation Myocardial Infarction in Women. Heart Lung Circ. 2023 Jun;32(6):696-701. doi: 10.1016/j.hlc.2023.04.002. Epub 2023 Apr 28.

Reply: Thank you very much for this important suggestion. We have now included and discussed briefly the InterTAK diagnostic score. Unfortunately, due to text size, we are unable to include all the requested studies, although we appreciate their relevance. We kindly request the understanding of the reviewer.

In the new version, we have now included a new table (Table 2) with this score. We have also included and discussed this in the text, as the reviewer suggested (page 19, lines 3-15): *"In order to better assess the probability of TTS and attemp to differentiate between patients with AMI, the Takotsubo International Registry has developed the InterTAK Diagnostic Score (112) (Table 2). This scoring system offers a user-friendly approach and can be swiftly computed at the bedside within an emergency context. Notably, it demonstrates a robust sensitivity in TTS diagnosis and exhibits a remarkable capacity to distinguish TTS from acute coronary syndrome with a high degree of specificity. When patients with a score of ≥50 were diagnosed as TTS, nearly 95% of TTS patients were correctly diagnosed. When patients with a score ≤31 were diagnosed as AMI, ~95% of patients were diagnosed correctly (112)."*

20. Prognosis – you should have another paragraph discussing about recurrent TTS

Reply: Thank you again for this important suggestion. We kindly refer to our response to point number 3 where the reviewer may corroborate how we have followed his/her suggestion.

21. Prognosis –There are conflicting reports regarding the long-term prognosis in patients with TTS who survive the index event. When longer term outcomes of patients with TTS were compared with those in ACS and non-cardiovascular disease (CVD) control cohorts, findings ranged from all-cause mortality in TTS being similar to the general population, to TTS outcomes as bad as for patients with STEMI. The conflicting observations may in part relate to methodological limitations, including not separating early in-hospital from post-discharge outcomes, and not using representative 'control' cohorts.
Looi et al recently compared the post-discharge outcomes of patients with TTS with two very large, real-world, matched cohorts of patients presenting with ACS and people without known CVD respectively. Patients discharge alive after TTS had twice the mortality risk of age- and gender-matched control patients drawn from a contemporaneous, representative population cohort. This finding was similar to a recent Swedish study which reported better survival in a control group drawn from a cohort with chest pain of unknown cause and normal coronary arteries.
Looi JL, Lee M, Webster MWI, To ACY, Kerr AJ. Postdischarge outcome after Takotsubo syndrome compared with patients post-ACS and those without prior CVD: ANZACS-QI 19. Open Heart 2018; 5: e000918.
Redfors B, Vedad R, Angeras O, Ramunddal T, Petursson P, Haraldsson I et al. Mortality in takotsubo syndrome is similar to mortality in myocardial infarction – a report from the SWEDEHEART registry. Int J Cardiol 2015; 185: 282–9.
I think you need to include these 2 studies and discuss about them.

Reply: Thank you for this important point. We have included in the text the following sentences (page 28, lines 15-24): *"Conflicting reports exist about the long-term prognosis of TTS survivors. Comparisons of TTS outcomes with ACS and non-cardiovascular disease control groups show varying results, from TTS having similar all-cause mortality to the general population to outcomes as severe as AMI. These discrepancies might stem from methodological limitations, including not differentiating in-hospital from post-discharge outcomes and lacking representative control cohorts. Looi et al. recently compared post-discharge outcomes of TTS patients with matched ACS and non-CVD cohorts. Surviving TTS patients exhibited twice the mortality risk of matched controls from a contemporary population (167)."*

22. Prognosis - The effect of stressors on the prognosis of TTS remains controversial due to scarcity of available data.
In addition to this study by Ghadri JR, Kato K, Cammann VL, et al. Long-Term Prognosis of Patients With Takotsubo 964 Syndrome. J Am Coll Cardiol. 2018;72(8):874–82 which suggests in-hospital outcomes in TTS patients with a preceding medical illness are worse than for patients with an emotional stressor or without an identified stressor, there are 2 other studies that should be included as well.
Brinjikji W, El-Sayed AM, Salka S. Inhospital mortality among patients with takotsubo cardiomyopathy: a study of the National Inpatient Sample 2008 to 2009. Am Heart J 2012; 164: 215 21.
Yerasi C, Koifman E, Weissman G, Wang Z, Torguson R, Gai J et al. Impact of triggering event in outcomes of stress-induced (Takotsubo) cardiomyopathy. Eur Heart J Acute Cardiovasc Care 2017; 6: 280–6.
Looi et al recently described the postdischarge mortality risk for those with TS associated with physical stress was approximately fourfold higher than those without known CVD, similar to those with MI. In contrast, those with TTS associated with emotional stress or no identifiable stress had a 5-year survival rate similar to people living in the community without known CVD.
Looi JL, Verryt T, McLeod P, Chan C, Pemberton J, Webster M et al. Type of stressor and medium-term outcomes after Takotsubo syndrome: what becomes of the broken hearted? (ANZACS-QI 59). Heart Lung Circ 2022; 31: 499–507.
All these should be incorporated into the section Prognosis.
I agree that TTS is not a benign condition but bear in mind that international studies have reported that late mortality after TTS is largely due to non-cardiac causes and appears to be related to the presence of comorbid disease rather than the TTS event itself. This is in contrast to deaths after ACS, which are frequently due to CVD.

Reply: Due to size restrictions in the text, which we have now exceeded following the numerous and highly valuable suggestions from our reviewers, we have only included the intriguing study by Looi et al in Heart Lung 2022. We kindly request the understanding of the reviewer.

Page 29, lines 3-8: *"However, patients with TTS triggered by emotional stress had a more favourable short- and long-term prognosis (165, 168). On the other hand, long-term mortality in patients with TTS triggered by physical activity, medical conditions, or procedures, is three times higher compared to those elicited by emotional triggers (134,168)."*

23. Summary – shouldn't you use the term Conclusion instead?

Reply: Completely agree. Thank you. Accordingly, we have made the change.

24. This statement "It manifests clinically as transient ventricular dysfunction without significant obstructive coronary artery disease, often closely resembling ACS." – significant obstructive coronary artery disease should be removed as CAD can coexist in TTS.

Reply: Thank you for this precision. We have changed "significant" for "culprit" as the Heart Failure Association–European Society of Cardiology Criteria states. As the reviewer wisely

pointed out and we stated in the manuscript: *"…patients may exhibit either normal coronary arteries on angiography or a degree of atherosclerosis that do not match the extent of left ventricular dysfunction or RWMA…"*. We fully agree, thank you.

Reviewer F

This review by Salamanca and Alfonso represents a thorough overview of Takotsubo Syndrome (TTS) pathophysiology, diagnosis and treatment.
The topic is of great interest, as the knowledge of the mechanisms leading to TTS is still incomplete and, consequently, there is lack of targeted therapies.

In general, the language quality is adequate and the dissertation is clear, interesting and comprehensive.

General reply: We deeply appreciate the reviewer's suggestions and comments. All of them were of major value in order to improve our work. Sincerest thanks.

However, in my opinion, there are some issues that should be addressed in order to raise the level of the manuscript:

1) The paper lacks in novelty, as there are already several similar papers in medical literature. However, the remarkable completeness of the overview provided from the authors is a point of strength that should be additionally stressed. Therefore, I suggest to expand the part of the echocardiographic diagnosis by citing the latest consensus document on multimodality imaging in TTS (Citro et al. "Multimodality imaging in takotsubo syndrome: a joint consensus document of the European Association of Cardiovascular Imaging (EACVI) and the Japanese Society of Echocardiography (JSE)" Journal of Echocardiography (2020) 18:199–224) and a recent paper which investigated the utility of left atrial strain, a novel echocardiographic parameters, to predict increased LVEDP and adverse events in this population (Iannaccone et al. "Left atrial strain analysis improves left ventricular filling pressures non-invasive estimation in the acute phase of Takotsubo syndrome", Eur Heart J Cardiovasc Imaging. 2023 May 31;24(6):699-707).

Reply: Many thanks for this important suggestion. We have expanded (briefly due to the current length of our work) the echocardiographic section (page 21, lines 15-23): "*Additionally, RV participation may be indicated by the reverse McConnell sign, indicative of biventricular ballooning (126). Speckle-tracking analysis reveals impaired circumferential strain in both longitudinal and radial directions, further characterizing the extent of myocardial dysfunction associated with TTS (126). Interestingly, a recent paper investigated the utility of left atrial strain in this population, lower left atrial reservoir and pump strain values were better predictors of left ventricular end-diastolic pressure than traditional echocardiographic indexes in the acute phase and were an independent predictor of adverse in-hospital outcomes (127).*"

2) Angiographic findings may be also of interests. In particular coronary slow flow may represent a marker of a more severe microvascular dysfunction. The author can cite and discuss PMID: 31924712.

Reply: Thank you for this interesting suggestion. Please, see (page 22, 5-8): *"). Interestingly, patients with TTS presenting with coronary slow flow have a worse clinical presentation with a higher rate of intrahospital complications and a poor long-term clinical outcome (131)."*

3) The authors did not sufficiently highlight an important possible clinical presentation/complication of TTS, that is the occurrence of ventricular arrhythmias, which deserves to be properly pointed out (i.e. Jesel L et al Ventricular arrhythmias and sudden cardiac arrest in Takotsubo cardiomyopathy: Incidence, predictive factors, and clinical implications. Heart Rhythm. 2018 Aug;15(8):1171-1178). Finally, I would recommend to discuss further the

importance of LVEDP as a relevant predictor of in hospital complications, also citing the study by Del Buono et al. “Left ventricular end-diastolic pressure predicts in-hospital outcomes in takotsubo syndrome.” (Eur Heart J Acute Cardiovasc Care. 2021;10(6):661-7).

Reply: You are correct, we completely oversighted this aspect. Thank you for these important suggestions.

Regarding the LVEDP issue, we have included and discussed the aforementioned paper (page 22, lines 12-13): *“Assessing LVEDP has been shown to be a reliable predictor of in-hospital complications (133).”*

Regarding the arrhythmic issue, we have included a subsection (page 25, lines 11-25; and page 25, line 23-page 26 line 21):

” *TTS complicated by life-threatening arrhythmias*

Life-threatening arrhythmias, including severe bradyarrhythmias or ventricular tachycardia and ventricular fibrillation, have the potential to arise early during the course of TTS (2, 155). Ventricular tachycardia or ventricular fibrillation can be observed in approximately 3.0–8.6% of cases and is related to significantly worse short- and long-term prognoses (155). These arrhythmic events tend to correlate with lower left ventricular ejection fraction (LVEF) at the time of presentation and a higher incidence of conduction disturbances. A majority of severe ventricular tachyarrhythmic episodes coincide with a prolonged corrected QT interval exceeding 500ms (2). For managing acute ventricular tachycardia, the approach involves magnesium sulfate and/or a short-acting BB. If there's sustained, pulseless ventricular tachycardia, direct current cardioversion might be necessary. Given the elevated prevalence of prolonged QT intervals in acute-phase TTS patients, the use of drugs that could potentially extend the QT interval should be avoided or immediately discontinued, and the use of amiodarone or sotalol needs to be evaluated for each case. In cases where torsades de pointes with QT prolongation emerge associated with bradycardia, temporary pacing should be considered. Persistent high-grade AV block warrants permanent pacemaker implantation. Interestingly, during the acute phase, ventricular arrhythmias are observed in TTS patients, without subsequent recurrence among survivors of hospitalization. However, it's important to note that significant conduction disorders tend to persist during long-term follow-up (155). ”

4) I suggest to add an explicative figure of the different treatment options in the acute phase and in the long- term follow-up or, in alternative, a table listing the different drugs investigated to address TTS and the results of the relative studies.

Reply: Following the reviewer’s suggestion we have added a figure of the different treatment options in the acute phase and in the long-term. Please, see figure 5.

“Figure 5. Treatment of Takotsubo syndrome. ACEi = Angiotensin-converting enzyme inhibitor , ARB = Angiotensin II receptor blocker , BB = Beta-blockers, IV = intravenous, LVOTO = Left ventricular outflow tract obstruction, LVSD = Left ventricular systolic dysfunction , IABP = Intra-aortic balloon pump, VA-ECMO = Veno-arterial extracorporeal membrane oxygenation, AV = Atrioventricular, RWMA = Regional wall motion abnormalities.”

5) Figure 3 and Figure 4 could be united in one figure, as they both display angiographic images of two different patterns of wall motion abnormalities in TTS.

Reply: We have united both figures. Thank you for the suggestion.

6) Finally, a recent experimental study demonstrated a role for coronary microvascular

dysfunction with abnormalities in flow regulation between the LV apex and base cause TTS. This interesting study can be discussed (PMID: 37170610, 34759123).

Reply: Thank you for your comment. We are indeed aware of that intriguing study, which we had already included in the initial version (page 11, lines 11-17): *"However, increasing myocardial blood flow through the use of chromonar, or inducing smooth muscle expression of Kv1.5 channels in TgKv1.5−/− mice, restored perfusion and normalized ventricular function between the apex and base (71). These interesting findings highlight the contribution of flow regulation abnormalities between the LV apex and base on the pathophysiology of TTS and also emphasize the potential of restoring normal perfusion to recover ventricular function."*

Reviewer G

I found this manuscript remarkably disappointing, especially given its immodest title: this review is neither comprehensive nor accurate.I think that the manuscript contains a number of very important omissions ant sheer errors.

General reply: We sincerely regret the reviewer's disappointment. This is a fascinating disease but unfortunately still of unknown etiology. The title in question aims to capture the reader's attention and persuade them to engage in critical reading. Please note its interrogative nature with this aim. We do not claim to address all the unresolved areas regarding Takotsubo syndrome (which are not few, as many of the reviewers have pointed out), but rather our genuine intent is to conduct a review that synthesizes a significant portion of current knowledge, highlighting well-known and relevant aspects as well as those that are more doubtful or controversial. In any case, we hope that the reviewer finds the changes we have made, aimed at improving our work, to be to their liking. It is worth mentioning that your commentaries and critiques have undoubtedly been invaluable for this improvement. Thank you very much for assisting us in this task.

Let me cover a few of the problems:-
Epidemiology:
(1) TTS should be divided into primary and secondary forms, with the second complicating extracardiac disease states (eg cerebral haemorrhage) and operations, as well as treatment with certain antidepressants which inhibit catecholamine reuptake into nerve endings. The fact that underlying phaeochromocytoma may result in presentation as TTS is totally missed, and instead readers are advised of the merits of beta-blocker therapy! The well-demonstrated association with antecedent malignancy is not even mentioned.
The "post-menopausal" aspect of epidemiology is mentioned, rather than ageing per se: there is very little evidence that menopause has anything at all to do with epidemiology. The incidence in ageing women is more like 10% than 5-6%.

Reply: Thank you very much for these important points.

We totally agree with the reviewer, and we have expanded the discussion of the primary and secondary forms (we kindly refer the reviewer to the responses and changes made in relation to the suggestions and comments of "reviewer B" (second point) and "reviewer C".).

We have also added references and commented on the pheochromocytoma issue (please, view the response to "reviewer B"; point 3).

The malignancies point has been now addressed by adding the following (page 5, lines 18-20):" Notably, the prevalence of neoplasms in patients with TTS is high, up to 12-17% in some registries. Cancer, either history or active, could be associated with an increased risk of adverse events in TTS (17,18)."

And finally, about the hormonal aspect, we have expanded our content. Please, kindly see the response to "reviewer D" (point 3).

(2) Pathogenesis: I think that the authors have almost no understanding of biochemical mechanisms, and simply have chosen to omit most papers of this type. "Not fully understood" perhaps refers to the authors! First, there is no mention that the early release of BNP/NT-proBNP is dramatically greater than that post AMI, and that this would normally have worked to suppress neutrophil production of superoxide anion, except that neutrophil NAD(P)H oxidase activity suppression by BNP is impaired. There is no mention of the fact that TTS is associated with impaired rates of microcoronary flow, and not surprisingly is associated with acute evidence of damage to the endothelial glycocalyx: this will result in non-laminar flow, increased vascular permeability to moncytes, polymorphs and fluid extravasation (relevant to hypotension!). The finding that TTS is associated with reduced levels of ADMA, increased platelet responsiveness to NO and with the generation of peroxynitrite from NO/02- is completely ignored, despite the fact that in a rat model interruption of this pathway limits negative inotropy.
Most importantly, the authors seem to think that plasma catechol levels represent the major determinants of extent of catechol signalling. They have chosen to ignore all data regarding impact (clinical!) of increased tissue catecholamine levels due to impaired reuptake, and especially the pioneering work of Paur et al (2012) related to biased post beta-2 signalling. (Hence the futility of beta-1 blockade.)

Reply: Thank you for this important and comprehensive comment. Respectfully, we believe that some considerations are beyond the scope of our review due to the extensive volume of articles addressing this issue. It is impractical to cite or reference every single one of them, and we deeply appreciate your understanding in this matter. Nevertheless, we have included the following valuable suggestions:

-The aforementioned article by Paur et al., which holds undeniable value (Page 7, line 25-page 8, line 10): *"Overall, an increased sympathetic stimulation and increased tissue catecholamine levels due to impaired reuptake appear to play a critical role in TTS. In this regard, some authors. proposed that elevated levels of circulating epinephrine prompt a shift in the internal pathway of signal transmission within ventricular cardiomyocytes, transitioning from G(s) protein to G(i) protein signaling via the beta(2)-adrenoceptor. While this transition safeguards against the proapoptotic consequences of intense beta(1)-adrenoceptor activation, it also has a detrimental impact on contractility. This impact seems most pronounced in the apical myocardium, which possesses the highest density of beta-adrenoceptors (39,40). However, the precise underlying mechanism of TTS remains elusive, leading to the open discussion of other pathophysiological theories.."*

- We have also added a significant point concerning cardiac biomarkers: (page 20, lines 21-22): *"…and significantly raised in TTS compared to AMI,…"*

- Please, kindly note that there is an entire subsection dedicated to microvascular circulatory dysfunction. Thank you very much for all your insights.

(4) The manuscript should contain a section about the very non-transient nature of TTS, including persistent inflammation and myocardial edema, development of fibrosis, persistent BNP/NT-proBNP elevation, subtle echo changes, impaired quality of life and ongoing mortality risk.

Reply: Thank you for this crucial comment. We totally agree and in this regard the reviewer may find now an entire subsection (page 15, line 11, page 16, line 2):

"*Long-term abnormalities: a syndrome that persists over time or exists prior to the current condition?*

Some authors have extensively studied the heart failure phenotype of TTS in predominantly symptomatic patients (98). This phenotype is characterized by preserved ejection fraction,

impaired cardiac energetic status, exercise limitation (reduced peak Vo2 and increased Ve/Vco2 slope during cardiopulmonary exercise testing), reduced apical myocardial anticlockwise rotation during systole with altered torsion and twist, and possibly microscopic fibrosis (94,98). Furthermore, cardiac biomarkers like B-type natriuretic peptide remain mildly elevated in the long-term (99). The persistence of long-term myocardial abnormalities raises the question of whether these abnormalities existed before the initial TTS event. This has led the authors themselves to wonder if this suggests the possibility of preexisting abnormalities that contribute to the predisposition for recurrent takotsubo events and even suggest the presence of an undiagnosed and subtle preexisting cardiomyopathy that becomes apparent during an acute stressful event (3).

(5) Line 201: The TAC model of "TTS" develops very slowly, and it is EXTREMELY doubtful that it represents any analog of TTS.

Reply: We appreciate the reviewer's comment. However, we only mention the TAC model in relation to an interesting TTS paper recently published in EHJ. The reviewer's observation, while entirely accurate, in our opinion, falls beyond the scope of our review. Thank you again for your insights and your understanding.