

Peer Review File

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REVIEWER A:

The manuscript under consideration investigates the diagnostic value of contrast-enhanced ultrasonography (cU), specifically contrast-enhanced transcranial Doppler (cTCD), contrast transthoracic echocardiography (cTTE), and contrast transesophageal echocardiography (cTEE), for patent foramen ovale (PFO). The study retrospectively analyzes data from patients diagnosed with PFO between October 2019 and January 2022.

The authors report the detectable rates of cTCD, cTTE, and cTEE, with respective sensitivity and specificity comparisons. The results suggest that the combination of cTCD and cTEE could be a favorable strategy for PFO diagnosis. However, several critical points need consideration before considering this manuscript for publication.

The article does not present any novel findings or significant advancements in the field. The study is retrospective and based on a limited number of patients, potentially undermining the generalizability of the results. Additionally, the absence of a comparative analysis with existing literature diminishes the manuscript's contribution to the current body of knowledge.

In summary, while the study explores an interesting aspect of PFO diagnosis, it fails to provide substantial novelty and is limited by its retrospective nature and small sample size.

Comment: The manuscript should undergo significant revisions, including a critical review of the literature, addressing limitations, and considering a more recent dataset, before it can be considered for publication in a reputable journal.

Reply: Right heart catheterisation with demonstration of a guidewire crossing the septum is the most accurate method to confirm the presence of PFO; but it is not suitable as a preliminary examination because it is invasive. Currently, various methods, including contrast-enhanced transcranial Doppler (cTCD), contrast transthoracic echocardiography (cTTE), and contrast transesophageal echocardiography (cTEE) have been used for the diagnosis of PFO-RLS. Various studies have investigated the diagnostic values of cTCD, cTTE, and cTEE for PFO-RLS. Each method has its advantages and disadvantages. Most previous studies have only compared the diagnostic values of two methods, and the results of the studies are inconsistent. In the past, cTEE was limited in the routine practices, and

frequent use of sedation affected the results of cTEE. Very few studies have compared the diagnostic values of the three methods at the same time. The anatomical features of PFO and the adjacent atrial septum have become key components for shared decision making in choosing the optimal management strategy for patients with PFO. The qualitative diagnosis and quantitative analysis of PFO-RLS is a key process in the interventional treatment of PFO occlusion; therefore, the accurate diagnosis of PFO and the quantitative diagnosis of PFO-RLS are of great importance. In addition, debate continues as to the best diagnostic strategy.

The sample size of our study is small, and we will continue to accumulate cases for further research.

Thank you for your careful review, we have modified our text as advised. (see Page 4, line 111-131)

REVIEWER B:

The manuscript entitled Diagnostic value of contrast-enhanced ultrasonography for patent foramen ovale is an original paper, which assessed the diagnostic value of contrast-enhanced ultrasonography, including cTCD, cTTE and cTEE, for PFO. This is an observational retrospective study.

The authors concluded that cTCD has the potential to be the first line investigation examination for detecting right to left shunt. The combination of cTCD and cTEE could provide a favorable strategy for the diagnosis of PFO and access in right-to-left shunt.

This manuscript is well written. It has some important messages for clinical practice. Therefore I consider that it deserves to be published. Thank you for the details of the technique of imaging methods used in this study. It is important for clinical practice. However, I have some minor comments/issues.

Comment 1: The number of patients is small.

Reply 1: The sample size of our study is small, and we will continue to accumulate cases for further research.

Comment 2: Do you really think that cTCD is easier and more accessible than cTTE? What are the disadvantages of this imaging method? Please comment this in discussions.

Reply 2: The high sensitivity of cTCD may be related to the stable images that are not affected by provocation maneuver and the ability of automated microbubbles monitoring software to detect small MBs that cannot be monitored by the ultrasonographer with the naked eye; and cTCD does not rely on tomography. But cTCD can not show the origin of the RLS and it only indirectly determines the origin through the timing of appearance of microbubbles in the MCA.

Thank you for the insightful comment. We have modified our text as advised.(see Page7,line220-230)

Comment 3: What is the reproducibility of cTCD? Is reliable this semi-quantitative classification criteria? This could be a major cornerstone of this study.

Reply 3: Recently,an in vitro and in vivo observational study showed that Agitated saline with 10% blood increases number and stability of microbubbles in detection right-to-left shunt(RLS) by contrastenhanced transcranial Doppler.And it showed that both the size and number of microbubbles were most stable in the agitated saline with 10% blood(10% BAS), which could be the reasons why the 10% BAS had the highest positive rate and RLS levels in c-TCD.In our study,we also find that satisfactory results of cTCD can be obtained with 10% BAS.cTCD has stable images that are not affected by provocation maneuver ,and cTCD does not rely on tomography.So cTCD has good repeatability.

The semi-quantitative classification criteria are not uniform but similar.We classified the RLS size using the quantification criteria of the Consensus Conference of Venice modified by Serena et al(1): minimum (more than 1 and less than 10 MBs), moderate (more than 10 and less than25 MBs), and massive (more than 25 MBs with showeror curtain pattern).The number of microbubbles is affected by numerous methodological variations including dose and type of contrast agent, use of smaller veins and needles, number of tests performed, and effectiveness of provocative maneuvers. And studies showed that the degree of RLS is not considered to be associated at all with the risk of future cerebrovascular events in patients with PFO(32,33). A correct RLS classification can be considered of minor importance(34).

Thank you for the insightful comment. We have modified our text as advised.(see Page6,line213-219; Page7,line257-262)

[1]. Serena J, Segura T, Perez-Ayuso MJ,et al. The need to quantify right-to-left shunt 'in acute ischemic stroke: a case-control study. Stroke1998;29:1322-8.

Comment 3:In table 2 cTCD and cTTE have the same specificity. How do you comment this?

Reply 3: cTCD and cTTE had exactly the same number of false positives(6 cases) and true negatives(18 cases) respectively in our study,so the specificity of both is 75%.RLS in six false positives were confirmed as pulmonary shunt by cTEE in our study.

Thank you for the insightful comment. We have modified our text as advised.(see Page7,line230-231; Page7,line239-240)

Comment 4:Page 4 line 31: What is ``microvesicles``?

Reply 4: Thank you for the insightful comment. We have modified the word .(see Page5,line163)

Reviewer C

Comment: The methods section describes the study design and data collection adequately, however, it lacks clarity in explaining the technical aspects of how the procedures contrast-enhanced transcranial Doppler (cTCD) were conducted, including specific protocols, equipment used, and any variations in techniques between the technicians.

could you please provide this data ?

Reply: The patient was placed in the supine position, and the gitated saline with blood was aspirated into one syringe, and the patient was asked to perform Valsalva exercises before exhaling, the Valsalva movement should be initiated at 5 s after injection of the contrast agent and maintained for 10 s, and the Doppler spectrum of the middle cerebral artery (MCA) was monitored for 1 minute (25). If the patient was not cooperating well, to better complete the stimulation of the movement, auxiliary abdominal pressure was applied, or the patient was asked to cough. At least two ultrasound examinations were performed with 5-minute intervals. All the examinations were performed by two senior ultrasonographers and one nurse.

Thank you for the insightful comment. We have modified the word .(see Page16,line467-469)

Reviewer D

I have read with great interest the article entitled “Diagnostic value of contrast-enhanced ultrasonography for patent foramen ovale” submitted in Journal of Thoracic Disease as an Original Article. This study sought to investigate the diagnostic value of contrast-enhanced ultrasonography (cU), including contrast-enhanced transcranial Doppler (cTCD), contrast transthoracic echocardiography (cTTE), and contrast transesophageal echocardiography (cTEE), for PFO. My comments are the following:

Comment1: Line 77: Add relevant references (10.1177/17562864221103459, 10.1007/s11910-017-0730-5.)

Reply 1: Thank you for the insightful comment. We have added relevant references as advised.(see Page3,line108; Page10,line326-329)

Comment 2: Introduction: It was not only RESPECT trial that showed benefit from PFO closure. Please data from other RCTs.

Reply 2: Thank you for the insightful comment. We have added relevant references as advised.(see Page3,line105; Page9,line313-316)

Comment 3: Line 110: Generally, R->L flow is not observed in PFO, but in ASDs.

Reply 3: Thank you for the insightful comment. We have added relevant exclusion criteria .(see Page5,line146-147)

Comment 4: Line 114-115: How many patients were excluded due to III?

Reply 4: 5 cases were excluded due to III in our study. A total of 147 consecutive patients were collected after exclusion according to exclusion criteria in our study.

Comment 5: It would be useful if the authors could explain us why TCD should be first line instead of bubble test TTE?

Reply 5: cTCD can be choice for primary screening test because of its high sensitivity, noninvasiveness and repeatability. The high sensitivity of cTCD may be related to the stable images that are not affected by provocation maneuver and the ability of automated microbubbles monitoring software to detect small microbubbles that cannot be monitored by the ultrasonographer with the naked eye;and cTCD does not rely on tomography.

Thank you for the insightful comment. We have modified our text as advised.(see Page8,line274-276)

Comment 6: If I had to choose between TTE and TCD, I would choose TTE because it can provide further information about potential cardioembolic sources (Left Atrium, Mitral Valve, LV apex, aorta, etc).

Reply 6: Transthoracic echocardiogram is usually performed routinely to examine the structure and function of the heart.And cTCD is superior to cTTE in detecting RLS in our study.

Comment 7: The authors should add a paragraph emphasizing on the novelty of their manuscript.In my opinion, the authors have performed an interesting study and have prepared a well-written manuscript. However, I do not see any novelty in this study.

Reply 7: Right heart catheterisation with demonstration of a guidewire crossing the septum is the most accurate method to confirm the presence of PFO;but it is not suitable as a preliminary examination because it is invasive.Currently, various methods, including contrast-enhanced transcranial Doppler (cTCD), contrast transthoracic echocardiography (cTTE), and contrast transesophageal echocardiography (cTEE) have been used for the diagnosis of PFO-RLS.Variou studies have investigated the diagnostic values of cTCD, cTTE, and cTEE for PFO-RLS. Each method has its advantages and disadvantages.Most previous studies have only compared the diagnostic values of two methods ,and the results of the

studies are inconsistent. In the past, cTEE was limited in the routine practices, and frequent use of sedation affected the results of cTEE. Very few studies have compared the diagnostic values of the three methods at the same time. The anatomical features of PFO and the adjacent atrial septum have become key components for shared decision making in choosing the optimal management strategy for patients with PFO. The qualitative diagnosis and quantitative analysis of PFO-RLS is a key process in the interventional treatment of PFO occlusion; therefore, the accurate diagnosis of PFO and the quantitative diagnosis of PFO-RLS are of great importance. In addition, debate continues as to the best diagnostic strategy.

Thank you for your careful review, we have modified our text as advised. (see Page 4, line 111-131)

Reviewer E

Comment 1: First of all, my major concern for the methodology of this study is the very small sample size of PFO- patients. The patient sample was almost PFO+ patients, which would result in higher estimate of sensitivity.

Reply 1: Thank you for your careful review. The sample size of our study is small, and we will continue to accumulate cases for further research.

Comment 2: Second, the title needs to indicate the clinical research design of this study such as a diagnostic test.

Reply 2: We have changed the title to “Diagnostic value of contrast-enhanced ultrasonography for patent foramen ovale detection”.

Comment 3: Third, the abstract is not adequate. The background did not directly indicate the potential reason for the controversy and the clinical needs to focus on cU and its subtypes. The methods need to describe the inclusion of both PFO+ and PFO- patients and the gold diagnostic criteria for PFO, as well as the calculation of diagnostic accuracy indicators. The results need to briefly describe the clinical characteristics of the patient sample and the numbers of subjects with and without PFO. Please also report the accurate P values for these comparisons. The conclusion needs to be made with cautions due to the methodology problems.

Reply 3: Thank you for your careful review. We have modified the abstract as advised. (see Page 2, line 63-81)

Comment 4: Fourth, the introduction needs to analyze the characteristics, strengths, and limitations of cTCD, cTTE, and cTEE in detail because the authors' findings showed that the three methods had different performance for the subtypes of PFO. The authors also need to analyze the methodology limitations of prior studies.

Reply 4: Thank you for your careful review. We have modified the introduction as

advised.(see Page4,line 111-131)

Comment 5:Fifth, the methodology needs to describe the sample size estimation procedures of this study, and details of subjects inclusion. The statistics should describe the calculation of diagnostic accuracy measures such as sensitivity and specificity. In the main text and the abstract, it is not correct to describe sensitivity rate and specificity rate. It is adequate to use sensitivity and specificity. The authors need to describe the 95% CIs of sensitivity and specificity in particular the small sample size of PFO- patients.

Reply 5: Thank you for your careful review. we have modified our text as advised.(see Page2,line 69-81; Page6,line 181-201;) And we modified table2 to describe the calculation of diagnostic accuracy measures such as sensitivity and specificity. (see Page14,line 430-435)

Reviewer F

1. Please check all abbreviations in the abstract, highlight box, and the main text, such as “MBs” in the abstract, et al. Abbreviated terms should be full when they first appear.

Reply : We have checked all abbreviations in the abstract.

2. The citation of Ref 24 in the main text was missing. Please indicate where you would like to cite Ref 24 in the main text, which should be cited between Ref. 23 and Ref. 25.

Reply : We have deleted this reference .

3. The citation of Ref 32 in the main text was missing. Please indicate where you would like to cite Ref 32 in the main text, which should be cited between Ref. 31 and Ref. 33.

Reply : We have deleted this reference .

4. The authors mentioned “studies...”, while only one reference was cited. Change “Studies” to “A study” or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

Most previous studies have only compared the diagnostic values of two methods (22), and the results of the studies are inconsistent.

Reply : We have added two references .(see Page4,line127)

5. ALL abbreviations used in each table/figure or table/figure description should be

defined in a footnote below the corresponding table/figure. Please check all figures/tables and provide correspondingly.
For example, cTCD in Figure 2.

Reply : We have modified the footnotes as advised.(see Page13,line423-424)

6. Ref. 23 and Ref. 26 are the same. Please revise.

Reply: We have modified the Ref. 26 .(see Page11,line375-377)