Peer Review File

Comment 1: First, the authors developed the diagnostic algorithms according to the size of the lesion, not diagnostic criteria. The title needs to clearly indicate this and the focus of diagnostic accuracy.

Reply 1: The title has been modified as advised.

Changes in the text: See Page 1, line 2-3; Page 3, line 2-3

Comment 2: Second, the objective of the abstract needs to briefly describe the clinical significance and knowledge gap of this research focus. The methods need to describe the inclusion of subjects and the outcome of interest of this study, i.e., benign and malignant lesions or different stage of the cancer, as well as how the diagnostic model was developed and validated. The results need to briefly describe the characteristics of the study sample. The conclusion needs comments for the clinical implications of the findings.

Reply 2: The Abstract section has been modified: The objective and conclusion have been modified as advised. We add the inclusion of subjects and the outcome of interest of this study as well as how the diagnostic model was developed and validated in methods. Besides, we added some data of training and validation sets in this study. We have added brief description of characteristics of the study sample in results.

Changes in the text: See Page 3 line 4 – Page 4 line 4

Comment 3: Third, in the introduction of the main text, please review what has been known on the application of CEUS for the diagnosis of benign and malignant lesions in the breast, and factors influencing the diagnostic accuracy, and analyze why the size of lesion is important for the diagnostic accuracy. Empirical data are needed to support this research focus.

Reply 3: The Instruction section of the main text has been modified as advised.

Changes in the text: Page 5 line 2 – Page 6 line 2

Comment 4: Fourth, in the methodology of the main text, please accurately describe the clinical research design of this study such as a diagnostic test, and the sample size calculation. The outcome of interest of this study need to be specific to stage of cancer or binary diagnosis of benign and malignant lesions. In statistics, please describe the statistical methods for calculating AUC, SEN, and SPE, as well as their threshold values for a good diagnostic test. Please ensure P<0.05 is two-sided.

Reply 4: We add description of this study of as a diagnostic test and the sample size calculation in the Methods section. That the outcome of interest of this study is binary diagnosis of benign and malignant lesions has been added. The statistics has been

modified as advised.
Changes in the text: Page 6 line 5-8; Page 9 line 3-8; Page 9 line 12-15; Page 9 line 22 – Page 10 line 5

Comment 5: Finally, several related papers need to be reviewed and cited: 1. He H, Wu X, Jiang M, Xu Z, Zhang X, Pan J, Fu X, Luo Y, Chen J. Diagnostic accuracy of contrast-enhanced ultrasound synchronized with shear wave elastography in the differential diagnosis of benign and malignant breast lesions: a diagnostic test. Gland Surg 2023;12(1):54-66. doi: 10.21037/gs-22-684. 2. Li SY, Niu RL, Wang B, Jiang Y, Li JN, Liu G, Wang ZL. Determining whether the diagnostic value of B-ultrasound combined with contrast-enhanced ultrasound and shear wave elastography in breast mass-like and non-mass-like lesions differs: a diagnostic test. Gland Surg 2023;12(2):282-296. doi: 10.21037/gs-23-51. 3. Jia C, Niu Q, Liu L, Li G, Jin L, Du L, Shi Q, Li F. Value of an expanded range of lesions on contrast-enhanced ultrasound for the diagnosis of hypervascular breast masses. Gland Surg 2023;12(6):824-833. doi: 10.21037/gs-23-165.
Reply 5: Above papers have been cited in text.
Changes in the text: Page 5 line 7; Page 14 line 10

Comment 6: In the abstract, in the sentence "M0 was not suitable for lesions ≤ 1.0 cm, but well applicable for lesions > 1.0 cm (0.844 vs 0.921, P = 0.029; 0.936 vs 0.921, P = 0.360; 0.928 vs 0.921, P = 0.711)", such as 0.844 vs 0.921, please clearly indicate what versus what?
Reply 6: The AUC values of M0 combined with BI-RADS in lesions ≤ 1.0 cm vs the AUC value of M0 combined with BI-RADS in all lesions was 0.844 vs 0.921. The Abstract section has been modified as advised.
Changes in the text: Page 3 line 19 – Page 4 line 1

Comment 7: In the research, the diagnostic criteria of CEUS in breast lesions was the critical topic. How about the recently applied diagnostic criteria of CEUS, or what were the defects of the criteria in breast lesions? Please state in the introduction.
Reply 7: There are some limitations in applying CEUS in the breast and a lack of uniform diagnostic criteria. This point has been added in the Introduction section.
Changes in the text: Page 5 line 7-8

Comment 8: In the methods, what was the "intuitive observation"? please state clearly.
Reply 8: This word was a misrepresentation and has been deleted from the text.
Changes in the text: Page 7 line 17

Comment 9: Whether there were differences of accuracy between benign and

malignant breast lesions by CEUS?

Reply 9: In this study, there is no differences of accuracy between benign and malignant breast lesions by CEUS (85.2 vs 89.5, P = 0.084). This content is not the focus of this study and is not included in the text.

Changes in the text: None

Comment 10: Whether there were effects of actuation duration of contrast agent on the diagnosis by CEUS? Please state in the discussion.

Reply 10: The bubbles half-life is tens of minutes in *vivo*, and the time for contrast agent bubbles to start infusing breast tissue and to fade varies from person to person. The characteristics of wash-in time and wash-out time of lesions in this study were compared with surrounding breast tissue to avoid the influence of contrast agent actuation duration. If the same patient needs re-examined by CEUS, the interval must be greater than 20 minutes. This part has been added in the Discussion and Methods section.

Changes in the text: Page 15 line9-14, Page 7 line 19-20

Comment 11: How to improve the diagnosis efficiency of CEUS for lesions 1.0 cm, or what were your suggestions? Please supplement in the discussion.

Reply 11: In order to improve the diagnosis efficiency of CEUS for lesions ≤ 1.0 cm, we established new CEUS diagnostic model for breast lesions ≤ 1.0cm and it has a good diagnostic performance than before. That has been added in text. When performing CEUS examine for breast lesions ≤ 1.0cm, more features should be paid attention to, such as wash-in time and enhancement margin.

Changes in the text: Page 12 line 10 – Page 13 line 4; Page 16 line 15-20

Comment 12: The MRI was listed in the abbreviations, but the MRI was not showed in the main text.

Reply 12: The MRI has been deleted in the abbreviations.

Changes in the text: None