Peer Review File

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Reviewer A

This manuscript describes a risk analysis and some clinical experience of treating red breast syndrome (RBS) in breast reconstruction that is associated with the use of human tissue acellular dermis matrix (ADM) allografts.

The analysis falls short on a number of points. First and most importantly, there are no tissue donor risk factors that are taken into consideration, on either donor characteristics or processing. There are other limitations that will be outlined below in specific comments:

Reply : Thank you for reviewing the article thoroughly. On reference 1, Nahabedian et al. insisted 'It is important to recognize that RBS is not product specific. It can occur with any ADM regardless of the degree of sterilization or the biologic source.' I think RBS has multifactorial etiology and patient's factors can be more important, but I will modify and add things you pointed out as much as possible.

1. Title should say "allograft" instead of implant. It is implanted, but is not an implant.
Reply 1: Breast reconstruction is mainly divided into a method using autologous tissue and a method using implants, and we conducted a study on the reconstruction method using implants. Since we mostly use acellular dermal matrix in reconstruction using implants, we did not indicate it in the title. If we could change the title, we would consider changing it from 'Risk factor analysis and clinical experience of treating red breast syndrome in implant-based breast reconstruction' to 'Risk factor analysis and clinical experience of treating red breast syndrome in acellular dermal matrix and implant-based breast reconstruction', as you suggested.
Change in the text: Title page line 3

2. Abstract should mention donor graft risk factors, e.g., processing, culture results, endotoxin results
Reply 2: I made new paragraph about donor graft risk factors in patients and methods.
Change in the text: Page 4. Line 71-78.

3. Line 44: what the "bacterial theory of RBS"?
Reply 3: On reference 16, Danino et al. performed scanning electron microscopy on

$1cm^2$ sized ADM in RBS patients. They found biofilm on ADM and insisted that despite there were no infectious signs, bacterial contamination could play some role on formation of RBS.

4. Line 46: There is nothing discussed about donor risk factors. A paragraph should be added to make clear to readers that dermis allografts come from human deceased donors, and that tissues are processed and tested differently depending on the supplying tissue bank. Surgeons also have different methods of soaking before implantation and even may do pre-implant cultures.
Reply 4: We made new paragraph about ADM in patients and methods.
Change in the text: Page 4. Line 71-78.

5. Line 56 methods should include ADM source, processing (irradiated vs chemical), dermis transport culture results, endotoxin screening results (if done), sterility cultures, etc.
Reply 5: We made new paragraph about ADM in patients and methods as you recommended.
Change in the text: Page 4. Line 71-78.

6. Line 77 RBS definition. RBS is an acute process, so should be limited by time post-op (e.g. 30 days), otherwise is more likely complications of non-incorporation or poor blood flow.
Reply 6. It usually occurs within several months. But actually there is no exact definition especially about the time period, and there are some reports about RBS after 9 months(reference 14) and even after 4 years(reference 16).

7. Line 77-86 post op antibiotic prophylaxis is not mentioned.
Reply 7. We added information about antibiotic use.
Change in the text: Page 3. Line 69.

8. Line 102 ADM is processed differently depending on the processor. Summarize these processing methods.
Reply 8. We added new paragraph about ADM.
Change in the text: Page 4. Line 71-78.

9. Line 106 what cases were unilateral or bilateral RBS? For each, did these cases have ADM from one donor bilaterally, or grafts from different donors in each breast? At least report if RBS were in grafts from same or different donors.
Reply 9. 4 RBS cases were from unilateral reconstruction and 4 cases were from

bilateral reconstruction. RBS occurred in one side only in bilateral reconstruction cases. Grafts from different donors were used in all bilateral reconstruction cases.
Change in the text: Page 4. Line 71-78. Page 5. Line 112-113.

10. Line 124 why exclude Alloderm and Myderm in analysis?
Reply 10: After consulting with a statistician, we reflected that there were too few cases and that excluding them would be better for analyzing the results.

11. Line 133-136 were there no biopsies done?
Reply 11: Biopsy was done in surgical intervention cases. We added information about it.
Change in the text: Page 6. Line 140-141.

12. Case presentations: how were these patients chosen for description? As outlined, presentation of RBS 3 and 8 months postop are atypical. Prepect recon is known for risk of lack of incorporation, and seroma formation is not typical of RBS. These are NOT RBS cases by most definitions.
Reply 12: We wanted to provide information on patients who required surgical intervention rather than those who were treated conservatively. And in two patients, the course of events was better documented photographically. About seroma, there is report that seroma can increase RBS by affecting angiogenesis and lymphangiogenesis.(Reference 1)

13. Line 142 good is misspelled
Reply 13: Corrected. Thanks.

14. Explain what “very ambiguous characteristics” means
Reply 14: Redness may have similar appearances, and even if there is infection, leukocytosis and elevated crp may not be seen, and if sensation is lost during mastectomy, pain may not be severe, so in cases of subclinical infection, differentiation may be difficult. In addition, clinical improvement should be observed for RBS diagnosis, but differentiation may not be easy when antibiotics are used for various reasons.

15. Line 164 biofilm formation does not necessarily mean “infectious” in terms of transmission from donor, is that what is being implied?
Reply 15: Despite the meticulous aseptic technique, we believe there is a small possibility that bacteria may begin to grow from the ADM, implant, or patient tissues, causing a focal infection.

16. Line 167: the authors don’t seem to understand the endotoxin hypothesis. Gram negative organisms have cell walls with endotoxin, which causes an inflammatory reaction whether the organisms are living or dead, or even in fragments. Sterilization (irradiation, chemicals) does nothing unless the endotoxin is removed.
Reply 16: Thanks for the comment. We deleted ‘and sterilization’ as you mentioned.
Change in the text: Page 8. Line 175.

17. Line 173 delete “highly”
Reply 17: Deleted as recommended.

18. Line 188-191 are the authors postulating that this is an infectious condition? From the donor, or elsewhere?
Reply 18: I think that in certain circumstances, infection may play a role, but I don't think we know exactly how bacterial implantation and growth would begin, whether it's through ADM, implant, patient tissue, or contamination during the surgical procedure.

19. Limitations are insufficient. See previous comments.
Reply 19: I've added some limitations based on what you pointed out.
Change in the text: Page 10. Line 231-233.

20. Conclusions are made which are not supported by data, especially about treatment. All authors can say is that RBS happens after ADM implant in breast recon.
Reply 20: Regarding treatment, what you said is correct, but since there are not many cases, it was difficult to conduct a comparative experiment. And since the treatment produced good results in the given situation, I think it is possible to comment that it can be helpful.

Reviewer B

The authors present a retrospective case series on implant-based reconstruction looking at the incidence of red breast syndrome. They identify a small cohort of cases the majority of improved with corticosteroids and antibiotics.

How as the ADM handled in the OR? Was it washed and soaked?

Reply: ADM was washed 5-10 times with betadine and then 5-10 times with normal

saline. And it was soaked in betadine until use.

Change in the text: Page 4. Line 71-78.

If RBS is felt to be a non-infectious etiology, why are antibiotics prescribed in the protocol? Similarly, if the cases improved with antibiotics, how can a subclinical infection be excluded for?
Reply: RBS usually shows a self-limiting course, but if it is not differentiated from subclinical infection, it can lead to implant removal or reconstruction failure, so using antibiotics is to prevent this. Usually, it is differentiated by the extent of pain and redness, leukocytosis, or crp, but in the case of subclinical infection, it is sometimes difficult to differentiate. However, as there may be cases where focal subclinical infection accompanies RBS especially after radiotherapy, I think it may be helpful to treat with steroid and antibiotics.

Can the authors comment on the processing of the different ADMs used? RBS has mainly become an entity of the past with new sterile ADM (not particularly new they have been around for several years as opposed to aseptic). With sterile ADM and soaking washing, RBS is not really a significant clinical problem these days.
Reply: All of them were new sterile, ready-to-use products. As you mentioned, the rate of RBS has decreased recently with the use of these products, but it has not completely disappeared yet, and cases are being published with new products(reference 1,3). I think it's still worth discussing.

Reviewer C

A nice review on a difficult topic
An explanation of rate of "RBS" may enhance the discussion
Although the literature is sparse ,this topic would be beneficial
Minor revision to include a discussion of 5.8% RBS rate.
Reply: Thanks for your nice comment. We added paragraph about the RBS rate as you commented.
Change in the text: Page 8. Line 178-184.