

June 14th, 2021

World Journal of Orthopedics

We have revised the manuscript entitled “Performance of Alpha-defensin Lateral Flow test after synovial fluid centrifugation for diagnosis of periprosthetic knee infection” according to the suggestions made by your referees. We hope these changes will meet with your approval.

Sincerely,

Rodrigo Abdo

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Periprosthetic joint infection (PJI) following total joint arthroplasty is a serious complication that causes severe morbidity and adds a major financial burden to the healthcare system. Although there is plenty of research on the alpha-defensin (AD) test, a meta-analysis consisting of only prospective studies investigating AD's diagnostic efficacy has not been performed. Additionally, some important subgroups (such as total hip arthroplasty (THA) and Total knee arthroplasty (TKA)) have not been separately analyzed, particularly regarding two commonly used versions of the AD test, the laboratory-based (ELISA) and lateral-flow (LF). Abdo et al. is an original research article aimed to conduct a prospective, cross-section diagnostic study to assess the performance of the alpha defensin and evaluate whether a prior synovial fluid centrifugation could lead the lateral flow performance to achieve comparable results to enzyme-linked immunosorbent assay (ELISA) during Periprosthetic joint infection (PJI) diagnosis after TKA. The primary outcome was to evaluate the sensitivity, specificity, and accuracy of the lateral flow test post fluid centrifugation. Secondly, assessed the performance of the alpha-defensin ELISA in the same population of study, and compared the results between both modalities. They found that HNP1-3 lateral flow test showed high sensitivity, specificity,

and accuracy after a prior synovial fluid centrifugation, achieving comparable results to ELISA. Considering the lower complexity of the lateral flow and its equivalent performance obtained in this condition, investigators concluded that a prior centrifugation might be added as a valuable step to enhance the PJI diagnosis.

Answer: We thank the reviewer-1 for the kind comments on our article and for raising important points to improve our work. We reviewed the manuscript and added the necessary information in this revised version accordingly.

Recommendations: 1. The title should clearly state “human alpha-defensins (HNP1-3)”. This is important to avoid confusion to readers that has nothing to do with “Alpha-defensin 5 or 6” which are Paneth cell specific and contributes to innate defense of the gastrointestinal mucosal surface. HNP1-3 triggers macrophage release of TNF-alpha and IFN-gamma, which act in an autocrine loop to enhance expression of CD32 and CD64 and thereby enhance phagocytosis.

Answer: We have now modified the title to fulfill the journal's criteria. Considering the 18-word limit and the fact that lateral flow test is a commercially available kit that uses HPNI-3. We believe that the term “human alpha-defensins (HNP1-3)” is already implicit in the test's name. Therefore, we kindly ask the reviewer and the Science editor to take into account this consideration. The modified title would be: “Performance of Alpha-defensin Lateral Flow test after synovial fluid centrifugation for diagnosis of periprosthetic knee infection”.

2. It is mentioned that there are two commercially available methods for the determination of synovial alpha-defensin i.e., (1) the quantitative laboratory-based ELISA, that requires a centrifuged synovial fluid to assess the concentration of alpha-defensin and (2) the qualitative lateral flow test. Carefully please check the specificity of the antibody used for the ELISA whether is specific to all HNP1-3 combined or is specific to a particular defensin. This is important due to the similarity in the peptide amino acid sequence, they only differ in ONE amino acid in the head and often do cross react.

Answer: The antibody used for the ELISA is specific to all HNP1-3 combined. We have now included in the Method Section (page 6) this important point raised by the referee about the specificity of the antibody.

3. Synovasure Diagnostics test support diagnosis of several conditions related to joint pain. Are you quantifying pool of HNP1-3? Is it possible to narrow down and use antibody specific for HNP1, HNP2, HNP3 and HNP4? This will bring this interesting study to a more focused work.

Answer: The Synovasure Diagnostics test - lateral flow test – quantifies a pooling of HNP1-3. Based on this, by using this commercially available test, we cannot quantify individually the HNP1, HNP2, HNP3 or HNP4.

(1) **Science editor:** 1 Scientific quality: The manuscript describes a Prospective Study of the Synovial fluid centrifugation prior to lateral flow test. The topic is within the scope of the WJO. (1) Classification: Grade C; (2) Summary of the Peer-Review Report: Research article aimed to conduct a prospective. The title should clearly state “human alpha-defensins (HNP1-3). The questions raised by the reviewers should be answered; (3) Format: There are 4 tables and 1 figure; (4) References: A total of 33 references are cited, including 2 references published in the last 3 years; (5) Self-cited references: There is no self-cited reference. 2 Language evaluation: Classification: Grade B. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form. No academic misconduct was found in the Bing search. 4 Supplementary comments: The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). The topic has not previously been published in the WJO. 5 Issues raised:

Answer: Considering that the Lateral flow test uses HNP1-3 to assess the alpha-defensin, we believe that the term “human alpha-defensins (HNP1-3)” is already implicit in the test’s name. Therefore, we kindly ask the reviewer and the Science editor to take into account this consideration.

(1) The language classification is Grade B. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>;

Answer: As suggested, the manuscript was revised by a native-English speaker. A certificate was uploaded along with the other required files. This article was initially revised by AJE; if the editors feel that another revision is needed, we can submit to AJE again, but we would need more time to accommodate the deadline.

(2) The title is too long, and it should be no more than 18 words

Answer: The title was modified to: "Performance of Alpha-defensin Lateral Flow test after synovial fluid centrifugation for diagnosis of periprosthetic knee infection".

(3) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Answer: The approved grant application form was uploaded in the system.

(4) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Answer: Original figure is now provided.

(5) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout;

Answer: PMID and DOI numbers are now included in the reference list.

(6) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text. 6 Recommendation: Conditional acceptance.

Answer: Article highlights are now provided.

(2) Company editor-in-chief: I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Orthopedics, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision by Authors. However, the quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>.