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Synovial Cell Count Before Reimplantation Can Predict the Outcome of Patients with Periprosthetic Knee Infections Undergoing Two-stage Exchange

--Manuscript Draft--

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AUTHOR RESPONSES TO REFEREE REMARKS

We suggest you complete your responses in this table prior to revising the manuscript.

Reviewer Remarks	**Authors' Responses** **•If you disagree with a reviewer's comment please state why.** **•If already in the text, so note; for all other comments, a revision of the manuscript in response to the reviewer comment is expected. For those, indicate "change made" in this column, and provide text in next column. Remember, if a reviewer has a question, a reader probably will, too.**	**Text Changes** **•Copy and paste the first few lines of any added text from the final version of your revised manuscript in this column.** **•Indicate the page numbers where all changes appear** **•Leave no responses blank; if no text change made, state why not.**
Editor-in-Chief (AU: I've pasted my email questions below, and your responses in the middle column. ED)		
1. There are concerns about the completeness and duration of follow-up of your study. Currently, this is set to 1 year and you don't say how many were lost. Normally, for reconstructive papers of this sort, our minimum follow-up is 2 years. So, if you could, please let me know: a. If the minimum follow-up is to remain at 1 year, what % (n) of patients who had the treatment in question were lost to follow-up before that time; and,b. If the minimum follow-up is re-set to 2 years, as should be possible (since the study period ended in	R. No patient was lost to follow-up, even after re-setting the follow-up to 2 years. (AU: Please ensure this is reported, and set the minimum follow-up to 2 years. ED)	We wrote: Abstract section, page 3, lines 17-18 '….remaining under follow-up in our institution for a period ≥96 weeks, were included in this study.' Page 7, lines 113-117 'Exclusion criteria .....a post-treatment follow-up duration of less than 96 weeks….'

’18) what % (n) of your patients would be lost?		
2. In addition, it’s probably best not to speak about "cure", especially at such short term (instead, perhaps, speak about absence of symptoms or signs of infection). But more importantly, there are concerns about how you defined this. Currently, it's "A cure was defined as the disappearance of all clinical, microbiological, and radiological evidence of PJI coupled with the normalization of the CRP levels during the 96-week follow-up period after the antibiotics were discontinued". However, it's seldom that clear. For instance, I'm curious if someone was asymptomatic and happy with his knee, but had a borderline high ESR, would this count as a "cure" or an infection (by your definition -- "disappearance of ALL ... evidence" – this should be considered an infection, but I suspect you did not count it this way). Please clarify?	R. Sure, patients can have borderline values of CRP and ERS during the first period after reimplantation, but if we set the evaluation up to 12 months or 24 months as you previously asked you can observe a long-term normalization of both CRP and ERS. In this case-series, the absence of CRP and ERS normalization was associated to implant reinfection as we diagnosed following current guidelines (AU: Please ensure this is reported in the paper. ED)	We deleted the term cure along the text and wrote ‘remitted’ (lines 164 and 166) and 'free of infection' at lines 109 and 158
Methods Editor (AU: I’ve pasted the questions below, but have not pasted your responses in the middle column since they were long and you may yet modify them; still, I thought your responses were good and feel free to use them in the revised manuscript. **Addressing all of the Methods Editor’s comments is required for publication**. ED)		
1. My first point relates to the use of “diagnostic accuracy” language throughout the paper and in particular the abstract. If the purpose is to evaluate the “diagnostic accuracies of SF white blood cell counts and	We agree with suggestion	We changed the terms 'diagnostic accuracy' with 'predictive value' in the text (lines 10, 39, 56, 70, 219, 233, 251).

neutrophil percentages", then the authors must say the accuracy in diagnosing what. Whereas using ROC curves is correct and referring to sensitivity and specificities is acceptable though slightly less sophisticated, diagnostic assumes we try to predict a state that exist at the time the maker (or diagnostic test) is measured. By definition, recurrence seems something happening in the future. So, it is not a matter of diagnosis, but rather prediction. Moreover, in diagnostic accuracy studies, one has to explain the reference standard, and there is no mention of reference standard, which is at odds with the STARD statement (which is the guideline applying to a diagnostic accuracy study). Reference 21 (a systematic review and meta-analysis) also mentions diagnostic accuracy, but not exactly what is diagnosed. Reference 10 (another systematic review and meta-analysis) mentions accuracy, but gives less emphasis to "diagnostic accuracy" ("to determine the accuracy of different tests used to detect persistent infection after the first stage, and/or predicting failure following reimplantation in patients with PJI"). References 9, 28, 31 do not focus on diagnostic accuracy either. To summarize, everything would be clearer if the paper focused on predicting infection recurrence (or persistent infection, but here, they mention recurrence). Another option would be to keep the diagnostic accuracy language, but then stick to STARD and be explicit on what is the underlying disease state "diagnosed" (e.g. a "remaining		

infection"), and what is used as a reference standard to ascertain it ("recurrence at a given time frame"). How would the authors modify the paper to address this?		
2. In terms of statistical methods, once a cut-off has been chosen based on some optimization rule (here Youden's index), then the properties of the test evaluated at that threshold in the same data are biased. (This is called optimism: since the cut-off giving the "best" values on the sample is selected, the performance is overestimated by the selection process). Methods such as cross-validation can be used to correct for this (the following reference is for survival, but the idea is the same: Farragi D, Simon R. A simulation study of cross-validation for selecting an optimal outpoint in univariate survival analysis. Stat Med 1996; 15: 2203–2213). How would the authors handle this?	We agree that quantifying the predictive ability of a model on the same data from which the model was developed (often referred to as apparent performance) will tend to give an optimistic estimate of the performance. Studies developing new prediction models should therefore always include some form of internal validation to quantify any optimism in the predictive performance (for example, alibration and discrimination) of the developed model. In this regard we think that the adoption of a bootstrap technique represents an appropriate solution.	We changed the statistical paragraph from line 182 to line 195 (pages 10-11). The text starts with: 'An internal validation method through bootstrapping method is used to obtain the optimal cutoff values.....'
3. On this particular point, the authors should be informed that the popular Youden index is not the best tool to determine a cut-off, because it assumes similar "costs" of false positives and false negatives. But they are not necessary the same. The Youden index does not account for the absolute probability of errors either. In that sense, it does not allow to maximize a relevant loss function (see Perkins et al. American Journal of Epidemiology 2006;163:670–675). This is not a major flaw, but may be either considered if a cut-off has to be determined, or simply discussed (I acknowledge that relative costs of false positives and false negatives may be often difficult to	In the clinical field there is a need for an optimal cut off on markers or tests for separating persons with a specific condition (disease) from those without this condition (healthy). The Youden index is calculated for each threshold c, and the value c*, which achieves a maximum, is referred to as the 'optimal' threshold. In the original article, by using the Youden's Index it was stated that the index "assumes false positives to be as undesirable as false negatives", this allows, i) incorrect classifications of healthy and sick people are equally costly, and ii) that prevalence does not play a role. As we will see, the first is only true in one specific situation [Niels Smits. A note on Youden's J and its cost ratio. BMC Medical Research	We changed the statistical paragraph from line 182 to line 195 (pages 10-11). The text starts with: 'An internal validation method through bootstrapping method is used to obtain the optimal cutoff values.....'

determine). Again, how would the authors handle this?	Methodology 2010, 10:89].	
4. By the way, "recurrence" and "persistence" are not synonyms, and more precise terminology is needed.	We agree with your suggestion	According to your suggestion, we used 'persistent or recurrent infection' each time we need (lines 23, 30, 42, 161, 203, 232, 236, 257, 290, 294).
5. The exclusion criteria on follow-up may lead to bias. Actually, since the inclusion period ended late 2018, all participants have a theoretical 2 years follow-up. Missing information may be informative, and should be accounted for in the analysis. At least the risk of bias should be mentioned and discussed.	In our institution patients remain in follow-up for at least 96 weeks after the second step of the 2-stage procedure. In this case, the last patient considered completed the procedure in the middle days of november, so all cases report the required follow-up	The minimum follow-up was 96 weeks after the second step of the 2-stage procedure. We have no missing information. See lines 18,117, 156,157
Reviewer #1		
1. I am comfortable reviewing the methods used in this article, but I am concerned that some reviewers and many readers may not be familiar with the use of Youden's J-statistic to determine the optimal cut-off value in receiver-operating-curves. The authors do not state this and they do not offer any explanation of how this statistical tool works. I imagine that this would be a point of contention among reviewers who many contend that the "most accurate" is highly subjective. In fact, there is considerable support in the medical literature and the recent arthroplasty literature for using Youden's J-statistic to determine optimal "cut-off" values for dichotomous tests, and I think that most arthroplasty surgeons do not yet appreciate the power of this statistical tool. This manuscript could be somewhat improved if the authors offered a short maybe one or	According to Editor in Chief (EIC) 's suggestions, Youden's J-statistic was not used to assess the validity of our test. We add an explanation of the test adopted in the materials and methods section.	Page 10 from line 182 e following starting with: 'An internal validation……'

two sentence explanation of this. Doing so is not necessary as it appears that authors used these statistical methods appropriately, but a short explanation might make this manuscript more accessible to a general audience. Maybe drawing the J-statistic threshold value as a line on the ROCs in figures could be helpful. Figure 1 should probably be divided into two separate figures for WBC count and PMN %.		
2. Methods - Sources of bias: Authors note a 96-week follow-up of CRP and ESR to establish a cure, however exclusion criteria were follow-up less than 1 year (52 weeks). What was the median and range of the follow-up interval for all patients in the cohort? How does this compare to the average interval at which treatment failure is diagnosed?	We agree with this comment. See reply to EIC	See lines 18, 117, 154, 156, 157
3. For how long did these patients continue antibiotics after the second-stage reimplantation? How many of these patients are on lifelong antibiotic therapy? How does this compare with the gold standard?	All patients discontinued the treatment 15 days after second step of the procedure. No patient received lifelong therapy. These procedures were performed according to suggestion derived from ICM-2013 and ICM-2018.	Page 7, lines 100-103 'None of the patients discontinued antibiotic treatment before definitive reimplantation because of side effects----- '.
4. Were any of these patients undergoing a second or third two-stage revision or were these all first two-stage revision patients? How might this impact results?	Study population is rather homogeneous regarding this point as all patients were at their first two-stage revision.	Page 6, line 90 'All patients were at their first 2-stage revision.'
5. Methods - General comments: Authors excluded patients with acute PJI, patients with clearly persistent infection, and patients that discontinued antibiotic therapy before reimplantation.	This is to reduce the risk of bias.	No change was done for this point
6. The authors included patients who had PJI as defined by the 2013 ICM criteria. These criteria were updated in 2018. I am	All patients enrolled during the first year of study, before 2018 ICM criteria were released fulfilled both 2013 and 2018	See page 6, line 80 '….. diagnosis of PJI based on the International Consensus Meeting on PJI (ICM) criteria, patients aged

not sure if readers would take objection to authors use of outdated criteria. Would all the patients included using 2013 criteria also meet the 2018 definition? If so, then it may deserve mention that all of these patients also met the current criteria.	criteria. We refer to both ICM in the text and no further change was done to avoid that the text could appear rather boring.	> 18 years, and a delayed infection to be included in the study [25, 26] (Both ICM 2013 and 2018 were in the references). Page 7, lines 111-112 'PJI was defined using the 2013 Philadelphia ICM diagnostic criteria as modified by 2018 ICM [25, 26]'. Page 14, line 268 '…as established by ICM 2018
7. The results are clear, but the discussion of osteomyelitis in the introduction and discussion section is off-topic. Authors should stick with discussion of the topics that are supported by the findings that they present.	We removed the section regarding osteomyelitis.	See lines 32, 42, 50, 217, 220, 255
8. Authors state that specimens obtained at reimplantation revealed bacterial growth in 6 patients (7%) and that there was no microbiological concordance. What was the fate of these patients? Did they all go on to treatment success and eradication of infection? Did they all go on to treatment failure?	All these patients had complete eradication of PJI	'All these patients were free of infection 96 weeks after the second step of the procedure.' (Page 7, lines 109-110)
9. Discussion – Limitations: Patients did not discontinue antibiotic treatment prior to reimplantation. This could be a source of controversy as the present standard is two-week antibiotic holiday followed by re-aspiration of spacer to prove that there is no PJI before reimplantation. Do the authors propose that their proposed cut-offs for synovial fluid analyses could replace this standard?	We chose this protocol according to previous investigations (Ascione et al J. Arthroplasty). We believe that these cutoffs report the highest performance in those that do not discontinue antibiotic therapy. No study assessed this argument previously.	Page 14, lines 252-254. 'For this reason, the cutoffs established in the present study can have the highest value in those adopting such therapeutic schedule, but the value in patients who observe an antibiotic holiday period needs to be assessed.'
10. Did the Husuma, Boelch , and MSIS studies include an antibiotic holiday or not? How does this impact the validity of the comparisons to these studies, and how does it impact the generalizability of the results of	We agree with the referee and evaluate this argument in the discussion section.	Page 15, lines 282-287. 'We proposed thresholds that performed .....'

this study? Authors note that their cutoff values are lower than those reported from previous studies and authors suggest that this may be associated with differing antibiotic regimens, but could it also be related to antibiotic duration / continuing antibiotics?		
11. Discussion - Context and Conclusions: For the first paragraph of the discussion, again, this study does not investigate curing osteomyelitis as an endpoint. That would require some histological data. For example Parvizi has presented some histologic data that shows that MRSA can actually become enveloped within osteons and this has been proposed as a mechanism of late treatment failure. This study compares differences in synovial fluid between patients who had successful 2-stage reimplantation at latest follow-up versus those that did not. The authors did not investigate osteomyelitis and pre-sent no data specific to this and the manuscript should be re-worded in the introduction and discussion to better reflect the data that the authors presented.	We removed the term osteomyelitis along all the manuscript.	See lines 32, 42, 50, 217, 220, 255
12. Furthermore, most of the first paragraph on the discussion section should be moved to a spot early in the introduction section. I suggest re-organizing the discussion and starting it this way "Criteria have not been proposed that enable accurate predictions of a PJI cure before definitive reimplantation in the interval between resection arthroplasty and reimplantation [1, 3-6, 19, 22, 29, 30, 34]. Three recent meta-analyses that evaluated the accuracy of different diagnostic tests	We eliminated most of the first paragraph and re-organized the section according to the suggestions.	Page 14, lines 249-254. 'This choice was based on.....'

used before the second stage of surgery concluded that no single test can be used alone to predict failed reimplantation beyond the second stage of surgery [10, 14, 21]…"		
13. I think that using Youden's J-statistic to determine optimal "cut-off" points for dichotomous tests is a super powerful tool that has significant support in the medial literature. And, I would agree that a report of that uses this tool to evaluate the usefulness of synovial fluid lab values potentially could be cited numerus times, could lead to changes in the consensus guidelines, and ultimately could benefit many patients. Therefore, this paper has significant potential, but it suffers from some aforementioned flaws. I think acknowledging the two-week antibiotic holiday as the standard, discussing the limitations of that approach and the reasons for shifting toward continuing antibiotics (no holiday) as presented by Ascione, Journal of Arthroplasty, 2019, and then noting that no one has yet presented cut-off values for aspiration while antibiotics are continued would firmly cemented the place of these findings in the current literature and the authors should present this in their introduction section.	We agree with these considerations that are reported in the discussion section.	Page 14, lines 247-254 'Another limitation can be given by the antibiotic schedule adopted……..'
14. It's a good paper, but I wouldn't want it to get dismissed by skeptical readers. For example, the first line of the abstract is somewhat unclear and might make some readers skeptical about the content that follows. Readers might ask "'adopted' by whom?" I would suggest authors revise this	The first sentence of the abstract was changed.	Page 2, lines 3-5. 'Although synovial fluid can be used to diagnose periprosthetic joint infections (PJI) effectively, only the cutoff values adopted at the time of PJI diagnosis have been standardized'

and formulate an air-tight first sentence for their paper.		
15. Similarly, I would recommend adding an alternative first sentence to the introduction section that focuses and the specific topic of the paper. Something like "2-stage exchange is the standard for treatment of TKA PJI in the US, however some controversy remains regarding optimal synovial fluid indicators of antibiotic spacer effectiveness." Likewise, the introduction seems to focus on treating the underlying osteomyelitis, but the study investigates treatment failure and prediction of treatment failure and presents no data on whether the osteomyelitis has been cured - such a study would involve presentation of some histological data. I would suggest author focus on "treatment failure" rather than "underlying osteomyelitis."	The introduction section now reports the arguments suggested and the part regarding osteomyelitis has been deleted.	Page3, lines 47-56 'Two-stage exchange is a widely used treatment for patients with periprosthetic joint infections (PJI) because it enables PJI to be treated……..'
Reviewer 2		
1. - the interval between the implant removal and reimplantation is rather long. today, there are many institutions that are doing only 2 weeks between the two stages with also good results. in theory, a complete cure of an underlying osteomyelitis is not necessary for the second operation, because a second debridement is then conducted. So, regarding the aim "to help finding the best timepoint for reimplantation" this needs to be discussed.	We followed Italian guidelines which consider 6-8 weeks of antibiotic therapy. ICM-2018 recommend an antibiotic treament for 2-6 weeks. 'to help finding the best.....' was removed	No change was reported
2. - also the sensitivity of the SF analysis might be influenced by the timepoint the samples are taken with regard to the index surgery (implant removal) - that needs to be	Aspirates were performed between 14 and 16 days prior to the definitive reimplantation to ensure that the risk of variability related to the timing was set to	No change was reported in the text

discussed as a potential limitation of the study. Do the authors have any information regarding the sensitivity of their tests with regard to the time line??	the minimum. We have no information about the sensitivity of the text with regard to the time line.	
3. - Overall the potential limitations are missing in the discussion.	A limitations paragraph was added	Page 13, lines 239-254. 'This study's findings should be interpreted in light of several limitations….'
4. - What is the potential reason your data is "better" in terms of sensitivity compared to others? different methods? larger patient group? please give more detailed information.	We have a very homogeneous population and our statistical analysis is very accurate.	Page 15, lines 277-280. 'Moreover, our statistical methodology, which used bootstrap methodology, is able to obtain accurate.....
5. Results: the last paragraph of the results section is too much a discussion. please separate the actual results from the interpretation and the comparison against other studies (which should be in the discussion)	We limit to the comparison of previously known cutoffs performance on our dataset in the result section and discuss the results in the discussion section, as the referee suggests.	Page 15, lines 282-287. 'We proposed thresholds that performed as well as or better than previously published thresholds [11,20,27]….'
6. please be more careful when concluding the importance of your results: Saying "an EXCELLENT tool to establish" appears a bit too positive, especially with regard to potential problems with short intervals for reimplantation. This point should also be clarified in the title, abstract and introduction that the study investigates 2stage exchange with LONG interval!	We agree with this suggestion, but we have to consider that the 6-week period considered is largely used in clinical practice and that shorter protocols need further investigations. The term excellent tool is not reported anymore.	See lines 219 and 273 where the term 'excellent' was removed

Synovial Cell Count Before Reimplantation Can Predict the Outcome of Patients with Periprosthetic Knee Infections Undergoing Two-stage Exchange

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Each author certifies that neither he or she, nor any member of his or her immediate family, has funding or commercial associations (consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research*® editors and board members are on file with the publication and can be viewed on request.

Ethical approval for this study was obtained from the Department of Infectious Diseases of the 'D. Cotugno' hospital.

This work was performed at D. Cotugno Hospital, Naples, Italy.

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Abstract

Background Although synovial fluid can be used to diagnose periprosthetic joint infections (PJI) effectively, only the cutoff values adopted at the time of PJI diagnosis have been standardized, and only few data are currently available about effectiveness of synovial fluid examination before definitive reimplantation.

Questions/purposes We asked: (1) What are the most appropriate thresholds for synovial fluid leukocyte counts (WBC) and neutrophil percentage (PMN percentage) in a patient group undergoing definitive reimplantation after an uninterrupted course of antibiotic therapy for chronic PJI? (2) What is the predictive value of our synovial WBC and PMN percentage threshold compared with previously proposed thresholds?

Methods In all, 101 patients with PJI were evaluated for inclusion from January 2016 to December 2018. Nineteen percent (19 of 101) of patients were excluded because of the presence of a chronic inflammatory disease, acute/late hematogenous infection, low amount of synovial fluid for laboratory investigations or infection persistence after spacer placement and adequate antibiotic therapy. Finally, 81% (82 of 101) of patients with a median (range) age of 74 years (48 to 92) undergoing two-stage revision for chronic TKAs infection, who were followed up at our institution for a period 96 weeks or more were included in this study. The patients did not discontinue antibiotic treatment before reimplantation and were treated for 15 days after reimplantation if intraoperative cultures were negative. No patient remained on suppressive treatment after reimplantation. Synovial fluid was aspirated aseptically with a knee spacer in place to evaluate the cell counts before reimplantation. Thirteen percent (11 of 82) of patients had persistent or recurrent infection, defined as continually elevated erythrocyte sedimentation rate or C-reactive protein levels coupled with local signs and symptoms or positive cultures. The synovial fluid WBC counts and PMN percentage from the 11 patients with persistent or recurrent PJI were compared with the 71 patients who were

believed to be free of PJI. Receiver operating characteristic (ROC) curve analyses assessed the predictive value of the parameters, and the areas under the (ROC) curves were evaluated. The sensitivities, specificities, and positive and negative predictive values were determined for the WBC count and PMN percentage. Patients with persistent or recurrent infection had higher median WBC counts (471 cells/μL versus 1344 cells/μL; $p < 0.001$) and PMN percentage (36% versus 61%; $p < 0.001$) than did patients believed to be free of PJI.

Results ROC curves analysis identified the best threshold values to be a WBC count of 934 cells/μL (sensitivity of 0.82 [95% CI 0.71 to 0.89] or more, a specificity of 0.82% [95% CI 0.71 to 0.89], as well as a PMN percentage of at least 52% (sensitivity 0.82 [95% CI 0.71 to 0.89] and specificity of 0.78 [95% CI 0.67 to 0.86]. We found no difference between the AUCs for the WBC count and the PMN percentage (0.87 [95% CI 0.79 to 0.96] versus 0.84 [95% CI 0.73 to 0.95]. Comparing the sensitivities and specificities of the synovial fluid WBC count and PMN percentage proposed by other authors, we find that a PMN percentage more than 52% showed better predictive value than previously reported.

Conclusion Based on our findings, we believe that patients with WBC counts of at least 934 and PMN percentage of 52% or more should not undergo reimplantation, but rather a repeat debridement, as their risk of persistent or recurrent PJI appears prohibitively high. The accuracy of the proposed cutoffs is better than previously reported.

Level of Evidence Level III, diagnostic study.

Introduction

Two-stage exchange is a widely used treatment for patients with periprosthetic joint infections (PJI) because it enables PJI to be treated with a spacer in place before definitive reimplantation [3, 5, 13, 24, 25, 27, 35]. There is no consensus about when to perform reimplantation during a two-stage exchange arthroplasty because no identified variables are consistently associated with infection eradication [10, 26]. Indeed, the disappearance of clinical signs and the normalization of serum biomarkers do not accurately identify patients at the lowest risk of infection recurrence [16, 17, 20, 32, 33]. Moreover, joint aspiration before definitive reimplantation and intraoperative bacterial sampling at the time of reimplantation predict successful procedures with low levels of accuracy, when cutoffs suggested at the time of diagnosis are adopted [9, 28, 31]. Three meta-analyses that evaluated the predictive value of different tests to guide the appropriate timing of reimplantation concluded that no single diagnostic test could definitively confirm that patients are free of PJI after the first stage and before reimplantation [10, 14, 21]. Therefore, multiple diagnostic tests are often used to determine risk of infection persistence or recurrence before reimplantation, but none of the tests is sufficiently accurate to exclude persistence or recurrence of infection after reimplantation. Leukocyte counts in synovial fluid aspirates taken with a spacer in place enable important preoperative assessments of an infection cure before definitive reimplantation. However, the cutoff values for the cell counts that predict reimplantation without further symptoms or signs of PJI with the greatest accuracy have not been established [11, 17, 20, 23, 33, 36].

We therefore asked: (1) What are the most appropriate thresholds for synovial fluid leukocyte counts (WBC) and neutrophil percentage (PMN percentage) in a patient group undergoing definitive reimplantation after an uninterrupted course of antibiotic therapy for chronic PJI? (2) What is the predictive value of our synovial WBC and PMN percentage threshold

compared with previously proposed thresholds?

Patients and Methods

Between January 2016 and December 2018, we evaluated and treated 101 patients with confirmed knee PJI. Known comorbidities relating to an increased infection risk were reported in 37% (37 of 101) of patients, and diabetes mellitus and chronic hepatitis were identified with the highest frequency. Twelve patients had a BMI more than 30 kg/m^2, and the remaining 89 had a BMI below 30 kg/m^2. We evaluated 101 patients with diagnosis of PJI based on the International Consensus Meeting on PJI (ICM) criteria. In this study, we included patients older than 18 years who had a delayed infection [25, 26]. We excluded two patients with chronic inflammatory joint diseases; nine patients with acute infections that appeared less than 90 days after the index procedure or late hematogenous infections with symptom durations of less than 3 weeks; three patients with inadequate amounts of synovial fluid for cultures and leukocyte counts; and five patients with persistent infection after spacer placement and adequate antibiotic therapy (a persistent infection was defined as continually elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels coupled with local symptoms or positive cultures yielding the same bacteria retrieved at the time of PJI diagnosis). No patient discontinued antibiotic therapy before revision surgery. Thus, of the original 101 patients, the final analysis included 82 patients with a median (range) age of 74 years (48 to 92). All patients were at their first two-stage revision.

Eighty-five percent (70 of 82) patients had positive microbiologic culture results. The main pathogens isolated were coagulase-negative staphylococci (46% [32 of 70], with 11 of these patients were infected with methicillin-resistant bacteria. *Staphylococcus aureus*, including nine methicillin-resistant strains, was isolated from 41% (29 of 70) of patients. Gram-negative bacteria were isolated from 13% (9 of 70) of patients, and *Pseudomonas aeruginosa* was cultured from four patients.

Among patients included in the study, 80% (66 of 82) had articulating spacers and 20% (16 of 82) had static spacers; the surgeon based their choice of spacers on evaluation of bone stock and soft tissue involvement. The median antibiotic treatment duration was 8 weeks (IQR 8 to 8). No patients discontinued antibiotic treatment before definitive reimplantation because of side effects, and all patients continued the antibiotic treatment for 15 days after reimplantation, until intraoperative cultures were available. We did not administer long-term chronic suppressive antibiotic therapy in any patient. The patients did not report any clinical signs that suggested active infections, and the ESR and CRP levels were below the upper normal limits before definitive reimplantation. The median interval from prosthesis removal to reimplantation was 8 weeks (IQR 8 to 8). Specimens obtained at reimplantation revealed bacterial growth in 7% (6 of 82) of patients. The bacteria isolated at prosthesis removal and reimplantation did not show microbiologic concordance in any patients. All these patients were free of infection 96 weeks after the second step of the procedure.

PJI was defined using the 2013 Philadelphia ICM diagnostic criteria as modified by 2018 ICM [25, 26]. The inclusion criteria were a diagnosis of PJI based on the ICM criteria, patients aged older than 18 years, and a delayed infection. The exclusion criteria were chronic inflammatory joint diseases, acute infections within 90 days after the index procedure, or late hematogenous infections with symptom duration of less than 3 weeks, inadequate amounts of synovial fluid for cultures and leukocyte counts, discontinued antibiotic therapy before revision surgery, a post-treatment follow-up duration of less than 96 weeks, and persistent infection after spacer placement and adequate antibiotic therapy, which was defined based on ongoing clinical symptoms and persistently elevated ESR and CRP levels that prevented the patients from undergoing the second-stage revision procedure.

Treatment Regimen

The two-stage exchange procedure adopted is described in detail elsewhere [4]. The Italian

PJI guidelines recommend a two-phase antibiotic treatment protocol of 2 weeks of intravenous therapy followed by oral targeted therapy for 6 weeks, when feasible, based on microbiologic test results [15]. Hence, antibiotic therapy began with parenteral antibiotics for 2 weeks after implant removal. When available, the synovial fluid cultures determined the selection of drugs administered before the infected implants were explanted. When synovial fluid culture results were negative, empiric antibiotic therapy was used, which comprised drugs that were active against gram-positive methicillin-resistant bacteria, until the microbiologic results from cultures of the periprosthetic tissues or implant sonication became available. The subsequent 6-week course of antibiotic therapy included oral drugs, when possible, which were selected based on the microbiologic evaluations. When all preoperative and intraoperative culture results were negative, combination regimens that contained a drug active against methicillin-resistant staphylococci (for example, cotrimoxazole or minocycline) were considered for first-line therapy after the parenteral antibiotic therapy. After completing a course of antibiotics, the patients underwent reimplantation while continuing antibiotic therapy. This was established based on reported clinical evidence [3]. Reimplantation was scheduled for patients whose CRP levels and ESR remained normal and who did not have any local symptoms preoperatively.

Scheduled Assessments

The ESR, CRP levels, and complete blood counts were assessed before the infected implant was removed and every 7 days for 2 weeks after spacer placement. Synovial fluid aspirations were scheduled at least 14 days before reimplantation to evaluate the leukocyte counts and establish cultures with the knee spacer in place. The aspirate was directly inoculated into two different vials: one containing ethylenediaminetetraacetic acid (either K2 or K3) for cell counting and the other (0.5 mL to 3.0 mL of aspirate) for inoculation of blood culture bottles (Bactec-Ped; bioMerieux). To be considered sterile, bottles were incubated for 14 days

beforehand. The synovial fluid samples collected in the ethylenediaminetetraacetic acid-coated tubes were transported to the laboratory and stored at room temperature, and their WBC counts and PMN percentage were determined within 3 hours using a hematology analyzer. At reimplantation, at least five periprosthetic tissue samples had been collected from all patients for microbiologic analyses. Brain-heart infusion broth (bioMerieux) was added to the specimens within 1 hour, and they were incubated for 24 hours at 37° C before terminal subculturing. After replacement of the prosthetic implant, the CRP levels and ESRs were assessed for 96 weeks. We defined the absence of PJI as the disappearance of all clinical, microbiologic, and radiologic evidence of PJI coupled with the normalization of CRP levels during the 96-week follow-up period after the antibiotics were discontinued. After the 96-week follow-up period, 87% (71 of 82) of patients were considered free of infection, and 13% (11 of 82) patients were not, based on our criteria.

Laboratory Values

Before reimplantation, the median synovial fluid WBC counts and PMN percentage were higher in patients who eventually had persistent or recurrent PJI than in patients who did not. The median WBC count in the patients who demonstrated persistent infection was 1344 cells/μL (IQR 934 to 2776 cells/μL) compared with 471 cells/μL (IQR 290 to 804 cells/μL) in patients whose infection was regarded as remitted ($p < 0.001$). The median PMN percentage was 61% (IQR 52% to 78%) in patients who demonstrated persistent infection versus 36% (IQR 28% to 51%) in those whose infection was regarded as remitted ($p < 0.001$) (Table 1).

Ethical Approval

Ethical approval for this study was obtained from the Department of Infectious Diseases of the D. Cotugno Hospital. The study was conducted in accordance with national and

institutional standards, and in accordance with the principles of the Declaration of Helsinki. The patients provided informed consent before they were included in the study.

Statistical Analyses

We used descriptive statistics for continuous variables, which we compared using the Mann-Whitney U test. Categorical variables are expressed as proportions, and we compared them using the Fisher exact or the chi-square test, as appropriate. Receiver operating characteristic curves (ROC), which depict relationships between true-positive results (sensitivity) and false-negative results (1-specificity), were constructed for the synovial fluid WBC counts and PMN percentage. The parameters' sensitivities, specificities, positive predictive values, and negative predictive values (NPVs) were calculated using 2 × 2 contingency tables. The areas under the ROC curves (AUCs) were assessed to better evaluate the parameters' accuracies. An AUC of 1 indicated 100% sensitivity and 100% specificity, while an area under the < 0.5 indicated a less useful test. An internal validation method through a bootstrapping method was used to obtain the optimal cutoff values for the overall WBC count and the neutrophil percentage. A total of 1000 bootstrap samples from the 82 patients were drawn with replacement in the original data. The advantage of this method is that the bootstrap-based ROC curves are much stable than those of the holdout or cross-validation, indicating a more stable ROC analysis. This is performed by considering a misclassification cost function (to be minimized) to assess the discriminatory ability of a cutoff point relied on the elements of the 2 × 2 confusion matrix, that is true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN), that is $cost_{FP}$_× FP + $cost_{FN}$ × FN. We assumed that a false negative result was five times more costly than a false positive. The empirical prevalence (equal to 0.13) was used to run the analysis. Furthermore, the sensitivities and specificities of the synovial fluid WBC count and PMN percentage at the obtained optimal thresholds were compared with results obtained from our patients according to the thresholds proposed by the

MSIS [27], Boelch et al. [11], and Kusuma et al. [20] using the McNemar test [18]. The AUCs were compared using the DeLong test [12]. A value of $p < 0.05$ indicated statistical significance. We used the R statistical software environment IBM SPSS software, Version 21.0.0.1 (IBM Corp) to construct the databases and conduct the statistical analyses.

Results

Predictive Value of Proposed Cutoffs for WBC and PMN Percentage

ROC curve analysis used to identify the best threshold values showed that a WBC of at least 934 cells/μL (proposed threshold) yielded a sensitivity of 0.82 (95% CI 0.71 to 0.89) and a specificity of 0.82 (95% CI 0.71 to 0.89) in predicting persistent or recurrent infection, whereas a PMN percentage of greater than 52% (proposed threshold) had a sensitivity of 0.82 (95% CI 0.71 to 0.89) and a specificity of 0.78 (95% CI 0.67 to 0.86). The AUC was 0.87 (95% CI 0.79 to 0.96) for the WBC count (Fig. 1) and 0.84 (95% CI 0.73 to 0.95) for PMN percentage (Fig. 2). Sensitivity for both synovial fluid parameters was 0.82 (95% CI 0.71 to 0.89), specificity was 0.82 (95% CI 0.71 to 0.89) for WBC and 0.78 (95% CI 0.67 to 0.82). A WBC count of at least 934 cells/μL combined with a PMN percentage no less than 52% had a sensitivity of 0.66 (95% CI 0.52 to 0.74), a specificity of 0.92 (95% CI 0.83 to 0.96), a PPV of 0.54 (95% CI 0.43 to 0.65), an NPV of 0.94 (95% CI 0.86 to 0.98), and an AUC of 0.70 (95% CI 0.60 to 0.95) (Table 2).

How Do These Values Compare with Previously Published Thresholds?

Comparing the sensitivities and specificities of the synovial fluid WBC count and PMN percentage at the obtained optimal thresholds with that obtained adopting for our dataset the thresholds proposed by the MSIS [27], Boelch et al. [11], and Kusuma et al. [20] using the McNemar test [18], we found no difference in the AUC between a WBC count higher than 934 cells/μL and a WBC count higher than 1102.5 cells/μL, as proposed by Kusuma et al.

[20] (0.87 [95% CI 0.79 to 0.96] versus 0.79 [95% CI 0.63 to 0.96]; p = 0.60). In contrast, a PMN percentage of 52% or more showed better predictive value than a percentage more than 80% and 72% (0.84 [95% CI 0.73 to 0.95] versus 0.58 [95% CI 0.38 to 0.76]; p < 0.001; and 0.84 [95% CI 0.73 to 0.95] versus 0.63 [95% CI 0.44 to 0.83]; p = 0.04, respectively) (Table 3).

Discussion

PJI is a complication of total joint arthroplasty that can occur postoperatively or as a delayed or late infection well after implantation. The two-stage exchange procedure is used for treating delayed PJIs, infections caused by multidrug-resistant bacteria, and those showing a sinus tract [3-6]. Although the two-stage exchange technique is largely standardized, several questions that remain about the procedure must be answered to increase its likelihood of success. Actually, no criteria have not been established to enable accurate predictions of a PJI persistence before definitive reimplantation and to establish the most appropriate interval between resection arthroplasty and reimplantation [1, 3-6, 19, 22, 29, 30, 34]. High levels of consensus regarding PJI diagnoses according to ICM [2, 25] and Musculoskeletal Infection Society [27] criteria have been obtained, but when the same criteria are applied to establish the absence of persistent or recurrent PJI before the second step of the two-stage procedure, their predictive value remains low, suggesting the need for better diagnostic tools and approaches [7, 8]. Based on our discoveries, we believe that patients with WBC counts greater than 934 and PMN percentage of 52% or more should not undergo reimplantation, but rather a repeat debridement, as their risk of persistent or recurrent PJI appears prohibitively high.

Limitations

This study's findings should be interpreted in light of several limitations. First of all, we

aimed to investigate a very selected group of patients with chronic PJI and without chronic inflammatory joint disease. This choice made the study population homogeneous and reduced some relevant biases but affected the possibility to apply the cutoffs obtained to the whole population of patients affected by PJI. Moreover, some patients report cofactors that can influence PJI outcome, such as diabetes or chronic liver disease. Although the sample appears to be well-balanced, its size precludes assessing the value of the cutoffs proposed in selected subpopulations and further investigations on the role of each of these factors have not been performed and are difficult to plan. Another limitation is the antibiotic schedule adopted, as our patients did not discontinue antibiotic treatment before reimplantation. We made this choice based on the results of a previous study which demonstrated that a better outcome could be obtained without antibiotic discontinuation before definitive reimplantation because of the low predictive value of bacterial investigations before definitive reimplantation [3]. For this reason, the cutoffs established in the present study may have the highest value in those adopting such therapeutic schedule, but the value in patients who observe an antibiotic holiday period must be assessed.

Predictive Value of Proposed Cutoffs for WBC and PMN Percentage

We found that WBC counts greater than 934 and cells/μL and PMN percentage of 52% were associated with a high risk of persistent or recurrent PJI. The exact value of synovial fluid WBC counts and PMN percentage for diagnosing persistent infections before reimplantation was not fully assessed previously. In fact, Bian et al. [10] reported extreme variations in the sensitivities and specificities of synovial fluid WBC counts and PMN percentage, when they were used to identify persistent infections. Newman et al. [23] and Zmistowski et al. [36] reported substantial elevations in synovial fluid WBC counts and PMN percentage in patients with persistent PJIs, suggesting their evaluation at the time of definitive reimplantation. Our findings agree with these results, confirming the link between the synovial cell count at

reimplantation and PJI recurrence because the synovial fluid WBC counts and PMN percentage were higher in patients with recurrent infections than in patients who underwent successful procedures ($p < 0.001$), but only 18% of these patients had WBC counts or PMN percentage above the limits required to diagnose PJI as established by ICM 2018 [26]. Hence, lower threshold values should be considered for patients with antibiotic spacers to exclude persistent infections. Some studies have attempted to determine the best cutoff values for synovial fluid WBC counts and PMN percentage in patients who observe a minimum 2-week antibiotic holiday period before reimplantation [11, 17, 23, 33, 36], but no study has assessed the usefulness of these synovial fluid parameters in patients who did not observe an antibiotic holiday period before reimplantation. Zmistowski et al. [36] determined that a WBC count of 640 cells/μL and a PMN percentage of 56% were excellent thresholds for diagnosing persistent infections, while Kusuma et al. [20] reported a synovial fluid WBC count of 1102 cells/μL and a PMN percentage of 71.5%. Our study is the first to assess the cutoffs to be used for patients who do not observe an antibiotic holiday period. Moreover, our statistical methodology, which used bootstrap methodology, obtained accurate measures of both bias and variants of the true error estimate, which makes the analysis more accurate than performed in other studies.

How Do These Values Compare with Previously Published Thresholds?

We proposed thresholds that performed as well as or better than previously published thresholds [11, 20, 27]. In the second step of our analysis we tried to apply the cutoffs derived by our investigation and by other studies to our data set. We found that our cutoff values for synovial fluid WBC and PMN percentage had a higher predictive value than achieved using MSIS [27] proposed thresholds. Furthermore, a PMN percentage more than 71.5% as reported by Kusuma et al. [20] showed lower specificity and AUC than our results.

Conclusion

In conclusion, our study's findings suggest that our synovial fluid cell count thresholds accurately predicted persistent or recurrent PJI showing a higher accuracy than previously reported cutoffs. Patients whose synovial fluid WBC counts and PMN percentage were above our cutoff values had a 94% probability of favorable outcome. Given that WBC counts of at least 934 cells/μL and PMN percentage no less than 52% were both reasonably sensitive and specific for a patient presenting later with persistent or recurrent PJI, we recommend that patients with these characteristics undergo a repeat debridement, as their risk of persistent or recurrent PJI appears prohibitively high.

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Legends

Fig. 1 A receiver operating characteristic curve was used to determine the most appropriate synovial fluid WBC count threshold.

Fig. 2 A receiver operating characteristic curve was used to determine the most appropriate synovial fluid PMN percentage threshold.

Table 1. Synovial fluid parameters in patients with and without a favorable outcome

Variable	Patients with infection (n = 11)	Patients without infection (n = 71)	p value
WBC count (cells/uL)	1344 (934-2776)	471 (290-804)	$p < 0.001$
PMN percentage	61 (52-78)	36 (28-51)	$p < 0.001$

Data are presented as median (IQR).

Table 2. Diagnostic parameters of synovial fluid WBC count and PMN percentage at the proposed threshold

Parameter	WBC count	PMN percentage	WBC count ≥ 934cells/μL, PMN percentage ≥ 52%
Proposed threshold	934 cells/μL	52%	
Sensitivity	0.82 (95% CI 0.71-0.89)	0.82 (95% CI 0.71-0.89)	0.66 (95% CI 0.52-0.74)
Specificity	0.82 (95% CI 0.71-0.89)	0.78 (95% CI 0.67-0.86)	0.92 (95% CI 0.83-0.96)
Positive predictive value	0.41 (95% CI 0.30-0.52)	0.36 (95% CI 0.26-0.547)	0.54 (95% CI 0.43-0.65)
Negative predictive value	0.98 (95% CI 0.89 0.99)	0.97 (95% CI 0.89-0.99)	0.94 (95% CI 0.86-0.98)
Area under the curve	0.87 (95% CI 0.79-0.96)	0.84 (95% CI 0.73-0.95)	0.70 (95% CI 0.60-0.95)

Table 3. Comparison of sensitivity, specificity, and AUC of proposed threshold versus published values

Parameter	MSIS threshold	MSIS p value vs proposed threshold	Kusuma et al. threshold	Kusuma et al. p value vs proposed threshold	Boelch et al. threshold	Boelch et al. p value vs proposed threshold
WBC count						
Threshold	3000 cells/μL		1102.5 cells/μL		3250 cells/μL	
Sensitivity	0.18 (95% CI 0.11-0.29)	0.14	0.73 (95% CI 0.62-0.82)	0.08	0.18 (95% CI 0.11-0.29)	0.14
Specificity	0.99 (95% CI 0.92-0.99)	0.04	0.86 (95% CI 0.76-0.92)	0.02	0.99 (95% CI 0.92-0.99)	0.04
AUC	0.58 (95% CI 0.39 - 0.78)	< 0.001	0.79 (95% CI 0.65-0.96)	0.60	0.58 (95% CI 0.39-0.78)	< 0.001
PMN percentage						
Threshold	80%		71.5%		NA	NA
Sensitivity	0.18 (95% CI 0.11-0.29)	0.14	0.36 (95% CI 0.26-0.48)	0.15		
Specificity	0.97 (95% CI 0.90-0.99)	0.04	0.90 (95% CI 0.81-0.95)	0.03		
AUC	0.58 (95% CI 0.38-0.76)	< 0.001	0.63 (95% CI 0.44-0.83)	0.04		

MSIS = Musculoskeletal Infection Society; AUC = area under the curve; NA = not available.

Figure 1

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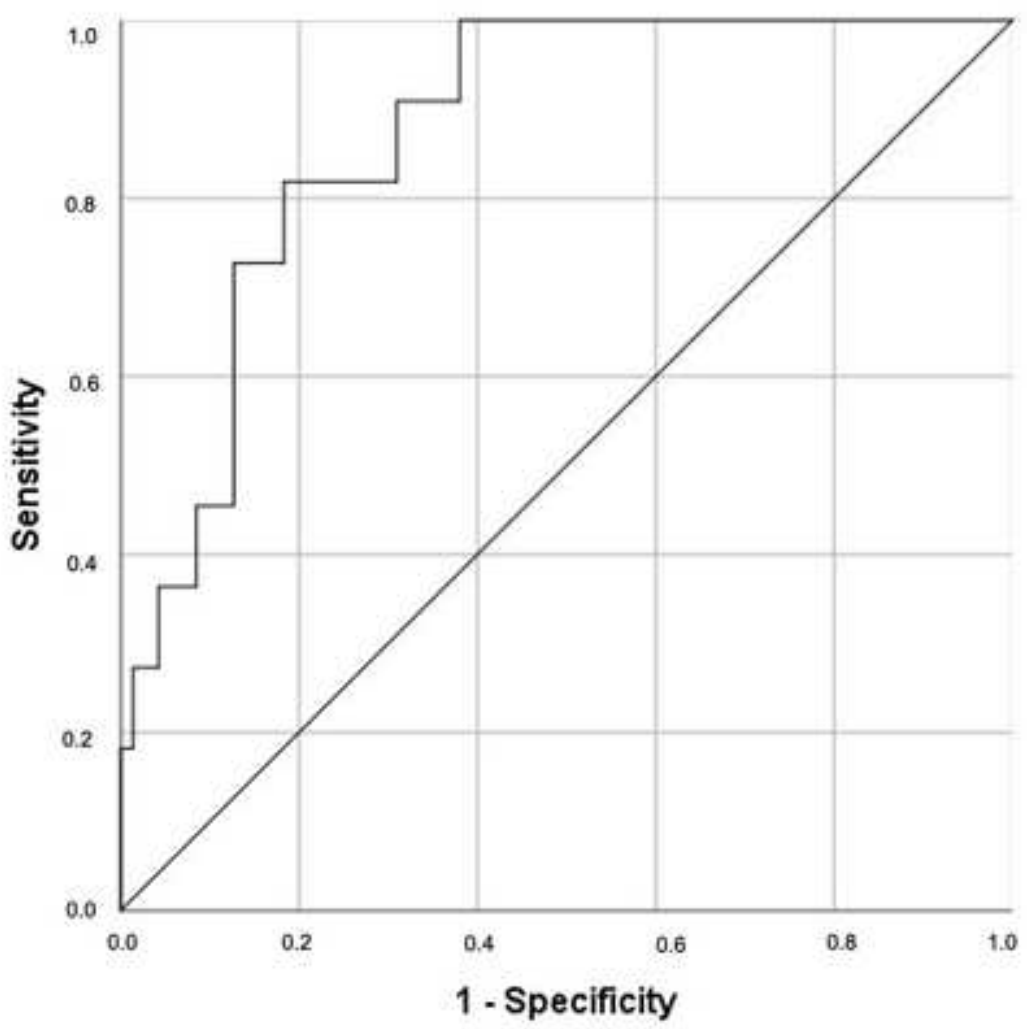

Figure 2

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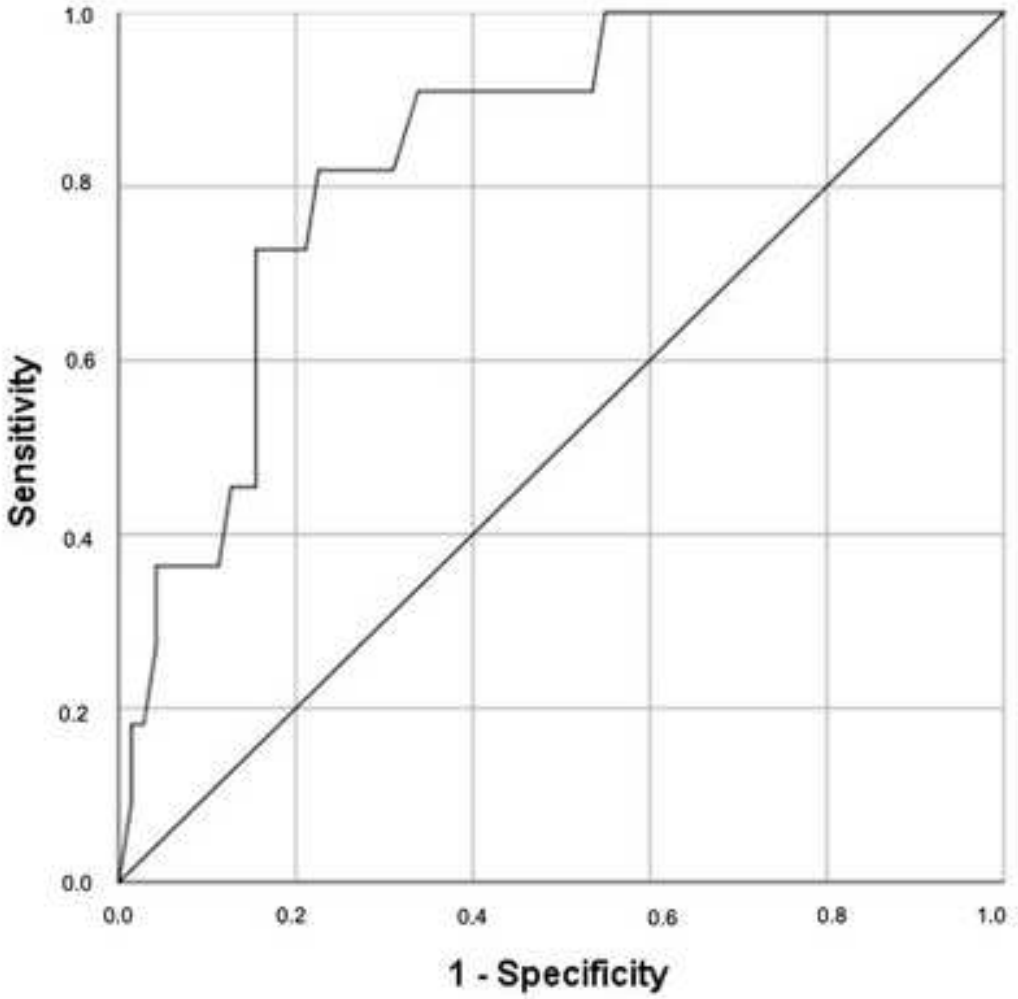

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STROBE Guidelines for authors of CORR

To be used by authors of all observational clinical studies published in CORR. For this purpose a cohort study (the term used by STROBE) is considered a longitudinal study typically reporting outcomes of treatment in one or more cohorts; a case-control study is one identifying factors in outcomes; a cross-sectional study is one to identify the prevalence of factors or characteristics in a population at a single point in time.

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Modifications: We added a fourth column for authors to check inclusion. You must include all items in your manuscript unless the information is not applicable. Information on the study cohort (Items 13 and 14 in the STROBE guidelines) should be provided in Patients and Methods, not in Results; we have omitted the portions of the STROBE guidelines related to Results and Discussion (see our guidelines). The STROBE guidelines were developed for epidemiological studies; "exposed" or "exposure" have been modified with the words "treated" or "treatment."

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	**Recommendation**	**Please insert check where included or N/A where not applicable**
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	INCLUDED
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	INCLUDED
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	INCLUDED
Objectives	3	State specific objectives, including any prespecified hypotheses	INCLUDED
Methods			
Study design	4	Present key elements of study design early in the paper	INCLUDED
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, treatment, follow-up, and data collection	INCLUDED
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	INCLUDED
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of treated and untreated *Case-control study*—For matched studies, give matching criteria and the number of controls per case	

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	INCLUDED
		(b) Give reasons for non-participation at each stage	INCLUDED
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on other treatments and potential confounders	INCLUDED
		(b) Indicate number of participants with missing data for each variable of interest	INCLUDED
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	INCLUDED
Variables	7	Clearly define all outcomes, treatments, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	INCLUDED
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	INCLUDED
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	N/A
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	INCLUDED
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	INCLUDED
		(*b*) Describe any methods used to examine subgroups and interactions	INCLUDED
		(*c*) Explain how missing data were addressed	INCLUDED
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	INCLUDED
		(*e*) Describe any sensitivity analyses	INCLUDED

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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