Author's response to reviews

Title: CD64 as a potential biomarker in septic arthritis

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Author's response to reviews: see over
Dear editorial board of Scandinavian Journal of Infectious Diseases.

Please find enclosed the revised manuscript: “CD64 as a potential biomarker in septic arthritis”, by Oppegaard O and Skodvin B et al., to be submitted as an Original Article to BMC Infectious Diseases. We appreciate the constructive comments from the peer reviewers, and have revised the manuscript accordingly. The comments are addressed below.

Reviewer 1 (Vasileios Fotopoulos):

MAJOR COMPULSORY REVISIONS
1. “In group 4 (acute arthritis) 8 patients were excluded due to uncertain final diagnosis. 4 patients presented with acute arthritis had spontaneous recovery without the administration of antibiotics. These last 4 were considered to be of non-bacterial etiology and were analyzed with group 3. This is probably wrong. If you can somehow rule out septic arthritis in one patient, this does not mean the patient suffers from rheumatic arthritis. There are numerous conditions other than rheumatic arthritis that mimic septic arthritis.”

R: We fully agree that excluding infection does not infer that the patient suffers from rheumatic arthritis, and that relocating the mentioned four cases to group 3 (rheumatic arthritis) is imprecise. However, the purpose of the present study was to investigate the inflammatory markers’ ability to discern infectious from non-infectious conditions. In this context we find it feasible to group these four cases with the other non-infectious cases (rheumatic arthritis and crystal induced arthritis) when calculating the inflammatory markers sensitivity and specificity for infectious disease. We have attempted to clarify this in the results section, fourth paragraph.

2. “In group 4 (acute arthritis), 26 patients had septic arthritis whereof 9 were related to prosthetic joints. There are a lot of researchers that would reject this study because the authors did not separate these two subgroups – native joint infections vs. prosthetic ones. According to literature, prosthetic joint infections behave in an entirely different way leading to more indolent clinical presentations or even normal laboratory findings [see Diagnosis and Management of Prosthetic Joint Infection Curr Opin Infect Dis 2012;25(6):670-676]. If there is a more indolent inflammatory response in prosthetic joint infections, this could jeopardize the credibility of the whole study against the use of CD64 as a biomarker in septic arthritis. However, the authors state that the values for CD64 and PCT did not differ significantly between patients with native vs. prosthetic joints. I would
like to see these data in the script in order to overcome this lack of segregation.”

R: The concern raised about the indolent clinical course of prosthetic joint infections (PJI) is appropriate. However, the clinical picture is quite varied, and is associated with the time since implantation. Traditionally PJI have been divided into early infections (<3 month after implantation), delayed (>3 months but <2 years) and late infections (>2 years). Early infections and late infections are associated with sudden onset of local joint inflammation and fever, especially so in hematogenous PJI. The delayed infections, on the other hand, usually present by subtle signs and symptoms, sometimes even not suggestive for infection. (See Prosthetic-joint-associated infections Best Practice & Research Clinical Rheumatology Vol. 20, No. 6, pp. 1045e1063, 2006; “Diagnosis and Management of Prosthetic Joint Infection: Clinical Practice Guidelines by the Infectious Diseases Society of America» Clinical Infectious Diseases 2013;56(1):e1–25; “Clinical manifestations and diagnosis of prosthetic joint infections” UpToDate.)

The PJIs included in the present study were all (with one exception, see 4) either early infections or haematogenously spread, and presented accordingly with symptoms of acute arthritis. We acknowledge the reviewers request for presentation of data for native vs prosthetic joints, see results-section, last sentence. The data can be presented in a separate figure if the editorial board prefers even if this is not our preference.

3.
“In Septic arthritis group there is one case of Mycobacterium abscessus infection and one case of Lyme disease. We know that mycobacterial infections cause chronic, slow progressive monoarthritis rather than acute septic arthritis (duration of symptoms 30 days > 2 weeks). On the other hand, the diagnosis of Borrelia-arthritis was based on history (recent tick exposure) and a high serum IgG-titer (IgM-titer just above cut-off). These do not necessary prove actual Borrelia arthritis. Borrelia can cause a type of reactive arthritis. Thus, it would be better if the authors excluded these two cases.”

R: Mycobacterial infections in general are, as commented, slow progressive and chronic in nature. Mycobacterium abscessus belongs to the Rapidly Growing Mycobacteria (RGM), defined by visible colony formations on Loewenstein-Jensen culture medium within 7days of subculture. RGM possess characteristics that have more in common with conventional bacteria than with slow-growing mycobacteria. (“Mycobacterium abscessus: an emerging rapid-growing potential pathogen” APMIS 2006;114:319–28.)

The patient concerned presented with an acute arthritis and fever, and was admitted to a local hospital 2 days after the debut of symptoms. We wish to clarify that the duration of symptoms of 30 days mentioned, reflects the time to acquisition of blood-samples for analysis of inflammatory markers and not to admission to hospital. The joint fluid grew bacteria, but the local microbiology-department had great difficulty identifying the microbe. When the etiological agent finally was established, the patient was transferred to our hospital, and inclusion was performed. The patient had been treated empirically for septic arthritis with cloxacillin at the local hospital, but had not received any antibiotics with anti-mycobacterial effect. The clinical picture
had not improved, and still resembled an acute arthritis. We have accordingly chosen to include this patient as an acute arthritis.

As correctly pointed out, the diagnosis of Borrelia arthritis is difficult. We support the recommendation from the reviewer, and have excluded the Borrelia case (uncertain final diagnosis).

4. “Again in table 2, there is one case of prosthetic joint infection by S.epidermidis with duration of symptoms greater than 2 weeks (30 days). This is not an acute arthritis. This case should be excluded too.”

R: We concur, and have accordingly excluded this case.

5. “A short clinical relevance should be discussed (Part of the conclusions should be included).”

R: Elements of clinical relevance has been added to the discussion.

6. “Except for the last sentence, the whole paragraph of the conclusions includes data that should have been part of the discussion. The conclusion should be based on data found in the manuscript itself.”

R: The conclusion section has been rewritten.

7. “Limitations of the study must be mentioned. Except for the subgroup analysis in group 2 (UTI) there is no other limitation of the current study mentioned. In view to the above remarks, the authors should clearly state the limitations of their study. There is no perfect study and certainly this one has a lot of controversial issues for a prospective study. Of course it is always difficult to deal with septic arthritis and its heterogeneity of the cases.”

R: As correctly remarked, the limitations of the study were not discussed in detail. We’ve added some segments to the discussion section to address this subject. See paragraph 6, discussion-section.

MINOR ESSENTIAL REVISIONS

1. “Group 3 (chronic rheumatic arthritis): This group consisted of patients with an acute disease flare. Perhaps it is better to erase chronic from the title of the group in order for the reader to understand what the authors want to pose – differentiation between acute septic and aseptic arthritis.”
R: We have changed the label to “flare of rheumatic arthritis”. It is important to avoid confusion with rheumatoid arthritis (RA), as our cohort comprised a multitude of rheumatic diseases (including rheumatoid arthritis).

2. “The authors should give more details in table 2 regarding CNS (CNS: S.epidermidis, S.capitis, and S.lugdunensis) and the affected joint.”

R: We have included the affected joint in table 2. The distinction between native and prosthetic joint and early vs late PJI is already presented. Likewise is the speciation of the CNS. The new table might be perceived as too expansive, but we leave this to the judgment of the editorial board.

3. “It would be better if the authors started the discussion with the most significant findings of the study.”

R: We agree, and the disposition of the discussion-section has been rearranged accordingly.

4. R: Spelling errors have been corrected.

DISCRETIONAL REVISIONS

1. “Subgroup analysis was conducted to evaluate the influence of the causative agent being CNS on the discriminatory power of the inflammatory markers. Although this kind of subgroup analysis is very interesting, I feel that the authors should have split the Septic arthritis group to Gram-positive vs. Gram-negative infections [This is not applicable due to small size of the Gram-negative infections] or high virulence bacterial infections vs. low virulence ones. This discrimination has a lot more value for the clinician rather than CNS vs. all other bacterial infections (some by low virulence bacteria too).”

R: We agree that the subgroup CNS may appear arbitrary, and that dividing into low-virulence and high virulence bacteria would be more meaningful for the clinicians. However, the distinction between low- and high-virulence is not clear-cut, and aside from CNS few of the microbes are unequivocally low-pathogenic. E.faecalis, Bordetella holmesii and Mycobacterium abscessus are possible candidates, but their level of pathogenicity in septic arthritis is not clearly defined. To avoid this challenge we opted to separate only the CNS, as this is a well-defined and homogenous group.
"One proposition to improve the overall impact of the study would be to investigate the effect of previous administration of antibiotics. I understand the size is rather small (only 6 out of 26), but it may reveal a tendency."

R: The impact of previous administration of antibiotic of the CD64-value would be interesting to document. The small size (5 out of 24), though, makes it difficult both to reveal a tendency, and almost impossible to demonstrate significance. Accordingly we found no statistical significant difference between the antibiotic-naive and previously antibiotic-exposed populations (p=0.90), nor any distinct tendency.

3. “The title though is a little too sharp. Since serum CD64 did not prove its sensitivity in septic arthritis I would change the title to: CD64 as a possible biomarker in septic arthritis."

R: The title has been changed to: “CD64 as a potential biomarker in septic arthritis”.

Reviewer 2 (Saul Faust):

2. “Are the methods appropriate and well described? Methods were appropriate with healthy and chronic disease controls. However, the cases included/excluded in group 4 are not clearly defined in terms of timescale or how this would affect the potential use as a clinical diagnostic marker. For example, 8 (of 69) were excluded from the analysis due to “uncertain final diagnosis” and 4 (of 69) subsequently recovered without antimicrobials so were analysed in group 3. From a clinical use perspective, this means the data appear stronger for CD64 than they would otherwise do – to be useful the data might have been better chosen as the “69 presenting with acute arthritis”. Only 26 had culture proven infection of whom only 12 had positive blood cultures (not an unusual situation in bone and joint infections where definitive microbiological diagnosis is commonly unproven in clinical practice without novel molecular diagnostics being used). 7 of the “included infections” lacked CD64 analysis.”

R: We agree that the exclusion of 8 (9) cases due to uncertain final diagnosis could potentially influence the calculated diagnostic accuracy of the inflammatory markers. This is the inevitable consequence of using a culture-based gold standard, and the etiology will remain elusive in some cases of acute arthritis. We’ve added a passage to the discussion-section to address this specific challenge. See discussion-section, paragraph 6. Concerning the four cases (previously) analyzed in group 3, see answer to reviewer 1, major compulsory revisions, answer 1.
4. “Does the manuscript adhere to the relevant standards for reporting and data deposition? It is not stated where the data are to be deposited.”

R: The data are deposited on the research-server at Haukeland University Hospital.

5. “…..However, the conclusions are perhaps too much in favour of CD64 as a potential clinically useful biomarker. From a clinical perspective, the new flow cytometry based test (CD64) under investigation has not been demonstrated to be of any great benefit compared to routinely available and cheap biomarkers (eg CRP), or potentially useful others (eg procalcitonin) especially due to the major limitation described: lack of sensitivity in localised infection.”

R: We concur, and have revised the manuscript accordingly. See conclusion-section.

6. “Are limitations of the work clearly stated?
The discussion describes the range of pathogens (gram positive, low virulence, negative blood culture) as potential difference to previous studies but in fact this is likely to be the case in pragmatic “real life.”

R: We agree.

We hope the editorial board will agree on the interest of this study.

Sincerely yours,

Oddvar Oppegaard and Brita Skodvin on behalf of the authors.

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Main article: 2416 words.
Abstract: 224 words