Author’s response to reviews

Title: Retention of metals in periprosthetic tissues of patients with metal-on-metal total hip arthroplasty is reflected in the synovial fluid to blood cobalt transfer ratio in the presence of a pseudotumour

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Author’s response to reviews:

Dear Editor,

Thank you for the review and the response on June 18th indicating that our manuscript “Retention of metals in pseudotumour tissue can reduce the diagnostic accuracy of blood metal scanning in metal-on-metal patients” could be considered for publication in BMC Musculoskeletal Disorders, upon revisions. We thank the editor and the reviewers for the insightful comments. We have gone through the comments and modified the manuscript accordingly. Responses to the reviewers’ queries and a detailed description of the modifications can be found below. The improved manuscript and images have now been uploaded for review. We will be happy to make any further changes if some aspect has not been answered thoroughly enough or there are points that remain unclear.

Thank you for your time and consideration; I look forward to hearing from you.

Yours sincerely,

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“Editor Comments:

The quality of the English used throughout your manuscript does not currently meet our requirements, as there are several incorrect sentence constructions and grammatical errors throughout obscuring the message the authors want to convey. We recommend that you ask a native English speaking colleague to help you copy-edit the paper. If this is not possible, you may need to use a professional language editing service. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication.

Reply: This manuscript has been checked by a professional language editing service.

It is important that your manuscript gives a clear and complete account of your study, and BMC supports reporting initiatives that contribute to this. Please adhere to the appropriate STROBE guideline for your methodology, and include a completed checklist with your revision as a supplementary file. You can see more information here: https://www.biomedcentral.com/getpublished/writing-resources/reporting-guidelines

Reply: A checklist of STROBE guidelines has been included as a supplementary file.

We operate a transparent peer review process for this journal where reviewer reports are published with the article but the reviewers are not named (unless they opt in to include their name).

Reviewer reports:

Reviewer 1: Interesting message but very chaotically presented. My impression is that the author, or authors, that wrote the paper are not orthopaedic surgeons or if they are they must be very junior. I suggest that you get it re-written by an experienced person. Preferably, someone that lived through the hard years when we were first faced with the issue of ARMD and its devastating consequences that lead to the follow up protocols that with minimal variations have been in place for most of the last decade.

Reply: Thank you for this valuable comment. The clinical aspects of MOM implants have been studied widely and the follow-up protocols and clinical practices have evolved through years of research and clinical experience. The wider objective of our research is to shed light on the biological aspects behind the adverse reactions, and this study is the first part in our series of biological and histological studies introducing our study population and its clinical features. We do acknowledge that therefore the cell biological background may affect the style of the manuscript even though three of the authors are experienced practicing orthopaedic surgeons.
The details of which aspects of the manuscript would need improvement were not defined. We addressed this by taking into account the remarks of the other two reviewers and added a section of dichotomous pseudotumour analysis. We have now described in the manuscript text that the pseudotumour subtype analyses should be interpreted as experimental and hypothesis generating. This has now been described in the methods, results and discussion. We have tried to clarify our conclusions and reword the title to highlight the main biological finding. We do apologize if we have not addressed all parts in need of revision. For this we would need a more detailed description of the insufficiencies.

Following changes has been made to the manuscript text:

Title: Retention of metals in periprosthetic tissues of patients with metal-on-metal total hip arthroplasty is reflected in the synovial fluid to blood cobalt transfer ratio in the presence of a pseudotumour

Background: The following sentence was added: “The clinical aspects of MOM implants have been studied widely and the follow-up protocols and clinical practices have evolved through years of research and clinical experience.”

Methods: “The distribution of metals was analysed in relation to the presence or absence of pseudotumours. The SF and ST Cr and Co concentrations were proportioned to blood Cr and Co concentrations by dividing the WB concentration by the SF or ST concentration and converting these to percentages.”

Results: “The concentration of metals (Cr and Co) in different tissues (WB, SF, ST) did not differ statistically significantly between patients with or without a pseudotumour (Supplementary file S5). The percentage of Co transferred from SF to WB was 1.7-fold higher in patients with than without a pseudotumour (p= 0.039) (Table 4.). No differences in the percentage of Co transferred from ST to WB or Cr transferred from SF or ST to WB were found (Table 4).”

Discussion: “In the presence of a pseudotumour the percentage of cobalt transferred from synovial fluid to whole blood was higher than in patients without a tumour. Since there were no differences between the groups in the SF or WB Co concentrations, there must be another source for the observed higher Co transfer. The pseudotumour retains some of the metals and adds to their transition to the circulation. It is known that cobalt is more readily transported to the blood and excreted in the urine, but chromium is accumulated in tissues [29, 33]. This is shown in the percentual transfer ratio of Co and explains why Co concentrations were higher than Cr in blood and the reverse was true for synovial fluid and soft tissue. This finding is important in the clinical perspective, since blood sampling is the standard method for ion level monitoring. In liquid samples (WB, SF) the measured concentration accurately reflects the absolute amount of metal in the tissue. In soft tissue biopsies, however, the sample may reflect only focal changes. We found interesting observational differences between the subgroups of pseudotumours. Soft tissue metal concentrations were higher in patients with type 1, type 2B or type 3 tumours and blood concentrations were highest in patients without a tumour (Figure 6). Although there were differences in metal concentrations between the Hart classes, the differences were not statistically significant. Therefore, these pseudotumour subtype analyses should be interpreted as experimental and hypothesis generating. There are previous studies examining the correlation between whole blood, synovial fluid or synovial tissue metal ion levels and histological findings or cellular parameters. Reito et al. [34] studied the possible diagnostic utility of joint fluid metal ion measurement for histological findings, but neither cobalt nor chromium were found to have good
predictive value. Lehtovirta et al. [35] found that whole blood, joint fluid and synovial tissue metal concentrations correlated poorly with histological findings. Yet, to our knowledge there are no previous studies examining the role of pseudotumours in the distribution of metals. In our opinion, this observation deserves to be investigated also in a larger cohort of patients. Previous studies, including our current study, examined only focal biopsies of the synovial/pseudotumour tissue, which may not reflect the total synovial metal load. The analysis of the whole pseudotumour, showing the total metal load, could give more accurate information and possibly show a stronger correlation with the blood concentrations.”

Conclusions: “This study, in agreement with earlier findings, shows the heterogeneous results of individual screening parameters, which should never be evaluated in isolation. High tissue metal load can be seen in patients with poorly implanted components. However, adverse tissue reactions are found even in patients with optimal implant orientation. While interpreting the results of our study, it should be noted that the study population was selected based on the need for revision surgery. Periprosthetic soft tissue shows retention of metals, increasing the patients’ total metal load. This is reflected at least to the WB/SF Co percentual transfer ratio. Due to our small sample size it is hard to formulate any solid and generalized recommendations, but multidisciplinary screening of these patients is mandatory. Future studies with thorough analysis of periprosthetic soft tissues may be able to reveal new markers to better understand these destructive processes.”

Reviewer 2: My biggest concern is that conclusion are being drawn from results that were not statistically significant (Lines 347-350): lots of emphasis of paper on findings that could be due to chance alone. Also appears in the abstract. Related to lines 234-251 & Table 4 nothing statistically different. Also relevant for discussion lines 292-322. Is the paper appropriately powered for this pseudotumour analysis?

Reply: Thank you for this valuable comment. The distribution of metals has now been analysed and discussed in relation to the presence or absence of pseudotumours. The SF and ST Cr and Co concentrations were proportioned to blood Cr and Co concentrations and the proportions were compared between patients with or without a pseudotumour. This has now been described in the methods, results and discussion.

Methods: (on lines 195-198) “The distribution of metals was analysed in relation to the presence or absence of pseudotumours. The SF and ST Cr and Co concentrations were proportioned to blood Cr and Co concentrations by dividing the WB concentration by the SF or ST concentration and converting this to percentages.”

Results: (on lines 259-263) “The concentration of metals (Cr and Co) in different tissues (WB, SF, ST) did not differ statistically significantly between patients with or without a pseudotumour (Supplementary file S5). The percentage of Co transferred from SF to WB was 1.7-fold higher in patients with than without a pseudotumour (p= 0.039) (Table 4.). No differences in the percentage of Co transferred from ST to WB or Cr transferred from SF or ST to WB were found (Table 4). “

Discussion: (on lines 327-355) “In the presence of a pseudotumour the percentage of cobalt transferred from synovial fluid to whole blood was higher than in patients without a tumour. Since there were no differences between the groups in the SF or WB Co concentrations, there must be
another source for the observed higher Co transfer. The pseudotumour retains some of the metals and adds to their transition to the circulation. It is known that cobalt is more readily transported to the blood and excreted in the urine, but chromium is accumulated in tissues [29, 33]. This shows in the percentual transfer ratio of Co and explains why Co concentrations were higher than Cr in blood and the reverse was true for synovial fluid and soft tissue. This finding is important in the clinical perspective, since blood sampling is the standard method for ion level monitoring. In liquid samples (WB, SF) the measured concentration accurately reflects the absolute amount of metal in the tissue. In soft tissue biopsies, however, the sample may reflect only focal changes. We found interesting observational differences between the subgroups of pseudotumours. Soft tissue metal concentrations were higher in patients with type 1, type 2B or type 3 tumours and blood concentrations were highest in patients without a tumour (Figure 6). Although there were differences in metal concentrations between the Hart classes, the differences were not statistically significant. Therefore, these pseudotumour subtype analyses should be interpreted as experimental and hypothesis generating. There are previous studies examining the correlation between whole blood, synovial fluid or synovial tissue metal ion levels and histological findings or cellular parameters. Reito et al. [34] studied the possible diagnostic utility of joint fluid metal ion measurement for histological findings, but neither cobalt nor chromium were found to have good predictive value. Lehtovirta et al. [35] found that whole blood, joint fluid and synovial tissue metal concentrations correlated poorly with histological findings. Yet, to our knowledge there are no previous studies examining the role of pseudotumours in the distribution of metals. In our opinion, this observation deserves to be investigated also in a larger cohort of patients. Previous studies, including our current study, examined only focal biopsies of the synovial/pseudotumour tissue, which may not reflect the total synovial metal load. The analysis of the whole pseudotumour, showing the total metal load, could give more accurate information and possibly show a stronger correlation with the blood concentrations.”

Another concern is that the authors mention controls which have not been described in the methods (first use of the word control(s) is in the results line 193).

Reply: The controls have now been defined in the methods section on line 182: “Synovial soft tissue samples from three patients undergoing primary hip replacement surgery were used as controls reflecting metal concentrations of synovial soft tissue that has not been exposed to a metal-implant.” and on line 192: “Synovial fluid samples from those three patients undergoing primary hip replacement surgery were used as controls reflecting metal concentrations of synovial soft tissue that has not been exposed to a metal-implant.”

Other more minor comments:

Abstract:
- "ceased their use" implies not used at all anymore. Metal resurfacing is still used for appropriate patients and hence this should be edited accordingly.

Reply: This has been rephrased in the abstract to: “dramatically reduced their use”.

Intro:
- Reference 6 referred to as recent, but is from 2005, which pre-dates much of the work on metal ions.

Reply: This has been rephrased to: “There are studies which explain these reactions by local toxic and hypersensitivity reactions to wear particles [1-6].”

- The quality of written English is generally acceptable as is. However the aim is not clear and should be re-worded (usual to have the effect of XXXX on YYYYY, but there is no "on" clause and hence is unclear).

Reply: The aim of the study has been rephrased as suggested. “The aim of this study is to describe the effect of acetabular component angles and pseudotumour formation on the blood metal ion levels, one of the clinical indicators of implant wear.”

Methods:
- It appears 3 patients were analysed differently (line 118: no MARS MRI, line 136: not clear if they were not tested for infection, or if they had infection, the wording could be interpreted either way). Also not clear if this is the same three patients. Is it appropriate to include these patients?

Reply: The analyses have now been clarified in the text on line 119 as follows: “MARS MRI was performed on 57 (95%) patients. There were three patients for whom MRI was not done, one patient had a cardiac pacemaker, one patient had a dorsal root nerve stimulator and one patient had severe symptoms requiring revision. Clinical findings during revision were abnormal in all those three cases.”

and on line 141 “In two cases we found one positive enrichment culture after ten days, one patient with Propionibacterium acnes in 1/3 cultures and another patient with Staphylococcus warneri in 1/4 cultures. Neither of these patients had infection before, during or after the MOM revision. These cultures were deemed to have been contaminations. One patient without previous signs of infection gave two positive enrichment cultures (10 days) out of five with Staphylococcus capitis. He developed a clinical infection after a few weeks latent time. The infection was treated with revision surgery and antibiotics.”

As described above the reason for not doing MRI was based on clinical indications and did not affect the analyses of metals in the tissues nor the analyses of the orientation of the implants. The positive bacterial cultures were post-operative findings that did not reveal any preoperative bacterial infections and thus the inclusion of these cases is justified. The three cases without MRI and three cases with positive bacterial cultures were different.

Statistical analysis:
- Was a power analysis done? The study appears to be under-powered for the analysis of pseudotumour types.

Reply: To our knowledge this is the first study addressing the effect of pseudotumour on the distribution of metals. This is a hypothesis generating experimental study, so data for power analyses were not available. The limited number of cases has now been taken into account by
analysing the effect of pseudotumour as a dichotomous variable by the presence or absence of a pseudotumour.

- Please clearly define the independent and dependent variables for the statistical analyses. Currently not clear. (lines 182-185)

Reply: The description of variables has now been clarified on lines 202-210 as follows: “A non-parametric Kruskal-Wallis test for independent samples was used for the evaluation of differences in metal concentrations between groups defined by implant cup angles (anteversion optimal/inclination optimal, AO/IO; anteversion optimal/inclination suboptimal, AO/IS; anteversion suboptimal/inclination optimal, AS/IO; anteversion suboptimal/inclination suboptimal, AS/IS) and pseudotumour type (no tumour, Hart 1, Hart 2A, Hart 2B, Hart 3). The Mann-Whitney test was used for evaluation of differences between metal concentrations (WB, SF and ST Co and Cr) and between percentual transfer ratios from SF or ST to blood in patients with or without a pseudotumour.”

Results:

- line 211: B-Co not defined - blood cobalt? Same with B-Cr. AO/AS/IS/IO also not defined in body of text, same for SF, ST, WB etc. Please define in main text, not just figure legends. Please check all acronyms defined at first use in body of text.

Reply: All acronyms have now been defined in the main text when first mentioned and described in the list of abbreviations.

- Tables 3 and 4. Please report the n per group in the table for clarity.

Reply: The number of cases in each group has now been defined in the tables as requested.

Discussion
- Line 265: is it possible to correlate the Ti data with the clinical notes on taper wear to confirm this hypothesis

Reply: The Ti data has been assessed with the clinical findings and the manuscript text has been modified accordingly on lines 296-299: “Some samples (synovial tissue n 13 and synovial fluid n 18) contained also elevated levels of titanium, which plausibly originates from the femoral taper. This hypothesis is supported by the fact that 18/33 cases of our study population with tarnished, discolored or worn femoral taper showed also elevated titanium levels (ST and/or SF).”

Reviewer 3: PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?
Yes - there is a clear objective
DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?  
Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?  
No - there are minor issues

STATISTICS - Is the use of statistics in the manuscript appropriate?  
Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?  
Yes - the author’s interpretation is reasonable

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound?  
If not, can revisions be made to make the work technically sound?  
Yes - current version is technically sound

PEER REVIEWER COMMENTS:

GENERAL COMMENTS:
This paper examined the relation between implant positioning (from radiographs), presence of pseudotumors (from MRI) and metal concentrations in tissues and blood of 60 patients with THA replacements.

This is a clinically important question since many patients operated decade(s) earlier necessitate an evidence based prognosis for their implants now.

The authors have done a great job I think. The paper reads very nicely. It is concise but detailed. Methods and data are perfectly described. The text is overall excellent and it was a real pleasure to read this work.

I was surprising to read that even with perfectly positioned implants the metal concentrations can be high and interested in that the concentrations depend on the pseudo tumor type.

REQUESTED REVISIONS:
Surprisingly the data relative to the second aim of the paper, ie "distribution of metals in relation to presence or absence of pseudotumors" is missing and is not discussed either. The authors have skipped this aim and went directly to analyze the effect pseudotumor type on metal concentrations. The analysis of metals vs dichotomised pseudotumor data (present absent) should be added and shortly discussed before the subanalysis with pseudotumor type.

Reply: Thank you for this valuable comment. The distribution of metals has now been analysed and discussed in relation to presence or absence of pseudotumours. The SF and ST Cr and Co concentrations were proportioned to blood Cr and Co concentrations and the proportions were
compared between patients with or without a pseudotumour. This has now been described in the methods, results and discussion.

Methods: “The distribution of metals was analysed in relation to the presence or absence of pseudotumours. The SF and ST Cr and Co concentrations were proportioned to blood Cr and Co concentrations by dividing the WB concentration by the SF or ST concentration and converting this to percentages.”

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Discussion: “In the presence of a pseudotumour the percentage of cobalt transferred from synovial fluid to whole blood was higher than in patients without a tumour. Since there were no differences between the groups in the SF or WB Co concentrations, there must be another source for the observed higher Co transfer. The pseudotumour retains some of the metals and adds to their transition to the circulation. It is known that cobalt is more readily transported to the blood and excreted in the urine, but chromium is accumulated in tissues [29, 33]. This shows in the percentual transfer ratio of Co and explains why Co concentrations were higher than Cr in blood and the reverse was true for synovial fluid and soft tissue. This finding is important in the clinical perspective, since blood sampling is the standard method for ion level monitoring. In liquid samples (WB, SF) the measured concentration accurately reflects the absolute amount of metal in the tissue. In soft tissue biopsies, however, the sample may reflect only focal changes. We found interesting observational differences between the subgroups of pseudotumours. Soft tissue metal concentrations were higher in patients with type 1, type 2B or type 3 tumours and blood concentrations were highest in patients without a tumour (Figure 6). Although there were differences in metal concentrations between the Hart classes, the differences were not statistically significant. Therefore, these pseudotumour subtype analyses should be interpreted as experimental and hypothesis generating. There are previous studies examining the correlation between whole blood, synovial fluid or synovial tissue metal ion levels and histological findings or cellular parameters. Reito et al. [34] studied the possible diagnostic utility of joint fluid metal ion measurement for histological findings, but neither cobalt nor chromium were found to have good predictive value. Lehtovirta et al. [35] found that whole blood, joint fluid and synovial tissue metal concentrations correlated poorly with histological findings. Yet, to our knowledge there are no previous studies examining the role of pseudotumours in the distribution of metals. In our opinion, this observation deserves to be investigated also in a larger cohort of patients. Previous studies, including our current study, examined only focal biopsies of the synovial/pseudotumour tissue, which may not reflect the total synovial metal load. The analysis of the whole pseudotumour, showing the total metal load, could give more accurate information and possibly show a stronger correlation with the blood concentrations.”

In the discussion lines 282-291, the message that even well placed cup can have elevated metals, maybe the authors could add briefly the concept that wear results from a badly placed cup relative to the femoral component.
Maybe in these patients with well placed cups but high metals, the cups may be well placed but the femoral component could have sub-optimal version, offset, or angle? Did the authors categorize the quality of femoral component placement? Can they discuss shortly this aspect of placement relative to the femoral component? It is friction after all. It requires two parts.

Reply: Thank you for this valuable comment. This aspect would have given an interesting perspective. Unfortunately, the femoral component placement was not evaluated in our study. The following sentence was added to the discussion on line 320: “In current study we did not measure the orientation of the femoral components, which affect the total component orientation and implant wear. “

In the conclusion line 346-347, "even optimal implant orientation does not prevent adverse tissue reactions" is a little alarming. This is a sub-group of patients that were selected because of the need for revision. The number of patients with well placed cups in this cohort should be put in perspective to the total number of patients with well placed cups who did not need revision.

Reply: The conclusion was partly rephrased on lines 386-391 as follows: “This study, in agreement with earlier findings, shows the heterogeneous results of individual screening parameters, which should never be evaluated in isolation. High tissue metal load can be seen in patients with poorly implanted components. However, adverse tissue reactions are found even in patients with optimal implant orientation. While interpreting the results of our study, it should be noted that the study population was selected based on the need for revision surgery.”

ADDITIONAL REQUESTS/SUGGESTIONS:
See above. Otherwise excellent.