Author's response to reviews

Title: A dose response analysis of a specific bone marrow concentrate treatment protocol for knee osteoarthritis

Authors:

Christopher J Centeno (centenooffice@centenoschultz.com)
Hasan Al-Sayegh (hasan@regenexx.com)
Jamil Bashir (jbashir@centenoschultz.onmicrosoft.com)
Shaun Goodyear (shaun.goodyear@msn.com)
Michael D Freeman (forensictrauma@gmail.com)

Version: 3 Date: 6 July 2015

Author's response to reviews: see over
Date: July 6, 2015

To:
Editorial Team
BMC Musculoskeletal Disorders

Dear Editor,

Please find attached our revised manuscript of the study titled “A dose response analysis of a specific bone marrow concentrate treatment protocol for knee osteoarthritis”. In this study, we suggest a cut-off point for nucleated cell dose within bone marrow concentrate injections used for treatment of knee osteoarthritis. We also provide a comparative analysis between low and high cell dose groups. We thank the reviewers for their comments, and have revised our manuscript to address their questions and concerns. Below, we copied and pasted all reviewers’ notes and provided our responses and corresponding changes.

Thank you very much for your time and consideration,
Jamil Bashir, MD

Responses to Reviewer number: 1

Reviewer’s Comment:
The authors contribute with their information on patients treated for knee OA with BMC cells in combination with PRP/PL. My main concern for this manuscript is the fact that BMC cells are not the same as MSC’s. The BMC does indeed contain MSCs as stated by the authors. But can the authors further explain why they think that the MSCs in the injections are responsible for the observed effect. By providing this information, this work will add important information to the field. Ideally the BMC injection should be compared to culture expanded MSC injections.

Response:
We added these paragraphs to the introduction and discussion sections to address this concern:

In the Introduction, we added the following: “In humans, nucleated cells are isolated from the aspirate of bone marrow that is typically harvested from the superior iliac crest of the pelvis via needle[9]. The composition of these nucleated cells is diverse including Mesenchymal Stem Cells (MSCs), Hematopoetic Stem Cells (HSCs), Monocyte Precursor cells, Macrophages, T cells, B cells, Dendritic Antigen Presenting Cells, Natural Killer Cells and Neutrophils[10-12]. The action of these cells, acting both in isolation and symbiotically, once introduced into arthritic joints may help improve pain and function by replenishing damaged joint structures and providing a mediation of catabolic immune response, thus alleviating the symptoms and progression of the disease[12, 13].”

In the Discussion, we added the following: “Conceptually, it makes sense that a higher BMC nucleated cell count equates to a better outcome than a lower cell count. Nucleated cell count is a proxy for the total number of MSCs, and MSCs are the cells that contribute to regeneration of intra-articular cartilage [26, 40]. Unlike studies that have focused on culture expanded MSCs, the number of these cells in BMC is much smaller [24]. Hence, dose may be more critical in some patients with poor MSC counts in bone marrow, which is common in older patients [21]. Additionally, BMC constituents including hematopoietic stem cells, T-lymphocytes, B-lymphocytes, monocytes, macrophages, epithelial progenitor cells and platelets, are all capable of producing growth factors and cytokines that together may support a microenvironment that promotes proliferation and functional differentiation of MSCs [41]. For example,
co-culture of MSCs with Monocytes has been shown to increase chondrogenic differentiation capacity [12]. Further studies examining the effect of varying cellular composition of applied fractions of marrow will allow for greater understanding of the complex interplay that ensues as the body undergoes tissue repair.

Reviewer’s Comment:
Furthermore, the treatment is combined BMC and PRP/PL injection. There is no control treatment involved. I think this treatment should be judged as a combined treatment. How do the authors know that the BMC cells are responsible for the observed effect? Was the injected volume always the same? Was the total amount of PRP/PL the same for both treatment groups?

Response:
To address this question, we added the following to the discussion section: “It is important to note that the observed treatment’s effect may be attributed to the platelet component of injections; PRP and PL have been shown in numerous studies to improve the symptoms associated with mild-moderate knee OA [42-44]. However, the clinical efficacy of PRP therapy is transient, and relief from pain and function improvement declines to baseline between 6-to-24 months after treatment [45, 46]. Further, the efficacy of PRP therapy is limited in moderate and severe OA, versus mild OA[7, 47, 48]. The significant difference in pain score associated with the higher cell group in this study reveals that varying MSC dose, within a standardized protocol, has an impact on treatment outcome.”

Reviewer’s Comment:
Was there a difference in side-effects or adverse effects?

Response:
We did not examine side effects in the current study.

Responses to Reviewer number: 2

Reviewer’s Comment:
Centeno at all evaluate a large cohort of patients treated with bone marrow concentrate with the question whether dose matters. Unfortunately, the number of patients per group for evaluation was rather low, since different clinical scales are available. Although the study could give some interesting initial indications, the conclusion is now overstated. The major problem I have with the study concerns the set up. The authors very elegantly use a method to determine the best dose to discriminate effects on pain (fig. 1). However, they then use the same patients and the same data to show that there is an effect on pain, indeed. This I think is a methodological flaw. The conclusion of the study should be that based on this method (in figure 1) this cell dose of 400 million cells can be the best to use but it needs further investigation. The second part of the study can maybe be used to further support this suggestion. This will need rewriting of the entire article including the title.

Response:
Conclusion was changed to the following: “Improved function and reduced pain was observed in patients treated with BMC injectate regardless of cellular dose; however, patients receiving a higher concentration of cells reported a better pain outcome in comparison with the lower dose group. These findings indicate that cell dose may be a factor governing clinical outcomes in autologous BMC treatment of knee OA. Further studies using randomized and placebo-controlled design are needed.”

Reviewer’s Comment:
Other remarks: Abstract: The statement on improved function and reduction of pain by BMC injection regardless of cellular dose can be misleading. I understand that there is an improvement but it is well known that patients improve when having surgery or an injection.

Response:
In this statement, we are trying to clarify that we have observed statistically significant changes in clinical scales in both groups, comparing pre- and post-treatment means.

Reviewer’s Comment:
Finally the last sentence states that further studies using a larger patient population may help elucidate these findings. I would like to see the need for prospective dose response RCT suggested here, rather than larger cohort study.

Response:
We added the following to the discussion: “Sufficiently powered randomized placebo-controlled trials are needed to validate and expand on these preliminary results.”

Reviewer’s Comment:
The conclusion of the study should be a “suggestion for a dose” rather than a hard conclusion.

Response:
We added the following to the abstract: “These preliminary findings suggest that cell dose may be an important factor governing clinical outcomes in autologous BMC treatment of knee OA. Further studies using a larger patient population may help elucidate these findings.”

Reviewer’s Comment:
Materials and methods: Please mention the total volume of bone marrow obtained per patient.

Response:
We edited the methods section to address this question: “On the day of the procedure, approximately 10–15 cc of whole bone marrow aspirate was harvested from 6 to 8 bone sites (approximately 3-4 on each side) of the patients’ Posterior Superior Iliac Crest.”

Reviewer’s Comment:
I understood the injection is performed in combination with surgery. The type of surgery can be a confounder in the study. Information about types of surgery in the different groups should be provided.

Response:
There were no surgeries involved in the current study.

Reviewer’s Comment:
Results: I would prefer to see results presented per patient rather than per procedure.

Response:
Data were gathered per procedures for this study.

Reviewer Comment:
Discussion: In general the discussion is well written and mentions the limitations of the study. In the third paragraph the authors state that “nuclear cell count is a proxy for the total number of MSCs”. This requires a reference.
Response:
Citations and references were added accordingly.

Reviewer Comment:
There is one thing that I missed in the discussion: In the introduction a paragraph is dedicated to the use of PRP. However, in the discussion of the article PRP is not mentioned at all. Discussion whether BMC is better or different from PRP would be helpful for the reader. Also some indication about whether or not PRP like product is present in the BMC would help.

Response:
We edited the discussion section to the following: “It is important to note that the observed treatment’s effect may be attributed to the platelet component of injections; PRP and PL have been shown in numerous studies to improve the symptoms associated with mild-moderate knee OA [42-44]. However, the clinical efficacy of PRP therapy is transient, and relief from pain and function improvement declines to baseline between 6-to-24 months after treatment [45, 46]. Further, the efficacy of PRP therapy is limited in moderate and severe OA, versus mild OA [7, 47, 48]. The significant difference in pain score associated with the higher cell group in this study reveals that varying MSC dose, within a standardized protocol, has an impact on treatment outcome.”

Reviewer’s Comment:
Figure 2: The x-axis needed an indication of x10E8, and an indication that this is number of nucleated cells injected per joint. Clear indication of a line or different colours between low and high cell number group would be helpful as well.

Response:
Graph was edited accordingly.