Author's response to reviews

Title: Evaluating regional blood spinal cord barrier dysfunction following spinal cord injury using longitudinal dynamic contrast-enhanced MRI: application in mouse

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Author's response to reviews: see over
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Anastasios Koutsos, PhD.
Senior Assistant Editor
BMC-series journals

Dear Dr. Koutsos,

We are submitting a revised version of the manuscript (MS) “Evaluating regional blood spinal cord barrier dysfunction following spinal cord injury using longitudinal dynamic contrast-enhanced MRI”. We found that the reviews were very helpful. We were also pleased that the reviewers chosen for evaluating the MS were expert in the topic covered by the MS. We responded the critiques one by one and made the required changes, as listed below. Spinal cord injury damages both neuronal and vascular structures, and leads to loss of blood spinal cord barrier integrity. In our previous efforts, we developed a pharmacokinetic model to quantify barrier permeability using dynamic contrast enhanced magnetic resonance imaging modality. In the current work, we expanded our efforts further. We 1) used spinal cord injury mouse model which is much more challenging in terms of longitudinal magnetic resonance imaging, 2) provided a computational algorithm to produce maps representing spatial variations in barrier permeability in injured cords, 3) also performed diffusion tensor imaging, and 4) demonstrated that vascular dysfunction and neuronal damage are closely related in injured cord. We also would like to note that the approach presented in the MS is directly applicable to clinics provided contrast enhanced data is gathered from human patients.

We believe the manuscript conforms to the journal style and the files are correctly formatted. We thank you for your considerations of this MS for publication in BMC Medical Imaging journal.

Sincerely Yours,

Mehmet Bilgen
**Reviewer:** Dr. Martin Burian

We thank Dr. Burian, an expert in experimental SCI research, for critically reading the manuscript (MS) and providing his suggestions to improve it. We are pleased to receive his views and feedback. Please find our responses to the reviews below.

**Major Compulsory Revisions**

*I have none.*

**Minor Essential Revisions**

1) The specification of the contrast agent used in the study (Materials and Methods) is somewhat misleading. Is the brand name really Gadolinium? This is the name of an element whose ion is present within the CA compound and which is responsible for the effect of CA to relaxation rates of the tissue and hence to the signal intensity. However, isn't the brand name of the contrast agent Magnevist?

   The brand of the contrast agent is Magnevist. We agreed and removed Gd from the text.

2) There are few small typos. A typical example is e.g. on p. 8, line 16 of the text: “Following an IV bolus injection, Gd is first leaks from plasma to the injured SC tissue ...“. However, the meaning of the text is clear. Please, read the whole text carefully.

   Thank you. This typo and others were corrected in the revised MS.

3) The Figure captions, starting on Figure 5 are shifted (at least in my pdf version). Caption for Figure 5 actually references Figs. 5 & 6, caption for Fig. 6 references Fig 7 and vice versa. Also the number of images is quite big. You may consider rearrangement of the images (grouping etc.).

   Thank you. We corrected these mismatches in the revised MS. As suggested, we removed one figure to reduce the number of figures.

**Discretionary Revisions**

1) Discussion spans over more than 2 pages. However, roughly half of it is actually a sound literature review with a little or no discussion. Shouldn't this be rather in Introduction?

   We agreed and moved/shortened the mentioned section.
Reviewer: Dr. David Hackney

We thank Dr. Hackney for critically reviewing the manuscript (MS) and providing his constructive suggestions to improve it. We are pleased to see that this MS has been evaluated by a world renowned expert in SCI. Please find our responses to the reviews below.

The authors report a detailed investigation of early blood spinal cord barrier response to mechanical injury. The technical quality of the images is excellent. However, the manuscript seems to be very short on data, and reads more as an extended description of methods, with little in the way of results. The heart of the data would appear to be the temporal response of the BSCB after injury. This data is summarized in a few figures, and seem to show an early dramatic increase in BSCB permeability, with subsequent reductions in ktrans. However, the remainder of the manuscript does not contribute to this evaluation. Instead, there are subjective and qualitative descriptions of findings, with little in the way of criteria. Thus, it is impossible to determine whether the inferences about primary versus secondary injury are correct, or supported by their results.

We spent significant amount of time to develop the required technical capabilities to acquire the presented high quality images. This work is a preliminary feasibility study. The MS aims to describe our method and data acquisition/processing tools in detail so that the approach can be implemented or adapted by other research groups for further investigations of injured SC in mouse models. In the revised MS, we eliminated the sections with subjective or qualitative arguments, and emphasized the results supported by quantitative evaluations. The time frame for the primary versus secondary events in injured SC has been described extensively in the literature using ex vivo techniques. This is beyond the scope of this work. The interferences made about the primary versus secondary injuries in the MS were provided based on the interpretation of the histology by Dr. Desouki, coauthor and pathologist in our institution. To comply with this comment, we removed the statements made about the primary and secondary events from the MS.

The manuscript would benefit from an extensive rewrite. The authors need to decide what data they wish to report, presumably the BSCB permeability data, then focus their presentation on these results.

Thank you for providing a clear guidance. We followed the suggestion and reorganized/rewrote the MS, and clarified the novel contributions and results.
On the rewrite, it would be important to edit the manuscript so that only the important information is included. The extended description of the permeability study seems to report a standard implementation of DCE. If there is something unique here the authors should make it clear what they believe this to be. If it is a standard implementation, then only a brief summary and reference are needed.

Performing experiments on mouse is a unique feature of this study. The pharmacokinetic model has been described earlier, but, the algorithm developed to produce permeability map from DCE-MRI data is new and deserves a full description in detail, which was moved to Appendix in the revised MS. Performing DCE-MRI and DTI on the same scan session and establishing the association between the permeability and diffusion tensor measurements is another important feature of the study. These are now clearly stated in this revision.

The authors have presented the BSCB permeability data from measures of 6 images approximately centered on the injury site. However, it is not clear how they identified the injury site. This approach also assumes that the effects of the injury extended only on these images, with no effects farther away. If this is not true, then this approach underestimates the extent of the lesion, and obscures the temporal evolution of the injury.

We identified the injury site from the anatomical and DCE-MR images. That is the reason why we included the coronal and horizontal images. The proposed method operates on DCE-MRI data from all available slices and produces permeability maps accordingly. It leaves up to the user to interpret and decide which slices to take into consideration. In this study, 14 contiguous slices were acquired with no gap in between. The BSCB permeability maps were computed for all slices. But, in the MS, the data from only six slices centering on the lesion were presented because the remaining slices showed insignificant or no barrier breakdown.

By imaging only twice, they assume they can characterize the permeability of the BSCB. But this will not produce accurate results if the wash in and wash out phases are different than they assume.

The proposed approach was derived from a two-compartment model typically used in DCE-MRI studies. We closely analyzed the resulting analytical formulas from this model and evaluated the time
asymptote near zero. This analysis yielded the expression \( k_{p-sc}(x, y, z) = \frac{1}{[C]_{p}(t = 0)} \frac{d[C]_{sc}(t, x, y, z)}{dt} \bigg|_{t=0} \).

This formula shows dependence of permeability on wash in, but not wash out phase of contrast agent. If the delivery is bolus, the formula should work well. This condition has been stated in the MS.

Omit figure 2.

Done.

Figure 3 displays the interesting finding of hyperintensity in the corticospinal track rostral, but not caudal, to the injury site. Since one might expect more rapid anterograde than retrograde degeneration, was this a reproducible finding, or observed only in the illustrated cord?

We agree. This was a unique case. We felt that it is worthy of presentation.

Figures 4, 5, and 6 can be omitted.

We respectfully disagree. We kept these figures in the revised MS to depict the contrast uptake with time and space. Since this work is first of its kind, we feel that the readers would benefit from the images presented in these figures.

Legend for figure 8 appears to describe figure 9.

Corrected.

The legend for figure 9 apparently describes the images displayed in figure 10. The legend states that white arrows denote vasculature whose BSCB became leaky due to secondary inflammatory processes. Unfortunately, the authors do not state how they concluded this. What in the image indicates BSCB damage? How do they know it is due to inflammatory secondary injury?
The figure legend was corrected. The cavity surrounding blood vessels are indicative of blood leakage and hence BSCB damage in injured SC. The damage of the BSCB is well demonstrated by the leakiness of the vascular endothelial lining in CD34 (an endothelial marker) stained sections (Figure 9, panel f in the revised MS) when compared to the well circumscribed, well localized staining in endothelial cells in intact vessels without leakage (Figure 9, panel c in the revised MS). Additionally, the presence of cavities and disruption of the continuity of vascular walls as shown in figures (7d, 8c and 8d and 9f) all are clear indication of disruption and leakage from vessels. The statement of causation of inflammatory changes as a secondary injury is a conclusion based on the literature accusing reactive species which induce cytotoxicity and consequently secondary insults of the BSCB. The source of the reactive species is mainly from inflammatory cellular infiltrate (please see the by reference Maikos JT and Shreiber DI; 2007, which was cited in the original MS). We appreciate the critics and agreed to remove the statements regarding the secondary inflammation from the text as well as the figure legend to eliminate any confusion.

*The legend for figure 10 apparently describes figure 11. The authors do not explain why this is included as well as figure 10, both appear to present the same findings.*

We included this figure because the stain used for these slices is in (b-d) is endothelial marker, CD34.

*The legend for figure 11 apparently describes figure 12. This portion of the study would benefit greatly from a more quantitative approach to analyzing the immunohistochemistry results.*

It was not clear to us what sort of quantitative IHC analysis had the reviewer in mind. We appreciate receiving further details of the suggested analysis. We would like to note that this MS was prepared to give the details of our DCE-MRI approach and computation algorithm, to demonstrate its feasibility in mouse model and to show its merits in terms of evaluating BSCB dysfunction. Efforts in our current projects are directed towards testing the efficacy of potential drugs in mouse models of SCI. These studies may take advantage of the tools described in this MS.

*The legend for figure 12 apparently describes figure 13.*

Corrected.
The legend for figure 13 apparently describes figure 14.

Corrected.

The legend for figure 14 apparently describes figure 15.

Corrected.

The legend for figure 15 apparently describes figure 16.

Corrected.

Given the extensive revisions required, it would be difficult to predict an overall score after these have been completed.

We hope we responded the critiques properly and the MS is now found acceptable for publication.