Author's response to reviews

Title: Trauma Induces Apoptosis in Human Thoracolumbar Intervertebral Discs

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Dear BioMed Central Editorial Team,

thank you very much for your letter from April 7th, 2006 including the reviewers' helpful comments and recommendations regarding our recently submitted manuscript (MS: 193361358948539). Enclosed, please find our revised version of the manuscript along with our rebuttal, including the responses to the reviewers. All changes within the respective sections have been marked in red ink.

We believe to have satisfactorily answered all the reviewers' comments and hope that the paper is now acceptable for publication in the BMC Clinical Pathology Journal.

Should you have further questions or comments regarding our revised manuscript, please do not hesitate to contact me at your earliest convenience.

Respectfully,

C.-E. Heyde, MD

Response to the Reviewers

Reviewer J.-B. Park:

1. We thank the reviewer for this helpful critique. However, recent publications have particularly emphasized the occurrence of apoptosis and apoptosis-related molecular changes in intervertebral discs from both idiopathic and neuromuscular scoliosis patients. For example, Chen and colleagues (Spine 2005; Mar 1; 30 (5): 519-524) reported IVD tissue from both the apex and non-apex discs to present with evident apoptosis and increased Fas/FasL expression. Moreover, in vivo studies presenting healthy control IVD tissue is extremely limited. In the very few cases, where healthy viable control IVD tissue (not cadaver samples) was accessible, IVD were obtained from primary vertebral tumours (also, please see comment to 3.).

2. Due to the nature of our study, trauma patients were not routinely assessed by MR imaging to determine degeneration in the affected segmental IVD. Furthermore, our clinical experience has shown that a graphical distinction between trauma-induced morphological changes and degeneration in MRI is extremely difficult, regardless of T-weighting. However, all three control samples were evaluated by MRI in regards to degeneration according to the classification described by Pfirrmann et al. (Spine 2001) prior to surgery. Accordingly, this has been revised in the Material and Methods section (page 5).

3. We agree with the reviewer's critique that microinfiltration of tumour cells may not be reliably excluded by MR imaging. Thus, our study additionally obtained histopathology on the respective control IVD specimens with all samples proving negative for microinfiltration. Accordingly, we have added clinical data as Table 2.

4. As stated in the Material and Methods section (page 7) all TUNEL analyses were performed on
cryo-sections. In addition to our experience with numerous other cell types (monocytes, lymphocytes, neutrophils and chondrocytes), this particular method has been reported to be an appropriate alternative to the commonly applied method of paraffin section analyses (Ahsan et al. J Orthop Sci 2001). We believe it is suitable for the purposes described in our study.

5. We apologize for this mistake. DNase I treated tissue specimens were used as positive controls. This has been revised in the Material and Methods section, accordingly.

6. As stated in the first paragraph of the Results section, a differentiation between the nucleus and anulus was not possible, due to the gross morphological damage in the majority of traumatic IVD tissue. Thus, we acknowledge this interesting aspect for future experimental approaches, but must resign in analysis of human tissue under given circumstances.

Reviewer H. Gruber:

1. The title has been changed to address the study's content more appropriately.

2. + 3. Clinical data has been added to give a brief overview in regards to fracture classification, concomitant diseases and respective disc segment analysis. Although we highly respect and acknowledge the reviewer's expertise in this field of research, we disagree with her opinion on whether our stated assumption of increased apoptosis can be made in trauma IVD specimens. Despite our limited data in control specimens, the age in control patients ranged from 44y to 47 years. In contrast, the majority of trauma patients (n=9) was below the age of 40y and only n=4 patients were above the age of 50y. We truly acknowledge that this fact does not solitarily justify our assumption. However, all three control samples were additionally graded by MRI analysis according to the method described by Pfirrmann (Pfirrmann et al. (Spine 2001)) with results indicating either no (n=2), or only very low disc degeneration (n=1). Due to the nature of acute patient treatment and our institutional guidelines, trauma patients were not routinely assessed by MR imaging prior to the respective surgical procedures. Furthermore, our clinical experience has shown that a graphical distinction between trauma-induced morphological changes and degeneration in MRI is extremely difficult, regardless of T-weighting. However, we agree with the reviewer that an age-matched animal study would support our data and emphasize the importance of our observational findings. Yet, we believe that this unique data from human specimens marks an interesting perspective to suggest novel experimental approaches and investigate the multitude of apoptosis-related molecular patterns in traumatic IVD.

4. All figure data is presented as mean value +/- standard error of the mean (SEM). This has been revised in the Material and Methods section.

Reviewer A. Hoyland:

1. Methods - We appreciate the reviewer's recommendation. Accordingly, respective data were added to further describe the method of real time RT-PCR in more detail. However, the applied primer sequences could not be supplemented to the Methods and Material section due to the use of validated assays from Qiagen(R). For a detailed description of the specific primer sequence, we must therefore refer to the company to provide respective information.

2. All p-values in the Results section have been corrected to state significance at p<0.05.

3. The magnification (x100) was added to the figure legend (Figure 1) as recommended. Furthermore, small images with a magnification of x400 were inserted to improve graphical demonstration of positive staining.

4. All graphs in Figures 5-7 were adjusted to log scale as recommended. In addition, we revised the Material and Methods section to state that all real-time RT-PCR data was normalized to SZ-actin (page 8).