

AGE-ASSOCIATED CHANGES IN THE CELL DENSITY OF THE HUMAN LUMBAR INTERVERTEBRAL DISC

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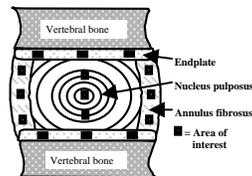
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INTRODUCTION: Despite intense investigation, the pathophysiology of disc degeneration is still not clear. Although it is widely accepted that the extracellular matrix of the disc undergoes significant modifications¹, little is known about the cellular changes in the discal matrix. Initial data suggest phenotypic alterations of the disc cells during degeneration.² However, changes of the cell density in the different regional compartments, such as nucleus pulposus (NP), annulus fibrosus (AF) and the end plates (EP), have not been investigated in depth. Since the disc cells are responsible for the proper maintenance of the discal matrix, their presence seems of crucial importance. Elucidating changes in both, the cell number and their phenotype are of basic interest for the understanding of disc pathophysiology and might lay the base for potential cell biologic interventions, such as cell transfer/transplantation. The current study presents a systematic morphometric analysis on a series of complete motion segments of human lumbar spines coming from cadavers of different ages.

METHODS: Study populations: Autopsy group (AG): A representative (with respect to age, sex and histological features) series of 30 disc motion segments (age range 0 – 86 years) was prepared out of an earlier described series of complete lumbar motion segments obtained from 49 cadavers at autopsy¹. The medical records provided no evidence of a history of spinal disease or back. In all cases a parasagittal section through the complete motion segment was prepared. This was fixed in 4-6% buffered formaldehyde, subsequently decalcified. The slices were then routinely embedded into paraffin and sections were prepared for routine histochemical stainings. Surgical group (SG): surgical samples were obtained from 12 patients (27 – 62 years, sex ratio 5 f and 7 m). All patients had undergone surgery for degenerative disc disease or protrusion. Clinical data on low back pain, related disability and imaging findings were available. The formalin-fixed tissue samples were embedded into paraffin as in the AG. Appropriate sections were used for routine histochemical stainings as indicated in the first group.

Assessment of disc degeneration: the extent of disc degeneration was determined on routine staining using the criteria established previously. Briefly, cellular organization, cleft formation, granular and mucoid matrix degeneration were determined and ranked. These criteria were then combined using a “histodegeneration score” (HDS)³.

Morphometric analysis: in the AG a morphometric analysis was designed to determine the cell number per field area (0.041 cm²). Each section was measured and the relative locations of the corresponding areas of interest were precisely defined (Fig. 1).



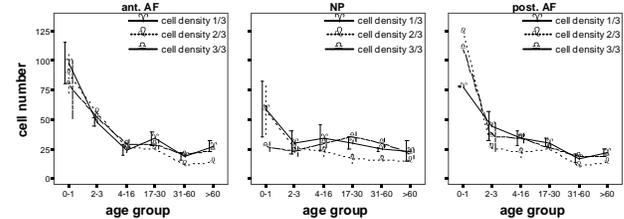
Immunohistochemistry: cells within the proliferation phase were identified by detecting the antigen Ki-67 (mab, MIB-1, DAKO, Hamburg, Germany). Ki-67 is exclusively expressed during the replication period and is absent in quiescent cells. The primary antibody was visualized using a sec. antibody complexed with the APAAP system as described. Lymphatic tissue was used as positive control and pre-immune serum was used instead of MIB-1 as negative control.

TUNEL assay: Apoptotic cells were detected using the TUNEL assay in sections from the SG. AG material showed false high labeling rates presumably due to post-mortal DNA fragmentation (data not shown). Paraffin sections were treated according to the manufacturer's instructions, developed with DAB and counterstained with hemalaun.

Statistics: All data for the HDS and cell density were evaluated using the Spearman ranking test with $p < 0.05$ being regarded as significant. The interrater reliability of the assessment of the cell density was assessed on 10 randomly selected specimens by two of the authors (A.G.N. and S.W.). Intraclass correlation coefficients for the two-way mixed effects were used to measure the rater reliability.

RESULTS: Disc cell density: In all EP regions, both of lower and upper EPs, anterior, middle and posterior regions, a comparable cell density was noted regardless of the different areas so that the values

were within the same range for each individual. There was, however, a considerable decrease in the cell density with increasing age. In prenatal EP 235 cells were identified per field. This value dropped significantly to 35 - 60 cells at 16 - 18 years of age, gradually decreasing to 10 - 20 cells in the old age group (86 years). A major decrease in the cell density was also observed in the anterior and posterior AF. However, the absolute cell densities were far lower compared to the EPs (especially for the prenatal and infantile discs). In the NP the changes in the cell density were less extensive. Furthermore, the NP material provided the highest variability of values. (Fig. 2)



Further analysis provided for all anatomic regions an indirect correlation between the occurrence of disc degeneration and cellular content.

Proliferation rate: no sample of the AG showed any signs of proliferative activity. 2 out of 12 samples of the SG showed focal chondrocyte proliferation in the NP (approx. 20 % in the respective fields). However, overall proliferation rate even in positive cases was < 1% of disc cells.

Cell apoptosis: none of the 12 samples in the SG investigated showed a single labeled cell. Neither necrotic nor apoptotic cells were found in those samples. **Statistics:** An inverse correlation between cell density and age or HDS was highly significant in all different anatomic regions, $p < 0.001$ or $p < 0.002$, respectively. The intraclass correlation coefficient for the assessment of the cell density showed an excellent rater agreement with a reliability coefficient $\alpha = 0.9342$.

DISCUSSION: This is the first study that describes the development of cellularity in human lumbar discs of defined age. Precise definition of the areas of interest accounted for differences between individuals, varying ages and disc sizes. Using this standardized approach we observed a highly significant drop in the cellularity of all disc structures from the fetal/early postnatal period to the juvenile/adolescent period. Since high cell numbers in the end plates, intermediate cell numbers in the AF and low cell numbers in the NP were found, the decrease in cellularity was differently extensive. The young to old adult discs revealed a further, though less pronounced decrease in cell numbers, for all anatomic regions investigated. Despite obvious cell proliferation in small localized areas as evidenced by clonal disc cell grouping, the overall number of disc cells dropped with ongoing adult age. Immunohistochemical detection of the proliferation-associated antigen Ki-67 revealed the absence of any proliferative activity in the autopsy samples and only focal proliferative activity in the biopsy material. Suggesting that the cell proliferation rate in the disc is very low and only by considerable lapse in time the „clonal proliferation“ of disc cells can occur. Detection of apoptotic cells using the TUNEL-assay in the SG samples, did not reveal any TUNEL-positive cells. Taken together, these observations suggest a very low cellular turnover on in disc tissue that nevertheless leads to a net decrease in the cell density in the intervertebral disc.

REFERENCES: 1) Nerlich A et al., Spine. 1997;22:2781-95 2) Nerlich a et al., Spine. 2002;27:2484-90 3) Boos N et al., Spine. 2002;27:2631-44

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