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Classification of Age-Related Changes in Lumbar Intervertebral Discs

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Study Design. A histologic study on age-related changes of the human lumbar intervertebral disc was conducted.

Objectives. To investigate comprehensively age-related temporospatial histologic changes in human lumbar intervertebral disc, and to develop a practicable and reliable classification system for age-related histologic disc alteration.

Summary of the Background Data. No comprehensive microscopic analysis of age-related disc changes is available. There is no conceptual morphologic framework for classifying age-related disc changes as a reference basis for more sophisticated molecular biologic analyses of the causative factors of disc aging or premature aging (degeneration).

Methods. A total of 180 complete sagittal lumbar motion segment slices obtained from 44 deceased individuals (fetal to 88 years of age) were analyzed with regard to 11 histologic variables for the intervertebral disc and endplate, respectively. In addition, 30 surgical specimens (3 regions each) were investigated with regard to five histologic variables. Based on the semiquantitative analyses of 20,250 histologic variable assessments, a classification system was developed and tested in terms of validity, practicability, and reliability. The classification system was applied to cadaveric and surgical disc specimens not included in the development of the classification system, and the scores were assessed by two additional independent raters.

Results. A semiquantitative analyses provided clear histologic evidence for the detrimental effect of a diminished blood supply on the endplate, resulting in the tissue breakdown beginning in the nucleus pulposus and starting in the second life decade. Significant temporospatial variations in the presence and abundance of histologic disc alterations were observed across levels, regions, macroscopic degeneration grades, and age groups. A practicable classification system for age-related histologic disc alterations was developed, resulting in moderate to excellent reliability (κ values, 0.49–0.98) depending on the histologic variable. Application of the classification system to cadaveric and surgical specimens demonstrated a significant correlation with age ($P < 0.0001$) and macroscopic grade of degeneration ($P < 0001$). However, substantial data scatter caution against reliance on traditional macroscopic disc grading and favor a histology-based classification system as a reference standard.

Conclusions. Histologic disc alterations can reliably be graded based on the proposed classification system providing a morphologic framework for more sophisticated molecular biologic analyses of factors leading to age-related disc changes. Diminished blood supply to the intervertebral disc in the first half of the second life decade appears to initiate tissue breakdown. [Key words: anulus fibrosus, endplate classification–lumbar–human, histology, intervertebral disc classification, nucleus pulposus]
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The human lumbar intervertebral disc (IVD) is a very complex joint structure that can be separated macroscopically into at least three distinct components: 1) the nucleus pulposus (NP) representing a centrally located gelatinous homogenous mass (in juvenile discs); 2) the anulus fibrosus (AF) consisting of concentrically organized layers of collagen fibrils, which contain the nucleus pulposus and; 3) the cartilaginous endplates (EP), which separate the nucleus pulposus and anulus fibrosus from the adjacent vertebral bone. Any disturbance of the integrity and interplay of one of the three structures can result in a compromised function of the intervertebral disc.

Like no other musculoskeletal tissue, the lumbar intervertebral disc undergoes very extensive destructive changes with age and degeneration.[7] The degree of this tissue destruction is closely linked to age, but different components of the disc undergo more extensive alterations than others.[10] Substantial individual differences can be observed in the sense that young individuals exhibit the disc of an elderly person and *vice versa*. Because of the extensive destructive changes that ultimately lead to an ankylosed motion segment,[44,45] many clinicians and researchers believe that the intervertebral disc is a predominant source of low back pain (LBP). We concur with Vernon-Roberts[44,45] that differentiating pure aging from degeneration is very difficult. For the purpose of this study the term "disc degeneration" is used to indicate the aforementioned changes without implying that disc degeneration is synonymous with a painful disc.

Although an increasing number of studies are focusing on the molecular mechanisms of disc degeneration and its therapeutic inhibition by gene therapy,[21,24,25,47] there is a paucity of studies on what actually represents

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disc degeneration in terms of histologic changes.[10,38–40,48] Many studies on the molecular mechanism of age-related disc changes[1,2,13,14,26,27,33,36,37] relate their findings on the molecular arrangement of the disc to a macroscopic assessment of disc degeneration. The heterogeneity of alterations within and between the different components of the disc may explain the data scatter observed in some studies. Advancement in the understanding of disc degeneration will be facilitated if more sophisticated biochemical and immunohistochemical data can be related to a histologic framework.

The minimal information on the histologic features of disc degeneration is derived from a few studies.[10,38–40,48] An extensive review has shown no studies focusing on a detailed, comprehensive, qualitative, and quantitative assessment of the histologic changes exhibited during the process of disc aging. Furthermore, there is no grading system that would allow alterations in the molecular biology of the IVD to be related to different degrees of tissue alteration and destruction.

In analyzing macroscopic and histologic sections of 180 human lumbar IVDs encompassing the whole range from fetal to senile age, this study aimed to

- provide a comprehensive semiquantitative assessment of the age-related histologic changes in lumbar IVD and EP,
- demonstrate that a macroscopic assessment of disc degeneration is insufficient because of the substantial heterogeneity of the alterations within and between the different IVD and endplate regions,
- develop a classification system for grading the histologic features of age-related disc changes based on an extensive semiquantitative histologic analysis,
- test the practicability, validity, and reliability of such a classification system.

■ Materials and Methods

Study Population.

Cadaver Specimens. For the purpose of this study, 54 human lumbar spines were harvested during routine autopsy from individuals without any known spinal disorders ranging in age from fetal to senile (88 years). All the individuals had died of acute causes such as acute trauma, acute poisoning, cerebral bleeding, sepsis, myocardial infarction or, in the case of infants, sudden infant death syndrome, acute trauma, or congenital heart defect. For none of the individuals did the medical reports (available at autopsy) mention a history of a relevant back problem (*e.g.,* previous in-hospital treatment, surgery, or invalidity).

Surgical Specimens. Furthermore, the study included 30 lumbar intervertebral disc specimens excised during surgery from 23 individuals undergoing a spinal intervention. The ages of the 11 males and 12 females ranged from 14 to 68 years. Clinical and MRI data (T1- and T2-weighted sagittal and T2W axial scans according to a standardized protocol[34]) had been prospectively collected on all the individuals, including the scoliosis patients. All the individuals with degenerative disc disease had a positive pain response during provocative discography at the index disc level, with a negative pain response at an adjacent MRI normal disc level.

Tissue Processing. All the spines were removed using an anterior approach, and osteotomies were performed at each pedicle level (L1 to S1) and at the vertebral levels of L1 and S1, respectively. Thus, the intact anterior spinal column with the anterior and posterior longitudinal ligament remaining attached to the specimen was obtained without mutilation of the cadaver. The removed specimens then were divided into motion segments, and midsagittal slices of each segment were obtained including parts of the adjacent vertebral body. Serial sagittal slices were processed in 10 cases to study variations in the coronal plane. All the sagittal slices were prepared for photodocumentation of the macroscopic appearance and further processed for histologic analyses.

All the slices were fixed in buffered 4% formaldehyde (pH 7.4) for 2 × 24 hours and subsequently decalcified in 0.1 mol/L EDTA (pH 7.2) over a 1- to 4-month period depending on the calcification of the osseous matrix of the vertebral bone. The decalcified complete sagittal disc slices were then embedded into paraffin as routinely performed. From the resulting blocks, paraffin sections (2–4 μm thick) were cut and placed on silanized glass slides for routine stainings (H&E, Masson-Goldner, Alcian blue-PAS) using standard histochemical protocols.[3,23]

Data Evaluation. All the intervertebral discs were evaluated macroscopically in conference by two of the authors (A.G.N. and N.B.), and the degree of disc degeneration (Figure 1) was ranked from Grade 1 (normal juvenile disc) to Grade 5 (severe degeneration) using the grading scheme by Thompson et al.[41] Based on a through review of the literature[7,9–12,20,32,49] and the authors' previous work,[3,23] histologic variables were selected for further data analyses (Table 1).

The cadaver specimens were divided into training (n = 150) an test (n = 30) samples. Ten age groups were determined based on theoretical grounds and group frequency of the specimens. The data analyses were accomplished in three steps: 1) The training sample was reviewed with regard to the presence and abundance of the histologic variables for the intervertebral disc and endplates by one of the authors (S.W.). Variations of the extent of the histologic variables with regard to age categories, disc degeneration, disc level, and regions were explored using univariate ANOVA. Simple relationships of the histologic variables with macroscopic disc degeneration and age groups were explored using the Spearman rank correlation test. The interrater reliability of the histologic assessment of the target histologic variables was assessed on 50 randomly selected specimens by two of the authors (S.W. and A.G.N.) using percentage agreement and kappa statistics. 2) Based on these evaluations, a classification system was developed including histologic variables that were applicable to cadaveric and surgical specimens. 3) The test set (n = 30) was then independently assessed by two pathologists (H.R. and C.W.) with an interest in musculoskeletal disorders and not involved in the development of the grading system. After a short training session, templates of the histologic variables were made available to the raters at the time of data assessment. Furthermore, the raters

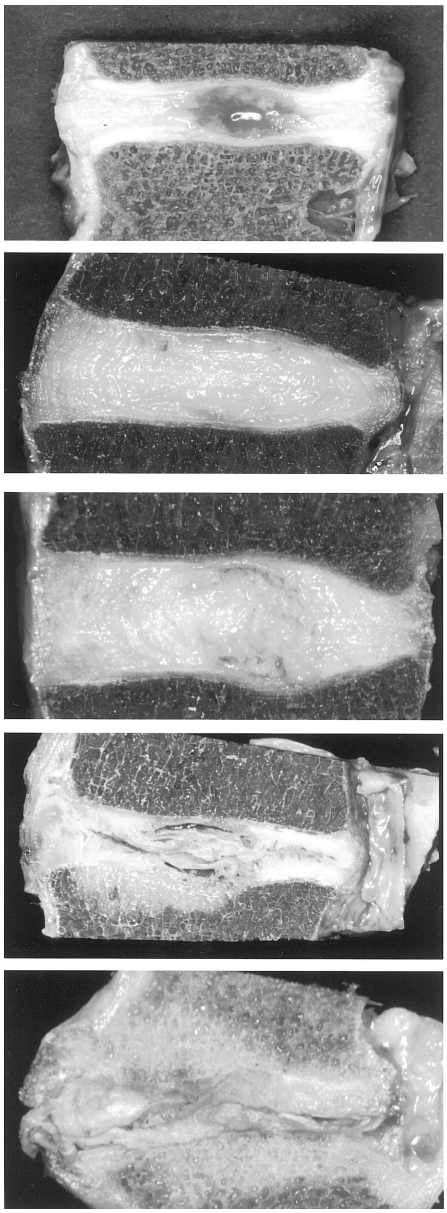


Figure 1. Macroscopic grading of age-related disc alterations according to Thompson et al.[41]

were asked to assess the histologic changes in 30 surgical specimens (3 random regions). Interrater reliability was again assessed by percentage agreement and kappa statistics. The resulting histologic scores were compared with age groups and macroscopic and MRI disc degeneration. The MRI assessment of disc degeneration was based on a recent paper.[34] The agreement was rated in terms of kappa statistics, according to Landis and Koch,[19] as follows: 0 to 0.2 (slight agreement), 0.21 to 0.4 (fair agreement), 0.41 to 0.60 (moderate agreement), 0.61 to 0.8 (substantial agreement), and 0.81 or more (excellent agreement). Absolute agreement would be 1. Frequency of disagreement was calculated for each grade. The validity of the grading system was assessed by evaluating the ability of the disc and endplate histologic markers to predict age and gross morphology based on disc degeneration. Specifically, the value of the various markers and the region of harvesting was assessed using CHAID-based decision tree analysis.[6,17]

■ Results

Sample Demographics.

Sample Group 1. From the data pool, 150 midsagittal complete lumbar motion segment slices harvested from 44 cadavers (15 females and 29 males) were randomly selected. They ranged in age from fetal to 86 years. The distribution across the disc levels was as follows: L1–L2 (n = 31), L2–L3 (n = 27), L3–L4 (n = 31), L4–L5 (n = 29), and L5–S1 (n = 32). The assessment of the macroscopic disc degeneration resulted in 48 discs rated as Grade 1, 47 rated as Grade 2, 37 rated as Grade 3, 11 rated as Grade 4, and 7 rated as Grade 5.

Sample Group 2. From the data pool, 30 additional complete lumbar motion segment slices were selected from 18 cadavers (8 females and 10 males). In this set, lateral (n = 6) and parasagittal (n = 6) slices were included in addition to midsagittal slices (n = 18). The ages of the individuals ranged from fetal to 88 years. The distribution across the disc levels was as follows: L1–L2 (n = 4), L2–L3 (n = 5), L3–L4 (n = 11), L4–L5 (n = 5), and L5–S1 (n = 5). The assessment of the macroscopic disc degeneration resulted in 6 discs rated as Grade 1, 3 rated as Grade 2, 9 rated as Grade 3, and 12 rated as Grade 4.

Sample Group 3. Thirteen patients underwent lumbar discectomy. Seven of these patients had a fusion operation for degenerative disc disease, and three underwent a scoliosis correction with anterior release. All the individuals with a discectomy or fusion operation had persistent back and leg pain despite an appropriate trial of nonoperative treatment. The ages of the 10 males and 3 females undergoing lumbar discectomy ranged from 21 to 68 years. Twelve patients underwent a one-level procedure, and the remaining patient had a two-level discectomy (10 at L5–S1 and 4 at L4–L5). The MRI grading of disc degeneration resulted in five discs classified as Grade 3 and nine discs classified as Grade 4.

Fusion operations were performed in six women and one man, who ranged in age from 32 to 46 years. The fusion operations were performed at L4–L5 (n = 5) and L5–S1 (n = 5). A two-level fusion was performed in three patients. All the patients with low back pain had unequivocal MRI findings of an abnormal disc at the index level. Grading of disc degeneration by MRI (in a conference of two readers) resulted in a classification of Grade 3 in three patients, Grade 4 in five patients, and Grade 5 in two patients. The three patients with scoliosis were 14 and 15 years of age, respectively. The specimens were obtained from Th12–L1 (n = 2), L1–L2 (n = 2), and L2–L3 (n = 2). The MRI assessment of disc degeneration resulted in a Grade 2 classification for all the patients.

Reliability of the Histologic Assessment

Interrater reliability estimates for the assessment of the histologic features in general showed good to excellent rater agreement. Details on the reliability estimates for

Table 1. Variables of Macroscopic and Histologic Assessment

Global disc appearance	
Macroscopic assessment (IVD, endplate, and adjacent bone)	Grade 1 = normal juvenile disc; Grade 2 = normal adult disc; Grade 3 = mild disc degeneration; Grade 4 = moderate disc degeneration; Grade 5 = severe disc degeneration
Intervertebral disc	
Cells (chondrocyte proliferation)	0 = no proliferation; 1 = increased cell density; 2 = connection of two chondrocytes; 3 = small size clones (several chondrocytes, grouped together, 3–7 cells); 4 = moderate size clones (8–15 cells); 5 = huge clones (>15 cells)
Multiple chondrocytes growing in small rounded groups or clusters sharply demarcated by a rim of territorial matrix	
Granular changes	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Eosinophilic-staining amorphous granules within the fibrocartilage matrix	
Mucous degeneration	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Cystic, oval, or irregular areas with an intense deposition of acid mucopolysaccharides (*i.e.,* sulphated glycosaminoglycans) staining dark blue with Alcian blue-PAS	
Edge neovascularity	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Newly formed blood vessels with reparative alteration	
Rim lesions	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Radial tears adjacent to the endplates	
Concentric tears	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Tears after the orientation of collagen fiber bundles in the anulus fibrosus	
Radial tears	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Radiating defects extending from the nucleus pulposus to the outer anulus lamellae parallel or oblique to the endplate (clefts)	
Notochordal cells	0 = absent; 1 = present
Embryonic disc cells	
Cell death	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Altered phenotype	
Scar formation	0 = absent; 1 = present
Amorphous fibrosus tissue without any differentiation	
Tissue defects	0 = absent; 1 = present
Voids within the tissue (*e.g.,* resulting from tissue resorption, probably filled with fluid *in vivo*)	
Endplate	
Cells	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Number of cells (chondrocyte clusters)	
Structural disorganization	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Focal disorganization of the cartilaginous matrix with clumping of chondrocytes	
Clefts	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Tears in the endplate	
Microfracture	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Disruption of the subchondral bone	
Neovascularization	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Vessels penetrating from the bone marrow into the endplate in conjunction with microfractures	
New bone formation	0 = absent; 1 = present
Bone islands within the cartilage	
Bony sclerosis	0 = absent; 1 = present
Formation of new bone	
Physiologic vessels	0 = absent; 1 = present
Obliterated vessels	0 = absent; 1 = present
Scar formation	0 = absent; 1 = present
Amorphous fibrosus tissue without any differentiation	
Tissue defects	0 = absent; 1 = present
Voids within the tissue (*e.g.,* resulting from tissue resorption, probably filled with fluid *in vivo*)	

IVD = intervertebral disc.

the various anatomic regions within the disc and endplate are shown in Table 2.

Semiquantitative Assessment of Histologic Age-Related Disc and Endplate Changes

The analyses were based on 150 complete lumbar motion segment slices (IVD and adjacent EP with vertebral bodies). Five regions (*i.e.,* anterior outer anulus, anterior inner anulus, nucleus pulposus, posterior inner anulus, and posterior outer anulus) were distinguished for the intervertebral disc and six regions (*i.e.,* anterior, central, and posterior upper and lower EP) for the EP.

The variation of the histologic variables across disc levels, regions, macroscopic degeneration grades, and age

Table 2. Interrater Reliability Estimates for the Assessment of Histologic Features of Disc Degeneration

Criteria	% Agreement	Weighted Kappa Value	Confidence Interval
Intervertebral disc (n = 50, 5 regions)			
Cells	80.8	0.871	0.836–0.907
Mucoid degeneration	80.0	0.826	0.779–0.873
Granular changes	92.8	0.932	0.900–0.963
Tears/cleft formation	94.4	0.955	0.931–0.978
Cell death	82.8	0.786	0.726–0.846
Cartilagineous endplate (n = 50, 6 regions)			
Cells	83.7	0.850	0.810–0.890
Cartilage disorganization	94.0	0.939	0.910–0.968
Cartilage cracks	91.7	0.869	0.814–0.924
Microfracture	96.0	0.846	0.761–0.930
Bony sclerosis	92.2	0.787	0.705–0.870
New bone formation	89.7	0.813	0.751–0.875

groups is presented in Table 3. The results of the correlation of the histologic variables with age and macroscopic degeneration grade are shown in Table 4. The temporal and regional variations in the histologic features (Figure 2A and 2B) with regard to disc degeneration are summarized in the following sections.

Group 0: Fetal Age (n = 9). In fetal discs, no histologic abnormalities are seen. However, it is noted that the EP

Table 4. Spearman Correlation Coefficients and Significance Levels for the Correlation of Histologic Criteria *Versus* Macroscopic Degeneration and Age Over All Disc Regions

	Macroscopic Disc Degeneration		Age Groups (n = 10)	
Criteria	Correlation Coefficient	*P*	Correlation Coefficient	*P*
Intervertebral Disc				
Cell	0.592	<0.0001	0.606	<0.0001
Granular changes	0.407	<0.0001	0.428	<0.0001
Mucous degeneration	0.637	<0.0001	0.692	<0.0001
Edge neovascularity	0.257	<0.0001	0.241	<0.0001
Rim lesions	0.199	<0.0001	0.191	<0.0001
Concentric tears	0.598	<0.0001	0.617	<0.0001
Radial tears	0.588	<0.0001	0.605	<0.0001
Notochordal cells	−0.258	<0.0001	−0.263	<0.0001
Cell death	0.450	<0.0001	0.493	<0.0001
Scar formation	0.231	<0.0001	0.172	<0.0001
Tissue defects	0.145	<0.0001	0.112	<0.0003
Endplate				
Cells	0.390	<0.0001	0.421	<0.0001
Cartilage disorganization	0.460	<0.0001	0.457	<0.0001
Cartilage cracks	0.487	<0.0001	0.482	<0.0001
Microfractures	0.371	<0.0001	0.335	<0.0001
Neovascularity	0.427	<0.0001	0.451	<0.0001
New bone formation	0.540	<0.0001	0.535	<0.0001
Bony sclerosis	0.509	<0.0001	0.512	<0.0001
Physiologic vessels	−0.549	<0.0001	−0.577	<0.0001
Obliterated vessels	0.015	<0.66	0.016	<0.64
Scar formation	0.079	<0.02	0.065	<0.05
Tissue defects	0.149	<0.0001	0.120	<0.0003

Table 3. Results of Univariate ANOVA for Age-Related Histologic Changes for the Intervertebral Disc and Endplate (n = 150) With Regard to Disc Level, Region, Macroscopic Degeneration Grade, and Age Groups

	Disc Level		Region		Degeneration Grade (1–5)		Age Groups (0–9)	
Criteria	F	P	F	P	F	P	F	P
Intervertebral Disc								
Cells	2.320	0.061	78.810	<0.0001	47.200	<0.0001	22.180	<0.0001
Granular changes	1.980	0.103	61.930	<0.0001	27.660	<0.0001	14.890	<0.0001
Mucous degeneration	0.850	0.495	30.510	<0.0001	41.070	<0.0001	23.530	<0.0001
Edge neovascularity	1.090	0.367	27.530	<0.0001	12.160	<0.0001	6.310	<0.0001
Rim lesions	0.270	0.898	15.780	<0.0001	12.620	<0.0001	4.910	<0.0003
Concentric tears	0.770	0.544	55.550	<0.0001	40.700	<0.0001	19.320	<0.0001
Radial tears	0.650	0.625	46.570	<0.0001	39.760	<0.0001	22.590	<0.0001
Notocordal cells	2.350	0.059	14.020	<0.0001	30.990	<0.0001	15.070	<0.0001
Cell death	0.480	0.754	119.430	<0.0001	38.130	<0.0001	21.990	<0.0001
Scar formation	1.070	0.377	0.990	0.417	2.400	0.061	1.020	0.444
Tissue defect	0.770	0.544	1.340	0.256	3.080	0.023	0.420	0.913
Cartilagineous Endplate								
Cells	0.350	0.843	14.300	<0.0001	8.930	<0.0001	3.330	0.005
Cartilage disorganization	1.270	0.288	14.770	<0.0001	20.020	<0.0001	10.120	<0.0001
Cartilage cracks	3.940	0.005	24.600	<0.0001	14.520	<0.0001	6.550	<0.0001
Microfractures	0.290	0.887	10.580	<0.0001	13.260	<0.0001	5.970	<0.0001
Neovascularity	1.480	0.215	8.560	<0.0001	14.130	<0.0001	6.980	<0.0001
New bone formation	2.920	0.025	4.960	0.0003	26.880	<0.0001	11.660	<0.0001
Bony sclerosis	0.510	0.726	8.720	<0.0001	17.920	<0.0001	9.130	<0.0001
Physiologic vessels	1.340	0.261	4.460	0.0007	45.040	<0.0001	51.010	<0.0001
Obliterated vessels	0.770	0.548	5.430	0.0001	3.890	0.008	4.500	0.0006
Scar formation	1.070	0.377	1.250	0.286	2.400	0.061	1.020	0.444
Tissue defect	0.790	0.535	1.190	0.315	3.030	0.025	0.430	0.910

ANOVA = analysis of variance.

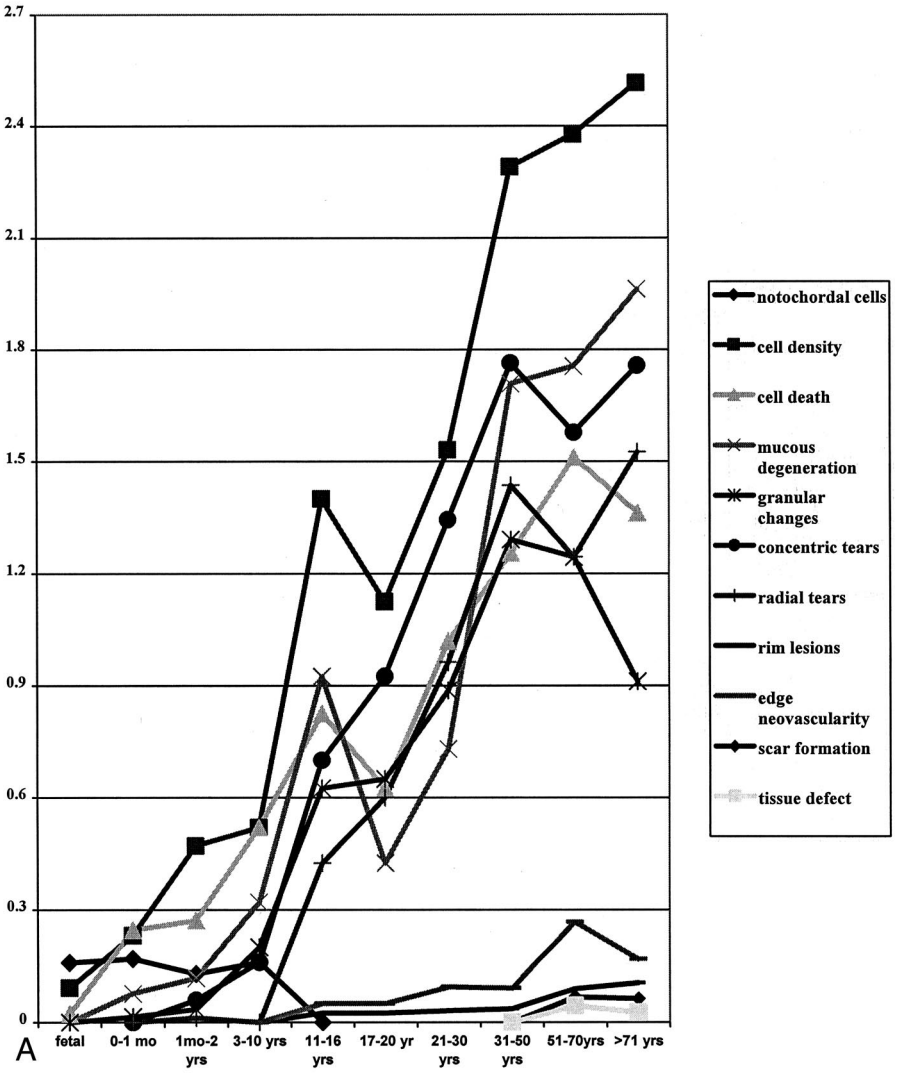


Figure 2. **A,** Temporal variation in histologic variables for the intervertebral disc. **B,** Temporal variation in histologic variables for the cartilaginous endplate.

Figure continues.

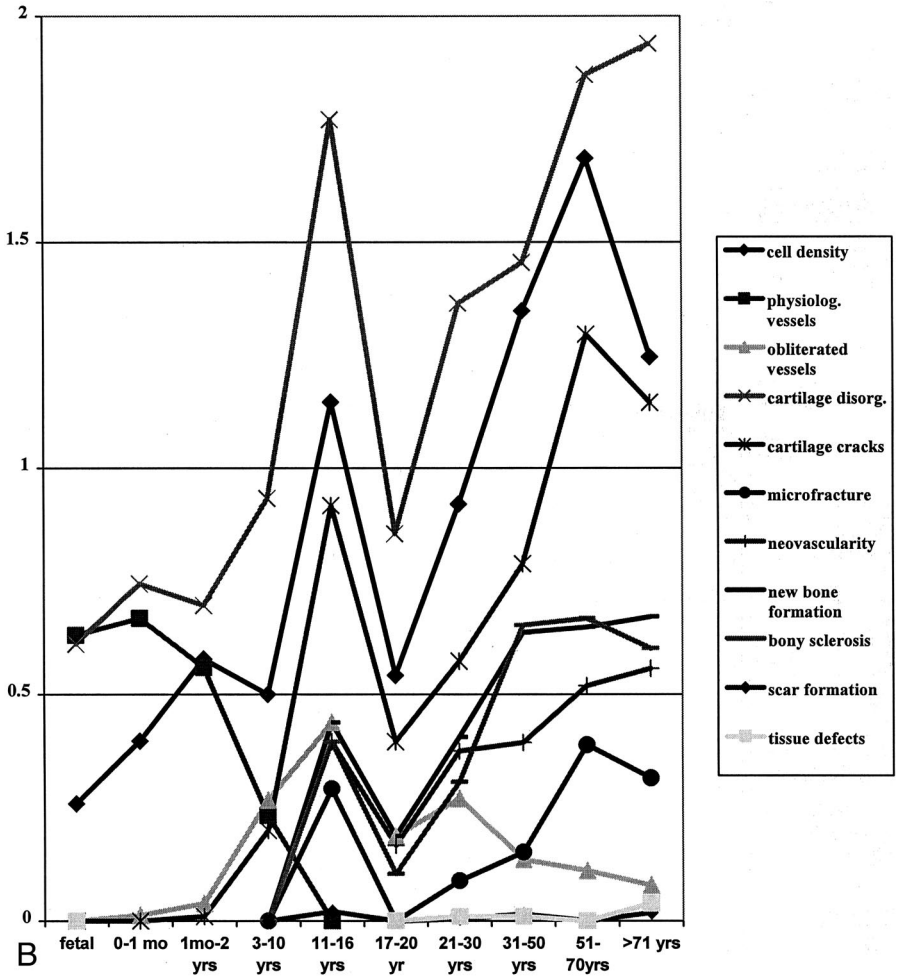
appeared slightly irregular, as compared with hyaline articular cartilage. In the nucleus pulposus, foci of abundant notochordal cells are seen. The fetal discs are vascularized by thin-walled, ectatic blood vessels.

Group 1: Ages 0 to 1 Months (n = 13). In the IVD of newborns, there is a slight increase in chondrocyte density, which is seen particularly in the nucleus pulposus. As compared with fetal disc, the rate of decayed cells is increased. Slight mucoid degeneration is encountered in the nucleus pulposus of 5 of 11 specimens in conjunction with chondrocyte proliferation (6/11). In the endplate, cell density and cartilage disorganization are increasing, as compared with fetal discs.

Group 2: Ages 2 Months to 2 Years (n = 17). In this age group, very mild cleft formation in the nucleus pulposus is occurring in 4 of 17 specimens. Similarly, granular changes are first seen in the nucleus pulposus (2/17). In the EP, there is a regression in the number of physiologic vessels, and areas with obliterated vessels are seen while cell density is increasing. Thus, the EP structure provides some disorganization.

Group 3: Ages 3 to 10 Years (n = 5). The key feature at this stage is a dramatic decrease of physiologic vessels in the EP. Conversely, the abundance of areas with obliterated vessels is increasing. With increasing structural alterations of the EP cartilage, the first cartilage cracks are seen. This process is most pronounced in the central EP. There is a substantial increase in cell death, chondrocyte density and proliferation, and granular changes, particularly in the NP.

Group 4: Ages 11 to 16 Years (n = 8). In this age group, unequivocal findings of tissue degradation can be observed. In the IVD, the substantial increase in cell death is associated with extensive chondrocyte proliferation. Structural alteration can be found abundantly in terms of cleft and radial tear formation. These alterations are most pronounced in the disc center. Notochordal cells and physiologic vessels disappear from the disc, and ar-



eas of obliterated vessels are therefore most pronounced at this stage. In the EP, cartilage cracks are frequently seen. In parallel, microfractures of the adjacent subchondral bone are present, which are associated frequently with new bone formation.

Group 5: Ages 17 to 20 Years (n = 8). As compared with Group 4, there is a steady increase in structural abnormalities such as clefting and tearing in the IVDs. Frequently, chondrocyte cloning can be observed adjacent to the structural abnormalities. There is an obvious decrease in chondrocyte proliferation, cell death, and mucoid degeneration while other abnormalities such as granular changes, clefts, and tears are still increasing in extent and frequency. Few rim lesions are seen first in this age group. Endplate abnormalities are very similar, but less pronounced than in Group 4.

Group 6: Ages 21 to 30 Years (n = 32). This stage is characterized by an increase in all histologic changes in the IVD and EP. Likewise, in the IVD, cell density, granular changes, mucoid degeneration, clefts and tears, and decaying cells are seen in increasing frequency and extent. There are few rim lesions associated with edge neovascularity. In the EP, abnormalities very similar to those in younger groups are seen, but in increasing numbers.

Group 7: Ages 31 to 50 Years (n = 11). This stage is characterized by a continued increasing frequency and extent of abnormalities in IVD and EP. Most pronounced are cell proliferation, mucoid degeneration of the extracellular matrix, granular changes in the IVD, and structural disorganization of the EP.

Group 8: Ages 51 to 70 Years (n = 9). During this stage, tissue alterations become most severe. Adjacent to significant tears and clefts, huge clones of hypertrophic chondrocytes can be found, indicating major cell proliferation. Clefts and tears are filled with granular material (granular changes). Edge neovascularity becomes most pronounced at this stage. In the EP additionally, microfractures and bone sclerosis are seen. In some of the specimens, scar formation and advanced tissue destruction resulting in tissue defects can be found.

Group 9: Ages Older Than 70 years (n = 38). In the elderly disc specimens, the structural abnormalities change more and more to scarlike tissue formation and large tissue defects. In this age group, frequently a distinction between the anatomic regions is no longer possible ("burnt-out appearance"). Therefore, distinct histologic features such as clefts and tears, cell proliferation, granular changes, cell death, and edge-neovascularity become

Table 5. Classification System for Grading Age-Related Histologic Changes in the Intervertebral Disc and Endplate

Intervertebral Disc	Endplate
Cells (chondrocyte proliferation) 0 = no proliferation, 1 = increased cell density 2 = connection of two chondrocytes, 3 = small-size clones (*i.e.,* several chondrocytes group together, *i.e.,* 2–7 cells) 4 = moderate size clones (*i.e.,* >8 cells) 5 = huge clones (*i.e.,* 15 cells) 6 = scar/tissue defects	Cells 0 = normal cellularity 1 = localized cell proliferation 2 = diffuse cell proliferation 3 = extensive cell proliferation 4 = scar/tissue defects
Mucous degeneration 0 = absent, 1 = rarely present, 2 = present in intermediate amounts 3 = abundantly present 4 = scar/tissue defects	Cartilage disorganization 0 = well-structured hyaline cartilage 1 = cartilage irregularities (obliterated vessels?) 2 = disorganized matrix with thinning 3 = complete cartilage disorganization with defects 4 = scar/tissue defects
Cell death 0 = absent, 1 = rarely present, 2 = present in intermediate amounts 3 = abundantly present 4 = scar/tissue defects	Cartilage cracks 0 = absent, 1 = rarely present, 2 = present in intermediate amounts 3 = abundantly present 4 = scar/tissue defects
Tear and cleft formation 0 = absent, 1 = rarely present, 2 = present in intermediate amounts 3 = abundantly present 4 = scar/tissue defects	Microfracture 0 = absent, 1 = present 2 = scar/tissue defects
Granular changes 0 = absent, 1 = rarely present, 2 = present in intermediate amounts 3 = abundantly present 4 = scar/tissue defects	New bone formation 0 = absent, 1 = present 2 = scar/tissue defects
	Bony sclerosis 0 = absent, 1 = present 2 = scar/tissue defects
Maximum: 22 points	Maximum: 18 points

less pronounced. In the EP, cartilage disorganization and new bone remain at the level of abnormalities, as seen in Group 8.

Development of a Histologic Classification System

Description. Based on the previous analyses, a grading system was developed involving the correlation coefficient with the outcome variables, rater reliability, and practicability of the assessment. The grading system is detailed in Table 5 as well as Figure 3A and 3B.

Reliability. Two independent raters not involved in the development of the classification system analyzed the interobserver reliability separately for the IVD, EP, and the surgical specimens. The reliability estimates (Table 6) ranged from substantial to excellent (weighted kappa, 0.493–977).

Validity. Decision tree analyses based on CHAID analyses were performed separately for the disc and endplate histology gradings. The resultant classification tables are summarized in Tables 7 and 8. In each case, the validity issue involved evaluating the ability of the histology markers to predict age and macroscopically determine disc degeneration. The results of these analyses were instructive on several levels. First, in predicting both age and macroscopic degeneration, grade disc histology markers were substantially more accurate than endplate markers. Second, in both the disc and endplate predictions of both age and macroscopic disc degeneration, the models showed significant relationships for a wide variety of the histologic markers across a wide range of locations within the disc and endplate, suggesting that both the nature and the harvest site of the histology markers are important aspects of prediction. Finally, the error rates (risk estimates) in the models may be interpreted as suggesting that histology patterns identify 16% to 31% of the cells demonstrating histology patterns that are not consistent with conventional assessments of the disc. If the histology classification is taken as the so-called gold standard, then the grading system identifies an important number of atypical conditions, in which the disc is graded as either older or younger than the chronological age, or as more or less degenerated than would be suspected by macroscopic evaluation.

■ Discussion

This is the first study systematically analyzing the histologic features of age-related changes in 180 complete lumbar motion segment slices obtained from 54 individ-

uals ranging in age range from fetal to 88 years. For each of the 180 discs, 11 histologic variables in the IVD and EP have been semiquantitatively investigated for five different regions (*i.e.,* anterior outer AF, anterior inner AF, NP, posterior inner AF, posterior outer AF) for the IVD and six regions (anterior upper and lower EP, central upper and lower EP, posterior upper and lower EP) for the EPs. Furthermore, 30 surgical specimens were explored considering three random regions with regard to five histologic variables. The 20,250 semiquantitative assessments of histologic variables have been statistically analyzed to explore the course of age-related disc changes. This thorough histologic analysis has resulted in an intriguing insight into the temporal course of age-related disc changes.

Only a few studies in the literature describe the histopathologic features of disc degeneration. The observations are variable due to both the inconsistency of the histologic parameters used for evaluation of disc degeneration and the obvious high individual and regional variability. In 1945, Coventry et al[9–11] provided the most comprehensive report to date on age-related and pathologic disc changes in complete transverse sections through lumbar motion segments. Accordingly, anular changes are characterized by a gradual loss of fine fibrous connective tissue meshwork and its replacement by increasingly hyalinized collagen fibers, the occurrence of fissures beginning in the third decade, cellular proliferation and enhanced cell death in the fourth decade, and finally the invasion of blood vessels along tears and clefts. In the nucleus pulposus of infants, residues of notochordal cell aggregates are replaced by proliferating chondrocytes, beginning in the second life decade. This is followed by the occurrence of tissue clefts, beginning in the fourth decade, and the progressive replacement of the nucleus by fibrous tissue from the fifth decade onward. Finally, Coventry et al[10] described the NP as extensively destroyed, with large spaces filled with amorphous material during the seventh decade. Most surprising are the descriptions of the changes in the EP. Here, the authors described the presence of vascular channels up to the third decade coinciding with progressive thinning and microscopic protrusions into the adjacent subchondral bone along with numerous clefts and fissures, starting in the fourth decade. Finally, the EP is replaced by fibrocartilage with focal necroses during the sixth and seventh decades.

These EP changes contrast with the observations by Wassilev and Kühnel,[46] who did not find EP vascularization beyond the fourth year of life. Unfortunately, these authors did not provide further information on the disc changes. Similarly, Saunders and Inman[40] described the IVD only as vascularized during the fetal and early postnatal periods, with progressive postnatal regression and atrophy of the vessels leading to scars and faults in the cartilage plate. These authors furthermore suggested that degeneration may occur as early as the third decade of life or even before.

Weidner and Rice[48] provided a comprehensive description of alterations in surgical specimens. These authors investigated the significance of edge neovascularity, granular changes, chondrocyte cloning, and fibrillation of fibrocartilage. The presence of these parameters was regarded as indicating disc degeneration. However, no age-related changes were identified. With respect to herniated material, only the edge neovascularity proved to provide discriminative information. Pritzker[35] analyzed the presence of mucous material in the IVD. He observed an increase in territorial positively stained matrix with age. In the EP, frequent cell death was noted. Regenerative changes in the pericellular matrix were followed by calcifications and microfractures of the adjacent subchondral bone.

In summary, the previous studies concur that there is a progressive loss of structure in all three anatomic subsettings of the IVD, finally leading to the development of a scarlike repair tissue. However, there are some interesting differences. The vascularization of the IVD is seen mostly to end with early infantile age (first decade), so that the nutritional supply is severely impaired with ongoing growth and enlargement of the disc. The occurrence of NP clefts and tears in Coventry's[9–11] study not before the fourth life decade contrasts with the current authors' observations of NP clefts and tears as early as the second decade.

Temporal Variations in Age-Related Histologic Changes

The systematic review of the histologic variables with the different regions of the IVD and EP provided clear evidence that significant age-related changes start at the end of the first life decade (Figure 2A and 2B). They became substantial in the first half of the second decade and affected the three anatomic regions to a variable extent. The EP alterations evidently preceded the NP changes, whereas the outer anulus fibrosus was pronouncedly affected only in elderly individuals. In addition, there was a high variability of changes within and across these anatomic regions.

A major hallmark of the current observations was the obliteration of blood vessels in the endplate of subjects between the age of newborns and about 10 years. Well in line is the finding that the abundance of obliterated EP blood vessels gradually increases between 1 month and 16 years. It is intriguing that this decrease in blood vessels is paralleled by a significant increase in the extent of cartilage disorganization, EP cell density, and cartilage cracks as well as microfractures. These changes all reach a first maximum in the group 11 to 16 years of age, when the disc alterations start to rise. These are characterized by a dramatic increase in clonal cell proliferation, mucous matrix degeneration, granular changes, and clefts and tears within the IVD. The NP is more severely affected in this age group than the AF.

Interestingly, the loss of vascularization falls in the age group of the most significant growth period for these

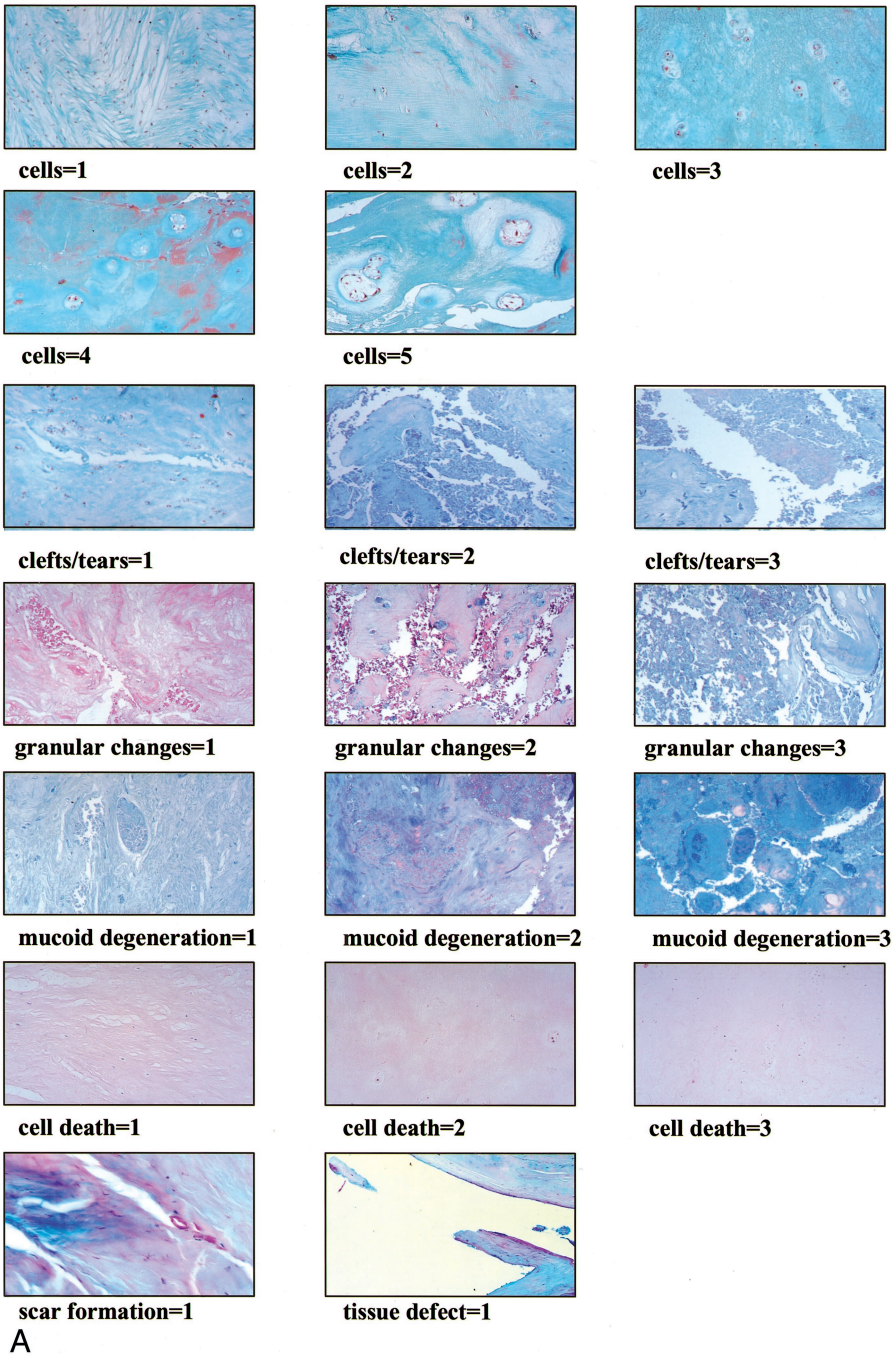
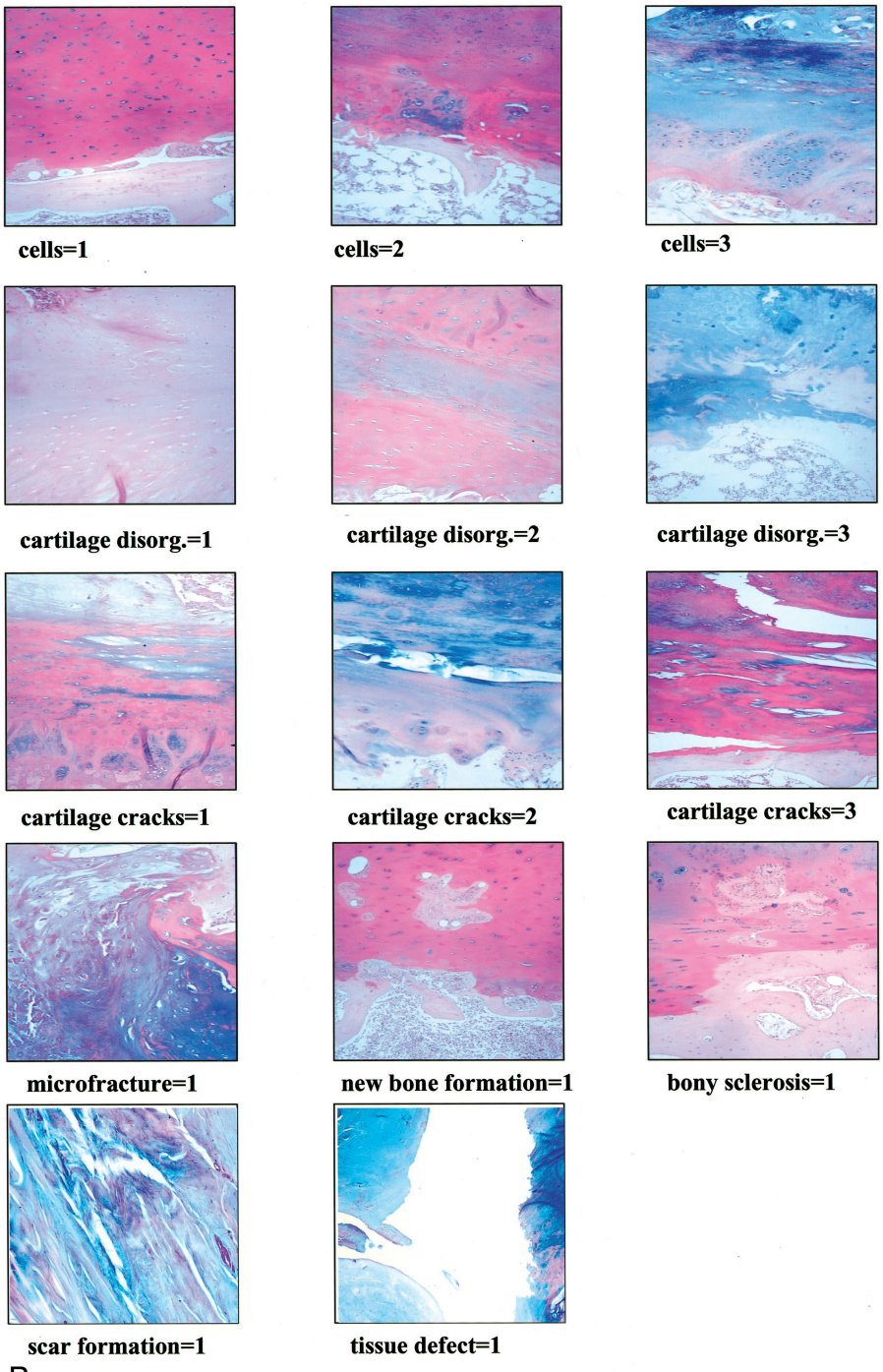


Figure 3. **A,** Histologic classification system for the intervertebral disc. **B,** Histologic classification system for the cartilaginous endplate.

Figure continues.

individuals. At that time, the nutritional status of the disc may become critical. In turn, the close contact of the EP to the extensive sinusoidal blood supply of the bone marrow makes it unlikely that the EP changes themselves are caused by altered diffusion. However, it may be assumed that during this period the growth of the disc leads to a significant elongation of diffusion distances, so that any nutritional deficiency in NP and AF may trigger tissue alteration. In the adult age groups, the extent of EP and disc alterations rises, reaching a maximum level between 51 and 70 years. Thereafter, most parameters either decrease or remain at the level of the younger age groups. The latter observations can be interpreted to represent a "burnt-out" stage, when complete disarrangement of disc and EP prevent further cell proliferation, cleft and tear formation, and mucous or granular matrix changes.

The traditional concept of a nutrition-related[15,22,28,42,43] initiation of disc degeneration can unequivocally be substantiated by the current histologic findings. This concept assumes that once skeletal maturity is reached and the epiphyses have closed in the vertebral bodies, nutrition of the disc cells becomes very critical, and exit diffusion routes for large macromolecules from the nucleus pulposus are limited. Horner and Urban[16] have provided clear evidence for the



critical effect of the nutrient supply on the viability of cells from the NP. Urban[42] has shown that a decrease in O_2 tension in the disc can lead to decreased matrix synthesis rates and a fall in intradiscal pH as a result of the increased lactate production. Factors such as decreased blood flow to the disc or a fall in endplate permeability, which will reduce O_2 tensions in the nucleus center, could have a detrimental effect on matrix metabolism and ultimately affect disc composition.[42] Declining disc nutrition, loss of proteoglycan organization and concentration, a decline in cell numerical density and synthetic activity, and increased degradative enzyme activity relative to matrix synthesis may lead to the loss of disc structure and function recognized as disc degeneration. The results of the current histologic assessments clearly support this notion by the coincidental disappearance of blood vessels from the endplate and the increase in matrix breakdown, as evidenced by the occurrence of cartilage cracks, microfractures in the EP, and tear and cleft formation in the NP.

Table 6. Interrater Reliability Estimates of Two Additional Readers Not Involved in the Assessment of Histologic Features of Disc Degeneration (Percentage Agreement/Weighted Kappa Values)

Criteria	Percent Agreement	Kappa Value	Confidence Interval
Intervertebral disc (n = 30, 5 regions)			
Cells	68.7	0.759	0.695–0.822
Mucoid degeneration	68.0	0.728	0.660–0.797
Granular changes	74.7	0.726	0.642–0.810
Tears/cleft formation	66.0	0.707	0.632–0.783
Cell death	85.3	0.806	0.727–0.885
Cartilagineous endplate (n = 30 specimens, 6 regions)			
Cells	57.2	0.493	0.398–0.588
Cartilage disorganization	76.1	0.780	0.718–0.843
Cartilage cracks	85.6	0.699	0.597–0.800
Microfracture	98.9	0.869	0.691–1.048
Bony sclerosis	95.0	0.741	0.580–0.902
New bone formation	98.9	0.977	0.947–1.009
Surgical specimen (n = 30, 3 regions)			
Cells	82.2	0.870	0.813–0.927
Mucoid degeneration	92.2	0.923	0.867–0.978
Granular changes	91.1	0.880	0.802–0.958
Tears/cleft formation	85.6	0.838	0.755–0.922
Cell death	84.4	0.773	0.662–0.884

Classification of Age-Related Changes

Based on the systematic semiquantitative analyses of the histologic variables, the authors developed a classification system for the IVD and endplate. Variables were considered to be included in the presence of a significant correlation with age and macroscopic disc degeneration. Histologic variables were excluded if they did not occur in all regions of the disc (*e.g.,* rim lesions and edge neovascularity). Because concentric tears (cleft formation) are predominantly found in the NP and radial tears are more prominent in the AF, the authors combined these two structural variables into the new variable, "max tearing" (including tears and clefts). In very advanced stages, regular disc tissue can be replaced by scar. Tissue destruction can even be so severe that a tissue defect occurs. To account for these features, scar and tissue defect was included as the most severe category for every variable where applicable. Similarly, the classification system for the endplate was developed.

During the evaluation of the authors' grading system it became evident that the application of this system is better suited for describing complete IVD sections than for describing surgical specimens. This is due to the variance of the alterations within the disc. Several parameters may be underscored with the surgical material, which are clearly evident in the complete sections. This is particularly true for any clefts and tears, which are often difficult to identify in the surgical material when small samples are extracted with major mechanical disruption of the disc tissue. Likewise, granular changes may have been lost during the tissue processing (washout), and cell death is difficult to assess correctly. Therefore, cellular parameters (cell density and proliferation) and matrix parameters (mucoid degeneration) can be regarded as appropriate for surgical disc material.

Table 7. Misclassification Matrix Based on Decision Tree Analysis

	Actual Category					
Predicted Category	1	2	3	4	5	Total
Predicting the macroscopic degeneration grade from disc histology*						
1	46	6	1	0	0	53
2	2	31	1	0	0	34
3	0	10	31	3	1	45
4	0	0	3	8	0	11
5	0	0	1	0	6	7
Total	48	47	37	11	7	150
Predicting age from disc histology†						
1	41	3	0	0	0	44
2	0	9	0	0	0	9
3	0	3	33	4	1	41
4	0	1	1	11	5	18
5	0	0	4	2	32	38
Total	41	16	38	17	38	150

* Risk estimate, 0.187; SE of risk estimate, 0.032.
† Risk estimate, 0.16; SE of risk estimate, 0.03.

In their review of the pathology of the intervertebral disc in 1940, Saunders and Inman[40] showed that the intervertebral discs are subject to continuous and progressive changes throughout life so marked that it is difficult to determine what is normal and what is pathologic. They further stated that degenerative phenomena are so frequent in supposedly healthy spinal columns at middle age that changes must be regarded for the most part as the outcome of age processes in an organ subjected to destructive forces of considerable magnitude. Differentiating normal aging from degeneration will therefore be very difficult to achieve because no "gold standard" is available. This implies that a proper assess-

Table 8. Misclassification Matrix Based on Decision Tree Analysis

	Actual Category					
Predicted Category	1	2	3	4	5	Total
Predicting the macroscopic degeneration grade from endplate histology*						
1	43	2	0	0	0	45
2	4	29	9	2	0	44
3	1	14	25	3	4	47
4	0	2	3	6	3	14
5	0	0	0	0	0	0
Total	48	47	37	11	7	150
Predicting age from endplate histology†						
1	41	2	0	0	0	43
2	0	6	2	0	0	8
3	0	4	24	3	4	35
4	0	0	0	0	0	0
5	0	4	12	14	34	64
Total	41	16	38	17	38	150

* Risk estimate, 0.314; SE of Risk Estimate, 0.038.
† Risk estimate, 0.3; SE of Risk Estimate, 0.038.

ment of the validity of the grading system is difficult to obtain. There is a consensus in the literature that disc tissue undergoes dramatic destructive and reparative changes with age. This relationship could be substantiated by the authors' systematic review. There is also a close correlation of the concomitant increase in histologic alterations and the macroscopically visible extent of tissue destruction. However, the current results clearly indicate that within gross categories of age and macroscopic alterations, substantial variation in the degree of histologic changes occur. Using decision tree analyses, the authors could demonstrate that some discs of young individuals exhibit very advanced alterations, whereas some discs of elderly individuals show only minor histologic changes (Tables 7 and 8). This implies that grading of disc material by age or macroscopic assessment is insufficient because of the substantial individual and regional variations, as shown in the current study (Tables 7 and 8). This may raise the question whether results from biochemical analyses are representative when they are assessed without a morphologic reference standard. The histologic classification system proposed in this study can serve as such a morphologic framework. However, the authors are fully aware of the preliminary character of this classification system, which deserves further vigorous testing and may require adjustments.

Research and Clinical Relevance

The key finding of this study is the morphologic evidence that the decrease in endplate blood vessels is detrimental to the maintenance of the structural integrity of the disc matrix. The current findings are in line with experimental studies on disc nutrition[15,16,28] and their impact on matrix metabolism.[16] The research and clinical relevance of this finding is that any attempt to prevent or repair the structural matrix destruction must target the vascular and nutritional supply at a very early age. Although very intriguing, recent attempts at gene therapy–related tissue engineering[21,24,25,47] are substantially hampered when the nutritional supply to the disc is not addressed. Based on the current histologic findings, tissue regeneration at a stage when disc alterations may become a source of LBP is even more difficult. The extent of histologic alteration in terms of tear and cleft formation in the IVD and microfractures in the EP already present at the end of the second life decade is a challenge to any tissue engineering and repairing attempt.[21,24,25,47] Because of the early and severe tissue alterations in lumbar IVD, the success of such concepts must be questioned with regard to their clinical relevance.

Recent studies have provided evidence of inflammatory reactions[8,18,29–31] within the disc and adjacent nerve root, which are likely to be important as a causative factor for back and leg pain development. Furthermore, it has been shown by MRI studies that even severe disc abnormalities remain asymptomatic in the majority of individuals.[4,5] These findings may direct future research efforts to convert a symptomatic disc alteration to an asymptomatic alteration by tackling the discal inflammatory reactions at a molecular level instead of attempting tissue repair.

■ Key Points

- This is the first systematic semiquantitative analyses of age-related morphologic changes in the intervertebral disc and cartilaginous endplate, which was based on 20,250 assessments of histologic variables.
- Significant temporospatial variations with regard to the presence and abundance of histologic disc alterations across levels, regions, macroscopic degeneration grades, and age groups have been demonstrated.
- The authors have developed and tested a practicable, valid, and reliable histologic classification system for lumbar discs, which can serve as a morphologic reference framework to allow for more sophisticated molecular biologic studies on the pathogenesis of aging and premature aging (degeneration) of the disc.
- This study provides clear histologic evidence for the detrimental effect of a diminished blood supply on the intervertebral disc that appears to initiate disc tissue breakdown, beginning in the first half of the second life decade.

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