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Histology of the ligamentum flavum in patients with degenerative lumbar spinal stenosis

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Abstract The degree of calcification as well as the structural changes of the elastic fibres in the ligamentum flavum in patients with degenerative lumbar spinal stenosis were evaluated and the results were compared to those of patients without spinal stenosis. In 21 patients (13 male, 8 female) with lumbar spinal stenosis the ligamentum flavum was removed, histologically processed and stained. The calcification, the elastic/collagenous fibre ratio as well as the configuration of the fibres were evaluated with an image analyzing computer. As a control group, 20 ligaments of 10 human corpses were processed in the same way. The results were statistically analysed using the Mann-Whitney-Wilcoxon test ($\alpha = 0.05$) and the t-test ($\alpha = 0.05$). Nearly all the ligaments of patients with lumbar spinal stenosis were calcified (average 0.17%, maximum 3.8%) and showed relevant fibrosis with decreased elastic/collagenous fibre ratio. There was a significant correlation between age and histological changes ($P < 0.05$). In the control group we only found minimal calcification in 3 of 20 segments (average 0.015%). No relevant fibrosis was found and the configuration of elastic fibres showed no pathologic changes. The results of this study illustrate the important role of histological changes of the ligamentum flavum for the aetiology of lumbar spinal stenosis.

Key words Lumbar spinal stenosis, ligamentum flavum, histology · Lumbar spinal stenosis, ligamentum flavum, calcification · Lumbar spinal stenosis, ligamentum flavum, elastic fibres

Introduction

Degenerative lumbar spinal stenosis may originate from formation of osteophytes and/or ligamentous hypertrophy. As the ligamentum flavum covers most of the posterior and lateral part of the lumbar spinal canal, morphological and histological changes merit special attention in the development of lumbar spinal canal encroachment [4]. Despite this fact, information concerning the calcification of the ligamentum flavum in the literature is relatively sparse, with most of the contributions addressing the problem of ligamentous hypertrophy [22, 25–27]. Other studies analyse the descriptive macroanatomy [8, 14, 15, 23] or the histology of fibre distribution and calcification of the ligamentum flavum [7, 11, 16–18, 24, 26] in a qualitative aspect, without correlation to the pathology due to degenerative changes.

The aim of this study was to quantify the degree of calcification and to document structural changes of the ligamentum flavum in lumbar spinal stenosis and to compare these data with normals.

Materials and methods

Twenty-one patients (13 men, 8 women; average age 60.7 years, range 44–84 years) underwent decompressive surgery of the spinal

canal because of signs and symptoms of degenerative lumbar spinal stenosis. The indication for the operation was based on clinical, neurophysiological and radiological findings. In standardised questionnaires, data concerning patients' history and clinical symptoms were gathered. The main clinical complaint was low back pain in 17 of 21 patients; 11 of these showed additional radicular symptoms, 4 patients had no low back pain, but only a neurogenic claudication. In all, 13 patients complained of neurogenic claudication (9 in combination with low back pain, 4 with no low back pain) with an average painfree walking distance of 150 (0–500) m. Eight patients reported not being restricted in their walking capacity, but showed marked stenosis on myelo-CT that was consistent with pain pattern and level of neurologic deficit (Table 1). All patients underwent radiological investigations with a combination of myelography and CT (myelo-CT) to confirm lumbar spinal stenosis. In five patients the stenosis was monosegmental, in 12 patients a narrowing of the spinal canal was diagnosed in two segments and in four patients in three segments.

Posterior decompression of the lumbar spine was performed with removal of the ligamentum flavum and osteophytes, with additional stabilization only in cases with instability or marked deformation. A total of 41 ligaments were harvested using a standardized technique [5 in monosegmental stenosis (5 × 1), 24 in bisegmental stenosis (12 × 2), and 12 in stenosis on three levels (4 × 3)]. Three ligaments were lost during the histological procedure, so 38 ligamenta flava were analysed (Table 2).

A ca. 3 × 3-mm piece was osteotomized to preserve the bony insertion of the ligamentum flavum for histological workup. The ligament was cut to a length of 1–1.5 cm. The biopsies were put into a solution of 4% formol (buffered) in order not to dissolve the calcification. After the histological procedure including dehydration, the samples were embedded in methylmethacrylate, stained with Giemsa, Orcein and van Kossa and cut into slices of 6 μm thickness.

Table 1 Clinical signs and symptoms and neurological defects in patients with spinal stenosis (n = 21)

	n
Neurogenic claudication	
With low back pain	9
Without low back pain	4
Low back pain	
With radicular symptoms	11
Without radicular symptoms	6
Neurologic defects	
Motorics	6
Sensitivity	12
Vibrationsensitivity	13
Reflexes	5
Pos. Lasègue	3

Table 2 Level of spinal decompression and harvesting of ligamentum flavum. The numbers in brackets are those after histological processing (loss of 3 ligaments)

Level	Mono-segmental stenosis	Bi-segmental stenosis	Tri-segmental stenosis	Total
L2/3	–	1	–	1
L3/4	–	10 (9)	2	12 (11)
L4/5	3	12 (11)	4	19 (18)
L5/S1	2	1	6 (5)	9 (8)
Total	5	24	12	41

Fig. 1 Giemsa staining (*left* = bone; *right* = ligament)

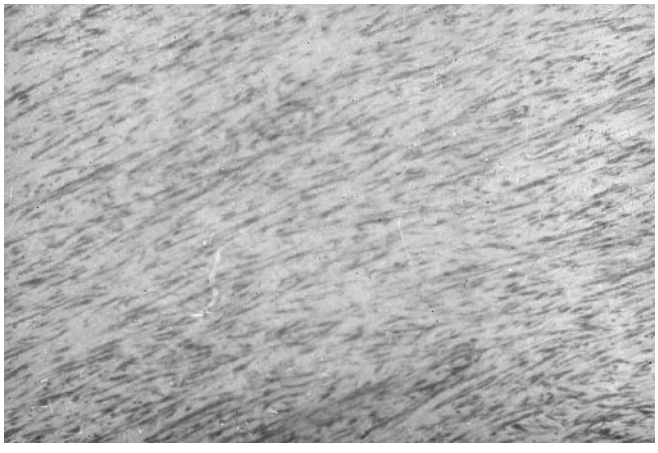

Fig. 2 Orcein staining of ligamentum flavum of controls shows strict parallel order of elastic fibres

Controls comprised ligamenta flava of 10 patients (5 male, five female; average age: 60.8 years, range 34–79 years), who died from causes unrelated to their lumbar spine and whose history, which was known in all cases, showed no evidence of spinal stenosis or other lumbar spine pathology. While taking the biopsies from the cadavers, macroscopic relevant narrowing of the spinal canal was excluded. The harvesting and processing procedure was identical to the above-described technique.

The following stains were used:

1. Giemsa: With this overview staining all important structures can be clearly identified. A quick and rough orientation is possible (Fig. 1).
2. Orcein: Elastic fibres are clearly identified as red fibres in contrast to green background (Figs. 2, 3).
3. van Kossa: The calcification of the ligament is shown selectively by black spots. Giemsa was used as background staining to identify the other structures (Fig. 4).

To determine the degree of calcification the calcified area per ligament stained with van Kossa was measured. An image analysing computer (IMCO, Kontron) was used. The images were digitized

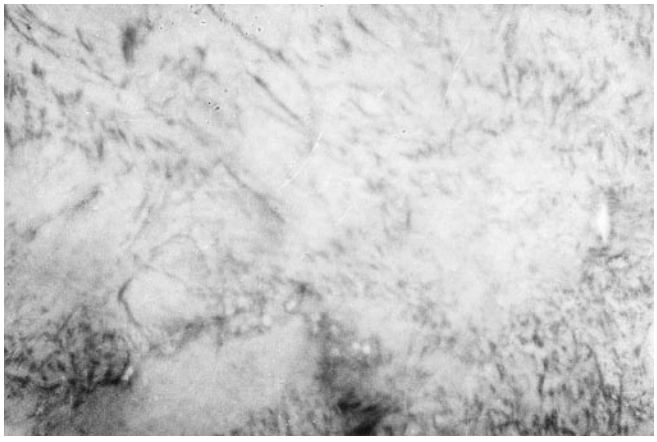

Fig. 3 Orcein staining of ligamentum flavum of spinal stenosis patients shows no parallel order of elastic fibres

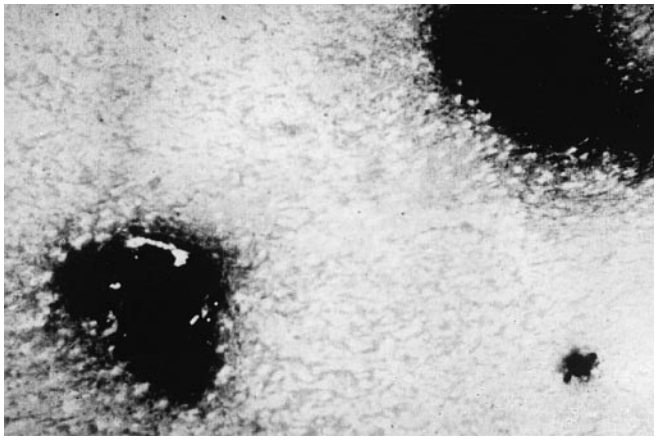

Fig. 4 van Kossa staining of ligamentum flavum in spinal stenosis: black spots indicate calcification

with an Ikegami video camera, each with an area of 512 × 512 pixels. Using this equipment, 256 grey values can be differentiated. Assisted by the software of Kontron (SMUI, Structured Multi User Interface), a structural analysis of the ligaments was possible. A Zeiss microscope with a magnification of 1.25 and a zoom factor of 1 was used to measure calcification. Each slide was divided into 2–5 sections. In each section the calcified area was measured and converted in a standardized way from pixels to square millimetres (512 pixels correspond to 4.58 mm). The total area of the ligament was measured, using a macro-switar lens (Kern) with the video camera. Again, pixels were converted to square millimetres (512 pixels correspond to 19.8 mm). With these two values, the percentage of calcification of each ligament was calculated.

The structural changes of the ligaments were also evaluated by using the image-analysing computer. The relevant parameters to be examined were: the amount of elastic fibres, the angle of orientation, and the area and the perimeter of the elastic fibres.

The statistical analysis was performed by the program RS/1 in MS-DOS. The final values, which are provided by the image analysis of a single field, are:

1. The number (n) of fibres within the field
2. The number ($n1$) of fibres oriented within the preferential orientation angle

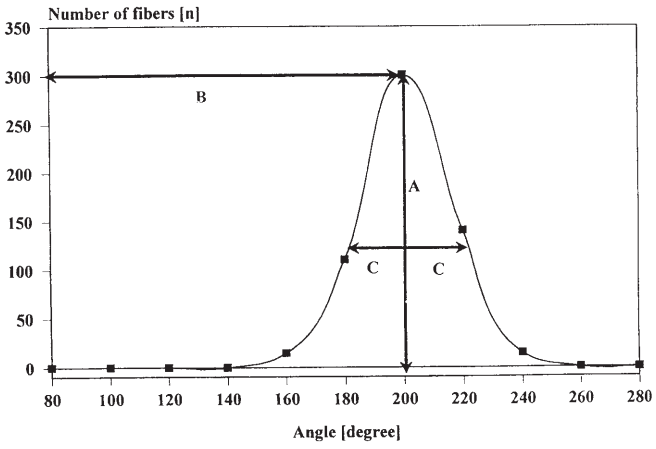

Fig. 5 Linear representation of the counts in relation to the angular orientation in control group (A = amplitude/maximal number of counts, B = preferential orientation angle, C = dispersion of the fibre orientation)

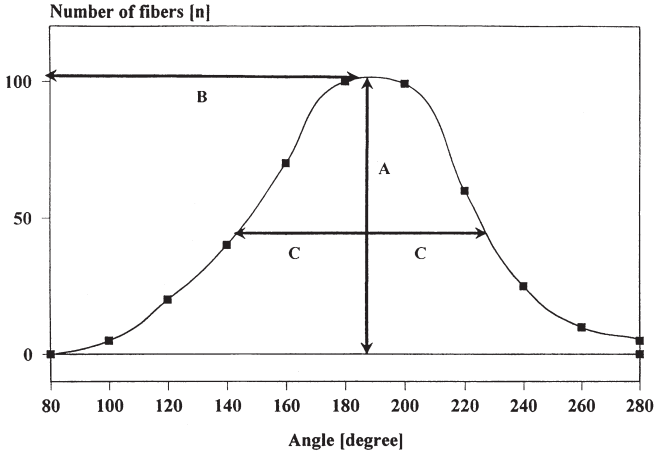

Fig. 6 Linear representation of the counts in relation to the angular orientation in spinal stenosis (A = amplitude/maximal number of counts, B preferential orientation angle, C = dispersion of the fibre orientation)

3. The average fibre area represented by the median of area values
4. The dispersion of area values represented by the lower quartile (Q1) and the upper quartile (Q2) of area values
5. The range of area values represented by the lowest (A1) and the highest (A2) area values
6. The width of the preferential angular orientation

As a strong linear correlation was observed between area and perimeter, one of these two variables is redundant, and therefore only the area was taken into account. The median and the quartiles instead of the mean value and the standard deviation were used as statistical parameters, because a strong skew was observed in the area values.

Statistical analysis of polar diagrams describes the preferential orientation using few relevant figures. If the polar values of the distribution counts are displayed linearly, the angular orientation is displayed on the x-axis and the number of counts per 20° class on the y-axis.

This plot seems to follow a normal (Gaussian) distribution (Figs. 5, 6). This is demonstrated by an appropriate curve fitting using the following equation:

$Y = \mathbf{A}\exp\,[-(X - \mathbf{B})^2/\mathbf{C}^2]$

where the three coefficients **A**, **B** and **C** represent the following:

A = amplitude/maximal number of counts
B = preferential orientation angle
C = dispersion of the fibre orientation

After this data reduction the results were statistically processed. The upper and lower 95% confidence bounds were defined. As statistical tests, the Mann-Whitney-Wilcoxon test ($\alpha = 0.05$) was used for non-normal distribution and the *t*-test ($\alpha = 0.05$) for normal distribution.

Results

Calcification

In patients with lumbar spinal stenosis, 35 of 38 ligaments were calcified (average 0.17%, maximum 3.8%, minimum 0%). As the distribution of age was heterogenous, we set the degree of calcification in relation to the age. Patients were classified into three age groups: I: those older than 68 years (15 biopsies), II: those aged 58–67 years (14 biopsies) and III: those younger than 58 years (9 biopsies). The percentage of calcification increased with age across the three groups (I > II > III) (Table 3). The results were statistically significant (Mann-Whitney-Wilcoxon test, $P < 0.05$).

In the control group, only 3 of 20 ligaments showed minimal calcification (average 0.015%, maximum 0.22%, minimum 0%). The ligaments were divided into the same three age groups as above. Only in age group I were calcified ligaments found. A relationship between age and degree of calcification could only be seen as a trend (the older the more calcified), but was statistically not significant ($P > 0.05$).

Compared to normals the average percentage calcification of the ligamentum flavum in lumbar spinal stenosis was significantly higher in our patients with lumbar spinal stenosis (0.015% vs 0.17%; $P < 0.05$).

Elastic fibres

The average number of elastic fibres per ligament was 9283 in controls (8106–12,255) and 3008 in spinal stenosis patients (2634–3543). A statistically significant reduction of elastic fibres in spinal stenosis to 32.4% of the control value was found (Student-*t* test $P < 0.05$; the average absolute number of fibres in controls was set 100%).

According to Menson and Fender the angles of the elastic fibres in non-degenerative ligamenta flava should remain approximately equal, i.e. the fibres should appear parallel [10]. To prove whether this parallel order exists, we measured every fibre angle in the ligament. The distribution of the counts of the angular orientation of the elastic fibres showed that the dispersion of the fibre orientation (**C** in Fig. 6) was 2.57 times wider in the ligamentum flavum of patients with spinal stenosis than in controls. By this equation it was proved that the parallel order of the elastic fibres of the ligamentum flavum is lost in degenerative lumbar spinal stenosis. A statistically significant correlation between age and histological changes concerning fibre orientation was found (*t*-test, $P < 0.05$).

The elastic fibres in the control group showed a strictly parallel orientation. A statistically significant correlation between age and histological changes could not be found in this group (*t*-test, $P > 0.05$; Mann-Whitney-Wilcoxon test, $P > 0.05$).

Table 3 Age-related percentage of calcification of the ligamentum flavum in patients with spinal stenosis and in the control group (*n* represents the number of ligaments)

Age group (years)	Spinal stenosis ($n = 38$)	Controls ($n = 20$)
70–75	0.336% ($n = 15$)	0.142% ($n = 6$)
60–69	0.082% ($n = 14$)	0% ($n = 8$)
< 60	0.032% ($n = 9$)	0% ($n = 6$)

Discussion

Relevant studies on ossification of spinal ligaments due to systemic diseases are mostly found in the Japanese literature. It is reported to occur predominantly in the thoracic [1, 9, 13] and cervical [12] spine, causing spinal stenosis, eventually associated with neurologic symptoms [2, 3, 13, 27]. Ossification of the ligamentum flavum in these cases is based on hypertrophy of the ligamentum flavum with cartilaginous tissue proliferation [13]. Calcification of the ligamentum flavum is reported to appear more often in combination with other degenerative changes of the spine. Avrahami et al. [1] indicates an incidence of 80% in a group of 30 patients with radiologically confirmed lumbar spinal stenosis. Baba et al. [2] reported the histologic findings of five patients who underwent lumbar decompressive surgery for cauda equina syndrome and radiculopathy secondary to degenerative stenosis associated with calcium deposition in the ligamentum flavum. Histology showed marked degeneration in elastic fibres and diffuse to massive calcium deposition in the ligamentum flavum. This was interpreted as being associated with the degenerative process in the ligament, and changes were suspected as causing or aggravating the neurological symptoms. A quantitative analysis of calcification was not performed. No information is given to describe the degree of degeneration of elastic fibres. Yoshida et al. [27] evaluated 45 cases of lumbar spinal stenosis by CT and pathologic and immunohistochemical studies. As controls, ten cases of acute disc herniation were used. Statistically significant differences in thickness and transverse area were found compared to the controls. The pathogeneses of the hyper-

tropied ligamenta flava were classified into three major groups: (1) fibrocartilage change due to proliferation of type II collagen, (2) ossification, and (3) calcium crystal deposition. Postacchini et al. [17] examined ligamenta flava obtained from nine patients with lumbar disc herniation and ten patients with lumbar spinal stenosis. The ligaments were studied at histological, histochemical, and ultrastructural levels. Controls comprised ligaments from six patients undergoing surgery for thoracolumbar fractures. In lumbar spinal stenosis, degenerating elastic fibres were seen occasionally, while calcification could be seen often. Histological findings concerning degeneration were observed in controls aged 50 or older; similar histological features could be found in patients with disc herniation of similar ages.

Calcification of the ligamentum flavum, as described in our study, represents a process of physiologic aging; we found an age-related percentage of calcification of up to an average of 0.142% in the group aged over 68 years in asymptomatics. Due to anatomical configuration and the close relation of dura and spinal nerves to the ligamentum flavum, it is obvious that this ligament may considerably contribute to the pathogenesis of lumbar spinal stenosis [1, 5, 6, 19–22, 27]. Our findings confirm that clinical symptoms of lumbar spinal canal stenosis are associated with a pathologic percentage of calcification of the ligamentum flavum; all patients with symptoms that were considered to be severe enough to indicate decompressive surgery showed deposits of calcium crystals in the ligament. As found in normals, the amount was in relation to age (statistically not significant, but a strong trend could be seen); however, compared to normals, the percentage of calcification was higher. This quantitative analysis of calcification of the ligamentum flavum proves that this degenerative process can cause sciatic or neurological clinical findings in lumbar spinal stenosis.

A strict parallel order of the elastic fibres in the ligamentum flavum shown by Menson and Fender [10] is characteristic of healthy specimen and our findings are consistent with these results. Our study also showed that this parallel order could no longer be found in the ligamenta flava in lumbar spinal stenosis. These findings are consistent with the results of Yoshida et al. [27]. Postacchini et al. [17] found age-related changes in the ligamentum flavum, too. These findings are consistent with our results. Thus, we think that in addition to the explanation of reduced elasticity of the ligamentum flavum, a concomitant increase of volume of the ligament due to this "disorder" and the reduction of elastic fibres may contribute to the pathogenesis of lumbar spinal canal stenosis.

Many authors [1, 2, 5, 6, 17, 19–22, 27] have previously described a relationship between changes of the ligamentum flavum and degenerative lumbar spinal stenosis. In this study, for the first time, the degree of calcification and the structural changes of the elastic fibres are quantified.

Conclusion

We conclude that the conflict of interest in available space in the spinal canal between neural structures and the surrounding osteo-ligamentous tissue is partly the result of degenerative changes of the latter. The degeneration of the ligamentum flavum involves histological changes with increased calcification and decreased number and loss of parallel order of the elastic fibres.

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