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IMMUNOMORPHOLOGICAL CHARACTERISTICS OF THE LIGAMENTUM FLAVUM IN THE LUMBAR SPINAL CANAL IN CASES OF DEGENERATIVE STENOSIS

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Relevance. Research into the morphological, cellular, and molecular mechanisms underlying hypertrophy of the ligamentum flavum and postoperative epidural fibrosis continues globally (4, 5). Studies on degenerative stenosis of the lumbar spinal canal primarily focus on improving clinical and radiological diagnostics, as well as surgical decompression techniques (1, 2, 3).

Objective. To investigate the immunomorphological profile of the ligamentum flavum in the lumbar spinal canal in the context of degenerative stenosis.

Material and Methods. This study is based on the examination and surgical treatment of 86 (40.2%) patients diagnosed with degenerative stenosis of the lumbar spinal canal accompanied by hypertrophy of the ligamentum flavum. In our retrospective comparative analysis, all patients were divided into subgroups based on the clinical course—favorable (54/86 patients; 62.7%) or unfavorable (32/86 patients; 37.3%)—depending on the progression of epidural scar tissue.

The reference group consisted of 40 (18.7%) patients who underwent surgery for a lumbar intervertebral disc herniation, with no MRI evidence of ligamentum flavum hypertrophy or clinically significant degenerative spinal stenosis.

Results and discussion. An initial assessment of the histological specimens revealed a progressive structural shift: the relatively well-preserved elastic architecture was gradually replaced by a dense collagenous matrix. In contrast, the reference tissue exhibited elastic fibers organized primarily into continuous, parallel bundles, interspersed with thin layers of collagen and a sparse cellular population. In cases with a favorable postoperative course, fibrous replacement was more pronounced, yet areas of a continuous elastic framework remained intact. Conversely, in the subgroup of

patients with an unfavorable postoperative course, the tissue was characterized by extensive collagenized zones, numerous ruptures and shortening of elastic fibers, increased cellularity, and focal inflammatory infiltration. The area of fibrosis increased progressively—from 21.24±1.35% in the reference group to 36.28±1.37% in the subgroup with a favorable postoperative course ($t=-7.81$, $p<0.001$)—while in patients with progressive epidural fibrosis, this figure reached an average of 47.90±1.73%. The difference between these two postoperative outcomes was 11.62% ($t=-5.27$, $p<0.001$), with the observed changes corresponding to an expansion of the collagenous component, which, in the subgroup with an unfavorable course, accounted for, on average, more than half of the total area examined.

The combination of reduced surface area and disrupted fiber continuity proved more informative than either feature alone, as it reflected not only the quantity of elastin but also the loss of its functional architecture; consequently, the collagen-to-elastin ratio increased in proportion to the extent of tissue remodeling.

The immunohistochemical profile of SPP1 expression showed a progressive increase in both the intensity and the extent of the positive reaction—ranging from the reference tissue, where only isolated, weakly positive cellular elements with faint cytoplasmic staining were observed, to the pattern associated with an unfavorable postoperative course.

In cases with a favorable outcome, SPP1 expression was moderate and focal; positive staining was seen in spindle-shaped cells distributed sparsely or in small clusters along segments of the fibrous stroma. Large areas showing little or no reaction persisted between these immunopositive zones, resulting in a non-uniform distribution of SPP1 without the formation of confluent, intensely stained fields. In contrast, samples from patients who subsequently developed progressive epidural fibrosis exhibited pronounced, widespread SPP1 expression.

In the unfavorable scenario, an intense cytoplasmic reaction was detected in numerous spindle-shaped cells, which were oriented primarily along densified fibrous bundles. These positive elements occupied a significant portion of the examined field and were accompanied by an increase in overall cellular density.

The density of CD68-positive cells increased from 28.07±1.06 cells/mm² to 35.12±0.97 cells/mm² ($t=-4.92$, $p<0.001$) and 42.16±1.14 cells/mm² ($t=-9.06$, $p<0.001$ compared to the reference group; $t=-4.72$, $p<0.001$ compared to the favorable clinical course). Even more notable were the changes in SPP1; its H-score in the unfavorable subgroup reached 105.35±3.12 units ($t=-6.12$, $p<0.001$ vs. the reference group)—a value 21.61 units higher than that observed in the favorable course subgroup ($t=-5.50$,

p<0.001). We interpreted the combination of dense macrophage infiltration and pronounced SPP1 expression as an indicator of an active immuno-fibrotic microenvironment; however, in the absence of double staining, SPP1 could not be attributed exclusively to macrophages. Concurrently, TGF-β1 and nuclear p-Smad3 levels showed a consistent, unidirectional trend: the TGF-β1 H-score was 69.23±2.68 units in the reference tissue, 95.14±2.70 units in the favorable course subgroup (t=-6.81, p<0.001), and 109.24±2.60 units in the unfavorable course subgroup. (t=-3.76, p<0.001), whereas for p-Smad3, the corresponding values were 61.14±2.51 units, 80.87±2.43 units (t=-5.65, p<0.001), and 95.70±2.69 units (t=-9.40, p<0.001 and t=-4.09, p<0.001, respectively); this consistency in direction supported a sequence leading from a local profibrotic signal to intracellular Smad-dependent activation. Subsequently, an expansion of the α-SMA-positive interstitial area reflected an increase in the proportion of cells with a myofibroblastic phenotype; this was most pronounced in cases with an unfavorable clinical course (t = -2.67, p = 0.010, compared to a favorable course). In these instances, the expansion was associated with a larger area of type I collagen. Type III collagen also increased, though the intergroup difference was less marked (t = -2.37, p = 0.020). The ratio of type I to type III collagen did not reach statistical significance in any of the pairwise comparisons (t = -0.71, p = 0.479 and t = -1.31, p = 0.196 when compared to the reference group for each subgroup; t = -0.71, p = 0.481 between subgroups of patients in the control group). Consequently, the unfavorable phenotype was determined primarily by the total volume of the collagen matrix and the intensity of myofibroblastic activity, rather than by a specific shift in the relative composition of the two collagen types studied. Furthermore, the distributions of five key tissue markers showed partial overlap between groups, precluding an artificial, absolute separation of patients. Nevertheless, the median values for the area of fibrosis, CD68, SPP1, and α-SMA consistently shifted toward higher values, while the residual area occupied by elastic fibers decreased.

Preserved elastic architectonics were observed in 26/40 (65.0%) patients in the reference group and in 12/54 (22.2%) patients with a favorable clinical course (χ^2 = 17.46, p < 0.001), but were entirely absent in the unfavorable subgroup. Conversely, diffuse collagenization was identified in 21/32 (65.6%) patients with progressive fibrosis—a frequency nearly double that observed in the group with a favorable course (12/54 patients; 22.2%; χ^2 = 8.45, p = 0.004). Similar trends were noted regarding a high macrophage component (found in 1/40 reference group patients, 13/54 patients with a favorable course, and 22/32 patients with an unfavorable course) and expanded α-SMA-positive areas (observed in 4/40 reference group patients, 17/54 patients with

a favorable course, and 21/32 patients with an unfavorable course). A combined "highly fibrotic" phenotype—characterized by an extensive area of fibrosis, elevated SPP1 and TGF-β1 levels, and an expanded α-SMA-positive interstitial zone—was present in 13 (40.6%) patients in the unfavorable subgroup ($\chi^2 = 19.83$, $p < 0.001$ vs. the reference group; $\chi^2 = 10.17$, $p = 0.001$ vs. the subgroup with a favorable course) and in only 6 (11.1%) patients with a favorable course ($\chi^2 = 4.75$, $p = 0.029$ vs. the reference group), while it was entirely absent in the reference group. ...patients confirmed that this might not be a matter of an isolated, incidental marker, but rather a coordinated cellular-matrix remodeling.

Thus, the initial state of the ligamentum flavum in the lumbar spinal canal—in cases where epidural fibrosis subsequently progressed—was characterized by a greater extent of fibrosis and collagen deposition, a marked loss of elastic fibers, increased CD68- and SPP1-associated activity, activation of the TGF-β1/Smad-dependent pathway, and an expansion of the myofibroblastic compartment. However, even with these immunohistochemical features, such tissue reflected only the phenotype present at the time of surgery; a detailed assessment of whether this phenotype persists following the removal of the ligament would require tracking the dynamics of systemic immunological markers.

Conclusions:

1. The initial state of the ligamentum flavum in the lumbar spinal canal, in cases where epidural fibrosis subsequently progressed, was characterized by a greater extent of fibrosis and collagen deposition.

2. There was a marked loss of elastic fibers, increased CD68- and SPP1-associated activity, activation of the TGF-β1/Smad-dependent pathway, and an expansion of the myofibroblastic compartment.

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