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CLINICAL CASE SERIES

Outcome of Lumbar Epidural Steroid Injection Is Predicted By Assay of a Complex of Fibronectin and Aggrecan From Epidural Lavage

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Study Design. A single-center, prospective, consecutive case series of patients undergoing epidural lavage before the treatment of radiculopathy due to lumbar disc herniation.

Objective. To determine whether a novel complex of fibronectin and aggrecan predicts clinical response to epidural steroid injection (ESI) for the indication of radiculopathy from lumbar herniated nucleus pulposus (HNP).

Summary of Background Data. ESI for lumbar radiculopathy due to HNP is widely used despite variable effectiveness for this indication. With increased attention aimed at cost containment, it would be beneficial to identify those in whom ESI may be helpful. There are currently no accurate diagnostic tests to predict response to ESI in back pain and sciatica syndromes. We have previously investigated biomarkers of disc degeneration associated with radiculopathy.

Methods. We embarked to determine whether a molecular complex of fibronectin and aggrecan predicts clinical response to ESI for the indication of radiculopathy from HNP. This prospective study was conducted at a single center and included 26 patients with radiculopathic pain and magnetic resonance imaging positive for HNP, who elected ESI. Epidural lavage with physiologic saline was performed immediately before ESI. The lavage fluid was assayed for the fibronectin–aggrecan complex (FAC) by using a heterogeneous sandwich enzyme-linked immunosorbent assay. The results were compared with the interval improvement in the physical component summary (PCS) score of the Medical Outcomes Study Short Form-36 instrument (SF-36) after injection compared with baseline.

Results. The mean improvement from baseline PCS in patients with the FAC was 22.9 (SD, 12.4) and without the complex was 0.64 (SD, 3.97; $P < 0.001$). Differences in total SF-36 improvement were also highly significant ($P < 0.001$). The presence of the FAC predicts a clinically significant increase in PCS after lumbar ESI by receiver-operating-characteristic analysis (area under the curve = 0.97; $P < 0.001$). There was no significant difference in age ($P = 0.25$), sex ($P = 0.84$), laterality ($P = 0.06$), lumbar spinal level ($P = 0.75$), or payer type (worker's compensation *vs.* private insurance; $P = 0.90$) between groups with and without the marker.

Conclusion. A molecular complex of fibronectin and aggrecan predicts response to lumbar ESI for radiculopathy with HNP. The biomarker is accurate, objective, and not affected by demographic or psychosocial variables in this series.

Level of Evidence. Diagnostic level of evidence II.

Key words: biomarker, epidural steroid injection, radiculopathy, herniated nucleus pulposus, fibronectin, aggrecan. **Spine 2011;36:1–6**

Protein biomarkers associated with lumbar disc disease have been studied as diagnostic indicators and therapeutic targets. A complex molecular and cellular cascade of disc degeneration has been elucidated, which involves inflammatory mediators (*e.g.*, cytokines, nitric oxide, and signal transduction pathways), structural proteins and their degradation fragments (*e.g.*, fibronectin, aggrecan, and collagens), and proteases/protease inhibitors (*e.g.*, matrix metalloproteinases [MMPs] and aggrecanases).[1–6] Numerous disease-modifying therapies have been proposed to intervene in this cascade, including antibody therapies,[7,8] stem cell and cellular therapies,[9] and gene therapies.[10]

Less attention has been given to molecular biomarkers that predict response to established treatments. Recently, cytokines biomarkers have been shown to predict response to lumbar epidural steroid injection (ESI) in patients with radiculopathy and herniated nucleus pulposus (HNP).[11] The identification of biomarkers predictive of response to ESI is important, since there is only fair evidence that ESI is moderately effective for short-term relief from radiculopathy with HNP.[12] If molecular biomarkers predict which patients respond better relative to all comers, more effective therapy is possible.

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Since cytokines are associated with response to ESI, molecular biomarkers associated with cytokine activity may also be predictive. Inflammatory cytokines are associated with structural matrix proteins and their degradation fragments, including fibronectin[13] and aggrecan,[14] in degenerative disease of the synovial joints. In degenerative disc diseases, the roles of fibronectin[5] and aggrecan[15] have also been confirmed, along with MMPs and tissue inhibitors of matrix proteinases.[16,17] Investigating the relationship among cytokines, fibronectin, and aggrecan in disc disease, we identified a molecular protein complex of fibronectin and aggrecan in degenerative discs.[18] The protein complex was isolated by mass spectrometry and immunologic methods. A heterogeneous enzyme-linked immunosorbent assay (ELISA) was developed for detection of the complex by using an antibody against one protein for capture and against the other protein for detection. Assay conditions were optimized to minimize background signal, and individual proteins were used as negative controls.[18]

In the present study, we sought to prospectively validate the fibronectin–aggrecan complex (FAC) as a biomarker for response to ESI for radiculopathy with HNP. We measured levels of the protein complex in the epidural space of patients undergoing lumbar ESI for radiculopathy with HNP. We assessed functional outcomes at baseline and after treatment with the physical component summary (PCS) subscale of the Short Form (SF-36). Our hypothesis was that the complex is present in patients with clinically significant functional improvement after ESI.

MATERIALS AND METHODS

Subjects

Independent institutional review board approval was obtained (Sterling, Inc, Atlanta, GA), and all patients provided informed consent for study participation. Patients considered as candidates for ESI were 18 years of age or older with a history of leg sensory complaints, primarily dictated by pain with associated sensory symptoms and/or low back pain for 2 weeks or more, who had failed expectant management with nonsteroidal anti-inflammatory drugs (NSAIDS), activity modification, and/or physical therapy. The leg symptoms were consistent with a dermatomal pattern due to radiculopathy in one or more lumbar nerve root distributions with magnetic resonance imaging evidence of HNP in the symptomatic distribution, with or without lumbar spinal stenosis. Distributional leg sensory symptoms were of acute onset with or without a clear inciting event but were not attributable to a history of baseline chronic pain in the same distribution. Inclusion criteria were as follows: acute onset of sensory symptoms with the primary complaint as pain, as well as varying sensory symptoms (*e.g.*, tingling and numbness) in one or more lumbar nerve root distributions; positive physical examination findings, including sensory findings consistent with a spinal nerve root, a positive straight leg raise test, and/or diminished patellar or Achilles deep tendon reflexes consistent with sensory symptoms; and magnetic resonance imaging of lumbar spine positive for HNP in a distribution correlating with physical examination. Exclusion criteria were as follows: plain radiography demonstrating severe loss of disc height, high-grade degenerative disc disease, and spondylolisthesis greater than grade I; a history of prior lumbar surgery or trauma and weakness in a consistent distribution (nonprogressive with strength at least four of five); red flags, including progressive weakness, bowel/bladder complaints, radiographic unknown mass, and unexpected weight loss; and diagnosis of inflammatory arthritides, crystalline arthropathies, or other rheumatologic diseases. Patients were recruited from the private practice of a single board-certified and fellowship-trained orthopedic spine surgeon during the period March 2008 to April 2009. SF-36 was self-administered at baseline and at final follow-up at a minimum of 1 week from injection. [AQ3]

Sample Acquisition, Storage, and Preparation

At the time of ESI, epidural lavage was undertaken before injection, as previously described.[19] Briefly, the patient was positioned prone on a radiolucent table, and monitored anesthesia was induced. After preparation with 1% povidone iodine, a 16-gauge spinal needle was placed from a caudal approach with the use of C-arm fluoroscopy in multiple planes. A nylon catheter was placed through the needle before removal, and the level of the catheter was localized by fluoroscopy to the disc space of interest. Epidural lavage was undertaken by injection/aspiration of approximately 3 cc of 0.9% normal saline without preservative by the use of a 10-cc syringe with luer-lock attachment. After lavage, epidural injection was undertaken with 80 mg of methylprednisolone acetate (Depo-Medrol, Pfizer Inc, New York, NY) in 2 cc of 1% lidocaine hydrochloride (Xylocaine, APP Pharmaceuticals, Schaumberg, IL) and 2 cc of 0.9% normal saline. The lavage fluid was aliquoted into a sterile polypropylene tube and frozen at −80°C until the time of sample analysis.[20] At the time of analysis, each patient sample was thawed to room temperature, clarified by centrifugation at 5000 g, and filtered by using 0.45-μm low protein-binding filter. The collected filtrate was immediately assayed as described later.

ELISA Analysis

A heterogeneous, sandwich ELISA was developed and validated on a prior series of patients.[18] This assay detects a protein complex of fibronectin and the aggrecan G3 domain. Briefly, antiaggrecan G3 domain antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in phosphate-buffered saline/tween 20/thimerosal was used to coat a 96-well microplate. The plate was treated with bovine serum albumin in the same buffer overnight at 4°C to block excess binding sites and then washed with six washes of phosphate-buffered saline/tween 20/thimerosal. The centrifuged and filtered sample was aliquoted at three serial dilutions in triplicate into the microplate and incubated for 1 hour to facilitate binding of the complex to the immobilized antibody. After washing six times with the wash buffer, antifibronectin antibody labeled with HRP (US Biologic, Swampscott, MA) was added and incubated for 1 hour. After six washes, the tetramethylbenzidine substrate was added and the reaction product measured by optical

density (OD) at 450 nm wavelength. Human fibronectin (BD Biosciences, San Jose, CA) at 10 μg/mL concentration was used as a negative control.

STATISTICAL METHODS

Data Analysis

Data were analyzed by *t* test, Wilcoxon rank sum test, Kolmogorov-Smirnov test, and two-by-two contingency table analysis. *A priori* power analysis was performed. The power of *t* tests is 84% for N = 26 subjects and α value of 0.05, assuming a very large effect size (Cohen d = 1.2). receiver-operating-characteristic (ROC) analysis was undertaken on the two-by-two contingency table, comparing SF-36 scores and molecular-complex data.

RESULTS

Twenty-six patients enrolled over the period March 2008 to April 2009. There were 19 men and 7 women with mean age of 45.9 ± 13.5 years. There was one symptomatic level for 24 patients (92.3%), and there were two symptomatic levels for 2 patients (7.7%). The most cranial disc level was L3–L4 for 4 patients (15.4%), L4–L5 for 15 patients (57.7%), and L5–S1 for 7 patients (26.9%). Symptoms were unilateral in 23 patients (88.5%) and bilateral in 3 patients (11.5%), including one of the patients with two symptomatic levels. There were 17 patients (65.4%) with private insurance and 9 patients (34.6%) with worker's compensation insurance.

Baseline SF-36 scores were collected on the day of injection for 23 patients (88.5%), 1 day before injection for 1 patient (3.8%), and 2 days prior for 2 patients (7.7%). Follow-up SF-36 scores were collected at a mean of 6.4 weeks (SD, 2.1 weeks; range, 10 days–10 weeks). The mean improvement in the PCS scale of the SF-36 instrument was 10.9 (SD, 14.3; range, −7 to 43), and the mean improvement in SF-36 total score was 9.6 (SD, 12.2; range, −5 – 44). For the purposes of further analysis, the treatment group was dichotomized into two groups: "responders" to treatment and "nonresponders" to treatment. Responders were defined as patients with a PCS improvement greater than 4.9, the minimum clinically important difference (MCID) for the PCS.[21] Nonresponders were defined as having PCS improvement less than the MCID. This definition was adopted to be independent of the biochemical assay and to divide the treatment group into responding and nonresponding subgroups. There were 13 (50%) responders with a mean PCS improvement of 21.9 (SD, 12.4; range, 6 – 43) after treatment, and 13 (50%) nonresponders with a mean PCS change of −0.08 (SD, 3.4; range, −7–4); the difference was highly significant, illustrating a clear dichotomy between responders and nonresponders ($P < 0.001$). Figure 1 illustrates these results.

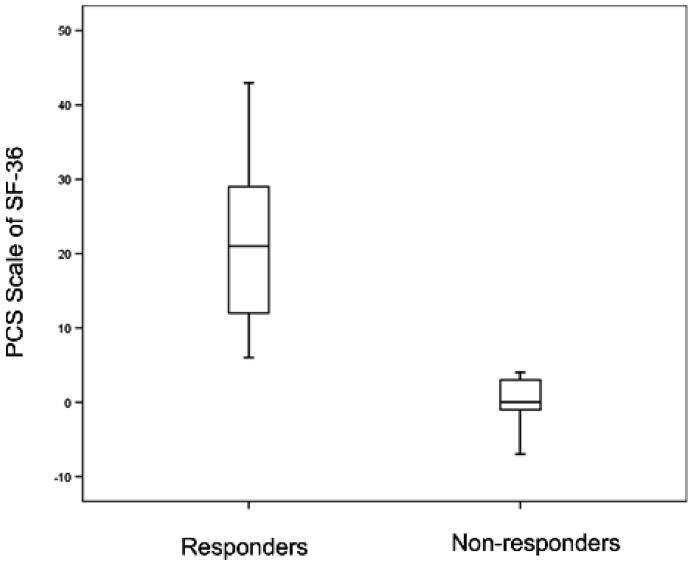

Figure 1. Box-and-whiskers plot comparing improvement over baseline PCS in responders *versus* nonresponders. Response is defined relative to the MCID for the PCS and is independent of the molecular assay. The y-axis is the PCS score. The center of the box is the median. The ends of the box are upper and lower quartiles. The whiskers are the maximum and minimum values. The differences are clinically and statistically significant ($P < 0.001$), indicating that responders and nonresponders clearly dichotomize into two distinct groups.

The FAC was below the limit of detection (LOD) of the heterogeneous ELISA assay[18] in 14 patients (50%). In the 12 patients with detectable concentrations, the OD at 450 nm had a mean value of 1.03 OD (SD, 1.92; range, 0.06–6.53 OD). Between groups with and without the complex, the mean difference in PCS score improvement was highly significant at 22.3 (95% confidence interval [CI] = 14.2–30.4; $P < 0.001$), and the mean difference in SF-36 total score improvement was highly significant at 20.4 (95% CI = 12.4–28.5; $P < 0.001$). Figure 2 illustrates these results. Between groups with and without the complex, there was no significant difference in age ($P = 0.25$), sex ($P = 0.84$), laterality ($P = 0.06$), lumbar spinal level ($P = 0.75$), or payer type (worker's compensation *vs.* private insurance; $P = 0.90$).

In this series, the presence of the FAC was a statistically significant predictor of response to ESI (improvement in PCS > MCID) (area under the curve = 0.97; $P < 0.001$). Table 1 illustrates these results. Figure 3 illustrates the ROC curve for the two-by-two contingency table analysis of the FAC *versus* PCS score, allowing the cutoff value of PCS score to vary.

DISCUSSION

The present study demonstrates that a FAC is present in the epidural lavage fluid of patients with radiculopathy and HNP and that the presence of the complex predicts clinically significant response to ESI. Advantages of this study include a prospective design, a well-validated clinical instrument, a prospectively defined MCID, a prospectively defined LOD, and well-defined inclusion/exclusion criteria for a common intervention and indication. The primary finding of the study is that a molecular complex of fibronectin and aggrecan is a biomarker for the painful state that is likely to respond to ESI.

It was recently reported that lavage fluid from the epidural space demonstrates immunoreactivity to interferon-γ and that the degree of immunoreactivity correlates with pain relief after ESI for radiculopathy with HNP.[11] The relationship between inflammatory cytokines and structural

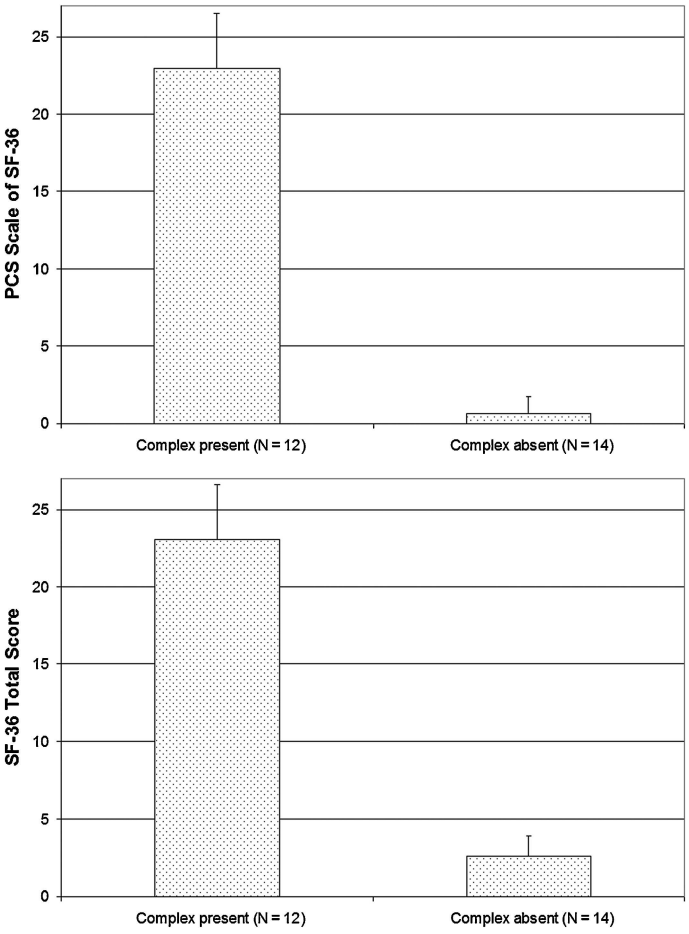

Figure 2. Bar graphs depicting improvement over baseline PCS (posttreatment minus pretreatment) (left) and the improvement over baseline SF-36 total score (posttreatment minus pretreatment) (right) for groups with the fibronectin–aggrecan complex present *versus* absent. The y-axis is the mean score of the clinical instrument. The error bars are the standard error of the mean. Both differences are clinically and statistically significant ($P < 0.001$).

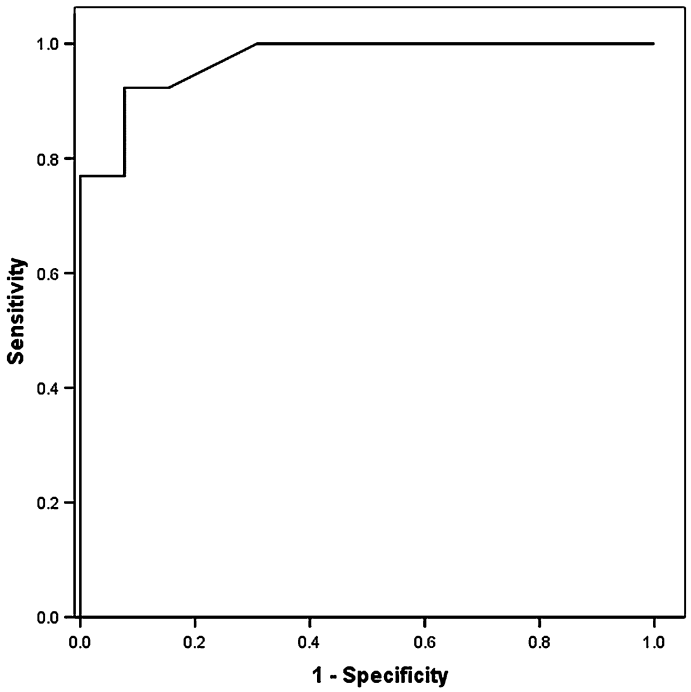

Figure 3. Receiver-operating-characteristic (ROC) curve for the two-by-two contingency table comparing the presence of the fibronectin–aggrecan complex to PCS score. The y-axis is the sensitivity and the x-axis is the specificity (also known as the false-positive rate). The curve is generated by allowing the cutoff value of PCS score to vary. ROC analysis is a graphical representation of the tradeoff between sensitivity and specificity inherent in any test. Here, the tradeoff is controlled by choosing a cutoff value for PCS. The difference between the ROC curve and the null hypothesis curve is highly significant ($P < 0.001$), indicating an effective test.

matrix proteins in the pathophysiology of degenerative joint diseases, though well established, continues to be elucidated. Inflammatory cytokines are associated with fibronectin and its fragments in degenerative disease of the intervertebral disc[5] and synovial joints.[13] Aggrecan and its fragments have also been implicated in degenerative disease of the intervertebral disc,[15] as have aggrecanases[16] and tissue inhibitors of matrix proteinases.[17] In synovial joints, aggrecan cleavage is associated with fibronectin fragments.[14] The molecular complex of fibronectin and aggrecan was first purified from the synovial fluid of painful knees with meniscal pathology. In the knee, this complex is associated with other proinflammatory cytokines and exhibits cross-immunoreactivity with interferon-γ in some commercial antibodies.[22] These observations point to a hypothetical role for the FAC as a matrix degradation product involved in an inflammatory cascade, which mediates pain in patients with radiculopathy and HNP. In summary, the relationships among cytokines and other inflammatory mediators, structural proteins and their cleavage products, and proteinases and their inhibitors is complex and still merits further investigation. [2,23]

The identification of biomarkers predictive of response to ESI for radiculopathy with HNP may be an important element in the continued utility of this treatment modality.

With current clinical selection criteria, there is only fair evidence that ESI is moderately effective for short-term relief from radiculopathy with HNP.[12] Evidence is poorer for patients with chronic symptoms or back *versus* leg pain,[24] and evidence is more limited regard-

TABLE 1. Two-By-Two Contingency Table for the Presence of the Fibronectin–Aggrecan Complex in the Responder and Nonresponder Groups to ESI

	Responders to Injection	Nonresponders to Injection
Fibronectin–aggrecan complex present	12	2
Fibronectin–aggrecan complex absent	1	11

ing improvement in self-reported functional outcome as opposed to pain relief.[25] Several clinical trials and animal data have shown no significant difference between infiltration with corticosteroids *versus* local anesthetic alone for radiculopathy and HNP,[26–28] a surprising result, given the pharmacologic half-lives of these drugs. Taken together, our findings raise the possibility that a subset of patients (1) have biomarker evidence of inflammation and/or pain, (2) are more likely to respond to depot steroid preparations (or other controlled delivery drugs) *versus* local anesthetic infiltration alone (without depot delivery), and (3) are more likely to respond vigorously with improved functional outcome after ESI. This investigation represents one step in establishing tools for improving response to ESI for radiculopathy.

The present study has several limitations. Sample acquisition by epidural lavage is a technically challenging clinical procedure that must be performed through a caudal approach with the use of a catheter, it and has not yet been successful with a transforamenal approach using a 22- to 24-gauge needle.[19] This necessitates ESI by the caudal approach as well, which may contribute to differential response *versus* a transforamenal approach. The procedure itself may result in a small volume of aspirate after injection/aspiration of a variable amount of diluent.[19] This factor, in conjunction with the biology of the disease process,[29] may result in a wide range of concentrations for biomarkers assayed, requiring a sensitive assay and inducing floor or ceiling effects typical of immunoassays.[11] In the present study, these issues are addressed with the use of a sensitive heterogeneous ELISA, though the lack of a synthesizable positive control makes absolute quantification impossible. A reference standard and positive control for the FAC must be synthesized in large scale to generate a standard curve by which the OD (at 450 nm optical wavelength) can be converted to an absolute concentration. In addition, different values for the LOD would yield different estimates of positive *versus* negative test values. Clinically, the degree of tension signs on physical examination was not quantitated as an examination correlate of acute inflammation. In the future, it may be possible to correlate the severity of tension signs with molecular markers of inflammation. At a practical level, there are several challenges in developing the current results into a clinically useful test. Currently, the sampling method is as invasive as the injection itself, requiring cannulation of the epidural space. It is conceivable that a serum marker could be developed or that a molecular imaging strategy could be employed. However, optimization of the current assay into a point-of-care test may be feasible, leveraging an epidural catheter and a bedside optical immunoassay instrument, allowing immediate results.

Nonetheless, we believe that the biomarker complex identified represents a bonafide and independent indicator of ESI-responsive patients. Large trials, possibly with different sampling and treatment protocols are warranted.

➢ Key Points

- ❑ There are currently no accurate diagnostic tests to predict response to ESI in back pain and sciatica syndromes.
- ❑ We investigated a FAC that we identified in the epidural space in patients who improved after ESI.
- ❑ The FAC predicts response to lumbar ESI for radiculopathy with HNP.

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