

Clinical Study

Does the presence of the fibronectin-aggrecan complex predict outcomes from lumbar discectomy for disc herniation?

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Abstract

BACKGROUND CONTEXT: Protein biomarkers associated with lumbar disc disease have been studied as diagnostic indicators and therapeutic targets. Recently, a cartilage degradation product, the fibronectin-aggrecan complex (FAC) identified in the epidural space, has been shown to predict response to lumbar epidural steroid injection in patients with radiculopathy from herniated nucleus pulposus (HNP).

PURPOSE: Determine the ability of FAC to predict response to microdiscectomy for patients with radiculopathy due to lumbar disc herniation

STUDY DESIGN/SETTING: Single-center prospective consecutive cohort study.

PATIENT SAMPLE: Patients with radiculopathy from HNP with concordant symptoms to MRI who underwent microdiscectomy.

OUTCOMES MEASURES: Oswestry disability index (ODI) and visual analog scores (VAS) were noted at baseline and at 3-month follow-up. Primary outcome of clinical improvement was defined as patients with both a decrease in VAS of at least 3 points and ODI >20 points.

METHODS: Intraoperative sampling was done via lavage of the excised fragment by ELISA for presence of FAC. Funding for the ELISA was provided by Cytonics, Inc.

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Conflict of interest is always a potential confounding factor in studies. Cytonics, Inc. funded the cost of the ELISA for this study (C). Authors including SRG and GJS are paid consultants for Cytonics, Inc., and GJS is a board member; however, SRG and GJS were blinded to the results until after data analysis had been completed by MWS. We felt this was the best way to ethically report and interpret our results without bias.

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RESULTS: Seventy-five patients had full complement of data and were included in this analysis. At 3-month follow-up, 57 (76%) patients were “better.” There was a statistically significant association of the presence of FAC and clinical improvement ($p=.017$) with an 85% positive predictive value. Receiver-operating-characteristic (ROC) curve plotting association of FAC and clinical improvement demonstrates an area under the curve (AUC) of 0.66 ± 0.08 ($p=.037$). Subset analysis of those with weakness on physical examination ($n=48$) plotting the association of FAC and improvement shows AUC on ROC of 0.81 ± 0.067 ($p=.002$).

CONCLUSIONS: Patients who are “FAC+” are more likely to demonstrate clinical improvement following microdiscectomy. The data suggest that the inflammatory milieu plays a significant role regarding improvement in patients undergoing discectomy for radiculopathy in lumbar HNP, even in those with preoperative weakness. The FAC represents a potential target for treatment in HNP. © 2013 Elsevier Inc. All rights reserved.

Keywords: Discectomy; Herniated nucleus pulposus; Biomarkers; Cytokines; Lumbar

Introduction

Although degenerative disc disease has been a long-standing topic of research, much remains to be learned about its pathogenesis and the complex interrelationship among intervertebral disc histopathology, symptoms, and treatment. Historically, research has focused on the physiology of common disc degeneration, but little, if any, correlation to symptoms and response to treatment was found [1,2]. More recently, the relationship between pain and molecular markers in degenerative musculoskeletal syndromes has garnered interest for their potential as novel diagnostic and therapeutic targets.

Relative to other degenerative disc disorders, clinical outcomes are best defined for lumbar disc herniation. Although some factors are recognized to increase the risk of poor outcomes [3,4], most patients are expected to improve with conservative treatment [5]. Overall, clinicians remain unable to reliably predict who will recover and who will not, and they have no accepted standard to determine when to offer surgery. Traditionally, the decision to offer discectomy has relied on the clinical course and the findings of magnetic resonance imaging (MRI) to confirm what are often nonspecific subjective complaints. This has fueled the search for more reliable markers of disease and better predictors of treatment outcomes.

Several cytokine biomarkers are known to be associated with lumbar intervertebral disc herniation, yet few have shown the ability to predict response to treatment. Recently, a cartilage degradation product, the fibronectin-aggrecan complex (FAC), was shown to be associated with pain from inflammation in various degenerative spine and joint conditions [6–8]. Interestingly, its presence was also shown to predict response to lumbar epidural steroid injection in patients with radiculopathy from disc herniation [7]. The goal of the present study was to determine the ability of FAC to predict response to microdiscectomy for patients with radiculopathy due to lumbar disc herniation.

Material and methods

Study set-up/patient selection

This was a single-center prospective cohort study of 92 consecutive patients who opted for microdiscectomy to treat lumbar or lumbosacral radiculopathy caused by a lumbar disc herniation. The study was HIPAA compliant; institutional review board approval was obtained and all study participants signed informed consent. Patients of all four spine surgeons practicing at the study site were included.

The selection criteria were kept to a minimum to best reflect what occurs in routine practice. Patients were considered for inclusion if they opted for a microdiscectomy to treat lower extremity radicular pain, and had an MRI of the lumbar spine showing a disc herniation in a location consistent with symptoms. The presence or absence of reflex changes and/or a motor deficit were tested and recorded but did not factor into the inclusion process.

Patients were excluded if they met any one of the following parameters: plain radiography demonstrating severe loss of disc height; high-grade degenerative disc disease; spondylolisthesis greater than grade I; a history of prior lumbar surgery or trauma; physical examination revealing weakness in a distribution inconsistent with the MRI; diagnosis of inflammatory arthritides, crystalline arthropathies, or other rheumatologic diseases; and red flags, including progressive weakness, bowel or bladder complaints, unknown radiographic mass, unexpected weight loss.

Sample acquisition, storage, and preparation

Microdiscectomy was completed following fluoroscopic localization by using a small incision at the representative level, with minimal partial laminectomy, and fragmentectomy, not subtotal discectomy. A “disc bath” was performed on the excised disc fragment by placing it into a petri dish and lavaging the fragment with approximately 3 mL of saline before aspirating the fluid. Each fluid sample

was aliquoted into a sterile polypropylene tube and frozen at -80°C until the time of sample analysis. At the time of analysis, each patient sample was thawed to room temperature, clarified by centrifugation at 5,000g, and filtered using 0.45 μm low-protein binding filter. The collected filtrate was immediately assayed as described below.

ELISA

A heterogeneous, enzyme-linked immunosorbent sandwich assay (ELISA) was developed and validated on a prior series of patients (18). This assay detects FAC, a protein complex of fibronectin and the aggrecan G3 domain. Briefly, antiaggrecan G3 domain antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in phosphate-buffered saline (PBS)/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, then washed with six washes of PBS/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, anti-fibronectin antibody labeled with horseradish peroxidase (US Biological, Swampscott, MA, USA) 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, USA) at 10 $\mu\text{g}/\text{mL}$ concentration was used as a negative control.

Outcomes assessment

Outcome metrics, including Oswestry Disability Index (ODI) and visual analog scores leg pain (VAS) were compiled at both baseline and at 3-month follow-up visits. The primary composite outcome was a determination of “clinical improvement.” Clinical improvement was defined as both a VAS reduction of three or more and ODI improvement (decrease) of at least 20 points. This was chosen to be in excess of previously determined minimal clinically improved difference (MCID) for spine outcomes [9,10].

The 3-month time point was chosen because we know that the overall natural history of herniated nucleus pulposus (HNP) is that patients will get better. By choosing the 3-month point, we were catching patients in the perioperative period when relief of the inflammatory effect would be most pronounced and most relevant to the study.

Statistical analysis

Statistical analysis was then completed by comparing the primary and secondary outcomes to the presence of FAC in the disc bath. The presence of weakness was correlated with the presence or absence of FAC. Data were

analyzed by Fisher’s exact test, Student’s *t* test, and receiver operating characteristic (ROC) curves.

Results

Ninety-two patients were enrolled between April 2009 and September 2011. Complications with the storage of samples and the protease used rendered the results not viable for the initial five patients. One patient died within the follow-up period from unrelated causes, and two patients were lost to follow-up, leaving 84 patients (91%) with 3-month follow-up data. Furthermore, 75 of 84 patients had VAS and ODI scores that would afford the opportunity to show clinical improvement according to our definition. The average age of the patient population was 40.8 years (± 1.5). There were 35 women and 40 men. Patient ODI and VAS scores significantly improved postoperatively from baseline for all groups and the average change of those who “improved clinically” was greater than those who did not (Table 1). A bell-shaped curve distribution was observed comparing the change in ODI and presence of FAC with the most common change in ODI in the range of 31 to 40 (data not shown).

Primary outcome measures show 57 (76%) of 75 patients “clinically improved” by 3 months according to our predetermined improvement thresholds for VAS and ODI. Of those who “clinically improved,” 77% (44/57) were FAC+ and a significant association ($p=.017$) was observed between the presence of FAC at the time of microdiscectomy and clinical improvement at 3-month follow-up. For the study population, 52 (69%) of 75 patients were identified as FAC+; 44 of 52 patients who were FAC+ “clinically improved” (positive predictive value [PPV] of 85% and a negative predictive value [NPV] of 43%).

ROC analysis was performed to determine the utility of FAC as a diagnostic test for response to surgery for HNP (“clinically improved”). FAC is a statistically significant test, with area under the curve (AUC)= 0.68 ± 0.06 ; $p=.006$ (Fig. 1). Subset analysis of patients presenting with weakness ($n=48$) demonstrates improved test performance with an AUC= 0.81 ± 0.07 ; $p=.002$ (Fig. 2).

Table 1
Comparison of outcome matrices

Outcome matrix	Preoperative	Postoperative	p Value	Average change	p Value
Overall cohort					
VAS	6.8 (2.1)	2.1 (2.0)	<.001	4.7 (2.5)	
ODI	52.9 (19.6)	13.3 (15.6)	<.001	39.6 (22.2)	
Clinically improved					
VAS	7.1 (2.0)	1.5 (1.6)	<.001	5.6 (2.0)	<.001
ODI	55.2 (20.3)	8.3 (9.8)	<.001	46.9 (18.7)	<.001
Not improved					
VAS	5.8 (2.3)	3.9 (2.2)	.02	1.9 (1.9)	
ODI	45.6 (15.5)	29.0 (20.0)	.01	16.6 (15.6)	

ODI, Oswestry disability index; VAS, visual analog score.
Average change was greater in the clinically improved group compared with the not improved group.

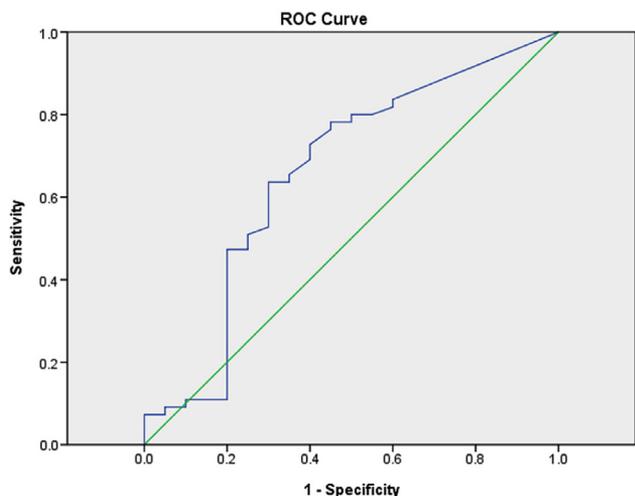


Fig. 1. Receiver operating characteristic (ROC) curve for the use of fibronectin-aggrecan complex (FAC) as a diagnostic test for patient improving from surgical management of lumbar herniated nucleus pulposus at 3-month follow-up (n=75). FAC is a statistically significant test, with area under the curve of 0.66 ± 0.077 ($p=.037$).

Eight patients were FAC+ but did not improve according to our parameters. Two of these patients experienced a recurrent disc herniation within the first 3 months postoperatively, whereas four of the others had continued pain and disability (Table 2).

Discussion

The results of this investigation demonstrate that the presence of FAC in disc samples from patients with radiculopathy is associated with clinical improvement following surgical microdiscectomy. This adds to previous work identifying the FAC in patients with radiculopathy who responded to epidural injection [7]. It is possible that this

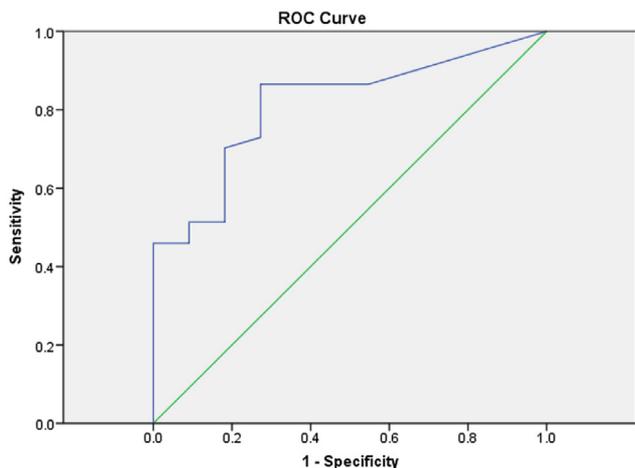


Fig. 2. Receiver operating characteristic (ROC) curve for the use of fibronectin-aggrecan complex (FAC) as a diagnostic test for patients with weakness improving from surgical management of lumbar herniated nucleus pulposus at 3-month follow-up (n=48). FAC is a statistically significant test, with area under the curve of 0.81 ± 0.067 ($p=.002$).

Table 2
Patients who were FAC positive who did not clinically improve

Patient	VAS, pre/postoperative	ODI, pre/postoperative	Circumstance
A	3/1	36/4	Needed to have no pain in order to be “better”
B	6/4	38/26	Right knee OA and underwent TKA same side as HNP symptoms
C	7/5	36/24	Chronic low back pain at 6 weeks all pain and disability related to back, not leg pain
D	6/8	78/64	Recurrent HNP
E	6/8	48/68	Recurrent HNP
F	10/5	48/36	H/o fibromyalgia and chronic low back pain
F	8/3	26/14	Vascular condition that gives patient leg pain ipsilateral to HNP pain and cause of disability
G	3/1	58/16	Needed to have no pain in order to be “better”

FAC, fibronectin-aggrecan complex; HNP, herniated nucleus pulposus; H/o, history of; OA, osteoarthritis; ODI, Oswestry disability index; TKA, total knee arthroplasty; VAS, visual analog score.

test may prove helpful in preoperatively guiding decisions for surgery in patients in whom the diagnosis is more challenging, or in those at greater risk of failed treatment, such as in the setting of diabetes or secondary remuneration. Moreover, this adds to the body of evidence that targeting prevention of complex formation in vivo may play some role in diminishing radiculopathy and serve as a potential therapeutic target. The current study bridges the gap between the presence of a biomarker in the inflammatory milieu and clinical outcomes. Previously, Golish et al. [7] established the presence of FAC in the epidural space and its association with clinical improvement of radiculopathy from epidural steroid injection for HNP. In fact, Dewing et al. [11] showed that predominance of leg pain over back pain is associated with improved response to surgery. The high PPV potentially affords the opportunity to provide patients postoperative prognostic data for recovery.

Our study reports similar clinical outcomes as a previous major trial [5,12]. The SPORT trial reported an ODI improvement of 37 points in 80% of discectomy patients. The patients in the present study who had clinical improvement had on average a 47-point change in ODI. Furthermore, we used a definition of clinical improvement that was in excess of the MCID [9,10]. Additionally, our defined outcome measure was a combination of two universally accepted outcome parameters (ODI and VAS). Our success rate (76%) was lower than other published work, likely secondary to the broad inclusion criteria and the threshold that we established for success at the study outset [13]. In fact, two patients with pre-/postoperative a ODI scores of 36/4 and 58/16, respectively, could not be categorized as improved according to our criteria because the VAS

spanned only 2 points (from 3 to 1). Two patients in this group (FAC+) had a recurrent disc herniation and underwent revision discectomy, and were not better at 3 months, although both were better by our criteria after the second procedure. Other patients had confounding conditions that, with stricter inclusion and exclusion criteria may not have been allowed entry into the study (Table 2).

The results of the subset analysis suggest that patients with preoperative weakness have a very strong association between presence of FAC and clinical improvement. This is an intriguing finding that the removal of the inflammatory component may be more related to outcomes, not necessarily the mechanical compression. In a rat model, intrathecal and perineural injection of a nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) decoy reduced mechanical allodynia and thermal hyperalgesia [14,15]. Additionally, injection of an NF- κ B decoy suppresses the effects of mechanical nerve root compression in a rat model [15]. This suggests that elimination of the inflammatory components of nerve root compression ultimately influence outcomes.

The relative contribution of inflammatory processes versus mechanical processes is evolving. Early biomarker studies identified HNPs with elevated levels of metalloproteinases, nitric oxide (NO), prostaglandin-E, and interleukin (IL)-6 [16]. Furthermore, the cells in the inflammatory milieu show increased production of tumor necrosis factor (TNF)- α , NO, IL-1, and cyclo-oxygenase-2 [17]. Recent literature has focused on the role of TNF- α in matrix degradation in relation to HNP [18–21]. This has been further supported by finding increased concentrations of TNF- α in the epidural fat of patients with HNP compared with controls without pain secondary to HNP [22]. Unfortunately, intravenous infusion with TNF- α inhibitors have not shown to significantly decrease pain caused by HNP [23]; however, epidural injection has shown promising results at 4-week follow-up [24] as well as injection of IL-6 inhibitors [25].

The pathophysiologic link from compression to inflammation suggests that compression initiates a biochemical response in the nerve tissue initiating alterations in the microvasculature, increased permeability, and upregulation of the inflammatory pathway [26]. Using a rat model, it has been shown that pain responses are highest with application of compression by disc material compared with mechanical compression, or presence of disc material alone [27]. To induce the increased cytokine production, both compression and chemical injury were required, compared with compression or chemical injury alone in a rat model [28].

Most studies have focused on TNF- α ; however, recent epidural studies did not find an association of clinical response to epidural steroid injection and the presence of TNF- α [7,29]. Instead they found a close association with FAC. To further support this association, in patients who had clinical responses to the ESI on repeat lavage the FAC was not present, but TNF- α was [29]. The evidence from the epidural studies and this discectomy study

demonstrate that FAC may be the most important biomarker in radiculopathy, and therapeutic targets should be designed appropriately.

As with any study, there are shortcomings. Bleeding, use of irrigation, identifying the disc extrusion and performing timely lavage, and other factors make it more difficult to obtain a reliable sample when identifying the herniation intraoperatively. The method of obtaining samples described previously involves placing the extracted disc material into a container with approximately 3 milliliters of saline. Over time, the hydrophilic nature of the disc causes fluid to be imbibed by the material and there may be variations in concentrations of mediators. Furthermore, we had broad inclusion criteria that allowed patients with potentially confounding pain generators in the study. This potentially altered the NPV, as it is likely there were false negatives secondary to multiple factors described previously. However, we feel this more accurately reflects a common spine practice and patient population. Because of our strict definition for “clinical improvement,” nine patients initially in the study had low preoperative ODI or VAS scores that even if they had values of 0 postoperatively, they would not be included as “clinically improved.” Therefore, they were excluded from statistical analysis because they would have skewed the results regardless of FAC status.

In conclusion, patients who are “FAC+” are more likely to demonstrate clinical improvement following microdiscectomy. The data suggest that the inflammatory milieu plays a significant role regarding improvement in patients undergoing discectomy for radiculopathy in lumbar HNP, even in those with preoperative weakness. This study suggests that FAC is an important biomarker in the pathway causing radicular pain from HNP and, therefore, is a potential therapeutic target.

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