Are Persistently Symptomatic Vertebral Compression Fractures Associated With Abnormal Inflammatory Profiles? A Prospective Study

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Study Design: A case-control study with prospectively collected samples for laboratory analysis in a series of patients with spinal fragility fractures and a series of patients without fracture who underwent fusion for LBP.

Objective: Was an exploratory data analysis for candidate cytokine biomarkers present in the fracture milieu of patients with persistent back pain associated with vertebral compression fracture.

Summary of Background Data: Lumbar and thoracic compression fractures are common. Little is known about the presence of inflammatory mediators within fractured vertebra in the clinical setting.

Methods: Thirty patients diagnosed with a single thoracic or lumbar compression fracture were treated with single level vertebroplasty. At the time of intervention, needle aspiration was carried out at the fractured level. A multiplexed bead assay was used to assess the presence of 27 different cytokines and inflammatory mediators. A control group consisted of needle aspiration samples of 30 lumbar vertebra from 13 patients with chronic pain but no fracture undergoing open instrumented fusion.

Results: Thirty patients with 30 fractures consisted of 23 female and 7 male with a mean age of 77.5 years (SD 13.6; range 42 to 97) and a mean of 3.9 weeks of pain (SD 3.1; range 1 to 12). The highest levels of inflammatory mediators were (in order): IL-1 receptor antagonist, PDGF, RANTES, IP-10, IL-8, and eotaxin. These mediators were present at concentrations > 200 pg/mL. Compared with controls with chronic pain, significant differences were present for 4 mediators: TNF, MIP-1b, IL-9, and IL-12. The panel of these 4 markers was 93.3% specific and 66.7% sensitive for fracture compared with the control group.

Conclusions: Inflammatory mediators are present in needle aspirates of symptomatic vertebral compression fractures. Some of these mediators show different levels than in patients with chronic pain but no fracture.

Level of Evidence: Diagnostic level of evidence II.

Key Words: spinal compression fracture, osteoporosis, cytokines, vertebral fracture

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The diagnosis of thoracic or lumbar vertebral compression fracture is often made by history, physical examination, and plain radiography, with MRI aiding in the assessment of acuity. However, it remains unclear why some patients who sustain a fracture experience significant pain whereas others remain relatively asymptomatic. Fracture biology involves the local production of inflammatory cytokines,[1] and cytokines are known mediators of neuropathic pain and nociceptor sensitization[2]; therefore, characterization of the cytokine profile of symptomatic vertebral compression fractures may provide insights into the biology of the painful state.

Further, cytokines and inflammatory mediators are potential biomarkers for response to fracture treatment, such as vertebral augmentation. The need for improved diagnostic and prognostic markers for response to vertebroplasty was highlighted by negative results from a large, multicenter randomized clinical trial.[3] In this study, inclusion criteria included the presence of thoracic or lumbar fractures at 1 to 3 levels with fracture age less than 1 year or positive T2 signal on MRI (for fractures of unknown acuity). There was no statistically significant difference in Roland Morris scores at 1 month, despite the study being sufficiently powered to detect a clinically significant difference in the primary outcome measure.[4] Although a number of possible explanations have been proposed, one possibility is that the standard diagnostic criteria do not sufficiently identify the group most likely to respond to treatment.[5] This raises the possibility that additional diagnostic criteria, including cytokine biomarkers, may enrich the treatment group for those likely to respond.

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The purpose of this study was an exploratory data analysis for candidate cytokine biomarkers present in the fracture milieu of patients with persistent back pain associated with vertebral compression fracture. The control group consisted of patients undergoing fusion for chronic pain in the absence of fracture. A commercially available, multiplexed immunoassay for cytokines involved in inflammatory processes was in conjunction with history, physical examination, plain radiography, and MRI. We hypothesized that inflammatory cytokines would be present in the fractured vertebra of patients undergoing vertebroplasty.

METHODS

Subjects

The design is a case-control study with prospectively collected samples for laboratory analysis. The protocol was approved by an independent Institutional Review Board, and all enrolled patients provide informed consent for study participation. Between the dates of April 2008 and September 2008, 43 total patients accepted enrollment from approximately 200 consecutive patients referred for evaluation. All patients underwent a history, physical examination, plain radiography, and MRI. Thirty (30) patients were treated by a single board-certified orthopedic spine surgeon who carried out vertebroplasty (JF). In addition, 13 patients were treated by a single board-certified orthopedic spinal who carried out spinal fusion for chronic pain in the absence of fracture (DRC).

The study cohort consisted of adult patients with acute back pain for less than 3 months identified as having an osteoporotic compression fracture as the cause of their symptoms. Indications for augmentation included the presence of symptoms on history, a physical examination positive for tenderness, the presence of fracture on plain radiography, and the presence of signal changes on MRI in an anatomic location consistent with the history and physical examination. The control group consisted of adult patients with chronic low back pain of greater than 3 months' duration in the setting of spondylolisthesis, spondylosis with severe loss of disc height and/or facet arthropathy.

Inclusion and Exclusion Criteria

Inclusion criteria: Patients >18 years old presenting with symptoms of recent-onset (< 3 mo) pain, either secondary to an acute event or a preexisting diagnosis of osteoporosis as determined by history, physical and imaging studies, and who satisfied indications for vertebral augmentation after an MRI of the spine.

Exclusion criteria: less than 18 years old, previously treated vertebral fracture past or current medical history of autoimmune disease (ie, rheumatoid arthritis). In addition, no patients involved in a worker's compensation claim or personal injury litigation were enrolled in the study.

Sample Acquisition

The standard operative procedure for performing vertebral augmentation through insertion of PMMA was carried out. However, upon entering and confirming position in the vertebral body, a sample was obtained through aspiration at the operative level, allowing the withdrawal of 1 to 2 mL of needle aspirate. For the control group, the standard operative procedure for posterior approach to the spine then instrumented fusion with pedicle screws and rods was carried out. However, upon entering and confirming entering the vertebral body with a pedicle finder under flouroscopy, a sample was obtained through aspiration at the operative level, allowing the withdrawal of 1 to 2 mL of aspirate. A sample was taken from each of the vertebral bodies that underwent fusion. All aspirates were immediately placed into 2 mL tubes containing 130 μL of protease inhibitor cocktail tablets (Roche Diagnostics, Indianapolis, IN) dissolved in pH 7.4 phosphate-buffered saline (PBS) (0.045 Tablet/mL sample) and frozen at -20°C on dry ice for shipping till storage at -80°C till the time of analysis.

Cytokine Analysis

The concentrations of 27 inflammatory cytokines and chemokines (IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, FGF basic, G-CSF, GM-CSF, IFNγ, IP-10, MCP-1, MIP-1a, MIP-1b, PDGF-bb, RANTES, TNFα, VEGF) were quantified in spine samples using a human 27-plex inflammatory cytokine panel and the BioPlex 200 System (Bio-Rad, Hercules, CA), following the manufacturer's protocol in a 96-well plate format. The choice of cytokines reflects the standard commercially available panel of mediators known to be involved in the biology of inflammation and leukocyte cell signaling. The assay uses antibodies linked to polystyrene beads containing different levels of fluorophores and has been validated against standard Elisa of human blood samples.[6]

Data Analysis

Data analysis was done with SPSS 12.0 (SPSS Inc, Chicago, IL) using Kolmogorov-Smirnov test, Wilcoxon rank sum tests and *t* tests with Sidak multitest correction, and binary logistic regression. An α value of 0.05 was considered statistically significant before multitest correction (0.0019 after multitest correction). For N = 60 total participants, the power of Wilcoxon rank sum tests with an α value of 0.05 is approximately 80% for a large effect size (d = 0.75) and for a very large effect size (d = 1.1) after multitest correction.

RESULTS

Thirty patients with 30 fractures consisted of 23 females and 7 males with a mean age of 77.5 years (SD 13.6; range 42 to 97) and a mean of 3.9 weeks of pain (std.dev 3.1; range 0 to 12). Thirteen patients (30 levels) with chronic pain but no fracture consisted of 9 female and 4 male with a mean age of 65.8 years (SD 12.9; range

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TABLE 1. Summary of Spinal Levels from Measurement of Inflammatory Markers From Patients With Vertebral Compression Fractures Versus Chronic Pain Without Fracture

Level	Fractures	Chronic Pain
T7	1	0
T8	1	0
T9	3	0
T10	2	0
T11	1	0
T12	4	0
L1	6	0
L2	4	4
L3	5	4
L4	1	9
L5	2	8
S1	0	5

38 to 82) and a mean of 91.6 weeks of pain (SD 80.6; range 10 to 200). Table 1 summarizes the spinal levels from which samples were obtained for both groups. There were significant differences in duration of pain ($P < 0.001$) and age ($P = 0.001$) but not gender (0.75) between the experiment and control groups.

Results for the cytokine measurements are summarized in Table 2. Wilcoxon rank sum tests with Sidak multitest correction was used to identify differences between inflammatory mediators in the fracture and chronic pain groups. Compared with controls with chronic pain, significant differences were present for MIP-1b, TNFα, IL-9, and IL-12. Binary logistic regression was used to fit a model for the diagnosis of fracture versus chronic pain from the four significantly different mediators. Table 3 is a two-by-two contingency table of the results. The panel of these 4 markers was 93.3% specific and 66.7% sensitive for fracture compared with the control group in a post hoc analysis.

TABLE 3. A Two-by-two Contingency Table Analysis of Classification by a Panel of 4 Biomarkers that is 93.3% Specific and 66.7% Sensitive for Fracture Compared with the Control Group in a post hoc Analysis

	Observed: Fracture	Observed: No fracture
Predicted: Fracture	20	2
Predicted: No fracture	10	28

DISCUSSION

This study shows that cytokines and inflammatory mediators are present in picogram/milliliter concentrations in the fractured vertebra of elderly patients undergoing vertebral augmentation for persistent pain. There are differences in the concentrations of several mediators relative to control patients undergoing instrumented

TABLE 2. Summary of Results From Measurement of 27 Inflammatory Markers in Acute, Symptomatic Vertebral Compression Fractures, and Chronic Pain Without Fracture

Marker	Fracture Mean (pg/mL)	Fracture Std. Dev. (pg/mL)	Chronic Pain Mean (pg/mL)	Chronic Pain Std. Dev. (pg/mL)	*P*
IL-1b	8.95	19.26	2.63	2.82	0.137
IL-1RA	2053.81	2766.61	1928.27	1815.87	0.657
IL-2	2.41	5.13	0.80	1.00	0.146
IL-4	2.81	8.44	0.52	0.51	0.316
IL-5	8.32	35.86	0.34	1.23	0.104
IL-6	152.84	368.11	10.87	17.09	0.016
IL-7	31.90	114.68	1.47	1.43	0.020
IL-8	222.12	587.87	0.27	1.28	0.006
IL-9	65.05	133.70	0.77	2.33	0.001*
IL-10	11.01	33.30	0.93	0.98	0.707
IL-12	19.65	63.73	0.13	0.73	0.001*
IL-13	34.68	115.09	1.20	0.85	0.098
IL-15	0.30	0.64	0.27	0.52	0.992
IL-17	0.33	0.71	0.40	2.19	0.030
Eotaxin	210.44	364.11	50.13	49.71	0.029
FGF basic	7.14	38.00	0.07	0.37	0.091
G-CSF	27.38	64.90	3.23	2.05	0.994
GM-CSF	3.11	10.47	0.00	0.00	0.005
IFN-g	174.19	474.11	13.40	8.19	0.185
IP-10	448.56	630.47	106.57	68.95	0.005
MCP-1	77.91	126.10	36.73	30.21	0.290
MIP-1a	0.60	1.57	0.20	0.76	0.233
MIP-1b	38.00	73.14	2.70	9.11	0.001*
PDGF-bb	1753.25	2179.32	1152.70	879.23	0.900
RANTES	1232.63	790.67	882.27	421.53	0.066
TNF-a	7.47	17.08	0.00	0.00	0.001*
VEGF	366.56	871.52	37.13	34.36	0.020

*P-values that are significant after multitest correction are marked.

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fusion for chronic pain in the absence of fracture, including TNFα, MIP-1b, IL-9, and IL-12.

The role of cytokines in the biology of fracture healing is well established. A classic study in the rat femoral fracture model elucidated the role of IL-1, IL-6, GM-CSF, and M-CSF in the first 3 weeks of fracture healing.[7] This work established cytokines as one component of the emerging biology of fracture healing, with structural proteins playing another molecular role, and multiple cell types fulfilling the cell biologic roles.[8] Throughout over a decade of further research, the fundamental role of cytokines as early mediators of the healing response has endured, and their temporal evolution relative to structural proteins, bone morphogenetic proteins,[9] and angiogenesis further defined.[1,10]

The role of cytokines in fracture-related pain has received less attention. Cytokines are known mediators of neuropathic pain[2] and are associated with the painful state in degenerative diseases of the spine[11,12] and synovial joints.[13] The role of cytokines in pain generation is best studied in the dorsal root ganglion (DRG),[14] but there is evidence of round-trip signaling between sites of injury and nociceptive neural circuits that involves cytokines.[15] Further, cytokines associated with spinal degeneration and injury may affect the DRG directly, owing to the proximity of spinal structures to the DRG.[14–16] There is some evidence of altered cytokine expression in delayed fracture healing,[17] which may be correlated with the painful state. Cytokines and their signaling pathways are involved in the biology of osteoporosis,[18] which may influence healing and pain in the setting of vertebral compression fractures. Overall, the relationship among cytokines in the fracture milieu, nociception, and successful healing in the setting of osteoporosis remains incompletely understood.

Tumor necrosis factor alpha (TNFα) is a ubiquitous cytokine well known in the spine for its role rheumatoid arthritis, ankylosis spondylitis, and other autoimmune diseases; however, it has been implicated in inflammatory conditions not exclusive to autoimmune or T cell-mediated processes.[19] TNFα is associated with bone loss syndromes including Paget disease in concert with the RANKL/OPG pathway.[20] TNFα has been associated with radiculopathic pain in the setting of herniated nucleus pulposus,[21] although its role in disc disease remains controversial.[22] Macrophage inflammatory protein 1 beta (MIP-1b) is a cytokine chemotactic for macrophages. It has been associated with the painful state in degenerative conditions of the synovial joints[13,23]; however, it was not detected in the epidural space of patients with radiculopathy and herniated nucleus pulposus.[11] Its role in spinal disease has received limited attention. Interleukin 9 (IL-9) is a cytokine most closely associated with eosinophils in allergic disorders[24]; however, the potential functions of this poorly studied mediator are unknown and the various roles of eosinophils and IL-9 may be broader than previously althought.[25–27] IL-12 and its homologs are intimately involved in the recruitment of T cells and coordinating the responses of antigen presenting cells and macrophages. For example, IL-12 is required for induction of interferon γ[28], which has been detected in diseases of the spine[11] and synovial joints.[13]

This study has several limitations. The control group consisted of patients undergoing instrumented fusion of the thoracic of lumbar spine for persistent pain without fracture. The use of this group as a control is suboptimal, and asymptomatic controls would have been preferable. However, deep ethical concerns regarding invasive procedures in asymptomatic volunteers prohibited this approach. An additional possible design is a case series of treated patients who are retrospectively stratified by improvement in a clinical instrument, such as Roland Morris Disability Score. However, the sample size needed to power a study to detect clinically significant differences in such clinical instruments is typically very large, and prohibitive for this exploratory analysis to identify candidate markers.[4,5] A further limitation is that the study group included patients with symptoms from 1 to 12 weeks' duration. A more uniform acuity of presentation might be preferable in yielding consistent results; however, the approach adopted here mirrors the difficulty of clinical decision making for patients actually presenting for clinical care as reflected in clinical trials for vertebroplasty.[3] Similarly, multiplexed assay of many more candidate markers would have been desirable, but would have necessitated a prohibitive sample size for meaningful interpretation. The experiment and control groups are significantly different regarding duration of pain and age, but these differences are inherent in the choice of control group and confound the positive results. Further prospective analysis will therefore be required to validate the candidate biomarkers identified in this exploratory analysis.

CONCLUSIONS

Cytokines are present in picogram/milliliter concentrations in the fractured vertebra of elderly patients undergoing vertebral augmentation for persistent pain in the 2 to 12 weeks time period after the onset of symptoms. There are differences in the concentrations of some mediators relative to control patients undergoing instrumented fusion for chronic pain in the absence of fracture.

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