

Diagnostic Utility of Cytokine Biomarkers in the Evaluation of Acute Knee Pain

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Background: The diagnosis of clinically important meniscal tears of the knee remains challenging, and it is unknown why only some injuries become painful. The role of inflammatory cytokines in generating pain following meniscal injury remains unclear. This study aimed to investigate the cytokine profile in patients with acute knee pain believed to be secondary to meniscal damage.

Methods: This prospective cohort study included thirty-two patients without rheumatoid arthritis who had knee pain for less than six months, with either an acute or insidious onset, and elected to have arthroscopic treatment after nonoperative management had failed. Twenty-three of these patients elected to have the contralateral, nonoperatively treated knee lavaged at the time of arthroscopy. Fifteen asymptomatic control subjects also contributed samples of knee joint fluid, for a total of seventy samples from forty-seven subjects. Lavage of the operatively treated, contralateral, and control knees was performed with the patient under regional anesthesia prior to arthroscopy, if applicable, by the infusion of sterile saline solution into the knee followed by the immediate withdrawal into a syringe. The concentrations of seventeen inflammatory cytokines and chemokines were measured with use of a multiplexed immunoassay panel. Preoperative magnetic resonance imaging findings and cytokine assay results were compared with intraoperative findings.

Results: Multivariate analysis of variance detected significantly greater concentrations of interferon gamma (IFN- γ); interleukins 2, 4, 6, 10, and 13 (IL-2, IL-4, IL-6, IL-10, and IL-13); monocyte chemoattractant protein-1 (MCP-1); and macrophage inflammatory protein-1 beta (MIP-1 β) in fluid samples from painful knees than in samples from nonpainful knees. Correlation analysis demonstrated a significant positive correlation between patient-reported pain scores and concentrations of IL-6 (Spearman $\rho = 0.7$), MCP-1 ($\rho = 0.8$), MIP-1 β ($\rho = 0.6$), and IFN- γ ($\rho = 0.6$). These four cytokines also demonstrated a positive correlation with each other ($\rho = 0.5$ to 0.7). The presence of IFN- γ , IL-6, MCP-1, or MIP-1 β performed as well as magnetic resonance imaging in the prediction of intraoperative findings.

Conclusions: Intra-articular concentrations of four inflammatory cytokines IFN- γ , IL-6, MCP-1, and MIP-1 β correlated to pain in patients with symptomatic meniscal tears in the knee but were markedly lower in asymptomatic normal knees and in asymptomatic knees with meniscal tears. These cytokines may be involved in the generation of pain following meniscal injury.

The diagnosis of clinically important meniscal tears of the knee remains challenging. Although the history and physical examination aids the diagnosis¹, magnetic resonance imaging is the mainstay of radiographic diagnosis. However, this modality identifies meniscal injury

in as many as 65% of asymptomatic individuals, calling into question not only the validity of magnetic resonance imaging as a primary diagnostic tool but also the correlation between abnormal meniscal anatomy and reported knee pain^{2,3}.

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It remains unclear why some patients who sustain meniscal injury experience pain while others remain asymptomatic. This phenomenon may reflect the local production of inflammatory cytokines, some of which might sensitize nociceptors, resulting in pain. The identification of intra-articular tumor necrosis factor- α (TNF- α) in patients with rheumatoid arthritis enabled the development of an effective pharmaceutical therapy by specifically blocking TNF- α ⁴. The identification of analogous cytokine(s) present in patients sustaining traumatic painful meniscal tears and/or symptomatic osteoarthritis could potentially serve as diagnostic biomarkers and therapeutic targets.

The current study was therefore designed to investigate the intra-articular inflammatory cytokine profile of patients with acute symptomatic meniscal damage in the knee compared with the contralateral knee and with knees of asymptomatic control subjects. A commercially available standard panel of cytokines traditionally involved in inflammation was evaluated, as these molecules are hypothesized to be invoked in a plethora of inflammatory processes. Magnetic resonance imaging findings, intraoperative findings, and cytokine concentrations were compared with and correlated to patient self-reported pain ratings. We hypothesized that a number of these inflammatory cytokines would be present in knees with acute meniscal tears resulting in pain but would be absent in radiographically similar but asymptomatic knees. Specifically, IL-6 (interleukin-6), IL-1 β , TNF- α , and MCP-1 (monocyte chemotactic protein-1) have all been implicated in knee inflammatory states by various *in vitro* and clinical studies, making their involvement in meniscal pain plausible *a priori*⁵⁻⁹.

Materials and Methods

Inclusion and Exclusion Criteria

Inclusion criteria were an age of eighteen years or older, knee pain of recent onset (less than six months), and physical examination findings and magnetic resonance imaging results consistent with meniscal pathology.

Exclusion criteria were an age of less than eighteen years, a recent history (within three months) of an intra-articular injection of a corticosteroid, and a past or current history of autoimmune disease (such as rheumatoid arthritis). In addition, no patient involved in a Workers' Compensation claim or personal injury litigation was enrolled in the study.

Subjects and Knee Lavage

This prospective cohort study was approved by the institutional review boards of Parkway Regional Medical Center and the University of Miami, Jackson Memorial Medical Center. The trial registration number is NCT00836966 (Clinicaltrials.gov). From June 2006 through June 2007, fifty subjects who met the above inclusion criteria were offered enrollment in the study and all fifty provided informed consent to participate. These subjects were selected from a cohort of approximately 200 consecutive patients referred for the evaluation of knee pain to two board-certified orthopaedic surgeons who were fellowship-trained in sports medicine. For all patients, a history was obtained, a physical ex-

amination was performed, and magnetic resonance imaging scans of the knee were acquired. The magnetic resonance imaging scans were read and interpreted by a single, independent, and experimentally blinded, board-certified radiologist. The study cohort consisted of adult patients who had knee pain for less than six months with either an acute or insidious onset and who had failed nonoperative treatment and elected to have arthroscopic management. Indications for surgery included the presence of mechanical symptoms; a physical examination with a positive McMurray sign or joint-line tenderness, or both¹; an absence of severe joint-space narrowing on plain radiographs^{10,11}; and grade-III signal changes on magnetic resonance imaging^{12,13} in an anatomic location consistent with the history and physical examination.

Eighteen subjects were later excluded from the study because of the presence of an anterior cruciate ligament tear and/or a large joint effusion (eleven patients), sample loss during shipment (five patients), or surgical exploration that was negative for meniscal injury despite positive findings on magnetic resonance imaging (two patients). Therefore, thirty-two subjects in the surgical group contributed data to the study.

Prior to arthroscopy and/or lavage, study patients were asked to rate the pain in both knees on a previously validated verbal pain-rating scale¹⁴ of 0 to 10 points, with a score of 0 indicating no pain and a score of 10, the worst pain imaginable. A knee with any score other than 0 was considered to have pain for the sake of grouping. These scores were later correlated to the intra-articular cytokine results. Patients deemed appropriate to undergo arthroscopic surgery as part of their treatment were invited to participate in the study and consented to intraoperative lavage of the involved knee or both knees. With the patient under regional anesthesia, a synovial fluid aspirate was obtained by means of lavage in the operatively treated knee or both knees, with use of approximately 15 mL of sterile physiologic saline solution, allowing the withdrawal of 3 to 5 mL of knee lavage aspirate. The aspirated fluid was then placed into 2-mL tubes containing 130 μ L of protease inhibitor cocktail tablets (Roche Diagnostics, Indianapolis, Indiana) dissolved in phosphate-buffered saline solution (0.045 tablet per milliliter of sample) with a pH of 7.4 and was frozen at -20°C temporarily until being shipped on dry ice to Stanford University, where samples were subsequently aliquoted and stored at -80°C . Intraoperative findings were catalogued, and any chondral defects were graded with use of the Outerbridge classification for subjects in the arthroscopy group¹⁵.

In addition to the surgical group, fifteen volunteers were enrolled in the study after consent was obtained for lavage of a knee without a recent injury or current pain and with no history of knee surgery. These individuals underwent office-based knee lavage. The skin was sterilized with 10% povidone-iodine solution and was anesthetized with a topical anesthetic. The knee lavage was performed as described above for intraoperative lavage.

The two orthopaedic surgeons who performed the knee lavage procedures and operations, as well as the radiologist, completed all aspects of the study in a manner blinded to the assay procedure and results.

Cytokine Analysis

The concentrations of seventeen inflammatory cytokines and chemokines (IFN- γ [interferon gamma], IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF [granulocyte-colony-stimulating factor], GM-CSF [granulocyte-macrophage colony-stimulating factor], TNF- α , IL-1 β , MCP-1, and MIP-1 β [macrophage inflammatory protein-1 beta]) were quantified in knee lavage samples with use of a human multiplex inflammatory cytokine panel and the Bio-Plex 200 System (Bio-Rad, Hercules, California), following the manufacturer's protocol in a ninety-six-well plate format. The choice of cytokines reflects the standard, prechosen panel available through Bio-Rad. This cohort was intended to represent typical inflammatory molecules in a variety of conditions that we hypothesized were also likely to be present in our study. This assay utilizes antibodies linked to polystyrene beads containing different levels of fluorophores and has been validated against standard enzyme-linked immunosorbent assays (ELISAs) of human blood samples¹⁶.

ELISA Cross-Validation

Although the Bio-Plex assay has been cross-validated against ELISA with use of human blood samples¹⁶, we performed another cross-validation experiment for two cytokines, with a majority of the knee lavage samples. Sample volume limitations prevented further cross-validation testing.

IL-6

Fifty-six samples were assayed with use of a human IL-6 Quantikine ELISA kit (D6050; R and D Systems, Minneapolis, Minnesota) according to the manufacturer's protocol.

MCP-1

Forty-six samples were assayed with use of a human MCP-1 ELISA kit (DCP00; R and D Systems) according to the manufacturer's protocol.

Statistical Analyses

Multiple analyses of variance were performed to compare mean concentrations of all seventeen cytokines between samples from asymptomatic knees and painful knees. Multiple hypotheses were tested in the analysis of data because of the multivariate nature of the dependent variables. The testing of multiple hypotheses can lead to false-positive results, a phenomenon that is encountered in many panel-type assays (for example, DNA microarrays and multiplexed bead immunoassays). This problem is mitigated by the use of multivariate analysis of variance, which partially accounts for this effect for any one test. However, p values in the range 0.005 to 0.05 should be viewed with some caution because of the multiple comparisons made. The asymptomatic knee group includes the contralateral knees of symptomatic subjects, which may violate the independence assumptions of multivariate analysis of variance. To address this important issue, we analyzed the data using a repeated-measures approach including a within-subjects effect in addition to the between-subjects effect. The within-subjects effect was not found to be significant by multi-

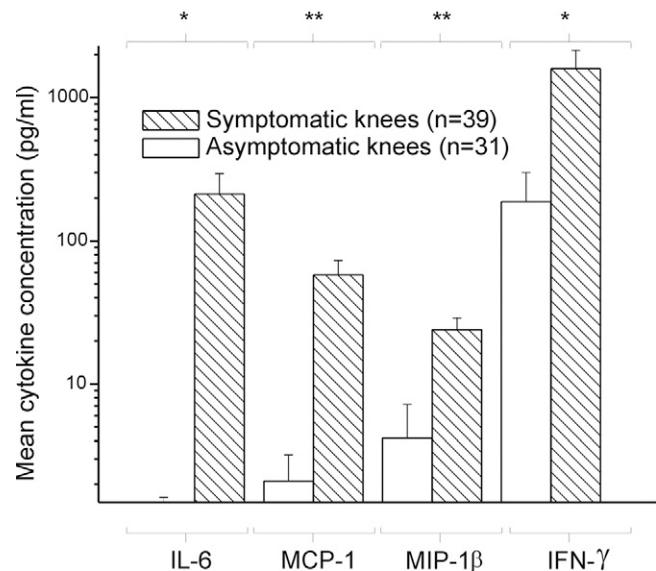


Fig. 1

The mean concentration of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1 β), and interferon gamma (IFN- γ) in knee lavage samples from thirty-one asymptomatic knees (open bars) with signs of meniscal injury (eight patients) or without such signs (twelve patients) on magnetic resonance imaging and thirty-nine subjects with knee pain and undergoing arthroscopic surgery for meniscal injury (hatched bars). All results are from a simultaneous multiplexed immunoassay with use of seventeenplex human inflammatory cytokine kits. The y axis is a log-scale representation of the mean cytokine concentration (pg/mL). Error bars represent the standard error of the mean. *p < 0.05. **p < 0.001 (multivariate analysis of variance).

variate analysis of variance with a single binary within-subjects factor (p > 0.05).

Correlation analyses of cytokine concentration compared with pain score or one cytokine concentration compared with another cytokine concentration were performed with use of Spearman correlation coefficient analysis. A p value of <0.05 was taken as significant.

Source of Funding

No external funding was used for this project.

Results

Cytokine analysis was completed for forty-seven subjects, consisting of thirty-two patients undergoing unilateral knee arthroscopy for acute pain and fifteen asymptomatic control subjects. Of the thirty-two patients who underwent knee arthroscopy and completed the study, twenty-three also provided a sample from the contralateral knee. Therefore, cytokine assay data were collected from a total of seventy knees. All patients undergoing arthroscopy had physical examination findings consistent with meniscal abnormality (joint-line tenderness and/or a positive McMurray sign) on the operatively treated side.

TABLE I Results for Detection of Intra-Articular Cytokines

	No. of Knees	IL-6*	MCP-1*	MIP-1 β *	IFN- γ *
Treatment group					
Arthroscopically treated knee	32	157 \pm 68.0 $\dagger\dagger$	57.4 \pm 18.9 $\S\#$	28.2 \pm 8.0 $\dagger\S$	1288 \pm 331**
Contralateral, asymptomatic knee	23	0.5 \pm 0.4**	10.3 \pm 4.9**	9.3 \pm 4.4**	1231 \pm 906**
Control knees	15	0.0 \pm 0.0	0.5 \pm 0.4	0.3 \pm 0.2	9.1 \pm 7.2
Symptoms					
Pain	39	214 \pm 83.0 $\dagger\dagger$	58.0 \pm 15.1 $\dagger\dagger$	24.2 \pm 5.0 $\dagger\dagger$	1558 \pm 556 $\dagger\dagger$
No pain	31	0.0 \pm 0.0	2.4 \pm 1.3	4.2 \pm 3.0	188 \pm 113
Preop. magnetic resonance imaging finding					
Positive for meniscal tear	44	189 \pm 75 $\S\S$	50.8 \pm 13.9 $\#\#$	22.9 \pm 4.9 $\#\#\#$	1359 \pm 505 $\S\S$
Negative	12	0 \pm 0	0.4 \pm 0.4	0.4 \pm 0.3	11.3 \pm 9.0

*The values are given, in picograms per milliliter, as the mean and the standard error of the mean and are calculated from immunoreactivity for interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1 β), and interferon gamma (IFN- γ) in knee lavage samples from all patients before and after pooling of data. \dagger Compared with control group, the difference was significant ($p < 0.05$). $\dagger\dagger$ Compared with contralateral group, the difference was significant ($p < 0.05$). \S Compared with the control group, the difference was significant ($p < 0.005$). $\#\#$ Compared with the contralateral knee, the difference was significant ($p < 0.005$). **Not significantly different from comparison made by magnetic resonance imaging finding alone (symptoms not taken into account.) $\dagger\dagger$ Compared with knees without pain, the difference was significant ($p < 0.05$). $\dagger\dagger\dagger$ Compared with knees without pain, the difference was significant ($p < 0.005$). $\S\S$ Compared with knees without a tear, the difference was significant ($p < 0.05$). $\#\#\#$ Compared with knees without a tear, the difference was significant ($p < 0.005$). $\#\#\#\#$ Compared with knees without a tear, the difference was significant ($p < 0.001$).

Pain Scores, Age, and Sex Profile of the Patients by Group

The mean self-reported pain score (and standard error of the mean) for the normal (volunteer) control knees, the operatively treated knees, and the nonoperatively treated, contralateral knees of the patients was 0 ± 0 , 6.3 ± 0.2 , and 0.7 ± 0.2 , respectively. The mean age of the normal control group, the operatively treated knee group, and the nonoperatively treated knee group was 43 ± 3 , 54 ± 3 , and 54 ± 3 years, respectively ($p > 0.05$, analysis of variance). The male-to-female ratio of the normal control group, the operatively treated knee group, and the nonoperatively treated knee group was 7:8, 23:9, and 17:6, respectively. The ratio of male to female was significantly different from 1:1 in the operatively treated and nonoperatively treated knee groups ($p < 0.05$).

Magnetic Resonance Imaging Findings by Group

Asymptomatic Volunteers

Fifteen subjects completed magnetic resonance imaging evaluation of the knee, which identified nonpainful meniscal abnormalities in three knees (the posterior horn of the medial meniscus was involved in one knee and the medial meniscus, in two).

Symptomatic Subjects—Operatively Treated Knee

All symptomatic, operatively treated knees had a meniscal injury identified on magnetic resonance imaging.

Symptomatic Subjects—Nonoperatively Treated Knee

Nine of the twenty-three patients who agreed to have lavage of the contralateral knee also completed magnetic resonance imaging of that knee. All nine of them were found to have a

degenerative meniscal abnormality (seven involved the posterior horn of the medial meniscus; one, the anterolateral portion of the medial meniscus; and one, the lateral meniscus). None of these knees were found to have an anterior cruciate ligament injury.

Intraoperative Findings

Of thirty-four patients who underwent knee arthroscopy, thirty-two had confirmation of the preoperative diagnosis of a meniscal tear on magnetic resonance imaging. However, for two knees, the operative exploration failed to confirm the meniscal injury identified by magnetic resonance imaging, representing possible false-positive findings.

Cytokine Assay Results

Cytokine data were grouped according to the presence or absence of acute knee pain. Results from all thirty-one nonpainful knees (fifteen normal controls and sixteen contralateral, nonoperatively treated knees) were grouped and compared with those from all thirty-nine painful knees (Fig. 1, Table I). Multivariate analysis of variance detected significantly greater mean concentrations of cytokines (and standard error of the mean) in lavage samples from painful knees compared with those from nonpainful knees for eight of the seventeen cytokines (in picograms per milliliter of knee lavage fluid) including IFN- γ (1558 ± 556 and 188 ± 113 , respectively; $p = 0.03$), IL-6 (214 ± 83.0 and 0.0 ± 0.0 ; $p = 0.026$), MCP-1 (58.0 ± 15.1 and 2.4 ± 1.3 ; $p = 0.002$), MIP-1 β (24.2 ± 5.0 and 4.2 ± 3.0 ; $p = 0.002$), IL-2 (5.6 ± 2.1 and 0.0 ± 0.0 ; $p = 0.023$), IL-4 (2.0 ± 0.7 and 0.2 ± 0.1 ; $p = 0.025$), IL-10 (3.9 ± 1.4 and

TABLE II Cytokine Analysis and Magnetic Resonance Imaging Compared with Arthroscopy for Detection of Painful Meniscal Tears*

Positive Finding on Magnetic Resonance Imaging (N = 44)	Arthroscopic Finding of Meniscal Tear		Sensitivity (95% Confidence Interval)
	Positive	Negative	
MCP-1			94% (79%-99%)
Present	30	0	
Absent	2	2	
IL-6			75% (57%-89%)
Present	24	0	
Absent	8	2	
MIP-1 β			81% (64%-93%)
Present	26	0	
Absent	6	2	
IFN- γ			75% (57%-89%)
Present	24	0	
Absent	8	2	
MCP-1 or MIP-1 β or IL-6 or IFN- γ			100% (89%-100%)
Present	32	0	
Absent	0	2	
Magnetic resonance imaging alone			100% (89%-100%)
Positive	32	2	
Negative	0	0	

*Forty-four knees in this study had a meniscal abnormality identified on a preoperative magnetic resonance imaging study by an independent, blinded radiologist. Of the forty-four patients, forty-two had a meniscal injury confirmed arthroscopically by an independent, blinded orthopaedic surgeon. Positive cytokine tests ("present") were then tallied with use of the definition that two standard deviations above the mean cytokine level in the asymptomatic normal control group represents a positive test. This analysis was performed for monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), macrophage inflammatory protein-1 beta (MIP-1 β), and interferon gamma (IFN- γ) separately, with resultant sensitivity ranging from 75% to 94%. The 95% confidence interval was calculated with use of the exact binomial method. In addition, if the analysis is performed as an "or" test, where the presence of any one of these three or four cytokines counts as a positive test, the diagnostic assay performs perfectly and outperforms magnetic resonance imaging in this small cohort.

0.1 \pm 0.0; $p = 0.016$), and IL-13 (3.4 \pm 1.3 and 0.3 \pm 0.3; $p = 0.036$) (Fig. 1).

A significant positive correlation was detected between patient-reported pain scores and concentrations of the following cytokines: IL-6 (Spearman $\rho = 0.7$; $p < 0.001$), MCP-1 ($\rho = 0.8$; $p < 0.001$), MIP-1 β ($\rho = 0.6$; $p < 0.001$), and IFN- γ ($\rho = 0.6$; $p < 0.001$). In addition, the concentration of these four cytokines was significantly correlated to each other ($\rho = 0.5$ to 0.7 ; $p < 0.001$) (MCP-1 compared with IL-6 and MIP-1 β). Although the p value reached significance in the covariant analysis for IL-2, IL-13, IL-7, IL-10, and G-CSF compared with reported pain scores, the correlation coefficient values were low ($\rho = 0.16$ to 0.26). With the numbers studied, there was no correlation between the duration of symptoms in the arthroscopy group and the cytokine concentration ($p > 0.05$). No significant differences in cytokine levels were observed with regard to sex ($p > 0.05$).

Within the operatively treated group, no significant difference was detected, with the numbers studied, between the mean concentration of cytokines measured in knee lavage

samples from the thirteen knees with moderate effusions compared with the nineteen knees with minimal effusions ($p < 0.05$).

The median Outerbridge classification score in the arthroscopy group determined intraoperatively was 3 (range, 0 to 4). A weak, negative correlation was observed between the Outerbridge score and intra-articular IL-2 ($p < 0.05$; $\rho = 0.39$); the correlation was not significant for any other cytokine.

Table II presents the sensitivity of IL-6, MCP-1, MIP-1 β , and IFN- γ alone or in a combination compared with magnetic resonance imaging of the knee alone.

ELISA Compared with Bio-Plex Cross-Validation Assays IL-6

The concentrations of IL-6 in knee samples, as determined by the Bio-Plex assay, were not found to differ significantly from those measured with use of standard ELISA ($p = 0.23$, Student paired t test), and a significant correlation was detected between the two assays (Pearson correlation coefficient, $r = 0.7$; $p < 0.001$; two tailed) (data not shown).

MCP-1

The concentration of MCP-1 determined by ELISA was, on the average, fivefold greater than that determined by the Bio-Plex assay, and the two assay means were significantly different ($p < 0.001$). However, relative to each other, the two assays were significantly correlated ($r = 0.9$; $p < 0.0001$; two tailed) (data not shown).

Discussion

We attempted to identify an intra-articular inflammatory cytokine profile that may assist the physician in evaluating patients with knee pain. We analyzed and compared the intra-articular aspirate of thirty-nine painful knees with that of thirty-one asymptomatic knees. In addition, these results were compared with magnetic resonance imaging findings from both groups of knees and the intraoperative findings in knees undergoing arthroscopy. Although we observed significantly greater mean concentrations of eight inflammatory cytokines in the painful knees compared with the nonpainful knees, four of these cytokines, IL-6, MCP-1, MIP-1 β , and IFN- γ , also had a significant positive correlation to self-reported pain scores and were present most consistently in painful knees. This cytokine profile was not present in knees with nonpainful meniscal abnormalities identified on magnetic resonance imaging.

It has been previously demonstrated in animal models that various inflammatory cytokines are elevated in response to tissue injury¹⁷⁻¹⁹, possibly related to leukocyte-mediated wound-healing mechanisms^{19,20}. This process may result in enhanced nociception or pain²¹⁻²³ by means of cytokine-induced sensitization of nociceptors²⁴⁻²⁶, which have been shown to innervate the outer third of the human meniscus²⁷. An acute response diminishing over time has been suggested, with several studies that have described cytokine proliferation in the acute phase of injury^{9,17,19,28}. It has recently been shown that IL-6 can sensitize nociceptive C-fibers innervating the rat knee joint²⁹, possibly by inducing the cyclooxygenase-prostaglandin-E₂ inflammatory pathway^{30,31}. There is also recent evidence that IL-6 may be involved in the transition from peripheral tissue injury and inflammation to central sensitization and thus pathological pain states³². However, the pattern of cytokine release has not yet been correlated to patient-reported knee pain.

We propose that a diagnostic test panel of these four cytokines fulfills criteria for diagnostic testing and meets the qualifications for validity proposed in the Sackett and Haynes model³³. The approach proposed by Sackett and Haynes centers on four fundamental questions or so-called phases that, they argue, any diagnostic test needs to address in order to achieve maximum validity. Although currently the mainstay of decision-making in the treatment of meniscal tears, magnetic resonance imaging clearly fails the first Sackett and Haynes criterion, that is, diagnostic test results in affected patients must differ from those in normal individuals. The fact that many physicians may base operative treatment on magnetic resonance imaging findings of knee pathology has been called into question by nu-

merous studies that have identified age-related meniscal changes as pathological^{3,34-36}.

When used as a diagnostic test to detect painful meniscal tears in the present study, the presence of MCP-1, IL-6, MIP-1 β , or IFN- γ alone was highly sensitive and specific (Table II). If the presence of any one of these cytokines is taken as positive (i.e., MCP-1 or IL-6, etc.), the sensitivity and specificity was 100%, outperforming magnetic resonance imaging compared with the so-called gold standard of intraoperative findings. This suggests that the cytokine test result might distinguish patients with and without painful meniscal tears among patients in whom it is clinically reasonable to suspect a meniscal injury. A larger study is necessary to confirm this preliminary finding, but these observations are promising for the development of a diagnostic test that is highly accurate, rapid, and inexpensive.

Although substantial effort was made to adhere to a standard sample collection and assay protocol, there was unavoidable variability in some sample handling parameters. Interindividual precision of the volume of sterile saline solution injected into and withdrawn from the knee joint was difficult to maintain. In addition, some knee lavage samples were more contaminated with blood than others. Although the injected volume of saline solution varied, depending on the amount that was required to enable withdrawal of a 2 to 3-mL fluid sample, it raised the possibility for bias toward greater dilution of cytokines in patients without an effusion compared with those with some effusion. However, this variation had no apparent pattern and thus was equally distributed among subjects in the operatively treated, nonoperatively treated, and control groups, who all had lavage performed in the same manner. Therefore, we do not believe that this factor introduced substantial bias or confounding effects. Furthermore, immunoassay measurements in the comparison of biological fluids are more likely to provide relative concentrations rather than absolute values. The lavage technique whereby saline solution was injected into the joint space was necessary since fluid aspiration is difficult or impossible in most patients unless a large effusion is present.

We believe there was a potential selectivity bias in the study for the participation of subjects who consented to have lavage of the contralateral knee; younger, healthier patients might be less likely to allow the healthy knee to be lavaged, for fear of a negative outcome. Indeed, a substantial number of subjects refused to have the contralateral knee lavaged. We recognize the inherent but unavoidable selection bias as approximately 28% of the patients in our study with a painful knee declined lavage of the other, nonpainful knee. It is likely that this bias, however, would tend toward reducing the difference between symptoms in the knee undergoing arthroscopy and those in the contralateral knee since younger, less symptomatic subjects would be less likely to volunteer the healthy knee for an invasive lavage procedure.

Finally, the asymptomatic knees include the contralateral knees of symptomatic subjects, which may violate the independence assumptions of multivariate analysis of variance.

To address this important issue, we analyzed the data using a repeated-measures approach including a within-subjects effect in addition to the between-subjects effect. The within-subjects effect was not found to be significant by multivariate analysis of variance with a single binary within-subjects factor ($p > 0.05$).

In conclusion, we observed a greater concentration of a combination of four inflammatory cytokines and/or chemokines, IL-6, MCP-1, MIP-1 β , and IFN- γ , in intra-articular lavage samples from painful knees of patients with an intraoperatively confirmed meniscal injury compared with lavage samples from nonpainful, control knees. These cytokines were nearly absent in the knees of fifteen normal, age-matched, asymptomatic control subjects. Compared with the findings of preoperative magnetic resonance imaging and intraoperative diagnosis, the assay of these four cytokines performed as well as or better than magnetic resonance imaging. These findings suggest that these cytokines may serve as the basis for further development of a diagnostic test. ■

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