# Predictors of Clinical Response to Intraarticular Hylan Injections — A Prospective Study Using Synovial Fluid Measures, Clinical Outcomes, and Magnetic Resonance Imaging

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***ABSTRACT.*** ***Objective.*** To evaluate synovial fluid (SF) and clinical and imaging predictors of clinical response in patients receiving intraarticular Hylan GF-20 injections.

***Methods.*** Thirty-two patients with mild to moderate osteoarthritis (OA) of the knee [OsteoArthritis Research Society International (OARSI) grades I–II] were followed over 6 months. SF and clinical and radiographic measures were assessed. Patella and tibial cartilage volume and cartilage defect scores were measured at baseline and 6 months using magnetic resonance imaging (MRI). The primary outcome measure was the relationship between SF measures and clinical response as defined by the OARSI-Outcome Measures in Rheumatology Clinical Trials responder criteria for OA ("High improvement" ≥ 50% improvement in pain or function; absolute change ≥ 20 NU on Western Ontario and McMaster University Osteoarthritis Index questionnaire). Secondary outcomes included MRI outcomes (change in cartilage volume and cartilage defect scores).

***Results.*** Fifteen patients achieved "High improvement." High baseline SF hyaluronic acid (HA) concentration was a statistically significant predictor of clinical response with odds ratio (OR) 6.04 ($p < 0.02$). HA concentration was divided into tertiles and fitted to a univariate regression model against clinical response. A baseline HA concentration value of > 2 mg/ml provided the greatest tradeoff between sensitivity and specificity with values of 60% and 77%, respectively, a likelihood ratio of 2.55, and OR of 4.88. Baseline clinical and radiological measures did not predict clinical response in this cohort with mild to moderate OA. Nineteen subjects had MRI at both timepoints. No change was noted in cartilage volumes or cartilage defect scores over 6 months. There was no association between baseline HA concentration and baseline cartilage volume.

***Conclusion.*** Baseline SF HA concentration predicts clinical response in patients receiving intraarticular Hylan. This has implications for the selection of patients who are likely to respond to this therapy. (First Release Feb 15 2008; J Rheumatol 2008;35:685–90)

*Key Indexing Terms:*
HYALURONIC ACID CLINICAL RESPONSE MAGNETIC RESONANCE IMAGING
OSTEOARTHRITIS KNEE

Osteoarthritis (OA) is a chronic disease characterized by gradual degradation of cartilage and failure of supporting joint tissues. It accounts for considerable morbidity in our community, with data from the Framingham study suggesting that symptomatic knee OA occurs in 6.1% of adults age 30 and over, and symptomatic hip OA in 0.7%–4.4% of US adults[1]. Current treatment strategies focus on symptomatic relief with paracetamol, nonsteroidal antiinflammatory drugs, neutraceuticals, and intraarticular agents, including glucocorticoids and hyaluronans. The American College of Rheumatology guidelines for the medical management of knee OA recommend use of intraarticular hyaluronans as second-line therapy for the treatment of this condition[2]. A recent Cochrane Review[3] concluded that hyaluronic acid (HA) showed superior efficacy compared to placebo for improvement in pain and function of knee OA. However,

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there are few data identifying patients most likely to respond. Data from a large retrospective Canadian study[4] showed radiographic grade of the knee to be a predictor of response, with significantly higher response rates in those with grade I–II OA than in those with endstage disease. While other studies have reported a similar outcome[5], a recent retrospective review of 155 patients with knee OA who had received Hylan GF-20 found that radiological grade did not have marked influence on treatment outcomes[6]. This study also reported improved outcomes in study recruits with moderate knee effusions compared to those with none. This contradicts findings from the large Canadian study[4], where efficacy of intraarticular Hylan was reduced in patients presenting with effusion.

In Australia, a single course (3 injections × weekly) of Hylan GF-20 costs ~ A$500. Hence there is a need to identify those most likely to benefit from this intervention. In addition, the effects of Hylan GF-20 on cartilage are not well defined. In OA, both synovial fluid (SF) viscosity and elasticity are impaired primarily due to a decrease in native HA concentration and molecular weight[7].

This was a prospective pilot study evaluating synovial SF and clinical and imaging outcomes of Hylan GF-20 injections in 32 patients with mild to moderate OA of the knee over 6 months. The effects of Hylan GF-20 on SF measures have been reported[7]. Our study focuses on potential SF and clinical and imaging predictors of response to Hylan GF-20 injections.

Ethics approval for our study was obtained from Royal North Shore Hospital Human Research Ethics Committee, and informed consent was obtained from all study recruits.

## MATERIALS AND METHODS

*Patients.* Patients were recruited through different avenues, including referrals from visiting rheumatologists, advertisements in the local newspapers, and self-referral, and our study conducted at a large tertiary referral hospital near the center of Sydney. We aimed to recruit 60 individuals aged between 40 and 80 years with mild to moderate [OsteoArthritis Research Society International (OARSI) grades I–II][8] knee OA. OA was determined radiologically on semi-flexed fluoroscopically controlled weight-bearing radiographs of the knees. Magnification was controlled for with a 5 mm ball bearing taped to the knee.

*Exclusion criteria.* Intraarticular steroid injections in the preceding 3 months, viscosupplementation in the preceding 12 months, inflammatory arthritis, crystal arthropathy, or large clinically significant knee effusions. Study subjects were asked to discontinue glucosamine and chondroitin sulphate at least 1 month prior to being screened for entry and remain off these agents for the duration of the study.

*Inclusion criteria.* At least 0.2 mL of SF must be aspirated (under ultrasound guidance) at baseline and at 3 months post Hylan GF-20 injection. This was deemed to be the smallest possible volume needed to conduct HA concentration and viscometric measurements. Failure to aspirate SF at 6 months did not result in exclusion from study analysis.

Endpoints

The primary outcome measure of our study is the relationship between SF measures and the likelihood of clinical response. SF measures included baseline SF volume, baseline HA concentration, baseline complex shear modulus (CSM), change in HA concentration, and change in CSM. Baseline HA concentration level predicting clinical response was determined. Successful clinical response was defined as "High Improvement" in pain and function at 3 and 6 months [≥ 50% improvement in pain or in function and absolute change ≥ 20 NU on Western Ontario and McMaster University Osteoarthritis Index (WOMAC) questionnaire] as defined by proposition D of the OARSI-Outcome Measures in Rheumatology Clinical Trials (OMERACT) responder criteria for OA[9]. The WOMAC questionnaire assesses the 3 dimensions of pain, function, and joint stiffness in knee and hip OA using a battery of 24 questions. Secondary outcome measures included magnetic resonance imaging (MRI) outcomes (change in cartilage volume and cartilage defect scores) at baseline and 6 months. Clinical outcomes measured included change in self-reported WOMAC scores of pain, stiffness, and physical function, visual analog scale (VAS) pain score, patient global score, and physician global score. The secondary outcome measures were also assessed for the likelihood of clinical response.

*Laboratory measures.* SF was collected from each study recruit by the same investigator and transported immediately on ice to the laboratory, where the samples were centrifuged for 10 min and the supernatant pipetted into 1.5 ml Eppendorf tubes and stored at –80°C until time of analysis. The samples were then thawed and analyzed in 4 batches of 8 patients each over a 6-month period using methods listed below. Samples were coded such that the investigator performing the analyses was blinded to study subject names and sequence of sample collection.

*Hyaluronan concentration*. The hyaluronan concentration of the human SF samples was determined by a modified micro-method of the uronic acid assay by Blumenkrantz and Asboe-Hanson[6]. The diluted SF samples were boiled with sulfuric acid/borax and a pink color developed by the addition of m-phenylphenol. The color intensity was then determined in a microtiter plate reader and compared with that of known standard concentrations of commercial hyaluronan preparations.

*Rheology measurements.* SF rheology was determined using a micro-Fourier rheometer. A small amount of fluid is placed on a metal plate, which is then subjected to compression over a range of experimental frequencies ranging from 0 to 20 Hz. A computer is then used to apply a Fourier conversion to the data. The data collected were expressed as complex shear modulus (CSM)[10]

$$G^*(w) = \sqrt{G'^2 + G''^2}$$

The rheometer expressed values of G' (elasticity) and G'' (viscosity) at 0.2 Hz intervals between 0 and 20 Hz. We used values at 1 Hz for all calculations, although the significance of the results did not differ whether calculations were done at 0.5, 1, or 10 Hz.

*Clinical evaluations.* Clinical measures were analyzed for change from baseline and for correlations with laboratory data. Study subjects were asked to complete the WOMAC questionnaire, VAS pain score, and patient global score at baseline and 3 and 6 months postinjection. A physician global score was also recorded at each visit. Patient diaries were maintained to ensure adherence to exclusion criteria.

*MRI assessment.* MRI of the treatment knee was performed at baseline and at 6-month followup. Knees were imaged in the sagittal plane using a 1.5 T whole-body MR unit (Signa, GE Medical Systems, Milwaukee, WI, USA) with use of a commercial transmit/receive extremity coil. The following image sequence was used: T1-weighted fat saturation 3-dimensional (3D) GRE, flip angle 30°, repetition time 40 ms, echo time 8 ms, field of view 150 mm, 54 partitions, 512 × 256 matrix, acquisition time 25 min; one acquisition, slice thickness 1.6 mm. Cartilage volumes and defects were assessed by 2 trained readers blinded to clinical and laboratory results. Cartilage volumes were assessed in a paired fashion but blinded to visit sequence.

*Knee cartilage volume assessment.* Cartilage volume was determined by manual segmentation using image processing on an independent work station using the software program Osiris (University of Geneva, Geneva,

Switzerland) as described[11]. Femoral cartilage volume was not assessed as it has been shown that 2 tibial sites and the patella site correlate strongly with this site[12,13]. One reader (CD) read all MRI and a second reader (AA) read 10 MRI to assess interobserver reproducibility. Intraobserver reliability was assessed on 10 MRI. Intraclass correlation coefficients (ICC) of agreement between readers was 0.99 for medial tibial, 0.99 for lateral tibial, and 0.99 for patella. The ICC for intraobserver reproducibility were 0.99 for medial tibial, 0.99 for lateral tibial, and 0.99 for patella.

*Knee cartilage defect assessment.* Cartilage defects (score range 0–4) were graded at medial tibiofemoral, lateral tibiofemoral, and patellar sites as described[14] (grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low signal intensity area with an intact surface and bottom; grade 2 = irregularities on the surface or bottom and < 50% loss of thickness; grade 3 = deep ulceration with ≥ 50% loss of thickness; and grade 4 = full-thickness chondral wear with exposure of subchondral bone). A cartilage defect had to be present in at least 2 consecutive sections. Cartilage defect scores at the medial tibiofemoral (range 0–8), lateral tibiofemoral (range 0–8), patellar (range 0–4), and whole (range 0–20) compartments were expressed. Changes in cartilage defects were calculated by subtracting the cartilage defect scores at baseline from the cartilage defect scores at followup. An increase in cartilage defect score of 1 or more was defined as an increase in cartilage defects, and a decrease in cartilage defect score of 1 or more was defined as a decrease in cartilage defects[15]. One reader (CD) read all MRI and a second reader (AA) read 20 MRI to assess interobserver reproducibility. Intraobserver reliability was assessed on 20 MRI. The ICC of agreement between readers were 0.86 for medial tibiofemoral, 0.83 for lateral tibiofemoral, and 0.96 for patella. The ICC for intraobserver reproducibility were 0.98 for medial tibiofemoral, 0.98 for lateral tibiofemoral, and 1 for patella.

*Statistical analysis.* Sample size estimates suggested that 30 subjects with sequential SF samples would be sufficient to detect a 10% change from baseline in the primary endpoint, SF measures. All results were entered into a MS Excel spreadsheet and then exported into Stata statistical software (Stata Corp., version 9). Changes in HA concentration and complex shear modulus pre- and post-Hylan GF-20 injection were calculated using the Wilcoxon signed-rank test and the paired Student's t test. Correlations were determined using Spearman's correlation coefficient. Univariate and multivariate logistic regression analyses were done using Stata for primary and secondary outcomes. The outcome variables entered into the model to assess predictors of high response included the various demographic, clinical, and SF measures. The mean and SD in change in cartilage volume over time for each of the knee compartments was calculated. Nonparametric testing for 2 related samples was used to compare cartilage defect scores between baseline and 6 months. MRI outcomes (cartilage volumes and cartilage defect scores) were compared between study subjects classified as OARSI responders and nonresponders. A p value < 0.05 and a 95% confidence interval (95% CI) not including the null point were regarded as statistically significant.

## RESULTS

A total of 60 subjects were screened and of these, 32 patients satisfied inclusion criteria with at least 0.2 ml of SF at baseline and 3 months post-Hylan GF-20 injections. There were 15 male and 17 female subjects, with a mean age of 65 years (range 42–87 yrs) and mean body mass index (BMI) of 29 kg/m$^2$ (range 23–40 kg/m$^2$). There were no statistically significant differences in any baseline characteristics between men and women (Table 1).

Twenty-eight subjects had insufficient SF at baseline and/or 3 months postinjection and were excluded from the study analysis as a result. The mean age and BMI of these 28 excluded recruits were 56 years (range 43–78 yrs) and 30.5 kg/m$^2$ (range 24–44 kg/m$^2$), respectively, with 15 women and 13 men. Only 5 of the excluded subjects had sufficient fluid at baseline for HA concentration analysis. There were no statistically significant differences in mean BMI, mean baseline VAS pain score, or radiographic severity between those recruits meeting inclusion criteria and those who failed. However, there were significant differences in mean age and mean baseline HA concentration between these groups. Mean baseline HA concentration (mean 2.29 mg/ml) was higher in those failing inclusion criteria (5 subjects). Response rates among those excluded from the study are not known, as VAS and WOMAC scores were not recorded at 3 months in this group. We have previously reported that baseline HA concentrations are inversely correlated with baseline VAS pain scores[7]. Hence, higher baseline HA may predict subjects who have milder disease. However, it is not clear if this would have affected the results as the numbers were small.

Of the 32 study recruits, 15 achieved "High improvement" as defined by the OARSI-OMERACT responder criteria. Univariate analysis was performed for all baseline characteristics thought to potentially influence response to Hylan GF-20 injections. High baseline HA concentration was a statistically significant predictor of clinical response with odds ratio (OR) of 6.04 (p < 0.02) whereas all other variables were not significant (Table 2).

*Table 1.* Baseline characteristics by sex. Values are expressed as mean (SD).

| Patient Characteristics | Male | Female |
|---|---|---|
| Study recruits, n | 15 | 17 |
| Body mass index, kg/m$^2$ | 29.4 (± 4.9) | 28.7 (± 4.9) |
| Minimal joint space narrowing, mm | 3.8 (± 2.0) | 3.2 (± 1.2) |
| Synovial fluid volume, ml | 3.6 (± 3.7) | 4.2 (± 4.2) |
| WOMAC pain, NU/100 | 34 (± 21.1) | 44 (± 18.0) |
| WOMAC function, NU/100 | 43 (± 21.8) | 49 (± 18.9) |
| Age, yrs | 64 (± 11.5) | 66 (± 9.5) |
| HA concentration, mg/ml | 2.1 (± 0.7) | 1.9 (± 0.6) |
| Complex shear modulus, Pa | 2.4 (± 2.4) | 1.7 (± 1.9) |

WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

*Table 2.* Correlation of baseline variables and high clinical response. OR and 95% CI describe univariate associations between each baseline variable and high OMERACT-OARSI clinical response.

| Variable | Odds Ratio | p | 95% CI |
|---|---|---|---|
| Baseline HA concentration, mg/ml | 6.04 | < 0.02 | 1.276–28.70 |
| Baseline synovial fluid volume, ml | 0.87 | < 0.18 | 0.701–1.07 |
| Age, yrs | 0.99 | < 0.75 | 0.924–1.06 |
| Sex | 0.61 | < 0.49 | 0.151–2.49 |
| Complex shear modulus, Pa | 1.11 | < 0.53 | 0.798–1.55 |
| Minimum joint space narrowing, mm | 1.39 | < 0.16 | 0.882–2.18 |

We have previously reported in this group a good correlation between baseline HA concentration and CSM (a measure of viscoelasticity) ($r = 0.59$, $p < 0.0005$)[7]. Also, there was no significant relationship between the changes in HA concentration and baseline values, and there was no significant correlation between changes in HA concentration and CSM at 3 months. There was no statistically significant difference in mean total SF volumes between both timepoints. Mean HA concentration increased by 13% ($p < 0.0008$) from baseline to 3 months. At 6 months, only 19 subjects had sufficient SF for HA concentration analysis, and mean HA concentration increased nonsignificantly by 10% ($p < 0.053$).

All variables were subsequently fitted to a multivariate regression model in which baseline HA remained statistically significant. However, there was significant interaction between several variables. Despite fitting interaction terms, the model was highly unstable due to the small number of study recruits and the presence of multiple variables leading to unacceptably high OR and very wide CI. HA concentration was then divided into tertiles and fitted to a univariate regression model against clinical response. The highest tertile was a strong predictor of clinical response (OR 18.26; $p < 0.13$) but CI were very wide due to small numbers (95% CI 1.87–178.56). We then set out to find a cutoff level for baseline HA concentration that would provide the greatest sensitivity, specificity, and positive predictive value for predicting a high clinical response to intraarticular Hylan GF-20 injections (Table 3). A baseline HA concentration value of 2 mg/ml provides the greatest tradeoff between sensitivity and specificity with values of 60% and 77%, respectively, a likelihood ratio of 2.55, and an OR of 4.88.

Due to technical difficulties, only 19 subjects had MRI available from both timepoints. Of these, 14 had sufficient SF at baseline and 3 months for SF analysis. Cartilage volumes did not change significantly when comparing baseline and 6-month results at any of the 3 sites, i.e., medial tibial, lateral tibial, and patella. Nor was any statistically significant difference observed in cartilage defect scores (medial tibiofemoral, lateral tibiofemoral, patellar, whole compartment) at baseline and 6 months. The mean rate of change of cartilage volume over 6 months was –1.05% (medial tibial), –1.73% (lateral tibial), and –1.63% (patella). There was no correlation between baseline HA and baseline MRI volumes and between baseline MRI measures and clinical response (data not shown).

*Table 3.* Sensitivity and specificity of baseline hyaluronic acid (HA) concentration ranges as predictors of OARSI-OMERACT class D response.

| Baseline HA* Concentration Cutoff Levels, mg/ml | Sensitivity, % | Specificity, % | Likelihood Ratio (+) | Positive Predictive Value, % |
|---|---|---|---|---|
| ≥ 1.5 | 87 | 29 | 1.23 | 52 |
| ≥ 2.0 | 60 | 77 | 2.55 | 69 |
| ≥ 2.5 | 27 | 94 | 4.53 | 80 |

* Normal range for HA concentration in healthy knees (2.5–4 mg/ml).

## DISCUSSION

OA is a heterogeneous condition causing significant morbidity. At present, there is no curative therapy for OA and hence there is a need to identify effective nonoperative options with good safety profiles. Intraarticular hyaluronan was approved in the US in 1997 and in other parts of the world since 1987. There have been several large randomized placebo controlled studies[16-22] suggesting superior efficacy of intraarticular hyaluronans over placebo, but there have also been several good quality randomized trials[23-26] showing no benefit of these interventions over both placebo or intraarticular steroid. More recently, 3 metaanalyses have been published[3,27,28]. They all report statistically significant treatment effects of intraarticular hyaluronan compared to saline injections, but differ with respect to calculated effect sizes and interpretation of clinical importance[29]. While part of the problem rests with adequacy of trial design, an overriding issue in the treatment of such a heterogeneous condition is the identification of subgroups most likely to benefit.

Several studies have attempted to identify clinical and imaging predictors of response to intraarticular hyaluronans. Early radiographic grade[4,5,26], both presence[6] and absence of effusion[4], and high baseline functional index[24] have been found to predict better response. These have yielded conflicting results as the quality of the trials has been variable.

To date, there have been no studies addressing the role of SF measures in predicting clinical response to intraarticular hyaluronan therapy. In our study, baseline HA concentration was a significant predictor of response and when further stratified into tertiles, the highest tertile was the strongest predictor of high response as defined by OARSI-OMERACT criteria. This is not surprising, as HA concentration has been described to fall with disease severity in knee OA[10,30]. Overall, 47% of the study population reported sufficient change in baseline clinical measures to be classified as OARSI-OMERACT responders at 3 months postinjection. Using a HA concentration cutoff of 2.0 mg/ml, the sensitivity and specificity of a positive clinical response was estimated to be 60% and 77%, respectively, with positive predictive value of 69%. In other words, for a given patient with baseline HA level > 2.0 mg/ml, there is a roughly 70% chance of having a high response to intraarticular Hylan injections. Given the relatively high financial cost of intraarticular Hylan injections, a specificity of 77% could be considered reasonable from the patient's perspective on the proviso that SF HA concentration was a readily available and easily performed test.

As there is a wide range of normal for SF HA concentration[30] (2.5–4 mg/ml), with concentrations declining after the

age of 30, stratifying HA concentration would allow more accurate prediction of response. One may expect HA concentration to correlate to other markers of disease severity such as minimum joint space narrowing on radiograph. However, we were unable to establish any significant correlation to other clinical variables including radiological severity, age, sex, SF volume on aspiration, and BMI. Notably, our study included only patients with mild to moderate radiological grades, in whom a greater response rate may be expected.

These rates of change of cartilage volume detected on MRI are lower than that expected with the natural history of OA[31-33]. However, it is not yet known whether the rates of change in cartilage volume are linear or phasic over time. In addition, the ranges obtained for rates of change at each site were very wide. In studies of intraarticular hyaluronan, only 2 studies have used MRI outcomes to evaluate structural damage to articular cartilage. In a 1-year, randomized single-blind trial, 24 patients were treated with hyaluronan while 16 patients were treated with a combination of hyaluronan and corticosteroid. Cartilage was assessed using a semiquantitative grading that had not previously been validated and cartilage volume was not assessed. MRI assessments were performed using a 1.5 T magnet. The authors report that no statistically significant progression was seen at the first year in either group[34]. In another study, Cubukcu, *et al* compared patients receiving hyaluronan and saline[35]. MRI of the patellofemoral cartilage was assessed using cartilage defect scoring similar to that we applied. Subjects also received gadolinium, and the MRI were evaluated for noncartilaginous changes such as meniscal abnormalities and ligamentous changes. However, the authors did not assess cartilage volume. They did not detect any change between the groups after treatment. There were a number of limitations with this study including the use of a 0.5 T magnet and followup assessment at 8 weeks. Jubb, *et al* assessed radiological joint space narrowing (JSN) in subjects receiving Hyalgan or placebo after 1 year. These patients received 3 courses of 3-weekly injections, and no significant difference was found between treatment groups at 1 year. However, those with radiologically milder disease at baseline had significantly reduced JSN compared to placebo[36]. In our study, previously validated techniques were used to assess cartilage defects and cartilage volumes. No progression in cartilage scores was found after treatment with intraarticular hyaluronan and the mean rates of change in volume were low over 6 months.

There are a number of limitations to our study. This study was open-labeled and uncontrolled, with small numbers. Whole-organ MRI assessment with MRI measures such as regional cartilage defects and ligament and meniscus integrity was not performed as this is still in development. The duration of followup for MRI assessment may not be optimal, and analysis at 1 year may be more appropriate in keeping with other structure-modifying studies. In addition, the range of radiological disease severity is narrow. Thus some predictors may not be identified due to type II error. However, this would serve to strengthen the positive finding of HA concentration within this group with mild to moderate knee OA. It cannot be determined whether HA concentration will predict response in a group with more advanced disease in whom the baseline HA levels may be lower. This would need to be tested in other studies. The lack of a control group also makes it difficult to draw firm conclusions regarding efficacy, but should not affect the SF measures, as the subjects had no knowledge of these and analyses were performed blinded to the response status and the time sequence of the sample. While our finding is significant, the most obvious limitation is the inability to perform a rapid and cheap test for SF HA concentration that would assist clinicians in deciding which patients were most likely to respond. It is labor intensive, requiring about 2 h for 1 to 10 SF samples. The costs will also be different in research and clinical settings. Further analysis of blood or urine biomarkers of OA is warranted and larger studies assessing MRI outcomes will be helpful.

Our study was designed as a pilot study to prospectively look for changes in clinical, SF, and imaging measures in a group of subjects with mild-moderate OA of the knee over 6 months. We found that a HA concentration cutoff of 2.0 mg/ml predicted OARSI-OMERACT clinical response with a sensitivity of 60% and specificity of 77%. Our findings support the general consensus that disease severity is likely to be a predictor of clinical response to intraarticular Hylan GF-20 injections, and to our knowledge this is the first study to investigate the role of SF measures as potential predictors of clinical response in the evaluation of intraarticular hyaluronan.

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