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Association between disease-specific quality-of-life and magnetic resonance imaging outcomes in a clinical trial of prolotherapy for knee osteoarthritis

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Abstract

Objective—To assess the relationship between knee osteoarthritis (KOA)-specific quality-of-life (QoL) and intra-articular cartilage volume (CV) in participants treated with prolotherapy. KOA is characterized by CV loss and multifactorial pain. Prolotherapy is an injection therapy reported to improve KOA-related QoL compared to blinded saline injections and at-home exercise but the mechanism of action is unknown.

Design—Two-arm (Prolotherapy, Control), partially blinded, controlled trial.

Setting—Outpatient.

Participants—37 adults with 3 months of symptomatic KOA.

Intervention—Prolotherapy: 5 monthly injection sessions; Control: blinded saline injections or at-home exercise.

Outcome Measures—Primary: KOA-specific QoL scores (baseline, 5, 9, 12, 26, 52 weeks; Western Ontario McMaster University Osteoarthritis Index, WOMAC). Secondary: KOA-specific pain, stiffness, function (WOMAC subscales), magnetic resonance imaging (MRI)-assessed CV (baseline, 52 weeks).

Results—Knee-specific QoL improvement among Prolotherapy participants exceeded that of Controls (17.6 ± 3.2 versus 8.6 ± 5.0 points, $p=0.05$) at 52 weeks. Both groups lost CV over time ($p<0.05$); no between-group differences were noted ($p=0.98$). While Prolotherapy participants lost CV at varying rates, those who lost the least CV (“Stable CV”) had the greatest improvement in pain scores. Among Prolotherapy, but not Control participants, the change in CV and the change in pain (but not stiffness or function) scores were correlated; each 1% CV loss was associated with 2.7% less improvement in pain score ($p<0.05$).

Conclusions—Prolotherapy resulted in safe, substantial improvement in KOA-specific QoL compared to Control over 52-weeks. Among prolotherapy participants, but not Controls, MRI-assessed CV change (CV stability) predicted pain severity score change, suggesting prolotherapy may have pain-specific disease-modifying effect. Further research is warranted.

Keywords

knee osteoarthritis; prolotherapy; dextrose; cartilage volume; magnetic resonance imaging

Knee osteoarthritis (KOA) is a significant public health challenge and leading cause of disability. It is common, expensive¹ and age-related²; by age 65, the majority of the population have radiographic evidence of osteoarthritis and 11% has symptomatic KOA.³ The etiology of pain and disability in KOA is not well known; recent data suggest that neurogenic inflammation involving intra-articular peptidergic receptors may have a pain-specific role in osteoarthritis.⁴ Location of pain fibers include the joint capsule, ligaments, synovium, bone, the outer edge of the menisci, and supportive extra-articular ligaments and tendons.^{5,6} To date, no assessment of these structures has identified a biomarker that reliably correlates with clinical outcomes and could predict KOA severity and monitor change.

A hallmark of KOA is intra-articular cartilage volume (CV) loss. Magnetic resonance imaging (MRI) may be the optimal imaging technique for monitoring longitudinal change in

CV as a measure of KOA severity and progression.⁷⁸ MRI-proven reversal of CV loss, CV stabilization or correlation between changes in clinical outcomes and CV over time after therapy would suggest KOA disease modification.

Prolotherapy is a complementary injection therapy for chronic musculoskeletal pain, including KOA, reported to stimulate healing and reduce pain associated with chronic soft tissue injury.⁹⁻¹¹ Hypertonic dextrose and morrhuate sodium are two commonly used prolotherapy injectants. Recent animal model studies have reported soft tissue strengthening¹² and enlargement¹³ of unclear significance after prolotherapy with both solutions compared to control injections. Our group recently completed two prospective uncontrolled trials and one randomized controlled trial (RCT) that reported significant, clinically meaningful improvements in self-reported knee-specific quality of life (QoL) after prolotherapy compared to baseline status¹⁴ and blinded and non-blinded control therapies,¹⁵ as assessed by the Western Ontario McMaster University Osteoarthritis Index (WOMAC).¹⁶ Participants reported high satisfaction, minimal, self-limited side effects and no adverse events. In spite of measurable clinical effects, prolotherapy's mechanism of action is not well understood and the association between clinical effects and intra-articular CV after prolotherapy has not been studied. Therefore, we evaluated a subsample of participants from two concurrent clinical trials of prolotherapy for symptomatic mild-to-moderate KOA using self-report and MRI to address the hypotheses that prolotherapy slows or reverses CV loss, or is associated with CV status, and is associated with improvement in WOMAC-based scores over 52 weeks.

Methods

The study protocol was approved by the University of Wisconsin Health Sciences Institutional Review Board.

Eligibility criteria and participant recruitment

Adults 40 to 76 years old participating in one of two concurrent clinical trials^{15,17} were invited to participate. They were recruited from the University of Wisconsin Sports, Rehabilitation and Family Medicine clinics and the community. Eligibility criteria and details of the methods for these two trials have been described.^{15,16} Briefly, these included a diagnosis of KOA as defined by clinical criteria (American College of Rheumatology)¹⁸ and augmented by identification of KOA by a radiologist on an existing radiograph obtained within five years prior to enrollment; tenderness of one or more anterior knee structures on physical exam conducted by the first author (DR); and self-reported moderate-to-severe knee pain for at least 3 months, defined as a score of “3” or more in response to the question “What has been the average level of your left/right knee pain been over the last week?” (0-6 ordinal response scale). In addition, eligibility criteria included willingness and ability (absence of standard MRI contraindications) to undergo MRI. The current study participants were followed from 2007 to 2009.

Study Design

Participants were concurrent participants in either a 3-arm RCT (Prolotherapy with dextrose versus blinded saline injections versus at-home exercise)¹⁵ or a single-arm uncontrolled study (Figure 1).¹⁷ Participants received either one of two similar prolotherapy protocols (“Prolotherapy”) or one of two control therapies (blinded saline injections or at-home exercise; “Control”). Participants enrolling in the clinical trials were serially offered MRI examinations at baseline and 52 weeks in addition to self-reported knee-specific assessment. The initial study design called for participants to be recruited from the RCT alone. However, midway through recruitment, MRI-related administrative issues resulted in the inability to

MRI-scan new RCT enrollees for four months. In order to achieve our a priori determined sample size, when scanning ability re-commenced, we opened recruitment to participants in the concurrent single arm uncontrolled study. The sample size and degree of blinding in the study arms therefore varied depending on a participant's initial study arm (Figure 1). In the current analysis, the radiologists interpreting the MRIs were blinded to group allocation and MRI study date.

Injection Intervention

The injection technique in each of two protocols was identical; solution type among Prolotherapy participants varied slightly (Table 1). Injections were performed at 1, 5 and 9 weeks post-entry, with optional additional sessions at weeks 13 and 17, per physician (JJP) recommendations and participant preference. Injections were performed using a published protocol.¹⁴ Injection participants were offered oral oxycodone prior to (one 5mg tablet) and after (eight 5mg tablets) injection and acetaminophen to treat injection-related pain.

At-home exercise intervention

Exercise group participants received an informational pamphlet about KOA (Visual Health Information™ www.vhikits.com/Default.aspx) depicting ten at-home knee exercises. These were demonstrated at baseline by the study coordinator. Participants were advised to begin exercises after enrollment (one session daily three times per week, 10 repetitions per exercise), gradually increase therapy as tolerated over 20 weeks (up to three sessions daily five times per week, 15 repetitions per exercise) and to continue exercising thereafter if desired.

Outcome measures

The primary outcome measure was change in KOA-related QoL as assessed by the composite score of the validated WOMAC questionnaire, made up of three subscales: pain, stiffness and function symptom severity.¹⁶ The WOMAC composite score, constructed as the weighted average of the subscale scores, ranges from 0 (worst) to 100 (best) KOA-related QoL,¹⁹ and has been shown to be responsive to change.¹⁶ The minimal clinically important improvement (MCII) on the WOMAC function scale for KOA has been reported as 9.1 points of change using a 0-100 mm visual analog scale.^{20,21} The WOMAC was collected prior to any procedure at baseline, 5, 9 and 12 weeks (in person), and 26 and 52 weeks (by phone).

The secondary outcome was CV as assessed by MRI expressed in cubic millimeters.²² Change in CV and its relationship to clinical change (expressed by WOMAC score change) in Prolotherapy versus Control group was the main focus of the current analysis. Assessment of CV within the knee joint was obtained using a sagittal spoiled gradient recalled-echo (SPGR) sequence with iterative decomposition of fat and water using least square estimates (IDEAL) fat-water separation (TR/TE: 10ms/4.4, 5.4, and 6.1ms, 14° flip angle, 0.4mm × 0.6mm × 1.0mm voxel volume, 5:00 min scan time) acquired on a 3.0 Tesla scanner (General Electric Healthcare, Waukesha, Wisconsin) using an 8-channel phased-array extremity coil (In Vivo, Orlando, Florida). CV measurements were performed using semi-automated cartilage segmentation software provided for research purposes by General Electric Healthcare on all articular surfaces in the medial, lateral, and patellofemoral compartments, and the total volume of cartilage in all compartments was used as the outcome measure. Cartilage segmentation was performed by an experienced radiology research assistant under the supervision of a fellowship-trained musculoskeletal radiologist (RK). Repeat CV measurements performed on 10 randomly chosen IDEAL-SPGR images

showed a coefficient of variation of 3.5% which is similar to previous reported precision values for CV measurements.²³

Other measures

Demographics, self-reported weight and height, and measures of KOA severity on knee radiographs were collected at baseline to characterize the sample and evaluate as covariates (age, gender, body mass index (BMI), and x-ray-derived KOA severity score). Adherence to at-home exercises was assessed by the question “In the past month, did you perform home exercises as directed? (yes/no)” administered by monthly mail-in logs for the first 20 weeks of the study.

Analysis

No studies have assessed the association between prolotherapy-related CV and clinical outcomes. Therefore the sample size (N=45) for the current study was by convenience; we obtained pre- and post-intervention MRIs from as many subjects in all 4 arms of the concurrent trials as resources allowed. The sample size of the parent 3-arm RCT was guided by two prior studies^{24,25} and powered to detect change in the composite WOMAC score; assuming minimal change in the control groups and minimal loss to follow-up, 32 participants per arm would provide 80% power to detect a 20% difference in mean composite WOMAC scores between control and prolotherapy participants ($p = 0.05$).

Data were analyzed using SAS® 9.1 statistical software (SAS Institute Inc.: Cary, NC). Descriptive statistics described outcomes at each time point. The analysis was per protocol and included all subjects who had both baseline and follow-up MRI evaluations. Repeated measures analysis of variance (five time points over the 52 week follow-up period) compared treatment groups on follow-up WOMAC total and subscale scores after adjusting for baseline scores, age, sex, and BMI. Statistical significance between treatment groups was assessed at each time point. Percent improvement in WOMAC scores was calculated as the change in total WOMAC score from baseline to 52 weeks relative to baseline score.^{26,27} The proportion of participants in each group who met the MCII benchmark of 12-point change on the WOMAC function score was calculated. CV change from baseline to 52-weeks was calculated as the total volume difference and the percent change in volume from baseline. A two-sample t-test compared change in CV at 52 weeks between prolotherapy and control participants. Using linear regression analysis, changes in composite and subscale WOMAC scores at 52 weeks were regressed on change in CV at 52 weeks to assess the degree to which changes in CV volume were associated with changes in WOMAC-assessed knee pain, stiffness and function. Separate regression models were constructed for prolotherapy and control subjects. Mean value $\pm$ SD was reported unless otherwise specified. Two-tailed p -value < 0.05 was established as a statistical significance level.

Results

Of the 60 persons offered MRI at enrollment, 45 agreed; no statistically significant differences were found in baseline demographics, and baseline and follow-up KOA severity variables among participants receiving either of the active therapies, or either of the control therapies. The two active and two control groups were therefore pooled as Prolotherapy and Control groups respectively (Figure 1). Eight participants did not receive a follow-up MRI; therefore, analyses included 37 participants.

The study sample consisted of 21 (57%) women (Table 2). Participants were on average 55.6 ± 7.1 years old (range 43-71 years); 27 (73%) were either pre-obese ($BMI \geq 25$ - 25.9 kg/m^2), or obese ($BMI \geq 30$ kg/m^2) (Table 2). Participants on average reported more than

five years of knee pain and most had failed at least one conservative therapy. Prolotherapy participants reported slightly more severe pain at baseline (lower WOMAC pain subscale score) than those in the Control group ($p=0.04$) but otherwise there were no significant differences between groups including Kellgren-Lawrence (K-L) severity scores. While K-L scores ranged from mild (1) to severe (4) there was no significant difference between the active and control groups in baseline K-L scores ($p=0.61$), nor was there a significant baseline difference within K-L subgroups (1-2/3-4 points) between active and control groups in baseline CV ($p=0.62/p=0.32$) or WOMAC pain scores ($p=0.15/p=0.32$).

Prolotherapy participants received 4.5 ± 0.9 injection sessions; saline control participants received 4.3 ± 0.8 injection sessions. Exercise participants returned an average of 77.4% exercise logs during the 20-week treatment period; 4 of the 5 Exercise group participants reported doing at-home exercises as directed.

Compared to baseline, both groups reported improved ($p<0.05$) composite WOMAC as well as pain and function subscale scores. Near-maximum improvement for the Prolotherapy group occurred at nine weeks and was maintained through 52 weeks (Table 3). Between-group comparisons showed that Prolotherapy participants compared to Control participants reported larger improvements in the WOMAC composite score at 9 weeks (17.4 ± 2.2 compared to 2.5 ± 3.2 points, $p=0.001$), 24 weeks (16.2 ± 3.1 compared to 6.6 ± 3.6 points, $p=0.04$), and 52 weeks (17.6 ± 3.2 compared to 8.6 ± 5.0 points, $p=0.05$) (Table 3). Sixty seven percent (16/24) of Prolotherapy participants improved by at least 9.1 points on the WOMAC function subscale score at 52 weeks compared to 46% (6/13) of Control participants. Improvement among Prolotherapy participants was consistent across the three WOMAC subscales. There were no adverse events or unexpected side effects.

MRI-based Cartilage Volume

CV and WOMAC scores were not correlated at baseline or follow-up time points. Participants in the Prolotherapy and Control groups lost, on average, 6.95% and 7.23% of CV, respectively compared to baseline status ($p<0.001$), without a difference between groups ($p=0.985$) (Table 3). Only 3 participants gained or did not lose CV, two in the Prolotherapy and one in the Control group. There was a correlation noted between CV change and change in the WOMAC-based pain subscale score over 52 weeks (Figures 2a, 2b). While Prolotherapy participants lost CV at varying rates, those who lost the least CV had the greatest improvement in pain scores (Figure 2b); in general, each 1% loss in CV resulted in 2.7% less improvement in WOMAC pain scores (correlation coefficient .44; $p=.033$). Prolotherapy participants with Kellgren-Lawrence score of 3-4 showed a stronger correlation coefficient of .66. However, among Controls, pain score change did not correlate to CV change (Figure 2a, correlation coefficient -.03; $p=.917$). There was no relationship between CV change and the change in WOMAC-assessed composite score, nor function or stiffness sub-scores, in either the Prolotherapy or Control groups.

Discussion

This pilot study, comparing effects of prolotherapy versus control therapy (saline injections or at-home exercise) for symptomatic KOA found 1) significant improvements in self-reported KOA-related QoL, pain and function severity; while reported MCII cutoff values vary,^{28,29} participants in the Prolotherapy group met or exceeded reasonable WOMAC-based reported MCII value for global, pain and functional scores;²⁹ 2) that neither prolotherapy nor control therapies resulted in cartilage growth or decreased rate of cartilage loss. However, 3) data do detect a correlation between MRI-assessed CV change and WOMAC-

based pain subscale score change in Prolotherapy, but not Control, participants at one year as demonstrated by a moderately strong correlation coefficient of .44 ($p=.033$).

Specifically, while both groups lost cartilage at similar rates, Prolotherapy participants who demonstrated less CV loss ("stable" cartilage) over the 52-week follow-up period also responded better clinically to prolotherapy, reporting dramatic improvement in WOMAC pain scores. This was true regardless of the baseline K-L scores. In fact, the relationship was even stronger among prolotherapy participants whose K-L scores were 3-4 (correlation coefficient .66). Prolotherapy seems to have a more robust pain-specific effect among participants who lost less cartilage overall during the 52 week follow-up period; that is, among those with more stable cartilage. Pain generators exposed to potential injectant effects included the intra-articular fat pad, synovium and menisci, and the extra-articular ligaments and tendons.⁵⁶ This finding suggests that prolotherapy may modify KOA directly through a pain-specific mechanism.

Clinically, Prolotherapy participants reported substantial, consistent improvements in WOMAC composite and pain and function subscale scores that were maintained over the 52 week follow-up period, compared to Control participants; 58% of the Prolotherapy participants improved by 12 or more points on the composite WOMAC score at 52 weeks. Analysis of the WOMAC data suggests that prolotherapy improves knee-related QoL and is consistent with the findings of three prior trials.¹⁴¹⁵¹⁷

The results of this study shed light on the possible mechanism by which prolotherapy effects patients with KOA. The similar rate of CV loss for patients in the Prolotherapy and Control groups suggests that prolotherapy, using the studied protocol, may not have a cartilage-protective effect. However, the significant relationship between improvement in WOMAC-assessed pain scores and CV loss among Prolotherapy participants suggests that prolotherapy may have a specific effect on pain-generating knee joint structures in the presence of less diseased cartilage than in more diseased cartilage. To date, experimental work regarding the biological effects of prolotherapy with dextrose and morrhuate sodium has focused on inflammation, and soft tissue size and strength.

A tissue-level inflammatory response in a rat knee ligament model has been reported for each solution.³⁰ Animal model data also suggest additional biological effects of morrhuate sodium and dextrose solutions compared to controls. Rabbit medial collateral ligaments injected with morrhuate sodium were significantly stronger (31%), larger (47%), and thicker (28%), and had a larger collagen fiber diameter (56%) than saline-injected controls.³¹³² Rat patellar tendons injected with morrhuate sodium were able to withstand a mean maximal load of 136% ($\pm 28\%$) – significantly more than the uninjected control tendon.¹² Injured medial collateral rat ligaments injected with 15% dextrose had a significantly larger cross-sectional area compared to both non-injured and injured saline-injected controls.³³ These mechanisms may partially explain the general improvement seen in prolotherapy participants but do not address the pain-specific response seen in this study.

Prolotherapy may decrease pain directly via an intra-capsular sensori-neural mechanism.³⁴ Studies have shown that articular tissue in several mammalian species is innervated by peptide-responsive sensory and autonomic fibers prior to and during OA, and that release of neuropeptides during OA is associated with local proinflammatory effects; this has been termed neurogenic inflammation.⁴ Afferent fibers expressing capsaicin-sensitive receptors (transient receptor potential vanilloid receptor-1, TRPV1) are under intense scientific scrutiny³⁵ and widely accepted as the fibers on which neurogenic arthritic pain depends.⁴ Dextrose may act on sensory afferent nerves associated with KOA in ways detectable to patients with stable cartilage as decreased pain. In a clinical trial, superficial dextrose

injections targeting sensory nerves have been reported to decrease trigger point-related pain more than lidocaine injections ($p < .01$).³⁶ In another study, participants with Achilles tendinopathy receiving exercise and dextrose prolotherapy injections targeting superficial sensory nerves reported improvement compared to exercise alone ($p = .007$).³⁷ The current data suggest that dextrose and/or morrhuate sodium may act on the TRPV1 or other receptors in KOA participants to reduce neurogenic inflammation and decrease subsequent pain; however, the specific mechanism by which this may occur is unknown.

These findings have clinical and research implications. Physicians can more confidently address this therapy with patients and colleagues, and may be more likely to learn and practice it for KOA. For researchers, the findings suggest that further investigation of mechanism of action involving pain-specific biomarkers may be fruitful avenues of exploration. Determination of the association between MRI-assessed CV and prolotherapy-related clinical outcomes will require confirmation in a larger trial that includes biomechanical and imaging measures to assess for potential disease modification.^{38,39} Clinical trials designed to optimize MRI assessment are also warranted, and may include analyzing changes not only in CV using conventional MRI sequences but also changes in various MRI parameters of cartilage composition and three-dimensional ultra-structure.

Limitations

Limitations of this study include a relatively small and heterogeneous sample size, though the groups were similar at baseline, and the effect size of prolotherapy proved adequate to detect substantial between-group differences, consistent with other studies.^{14,15} The use of Kellgren-Lawrence criteria for baseline radiological assessment of KOA severity is controversial. The Kellgren-Lawrence score, however, is likely to remain an important measure for gauging disease severity in symptomatic patients.⁴⁰ The age of the x-rays (up to five years pre-enrollment) may limit comparison of KOA severity between groups at baseline, though the similarity of baseline MRI-based CV (Table 2) suggests similar overall joint-space severity. Self-reported height and weight and non-reporting of baseline activity level could limit the accuracy BMI and overall comparability of groups. The measurement of CV as a measure targeting mechanism of action is relatively new; and the clinical utility of the association between the degree of clinical improvement (pain, stiffness and function) and MRI-assessed CV over time, while promising, is not formally validated. Strengths include pragmatic clinical assessment using validated, patient-oriented outcomes, state of the art CV assessment, and robust, consistent results.

Conclusions

Prolotherapy resulted in safe, significant improvement in knee-specific QoL, including pain severity and function, over the 52-week follow-up period. Among Prolotherapy participants, MRI-assessed CV stability predicted improvement in pain severity scores, suggesting prolotherapy may have a sensori-neural, pain-specific mechanism of action.

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Abbreviations

KOA	knee osteoarthritis
RCT	randomized controlled trial
BMI	body mass index
WOMAC	Western Ontario McMaster University Osteoarthritis Index
KPS	knee pain scale

Figure 1. Screening, Enrollment and Randomization

Figure 2a. Regression of Change in Pain Score on Change in Cartilage Volume (Control Participants)

Among Control participants, data show no correlation between change in cartilage volume and change in the WOMAC-based pain subscale score over 52 weeks ($y = -0.0118x + 0.1915$, $R^2 = 0.001$) where y is the equation of the regression line and R^2 is the percent variation explained by the data.

Figure 2b. Regression of Change in Pain Score on Change in Cartilage Volume (Prolotherapy Participants)

Among Prolotherapy participants, data show a correlation between change in cartilage volume and change in the WOMAC-based pain subscale score over 52 weeks ($y = 0.1338x + 0.7236$, $R^2 = 0.1895$) where y is the equation of the regression line and R^2 is the percent variation explained by the data.

Table 1
Study Interventions per Group

	Prolotherapy		Control Therapies	
	Dextrose	Dextrose-Morrhuate	Saline Control Injection	Home Exercise
Intervention	<p>Intra-articular: 6.0 mL of solution in a single injection was performed using an inferomedial approach.</p> <p>Extra-articular: Up to 15 sub-dermal injections were placed and 0.5 mL of solution was injected using a peppering technique with a 25-gauge needle at each ligament-bone insertion. Each puncture site allowed for placement of solution at as many as 3 ligament-bone insertions using the technique of skin sliding (withdrawing the needle to just below the skin and reinserting into an adjacent area without removing from the initial puncture site) allowing for the peri-articular placement of up to 22.5 mL of solution.</p>			Participants were advised to begin exercises (three sessions per week, one session daily, 10 repetitions per exercise), gradually increase therapy as tolerated over 20 weeks (five sessions per week, three times daily, 15 repetitions per exercise) and continue them thereafter if desired.
Injection	Active Injection	Active Injection	Control Injection	Exercise
Recipe	<p>Intra-articular:</p> <p>25% Dextrose: 5 mL 50% Dextrose 5 mL 1% Lidocaine</p> <p>Extra-articular:</p> <p>15% Dextrose: 6.75 mL 50% Dextrose 4.5 mL 1% Lidocaine 11.25 mL 0.9% Saline</p>	<p>Intra-articular:</p> <p>25% Dextrose: 5 mL 50% Dextrose 5 mL 1% Lidocaine</p> <p>Extra-articular:</p> <p>5% morrhuate sodium/15% dextrose solution: 3 mL 50% Dextrose 2 mL 5% morrhuate sodium 2 mL 0.9% saline 3 mL 1% Lidocaine</p>	<p>Intra-articular:</p> <p>5 mL 0.9% Saline 5 mL 1% Lidocaine</p> <p>Extra-articular:</p> <p>18 mL 0.9% Saline 4.5 mL 1% Lidocaine</p>	Exercise group participants received an informational pamphlet about KOA (Visual Health Information™ http://www.vhikits.com/Default.aspx) depicting 10 at-home knee exercises demonstrated by the study coordinator at baseline.
Blinded	Yes	No	Yes	Not Applicable

Table 2
Baseline Subject Characteristics (n=37)

	Prolotherapy	Control	P value
Total in grouped analysis	24	13	
Female, n (%)	13 (54.2%)	8 (61.5%)	.67
Age, years, mean (SD)	54.5 (6.8)	57.4 (7.6)	.25
Duration of Knee Pain, months, mean (SD)	85.6 (77.4)	82.2 (60.2)	.89
BMI, kg/m ² , n (%)			.88
25	6 (25.0%)	4 (30.8%)	
26-30	9 (37.5%)	4 (30.8%)	
31+	9 (37.5%)	5 (38.5%)	
Prior Knee Intervention, n (%)			
Arthroscopic surgery	13 (54.2%)	4 (30.8%)	.17
Physical Therapy	5 (20.8%)	4 (30.8%)	.41
Hyaluronic acid injection	1 (4.2%)	0 (0%)	.47
Corticosteroid injection	2 (8.3%)	1 (7.7%)	.95
Glucosamine	12 (50.0%)	3 (23.1%)	.11
WOMAC, mean (SD)			
Composite Score	59.9 (12.2)	67.0 (10.8)	.09
Pain	62.3 (12.9)	71.9 (13.5)	.04
Stiffness	54.7 (18.0)	60.6 (14.3)	.32
Function	62.6 (12.9)	68.5 (11.3)	.19
X-ray Kellgren-Lawrence OA severity score (0-4) of treated knees *			.61
1-2 score (mild OA)	15 (62.5%)	7 (54.8%)	
3-4 (moderate to severe OA)	9 (37.5%)	6 (45.2%)	
Cartilage Volume (mm ³)	24.6 (5.7)	22.2 (5.2)	.22

Abbreviations: SD, standard deviation; BMI, body mass index; WOMAC, Western Ontario McMaster University Osteoarthritis Index;

* Existing knee radiographs were obtained for the more severely affected knee for each participant. 32 subjects' radiographs were available for Kellgren-Lawrence scoring.

There was no difference between groups at baseline except for the WOMAC pain sub-score; untreated participants had slightly less severe pain scores (p=0.04) than treated participants.

Table 3

Change in WOMAC Composite and Subscale Scores, and Cartilage Volume.

Measure	Week 5 Prolo N=24 Control N=13	Week 9 Prolo N=24 Control N=12	Week 12 Prolo N=24 Control N=13	Week 24 Prolo N=24 Control N=13	Week 52 Prolo N=24 Control N=13
WOMAC Composite (SE) ¹					
Treatment	6.9 (3.0)	17.4 (2.2) **	14.2 (2.7)	16.2 (3.1) *	17.6 (3.2) *
% Change	22.2	43.4	35.4	40.4	43.9
Control	5.4 (3.0)	2.5 (3.2) **	7.0 (3.4)	6.6 (3.6) *	8.6 (5.0) *
% Change	16.4	7.6	21.2	20.0	26.1
WOMAC Subscales (SE) ¹					
Pain					
Treatment	11.0 (2.8)	17.1 (2.7) ***	15.4 (3.0) *	16.5 (3.4) **	18.1 (3.8) **
Control	3.9 (3.1)	1.1 (4.0) ***	3.5 (3.9) *	2.7 (4.3) **	4.6 (5.0) **
Stiffness					
Treatment	5.2 (4.4)	17.2 (2.9) *	13.0 (3.4)	15.1 (3.5)	16.1 (3.6)
Control	6.7 (4.1)	1.9 (4.4) *	9.6 (4.4)	9.6 (4.4)	11.5 (5.9)
Function					
Treatment	10.4 (3.2)	17.9 (2.4) **	14.3 (2.8)	17.1 (3.1) *	18.6 (2.9) *
Control	5.7 (2.7)	4.6 (2.7) **	7.8 (2.8)	7.5 (3.3) *	9.8 (4.8) *
CV Change (SE) ¹ in mm ²					
Treatment					-1.66 (1.42)
% CV change from baseline					-6.95% (6.44)
Control					-1.65 (1.30)
% CV change from baseline					-7.23% (4.93)

Repeated measures analysis of variance compared between-group composite WOMAC scores, subscale WOMAC scores and cartilage volume after adjusting for baseline scores, age, sex, and body mass index.

* p<.05,

** p<.01,

p<.001

Abbreviations: Prolo, Prolotherapy; N, number; WOMAC, Western Ontario McMaster University Osteoarthritis Index; SE, standard error; CV, cartilage volume; mm, millimeters