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REVIEW ARTICLES

Efficacy of prolotherapy in pain control and function improvement in individuals with lateral epicondylitis: a systematic review and meta-analysis

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ABSTRACT

Aim: The objective of this study was to evaluate the efficacy of prolotherapy when treating individuals with lateral epicondylitis through a systematic review and meta-analysis.
Methods: The search for articles was carried out in electronic databases including PUBMED, CENTRAL, WEB OF SCIENCE, SCIELO and Google Scholar, published up to July 2021. We used the following keywords: prolotherapy OR proliferation therapy OR hypertonic dextrose injections AND tennis elbow OR lateral epicondylitis. The effectiveness was expressed as mean difference or standardized mean difference (SMD and 95% CI).
Major results: Nine clinical trials that used prolotherapy in the treatment of lateral epicondylitis were included. In the pooled analysis, prolotherapy was effective in pain control in the medium (SMD= -0.85, 95% CI -1.29 to -0.41) and long terms (SMD= -1.05, 95% CI -2.06 to -0.03). It was also effective in improving function in the medium term (SMD= -1.21, 95% CI -1.64 to -0.78).
Conclusions: Prolotherapy was effective for reducing pain in the medium and long terms, as well as for improving function in the medium term, in individuals with lateral epicondylitis. However, the quality of evidence was only moderate. More studies with a low risk of bias are necessary to further clarify the efficacy of prolotherapy in patients with lateral epicondylitis.

Keywords: Prolotherapy; Proliferation therapy; Lateral epicondylitis; Tennis elbow.

INTRODUCTION

Lateral epicondylitis (LEPC) is a common cause of pain in the upper limb[1]. The general incidence per year is 3.4 cases per 1000 inhabitants and it increases considerably with age; additionally, it is more frequent in women [1]. Conservative treatment modalities are the most frequently used including non-steroidal anti-inflammatory drugs, orthotic devices, physical therapy and injections; while surgery is required less frequently[2].

The most widely used physical therapy modalities for treating LEPC are exercise[3], ultrasound[4], LASER therapy[4], and shock wave therapy[5, 6]. Injections with corticosteroids are perhaps the most widely invasive treatment used for LEPC[3, 7]. Other injections include platelet rich plasma[7], hyaluronic acid[8, 9], prolotherapy[9-11] and botulinum toxin[9, 11].

George Hackett, defined the term prolotherapy (PRT) and developed the technique based on injections with an irritating solution in the ligament-bone or tendon-bone system or in the intra-articular space, which is performed repeatedly at established intervals with the objective of favoring the repair processes. Irritants such sodium morrhuate, glycerin or phenol have been used; however, the most common PRT agent used in clinical practice is the hypertonic dextrose with concentrations ranging from 15% to 25%, which is considered effective and with less side effects than other irritants[12].

PRT has been reported to be effective in the treatment of knee osteoarthritis[13], tendinopathies of the lower limb[14], as well as upper limb tendinopathies such rotator cuff disease[15] and lateral epicondylitis[10], where clinical improvement without adverse effects has been reported. Basic science have shown that hypertonic dextrose generates trophic effects on the tendon, such as increase fibroblast proliferation and increase in collagen production, and extracellular matrix in treated

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tendons[16, 17].

Despite the fact that PRT has been used for treating LEPC and its technique is simple[18], its efficacy is not clear yet. Although some reviews have included studies that used PRT in individuals with LEPC[9-11], few studies have been included in these reviews and the evidence is poor. As new studies have been published, it is necessary to reevaluate the evidence on the efficacy of PRT in the treatment of LEPC.

The primary objective of this study was to evaluate the efficacy of PRT with hypertonic dextrose (combined or not with other irritants) for reducing pain and improving function in individuals with LEPC through a systematic review and meta-analysis; the secondary objectives were to describe the characteristics of the treatment and its adverse effects.

The PICOS strategy used in the study is described below:

(P) Patients: Individuals with clinical diagnosis of LEPC who referred pain and alterations in functionality.

(I) Intervention: Prolotherapy with Hypertonic Dextrose.

(C) Control: Rest and wait, non-invasive treatments or infiltrations of other substances.

(O) Outcomes: Efficacy in reducing pain and improving function.

(S) Study design: Clinical trials.

METHODOLOGY

The methodology used in this work was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[19] for the presentation of a systematic review and a meta-analysis, and it was registered at the International prospective register of systematic reviews (PROSPERO), ID number CRD42021282150.

Methods and Search Strategy

Articles of interest were identified in electronic databases, using a search period up to July 2021. The databases used were PUBMED, Cochrane Central Register of Controlled Trials (CENTRAL), WEB OF SCIENCE, SCIELO and gray literature such as Google Scholar. The search terminology included MESH terms, ENTRY terms and similar terms: ("prolotherapy" or "prolotherapies" or "proliferation therapy" or "dextrose injection" or "dextrose infiltration") AND ("tennis elbow" or "lateral epicondylitis" or "lateral-humeral epicondylitis"), with multiple combinations between them. The search comprised all the manuscripts reported in literature without language restrictions. The search formulas for each database are detailed in supplementary material (Supplemental Digital Content).

Types of studies

This review included randomized clinical trials that used PRT with hypertonic dextrose as a therapeutic intervention for treating individuals with LEPC. Review studies, clinical comments, observational studies such as cases - controls, cases series and one-case reports were excluded. The studies selected had to describe in detail the intervention carried out, the forms of evaluation and the results. For inclusion in the quantitative analysis, the studies had to express the results in terms of mean and standard deviation.

Participants

The selected studies included individuals with LEPC with the following criteria:

- Adults of at least 18 years of age.
- Both sexes.
- Clinical diagnosis of lateral epicondylitis: pain or tenderness over the lateral epicondyle on palpation or resisted wrist extension expressed in units of the Analogous Visual Scale (VAS) and Functional alterations evaluated in self-reported score of validated functionality scales for elbow and upper limb.
- Individuals in experimental groups treated with hypertonic dextrose injections.
- Individuals in control groups treated with placebo or other interventions.

Type of interventions

The selected studies included individuals with LEPC treated with PRT against other interventions. The criteria for the type of intervention used in the PRT groups were the following:

- One or more treatment sessions of PRT.
- The PRT solution consisted of hypertonic dextrose alone or in combination with other irritants such sodium morrhuate, glycerin or phenol. No studies were included where PRT was performed only with irritating substances (such sodium morrhuate, glycerin or phenol), since the objective of this revision was to evaluate the effects of the PRT with hypertonic dextrose.
- The injections were applied in the epicondyle region and adjacent areas such as supracondylar edge, annular ligament and insertion of the extensor carpal muscles.
- The injection was performed with anatomical technique or under ultrasound guidance.

Individuals in the control groups were treated with watch and wait, physiotherapy (PH), shock wave therapy (SWT) or infiltrations of other substances such as saline solution (SS), corticosteroids (CT) or hyaluronic acid (HA). Co-interventions were allowed as long as they were uniform in all groups.

Evaluation of the risk of bias of the included studies

Two investigators independently assessed the risk of bias of each study include. Disagreements were solved by consensus and the opinion of a third investigator. The evaluation of clinical trials was based on the Cochrane Handbook for Systematic Reviews recommendations, version 5.1, which includes seven domains and the risk of bias for each domain is classified as low, high, or uncertain [20]. The risk of bias for each trial was considered high, uncertain (some concerns about the result) or low risk, according to the results of each domain. The quality of the evidence for the outcomes evaluated was determined with the GRADE system[21].

Evaluation of Eligibility and Data Extraction

Two reviewers independently examined titles, abstracts and full texts, then determined the eligibility of each study (AVPI, TZCA). Disagreements were solved by consensus and the opinion of a third reviewer (CARG). For the eligible studies, data were extracted independently and included: name of first author, year, study design, risk of bias, clinical configuration, characteristics and number of the participants, characteristics of the interventions, results, duration of follow-up and adverse effects.

Outcomes

The primary outcomes were: efficacy of PRT for pain control and function improvement. Pain control was measured in terms of the VAS. Improvement in function was measured in terms of validated function scales such as the Disability of Arm and Shoulder Score (DASH) or the Patient Rated Tennis Elbow Evaluation Score (PRTEE). Both outcomes were evaluated according to the follow-up time at immediate term ($\leq$ 4 weeks), short term (5 - 11 weeks), medium term (12 - 23 weeks) and long term ($\geq$ 24 weeks).

The secondary outcomes were: the characteristics of the treatment and the adverse effects described on data provided in the included studies.

Statistical analysis

In the quantitative analysis, the efficacy of PRT was evaluated by analyzing the reduction pain and improvement of function according to the follow-up time. The effect magnitude was calculated comparing the PRT group with a control group, evaluating the mean of change, according to what was reported for each follow-up period in the included studies. For the studies where the results were not reported in terms of mean and standard deviation, the RevMan Calculator was used to estimate them from the data reported.

The efficacy of PRT in reducing pain was assessed with different measurements of the visual analogue scale, so the magnitude of the effect was measured with mean difference (MD) or standardized mean difference (SMD) and 95% confidence intervals (95% CI), according to the scales used in each pooled analysis. The efficacy of PRT in function improvement was evaluated with validated scales of functionality for the elbow and upper limb, therefore the magnitude of the effect was measured with a standardized mean difference (SMD) and 95% confidence intervals (95% CI). In both outcomes, the random-effects model for combining data was used, taking into account the clinical and statistical heterogeneity of the included studies.

The statistical heterogeneity was assessed in each meta-analysis using I^2 and Chi^2 statistics and Tau^2. Statistical Heterogeneity was considered when I^2 was greater than 50% and either Tau^2 was greater than zero or there was a low P value (less than 0.10) in the Chi^2 test for heterogeneity.

To evaluate the stability of the results in this meta-analysis, sensitivity analyses were performed. For this, an analysis was carried out excluding studies with a higher risk of bias or excluding studies with less or greater statistical weight. The publication bias was evaluated graphically by Begg´s funnel plots and the asymmetry was considered as a significant presence of bias. To evaluate the characteristics of the treatment and adverse effects, they were summarized in descriptive measures, according to data provided in the included studies. The meta-analysis was performed using the Review Manager 5.4 Software.

RESULTS

Study Characteristics and Demographics

A total of 244 citations were identified; 80 duplicates were excluded. The titles and abstracts of the remaining 164 studies were reviewed in detail and 144 were excluded, as they were basic studies, or used other treatments for LEPC, or treated other pathologies with PRT. Of the 20 remaining studies, 11 more were excluded for the following reasons: review studies (n = 3), case series (n = 1), reports of one case (n = 1), protocols (n= 3), clinical comments (n = 1), clinical trials that did not report sufficient data (n= 1), clinical trials that included the same participants of other publications under a different name (n=1). Finally, nine clinical trials were included in qualitative analysis [22-30]. The flowchart of the systematized search is shown in Figure 1.

In the analysis of risk of bias, three studies had unclear risk of bias and six studies had a high risk of bias (Figure 2).

This systematic review comprised 190 individuals with clinical diagnosis of LEPC treated with PRT and 225 controls. The average age in the groups treated

with PRT was 46 years and in the control groups 47.7 years. The design characteristics, interventions and results of the included studies are summarized in Table I.

The groups treated with PRT used a solution of hypertonic dextrose alone[24-30] or combined with other irritants such as sodium morrhuate, phenol or glycerin[22-24]. The control groups received watch and wait[24], PH[25], SWT[26, 30] or injections with CT[23, 28,] SS 22, 27 or HA[29] as treatments.

Eight studies included individuals with a diagnosis of LEPC of more than 3 months of evolution[22-24, 26-30,] only in one study the participants had less than a month of evolution[25]. For pain analysis, four studies[24, 26, 28, 30] performed immediate term follow-up (≤ 4 weeks), five studies[22,24-26,29] performed short term follow-up (5 - 11 weeks), seven studies[22-25, 28-30] performed medium term follow-up (12 - 23 weeks) and three studies[23, 25, 30] performed long term follow-up (> 24 weeks). For the analysis of function, three studies[24, 26, 28] performed immediate term follow-up (≤ 4 weeks), four studies[24-26, 29] performed short term follow-up (5 - 11 weeks), five studies[23-25, 28, 29] performed medium term follow-up (12 - 23 weeks) and two studies[23, 25] performed long term follow-up (≥ 24 weeks).

In all the studies included[22-30] the use of non-steroidal anti-inflammatory drugs during treatment or follow-up was restricted; the use of analgesics such as acetaminophen or tramadol was allowed in case of post-infiltration pain. In two studies[27, 28] the groups included were treated uniformly with an exercise program as a co-intervention. The studies did not report any other co-intervention during treatment or follow-up.

The quantitative analysis included eight clinical trials[22-26, 28-30] that reported their results with the statistical data necessary for their inclusion. One study[27] did not report enough data to be included in quantitative analysis.

Meta - analysis of the efficacy of Prolotherapy for reducing pain in patients with lateral epicondylitis

To analyze the efficacy of PRT in pain reduction, a meta-analysis was performed by follow-up time, and a sub-analysis by type of solution used in the PRT group.

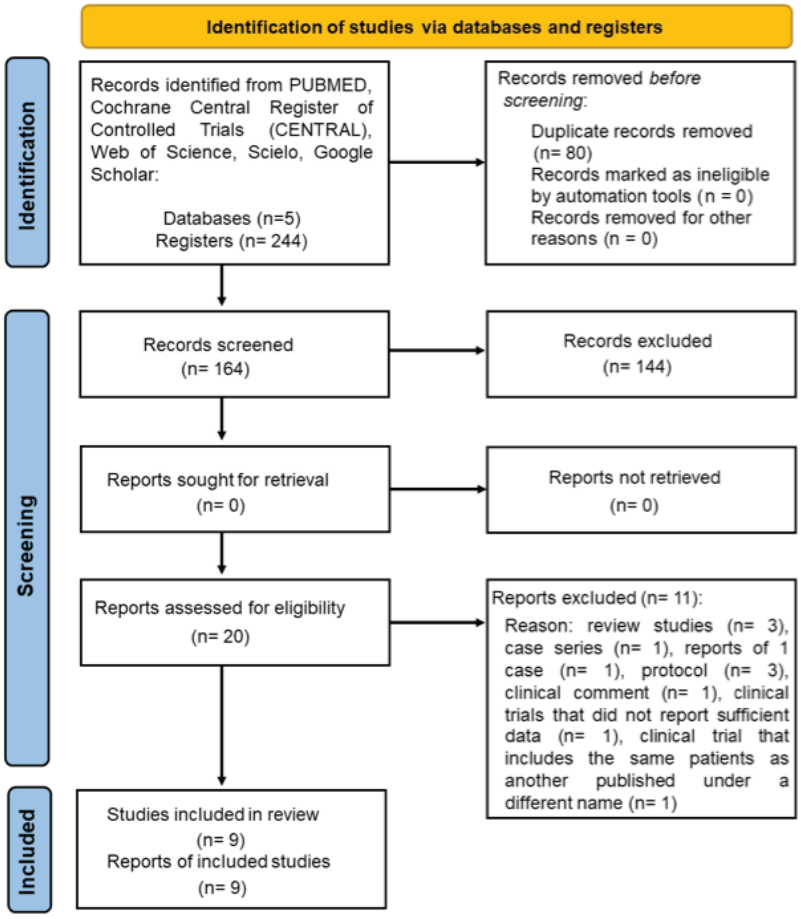

Figure 1. Systematic Review's Flow Diagram.

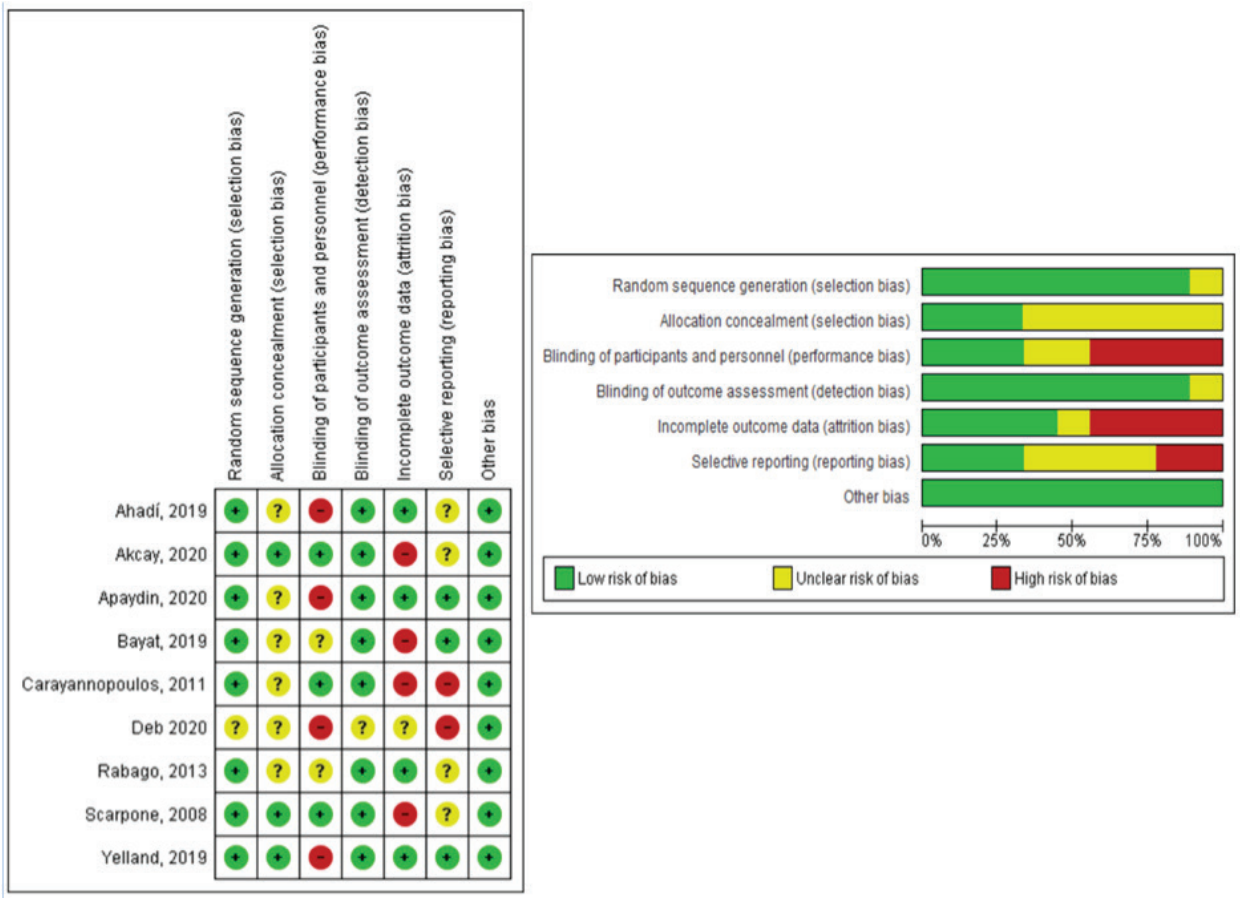

Figure 2. Summary of the risk of bias assessment of the included clinical trials, according to the Cochrane Handbook for Systematic Reviews recommendations.

Immediate term follow-up: Four studies (five groups) were analyzed. In the pooled analysis, no statistically significant difference in pain reduction was found when comparing PRT with controls (SMD= -0.21, 95% CI -1.05 to 0.64, p (z) 0.64, I^2=86%) (Figure 3A).

Short term follow-up: Five studies (six groups) were analyzed. In the pooled analysis, no statistically significant difference in pain reduction was found when comparing PRT with controls (SMD= -0.09, 95% CI -0.73 to 0.55, p (z) 0.79, I^2=78%) (Figure 3B).

Medium term follow-up: Seven studies (eight groups) were analyzed. In the pooled analysis, a statistically significant difference in pain reduction was found when comparing PRT with controls (SMD= -0.85, 95% CI -1.29 to -0.41, p (z) 0.0001, I^2=65%) in favor of the PRT groups (Figure 3C).

Long term follow-up: Three studies were analyzed. In the pooled analysis, a statistically significant difference in pain reduction was found when comparing PRT with controls (MD= -1.05, 95% CI -2.06 to -0.03, p (z) 0.04, I2=87%) in favor of the PRT groups (Figure 3D).

Meta - analysis of Prolotherapy efficacy in function improvement of patients with lateral epicondylitis

To analyze the efficacy of PRT for improving function, a meta-analysis was performed by follow-up time.

Immediate term follow-up: Three studies (four groups) were analyzed. In the pooled analysis, no statistically significant differences were found in the improvement of function between the groups treated with PRT and the control groups (SMD = -0.27, 95% CI -1.23 to 0.69, p (z) 0.58, I^2=82%) (Figure 4A).

Short term follow-up: Four studies (five groups) were analyzed. In the pooled analysis, no statistically significant differences were found in the improvement of function between the groups treated with PRT and the control groups (SMD= -0.23, 95% CI -0.95to 0.58, p (z) 0.98, I^2=95%) (Figure 4B).

Medium term follow-up: Five studies (four groups) were analyzed. In the pooled analysis, no statistically significant differences were found in the improvement of function between the groups treated with PRT and the control groups (SMD= -0.71, 95% CI -1.49 to 0.06, p (z) 0.07, I^2=84%) (Figure 4C).

Table I. Characteristics of the study design, interventions, evaluations, results and side effects.

Author, year, design	Intervention	Evaluations and results	Side effects
Scarpone, et al (22). 2008. Clinical trial. 20 patients with clinical diagnosis of lateral epicondylitis, with more than 6 months of evolution and failure of previous treatments such as NSAIDs, physical therapy and corticosteroid injections.	PRT GROUP: 10 patients aged 48.2 (9.5) years, treated with 3 prolotherapy sessions with a mixture of 10.7% dextrose, sodium morrhuete and lidocaine, applying it with a multi-injection scheme in lateral epicondyle, supracondylar ridge and annular ligament, every 4 weeks. SS GROUP: 10 patients aged 47.7 (8.6) years, treated with same treatment scheme as the PRT group was applied, but using only saline solution	Pain was assessed with VAS at 8 and 16 weeks. Baseline / 8 weeks / 16 weeks VAS PRT 5.1 (0.8) / 3.3 (0.9) / 0.5 (0.4) SS 4.5 (1.7) / 3.6 (1.2) / 3.5 (1.5)	Pain, erythema, and irritation in 2 patients in the PRT group, self-limited. There were no major side effects.
Carayannopoulos, et al. (23). 2011. Clinical trial. 17 patients with clinical diagnosis of lateral epicondylitis, with more than 3 months of evolution.	PRT GROUP: 8 patients aged 46 years, treated with two prolotherapy sessions with a mixture of 12.5% dextrose, 1.2% phenol, 12.5% glycerin, sodium morrhuate and procaine, applying it with a multi-injection scheme in insertion of extensor carpal tendons in lateral epicondyle, annular ligament and insertion of radial collateral ligament in the radial tubercle, with monthly frequency. CT GROUP: 9 patients aged 49 years, treated with two injections in the epicondyle region with 40mg. of methylprednisolone acetate combined with procaine, with monthly frequency.	Pain was assessed with VAS and functionality with DASH score at 12 and 24 weeks. Baseline / 12 weeks / 24 weeks VAS PRT 3.63 (2.00) / 1.25 (1.60) / 1.00 (2.39) CT 3.28 (2.64) / 1.83(2.85) / 1.72 (4.41) DASH Score PRT 30.41 (13.21) / 10.52 (16.93) / 8.65(17.15) CT 26.48 (15.95) / 13.15 (16.46) / 10.92 (18.54)	Loss of 1 patient in the PRT group due to excessive pain in the first application. No other side effects were reported.
Rabago et al (24). 2013. 31 patients with clinical diagnosis of lateral epicondylitis, with more than 3 months of evolution and failure of previous treatments such as NSAIDs, physical therapy and corticosteroid injections.	PRT DEX GROUP: 10 patients aged 50.4(6.8) years, treated with three prolotherapy sessions with a mixture of 20% dextrose and lidocaine, applying it with a multi-injection scheme in insertion of extensor carpal tendons, lateral epicondyle, and annular ligament, with monthly frequency. PRT DEX/SM GROUP: 10 patients aged 42.6 (9.8) years, treated with three prolotherapy sessions with a mixture of 10.7% dextrose, sodium morrhuate and lidocaine, applying it with a multi-injection scheme in insertion of extensor carpal tendons, lateral epicondyle and annular ligament, with monthly frequency. CONTROL GROUP: 11 patients aged 51.7 (6.8) years, in whom modifications in lifestyle and work activities were indicated.	Pain was assessed with VAS and functionality with PRTEE score at 4, 8 and 16 weeks. Baseline / 4 weeks / 8 weeks / 16 weeks VAS PTR DEX 24.2 (8.53) / 16.2 (8.22) / 15.5 (9.48) / 13.60(11.38) PTR DEX/SM 20.8 (9.48) / 20.4 (9.17) / 16.7 (10.75) / 7.90(12.64) CONTROL 24.8 (8.62) / 22.4 (8.29) / 23.2 (9.61) / 20.9 (11.60) PRTEE PRT DEX 16.4 (12.33) / 11.1 (9.48) / 1.6 (9.80) / 9.1(11.70) PRT DEX/SM 18.1 (13.28) / 16.6 (10.45) / 13.3 (11.06) / 7.3 (13.59) CONTROL 26.0 (11.60) / 22.2 (9.28) / 23.2(9.94) / 20.6 (11.93)	Mild to moderate, self-limited pain that resolved within 1 week in PTR DEX group. Moderate to severe pain, self-limiting up to 3 weeks in PTR DEX/SM group.

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Table I. *continuation*

Author, year, design	Intervention	Evaluations and results	Side effects
Yelland, et al. (25). 2019. 120 patients with clinical diagnosis of lateral epicondylitis, with more than 3 weeks of evolution.	PRT GROUP: 40 patients aged 49.2 (7.2) years, treated with four prolotherapy sessions with a mixture of 20% dextrose and lidocaine, applying it with a multi-injection scheme in insertion of extensor carpal tendons, lateral epicondyle, annular ligament and radial collateral ligament with monthly frequency. PH GROUP: 40 patients aged 51.0 (9.0) years, treated with physical therapy consisting of four sessions of manual therapy at weekly intervals and exercise program at home. PRT/PH GROUP: 40 patients aged 47.8(7.0) years, treated with a combination of prolotherapy and physiotherapy, with the same regimens as in the PRT and PH groups.	Pain was assessed with VAS and functionality with PRTEE score at 6, 12, 26 and 52 weeks. VAS: PRT 2.0 (1.6); 1.9 (2.0); 0.8 (1.3); 0.3 (0.7); 0.2 (0.5). PH 2.1 (2.0); 1.5 (1.5); 1.0 (1.5); 0.8 (1.3); 0.2 (0.6). PRT/PH 1.8 (1.5); 1.3 (1.9); 0.8 (1.2); 0.5 (1.7); 0.2 (0.5). PRTEE: PRT 31.6 (10.3); 24.5 (14.6); 18.2 (13.5); 8.9 (8.2); 4.9 (7.4). PH 33.5 (10.0); 19.7 (14.3); 12.2 (12.4); 9.3 (10.4); 4.4 (7.0). PRT/PH 31.3 (10.8); 18.3 (12.2); 12.4 (10.1); 8.2 (10.5); 3.9 (5.5). (Baseline; 6weeks; 12weks; 26weeks; 52weeks)	No difference between groups was reported for minor adverse effects such as self-limited pain. For significant adverse effects, it was reported that in the PRT group, 1 patient developed Posterior Interosseous Neuropraxia that resolved within 3 months. PH Group was reported without significant adverse effects. Recurrence of pain at 52 weeks: PRT group: 14.7% PH group: 23.5% PRT / PH group: 31.3%.
Ahadi, et al. (26). 2019. 33 patients with clinical diagnosis of lateral epicondylitis, with failure of previous treatments such as NSAIDs, physical therapy and corticosteroid injections.	PRT GROUP: 17 patients aged 46 years, treated with a session of prolotherapy with 20% dextrose without local anesthetics, in the insertion of carpal extensors tendons. SWT GROUP: 16 patients aged 47 years, treated with three sessions of shock wave therapy (2000 impulses at an intensity of 1.5 Bars at 10 Hertz), in the lateral epicondyle region, with weekly frequency.	Pain during movement was measured with VAS and functionality with DASH score at 4 and 8 weeks. (Baseline; 4 weeks; 8 weeks) VAS: PRT 7.35 (1.93); 5.71 (2.06); 5.47 (2.18). SWT 6.13 (1.28); 3.19 (2.00); 2.60 (1.6). DASH: PRT 47.82 (19.70); 39.67 (17.72); 37.39 (18.14). SWT 41.84 (12.16); 22.25 (14.28); 23.13 (12.80).	It was reported that there were no significant side effects in both treatment groups.
Akcay, et al (27). 2020. 50 patients with a clinical diagnosis of lateral epicondylitis, with more than 3 months of evolution and failure of previous treatments such as NSAIDs, physical therapy and corticosteroid injections.	PRT GROUP: 23 patients aged 48.1 (8.9) years, treated with three sessions of prolotherapy with 20% dextrose without local anesthetics, applying it with a multi-injection scheme in lateral epicondyle, supracondylar ridge and annular ligament, every 4 weeks. Home-based exercise program was given to subjects 4 weeks after the first injection SS GROUP: 27 patients aged 46.7 (8.3) years, treated with same treatment scheme as the PRT group was applied, but using only saline solution. Home-based exercise program was given to subjects 4 weeks after the first injection	Pain was assessed with VAS and functionality with PRTEE score at 4, 8 and 12 weeks. (Baseline; 4 weeks; 8 weeks; 12 weeks) VAS: PRT 9.0 (8.0–10.0); 6.0 (4.0–9.0); 4.0 (2.0–7.0); 3.0 (1.0–6.0). SS 9.0 (8.0–10.0); 7.0 (5.0–8.0); 5.0 (4.0–7.0); 4.0 (3.0–6.0). PRTEE: PRT 75.0(65.5–79.5); 51.5(42.0–71.5); 34.5(20.0–66.5); 22.5(13.5–67.0). SS 67.0(57.0–80.5); 57.0(42.5–76.0); 45.0(34.0–61.0); 39.5(27.0–63.0)	Injection site pain was reported. There were no major side effects.

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Table I. *continuation*

Author, year, design	Intervention	Evaluations and results	Side effects
Bayat, et al. (28), 2020. 28 patients with clinical diagnosis of lateral epicondylitis, with more than 3 months of evolution.	PRT GROUP: 14 patients aged 46.2 (6.4) years, treated with a session of prolotherapy with 16% dextrose and local anesthetics, at the point of maximum pain in the lateral epicondyle. It was indicated to carry out an exercise program at home. CT GROUP: 14 patients aged 50.7 (7.5) years, treated with an injection in the epicondyle region with 40mg. of methylprednisolone acetate combined with local anesthetics. It was indicated to carry out an exercise program at home.	Pain was measured with VAS and functionality with DASH score at 4 and 12 weeks. Baseline / 4 weeks / 12 weeks VAS PRT 7.3 (1.5) / 5.4(3.3) / 2.9(2.6) CT 7.2 (1.8) / 5.7(1.9) / 5.3(3.2) DASH PRT 43.2 (20.8) / 24.3(24.8) / 14.8(21.6) CT 52.2(16.4) / 34.9(10.7) / 34.7(12.3)	In the PRT Group, it was reported that there were no side effects in any patient. In the CT Group it was reported that there was post-treatment pain and stiffness in 3 patients.
Apaydín, et al. (29). 2020. 32 patients with a clinical diagnosis of lateral epicondylitis, with more than 6 months of evolution.	PRT GROUP: 16 patients aged 43.3 (7.4) years, treated with three sessions of prolotherapy with 15% dextrose and local anesthetics, applying it with a multi-injection scheme in lateral epicondyle, extensor carpal tendons, annular ligament, and radial collateral ligament every 3 weeks. HA GROUP: 16 patients aged 45.6 (4.7) years, treated with an injection of hyaluronic acid (30 mg/2 ml, 1500 KDaltons) at the point of maximum pain in lateral epicondyle.	Pain was measured with VAS and functionality with DASH score at 6 and 12 weeks. Baseline / 6 weeks / 12 weeks VAS PRT 4.94 (2.0) / 2.12 (1.3) / 1.06 (0.8) HA 5.19 (1.1) / 3.25 (1.9) / 2.44 (1.7) DASH PRT 53.2 (18.7) / 20.6 (11.7) / 9.7 (6.4) HA 53.1 (12.5) / 27.9 (11.1) / 24.7 (10.1)	PRT group: 2 patients developed post-application pain that was self-limited in 1-2 days. Group HA: 3 patients developed post-application pain, which was self-limited in 1–2 days.
Deb, et al. (30). 2020. 84 patients with a clinical diagnosis of lateral epicondylitis, with more than 6 months of evolution and failure of previous treatments.	PRT GROUP: 42 patients aged 30-50 years, treated with a session of prolotherapy with 25% dextrose with local anesthetics, in the insertion of carpal extensors tendons and points of maximum pain in the epicondyle region. SWT GROUP: 42 patients aged 30-50 years, treated with three sessions of shock wave therapy (2000 impulses at an intensity of 1.9 Bars at 10 Hertz), in the lateral epicondyle region, with weekly frequency.	Pain was assessed with VAS at 4, 12 and 24 weeks. Baseline / 4 weeks / 12 weeks / 24 weeks VAS PRT 7.57 (0.67) / 5.36(0.82) / 3.17(1.03) / 1.45 (0.59) SWT 7.57 (0.50) / 6.26 (0.77) / 4.45 (1.27) / 3.07 (0.92)	It was not reported if there were any side effects.

PRT= Prolotherapy, DX=dextrose, SS= solution saline, CT= corticosteroid, PH= Physiotherapy, SM= Sodium Morrhuate, HA= Hyaluronic Acid, SWT= shock wave therapy, VAS=Analog Visual Scale, DASH: Disability of Arm and Shoulder Score, PRTEE= Patient Rated Tennis Elbow Evaluation Score; mg =milligrams; ml=milliliters; NSAID = nonsteroidal anti-inflammatory drugs.

Long term follow-up: Two studies were analyzed. In the pooled analysis, no statistically significant differences were found in the improvement of function between the groups treated with PRT and the control groups (d = -0.06, 95% CI -0.45to 0.34, p (z) 0.78, I^2=0%) (Figure 4D).

Sensitivity analyses

In the sensitivity analyses of the studies that evaluated pain control in the medium term, no individual study showed a significant influence on the pooled results (Supplementary Figure 1); statistical significance was maintained in favor of the groups treated with PRT, despite excluding studies with a higher risk of bias (Figure S1A) or studies with less or greater statistical weight (Figures S1B and S1C). When the studies with less and greater statistical weight were excluded, statistical heterogeneity was totally eliminated (d = -0.90, 95% CI -1.19 to -0.61, p (z) 0.00001, I2=0%) (Figure S1D).

In the sensitivity analyses of the studies that evaluated improvement in function in the medium term (Supplementary Figure 2), when studies with higher risk of bias and with lower and higher statistical weight were excluded, the statistical significance was in favor of the groups treated with PRT and the statistical heterogeneity was eliminated (d = -1.21, 95% CI -1.64 to -0.78, p (z) 0.00001, I^2=0%) (S2A). This also occurred when only the study with the highest statistical weight was excluded (S2B), but not when the study with the lowest statistical weight was excluded (S2C).

In the evaluation of the publication bias (Supplementary Figure 3), no asymmetry was found in the Begg´s funnel plots when reduction in pain (S3A) and improvement in function (S3B) were evaluated in the medium term.

Characteristics and dosage of Prolotherapy

Regarding the type of solution used in PRT, three studies[22-24] used a solution containing hypertonic dextrose (concentration 10.7% to 12.5%) combined with other irritants such as sodium morrhuate, phenol or glycerin and local anesthetics. In seven studies[24-30] the application of PRT contained hypertonic dextrose alone (concentration 15% to 25%) combined or not with local anesthetics; the most commonly used dextrose concentration was 20%.

In the sub-analyses performed according to the solution used in the PRT groups, it was found a statistically significant difference for pain reduction in the medium term in the studies that used PRT with dextrose alone (without other irritants) (d = -0.71, 95% CI -1.15 to -0.28, p (z) 0.001, I^2=59%) (S4A), but not in studies that used PRT with dextrose plus other irritants (such as sodium morrhuate, glycerin and phenol) (d = -1.23, 95% CI -2.48 to 0.02, p (z) 0.05, I^2=77%) (S4B).

In relation to the PRT schemes used, in seven studies[22-25, 27, 29, 30] the application scheme involved multiple injections in the same session, more frequently applied in the lateral epicondyle, extensor carpal tendons, annular ligament, supracondylar ridge and radial collateral ligament. In two studies[26, 28] the application scheme was a single injection at the point of greatest pain at the insertion of the extensor tendons in the lateral epicondyle.

In relation to the number of treatments applied to each patient, six studies[22-25, 27, 29] used 2 to 4 sessions per patient, with 3 sessions being the most frequent; the application frequency was every 3 to 4 weeks. In three studies[26, 28, 29] the treatment consisted of a single session per patient.

Adverse effects

One study[28] reported that there were no adverse effects in the groups treated with PRT; seven studies [22-27, 29] reported minor adverse effects such as self-limited pain or erythema in groups treated with PRT. In control groups treated with CT[28] and HA[29], self-limited pain was also present after the applications.

Six studies[22, 24, 25, 27-29] reported that there were no major adverse effects; in two studies however, major adverse effects were reported in the groups treated with PRT including excessive pain[23] and neuropraxia of the posterior interosseous nerve in one patient[25].

DISCUSSION

Previous review studies discussed the efficacy of PRT when treating LEPC [9-11]. Dong *et al*[9] already performed a meta-analysis, however, they only included two studies that used PRT. Our review on the other hand, included nine clinical trials in which PRT was used for treating patients with LEPC and the results of our meta-analysis indicated that the application of PRT in individuals with LEPC has a significant effect on reducing pain in the medium and long terms when compared with other interventions, but not in the immediate or short terms. PRT also had a significant effect on improving function in the medium term, but not in the immediate or short terms. Furthermore, our results did not show statistical heterogeneity when we performed the sensitivity analysis.

Four studies compared PRT with non-invasive treatments. Rabago *et al*[24] compared a PRT group with a "watch and wait" group, and PRT was more effective in the short and medium terms for improving function and reducing pain, but not in the immediate term. Other treatments such as PH and CT injections have been effective in pain management in comparison with "watch and wait" in LEPC[3, 4, 9, 11]. Yelland *et al*[25] compared PRT with a PH program; their analysis indicated that there were no differences between groups

regarding pain reduction or function improvement in the short and medium terms; in the long term however, a difference was found in favor of the PRT group for pain reduction. PH modalities such ultrasound[4], LASER therapy[4] and manual therapy[3] have shown to be more effective than placebo for reducing pain in the short term in patients with LEPC. PH modalities have also been compared with CT injections, where corticosteroids are more effective in controlling pain only in the short term[11]. Exercise programs such as stretching and eccentric strengthening exercises are more effective than placebo for reducing pain and improving function in the short, medium and long terms[3]. Ahadi *et al*[26], compared PRT with SWT, and observed that SWT was more effective for reducing pain and improving function; nonetheless, they only performed immediate and short term follow-ups. Similarly, Deb *et al*[30] compared the same interventions and PRT was more effective in controlling pain in the immediate, medium and long terms. When treating LEPC, SWT has been an effective therapy for reducing pain and improving function when compared with placebo or other PH modalities[5] as well as CT injections[6]. In five studies[22, 24, 26, 27, 30,] PRT was applied after other therapies (such as nonsteroidal anti-inflammatory drugs or physical therapy) failed, and it was observed that PRT improved function and reduced pain, when those treatments had failed; these results suggest that PRT could be an optional treatment for LEPC, particularly when non-invasive treatments do not provide the expected benefits.

Five studies compared PRT with others injections. For instance, Scarpone *et al*[22] and Ackay *et al*[27] compared PRT injections with SS, in both studies PRT was more effective for controlling pain in the medium term. On the other hand, Carayannopoulos *et al* [23] and Bayat *et al*[28] compared PRT with CT injections. In the Carayanapoulos study, no significant differences were found between both interventions in the medium and long terms, while in the Bayat study, pain reduction and function improvement were found in favor of the PRT treatment in the medium term. On the other hand, CT injections have been effective for controlling pain in the short term when compared with no-intervention or non-steroidal anti-inflammatory drugs[3], PH modalities[11] and injections with platelet-rich plasma[7] in patients with LEPC; nevertheless, their efficacy gradually decreases in the medium and long terms. CT has anti-inflammatory effects that provide benefits in the short term; however, it has been suggested that its repeated use can generate deleterious effects on the tendon, such as a decrease in extracellular matrix synthesis (especially in type I collagen), disorganization and even collagen necrosis[31]. In contrast, it has been reported that PRT with dextrose increases fibroblast proliferation, collagen production and extracellular matrix in treated tendons[16, 17]. Apaydín *et al*[29] compared PRT with HA injections and observed that PRT was more effective for controlling pain and improving function in the medium term; both interventions appeared to have a similar mechanism of action, since both HA[8] and PRT[16, 17] have a possible trophic effect on the tendon. Previous studies[8] have reported that a single injection of HA is enough to generate favorable effects in patients with LEPC. Furthermore, in four studies included in our review[22, 24, 26, 27,] PRT was used when injections with CT had failed. Our results suggest that PRT could be an alternative to corticosteroid injections, especially when a single corticosteroid injection does not reduce/eliminate pain. On the other hand, when comparing PRT with HA injections, more studies are necessary to have better evidence, since we included only one study that performed this comparison.

Regarding the characteristics of PRT treatments in patients with LEPC, Van Pelt[18] proposes a treatment scheme with multi-sessions and multi-injections involving at least the following areas: insertion of extensor tendons in the lateral epicondyle, annular ligament, supracondylar ridge and additional pain points. In our review, only 2 studies[26, 28] did not comply with this scheme, and in one of them[26] it was reported that PRT was clearly less effective than the intervention used in the control group. On the other hand, Van Pelt[18] indicated that the solutions used in PRT can have dextrose alone or a combination with other irritants such as sodium morrhuate. In our review, three studies used combined solutions of dextrose with other irritants[22-24] and six studies[25-30] used a solution of dextrose alone. In our sub-analysis, we observed that PRT that used dextrose without other irritants was more effective than interventions used in the control groups for pain relief in the medium term; nonetheless, this was not observed in groups that used dextrose with other irritants. Therefore, it is possible that the application of PRT with dextrose alone is enough to generate beneficial effects.

With regards to adverse effects, in the groups treated with PRT and the groups treated with other injections such as HA or CT, pain was present during or after the application as the most frequent minor adverse effect. Although neuropraxia of the radial nerve occurred in one patient who received PRT, it is widely known that directing the needle above the radial head should be avoided in order to prevent possible injury to the radial nerve[18], which suggests that the adverse event was more related to the application technique than to the solution used.

It is important to note that our study has some limitations. The studies included in this systematic review have a high or uncertain risk of bias, which limits the evidence provided. Equally, the studies included pres-

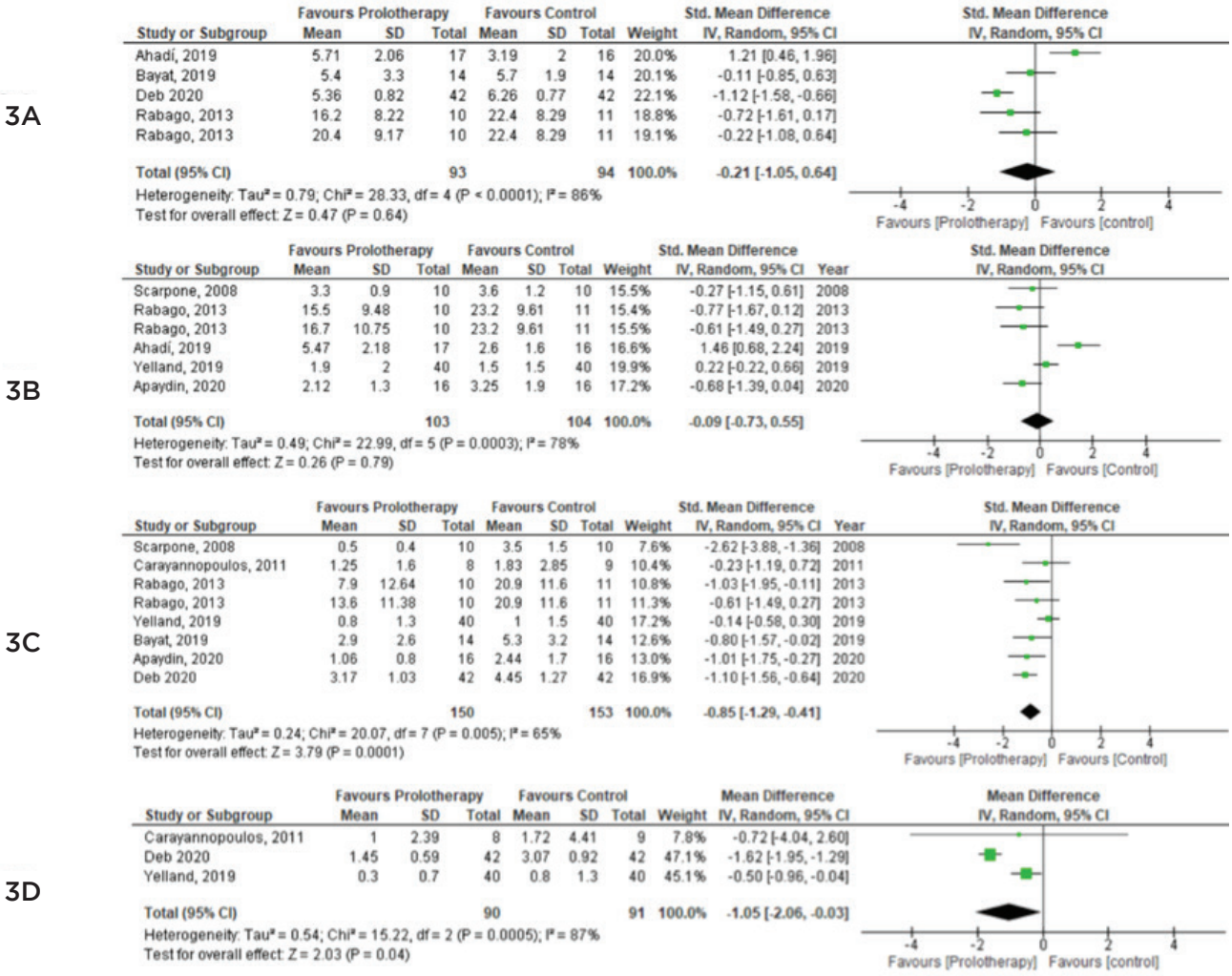

Figure 3. Forest plot of Prolotherapy for pain reduction: A) Immediate-term; B) Short-term; C) Medium-term; D) Long-term.
SD = standard deviation; CI = Confidence interval; Std = standardized; P = P-value; Z = Z-value; I^2 = I^2 Statistics; Chi^2 = Chi^2 Statistics
Tau^2 = Tau^2 Statistics

ent clinical heterogeneity, with non-standardized treatment schemes for PRT, variations in the substances used in PRT, variations in the concentration of dextrose used and in the number of sessions; however, by performing a sensitivity analysis, statistical heterogeneity can be eliminated.

When the Grade system was used to evaluate the quality of evidence, we found that PRT was not more effective than other interventions used in the control groups in reducing pain and improving function in the immediate and short terms, with low quality of the evidence (GRADE low ⊕⊕⊖⊖), given the presence of factors such as limitations in the design and execution of the study (risk of bias), and statistical heterogeneity. In the medium term, PRT was more effective than other interventions used in the control groups in reducing pain and improving function, with moderate quality of the evidence (moderate GRADE ⊕⊕⊕⊖) given the presence of limitations in the design and execution of the study (risk of bias). In the long term, PRT was more effective than other interventions used in the control groups for reducing pain, but not for improving function, with low quality of the evidence (GRADE low ⊕⊕⊖⊖), given the presence of limitations in the design and execution of the study (risk of bias), and statistical heterogeneity. Although our results suggest that PRT is an effective treatment for pain reduction and function improvement in the medium term in patients with epicondylitis, more clinical trials with low risk of bias and adequate standardization of treatment schemes are necessary to corroborate these results and improve the quality of the evidence.

CONCLUSIONS

This meta-analysis indicates that PRT is an effective treatment for reducing pain and improving function in

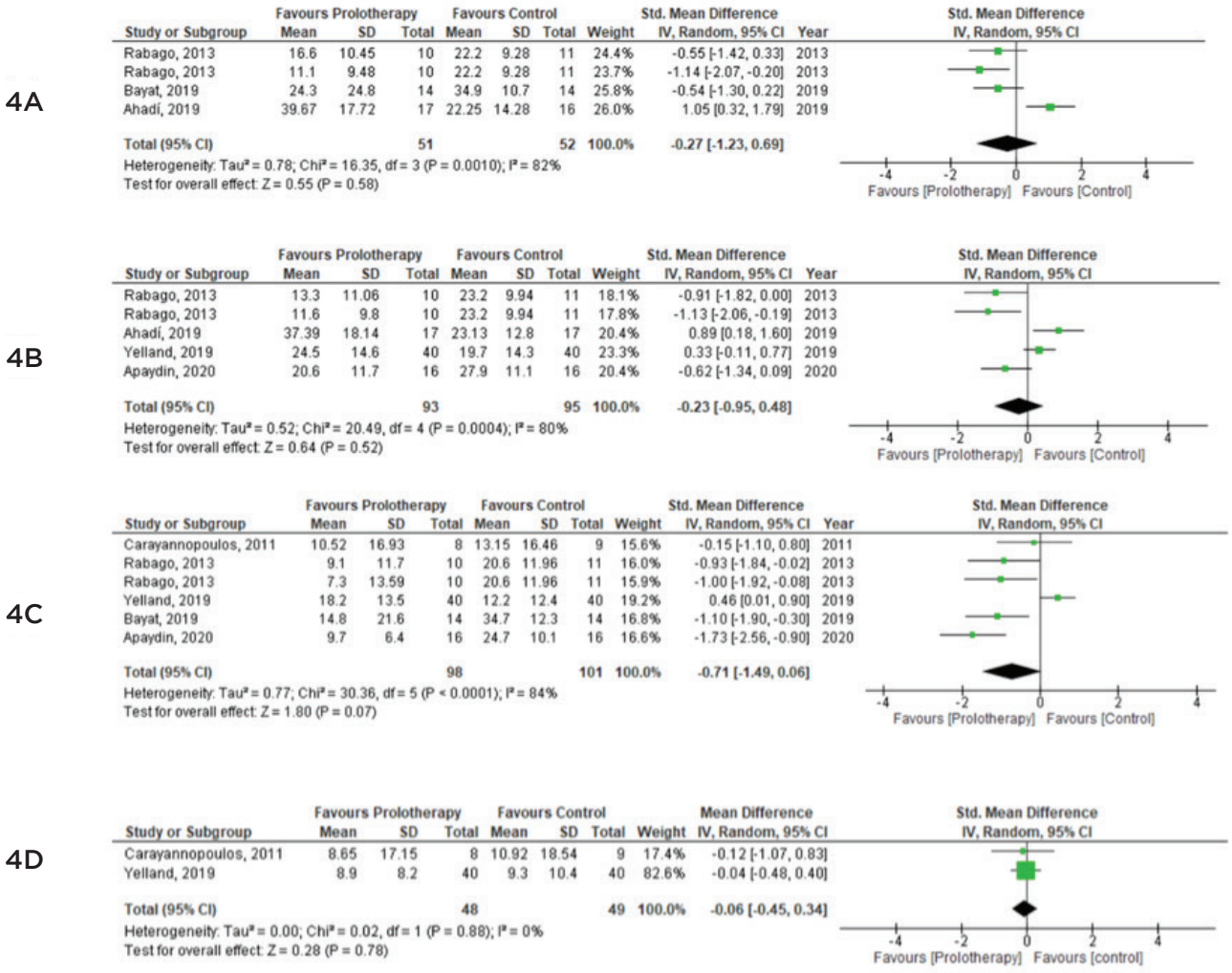

Figure 4. Forest plot of Prolotherapy in improvement in function: A) Immediate-term; B) Short-term; C) Medium-term; D) Long-term.
SD = standard deviation; CI = Confidence interval; Std = standardized; P = P-value; Z = Z-value; I^2 = I^2 Statistics; Chi^2 = Chi^2 Statistics; Tau^2 = Tau^2 Statistics

the medium term in patients with LEPC, with a moderate quality of the evidence. Our results suggest that PRT may be an option to non-invasive treatments or CT injections, when the expected benefits are not achieved. Apparently, PRT procedure should include multi-injections and multi-sessions regimens to maximize its effectiveness, as well as the use of dextrose alone without other irritants to achieve beneficial effects. PRT generates minor adverse effects such as self-limited pain; nonetheless, adequate training and adherence to the technique should be enough to avoid major adverse effects.

Despite the favorable results, the risk of bias found in the included studies caused a moderate quality of evidence. Clinical trials with a low risk of bias and adequate standardization of treatment schemes are required to confirm the efficacy of PRT and to increase the evidence provided in this meta-analysis.

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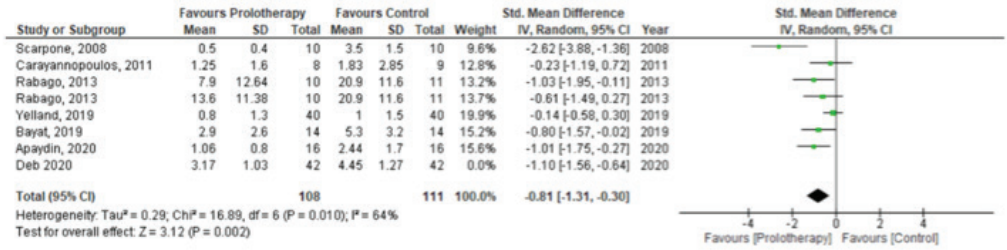
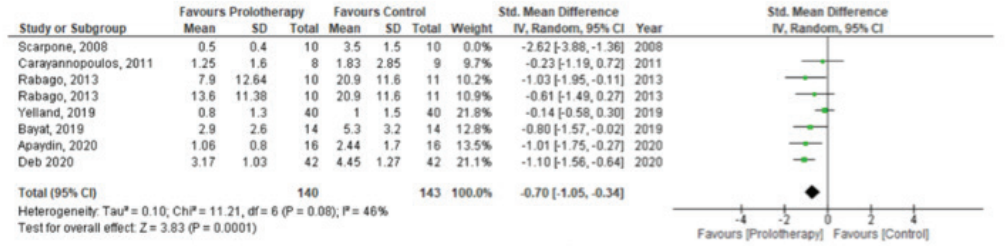
SUPPLEMENTARY MATERIAL

S1A



S1B



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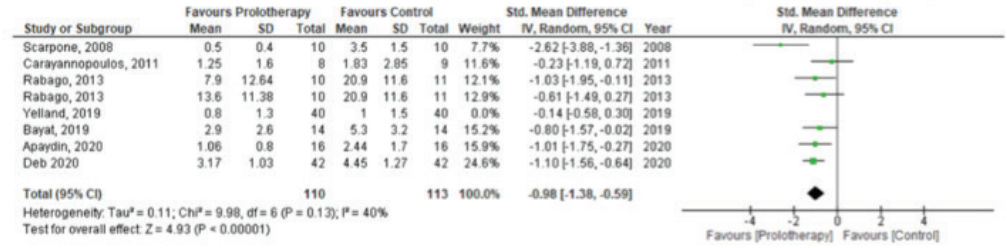

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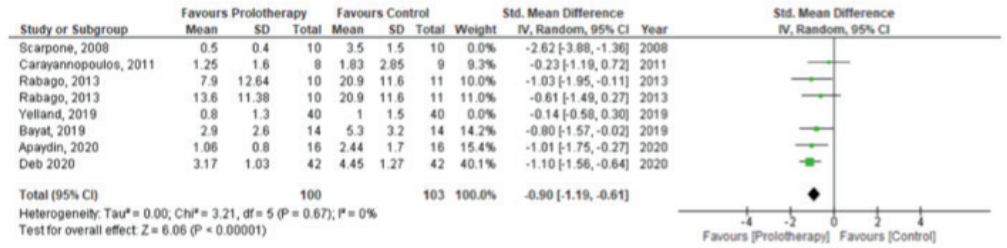
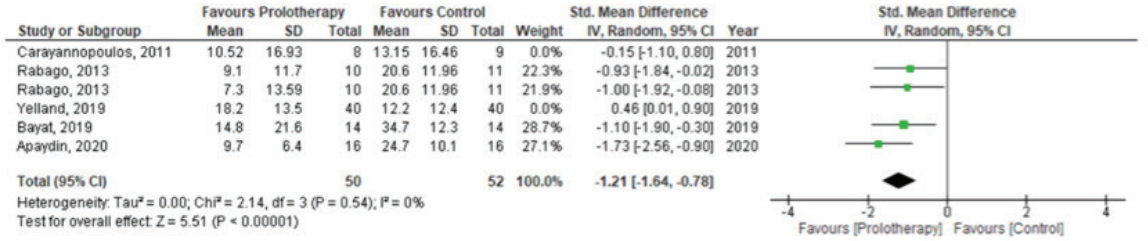
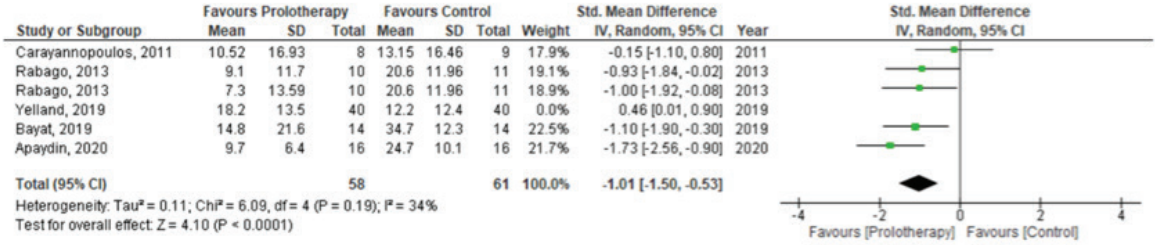


Supplementary Figure 1. Sensitivity analysis for medium-term pain reduction. Removing studies with: higher risk of bias (S1A); less statistical weight (S1B); greatest statistical weight (S1C); less and greater statistical weight (S1D).

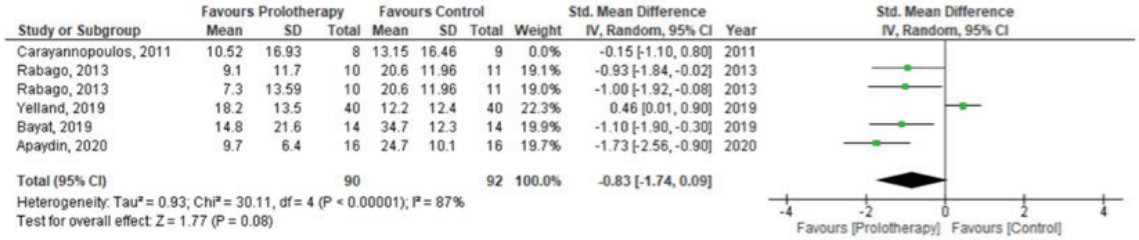
S2A



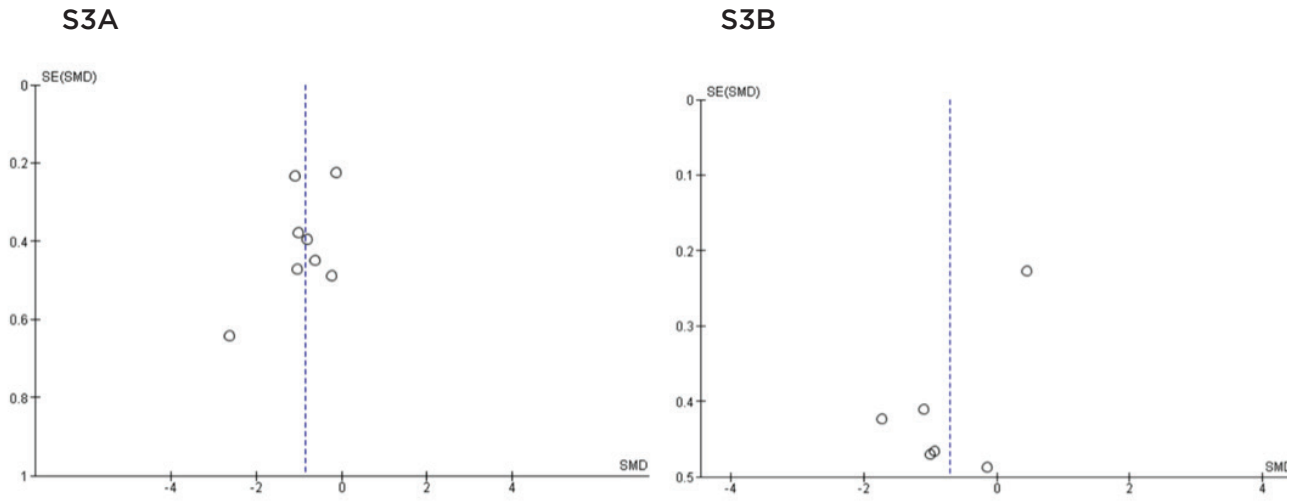
S2B



S2C



Supplementary Figure 2. Sensitivity analysis for functional improvement in the medium term. Removing studies with: less and greater statistical weight (S2A); greatest statistical weight (S2B); less statistical weight and higher risk of bias (S2C).

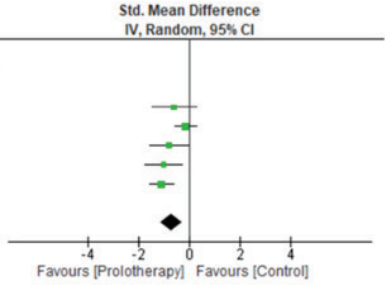


Supplementary Figure 3. Evaluation of the publication bias with Begg's funnel plots for pain reduction (S3A) and improvement in function (S3B) in the medium term.

S4A

Study or Subgroup	Favours Prolotherapy Mean	SD	Total	Favours Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Scarpone, 2008	0.5	0.4	10	3.5	1.5	10	0.0%	-2.62 [-3.88, -1.36]	2008
Carayannopoulos, 2011	1.25	1.6	8	1.83	2.85	9	0.0%	-0.23 [-1.19, 0.72]	2011
Rabago, 2013	7.9	12.64	10	20.9	11.6	11	0.0%	-1.03 [-1.95, -0.11]	2013
Rabago, 2013	13.6	11.38	10	20.9	11.6	11	14.5%	-0.61 [-1.49, 0.27]	2013
Yelland, 2019	0.8	1.3	40	1	1.5	40	26.0%	-0.14 [-0.58, 0.30]	2019
Bayat, 2019	2.9	2.6	14	5.3	3.2	14	16.7%	-0.80 [-1.57, -0.02]	2019
Apaydin, 2020	1.06	0.8	16	2.44	1.7	16	17.5%	-1.01 [-1.75, -0.27]	2020
Deb 2020	3.17	1.03	42	4.45	1.27	42	25.3%	-1.10 [-1.56, -0.64]	2020
Total (95% CI)			**122**			**123**	**100.0%**	**-0.71 [-1.15, -0.28]**	

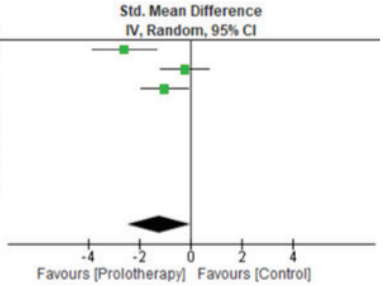
Heterogeneity: Tau² = 0.14; Chi² = 9.83, df = 4 (P = 0.04); I² = 59%
Test for overall effect: Z = 3.20 (P = 0.001)



S4B

Study or Subgroup	Favours Prolotherapy Mean	SD	Total	Favours Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Scarpone, 2008	0.5	0.4	10	3.5	1.5	10	30.2%	-2.62 [-3.88, -1.36]	2008
Carayannopoulos, 2011	1.25	1.6	8	1.83	2.85	9	34.7%	-0.23 [-1.19, 0.72]	2011
Rabago, 2013	7.9	12.64	10	20.9	11.6	11	35.2%	-1.03 [-1.95, -0.11]	2013
Rabago, 2013	13.6	11.38	10	20.9	11.6	11	0.0%	-0.61 [-1.49, 0.27]	2013
Yelland, 2019	0.8	1.3	40	1	1.5	40	0.0%	-0.14 [-0.58, 0.30]	2019
Bayat, 2019	2.9	2.6	14	5.3	3.2	14	0.0%	-0.80 [-1.57, -0.02]	2019
Apaydin, 2020	1.06	0.8	16	2.44	1.7	16	0.0%	-1.01 [-1.75, -0.27]	2020
Deb 2020	3.17	1.03	42	4.45	1.27	42	0.0%	-1.10 [-1.56, -0.64]	2020
Total (95% CI)			**28**			**30**	**100.0%**	**-1.23 [-2.48, 0.02]**	

Heterogeneity: Tau² = 0.94; Chi² = 8.73, df = 2 (P = 0.01); I² = 77%
Test for overall effect: Z = 1.93 (P = 0.05)



Supplementary Figure 4. Forest plot of Prolotherapy for controlling pain in the medium–term, according to the type of solution used. S4A) studies using dextrose alone; S4B) studies using dextrose plus other irritants.