

# The Efficacy of Intra-Articular Botulinum-Toxin Injection in Knee Osteoarthritis: A Systematic Review and Meta-analysis

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## DOI:

10.32098/mltj.03.2021.21

## LEVEL OF EVIDENCE: 1A

## SUMMARY

**Objective.** This investigation aimed to pool and discuss the existed literature on the efficacy and safety of intra-articular injection of Botulinum toxin type A (BTA) for knee osteoarthritis (OA) treatment focusing on different doses and comparing with other injectable agents.

**Methods.** *Data sources:* a systematic review through PubMed, Scopus, Google Scholar and Cochrane Central Register of Controlled Trials was conducted to identify all English-language studies published before November 2019. *Study Selection:* eligibility included adults suffering from knee osteoarthritis with intra-articular BTA injection as intervention. *Data Extraction:* after removing duplications and excluding non-eligible articles, data of final included studies were extracted including outcome measures; follow up time-points; and details of interventions in all groups including dose, duration, frequency and number of sessions. Included studies were quality appraised using PEDro (Physiotherapy Evidence Database) score. *Data synthesis:* the gathered data was extracted and analyzed in RevMan 5.3 software using random or fixed-effect models, as effect size measures such as raw or standardized mean difference (MD or SMD). Only six studies provided suitable data for meta-analysis with a total number of 459 patients. All studies included, except for one RCT that had fair quality, obtained good and high quality scores. The present data revealed that the included patients experienced significant pain relief immediately about 2 weeks after BTA intra-articular injection and this response remained significant even after 6 months (MD = 1.53 (95% CI: 0.94-2.11) and 1.71 (95% CI: 1.35-2.07), respectively).

**Results.** Also, this review proved that the short-term analgesic effect of BTA-corticosteroid was better than BTA alone, not only for pain control based on VAS (MD = 3.73 (95% CI: 2.60-4.86),  $P < 0.0001$ ), but also for WOMAC index (MD = 21.20 (95% CI: 14.14-28.26),  $P < 0.00001$ ). Additionally, this review find supportive evidence for the analgesic effect of low-dose BTA rather than higher doses (more than 200 IU).

**Conclusions.** The pooled data proved that a combination of BTA-corticosteroid achieved maximum efficacy for both pain relief and functional improvement within short and long-term follow up in comparison to both alone. Also, BTA is a safe and effective choice for clinical response with the most pain-relief effects 2-3 months after injection and the most effective dose of 200u among knee OA patients.

## KEY WORDS

*Conservative Treatment; Botulinum Toxins type A; Triamcinolone Acetonide; Hyaluronic acid; knee osteoarthritis.*

## ABBREVIATIONS

RCT: Randomized Controlled Trial  
 VAS: Visual Analog Scale  
 MD: Mean Difference  
 BTA: Botulinum Toxin Type A  
 HA: Hyaluronic Acid  
 AEs: Adverse Events  
 WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index  
 DALYs: Disability-Adjusted Life Years  
 YLDs: Years Lived with Disability  
 OA: Osteoarthritis  
 KLS: Kellgren Lawrance Scale  
 TENS: Transcutaneous electrical nerve stimulation  
 LLLT: Low Level Laser Therapy  
 HILT: High-Intensity Laser Therapy  
 PRP: Platelet-Rich Plasma  
 IA: Intra-Articular  
 CI: Confidence Interval  
 BTA + C: Botulinum toxin type A + Corticosteroid

## INTRODUCTION

Knee osteoarthritis (OA) as a common condition, contributes for global disability and high DALYs (1). Epidemiologic studies showed high prevalence of disease in females aged over 50 and YLDs for hip and knee OA has increased from 10.5 million in 1990 to 17.1 million in 2010 (2). Pain is a major clinical problem of osteoarthritis which not only impairs patient function, but also impress health-related quality of life (3).

OA occurs when the joint loses cartilage and the bone grows to compensate this loss to reduce joint degradation acceleration which cause other complications additionally (4). Recent studies emphasized more comprehensive view of OA, suggesting a disease of the whole joint involving synovia and bone marrow in addition to cartilage (5). Surprisingly, both clinical and experimental studies broke the borders with narrow point of view to joint and recently suggested changes of the nociceptive processing in the spinal cord, brainstem, and thalamocortical system (6, 7). Furthermore, there are growing evidence toward neuropathic pain components in OA especially in individuals with minor joint changes but with high levels of pain refractory to analgesic treatment (8). Despite recent progression in surgical techniques of knee OA management, conservative treatments still have their fans even for severe stages (9). Beyond the pharmacological treatments, non-pharmacological options including exercises, physical agent modalities and intra-articular injections are highlighted in studies (10).

A recent meta-analysis showed that intra-articular corticosteroids appear to be relatively more effective for pain than intra-articular hyaluronic acid (HA) within the first month of treatment. However, beyond week 8, hyaluronic acid has greater efficacy (22, 23). The results of another meta-analysis two years after PRP injection showed the superiority of PRP versus HA (25).

Intra-articular injection of interleukin-1 receptor antagonist, Anakinra, failed to confirm beneficial effects on knee pain, function, stiffness, or cartilage turnover in patients with knee OA (31). In general biologic treatment is not recommended for patients with OA based on the lack of evidence concomitant with the economic burden. Dextrose prolotherapy had a superior effect compared with local anesthetic injection and exercise regarding studies (32, 33). In a review with inconclusive low quality studies, prolotherapy with hypertonic dextrose was showed to be more effective than infiltrations with hyaluronic acid, ozone or radiofrequency and less effective than PRP, with beneficial effect in the short, medium and long term (34). Based on another meta-analysis on intra-articular ozone injection, its efficacy was significantly superior to placebo for short time in mild to moderate knee OA (35).

BTA is a neurotoxin produced by the bacterium *Clostridium botulinum* which blocks acetylcholine release into the synaptic cleft causing muscle paralysis (37). However, growing evidence supports a potent role of intra-articular use of BTA as a pain killer in various types of musculoskeletal conditions with preference in refractory ones (38). Although the exact mechanisms of pain modulation by BTA in OA is not clear, mechanisms such as directly decreasing peripheral sensitization by suppression in secretion of neurotransmitters leading to indirectly decreasing central sensitization, substance-P, calcitonin gene-related peptide (CGRP) and glutamate release inhibition resulting in nociception block has been raised (39-41).

Safety of BTA injection was reviewed through a meta-analysis in 2004 and finally focal weakness was the only adverse event founded. Fortunately, none of these complications occurred in intra-articular injections (42). A systematic review with meta-analysis evaluating intra-articular injections of BTA for refractory joint pain showed improvements in pain and function after 4 and 8 weeks compared with control group through different joint pathologies (43). Additionally, recent systematic review with meta-analysis confirmed usefulness of BTA injection in comparison to placebo, for knee OA, pooling data from 315 patients. They did not compare BTA effects with other injectable agents and did not evaluate different BTA dosages (44). OARSI guidelines, recently developed for the non-surgical management of knee osteoarthritis, did not recommend BTA due to

limited evidence (45). However, review of mentioned literature suggests BTA as potential option for knee OA treatment. Therefore, we aimed to evaluate efficacy and safety of BTA versus other alternatives focusing on different BTA doses through published RCTs.

## MATERIALS AND METHODS

### Inclusion and exclusion criteria

Two authors (M.H and E.K) independently reviewed four large databases: PubMed, Google Scholar, Scopus, and Cochrane Central Register of Controlled Trials from the database inception; furthermore, the website of [www.clinicaltrials.gov](http://www.clinicaltrials.gov) was searched for any ongoing trials. Only English language studies that had been finished before November 2019 were included. In case of disagreement, the final decision was made by a senior author (M.A). Researchers screened all 56 study titles and abstracts to identify relevant studies. Duplications were removed and 37 records remained. After reviewing them, 31 studies were excluded because of non-RCT design or lack of other eligibility criteria (**figure 1**). At last, only six RCTs remained with a total number of 459 patients.

### Primary and secondary outcome measures

Studies reporting one of our primary outcome measures were eligible for meta-analysis. Six studies had reported pain intensity based on VAS or Numeric Rating Scale (NRS); four ones utilized WOMAC to explain their patients' functional status; and only two RCTs used SF-36. On the other hand, Lequesne index, 40-meter timed walk, PGIC, and Oxford knee score, each one was used by only one study. Moreover, adverse events related to BTA injection were assessed.

### Data extraction

Data of all five included RCTs were extracted using a standardized form, which include the following: study title, first author and publication year; study design and setting; sample size and demographics of participants; outcome measures; follow up time-points; and details of interventions in all groups including dose, duration, frequency and number of sessions (**table II**).

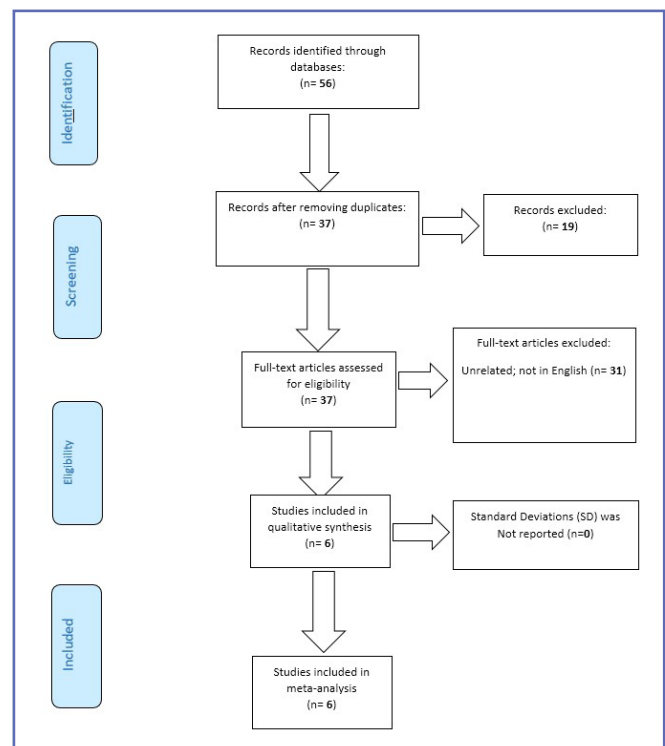
### Quality assessment

Two reviewers (N.M and S. RD) evaluated the quality of each study using PEDro (Physiotherapy Evidence Database) score. This scoring system contains 11 domains

(**table I**). We assessed risk of bias for each domain of the seven included studies. For each domain, a score of “+” or “-/?” indicates a low risk or high risk of bias, respectively. Five of our included studies achieved good quality (score > 6) and only one RCT had the fair (score = 4) quality (45).

### Registry and analysis

We have registered this review in International prospective register of systematic reviews (with ID review registry: CRD42020176671). The gathered data was extracted and analyzed in RevMan 5.3 software (Cochrane Collaboration, Oxford, UK) using effect size measures such as raw or standardized mean difference (MD or SMD). Also, the heterogeneity was assessed using  $I^2$ ; a value of less than 25% was considered as low heterogeneity and a value of more than 75% as high heterogeneity. The value of 0.05 was considered as the significance level for all our tests. Both random and fixed-effect models were used accordingly to calculate results. Sub-group analysis was also done to compare the results of different interventions at different time-points, as well as to compare the different outcome-measures.



**Figure 1.** Standard PRISMA study flow-diagram.

## RESULTS

### Descriptive results

#### *Andrea J. Boon; USA - 2010*

This study aimed to evaluate the effectiveness and safety of intra-articular BTA injection through a double blind randomized controlled trial. Sixty knee osteoarthritis patient with moderate pain (VAS > 6) and functional impairment, resistance to exercise and oral medications were included. Those knee OA patients who had grades 1 or 4 of Kellgren Lawrance scale (KLS) were excluded. All participants were assessed at week 8 post-injection; but only 32 subjects participated at week 26. Patients randomly received either of low dose BTA (100u) or high dose BTA (200u). The primary outcome measuring tool was VAS, while the secondary ones were WOMAC, SF-36, 40-meter timed walk and the complication rate of intervention. Findings of this study suggested intra-articular BTA injection as an alternative treatment for knee OA management (46).

One of the main strengths of the present RCT from USA was use of corticosteroid injection as the standard treatment for the control group and evaluation of both high and low dose BTA effectiveness. Application of different measuring tools and longtime follow up were the other strengths. They did not use imaging parameters or biochemical markers after injection to assess the efficacy. The also, did not evaluate biomechanical characteristics of knee muscles to detect possible adverse effects of BTA.

#### *Hsieh Lin-fen; Taiwan - 2016*

This randomized controlled trial investigated the effects of IA BTA injection on patients with knee OA through a landmark-guided or blind approach. Forty six patients with symptomatic knee OA (mostly KLS grades 2 and 3) were randomly assigned in two parallel treatments; the

first group received BTA (100u) injection (n = 21), and the control group who obtained education only (n = 20). The main outcomes including pain and functional status were evaluated using WOMAC, Lequesne index and VAS with-in short time (1 week after treatment) and long time (6 months later). Both VAS and WOMAC scores significantly decreased at short- and long-term follow ups compared to the control group.

As some shortcomings, it should be noted that the current RCT was a single-blinded one; furthermore, patients in the control group did not receive any injection which might cause placebo effect of needle in the intervention group. Using land-mark instead of ultrasound guidance is the other limitation. As the two main strengths, having both short- and long-term follow ups and the stratification of different KLS grades should be mentioned (47).

#### *Xiao Bao; China - 2018*

This investigation was a single-blinded randomized controlled trial evaluating 60 knee OA patients. Participants randomly divided into 3 categories; the first group received saline 0.9% injection as the control group. The second and third groups assigned to BTA (100u) and hyaluronic acid injection, respectively. Injections were performed using the ultrasound-guidance via in-plane method. After injection, all patients were educated and instructed to perform strengthening and balance exercises. Exercises were accomplished 5 times a week, for 8 weeks with 30-45 minutes sessions under the supervision of experienced physiotherapist. All participants were reassessed after 4 and 8 weeks follow up by a physiotherapist who was not aware of patients` groups (48). Mean age of participants were 65.9 years and only grades 2 KLS and above were included. In addition to knee x-ray and MRI, the other measuring tools were VAS, WOMAC and SF-36 reassessed after 4 and 8 weeks of follow up.

**Table I.** Quality assessment of included studies using PEDro score.

Study [Author's name, year]	1	2	3	4	5	6	7	8	9	10	11	PEDro Score
<b>Andrea J. Boon, 2010</b>	+	+	+	+	+	+	?	+	?	+	+	9
<b>Lin-Fen Hsieh, 2016</b>	+	+	+	+	?	?	?	+	?	+	+	7
<b>Xiao Bao, 2018</b>	+	+	+	+	?	+	?	+	?	+	+	8
<b>Timothy E. McAlindon, 2018</b>	+	+	?	+	+	+	+	+	+	+	+	10
<b>Divya Shukla, 2019</b>	+	?	?	+	-	?	?	?	?	+	+	4
<b>Jamile G. Mendes, 2019</b>	+	+	+	+	+	-	+	+	+	+	+	10

1: eligibility criteria specified; 2: patients randomized to groups; 3: concealment of allocation; 4: groups similar at baseline; 5: patients blinded; 6: practitioners administering intervention blinded; 7: assessors blinded; 8: measurements of key outcomes obtained from > 85% of patients; 9: intention to treat analysis; 10: statistical comparisons between groups; 11: point measures and measures of variability provided. Interpretation: scores 4-5: fair quality; scores 6-8: good quality; scores > 8: high quality; (+) Criterion clearly satisfied; (-) Criterion not clearly satisfied; (?) Unclear whether criterion was satisfied.

**Table II.** Characteristics of included studies.

Authors, year	Study design	Type of intervention	Number (% females)	Pain duration	Follow-up intervals	Outcome measures
<b>Andrea J. Boon; USA-2010</b>	Double-blind RCT with 3 groups	* methylprednisolone * BTA (100u) * BTA (200u)  - US-guided single injections	60 (58%)	97 months	8, 12, 26 weeks	-VAS -WOMAC -SF-36 -40-meter timed walk
<b>Lin-Fen Hsieh; Taiwan-2016</b>	Single blind RCT with 2 groups	* n = 21 (BTA 100u) * n = 20 (education alone)  - Two groups received acetaminophen - Blind single injections	46 (61%)	≥3 months	1 week  24 weeks	-VAS (pain) -Lequesne -WOMAC
<b>Xiao Bao, China-2016</b>	Single blind RCT with 3 groups	* Saline * BTA * HA  - Five sessions of HA injection - single BTA injection - US-guidance	60 (47%)	32 months	4, 8 weeks	-WOMAC -VAS -SF-36
<b>Timothy E. McAlindon; USA-2018</b>	Double-blind RCT with 2 groups	* BTA 200 * BTA 400 U * Placebo (saline)  - US-guidance	158 (60%)	≥3 month	1, 4, 8, 16, 20,24 weeks	-NRS -WOMAC -PGIC
<b>Divya Shukla; India-2019</b>	RCT with 2 groups Open-label Trial	* BTA 100u + Triamcinolone * Triamcinolone alone  - Fluoroscopy-guidance	30 (67%)	NR	Day 1  2, 4, 6, 12, 24 weeks	-VAS -Oxford knee score
<b>Jamile G. Mendes; Brazil-2019</b>	RCT with 3 groups	* BTA 100u * Triamcinolone 40 * Placebo (Normal saline)	105 (91.4%)	7.9 years	4, 8, 12 weeks	-WOMAC -VAS -SF-36 -TUG -ROM -6 minute Walk test

Researchers found a statistically significant difference for VAS and WOMAC improvement, as well as physical and mental components of SF-36 questionnaire at the 4<sup>th</sup> and 8<sup>th</sup> weeks between the BTA versus the control or even HA groups. There was not any change in regard to X-ray and MRI parameters during this follow up period. Finally, they concluded that BTA injection plus to therapeutic exercise could remarkably alleviate pain and improve function of knee OA patients, even in comparison to HA. As the main strength, this RCT used an objective parameter (imaging) for evaluation of BTA efficacy. Additionally, supervision of exercise would destroy possible bias of unequal exercise

among all participants. On the other hand, the main shortcoming of this research was lack of long-term follow up. Furthermore, the control group and BTA groups received only one injection session, while the HA injection was repeated for 5 times which might make comparison of the groups difficult.

#### **McAlindon E. Timothy; USA – 2017**

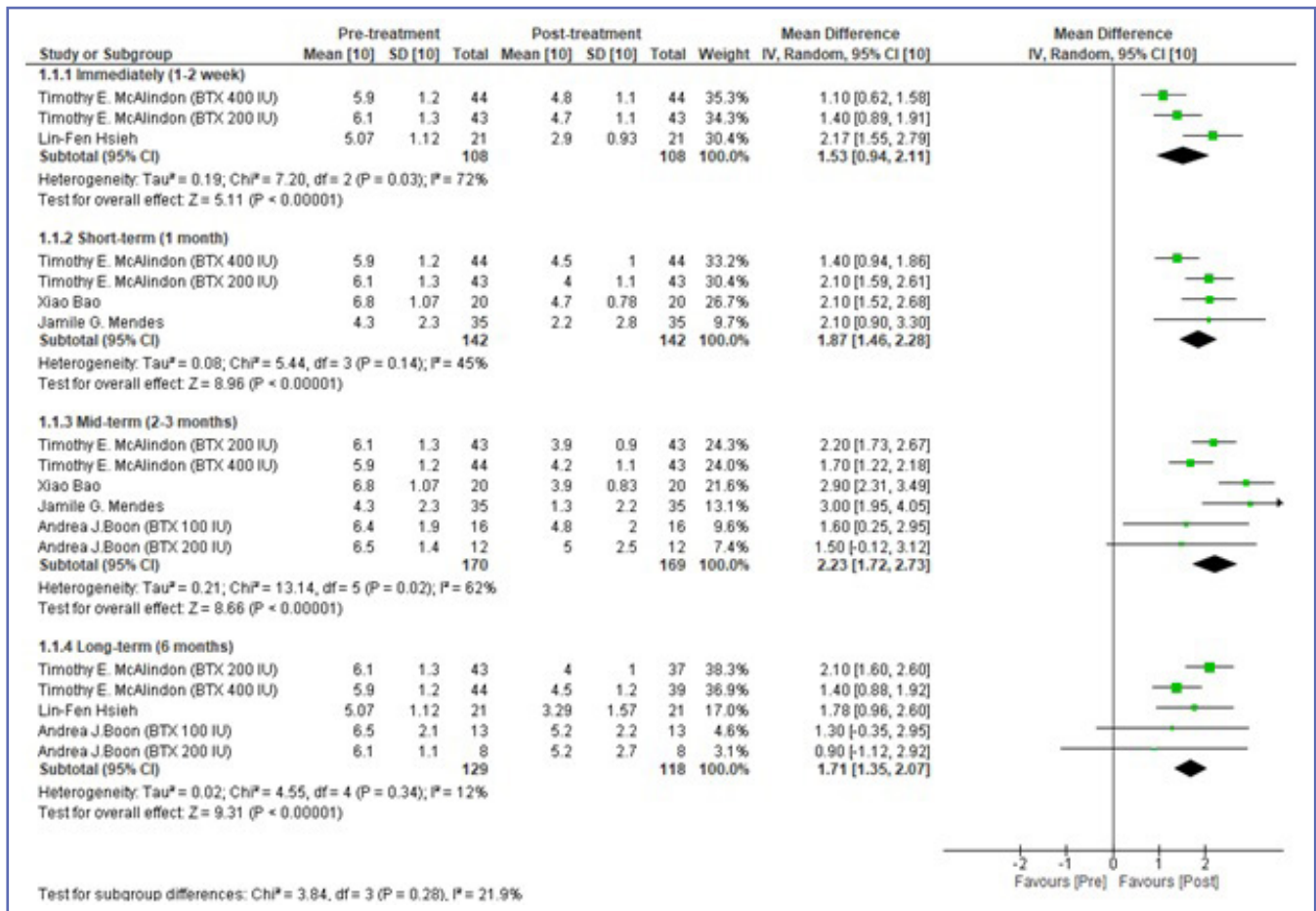
This large investigation, as a multi-centric double-blinded randomized controlled trial, was conducted in adults with knee OA for evaluation of IA-BTA effectiveness (400 u and 200 u). Patients who obtained the score of DETECT ques-



tionnaire less than 12 were considered to having nociceptive pain. Knee radiographs showed grades 2 and 3 of KLS for all patients. Then, participants were randomly divided into three groups of IA-BTA 400u, 200u and placebo (saline 0.9%) on a 1:1:2 ratio, respectively. They used 7-day average worst pain score and WOMAC index for outcome evaluation at the 8<sup>th</sup> week. Among the 176 enrolled patients, 158 subjects completed the study. Although the average pain score was decreased in all groups significantly, they did not observe significant difference between the groups. Researchers reported adverse events in 3.4% which was not dose dependent. The most important adverse events were new-onset or worsening arthralgia and nasopharyngitis. There was no statistically significant difference in muscle strength of leg or knee muscles after treatment. Despite excellent quality and relatively higher sample size compared to other included RCTs, application of PD-Q screening (for nociceptive pain identification) tool which had been validated for low back pain patients only, is questionable (49).

**Shukla D. et al. - 2018**

This study was a clinical randomized trial evaluating the effectiveness of triamcinolone in conjunction with BTA versus triamcinolone alone for knee OA managements. They included 30 patients who had less than 2 mm joint space in their knee X-ray. Therapeutic agent injection was applied after local anesthetic injection under the fluoroscopy-guidance. Assessment tools consisting VAS and Oxford knee score were applied after 1 day, 2 weeks, 4, 6, 12, and 24 weeks post-injection. Researchers detected a statistically significant pain improvement in combination therapy group comparing pre and post values, but not about triamcinolone group. The onset of BTA efficacy plus to triamcinolone was recorded within 2 weeks after injection and lasted for 6 months. Furthermore, a functional status improvement was observed at 4 weeks and 6 months for the combination therapy group, compared to triamcinolone group. They concluded that intra articular BTA in conjunction with corticosteroid injection could cause much better pain relief and function-



**Figure 2.** Forest plot of pain reduction of intra-articular BTA based on VAS at the different time-points (pre-post comparison).

al improvement among knee OA patients with long lasting effects, compared to steroid injection alone. As the weakness, researchers did not utilize a common classification system of knee OA severity; then comparison of their results against other studies would be problematic. They did not explain about randomization mechanism, nor about blinding. Another unclear question is why the authors ruled out patients with joint effusion and included only those with dry tap. On the other hand, the main strength of investigation was very close and frequent follow up time-points (50).

#### **Mendes JG. et al. - 2019. (51)**

Brazilian researchers compared the efficacy of intra-articular BTA 100u injection, and triamcinolone hexacetonide 40mg, versus normal saline among patients with primary knee OA. Patients were assessed at baseline and at 4, 8, and 12 weeks using VAS for pain during movement (VASm) and at rest (VASr), WOMAC questionnaire, 6-minute walk test, Timed Up and Go (TUG) test, Short Form (SF)-36 questionnaire, ROM, and ultrasound (US) measurement of synovial hypertrophy. Eventually they concluded that corticosteroid had a better efficacy effect than that of BTA or normal saline throughout short-term (four weeks) follow-up, in terms of VAS, WOMAC, and US measurement of synovial hypertrophy. However, at 12 weeks, there was no difference between the effectiveness of three interventions.

### **Meta-analysis results**

#### **Analgesic effect of BTA injection**

This review demonstrated the therapeutic trajectory for BTA efficacy in terms of VAS reduction during 6 months follow-up. According to **figure 2**, the pooled effect size for all kinds and doses of BTA injection was about MD = 1.53 (95% CI: 0.94-2.11), resulted from the comparison of pre- and post-treatment values within the 1-2 weeks after injection. This value rose to 1.87 (95% CI: 1.46-2.28), and reached to 2.23 (95% CI: 1.72-2.73) at 4-weeks and 2-3 months follow-up, respectively. Eventually, it decreased to 1.71 (95% CI: 1.35-2.07) till 6 months post-injection. It should be noted that all these MD values were statistically significant ( $P < 0.0001$ ). Therefore, we could state that knee OA patients experienced significant pain relief with BTA intra-articular injection, compared to baseline level, and this response remained significant even after 6 months.

#### **Non-analgesic effects of BTA injection**

Six trials contributed to the meta-analysis of function-related outcomes. Five RCTs used WOMAC and another one applied Oxford knee index. After putting them together,

the total effect size (MD) for short-term efficacy within 1-2 months was 14.04 (95% CI: 11.02-17.06) with heterogeneity score ( $I^2$ ) of 52% indicating acceptable heterogeneity among the trials. The pooled effect size of the long-term efficacy after 6 months was 12.13 (95% CI: 7.31 to 16.95) showing good but less effectiveness in comparison to short-time values. We also attempted to present the therapeutic effectiveness of BTA injection in either of the subscales of patients' function and stiffness for short and long-term period; however, the current data was not sufficient and the meta-analysis precluded.

#### **The different dosages of BTA versus other injection choices (short-term efficacy)**

**Figure 3** has shown comparison of short-term efficacy of BTA with different dosage (100u, 200u, and 400u) versus other alternatives including the combinational regimen of corticosteroids-BTA, corticosteroids alone, HA, saline and placebo (education only). According to our pooled results, it seems that BTA-100u should be considered as the most appropriate dosage in order to achieve rapid pain relief (MD = 2.38 (95% CI: 1.91-2.85),  $P < 0.0001$ ) and increasing BTA dosage did not provide further analgesic effects during this period (MD = 2.05 (95% CI: 1.56-2.53) for BTA-200u). Even, higher doses might have lower effect (MD = 1.40 (95% CI: 0.94-1.86) for BTA-400u). It should be noted that both of BTA-100 and BTA-200u injections provided better results than HA injection (1.90 (95% CI: 1.34-2.46)). However, BTA-400u achieved a remarkably smaller analgesic effect than HA and other BTA doses.

On the other hand as has been depicted in **figure 4**, BTA-400u interestingly had the best effect for short-term improvement of functional status, as the main subscale of WOMAC index, as well as other parts; i.e. joint stiffness and pain (MD = 16.90 (95% CI: 11.28-22.52),  $P < 0.00001$ ). This value was much better than the effectiveness of the other two doses of BTA (MD = 14.43 (95% CI: 10.53-18.33) of BTA-100u and MD = 12.46 (95% CI: 7.02-17.89) of BTA-200u). Among all injection choices, BTA combined with corticosteroids proved to have the highest short-term effectiveness not only for pain control based on VAS (MD = 3.73 (95% CI: 2.60-4.86),  $P < 0.0001$ ), but also for WOMAC index (MD = 21.20 (95% CI: 14.14-28.26),  $P < 0.00001$ ).

#### **The different dosages of BTA versus other injection choices (long-term efficacy)**

**Figure 5** has compared long-term effectiveness of BTA injection for pain reduction through different dosage (100 u-200 u-400 u) versus the other common treatments including mixed BTA-Corticosteroid (BTA + C), saline and placebo (education only). Meta-analysis of the pooled data proved that BTA-200 u was the most effective dosage with remark-

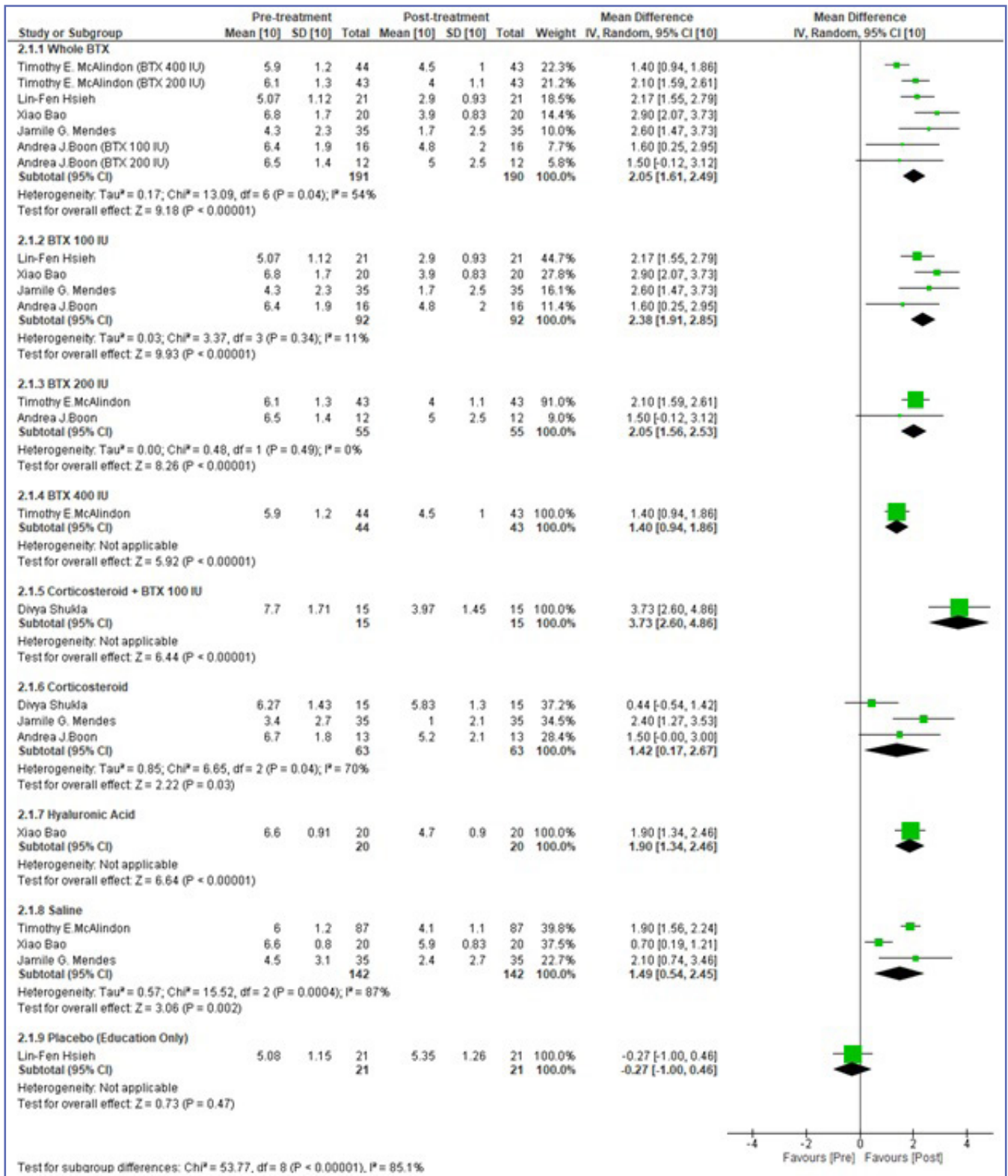


Figure 3. Forest plot of short-term efficacy of the different treatments based on VAS (before 2 months follow up).



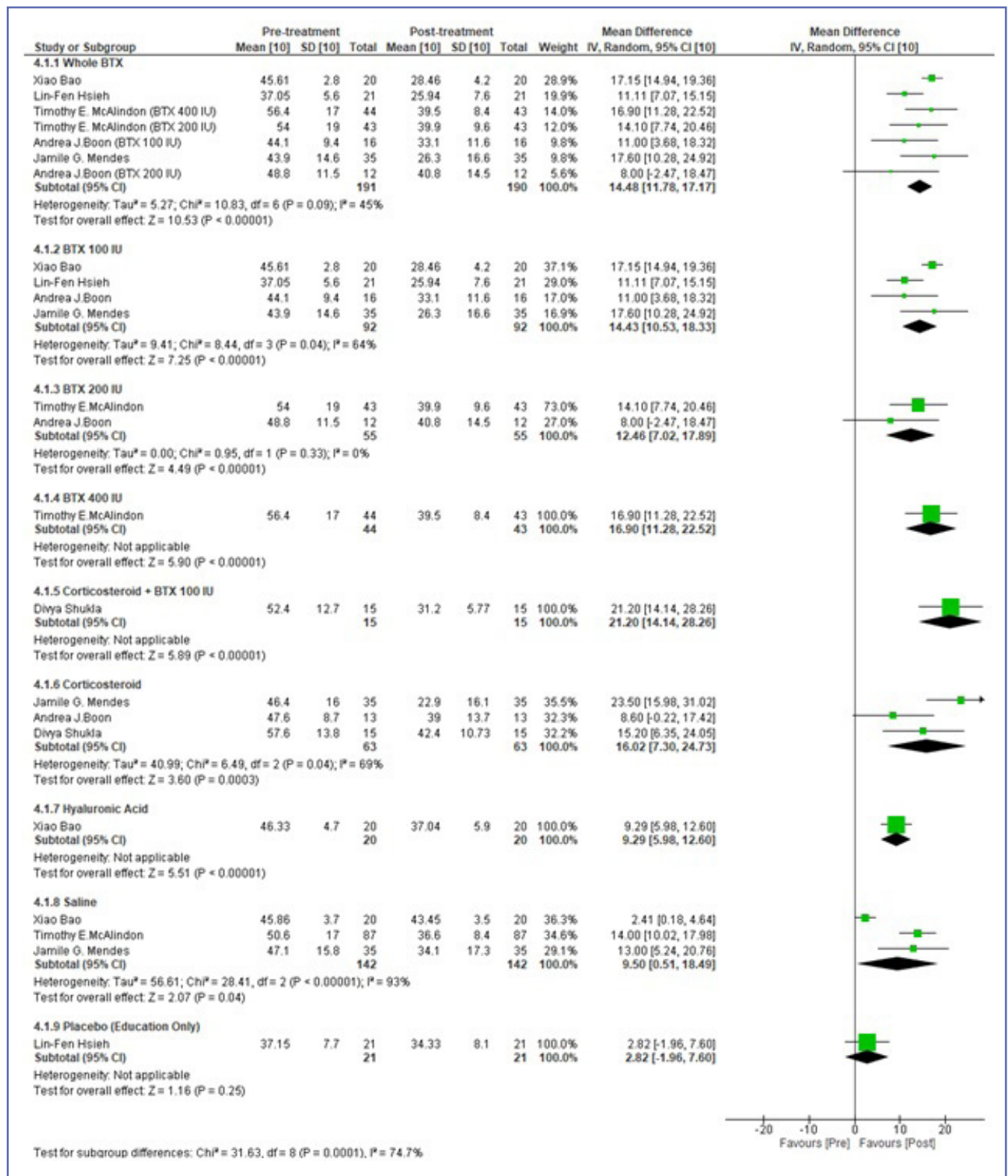


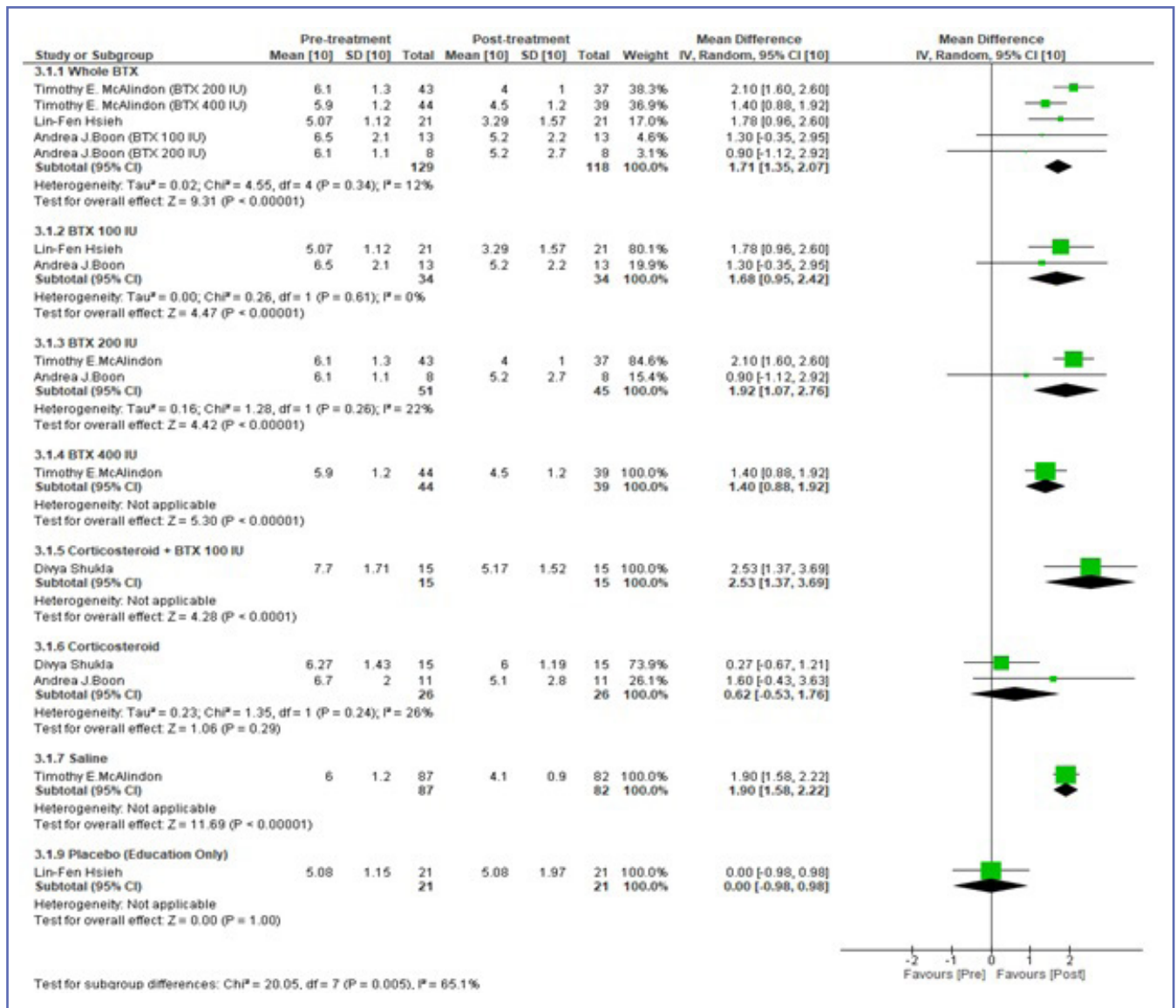
Figure 4. Forest plot of short-term efficacy of the different treatments based on WOMAC (before 2 months follow up).

able pain control until 6 months (MD = 1.92 (95% CI: 0.9, 2.96), P = 0.0002). Again, BTA-corticosteroid combination showed the best MD among all injection choices for both pain relief (figure 5) and functional status (figure 6) improvement (MD = 2.53 (95% CI: 1.37-3.69), P < 0.0001; and MD = 25.00 (95% CI: 18.21-31.79), P < 0.00001). Additionally, the pain-relief effect of saline injection (MD = 1.90 (95% CI: 1.58-2.22), P < 0.0001) was better than BTA-100 u and 400 u in long-term follow up (MD = 1.68 and 1.40, respectively). The latter results were in line with short-term evaluation of pain relief. In both figures 3 and 5, lower doses of BTA were

associated with higher MD values rather than BTA-400u. In contrary, when we take function and joint stiffness into account, high dose BTA (400 u) achieved more effectiveness than lower doses in both short- and long-term follow-ups (MD = 16.90 and 15.90, respectively).

**Intra-articular BTA safety**

There were no statistically significant differences between the BTA and controls for any adverse effects (RR = 0.66 (0.19, 2.27), P = 0.51). No subject withdraw from the studies evaluated in this meta-analysis.



**Figure 5.** Forest plot of long-term efficacy of the different treatments based on VAS (after 6 months follow up).

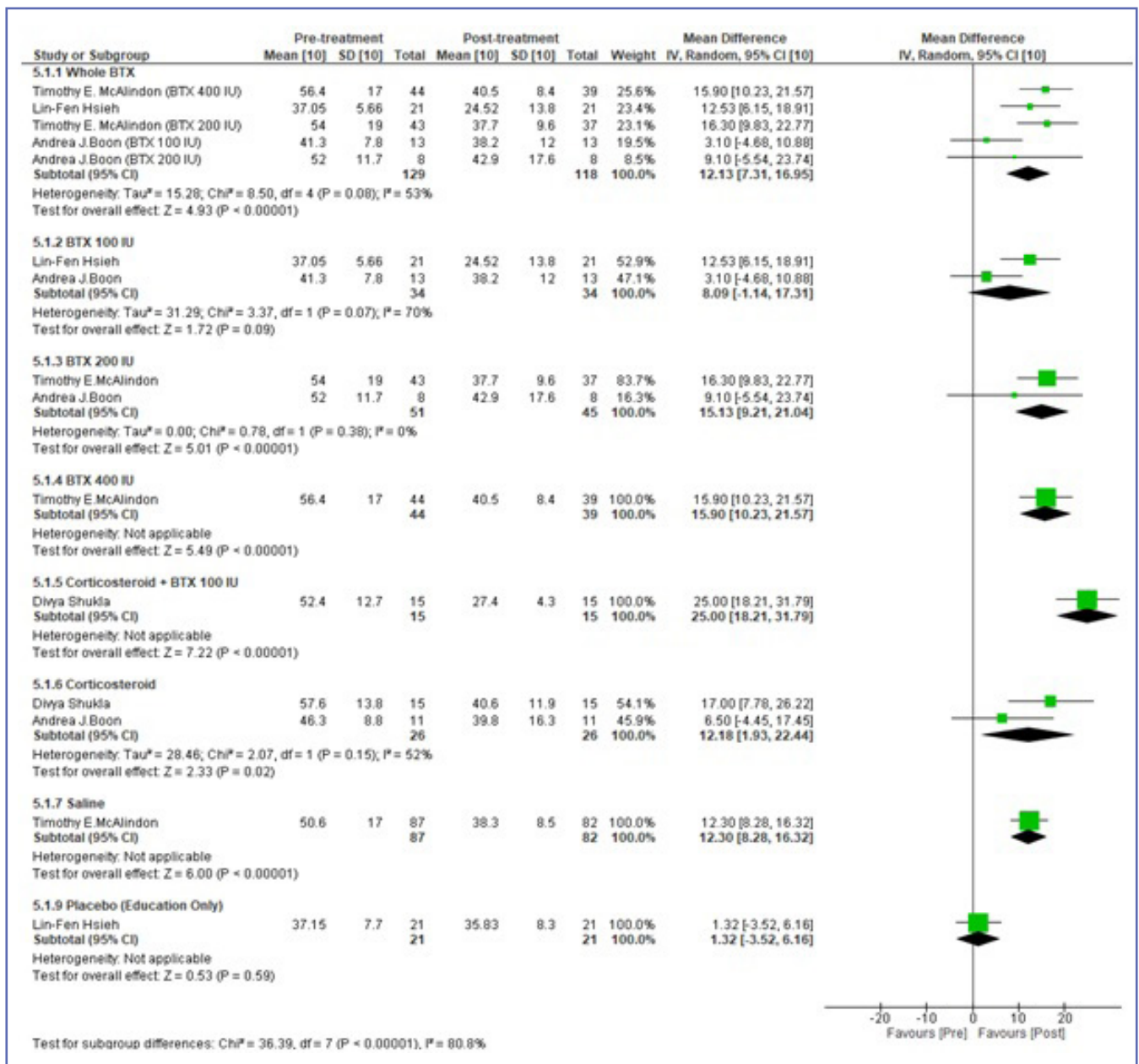


Figure 6. Forest plot of long-term efficacy of the different treatments based on WOMAC (after 6 months follow up).

## DISCUSSION

All studies included, except for one RCT that had fair quality (46), obtained good and high quality scores in PEDro assessment tool. Fortunately, all studies provided point measures and measures of variability in addition to statistical comparisons between groups. Four studies selected patients based on KLS grade 2-3 (47-51); but one study

used another radiographic parameter (joint space less than 2 mm) for case selection (46).

As we previously described, except for VAS which was applied in five articles (47-51), different studies have administered various types of tools to assess their outcomes including WOMAC for five studies (47-51), SF-36 for three studies (47, 49, 51) and 40-meter timed walk, Lequesne,



NRS, PGIC, Oxford knee score just in one study (46-48, 50). Furthermore, BTA injection was compared against HA only in one article (49); and versus corticosteroids in three studies (46, 47, 51). While five investigations evaluated the efficacy of low-dose (100 or 200u) BTA injection (46-49, 51), only one RCT utilized high-dose (400u) injection (50). Additionally, control groups were heterogeneous including exercise education (48), saline injection (49, 50, 51) or intra-articular corticosteroid (46, 47, 51). All studies used guide for their injections (ultrasound (47, 49, 50) or fluoroscopic (46)), again except for one article, which selected blind injection (48). To summarize, there was not any homogeneity among the included studies, especially in their interventions. However, we tried to pool results using random-effect models to conduct a meta-analysis.

We found the most pain-relief effects of BTA injection approximately 2-3 months after injection. Wu et al. in their meta-analysis on the efficacy of BTA injection for any kind of refractory joint pain, demonstrated a higher effectiveness in 4 weeks in comparison to 8 weeks (43). Inversely, Zhai et al. found the better pain relief of BTA injection among knee OA patients in long-term (6 months) than short-term (44). It should be emphasized that Wu's study was conducted on different pathologies including capsulitis, post total knee arthroplasty pain and OA through different joints including ankle, shoulder and knee. This might explain an existed gap between their results versus ours. There are two points to pay attention about pattern of MD change through immediate to long-term follow up (**figure 1**). Firstly, Divya's study used BTA with triamcinolone (46), which can cause rapid pain-relief effects, probably due to corticosteroids effect. Therefore, we performed sensitivity analysis to see the effects of this study and the results were almost the same. Secondly, lower MD in week 1 and 6 months could be due to Lin-Fen's study which applied landmark-guided BTA injection (48), possibly with less accuracy. Therefore, we did sensitivity analysis by deleting this study and again the results did not change.

We found that 100u and 200u BTA were the most effective doses for short- and long-term pain control. This finding is in contrast to Zhang et al. findings which emphasize the more effectiveness of higher doses (52). Although our results proved that there was no need to apply higher doses of BTA, we should be cautious to conclude definitely, because data on amount of physical activity and time from knee pain onset was not declared in most studies which could influence our findings in the setting of limited number of studies. For example, patients with more severe sarcopenia tolerate more pain due to lack of joint support from muscles and probably, we find the accumulation of mentioned patients in 200 u BTA injected studies. Furthermore, any increasing in BTA dose could result in patients' concern about possible side effects

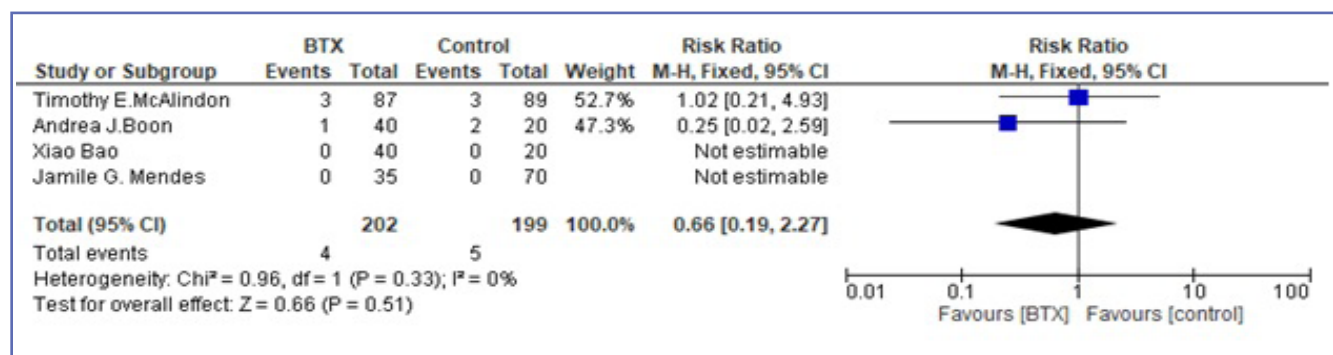
such as local weakness in muscles. Of note, this side effect was not reported through our literature review (46-50).

Our comparison suggested that BTA plus to corticosteroids was the most effective combination for knee OA treatment in terms of pain reduction; however, these results were extracted from a study with fair quality (46). Comparing the effect size of the mentioned combination in terms of MD versus MD of BTA injection alone, showed better pain relief for BTA+C. Actually as the **figures 3** and **5** have depicted, it was the most effective treatment during both short- and long-term periods. On the other hand, the pooled result of corticosteroid long-term efficacy in pain was not statistically significant at all (**figure 5**). Unfortunately, there was not any study comparing HA and BTA in long-term follow up. However, the short-term efficacy for pain relief was almost similar between the whole BTA (MD = 1.99) and HA (MD = 1.90) injection (**figure 3**).

Certainly, the main side finding of this review was the interesting short and long-term effectiveness of saline injection for pain reduction with MD = 1.31 and 1.90, respectively. Saltzman *et al.* in their meta-analysis of level-1 studies (36) confirmed our findings (MD = 1.21; (95% CI: 0.3-2.0); P = 0.007) for 3 month and 1.66 (95% CI: 1.2-2.1); P < 0.00001 for 6 months follow up). However, short-term improvement in functional status was not statistically significant (MD = 8.08 in **figure 4**).

WOMAC scale consisting pain, joint stiffness and functional status related subscales, improved at both short and long-term follow-ups of post BTA injection. The most remarkable change in WOMAC was interestingly found in 400u dosage. While, taking into account pain-related pooled findings, high dose injection of BTA was not more effective than low doses. Considering these two facts simultaneously showed that WOMAC improvement was not related to its pain subscale, but it was mainly because of changes in functional domain, and to a less degree, joint stiffness. Unfortunately, subscales-comparison was not achieved due to shortage in subscales' data reported in different studies. It seems higher doses of BTA are more efficacious than low doses for function and joint stiffness improvement. This finding implies more unrecognized pathophysiology of BTA effects in OA process, rather than the only analgesic effect. Our results about functional improvement following BTA injection were in line with Wu *et al.* study (43). Perhaps one of the most notable findings from this meta-analysis was the remarkable efficacy of BTA-corticosteroid combination. As far as we know, this study is the first review that has evaluated effects of BTA-corticosteroid mixture at short and long-term follow up. We found the mentioned combination more effective than BTA or corticosteroid, alone. Additionally, our data confirmed that the BTA intra-articular injection for knee OA could be considered as safe as other common alternatives.





**Figure 7.** Forest plot of comparison of treatment-related adverse events (AEs) between the groups.

One unique aspect of this meta-analysis was comprehensive analysis of data including different time course evaluation post-injection, different doses of injection, and different injectates comparison. Due to limited numbers of RCTs, we were not able to detect the effect size variations depending on trial quality, allocation concealment, intention-to-treat analysis, blinding mechanism, funding reporting, and publication bias status. Another problem in trying to pool study results was the considerable variety of assessment times and scales. Future trials should apply common outcome measuring tools and provide enough data on their mean and SD values at different time-points. Also, cost-effective analysis and economic evaluation should be performed to better compare BTA injection versus other common alternatives.

## CONCLUSIONS

The pooled data proved that a combination of BTA-corticosteroid achieved maximum efficacy for both pain relief

and functional improvement within short and long-term follow up in comparison to both alone. Also, BTA is a safe and affective choice for clinical response with the most pain-relief effects 2-3 months after injection and the most effective dose of 200u among knee OA patients. The study meets the ethical standards of the journal (53).

## CONTRIBUTIONS

SZE & MA contributed in idea formation. EK & MH contributed in search and data extraction. ShR & NM contributed in methodological evaluation of included studies and manuscript draft preparation. ShR did meta-analysis and all authors contributed in concept discussion and manuscript revision.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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