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ORIGINAL ARTICLE

Comparison of the clinical effectiveness of activated and non-activated platelet-rich plasma in the treatment of knee osteoarthritis: a systematic review and meta-analysis

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Abstract

Introduction/objectives Platelet-rich plasma (PRP) has shown to be clinically effective in the treatment of knee osteoarthritis (OA). Notwithstanding, some inconsistences remain due to methodological differences in PRP preparation such as the use (or not) of activation strategies. We aimed to evaluate whether the use of non-activated PRP would be as effective as activated PRP in patients with knee OA.

Method All randomized, placebo-controlled trials were identified through a search in MEDLINE, EMBASE, Scopus, and Web of Science up to June 2022. Pre- and post-injection pain and function scores were collected. The meta-analysis was conducted with a random-effects model and generic inverse variance method. Effect sizes were estimated using standardized mean differences (SMD).

Results Fourteen clinical trials involving 1292 subjects were included for meta-analysis. Exogenous activation of PRP revealed a significant pain relief (SMD, − 1.05 [95% CI − 1.58 to − 0.52]; $p = 0.0001$) and a significant functional improvement (SMD, − 1.21 [95% CI − 1.75 to − 0.67]; $p < 0.0001$) unlike studies describing the use of a non-activated PRP. The sensitivity analysis indicated that the effect size for both outcomes was not influenced by a single study.

Conclusions The results of this systematic review suggest that the use of an exogenously activated PRP is more effective in improving both pain and functional scores in patients with knee OA.

Key Points
- *Results from meta-analysis suggest that exogenously activated PRP is clinically more effective than non-activated PRP.*
- *The use of an activated PRP was more frequently reported by the included studies.*
- *The most frequent method for activation was the use of calcium chloride ($CaCl_2$).*

Keywords Calcium chloride · Knee osteoarthritis · Meta-analysis · Pain · Platelet-rich plasma · Systematic review · Thrombin

Introduction

Osteoarthritis (OA) is a chronic, debilitating, and degenerative joint disease, which affects approximately 10% of the population. It occurs more frequently in people older than 45 years of age and has been shown to significantly affect quality of life [1–3]. Treatment of symptomatic knee OA typically begins with non-invasive interventions such as changes in lifestyle and systemic non-steroidal anti-inflammatory drugs (NSAIDs). However, the uncertainty on their therapeutic effectiveness and the probable appearance of side effects after regular consumption make intra-articular injections of platelet-rich plasma (PRP) or viscosupplementation more attractive for patients and treating physicians [4, 5].

PRP from patients own blood is a feasible and economical source of growth factors which have shown chondrogenic potential in addition to modulating inflammation [4].

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Besides the intrinsic variation of being a biological product, PRP can be generated from different protocols, sometimes without clearly establish if platelets were effectively concentrated, or whether premature activation occurs, causing inconsistencies in patient outcomes and challenging the practicality of PRP clinical applications [6, 7].

Although efforts have been made to find out which characteristics of the PRP formulations, as well as of the treated patients, are the ones that result in a greater therapeutic benefit [8–10], there are still some unclear aspects of PRP therapy for knee OA. In this regard, exogenous (or endogenous) activation of platelets may also account for heterogeneity observed.

Several reports, including systematic reviews and meta-analysis, have concluded that PRP was found to be an effective and safe biological approach in the treatment of knee OA compared with other intra-articular injections [11–13]. While some randomized clinical trials assessed the effect of activated PRP therapy for knee OA [14, 15], none of these studies has directly compared the outcome of the activated PRP against the non-activated PRP.

The purpose of this systematic review and meta-analysis is to evaluate whether the use of non-activated PRP would be as effective in patients with knee OA, compared to studies that used activated PRP as treatment.

Methods

The systematic review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [16], and it was guided by a registered protocol (PROSPERO registration: CRD42022320169).

Information sources and search strategy

The search strategy was designed by an experienced librarian in collaboration with the investigators of the study. A combination of MeSH terms (knee osteoarthritis, platelet-rich plasma, PRP, autologous conditioned plasma, nonactivated, no activated, non-activated PRP, activated-PRP, non-active, reactivity, active*, intra-articular, injection) and text words were selected to find original articles or abstracts, in any language, including patients with a diagnosis of knee OA. The MEDLINE, EMBASE, Scopus, and Web of Science databases were searched from inception to June 2022.

Eligibility criteria

Studies were screened for inclusion according to the following criteria:

- Study design: Randomized controlled trials (RCT, parallel or cross-over).
- Population: Patients clinically and radiographically diagnosed with knee OA defined by any recognized diagnosis criteria (Kellgren-Lawrence or Ahlback classification).
- Intervention: Intra-articular injection of PRP or any derivative (with or without exogenous activation).
- Comparator: Placebo (normal saline solution).
- Outcomes: Pain relief and functional improvement assessed by validated questionnaires or scales (i.e., visual analog scale [VAS], Western Ontario and McMaster Universities Arthritis Index [WOMAC], International Knee Documentation Committee [IKDC], Knee injury and Osteoarthritis Outcome Score [KOOS]).

A minimum of one review outcome was considered sufficient for a study to be included in the review. Studies were excluded if a full-text was not available, did not include a control group, or were duplicated. We considered studies with a minimum follow-up of 12 weeks. There was no language restriction, and studies with relevant missing data regarding the outcomes of interest were also excluded.

Study selection process

Two reviewers screened the titles, abstracts, and full-text of manuscripts for eligibility in a 2-step approach. In the first step, the reviewers screened only the titles and abstracts of the studies. Studies approved by at least one reviewer were included. A full-text screening (step 2) was conducted to determine the inclusion of relevant studies. The same inclusion criteria were used for both screening phases; in this step, disagreements were resolved by consensus with a third reviewer. A chance-adjusted agreement was quantified using the kappa statistic after each step [17]. We used the Distiller Systematic Review Software (DistillerSR, Evidence Partners, Ottawa, Canada) for the data management during the selection process.

Data collection process

Data were extracted independently and in duplicate using a standardized digital data extraction format. Eligible studies were reviewed, and the following data were extracted: (1) first author name; (2) publication year; (3) follow-up; (4) number of participants in the intervention and control groups; (5) intervention arms; (6) number of injections; (7) OA classification; (8) time between injections; (9) injected volume; (10) type of PRP used; (11) activation method; (12) age, gender, and body mass index of the study participants; and (13) pain and functional scores at baseline and follow-up.

Risk of bias in individual studies

A systematic assessment of bias in the included studies was performed using the Cochrane Risk of Bias Tool version 2

(RoB 2.0), which covers the following domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in the measurement of the outcome, and bias in the selection of the reported result [18]. There are five possible answers for each domain (yes, probably yes, no, probably not, and no information), and according to the answers, an algorithm classifies the risk of bias as low, some concerns, or high.

Quantitative data synthesis

The meta-analysis was performed using the Review Manager statistical software (RevMan [Computer program], version 5.4.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) and the Comprehensive Meta-Analysis version 3 software (Biostat, Englewood, NJ 2013). For each study, a summary of the intervention effect was estimated by standardized mean differences (SMD) and 95% confidence intervals (CI) for pain (VAS, WOMAC Pain, EQ-VAS) and functional outcomes (WOMAC, IKDC, KOOS), respectively. Because of the different metrics used to evaluate pain and functionality scores, SMD was used for effect size estimation. Net changes in measurements were calculated as follows: measure at the end of follow-up minus measure at baseline. The mean change from baseline was used for analysis. When numerical values were only available in figures (results presented as graphs or charts), the data were extracted with the GetData (Graph Digitizer) software version 2.26 (http://getdata-graph-digitizer.com/). When only the standard error of the mean (SEM) was reported, the standard deviation (SD) was estimated using the following formula: $SD = SEM \times \text{sqrt}\ (n)$, where n is the number of subjects. If the outcome measures were reported in median and interquartile range (or 95% CI), mean and SD values were estimated with the methods described by Hozo et al. [19] and Wan et al. [20]. If not able to obtain the SD of a record after trying to contact the study authors, we used the range rule of thumb method to estimate the missing SD. This method estimates that the SD is a quarter of the range of a determined variable [20]. Finally, the SD of the mean difference was calculated using the following formula: $SD = \text{square root}\ [(SD_{\text{pre-treatment}})2 + (SD_{\text{post-treatment}})2 - (2R \times SD_{\text{pre-treatment}} \times SD_{\text{post-treatment}})]$, assuming a correlation coefficient (R) of 0.5. When a study with multiple intervention groups were correlated, the PRP intervention arms were combined to create a single pair-wise comparison [21].

Summary measures

The meta-analysis was conducted using a random-effects model and the generic inverse variance method. The exploration of consistency, focused on the heterogeneity of the studies, was examined by applying Cochrane's Q statistic test, and a p value < 0.05 was considered statistically significant. Additionally, the I^2 statistic was used, considering 0–25% of heterogeneity between studies as unimportant, > 25–50% as moderate, and > 50% as important heterogeneity. Lastly, we performed a sensitivity analysis to evaluate the influence of individual studies on the overall effect size using the leave-one-out method (i.e., removing one study each time and repeating the analysis) [20, 22].

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry and Egger's weighted regression tests. The Duval and Tweedie "trim and fill" method was used to adjust the analysis for the effects of publication bias for potentially missing studies.

Results

Search output

The search strategy identified 2184 publications. A total of 2147 studies did not meet the inclusion criteria and were excluded and 1 study was not retrieved. Subsequently, 36 full-text articles were reviewed for eligibility, and 22 were excluded for the following reasons: not being an RCT (12), not including a placebo (saline) group (6), not evaluating the outcomes of interest (3), and presenting incomplete data interfering with the analysis (1). The resultant 14 clinical trials were selected and included in the present meta-analysis. The complete workflow is shown in Fig. 1.

Characteristics of the included studies

Data suitable for analysis were reviewed and analyzed from 1292 subjects (746 in the PRP arm and 546 in the placebo arm). The range of publication dates of the studies was from 2008 [23] to 2022 [24]. The geographic region where the studies were conducted was quite heterogeneous. All subjects enrolled in the studies included had a confirmed diagnosed of knee OA. The final follow-up of patients in each study ranged from 24 weeks [25] to 24 months [26]. The most frequent PRP formulation reported by the different trials based on the Mishra [27] and PAW [28] classifications were the 4B and the P2-Bb, respectively. The included studies were divided into two categories according whether there was an activation method in the PRP previous application or not. A total of nine studies included an activation method [5, 23, 26, 29–34], being the use of calcium chloride ($CaCl_2$) the most frequently reported [5, 30–33]. Five studies did not

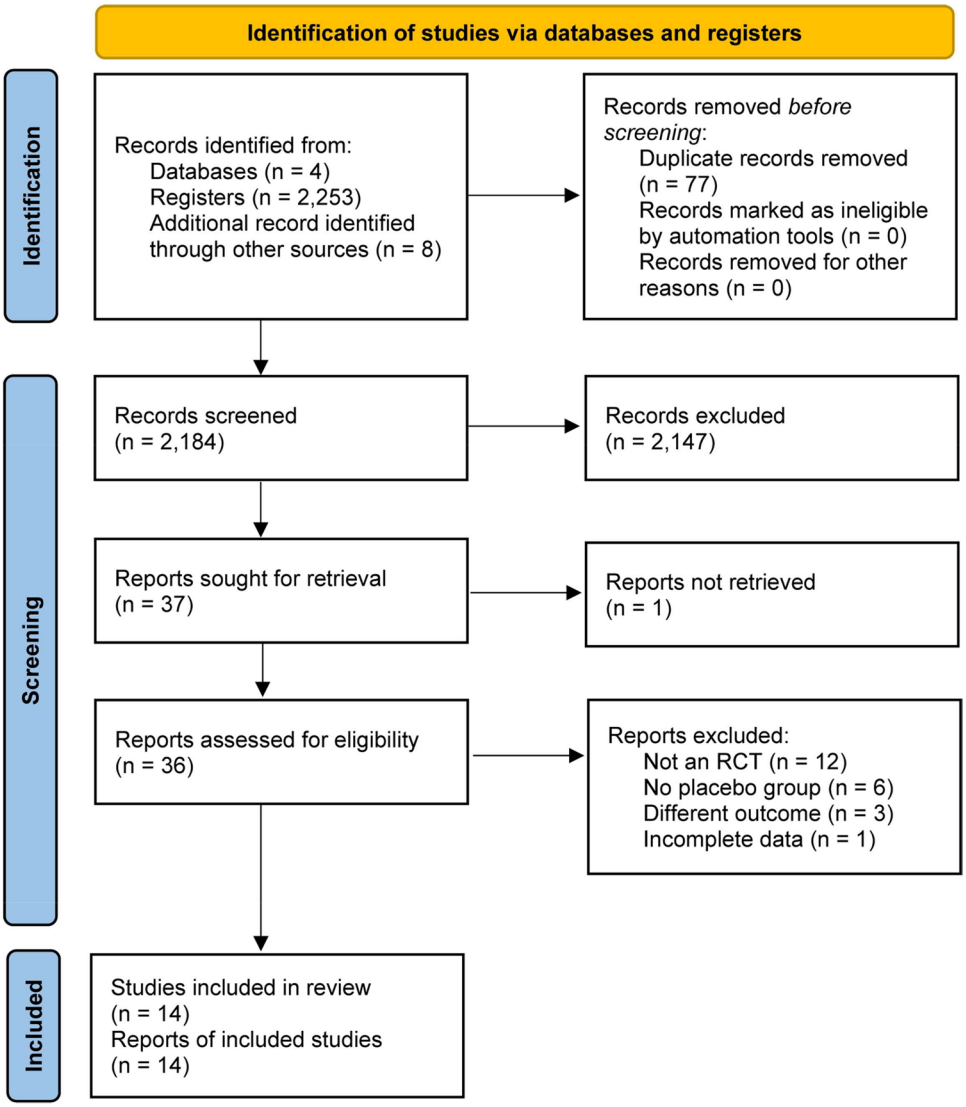

Fig. 1 Flow diagram of the study selection process

reported an activation method for their PRP therapy [24, 25, 35–37]. Detailed information of study characteristics and patients is depicted in Table 1.

Risk of bias assessment

For the randomization process domain, eight studies [26, 29–31, 33–36] were classified as some concerns; the rest of the studies had a low risk of bias. Seven studies were classified as some concerns in the domain related to deviations from intended interventions [26, 30, 31, 33–36] and the rest of the studies has a low risk of bias. All the studies had a low risk of bias regarding the missing outcome data and the measurement of the outcome domains. Eleven studies [5, 23–26, 30–33, 35, 36] had some concerns for the selection of the reported results, while the rest has low risk of bias. Finally, one study was judged to be at an overall low risk of bias [37] and thirteen studies were classified as some concerns [5, 23–26, 29–36]. The complete risk of bias assessment is shown in Fig. 2.

Greater clinical effectiveness of activated PRP formulations

A total of 12 and 13 studies reported pain and functional outcomes, respectively, and 9 of them reported and activation method in the PRP preparation/application. The meta-analysis revealed and overall significant improvement of both pain (SMD, − 0.92 [95% CI − 1.39 to − 0.44]; $I^2 = 92\%$; $p = 0.0001$; Fig. 3) and function (SMD, − 0.78 [95% CI − 1.27 to − 0.29]; $I^2 = 91\%$; $p = 0.002$; Fig. 4), favoring PRP over placebo.

Table 1 Characteristics of the included studies

Author	Country/region	Follow-up	*n*	Intervention arms	Number of injections	OA classification (Kellgren-Lawrence)	Time between injections	Injected volume (mL)	Type of PRP used		Activation method	Age, years	Female, *n* (%)	BMI (kg/m^2)	Pain score at baseline (VAS)	Functional score at baseline
									Mishra†	PAW‡						
Baltzer et al. 2009	Germany	26 weeks	134	ACS	6	2–3	2 per week	2.0	4B	P1-x-Bb	$CrSO_4$-coated glass beads	53.8 (12.2)	65 (48.5)	ND	69.6 (13.1)	5.2 (2.3)†††
			135	HA	3		1 week					57.4 (12.0)	74 (54.8)		68.3 (12.8)	5.2 (2.0)†††
			107	Placebo	3		1 week					60.3 (10.7)	68 (63.6)		66.3 (14.5)	5.2 (2.1)†††
Dório et al. 2021	Brazil	24 weeks	20	PRP	2	2–3	2 weeks	1.4–5.0	3B	P3-Bb	No activation	66.4 (5.6)	19 (95.0)	28.3 (4.1)	6.1 (1.6)	52.9 (15.5)††
			21	Plasma	2							66.1 (7.5)	19 (90.5)	28.0 (3.1)	5.9 (1.4)	46.7 (14.3)††
			21	Placebo	2							62.5 (8.1)	19 (90.5)	27.6 (3.8)	6.6 (1.4)	52.3 (15.9)††
Elik et al. 2020	Turkey	6 months	30	PRP	3	1–3	1 week	4.0	2A	P3-x-Aa	$CaCl_2$	61.3 (7.9)	29 (96.7)	30.4 (4.5)	3.9 (2.1)	56.4 (18.7)††
			27	Placebo	1		NA					60.2 (6.8)	24 (88.9)	30.7 (4.0)	4.9 (1.7)	57.4 (15.1)††
Eroğlu et al. 2017	Turkey	6 months	18	PRP	3	1–3	3 weeks	6.9	2B	P2-x-Aa	$CaCl_2$	64.2 (6.4)	15 (83.3)	29.2*	6.9 (4.2)#	33.3 (13.7)††
			20	Prolotherapy	3							66.0 (5.8)	19 (95.0)	28.7*	7.0 (4.2)#	33.5 (13.7)††
			20	Placebo	3							62.0 (6.5)	19 (95.0)	29.5*	7.1 (4.2)#	32.7 (14.0)††
Ghai et al. 2019	India	6 months	10	PRP	1	1–2	NA	8.0	4B	P2-x-Bb	$CaCl_2$	49.8 (9.4)	15 (75.0)	ND	8.4 (0.9)	37.5 (3.0)††
			10	Placebo	1										7.2 (0.9)	26.7 (2.9)††
Görmeli et al. 2017	Turkey	6 months	39	PRP3	3	1–4	1 week	2.0	4A	P3-x-Bb	$CaCl_2$	53.7 (13.1)	23 (58.9)	28.7 (4.8)	50.6 (4.5)##	40.3 (4.2)§§
			44	PRP1	3							53.8 (13.4)	25 (56.8)	28.4 (4.4)	49.3 (5.5)##	40.9 (5.8)§§
			39	HA	3							53.5 (14.0)	22 (56.4)	29.7 (3.7)	50.8 (4.8)##	40.8 (4.9)§§
			40	Placebo	3							52.8 (12.8)	20 (50.0)	29.5 (3.2)	50.3 (4.9)##	40.6 (4.7)§§
Lewis et al. 2022	Australia	12 months	47	PRP1	3	1–2	3 weeks	4.6–6.0	3A	P2-Bb	No activation	55.1 (12.6)	27 (57.4)	29.3 (6.7)	ND	60.9 (5.9)¶¶
			27	PRP3	3							59.4 (8.9)	18 (66.7)	29.7 (6.1)		54.0 (9.1)¶¶
			28	Placebo	3			5.0				60.1 (9.3)	16 (57.1)	29.9 (5.5)		63.8 (7.7)¶¶
Lin et al. 2019	Taiwan	12 months	31	PRP	1	1–3	NA	2.0	3B	P2-Bb	No activation	61.2 (13.1)	22 (71.0)	24.0 (2.6)	ND	52.8 (18.1)††
			29	HA	1							62.5 (9.9)	19 (65.5)	26.3 (3.0)		52.7 (18.1)††
			27	Placebo	1							62.3 (11.7)	17 (63.0)	25.0 (3.1)		48.6 (16.9)††
Patel et al. 2013	India	6 months	52	PRP1	1	1 – 3§	NA	8.0	4B	P1-x-Bb	$CaCl_2$	53.1 (11.6)	16 (59.3)	26.3 (3.2)	4.5 (0.6)	49.6 (17.8)††
			50	PRP2	2		3 weeks					51.6 (9.2)	20 (80.0)	25.8 (3.3)	4.6 (0.6)	53.2 (16.2)††
			46	Placebo	1		NA					53.7 (8.2)	17 (73.9)	26.2 (2.9)	4.6 (0.6)	45.5 (17.3)††
Smith 2016	USA	12 months	15	ACP	3	1–3	1 week	3.0–8.0	3A	P2-Bb	No activation	ND	ND	ND	10.0 (2.0)#	47.0 (11.9)††
			15	Placebo	3										11.0 (2.0)#	46.0 (12.8)††
Tucker et al. 2021	USA	12 months	11	PRP	1	2–3	NA	5.0	4B	P2-Bb	No activation	57.2 (3.9)¶	3 (66.7)	29.1 (2.1)¶	37.9 (9.2)¶	43.4 (8.4)¶ ‡‡
			6	Placebo	2		10 days					57.5 (1.8)¶	4 (27.3)	30.9 (1.5)¶	48.7 (9.8)¶	48.9 (7.7)¶ ‡‡
Wu et al. 2018	Taiwan	6 months	20	PRP	1	1 – 2§	NA	4.0	2A	P4-x-Aa	Calcium gluconate	63.3 (6.8)	15 (75.0)	24.1 (2.9)	23.1 (1.9)#	89.6 (8.1)††
			20	Placebo	1							63.3 (6.8)	15 (75.0)	24.1 (2.9)	19.1 (1.4)#	72.0 (6.6)††
Yang et al. 2008	Netherlands	12 months	73	ACS	6	1–3	3 weeks	2.0	4B	P1-x-Bb	$CrSO_4$-coated glass beads	54.0 (11.0)	31 (42.5)	27.0 (5.0)	59.9 (20.7)	55.1 (17.8)††
			67	Placebo	6							53.0 (11.0)	30 (44.8)	28.0 (14.0)	62.6 (18.7)	50.8 (15.9)††

Table 1 (continued)

Author	Country/region	Follow-up	*n*	Intervention arms	Number of injections	OA classification (Kellgren-Lawrence)	Time between injections	Injected volume (mL)	Type of PRP used		Activation method	Age, years	Female, *n* (%)	BMI (kg/m^2)	Pain score at baseline (VAS)	Functional score at baseline
									Mishra†	PAW‡						
Yurtbay et al. 2021	Turkey	24 months	62	PRP1	1	1–3	NA	5.0	2A	P4-x-Aa	$CaCl_2$	53.3 (13.0)	21 (33.9)	31.1 (5.5)	7.0 (1.7)	64.5 (1.7)¶¶
			59	Placebo1	1							56.3 (10.5)	11 (18.6)	30.7 (4.5)	7.0 (1.7)	63.3 (16.9)¶¶
			63	PRP3	3		1 month					57.4 (8.8)	9 (14.3)	30.7 (4.6)	7.1 (1.4)	59.9 (17.8)¶¶
			53	Placebo3	3							53.5 (11.3)	18 (34.0)	29.2 (4.8)	6.8 (1.8)	66.4 (14.6)¶¶

Data are represented as mean (standard deviation) unless otherwise indicated

†Mishra classification for PRP (Mishra et al. 2012). Type 1A = increased white blood cells (WBC), no activation, platelet count ≥ 5 times baseline; type 1B = increased WBC, no activation, platelet count < 5 times baseline; type 2A = increased white blood cells (WBC), activated, platelet count ≥ 5 times baseline; type 2B = increased white blood cells (WBC), activated, platelet count < 5 times baseline; type 3A = minimal WBC, no activation, platelet count ≥ 5 times baseline; type 3B = minimal WBC, no activation, platelet count < 5 times baseline; type 4A = minimal WBC, activated, platelet count ≥ 5 times baseline; type 4B = minimal WBC, activated, platelet count < 5 times baseline

‡PAW classification for PRP (Delong et al. 2012). Platelet counts: P1 = less than or equal to baseline levels; P2 = baseline to 750,000; P3 = 750,000–1,250,000; P4 = greater than 1,250,000. Total WBC: A = above baseline, B = below or equal to baseline. Neutrophil count: a = above baseline, b = below baseline. Activation method: X = exogenous

§Ahlback classification for knee osteoarthritis

¶Mean (SEM)

*Mean only

#WOMAC, pain

††WOMAC, total

‡‡WOMAC, function

§§IKDC

¶¶KOOS

##EQ-VAS

†††WOMAC, total (range 0–10)

Abbreviations: *PRP*, platelet-rich plasma; *BMI*, body mass index; *OA*, osteoarthritis; *NA*, not applicable; *ND*, no data; *ACP*, autologous conditioned plasma; *APS*, autologous protein solution; *ACS*, autologous conditioned serum; *VAS*, visual analog scale; *WOMAC*, Western Ontario and McMaster Universities Arthritis Index; *IKDC*, International Knee Documentation Committee; *KOOS*, Knee injury and Osteoarthritis Outcome Score

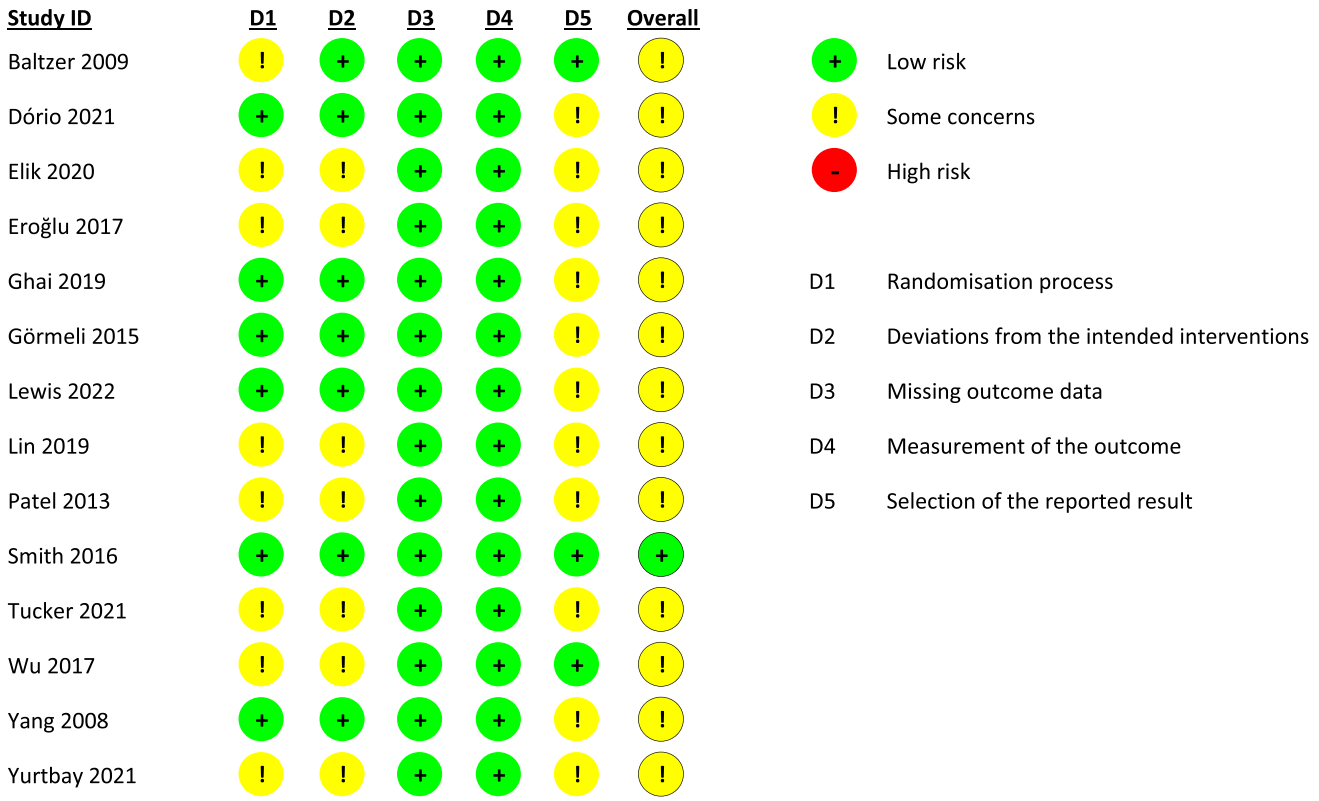

Fig. 2 Risk of bias assessment for the included studies

Subanalysis for pain assessment was performed for studies describing PRP therapy with an activation method (SMD, −1.05 [95% CI −1.58 to −0.52]; I^2 = 93%; p = 0.0001) and with no activation method (SMD, −0.42 [95% CI −1.63 to 0.80]; I^2 = 85%; p = 0.50), showing a significant pain relief for studies with an activation method (Fig. 3). The sensitivity analysis revealed that the effect of PRP was not affected after removing any study (Supplementary Table 1).

Similarly, the subanalysis showed a significant functional improvement in studies using PRP with an activation method (SMD, −1.21 [95% CI −1.75 to −0.67]; I^2 = 91%; p < 0.0001) and no significant functional improvement for those studies including patients treated with a non-activated PRP (SMD, 0.13 [95% CI −0.57 to 0.83]; I^2 = 80%; p = 0.71; Fig. 4). The sensitivity analysis indicated that the effect of PRP was not influenced by a single study (Supplementary Table 2).

Publication bias

Publication bias analysis showed asymmetric funnel plots suggesting evidence for potential bias. The assumed asymmetry was corrected by imputing potentially missing studies using the "trim and fill" method (Fig. 5). However, Egger's regression test suggested the absence of publication bias in the meta-analyses of pain (p = 0.517) and functional scores (p = 0.399). Accordingly, Begg and Mazumdar rank correlation test suggested no publication bias for both pain (p = 0.428) and functional scores (p = 0.537) (Supplementary Table 3).

Discussion

The results of the present meta-analysis confirm that PRP is an effective choice of treatment for knee OA, as indicated by previous systematic reviews and meta-analyses [38–40]. To answer our research question, we compared the clinical effectiveness between PRP protocols that included exogenous platelet activation methods with that from no activation approaches in randomized placebo-controlled trials. For both pain and functional outcomes, the main finding suggests that the PRP with an activation method was more effective than the PRP with no activation method. Notably, in the studies where PRP was not activated, no significant clinical improvement neither in pain nor in functional scores compared to placebo was reported.

Nevertheless, there are still multiple unclear factors that must be addressed to establish a therapeutic scheme and reduce the wide range of existing PRP formulations and heterogeneous results. Within the areas that have been addressed, there is evidence showing that a triple PRP

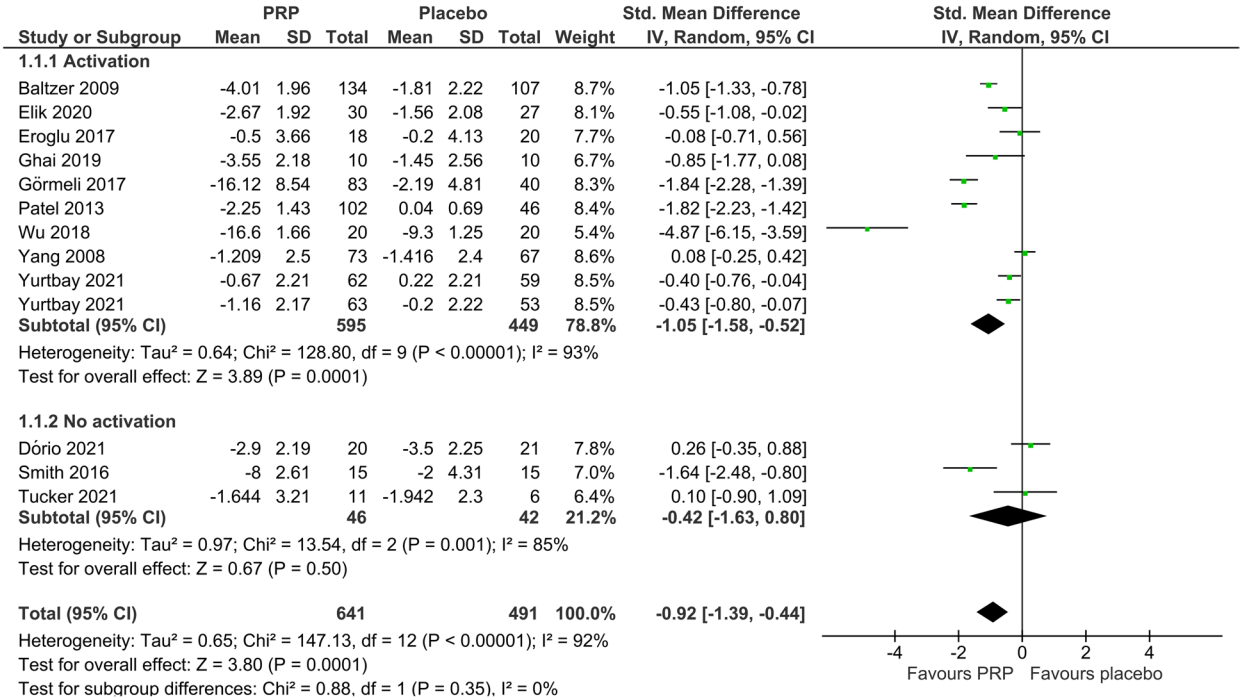

Fig. 3 Forest plot displaying the effect size (SMD) and 95% CI for activated and non-activated PRP in pain improvement

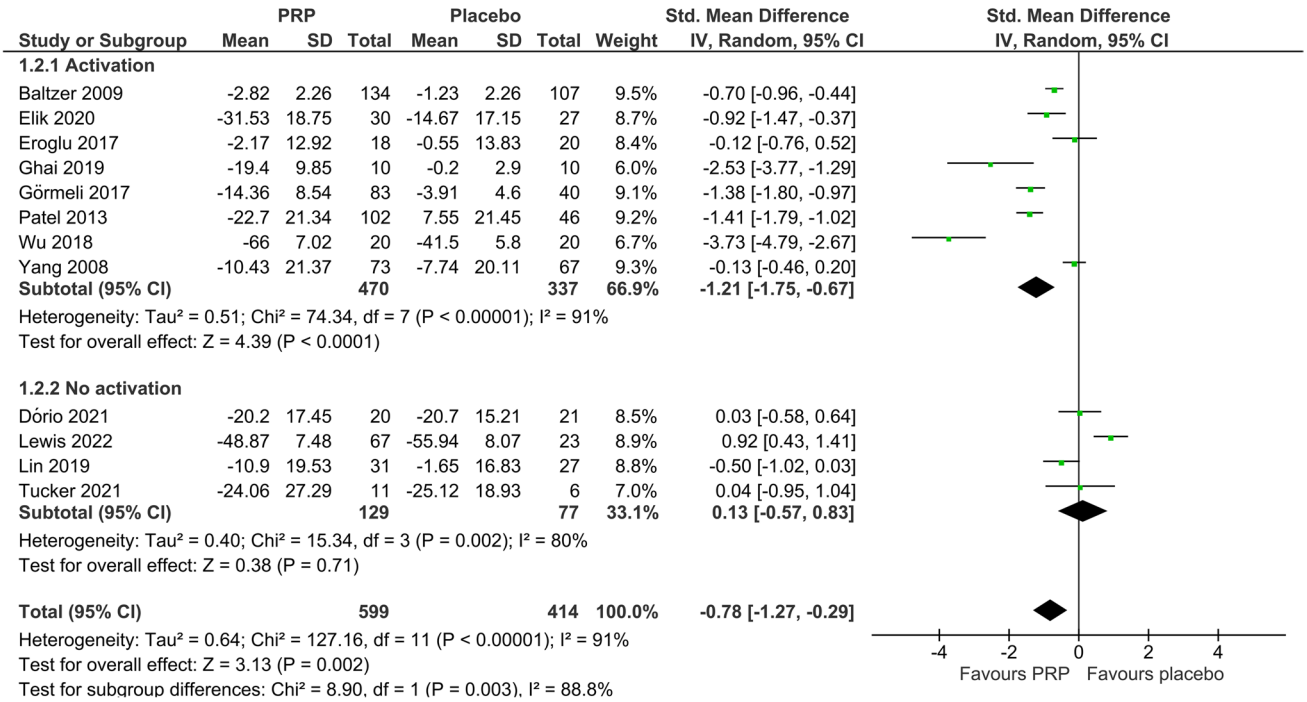

Fig. 4 Forest plot displaying the effect size (SMD) and 95% CI for activated and non-activated PRP in functional improvement

injection approach is more effective than a single injection when evaluating patient reporting outcomes [38]. There has also been suggested that PRP is an effective treatment for any stages of the disease in knee OA [10], although a long lasting therapeutic effect is referred for early stages [41]. While LP-PRP (leukocyte-poor PRP) had been recommended for

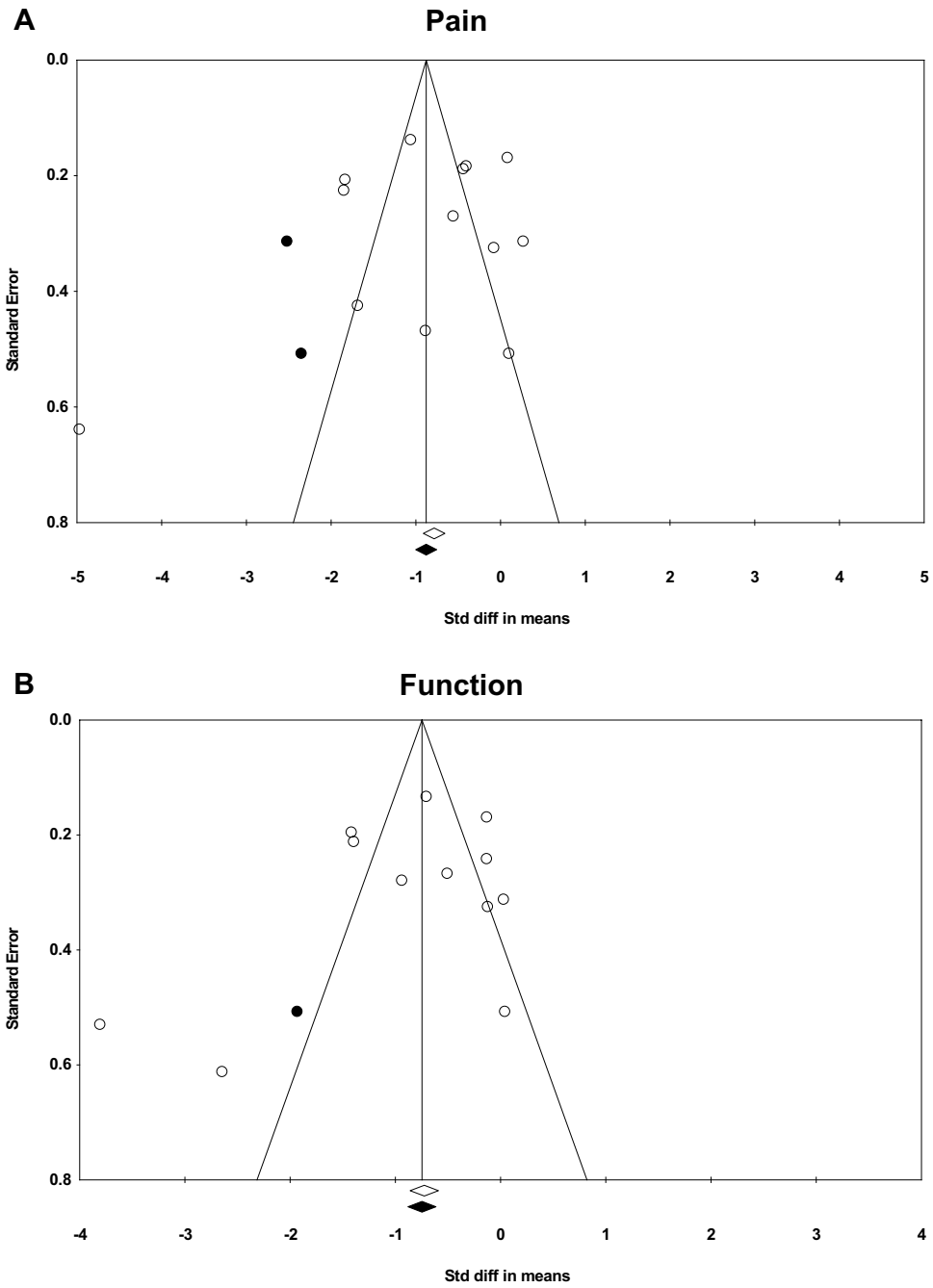

Fig. 5 Funnel plot representing publication bias analysis for **A** pain and **B** function

knee OA over a LR-PRP (leukocyte-rich PRP), recent evidence indicate that both preparations produced similar clinical improvement and complications after 1 year [9, 42]. Yet, the effect or consequences of leukocyte concentration at the level of the joint microenvironment remains to be definitively elucidated.

Platelets can be exogenously activated, resulting in rapid thrombus formation. Growth factors are thought to elute slowly from the clot over several days, which could lead to a sustained release of biologically active molecules. However, the effects of clot in the knee microenvironment are not well documented and clots can rapidly degrade within the joint [28]. Most common exogenous activators are bovine thrombin and calcium chloride. The use of bovine thrombin can cause complications related to the formation of antibodies that can lead to immune-mediated coagulopathy, whereas the use of calcium chloride can avoid this risk by initiating the formation of autologous thrombin from prothrombin [43]. Alternatively, platelets can be activated endogenously through contact with type I collagen receptors providing a slower aggregation of platelets and natural release pattern of growth factors [6, 28].

The thrombin which is now in disuse due to the high risk of generating coagulopathies causes a rapid aggregation of platelets generating a decrease in the total amount of available growth factors over time at the tissue site that may be counterproductive [28, 44]. Alternatively, calcium-based activators cause slow activation and progressive release of platelet content. With this prolonged effect, endogenous thrombin is accumulated gradually which allows a slower release of growth factors over a 7-day period, promoting cell migration and healing [28, 45].

In this systematic review, seven out of the nine studies that included exogenous platelet activation used calcium chloride [5, 26, 30–33] and calcium gluconate [34], the latter being the one with the best results in this meta-analysis. Both Baltzer et al. [29] and Yang et al. [23] used the same PRP activator in their studies ($CrSO_4$-coated glass beads); however, their results differ regarding proving superiority of PRP compared to placebo, since the first study shows an improvement in both pain and function at 2-year follow-up, the second did not met the same objective at 1-year follow-up, resulting in no significant difference for pain or functional outcomes. Meanwhile, Wu et al. [34] was the only study that used calcium gluconate as activator, which had significantly better improvement than placebo in pain, stiffness, and disability in patients. This study highlights the relationship of improved pain to greater leg muscle exercise and better long-term results. However, the sample size was small (40 knees), and the results should be taken with caution.

Among the studies that used $CaCl_2$ to activate PRP, Patel et al. [33] and Ghai et al. [32] suggested that a single dose would be enough for early stage of knee OA; this result was supported by Eroglu et al. [31] that applied three PRP injections within a 3-week interval and failed to show superior improvement over placebo. Nonetheless, another two trials in which a triple-dose scheme with a weekly interval was used indicated a superior clinical efficacy of PRP compared to placebo in early knee OA [5, 30]. It would be worthwhile to investigate in greater depth the ideal timing between injections for greater clinical efficacy considering a specific stage of the disease.

For trials using non-activated PRP, only the study by Smith [37] showed a statistically significant improvement in pain; the rest failed to showed a clear superiority over the placebo group for pain or functional sores. Interestingly, a formulation with a leukocyte concentration below baseline was used in studies reporting no activation of PRP. As already described, the presence of leukocytes might have no influence in the clinical outcome of patients, so the platelet activation may play an important role.

This study has some limitations. There were a limited number of studies included after a systematic review of the available scientific literature. Because of the small number of included studies, the number of studied participants was low (746 participants in the PRP arm). We obtained almost twice as many studies reporting an activation method as those reporting no PRP activation. Notably, most studies (11) reported the restriction of the NSAIDs consumption (during the study period or at least the immediate period after the intervention) and the use of paracetamol as rescue medication. In only one study the consumption of NSAIDs for severe knee OA was allowed [5, 23, 25, 26, 29–33, 35, 36]. However, no information is provided regarding how many patients used such medications or the frequency they were consumed, which prevent a further assessment. Finally, the heterogeneity in patient OA severity, types of PRP, follow-up time, and number of injections used between studies are factors to be considered since they all may have account as potential sources of heterogeneity.

The results of this study suggest that the use of an activation method in the application of PRP is more effective in improving both pain and functionality in patients with knee OA. However, we consider that the body of evidence supporting this assumption is still insufficient, and future research on this specific topic is needed to confirm our results.

Appendix 1. Search strategies used for the identification of potential records of interest in the different databases.

Embase / Ovid MEDLINE(R)

1 exp knee osteoarthritis
2 exp thrombocyte rich plasma
3 ("nonactivated " or "Non-activated" or "no activated" or "non-activated PRP" or "activated-PRP" or "non-active" or "reactivity" or "activ*").mp. [mp = ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, nm, ox, px, rx, an, ui, sy]
4 exp intraarticular drug administration
5 ("intra-articular injection" or "intra articular injections" or "intraarticular Injections" or "intra-articular PRP injection" or "intra-articular platelet-rich plasma injection" or "Intra-articular Autologous Conditioned Plasma Injections" or "intra-articular (IA) injection").mp. [mp = ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, nm, ox, px, rx, an, ui, sy]
6 4 or 5
7 ("Knee Osteoarthritides" or "Osteoarthritis, Knee" or "Knee Osteoarthritis" or "Osteoarthritis of Knee" or "Osteoarthritis of the Knee").mp. [mp = ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, nm, ox, px, rx, an, ui, sy]
8 1 or 7

9 ("platelet-rich plasma" or "Plasma, Platelet-Rich" or "Platelet Rich Plasma" or "PRP" or "platelets").mp. [mp = ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, nm, ox, px, rx, an, ui, sy]
10 2 or 9
11 6 and 8 and 10
12 3 and 11

Scopus

("Knee Osteoarthritides" OR "Osteoarthritis, Knee" OR "Knee Osteoarthritis" OR "Osteoarthritis of Knee" OR "Osteoarthritis of the Knee") AND ("platelet-rich plasma" OR "Plasma, Platelet-Rich" OR "Platelet Rich Plasma" OR "PRP" OR "platelets") AND ("nonactivated " OR "Non-activated" OR "no activated" OR "non-activated PRP" OR "activated-PRP" OR "non-active" OR "reactivity" OR "activ*") AND ("intra-articular injection" OR "intra articular injections" OR "intraarticular Injections" OR "intra-articular PRP injection" OR "intra-articular platelet-rich plasma injection" OR "Intra-articular Autologous Conditioned Plasma Injections" OR "intra-articular (IA) injection").

Web of Science

1"Knee Osteoarthritides" OR "Osteoarthritis, Knee" OR "Knee Osteoarthritis" OR "Osteoarthritis of Knee" OR "Osteoarthritis of the Knee".

2"platelet-rich plasma" OR "Plasma, Platelet-Rich" OR "Platelet Rich Plasma" OR "PRP" OR "platelets".

3"nonactivated " OR "Non-activated" OR "no activated" OR "non-activated PRP" OR "activated-PRP" OR "non-active" OR "reactivity" OR "activ*".

4"intra-articular injection" OR "intra articular injections" OR "intraarticular Injections" OR "intra-articular PRP injection" OR "intra-articular platelet-rich plasma injection" OR "Intra-articular Autologous Conditioned Plasma Injections" OR "intra-articular (IA) injection".

5. (((#1) AND #2) AND #3) AND #4

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Compliance with ethical standards

Disclosures None.

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