What is the clinically significant ideal mesenchymal stromal cell count in the management of osteoarthritis of the knee? — Meta-analysis of randomized controlled trials

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ABSTRACT

Study design: Meta-analysis.

Objectives: We aim to identify the clinically significant ideal Mesenchymal Stem Cell (MSC) count in the management of osteoarthritis of knee from Randomized Controlled Trials (RCTs) available in the literature.

Materials and methods: We conducted independent and duplicate electronic database searches including PubMed, Embase, Web of Science, and Cochrane Library till August 2021 for RCTs conducted in the management of knee osteoarthritis using MSC therapy specifying the quantity of MSCs delivered. We categorized the studies based on the MSC count utilized in them into four groups namely $<1 \times 10^7$ MSCs (Group I), $1-5 \times 10^7$ MSCs (Group II), $5-10 \times 10^7$ MSCs (Group III), and $>10 \times 10^7$ MSCs (Group IV). Visual Analog Score (VAS) for Pain, Western Ontario McMaster Universities Osteoarthritis Index (WOMAC), Lysholm score, Knee Osteoarthritis Outcome Score (KOOS), and adverse events were the outcomes analyzed. Analysis was performed in R-platform using OpenMeta [Analyst] software.

Results: 14 studies involving 564 patients were included for analysis. We noted incremental decrease in the VAS with increasing dosage of MSCs at 12 months [Group I, WMD = 2.641(p = 0.854); Group II, WMD = −4.853(p = 0.379); Group III, WMD = −12.154 (p = 0.316); Group IV, WMD = −15.935(p = 0.116)], and 24 months [Group I, WMD = −6(p = 0.001); Group II, WMD = −15(p = 0.001); Group IV, WMD = −20(p = 0.001)]. We also noted incremental improvement in the WOMAC, KOOS with increasing dosage of MSCs at 12 months [Group I, WMD = 7(p = 0.001); Group II, WMD = 28(p = 0.001); Group IV, WMD = 30(p = 0.001)] and [Group II, WMD = −2.562(p = 0.676); Group III, WMD = 7.670(p = 0.099); Group IV, WMD = 13.475(p = 0.261)] respectively. However, we noted significant reduction in the Lysholm score in Group IV, compared to the others at 12 months (WMD = −12.5, 95%CI[−25.883,0.883]) and 24 months (WMD = −6.6, 95%CI[−23.596,10.396]). We did not find any significant increase in the adverse events with incremental dosage of MSCs in any of the groups compared.

Conclusion: Compared to the four dosage groups of MSCs analyzed, Group III showed consistent significant improvement in pain and functional outcomes analyzed compared to the other groups. Hence, we recommend a cell volume of $5-10 \times 10^7$ cells to be delivered to the target site to obtain superior benefits out of the procedure. However, we urge future trials of sufficient quality to validate our findings to arrive at a consensus on the ideal count of MSCs to be delivered in the cellular therapy for knee osteoarthritis.

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1. Introduction

Due to its versatile nature, global researchers considered the Mesenchymal Stromal Cells (MSCs) as a potential regenerative tool for cartilage repair in osteoarthritis (OA) knee. Lopa et al. stated MSCs as *'medicinal signaling cells'* since they could sense the nature of their biological milieu and secrete appropriate cytokines, pro-angiogenic, anti-apoptotic, and anti-inflammatory molecules to maintain the native homeostasis.[1] Hence, the application of MSCs into the knee joint plays a major role in cartilage regeneration and rejuvenation.

Although bone marrow and adipose tissue were considered to be the common source of these MSCs, the quantity of MSCs present in either bone marrow aspirate concentrate (BMAC) or stromal vascular fraction (SVF) is usually lower when compared to their culture-expanded clinical-grade MSCs or MSCs expanded from any sources.[2,3] However, the lower number of MSCs in the concentrate does not imply an inferior nature of the treatment modalities that employ them. A successful outcome of MSC-based cellular therapy in OA knee relies not only on the quantity but also on the nature of the source, and quality of MSCs.[4] The transplanted MSCs should be of sufficient potential to regenerate the repaired cartilaginous tissue.

Various researchers have used various doses of MSC count (ranged from 5×10^6 to 150×10^6 MSCs) for regenerating the cartilage.[5–7] Vangsness et al. demonstrated that 50×10^6 and 150×10^6 intra-articular allogenic bone marrow MSCs (BM-MSCs) had regenerated the cartilage and meniscus along with a significant pain reduction in the patients with OA knee following partial medial meniscectomy.[7] Koh et al. observed a significant improvement in the functional knee score after 26 months of follow-up with 1.18×10^6 adipose tissue-derived MSCs (AD-MSCs) along with platelet-rich plasma.[8] Pers et al. reported the functional improvement in OA knee with low (2×10^6) dose than medium (10×10^6) and high (50×10^6) doses of autologous AD-MSCs.[9] Jo et al. demonstrated significant functional improvement in OA knee pain after 2 years of follow-up with MSC dose of 100×10^6 cells compared to 10×10^6 cells and 50×10^6 cells.[10] Jo et al. reported that hyaline-like articular regeneration with 100×10^6 AD-MSCs than 10×10^6 and 50×10^6 AD-MSCs.[11]

Due to varying doses of MSCs used by various researchers, it is a need for an hour to perform dose-escalation clinical trials to identify the optimal therapeutic dose for cartilage regeneration. To standardize the treatment of knee osteoarthritis with MSC-based cellular therapy, we aim to identify the clinically significant ideal cell count needed to be injected in the management of osteoarthritis of the knee.

2. Materials & methods

This meta-analysis was conducted following the guidelines of the Back Review Group of Cochrane Collaboration[12] and reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[13]

2.1. Search strategy

Two reviewers performed an independent electronic literature search for studies evaluating the safety and efficacy of stem cell therapy for spinal cord injury. We searched the following databases: PubMed, Embase, Web of Science, and the Cochrane Library up to August 2021. No language or date restrictions were applied. Keywords used for the search were as follows: "Knee Osteoarthritis", "Knee Degeneration", "Stem Cell Therapy" and "Mesenchymal Stem Cells", "Bone marrow", "Adipose". A sample search

strategy used in one of the included databases was presented in Supplementary File 1. The reference list of the selected articles was also searched to identify studies not identified in the primary search. As per the inclusion and exclusion criteria, eligible studies were included for meta-analysis. The discrepancy between the authors was resolved through discussion until a consensus was obtained. A detailed study selection flow diagram is given in Fig. 1.

2.2. Inclusion criteria

Studies were included for quantitative review if they met the following PICOS criteria:

Population: Patients with knee osteoarthritis.
Intervention: MSC-based cellular therapy with clear reporting on the amount of MSCs delivered to the target site.
Comparator: Control.
Outcomes: Visual Analog Score (VAS) for Pain, Western Ontario McMaster Universities Osteoarthritis Index (WOMAC), Lysholm Knee Scale (Lysholm), Knee Osteoarthritis Outcome Score (KOOS), and adverse events.
Study Design: Randomized Controlled Trials.

2.3. Exclusion criteria

Trials were excluded if they had the following characteristics:

1. RCTs without a clear mention about the quantity of the MSCs delivered to the intervention group.
2. RCTs mentioning only the quantity of mononuclear cells without a clear quantification of the MSCs within them.
3. Observational studies and interventional studies without a comparator group.
4. Animal studies involving stem cell therapy for knee osteoarthritis models.
5. Review articles and in-vitro studies involving stem cell therapy.

2.4. Data extraction

Two reviewers retrieved independently relevant data from articles included for analysis. Following data were extracted:

1. *Study characteristics:* year of publication, authors, country, level of evidence, number of patients enrolled.
2. *Baseline characteristics:* mean age, gender proportions, Kellgren Lawrence grade of osteoarthritis, Source of MSC utilized, intervention for both the groups, amount of MSCs delivered, follow-up duration, and assessment parameters utilized.
3. *Efficacy Outcomes:* VAS for pain, Functional outcomes like WOMAC score, Lysholm score, and KOOS.
4. *Safety Outcomes:* Adverse events in the included studies.

We categorized the studies included into four groups based on the quantity of the MSCs utilized in them. We categorized the studies using a low volume of MSCs ($<1 \times 10^7$ cells) as Group I, a moderate volume of MSCs ($1\text{-}5 \times 10^7$ cells) as Group II, high volume of MSCs ($5\text{-}10 \times 10^7$ cells) as Group III, and a very high volume of MSCs ($>10 \times 10^7$ cells) as Group IV for analytical purposes. Any disagreement in data collection was resolved until a consensus was attained by discussion.

2.5. Risk of bias and quality assessment

The methodological quality of the included studies was assessed independently by two reviewers using The Cochrane
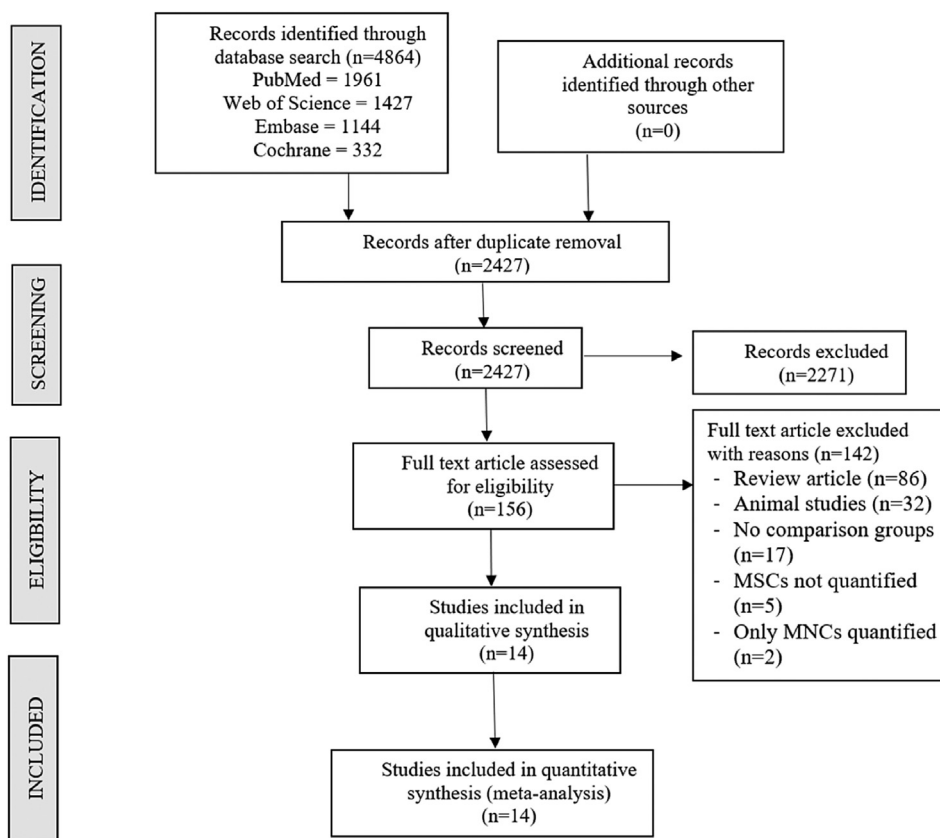
Fig. 1. PRISMA flow diagram of the included studies.

Collaboration's ROB2 tool for randomized studies which has five domains of bias assessment including randomization process, deviation from intended intervention, missing outcome data, measurement of the outcome, and selection of the reported results.[14]

2.6. Statistical analysis

Meta-analysis was conducted in the R platform with OpenMeta [Analyst].[15] For dichotomous variable outcomes, risk ratio (RR) with 95% Confidence Interval (CI) was used and for continuous variable outcomes, weighted mean difference (WMD) with 95% CI was used. Heterogeneity was assessed using the I^2 test.[16] If $I^2 < 50\%$ and $p > 0.1$, we used a fixed-effects model to evaluate, otherwise, a random-effects was used. A p-value <0.05 was considered significant. Sensitivity analyses were performed to explore the source of heterogeneity when it existed. Publication bias was analyzed with a funnel plot and normal quantile plot for the outcomes in the included studies and Egger's regression test.

3. Results

3.1. Search results

Electronic database search resulted in 4864 articles which after initial screening for duplicate removal gave a total of 2427 articles. Title and abstract screening were done in those 2427 articles and 2271 of them were excluded. 156 articles qualified for full-text review of which 142 were excluded. We included 14 studies[5–7,17–27] with 564 patients into the analysis. PRISMA flow diagram of study selection is given in Fig. 1. 6/14 studies[19,20,24–27] utilized MSC from adipose tissue, of which 1 used allogenic source and rest 5

studies utilized autogenous source of AD-MSCs. 8/14 studies[5–7,17,18,21–23] utilized MSC from bone marrow, of which 3 used allogenic sources, and the rest 5 studies utilized autogenous sources of BM-MSCs. There was no uniformity among the included studies for the outcome measures utilized. The general characteristics of the studies included were given in Table 1. The protocol of intervention used in the case and control groups along with the measures of outcome assessment were given in Table 2.

3.2. Quality assessment

The methodological quality of the included studies evaluated as per the RoB2 tool was presented in Fig. 2. None of the included studies had a high risk of bias to be excluded from the analysis.

3.3. Efficacy Outcomes

3.3.1. Visual analog scale for pain

We analyzed 2 studies[19,26] in Group I, 5 studies[5–7,18,22] in Group II, 5 studies[5,6,20,24,25] in Group III, and 3 studies[6,7,20] in Group IV reporting the VAS outcome at 6 months. There was a significant heterogeneity observed between the included studies. ($I^2>80\%$, $p < 0.001$). Hence, the random-effects model was used for analysis across all time points. On analysis, we noted an incremental reduction in the VAS with the increase in the dosage of the MSCs delivered. However, a significant reduction in VAS score was noted compared to their controls at 6 months only in Group III (WMD $= -15.346$, 95% CI [-28.691, -2.000], $p = 0.024$); Fig. 3A.

We analyzed one study[19] in Group I, 4 studies[5–7,17] in Group II, 4 studies[5,6,20,24] in Group III, and 3 studies[6,7,20] in Group IV reporting the VAS outcome at 12 months. There was a significant

Table 1
Characteristics of included studies.

Sl. No	Authors	Year	Country	Nature of Study	Kellgren Lawrence Grade	Sample size	Treatment/ Control	Mean Age (SD)		Male/Female		MSC Type	MSC Source	Follow-up (months)
								Treatment Group	Control Group	Treatment Group	Control Group			
1	A Vega	2014	Spain	RCT	II, III, IV	30	15/15	56.6 ± 9.24	57.3 ± 9.09	06/09	05/10	BM	Allo	12
2	CT Vangness Jr	2014	USA	RCT	NR	55	36/19	44.6 ± 9.82	47.8 ± 8	25/11	13/06	BM	Allo	24
3	D Garay Mendoza	2017	Mexico	RCT	NR	61	30/31	55.57 ± 12.02	59.32 ± 10.85	07/23	09/22	BM	Auto	6
4	D Kuah	2018	Australia	RCT	I, II, III	20	16/4	50.8 ± 7.29	55.0 ± 10.42	11/05	01/03	AD	Allo	12
5	J Freitag	2019	Australia	RCT	II, III	30	20/10	54.6 ± 6.3	51.5 ± 6.1	11/09	01/09	AD	Auto	12
6	JM Lamo-Espinosa	2016	Spain	RCT	II, III, IV	30	20/10	65.9	60.3	12/08	07/03	BM	Auto	12
7	KL Wong	2013	Singapore	RCT	NR	56	28/28	53	49	15/13	14/14	BM	Auto	24
8	M Emadedin	2018	Iran	RCT	II, III, IV	43	19/24	51.7 ± 9.2	54.7 ± 5.3	12/07	15/09	BM	Auto	6
9	PK Gupta	2016	India	RCT	II, III	60	40/20	58.10 ± 8.23	54.90 ± 8.27	12\28	4\16	BM	Allo	12
10	S Wakitani	2002	Japan		I, II	24	12/12	NR	NR	NR	NR	BM	Auto	16
11	TDX Tran	2019	Taiwan	RCT	II, III	33	15/18	58.2 ± 5.70	59.0 ± 6.04	03/12	05/13	AD	Auto	24
12	WS Lee	2019	South Korea	RCT	II, III, IV	24	12/12	62.2 ± 6.5	63.2 ± 4.2	03/09	03/09	AD	Auto	6
13	YG Koh	2012	South Korea	RCT	IV	50	25/25	54.2 ± 9.3	54.4 ± 11.3	08/17	08/17	AD	Auto	16
14	YG Koh	2014	South Korea	RCT	I, II, III	44	23/21	52.3 ± 4.9	54.2 ± 2.9	06/17	05/16	AD	Auto	24

AD — Adipose derived; Allo — Allogenic; Auto — Autologous; BM — Bone Marrow derived; MSC — Mesenchymal Stem Cell; NR — Not Reported; RCT — Randomized Controlled Trial; SD — Standard Deviation; USA — United States of America.

Table 2
Stem cell transplantation protocol of the included studies.

Study	MSC Type	MSC Source	MSC Preparation	MSC count (10^7 cells)	Treatment group Intervention	Control group Intervention	Outcome measures
A Vega	BM	Allo	CE-BMMSC	4	sIA Injection of MSC	sIA Injection of 60 mg HA	VAS, WOMAC
CT Vangness Jr	BM	Allo	CE-BMMSC	5/15	sIA Injection of MSC + 20 mg HA	sIA Injection of 20 mg HA	VAS, Lysholm Score
D Garay Mendoza	BM	Auto	BMC	2	600 μg/day G-CSF for 3 consecutive days before the procedure + sIA Injection of MSC	Oral acetaminophen 500 mg every 8 h for 6 months	VAS, WOMAC
D Kuah	AD	Allo	CE-ADMSC	0.39−0.67	sIA Injection of MSC	Placebo sIA Injection of cell culture media and cryopreservative	VAS, WOMAC, MRI assessment
J Freitag	AD	Auto	CE-ADMSC	10/20	sIA Injection of MSC ± 2nd injection at 6 months	Conservative management	VAS, WOMAC, KOOS, MRI assessment
JM Lamo-Espinosa	BM	Auto	CE-BMMSC	1/10	sIA Injection of MSC + 60 mg HA	sIA Injection of 60 mg HA	VAS, WOMAC, MRI assessment
KL Wong	BM	Auto	CE-BMMSC	1.46	HTO + Microfracture + sIA Injection of MSC + 20 mg HA	HTO + Microfracture + sIA Injection of 20 mg HA	Tegner Score, Lysholm Score
M Emadedin	BM	Auto	CE-BMMSC	4.1	sIA Injection of MSC	Placebo sIA Injection of Normal Saline	VAS, WOMAC
PK Gupta	BM	Allo	CE-BMMSC	2.5/5/7.5/15	sIA Injection of MSC + 20 mg HA	Placebo sIA Injection of 20 mg HA	VAS, WOMAC, MRI assessment
S Wakitani	BM	Auto	CE-BMMSC	1	HTO + Microfracture + sIA Injection of MSC	HTO + Microfracture + Placebo injection	MRI assessment, HSS Knee rating scale
TDX Tran	AD	Auto	SVF	10	Arthroscopic micro fracture + sIA Injection of MSC	Arthroscopic micro fracture	WOMAC, MRI assessment
WS Lee	AD	Auto	CE-ADMSC	10	sIA Injection of MSC	Placebo injection with Normal Saline	WOMAC, MRI assessment
YG Koh	AD	Auto	SVF	0.189	Arthroscopic debridement + sIA Injection of MSC + PRP	Arthroscopic debridement + PRP	VAS, Tegner Score, Lysholm Score
YG Koh	AD	Auto	CE-ADMSC	0.411	HTO + sIA Injection of MSC + PRP	HTO + PRP	VAS, Lysholm Score

AD — Adipose derived; Allo — Allogenic; Auto — Autologous; BM — Bone Marrow derived; BMC — Bone Marrow Concentrate; CE-ADMSC — Culture Expanded Adipose Derived MSC; CE-BMMSC — Culture Expanded Bone Marrow MSC; HA — Hyaluronic Acid;; HSS — Hospital for Special Surgeries; HTO — High Tibial Osteotomy; IA — Intra-articular; IKDC — International Knee Documentation Committee, KOOS — Knee Osteoarthritis Outcome Score; PRP — Platelet Rich Plasma; MRI — Magnetic Resonance Imaging; MSC — Mesenchymal Stem Cells; sIA — Single Intra-articular; SVF — Stromal Vascular Fraction; VAS — Visual Analog Score; WOMAC — Western Ontario Mc-Master Universities Osteoarthritis Index.

heterogeneity observed between the included studies. (I^2>80%, p < 0.001). Hence, the random-effects model was used for analysis across all time points. On analysis, we noted a similar incremental reduction in the VAS with the increase in the dosage of the MSCs delivered as noted at 6 months. However, a significant reduction in VAS score was not noted compared to their controls at 12 months in any of the groups analyzed (Fig. 3B).

We analyzed one study[27] in Group I, one study[7] in Group II, and one study[7] in Group IV reporting the VAS outcome at 24 months. There was a significant heterogeneity observed between the included studies. (I^2>80%, p < 0.001). Hence, the random-effects model was used for analysis across all time points. On analysis, we noted a similar incremental reduction in the VAS with the increase in the dosage of the MSCs delivered as noted at 6, 12 months.
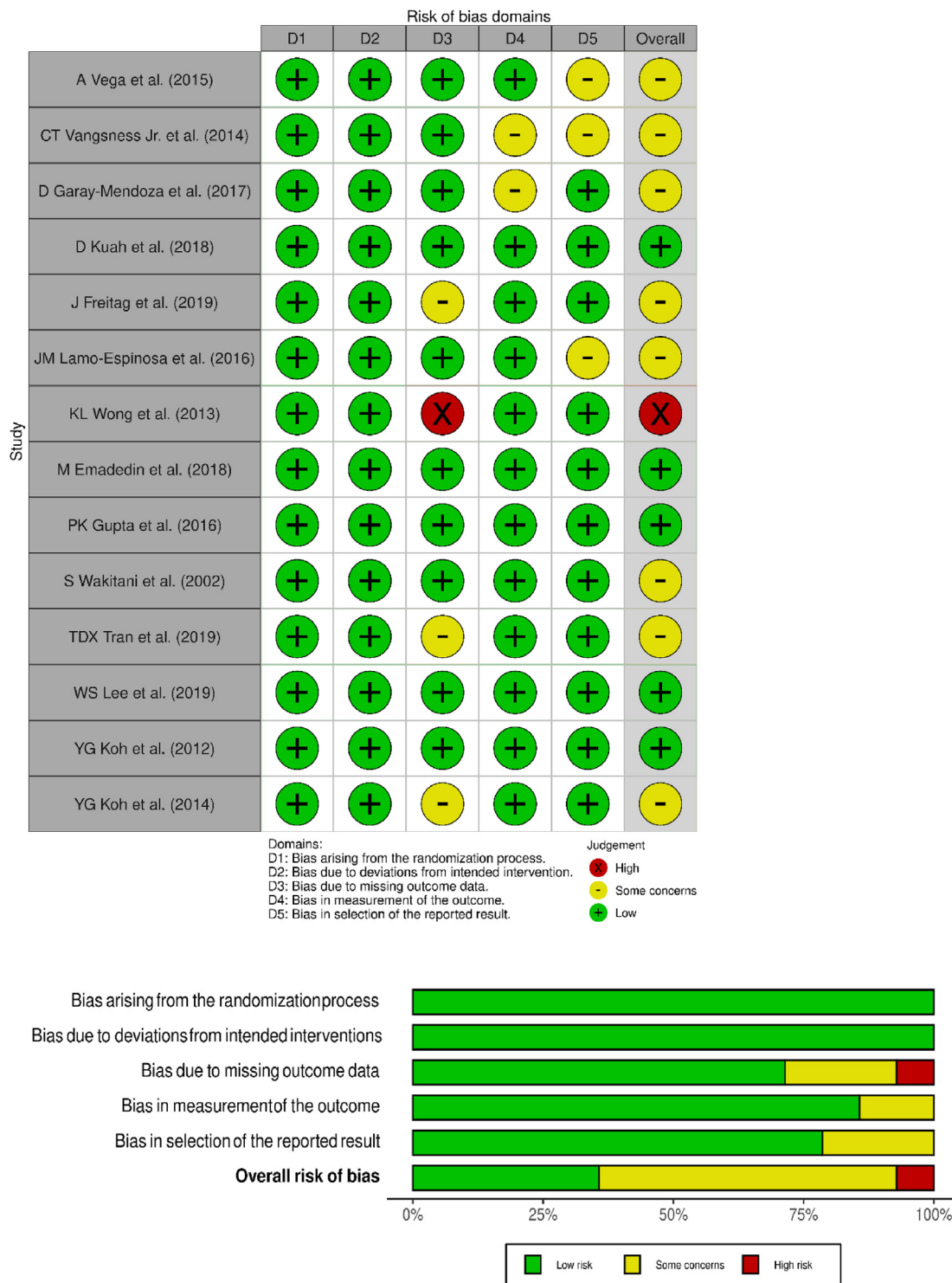
Fig. 2. Methodological quality and risk of bias assessment of all the included studies.

Moreover, a significant reduction in VAS score was noted compared to their controls in Group I (WMD = −6, 95% CI [−9.079, −2.921], p < 0.001), Group II (WMD = −15, 95% CI [−18.223, −11.777], p < 0.001), and Group IV (WMD = −20, 95% CI [−22.641, −17.359], p < 0.001) at 24 months (Fig. 3C) respectively.

On critical analysis of the pain reduction potential of the dosage groups compared, it is noted as shown in Fig. 4 that despite the lack of significance in the pain reduction at 12 months, we noted a dose-response relationship in the VAS outcome compared to the controls. Although all the groups produced significant results around 24 months, consistent results were delivered by Group III (5-10 × 10^7 cells). However, the inconsistencies in the results of the other groups could also be accounted to the heterogeneity in the studies included for analysis.
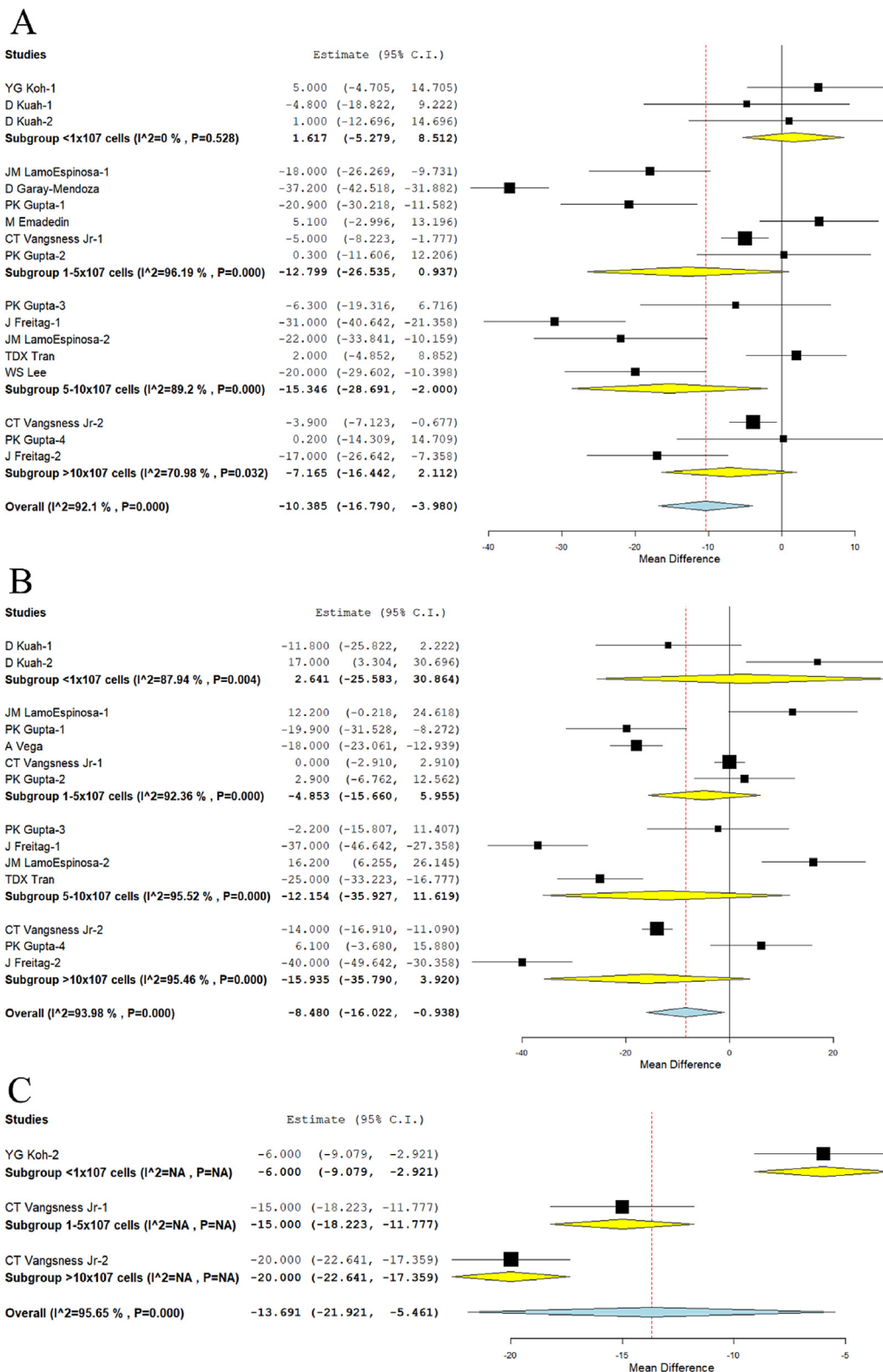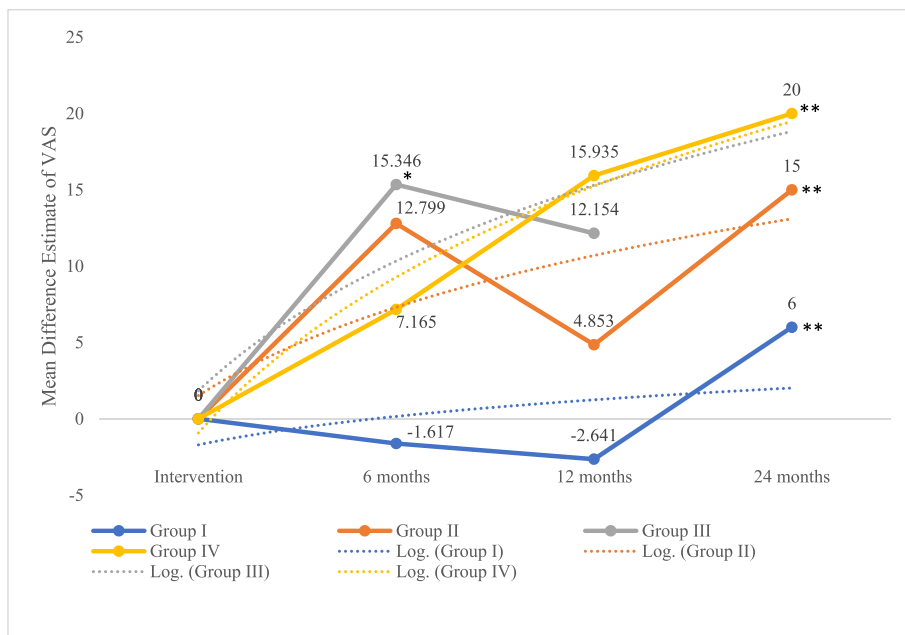
Fig. 3. Forest plot of the included studies comparing various doses of MSCs compared to their controls. A: VAS at 6 months; B: VAS at 12 months; C: VAS at 24 months.

3.3.2. KOOS, WOMAC & lysholm score

We analyzed the quality of life outcomes such as KOOS reported in one study[27] of Group I, one study[20] of Group III, and one study[20] of Group IV. There was a significant heterogeneity observed between the included studies. (I^2 > 80%, p < 0.001). Hence, the random-effects model was used for analysis across all time points. On analysis, we noted significant improvement in the outcomes scores across all the groups (p = 0.020) in an incremental fashion with WMD = 7.2, 95% CI [3.440,10.960] in Group I, WMD = 28, 95% CI [13.544,42.456] in Group III, and WMD = 30, 95% CI

*- significance p<0.05; **- significance p<0.001

Fig. 4. Dose-response relationship in pain reduction across incremental dosage groups of MSCs at various timepoints based on VAS score.*- significance p < 0.05; **- significance p < 0.001.
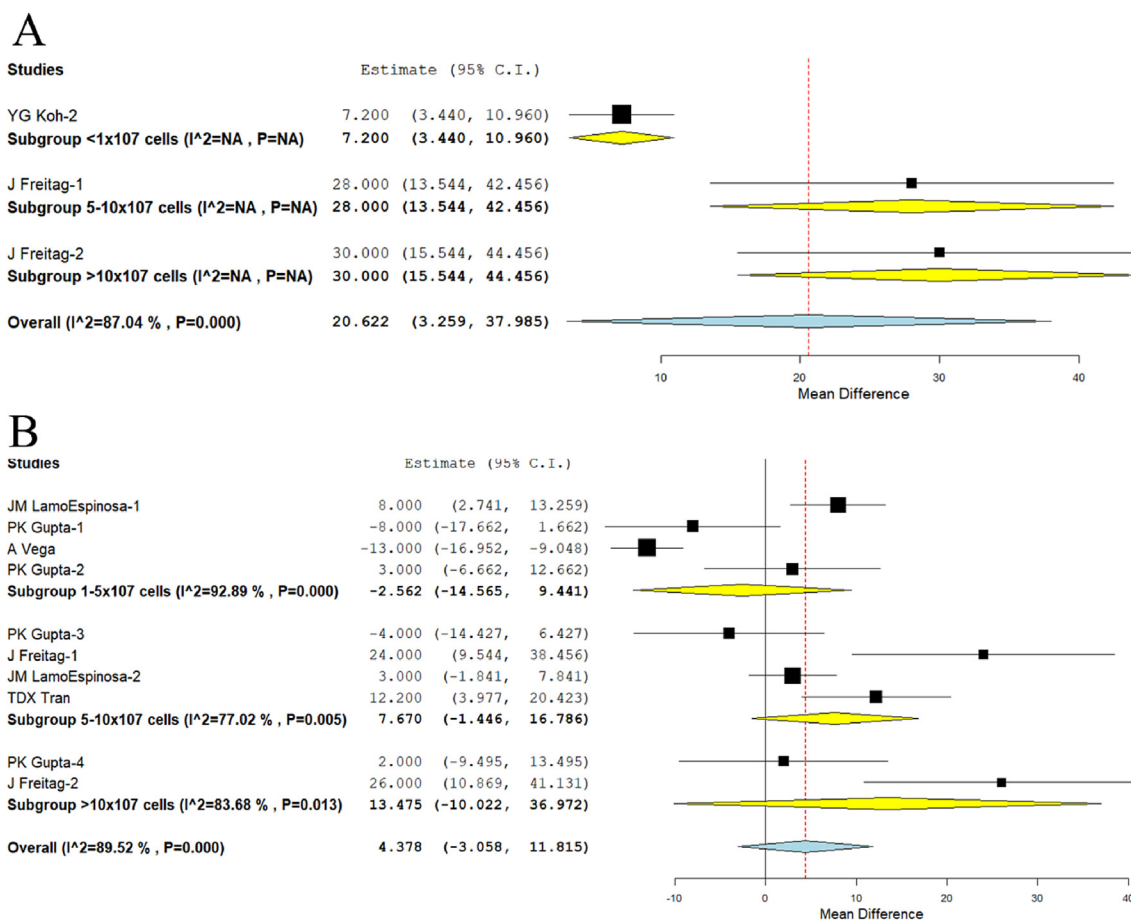

Fig. 5. Forest plot of the included studies comparing various doses of MSCs compared to their controls. A: KOOS at 12 months, B: WOMAC at 12 months.

[15.544,44.456] in Group IV respectively as shown in Fig. 5A.

We analyzed the functional outcome parameter such as WOMAC reported in three studies[5,6,17] of Group II, four studies[5,6,20,24] of Group III, and two studies[6,20] of Group IV. There was a significant heterogeneity observed between the included studies. (I^2>80%, p < 0.001). Hence, the random-effects model was used for analysis across all time points. On analysis, although we did not note a significant improvement in the outcomes scores across the groups analyzed (p = 0.249), we noted an incremental dose-response relationship in the improvement of WOMAC score with the increasing dose of the MSCs delivered, WMD = −2.562, 95% CI [−14.565,9.441] in Group II, WMD = 7.670, 95% CI [−1.446,16.786] in Group III, and WMD = 13.475, 95% CI [−10.022,36.972] in Group IV, as shown in Fig. 5B.

With regards to the lysholm score, we analyzed one study[26] in Group I, two studies[7,21] in Group II, one study[23] in Group III, and one study[7] in Group IV reporting the outcome at 12 months. There was a significant heterogeneity observed between the included studies. (I^2>80%, p < 0.001). Hence, the random-effects model was used for analysis across all time points. Although we did not note significant improvement across any of the groups analyzed, we noted an incremental response in Group I (WMD = −1.300, 95%CI [−12.095, 9.495]), Group II (WMD = 5.008, 95%CI [−0.016, 10.032]), and Group III (WMD = 6.300, 95%CI [−3.673, 16.273]). We noted a significant decrease (p < 0.001) in the outcome of Group IV (WMD = −12.500, 95%CI [−25.883, 0.883]) compared to the other groups analyzed as shown in Fig. 6A. Moreover, on analysis of the lysholm score reported at 24 months, we also noted similar significant deterioration (p < 0.001) in the outcome of Group IV (WMD = −6.600, 95% CI [−23.596, 10.396]) compared to the other

groups analyzed. We also noted significant improvement in the outcome compared to the controls in Group II (WMD = 5.600, 95% CI [0.600, 10.599]; p = 0.028) while data was not available for Group III at the specified time point analyzed for the given outcome score as shown in Fig. 6B.

On overall analysis of the KOOS, WOMAC, and Lysholm score, it is noted that incremental dose-response relationship in the outcome score was noted from Group I-III while Group IV demonstrated an inverse relationship compared to the other groups analyzed.

3.3.3. Safety

Ten studies[5−7,17,19−22,25,26] involving 400 patients reported adverse effects with low heterogeneity among the included studies across all the doses utilized. (I^2 = 0.0%, p = 0.992). Hence, a fixed-effects model was used for analysis. Across all the groups analyzed, we did not note any significant increase in the adverse events compared to the controls (RR = 2.117, 95% CI [0.837, 5.356], p = 0.113; Fig. 6). No major serious adverse events with permanent effects such as death, tumor, or immune reaction to the intervention were noted during follow-up in either of the doses of MSCs utilized. We also noted that the least estimate of the risk of adverse events in Group III (RR = 1.194, 95% CI [0.177, 8.037], p = 0.856; Fig. 7).

3.3.4. Sensitivity analysis

A sensitivity analysis was performed in each analysis. All the results (VAS for Pain, KOOS, Lysholm, and adverse events) were not significantly altered by sequentially omitting each study in the meta-analysis. On the other hand, the consistency of the results was
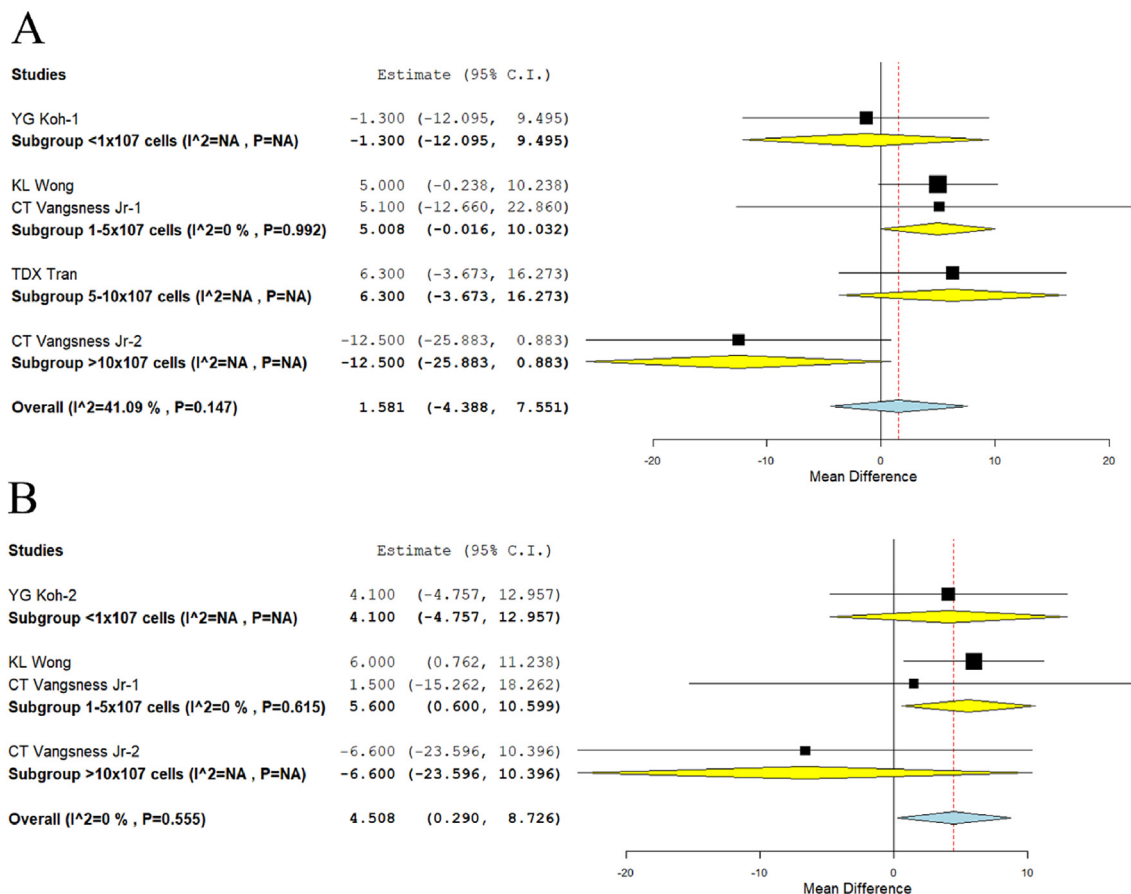

Fig. 6. Forest plot of the included studies comparing various doses of MSCs compared to their controls. A: Lysholm score at 12 months, B: Lysholm score at 24 months.
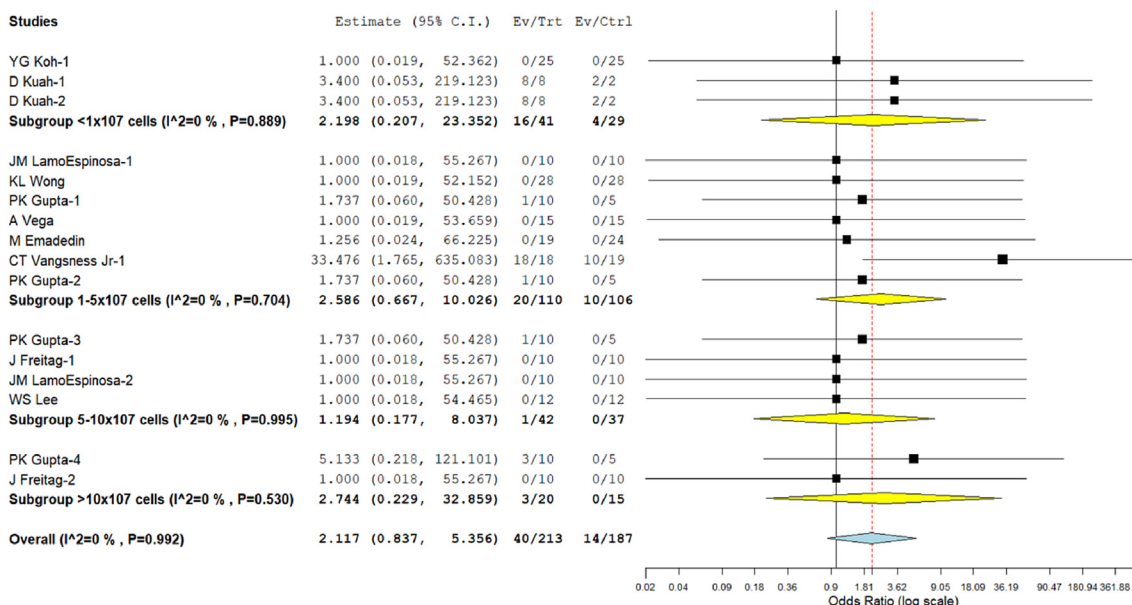
Fig. 7. Forest plot of the included studies comparing adverse events across incremental dosage groups of MSCs compared to their controls.

maintained after reanalysis by changing to the random-effects model.

3.3.5. Publications bias

Publication bias was analyzed utilizing the funnel plot, normal quantile plot, and Egger's regression test for the meta-analysis performed. There was no evidence of publication bias by funnel plot and normal quantile plot as shown in Fig. 8 or by Egger's regression test (p = 0.564). All the studies lied close to the 95% CI and no significant heterogeneity was noted in the distribution of the studies about the axes, implying minimal publication bias.

4. Discussion

When MSCs are injected locally, they modulate the joint environment and halt the progression of the disease process in OA knee. With a short in-vivo half-life, MSCs release the biomicromolecules inside the joint and alters the disease environment effectively.[28] The quantification of the survival time of MSCs inside the joint which is hypoxic, with low pH, and rich inflammatory mediators, to produce a therapeutic effect depends on the number of MSCs delivered into them. The dose-response relationship in MSC therapy depends not only on the cellular count but also on a vast grade of complex factors which makes it difficult for global researchers to standardize the therapy for optimal functional outcome. However, the clarity in the dose of MSCs was poorly understood as it represents the total number of MSCs for each shot of injection or the number of injected MSCs per kg body weight. To add to the ambiguity, some studies estimate the entire mononuclear cell count in dosage calculation which includes a heterogeneous population of cells apart from MSCs. Variability is also noted in the dosage frequency utilized for cartilage regeneration irrespective of the source of MSCs, cultured or uncultured, and autologous or allogeneic in origin. Upon utilization of the culture-expanded cells, there is no consensus in the available literature regarding the ideal and effective number of passages needed to obtain the optimal cell population with the required characteristics.[29] Identification of the minimum effective count of MSCs required for the healing cascade and definitive outcomes in terms of regeneration was suggested

since the larger the doses of MSCs transplanted into the joint, the greater the competition for nutrients for their survival within the grafted area occurs.[30]

MSCs act by direct cell-to-cell contact (membrane-bounded proteins and receptors) and by paracrine signaling (cytokines, chemokines, and trophic factors) in the transplanted environment.[31] Under physiological conditions, MSCs reside in the perivascular niche whereas they get activated upon signals from injured cartilage to re-establish the joint homeostasis. As the result, MSCs limit inflammation and apoptosis thereby increasing the reparative capacity of the recipient site.1 The priming of MSCs in OA synovial fluid which contains INF-γ and TNF-α increases the concentration of indoleamine 2,3-dioxygenase and interleukin-6 (IL-6) expression. These MSCs derived mediators inhibit the proliferation of T cells, downregulate IL-1β and ADAMTS5, and upregulate interleukin 1 receptor antagonist (IL-1Ra) and suppressor of cytokine signaling (SOCS-1).1.

Allogenic products namely CARTISTEM (allogenic cord blood-derived MSCs $-$ 2.5 $\times$ 10^6 cells/500 μL/cm^2 area of knee cartilage),[32] Stempeucell (allogenic ex-vivo cultured pooled human BM-MSCs $-$ 2 $\times$ 10^8 cells cryopreserved and stored in 15 mL cryobags),[6] and JointStem (autologous AD-MSCs $-$ 10 $\times$ 10^7 cells)[33] have launched the MSC derived products with a definite dosage for cartilage injuries.

Similar to the work of Herningou et al.[34] on the estimation of the native load of MSCs in the femoral head which would be necessary to restore in cellular therapy for its osteonecrosis, no such study is available to date on the OA knee. Hence estimation of the ideal cell count by stratification of the studies based on the cell counts utilized and reported outcomes became the necessary analysis.

4.1. Main finding

We made a stratified analysis of all the available RCTs using MSC therapy based on the MSC count delivered at the target site for knee osteoarthritis and found that.

1. An incremental decrease in the VAS with increasing dosage of MSCs at 12 months [Group I, WMD = 2.641 (p = 0.854); Group II,
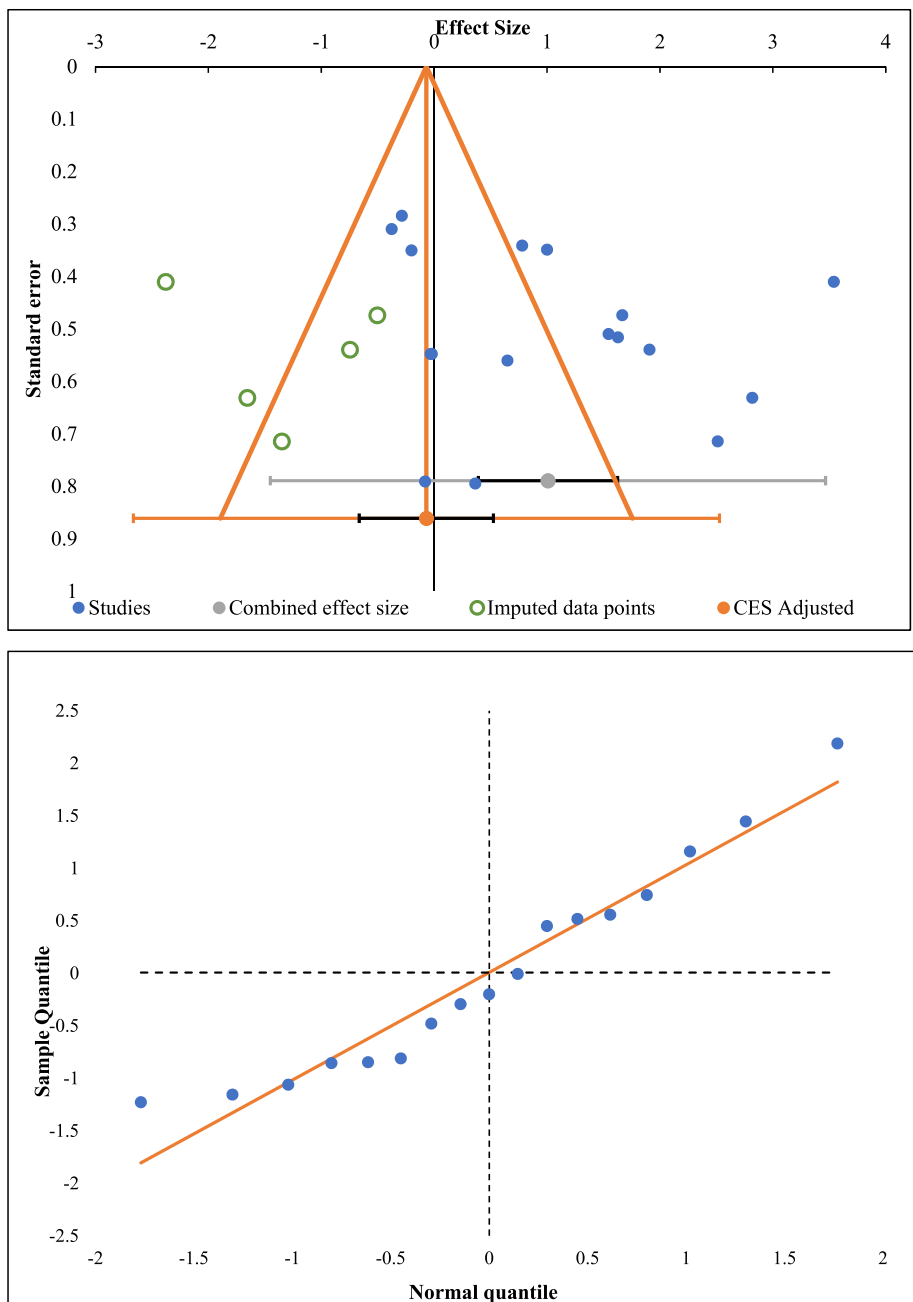
Fig. 8. Publication bias assessment with funnel plot and quantile plot for Visual Analog Score at 6 months in the included studies.

WMD = −4.853 (p = 0.379); Group III, WMD = −12.154 (p = 0.316); Group IV, WMD = −15.935 (p = 0.116)], and 24 months [Group I, WMD = −6 (p = 0.001); Group II, WMD = −15 (p = 0.001); Group IV, WMD = −20 (p = 0.001)]. We also noted significant incremental increase in the KOOS with increasing dosage of MSCs at 12 months [Group I, WMD = 7 (p = 0.001); Group II, WMD = 28 (p = 0.001); Group IV, WMD = 30 (p = 0.001)]. However, we noted significant reduction in the lysholm score in Group IV, compared to the others at 12 months (WMD = −12.5, 95%CI[−25.883,0.883]) and 24 months (WMD = −6.6, 95%CI[−23.596,10.396]).

2. On comparing the relative improvement between the groups with various parameters such as VAS, KOOS, and Lysholm score, Group III (5-10 × 10^7 cells) gave a consistent improvement in the

outcome parameters analyzed without significant deterioration compared to the other groups at various time points analyzed.

3. We did not find any significant increase in the adverse events with an incremental dosage of MSCs in any of the groups compared.

MSCs exhibit a varied spectrum of immunoregulating capabilities on both innate and adaptive immune systems. Due to the prevailing inflammatory environment, specific toll-like receptor (TLR) ligands affect the MSC-based cellular therapy in the management of OA knee.[35–37] In the early stages of OA knee, TLR2/4 on MSC surface may result in polarization into MSC1, which promotes further immunogenic response to protect joint tissues in OA pathogenesis.[36] In later stages of OA knee, TLR3 accumulation leads

to polarization into MSC2 which further prevents articular surface damage in the OA knee.[36] Failure of inflammatory attenuation leads to the continuation of the vicious cycle in OA pathogenesis.

In such a pro-inflammatory milieu, the quiescent resident MSCs are attenuated and may not be induced in the lines of regeneration of the articular cartilage and hence infusion of MSCs into the knee joint may not only repair and regenerate the joint cartilage but also helps in stimulating the resident MSCs to attain joint homeostasis in favor of regeneration. MSCs suppress host immune responses by inhibiting immune cells proliferation and maturation in a non-MHC restricted manner.[38] MSCs are considered to be hypoimmunogenic, which express low levels of HLA 1 and are deprived of HLA class II and co-stimulatory molecule expression.[39,40] Numerous studies have also demonstrated MSCs to suppress the activity of a broad range of immune cells, including T cells, natural killer cells, dendritic cells, B cells, neutrophils, monocytes, macrophages.[41]

There is no consensus on the viability of MSCs to exert immunomodulatory effects in the local or systemic environment. Once administered, MSCs undergo radical biological and morphological changes and disappear from the site of injection within few days of intra-articular injection.[11] Despite the disappearance of administered MSCs from the site, they elaborate the cytokines and chemokines which work with paracrine signaling and induces the quiescent and resident MSCs to enhance the significant therapeutic effects.[42] Hence the dead MSCs create an immunosuppressive or immune-privileged environment by secreting IL-10 and TGF-β and inhibition of lipopolysaccharide (LPS) stimulated macrophages by the secretion of IL-1β and TNF-α.[43] Indirectly, phagocytes eliminate MSCs and result in hyporesponsiveness to LPS and switching off the immunological cellular environment to an anti-inflammatory environment.[44] Upon cell-to-cell contact, T cells release perforin and granzyme B and induce apoptosis of infused MSCs.[45] IDO, a soluble factor, secreted by phagocytes is responsible for immunomodulation.[42] Mancuso et al. emphasized that apoptotic MSCs retrieved from OA knee exhibited higher immunosuppressive effects than viable MSCs.[46]

Complement cascade induces phagocytosis and recruit progenitor cells to the site of injury. Complement systems exhibit an interplay between MSCs to influence the biological features of engrafted cells. When a complement molecule binds to MSC, it induces phagocytosis by macrophages and in turn, M2 polarization occurs to establish a tolerogenic environment.[47] With the rapid clearance of MSCs from the systemic circulation, a therapeutic response being observed was due to the interplay between complement and monocyte-phagocyte system.[48] MSCs attenuates autophagy and promote cellular survival, proliferation, differentiation, and restore the functional tissues in the degenerative environment.[49] Senescent MSCs exhibit minimal proliferation, differentiation, immunoregulatory, and secretory ability in OA knee.[50]

4.2. Future directives

Our study gives a baseline range of MSCs ($5\text{-}10 \times 10^7$ cells) needed to demonstrate an effective and favorable outcome from MSC-based therapy for OA knee. However, appropriate dose-escalation studies of sufficient power and duration must be carried out to validate the findings of our study and arrive at a definitive consensus on the prevailing ambiguity in the volume and count of MSCs needed in the management of knee osteoarthritis. Although commercial vendors have their proprietary methods of dosage of MSCs, an objective and conventional method of estimation of the appropriate number of MSCs needed in the management of the disease needs to be estimated. Despite identifying the appropriate number of MSCs needed, objective measures to identify their regenerative potential with colony-forming unit (CFU) assays are also needed which is not provided in any of the included studies. Hence, we recommend reporting future trials of MSCs in OA knee to report not only the outcome of the MSCs used but also the efficacy of the source utilized based on the measures such as CFU assays.

4.3. Limitations

Our analysis has some limitations. Blinding was not established in most of the studies which might invite room for treatment bias from patients or observers. Heterogeneity was observed in most of the outcomes reported across the studies which might be due to the variability in the treatment protocols followed in the individual studies as shown in Table 2. Moreover, patients in various stages of the disease process were included in the studies which might also contribute to the heterogeneity of their results. Hence a large multicentric trial with standardized dosage and frequency protocol with established outcome assessment measures, without any adjuvant procedures is needed to further confirm the results of our analysis.

5. Conclusion

Compared to the four dosage groups of MSCs analyzed, Group III showed consistent significant improvement in pain and functional outcomes analyzed compared to the other groups. Hence, we recommend a cell volume of $5\text{-}10 \times 10^7$ cells to be delivered to the target site to obtain superior benefits out of the procedure. However, we urge future trials of sufficient quality to validate our findings to arrive at a consensus on the ideal count of MSCs to be delivered in the cellular therapy for knee osteoarthritis.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcot.2021.101744.

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