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REVIEW

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Cell therapy as a treatment of secondary lymphedema: a systematic review and meta-analysis

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Abstract

Background: Lymphedema, the accumulation of interstitial fluid caused by poor lymphatic drainage, is a progressive and permanent disease with no curative treatment. Several studies have evaluated cell-based therapies in secondary lymphedema, but no meta-analysis has been performed to assess their efficacy.

Methods: We conducted a systematic review and meta-analysis of all available preclinical and clinical studies, with assessment of their quality and risk of bias.

Results: A total of 20 articles using diverse cell types were selected for analysis, including six clinical trials and 14 preclinical studies in three species. The meta-analysis showed a positive effect of cell-based therapies on relevant disease outcomes (quantification of edema, density of lymphatic capillaries, evaluation of the lymphatic flow, and tissue fibrosis). No significant publication bias was observed.

Conclusion: Cell-based therapies have the potential to improve secondary lymphedema. The underlying mechanisms remain unclear. Due to relevant heterogeneity between studies, further randomized controlled and blinded studies are required to substantiate the use of these novel therapies in clinical practice.

Keywords: Stem cells, Lymphatic vasculature, Lymphedema, Regeneration, Regenerative medicine, Systematic review, Meta-analysis

Background

As knowledge on the diverse lymphatic vasculature roles in health and disease progresses, it increases the lymphatic vessel relevance in understanding the physiopathology of a number of diseases [1]. Lymphedema is a chronic edema, lasting more than three months, due to the accumulation of interstitial fluid caused by poor lymphatic drainage [2]. Secondary lymphedema is due to obstruction or infiltration of the lymphatic vessels by tumors, infections (recurrent lymphangitis), obesity, surgery or overload and saturation of the lower limb venous system [3]. The most frequent cause in undeveloped countries is filariasis, while in developed countries, it is iatrogenic due to radiotherapy or surgery related to the management of malignant neoplasms (breast cancer, malignant melanoma, gyneco-urological cancer) [4]. Approximately, 30% of women with breast cancer and 20% of melanoma patients who have axillary and inguinal lymph nodes removed, develop lymphedema [5, 6].

The accumulation of lymph in the interstitial tissue leads to remodeling of the skin and subcutaneous tissue and the accumulation of fibroadipose tissue [7]. The chronic form of lymphedema is characterized by swelling, fibrosis, accumulation of adipose tissue and infiltration of immune cells. Clinically, it can be classified into

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four stages: in stage 0, the condition is considered subclinical; swelling is not present. In stage I, edema is mild; fluid accumulates throughout the day but resolves overnight. In stage II, lymphedema is always present, but varies in severity. Stage III disease is characterized by persistent moderate-to-severe edema in the affected limb [8].

Lymphedema is a progressive and permanent disease for which there is no curative treatment. The standard treatment is physiotherapy (lymphatic drainage and compression bandaging) [9], although other treatments used include pharmacotherapy and surgery. More recently, reconstructive microsurgery (lympho-venous anastomosis, lymphatic vessel transplantation and autologous lymph node transplantation) has been proposed as an alternative [10–12].

Other potential therapies are still in development, e.g., the therapeutic potential of different growth factors, which would facilitate the regrowth of damaged, dysfunctional or obliterated lymphatics, has been investigated [13, 14]. Among them, the role of vascular endothelial growth factor VEGF-C as a stimulant of lymphangiogenesis and mediator of lymphatic endothelial cell growth and viability has been studied [15], as well as fibroblast growth factor-2 and hepatocyte growth factor [16]. Also, the use of gene therapy via adenovirus, plasmids or even direct application of recombinant VEGF-C has been described to reduce edema in different preclinical models [17–19]. However, there are currently many unresolved questions, such as the lifespan of recombinant proteins, the time-limited action of gene therapy, as well as the side effects of growth factors on the blood vasculature and on the development of new tumors [18, 20].

In the last decade, cell therapy with differentiated or progenitor cells has emerged as a new research target in the therapy of secondary lymphedema [21, 22]. Although the cellular pathways through which stem cell therapy could help lymphedema patients are unclear, in vitro studies indicate that stem cells may differentiate into lymphatic endothelial-like cells under in vitro culture conditions and can improve interstitial fluid drainage when injected in vivo [13]. Stem cells have a wide range of therapeutic effects in terms of anti-inflammation, antifibrosis, anti-oxidative stress, as well as promoting the regeneration of different tissues. These properties could promote the regeneration of lymphatic vessels, rebuild lymphatic circulation and successfully treat lymphedema. Currently, several clinical and preclinical studies have evaluated the therapeutic potential of using lymphatic endothelial progenitor cells (LEPCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) or mesenchymal stromal cells (MSCs) in the regeneration of lymphatic vessels. These results suggest that stem cell therapy is feasible and may promote recovery in patients with secondary lymphedema. However, stem cell transplantation has not been fully evaluated for the treatment of secondary lymphedema in clinical settings. In the present study, a meta-analysis of the available data was performed to evaluate the safety and efficacy of stem cell therapy for the treatment of secondary lymphedema.

Methods

We conducted a systematic review according to the Cochrane method [23] and SYRCLE guideline [24], and the results are reported in accordance with PRISMA guidelines [25]. The protocol for this review was registered on the International prospective register of systematic reviews website (https://www.crd.york.ac.uk/prospero/) with two separate IDs (CRD42020180348 for preclinical studies and CRD42019130951 for clinical studies).

Search strategy and literature selection

Studies of cell therapy as a treatment of secondary lymphedema were identified from Medline, Web of Science, EMBASE, and The Cochrane library with no language or time restrictions using these search terms: lymphedema, lymphoedema, lymphangiogenesis, lymphatic diseases, lymphatic vessels, lymph nodes, stem cells, stromal cells, mesenchymal stem cells, cell- and tissue-based therapy, cell transplantation, and regenerative medicine. We identified all relevant studies or trials regardless of language or publication status (published, unpublished, in press, and ongoing). Two independent searches were conducted on January 2021, one with the inclusion criteria: pre-clinical studies and all animal models, and the other one with the inclusion criteria: clinical trials and prospective controlled studies in human.

After developing a search strategy for each database and collecting the citations, the search results were combined. The first selection was made using only the title and abstract of the studies. To avoid biases in the selection process, two observers independently screened articles for relevance. The criteria used for the first screening were based on the search components: (SC1) intervention (only cell therapies were included); (SC2) disease of interest (secondary lymphedema); (SC3) type of study (only pre-clinical studies, randomized controlled clinical trials and prospective controlled studies were included. The ex vivo studies, in vitro studies, or in silico studies were not included. Non-intervention studies, no control group, co-intervention studies and studies with other outcomes were not included); and (SC4) publication types (reviews and conference abstracts were not included). Only clearly irrelevant citations were removed. Citations resulting from the first screening underwent a

second screening based on the predefined inclusion and exclusion criteria. Throughout the potentially relevant article selection process, the reasons for the removal of citations were documented and reported to facilitate transparency and to independently examine the accuracy of the study removal. Two independent reviewers performed all stages of the review process. Discrepancies were resolved by consensus. The flow diagram of search strategy and literature selection is shown in Fig. 1 for preclinical studies and in Fig. 2 for clinical studies.

Assessment of study quality and risk of bias

Quality and risk of bias was assessed for clinical trials by use of Cochrane's risk of bias tool [26], and for non-randomized studies by use of NewcastleOttawa's risk of bias tool [27]. For preclinical studies, we used SYRCLE Risk of Bias tool [28]. Two authors independently assessed the risk of bias of the included studies. A third author was consulted to resolve discrepancies related to risk of bias.

Besides, to overcome the fact that there were too many items classified as "unclear" because of the poor description of details on experimental design and methods, we included three items as other bias: (a) inappropriate influence of funders, (b) mention of randomization at any level, and (c) mention of blinding at any level. For inappropriate influence of funders, "Yes" indicated non-industry source of funding, no funding,

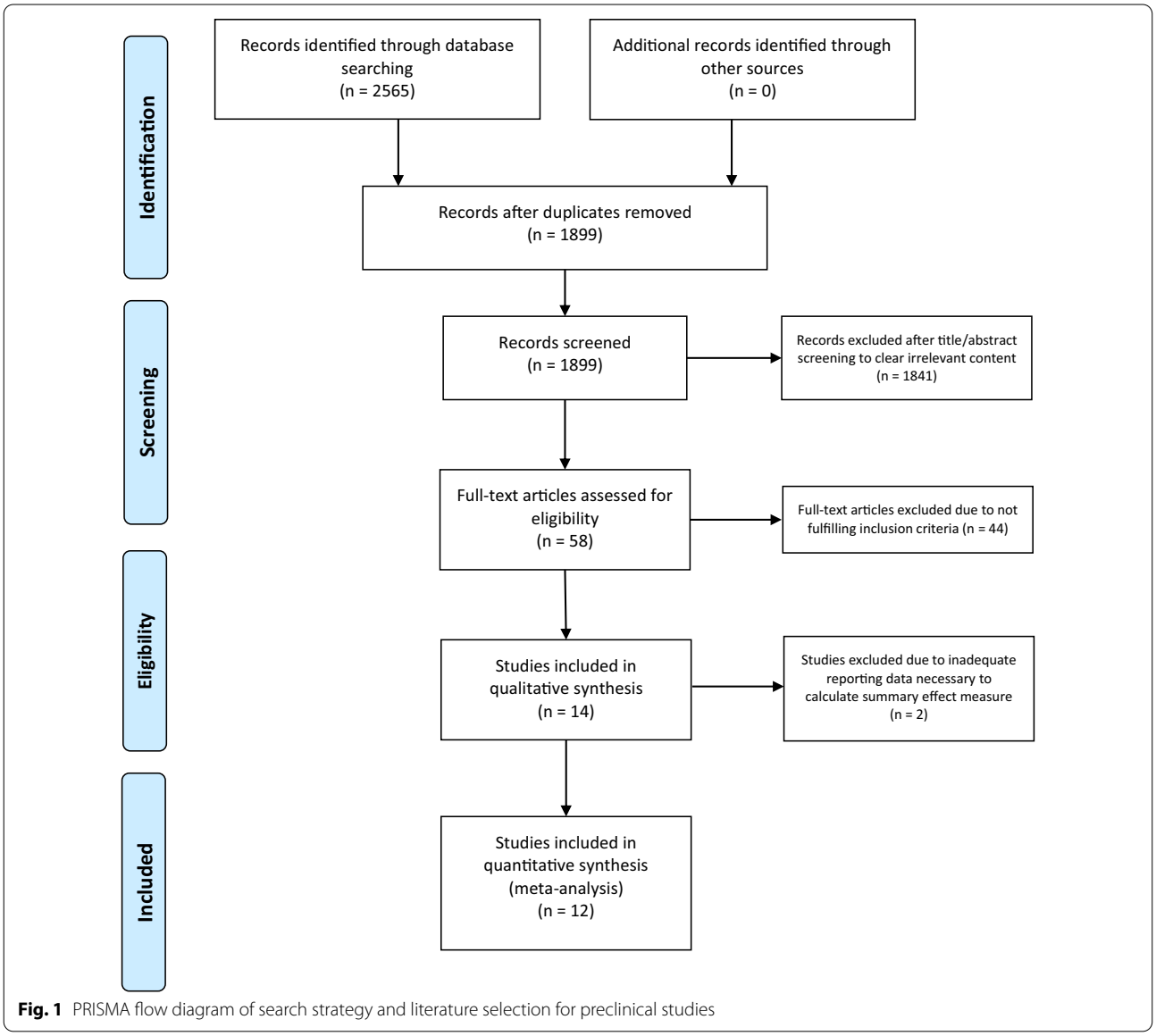


Fig. 1 PRISMA flow diagram of search strategy and literature selection for preclinical studies

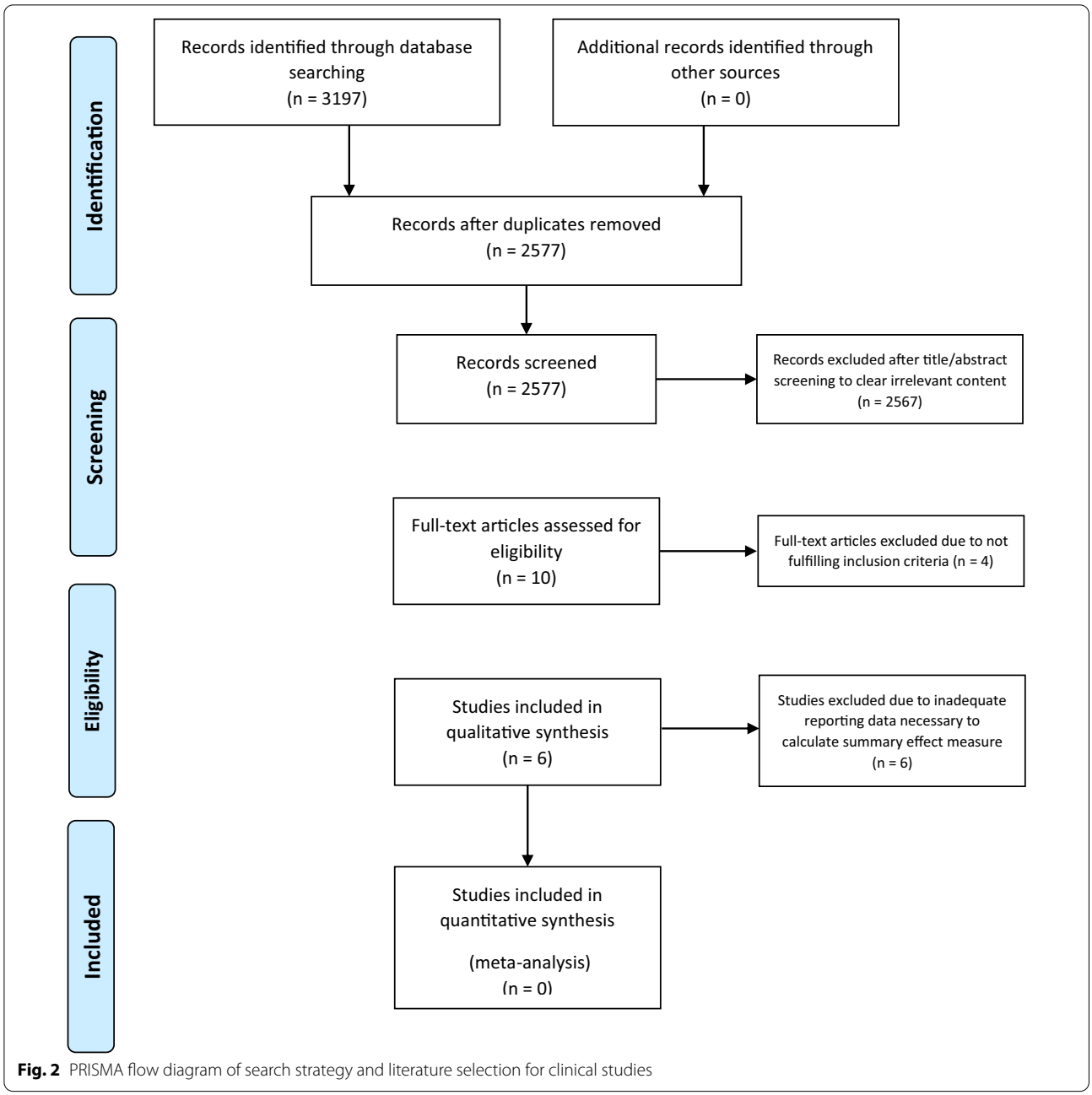


Fig. 2 PRISMA flow diagram of search strategy and literature selection for clinical studies

or no conflict of interest, "No" indicated the study was funded by industry- or author-mentioned conflict of interests, "unclear" indicated funding source or conflict of interest was not mentioned. For mention of randomization or blinding, "Yes" indicated reported and "No" indicated unreported.

An overall score was calculated by adding all the items scores as yes equals one, while no and not applicable equal zero. A score was given for every paper to classify them as poor, fair, or good conducted studies, where a score from 0 to 5 was considered poor, 6–9 as fair, and 10–14 as good.

Data extraction

For clinical studies, details about the study design, cell type, primary outcome assessment, follow-up time and results were extracted.

Data on animal model characteristics (animal species, total sample size, total groups, number of animals in control group, number of animals in intervention

Table 1 Cell-therapy for secondary lymphedema: clinical studies

Year	References	Study type	Edema location	Cell type/dose	Follow-up/assessment	Results	Conclusions
2008	Hou et al. [29]	Prospective controlled study	Upper limb	Freshly isolated bone marrow stromal cells N/A	12 months/Circumference measurements, volume of edema, pain in arm	BM-MSCs reduce the volume and % volume of lymphedema, and reduce the amount of pain caused by edema	Autologous BM-MSCs transplantation for the treatment of breast cancer-related arm lymphedema is effective and feasible
2011	Maldonado et al. [30]	Prospective controlled study	Upper limb	Freshly isolated bone marrow stromal cells ($7 - 56 \times 10^7$)	3 months/Circumference measurements, chronic pain, arm mobility and sensory loss	BM-MSCs reduce the volume of lymphedema. Chronic pain and sensitivity are markedly improved	The use of localized injections of BM-MSCs appears to be helpful in the management of lymphedema secondary to radical mastectomy
2016	Toyserkani et al. [31]	Nonrandomized clinical trial	Upper limb	Freshly isolated autologous adipose-derived stromal cells (4.07×10^7)	4 months/Circumference measurements, dual-energy X-ray absorptiometry scans, adverse events	ADSCs do not reduce the volume of lymphedema. Patients reported a decrease in symptoms over time. Five patients reduced their use of conservative treatment	ADSC-assisted lipotransfer is safe during the 4-month follow-up period and can alleviate symptoms of breast cancer-related lymphedema, minimizing the need for conservative treatment
2017	Toyserkani et al. [32]	Nonrandomized clinical trial	Upper limb	Freshly isolated autologous adipose-derived stromal cells (5.37×10^7)	6 months/Circumference measurements, dual-energy X-ray absorptiometry scans, patient-reported outcome and safety questionnaire assessment	ADSCs do not reduce the volume of lymphedema. Patients reported a decrease insymptoms over time. Five patients reduced their use of conservative treatment	ADSC-assisted lipotransfer is safe during the 6-month follow-up period and can alleviate symptoms of breast cancer-related lymphedema, minimizing the need for conservative treatment
2018	Ismail et al. [33]	Randomized controlled trial	Lower limb	Freshly isolated bone marrow stromal cells N/A	6 months/Circumferential measurements, heaviness and pain improvement, Immunohistochemical staining (lymphangiogenesis), recurrence of lymphedema	BM-MSCs reduce edema circumference as well as pain relief and improvement in walking ability. Increase in the number of lymphatic capillaries	BM-MSCs treatment can achieve improvement of symptoms in patients with chronic lymphedema
2019	Toyserkani et al. [34]	Nonrandomized clinical trial	Upper limb	Freshly isolated autologous adipose-derived stromal cells (5.41×10^7)	12 months/Circumference measurements, dual-energy X-ray absorptiometry scans, Patient-reported outcome and safety questionnaire assessment, lymphoscintigraphy changes	ADSCs do not reduce the volume of lymphedema. Patients reported a decrease in symptoms over time. Five patients reduced their use of conservative treatment	ADSC-assisted lipotransfer is safe during the 12-month follow-up period and can alleviate symptoms of breast cancer-related lymphedema, minimizing the need for conservative treatment

Table 2 Cell therapy for secondary lymphedema: non-clinical studies

Year	References	Animal model	Groups	Cell type/number	Implantation methods	Follow-up/ Assessment	Results	Conclusions
2009	Conrad et al. [35]	Mouse tail	2 groups (n = N/A for each group): Control, MSC	Allogeneic up to 3 passages BM-MSC ($p53^{-/-}$)/1 × 10^7	Subcutaneous	56 days/Circumference measurements, lymphatic drainage, neolymphangiogenesis (immunohistochemical staining)	(1) In stem cell-treated animals, a marked reduction in the edema was observed (2) Restoration of lymphatic drainage	The administration of BM-MSCs in vivo may contribute to the reduction in lymphatic edema
2011	Hwang et al. [36]	Mouse hindlimb	5 groups (n = 5): Sham, control, hydrogel alone, hADSC, hADSC + hydrogel	PKH-26-labeled hADSC/VEGF-C hydrogel/N/A	Subcutaneous	28 days/Circumference mesurements, lymphatic vessels (immunohistochemical staining)	(1) Significantly decreased dermal edema depth (2) Significantly greater lymphatic vessel regeneration	Co-administration of hADSCs and VEGF-C hydrogel has a substantial positive effect on lymphangiogenesis
2011	Zhou et al. [37]	Rabbit Hindlimb + IR	4 groups (n = N/A): Control, VEGF-C, BM-MSC, BM-MSC + VEGF-C	Allogeneic 3 passages BM-MSC + VEGF-C/1 × 10^7	Intramuscular	6 months/Limb volume changes, Immunohistochemical staining of lymphatic vessels, western blot analysis for VEGF-C	(1) Reduce limb volume at 6 months (2) Significant greater lymphatic vessel at 28 days	BM-MSC transplantation and VEGF-C administration could enhance the therapeutic effect of each other
2012	Shimuzu et al. [38]	Mouse tail	5 groups (n = 12): Sham, PBS, VEGF-C, BM-MNC, ADSC	Freshly isolated ADSCs/2 × 10^6	Subcutaneous	28 days/Tail diameter, lymphatic vessels diameter (H-E), lymphatic vessels (immunohistochemical staining), bone marrow-derived CD11b + macrophage kinetics assay	(1) Lymphedema was improved significantly by local injection of ADSCs (2) High lymphatic capillary density (3) Enhance recruitment of bone marrow-derived M2 macrophages, which serve as lymphatic endothelial progenitor cells	Implantation of autologous ADSCs could be a useful treatment option for patients with severe lymphedema via enhanced lymphangiogenesis
2013	Park et al. [39]	Mouse Hindlimb + IR	4 groups (n = 8): Control, Surgery, Surgery + IR, Cell therapy	Allogeneic muscle-derived stem cell + hLEC /1 × 10^7	N/A	56 days/Water displacement volumetric analysis, lymphoscintigraphy, lymphatic vessels (immunohistochemical staining),	(1) Attenuation of hindlimb volume (2) High lymphatic vessel density (3) Restore of the lymphatic flow	Stem cell lymphangiogenesis seems to be a promising approach

Table 2 (continued)

Year	References	Animal model	Groups	Cell type/number	Implantation methods	Follow-up/ Assessment	Results	Conclusions
2014	Kawai et al. [40]	Nude rat tail	4 groups: hLEC ($n=18$), hDMEC ($n=8$), Control ($n=19$), sham ($n=5$)	Human dermal microvascular endothelial cells (hDMEC) and human lymphatic endothelial cells (hLEC)/5×10^6	Wound/on postoperative days 1, 4, 7, 11 and 14	36 days/Circumference mesurements, indocyanine green fluorescence lymphography, thickness of epidermis (H-E), lymphatic vessels (immunohistochemical staining)	(1) In hLEC-treated animals, the circumference, lymphatic flow, and thickness of the skin became thinner (2) High lymphatic vessel density (3) hLECs are incorporated into the new vessels	Cell transplantation therapy using human LECs improved secondary lymphedema
2015	Ackermann et al. [41]	Mouse tail	3 groups ($n=10$): Control, PRP, ADSC	Allogeneic 3 passages ADSC vs platelet-rich plasma (PRP)/N/A	N/A	14 days/Wound healing analysis, tail diameter, real-time laser Doppler imaging for perfusion, lymphatic vessels (immunohistochemical staining)	(1) PRP and ADSC show a significantly increased epithelialization (2) High lymphatic vessel density in PRP group (3) Significant enhance perfusion of wounds treated by PRP and ADSC	PRP induces higher lymphangiogenesis than ADSCs
2015	Yoshida et al. [42]	Mouse Hindlimb + IR	5 groups ($n=20$): Sham, control, ADSC 10^4, ADSC 10^5, ADSC 10^6	Allogeneic up to 5 passages ADSC/1×10^4, 1×10^5, 1×10^6	N/A	16 days/Circumferential measurement, lymphatic flow assessment, quantification of lymphatic vessels (immunohistochemical staining and EGFP)	(1) The numbers of lymphatic vessels were significantly increased (2) ADSCs are not detected in lymphangiogenesis	ADSCs can restore the lymphatic vascular network in secondary lymphedema with increased collecting vessels
2016	Gousopoulos et al. [43]	Transgenic mice tail	2 groups ($n=5$) Control, T_{reg}	Regulatory T Cells (T_{reg})/$0.8–0.9 \times 10^6$	Intravenous	14 or 42 days/Tail volume, lymphatic vessels (immunohistochemical staining), RT-PCR, flow cytometry	(1) Reverse all of the major hallmarks of lymphedema, including edema, inflammation, and fibrosis (2) Promote lymphatic drainage function	T_{reg} application constitutes a potential new curative treatment modality for lymphedema

Table 2 (continued)

Year	References	Animal model	Groups	Cell type/number	Implantation methods	Follow-up/ Assessment	Results	Conclusions
2017	Hayasida et al. [44]	Mouse Hindlimb + IR	4 groups ($n = 5$): Control, VLNT, ADSC, ADSC + VLNT	Allogeneic 1–3 passages ADSC and vascularized lymph node transfers/1×10^4	Subcutaneous	14 days/Volumetric analysis of edema, near-infrared video camera system for lymphatic flow assessment, B16 mouse melanoma cells for lymphatic vessel and lymph node function, lymphatic vessels (immunohistochemical staining)	(1) ADSC + VLNT reduce the edema at 14 days (2) Increase the number of lymphatic vessels (3) Accelerate the lymphatic drainage to the venous systems	Combined ADSC and vascularized lymph node transfer treatment in secondary lymphedema may effectively decrease edema volume and restore lymphatic function
2018	Beerens et al. [45]	Nude mouse Skin flap model/Nude mouse Lymph node transplantation model	(1) Skin flaps groups (PBS $n = 10$/mMAPCs $n = 6$/hMAPCs $n = 6$) (2) Lymph node transplantation groups (PBS $n = 10$/hMAPCs 1n = 10/hMAPCs2 $n = 6$)	Allogeneic MAPCs/0.5×10^6 in lymph node transplantation model; 1×10^6 in skin flap model	Subcutaneous	16 weeks/lymphography, lymphatic vessels (immunohistochemical staining)	(1) Restored lymph drainage across skin flaps (2) Reconnected transplanted lymph nodes to the host lymphatic vessel	MAPC transplantation represents a promising remedy for lymphatic system restoration at different anatomical levels and hence an appealing treatment for lymphedema
2020	Bucan et al. [46]	Mouse Hindlimb + IR	3 groups ($n = 15$): Control, SVF, ADSC	Freshly isolated ADSCs vs stromal vascular fraction/1×10^6	Subcutaneous	8 weeks/CT and SPECT lymphoscintigraphy for volumetric measures, lymph vessel morphometry	(1) Treatment with ADSC did not reduce the edema at 8 weeks (2) lymph vessel lumen decreased when treated with ADSC	ADSC did not improve secondary lymphedema in this animal model
2020	Dai et al. [47]	Mouse Hindlimb	4 groups ($n = 5$): Control, ADSC unsorted, ADSC Pod+, ADSC Pod−	Freshly isolated ADSCs (Pod+, Pod−) / 2×10^6	Subcutaneous	10 weeks/Limb volume change, lymphatic vessels (immunohistochemical staining)	(1) More attenuation of hindlimb volume in Pod + cells (2) High lymphatic vessel density	The podoplanin-positive cells possessed lymphatic paracrine and differentiation abilities and may represent LEPCs
2020	Ogino et al. [48]	Mouse Hindlimb + IR	3 groups ($n = 6$): Control without IR, Control with IR, ADSC	Allogeneic 2–4 passages ADSCs/7.5×10^5	Subcutaneous	14 days/lymphatic vessels (immunohistochemical staining), picrosirius red staining for fibrosis	(1) ADSC transplantation accelerated LEC proliferation and increased lymphatic vessel numbers (2) ADSC mitigated fibrosis	ADSC transplantation contributes to lymphedema reduction by promoting LEC proliferation, improving fibrosis and increasing the number of lymphatic vessels

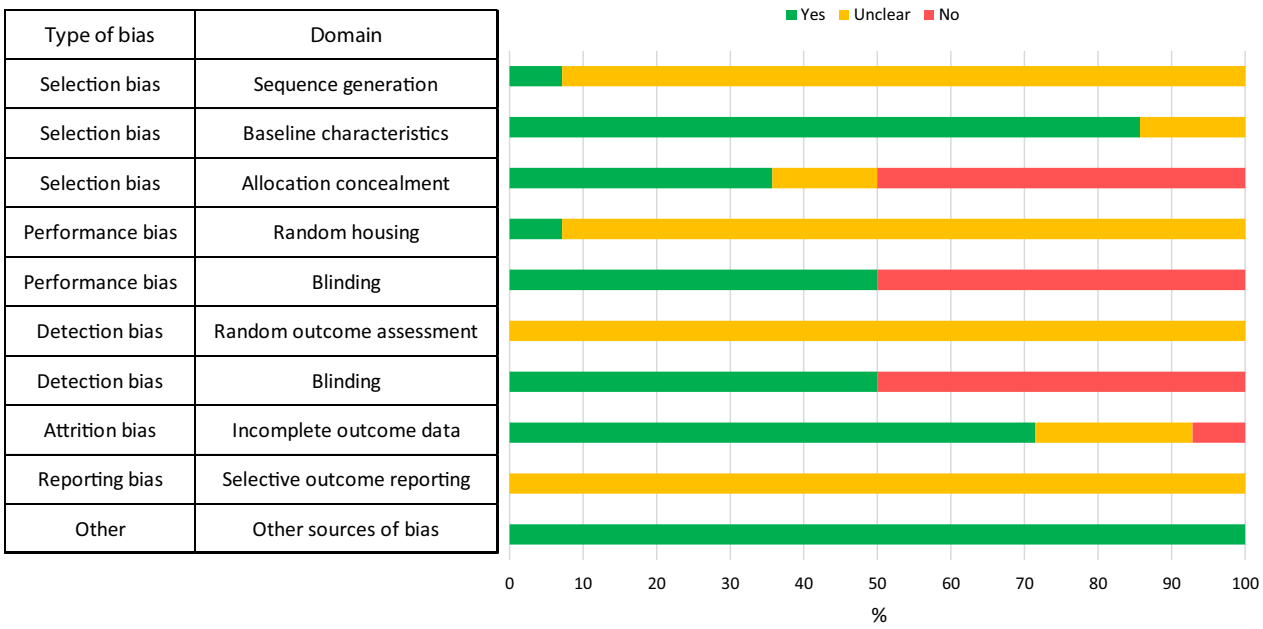


Fig. 3 Assessment of bias in 14 animal studies using the SYRCLE risk of bias tool

group), lymphedema model (tail, hindlimb, etc.), cell administration characteristics (cell type, source, as well as administration route, dose, timing and anatomical site of intervention), and primary outcome measures (evaluation of the lymphatic flow, quantification of edema, density of lymphatic capillaries and tissue fibrosis) were extracted.

For included articles, all independent comparisons were identified. Replications were also collected separately. Information on primary outcome was extracted from both text and graphs, when raw data or mean/median/incidence, SD/SE were reported or recalculated. In several studies, the results were adapted to be able to be analyzed with the rest of the studies. Gsys 2.4.6. software (Hokkaido University Nuclear Reaction Data Centre) was used to obtain values from graphs. When the number of animals was reported as a range, the lowest group size was collected. When no clear data could be extracted, the report was excluded from further meta-analysis.

Statistical analyses

Quantitative analysis was conducted using Review Manager (RevMan) version 5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). Treatment effects were first calculated separately for each study outcome. For all analyses, a random effect, inverse variance model was used to calculate standardized mean differences (SMD) and 95% confidence intervals (CI). Because most animal experiments use fewer than ten animals per group, we used Hedge's G effect sizes (which is based on Cohen's D but includes a correction factor for small sample size bias) for SMD analyses. The effect of heterogeneity (I^2) was used to measure the degree of inconsistency across pooled studies due to variability rather than chance, with larger values indicative of high heterogeneity (0–25% is considered to reflect very low heterogeneity; 25–50% reflects low heterogeneity; 50–75% reflects moderate heterogeneity; > 75% reflects high heterogeneity). Considering the anticipated heterogeneity, random effects models were used to conducted meta-analysis. Mean effect size, 95% confidence intervals (95% CI), significance, weight and forest plots were analyzed by the inverse variance method and the standard mean differences. The possibility of publication bias was assessed by analyzing funnel plot asymmetry (with trim-and-fill). The trim-and-fill method provides an estimate of the number of missing studies, and also provides an estimated intervention effect 'adjusted' for the publication bias (based on the filled studies). Finally, to explore sources of heterogeneity, stratified meta-analysis and meta-regression were performed.

Results

A total of 20 articles were selected for analysis. Six of these were clinical studies [29–34], including a randomized clinical trial, three nonrandomized clinical

Quantification of edema:

Study	Experimental Group			Control Group			Hedges'g (SMD)	SEg	95%CI-Lower	95%CI-Upper	weight(%)
	N1	Mean1	Sd1	N2	Mean2	Sd2					
Hwang et al.	5	3.31	0.15	5	4.12	0.16	4.718	1.200	2.366	7.069	8.302
Shimuzu et al.	12	3.58	0.26	9	4.58	0.26	3.692	0.710	2.301	5.083	9.833
Park et al.	8	236.20	37.80	8	249.94	45.50	0.311	0.476	-0.622	1.243	10.401
Kawai et al.	18	30.15	1.07	19	33.83	1.00	3.480	0.517	2.467	4.493	10.314
Ackermann et al.	10	29.90	3.93	10	40.44	16.20	0.857	0.449	-0.024	1.737	10.455
Yoshida et al.	20	8.50	3.90	20	51.20	4.16	10.380	1.201	8.025	12.734	8.297
Gousopoulos et al.	5	301.44	27.90	5	363.73	14.76	2.521	0.803	0.948	4.094	9.568
Hayasida et al.	5	0.25	0.01	5	0.38	0.07	2.258	0.762	0.764	3.752	9.685
Beerens et al.	6	1.28	0.13	10	2.08	0.49	1.889	0.591	0.729	3.048	10.141
Bucan et al.	15	163.90	16.20	15	164.90	10.00	0.072	0.355	-0.624	0.769	10.623
Dai et al.	5	363.45	8.24	5	547.55	10.21	17.923	4.048	9.989	25.858	2.380

	Hedges'g (SMD)	SEg	95%CI	z score	p value	Heterogeneity
Random Effect Model	3.18	0.706	[1.798,4.567]	4.506	7.00E-06	I^2=92.0%, Tau^2=4.57

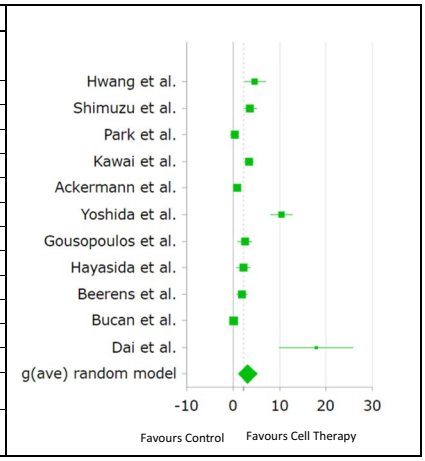
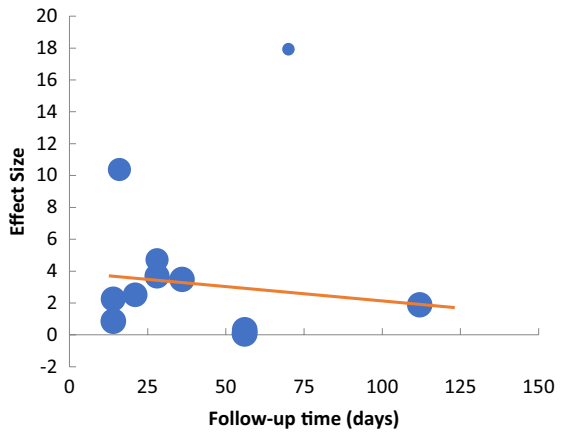


Fig. 4 Forest plot of the effects of cell therapy on the edema reduction



	B	SE	CI LL	CI UL	β	Z-value	p-value
Intercept	3.93	1.24	1.17	6.69		3.17	0.002
Slope	-0.02	0.03	-0.07	0.04	-0.15	-0.71	0.475

Analysis of variance	Sum of squares (Q*)	df	p	Mean square	F-Value	p-value
Model	0.51	1	0.475	0.51	0.20	0.667
Residual	23.29	9	0.006	2.59		
Total	23.80	10	0.008			

Combined effect size	3.21
T^2 (method of moments estimation)	4.96
R^2	2.15%

Fig. 5 Meta-regression analysis of follow-up time on effect of the cell therapy on the edema reduction

trials and two prospective controlled studies (Table 1). A case report and an observational study were excluded from the analysis. Five of them studied the effect of cell therapy on the upper limb, while the other studied lower limb edema. Mesenchymal stromal cells (MSCs) of different origins were used: three studies used bone marrow-derived MSCs (BM-MSCs) and the remaining three used adipose-derived MSCs (ADSCs). The follow-up period ranged from 3 to 12 months.

Fourteen animal studies [35–48] were included in the analysis (Table 2). These studies included three different animal models (mouse, rat and rabbit). In murine models, tail, hind limb, back skin flap, or lymph node transplantation was used. In rabbit models, hind limb was used. The cell types used included stem or progenitor cells (BM-MSCs, ADSCs, muscle-derived stem cells and multipotent progenitor cells) and differentiated cells (lymphatic endothelial cells and T_{reg} cells), and the number of cells used ranged from 10^4 to 10^7. The follow-up period ranged from 14 days to 6 months.

Assessment of study quality and risk of bias

The study design, including details of the method of randomization of subjects to treatment groups, criteria for eligibility in the study, blinding, method of assessing the outcome, and handling of protocol deviations are important features defining study quality. Due to the high risk of bias (data not shown) and the fact that only one of the human studies included was a properly blinded randomized controlled trial, a meta-analysis was not performed for clinical studies.

None of the pre-clinical studies had published protocols nor were registered with CAMARADES (University of Edinburgh, Scotland). Therefore, the selective outcome reporting item on the SYRCLE tool was

Table 3 Subgroup analyses of the effects of cell therapy on secondary lymphedema

Subgroup	Experiments (N)	Hedges' G (SMD)	SEg	95%CI-Lower	95%CI-Upper	z score	*p* value	Heterogeneity %
(1) Edema								
All studies	11	3.183	0.706	1.798	4.567	4.506	0.000	92.250
Animal model								
Tail model	5	3.330	0.963	1.442	5.217	3.458	0.001	88.307
Hindlimb model	5	3.329	1.315	0.751	5.907	2.531	0.011	95.132
Cell type								
Stem or progenitor cells (BM-MSC, ADSC, MAPC)	9	3.282	0.831	1.652	4.911	3.948	0.000	92.932
Differentiated cells (LEC, T_{reg})	2	3.197	0.438	2.339	4.055	7.305	0.000	0.989
(2) Lymphatic vessels								
All studies	10	6.348	1.139	4.115	8.581	5.571	0.000	92.650
Animal model								
Tail model	4	6.661	2.157	2.434	10.889	3.089	0.002	95.592
Hindlimb model	5	5.736	1.243	3.299	8.173	4.613	0.000	84.502

scored as "unclear." There was insufficient information reported for many of the remaining nine questions which were scored as "unclear." Several studies reported any randomization, although details were not given. 50% reported any blinding, either of investigators, animal handlers or outcome assessors. Overall, all studies had significant risks of bias according to the SYRCLE tool (Fig. 3), but these were not sufficiently remarkable as to be excluded from any analyses. Only two studies (Conrad et al. and Zhou et al.) did not report sample size for control and intervention groups, and thus those studies were not included in the meta-analysis.

Meta-analysis and effect evaluation

Meta-analysis was performed for outcomes that had data in at least three studies. The outcomes analyzed were: quantification of edema, density of lymphatic capillaries, evaluation of the lymphatic flow, and tissue fibrosis.

- Quantification of edema

Eleven studies were included to investigate the effect of cell therapy treatment on edema reduction in secondary lymphedema. The pooled estimate showed a significant decrease in edema (SMD 3.18; 95% CI 1.798, 4.567 ($p < 0.001$); however, between-study heterogeneity was very high ($I^2 = 92\%$; Fig. 4). Subgroup analysis as a function of the animal model used did not reduce heterogeneity. Subgrouping as a function of cell type indicated a similar reduction in edema with stem or progenitor cell treatment than differentiated cell treatment, with no evidence of heterogeneity in this subgroup (Table 3). Random effect meta-regression analysis was applied to estimate functional relationship of effect size on follow-up time. The regression coefficient was -0.02, and it was statistically insignificant ($p > 0.05$). These results indicated that the effect of follow-up time on the effect size was insignificant. Consistently, a linear relationship was not found (Fig. 5).

- Density of lymphatic capillaries

Ten studies were included to investigate the effect of the cell therapy treatment on the lymphatic regeneration in secondary lymphedema. The overall pooled analysis showed a significant increase in lymphatic vessel density in experimental group versus control group (SMD 6.35; 95% CI 4.115, 8.581; $p = 0.00$). However, the test for heterogeneity was significant ($I^2 = 93\%$; Fig. 6). Subgroup analysis as a function of animal model did not show differences between groups and did not reduce heterogeneity. Analysis as a function of cell type could not be carried out due to the small number of studies (Table 3). Random effect meta-regression analysis was applied and a regression coefficient of 0.03 was found, which was statistically insignificant ($p > 0.05$). These results indicated that follow-up time does not explain the heterogeneity found between the studies (Fig. 7).

- Evaluation of the lymphatic flow

Four studies were included to investigate the effect of the cell therapy treatment on the lymphatic perfusion restoration in secondary lymphedema. The pooled estimate suggested a significant improvement of lymphatic perfusion (SMD 2.49; 95% CI 0.583, 4.394 ($p = 0.01$); $I^2 = 88\%$, Fig. 8). Due to the limited availability of data,

Density of lymphatic vessels:

Study	Experimental Group			Control Group			Hedges'g (SMD)	SEg	95%CI-Lower	95%CI-Upper	weight(%)
	N1	Mean1	Sd1	N2	Mean2	Sd2					
Hwang et al.	5	13.96	2.17	5	49.12	2.83	12.594	2.873	6.962	18.226	6.761
Shimuzu et al.	9	6.31	1.20	12	34.60	2.30	14.179	2.228	9.811	18.546	8.160
Park et al.	8	0.83	0.25	8	2.98	0.69	3.923	0.839	2.278	5.567	11.145
Kawai et al.	19	10.28	0.76	18	15.40	1.32	4.685	0.633	3.445	5.925	11.443
Ackermann et al.	10	2.50	0.30	10	2.80	0.60	0.606	0.439	-0.255	1.466	11.657
Yoshida et al.	20	6.70	1.52	20	33.00	4.01	8.501	1.000	6.541	10.460	10.869
Hayasida et al.	5	7.40	0.90	5	11.50	1.40	3.147	0.906	1.370	4.923	11.034
Beerens et al.	10	0.21	0.05	6	0.87	0.11	8.104	1.514	5.138	11.071	9.809
Dai et al.	5	2.00	0.32	5	10.33	1.08	9.446	2.188	5.158	13.735	8.252
Ogino et al.	6	7.14	0.52	6	9.54	0.55	4.139	0.999	2.181	6.097	10.871

	Hedges'g (SMD)	SEg	95%CI	z score	p value	Heterogeneity
Random Effect Model	6.35	1.139	[4.115,8.581]	5.571	0.00E+00	I^2=93.0%, Tau2=10.94

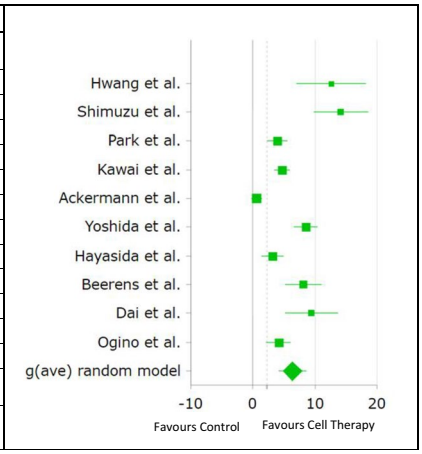


Fig. 6 Forest plot of the effects of cell therapy on the lymphatic regeneration

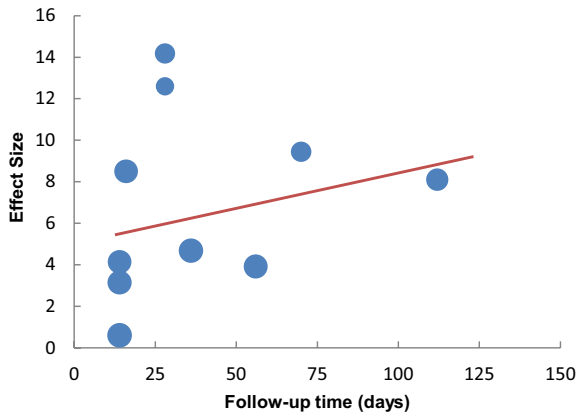
it was not possible to conduct subgroup analyses. Using random effects meta-regression analysis, the regression coefficient was -0.04, which was not statistically significant ($p > 0.05$). The results indicated that follow-up time does not explain the heterogeneity between studies (Fig. 9).

- Tissue fibrosis

Only three studies were included to investigate the effect of cell therapy treatments on the fibrosis reduction in secondary lymphedema. The analysis of the effect size showed a significant reduction in the fibrosis (SMD 4.39; 95% CI 1.439, 7.352 ($p < 0.01$); $I^2 = 82\%$, Fig. 10). Subgroup analysis was not carried out due to the small number of studies included. The regression coefficient was found to be -0.19 and statistically insignificant ($p > 0.05$) using random effect meta-regression analysis. The study's heterogeneity was not explained by the follow-up period, according to the findings (Fig. 11).

Publication Bias

The publication bias evaluation (Funnel plots) for the meta-analysis of lymphatic regeneration (ten studies) is shown in Fig. 12. After adjusting for missing studies, we found that the point estimate of the overall effect size continued to show a positive effect in favor of cell therapy (SMD 5.65 [CI 95% 2.48–8.83]). No significant publication bias was observed for edema reduction, lymphatic perfusion restoration and fibrosis reduction. This confirms that if there were a publication bias, the effect of cell therapy on secondary lymphedema would not be modified.



	B	SE	CI LL	CI UL	β	Z-value	p-value
Intercept	5.02	1.73	1.09	8.94		2.89	0.004
Slope	0.03	0.04	-0.05	0.12	0.26	0.95	0.342

Analysis of variance	Sum of squares (Q*)	df	p	Mean square	F-Value	p-value
Model	0.90	1	0.342	0.90	0.59	0.465
Residual	12.29	8	0.139	1.54		
Total	13.19	9	0.154			

Combined effect size	6.30
T^2 (method of moments estimation)	9.81
R^2	6.84%

Fig. 7 Meta-regression analysis of follow-up time on effect of the cell therapy on the lymphatic regeneration

Evaluation of the lymphatic flow:

Study	Experimental Group			Control Group			Hedges'g (SMD)	SEg	95%CI-Lower	95%CI-Upper	weight(%)
	N1	Mean1	Sd1	N2	Mean2	Sd2					
Kawai et al.	5	1.92	0.20	5	0.81	0.02	7.100	1.687	3.793	10.407	16.068
Ackermann et al.	10	171.00	9.00	10	150.00	9.00	2.235	0.555	1.146	3.323	28.269
Gousopoulos et al.	5	12990.50	3513.90	5	5349.00	1805.00	2.471	0.795	0.913	4.029	25.777
Bucan et al.	15	0.75	0.04	15	0.73	0.07	0.265	0.357	-0.435	0.964	29.886

	Hedges'g (SMD)	SEg	95%CI	z score	p value	Heterogeneity
Random Effect Model	2.49	0.972	[0.583,4.394]	2.560	1.05E-02	I^2=88.0%, Tau2=3.04

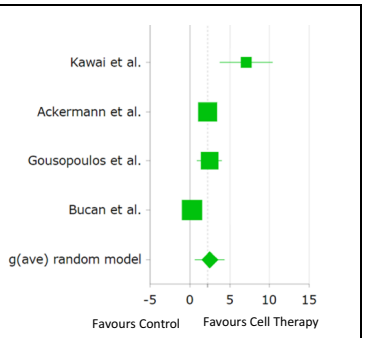
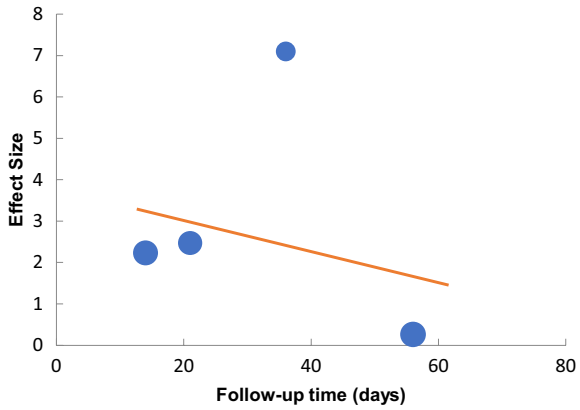


Fig. 8 Forest plot of the effects of cell therapy on the lymphatic perfusion restoration



	B	SE	CI LL	CI UL	β	Z-value	p-value
Intercept	3.77	2.30	-3.56	11.09		1.64	0.102
Slope	-0.04	0.06	-0.24	0.17	-0.29	-0.59	0.556

Analysis of variance	Sum of squares (Q*)	df	p	Mean square	F-Value	p-value
Model	0.35	1	0.556	0.35	0.17	0.717
Residual	4.00	2	0.135	2.00		
Total	4.35	3	0.226			

Combined effect size	2.57
T^2 (method of moments estimation)	3.95
R^2	7.98%

Fig. 9 Meta-regression analysis of follow-up time on effect of the cell therapy on lymphatic perfusion restoration

Discussion

In the present study, we performed a systematic review and meta-analysis to evaluate the safety and efficacy of stem cell therapy for the treatment of secondary lymphedema, both in preclinical and clinical studies. We found that cell therapy proved to generate a robust beneficial effect in animal models of secondary lymphedema. Although several in vitro and in vivo studies have reported beneficial effects of cell therapy against secondary lymphedema [21, 49, 50], a formal meta-analysis that assesses the regenerative activity of cell therapy in animal models of secondary lymphedema had not been performed.

Animal studies are critical for understanding disease processes and assessing the safety and effectiveness of treatments. Animal trials, however, are inherently heterogeneous, even more than clinical trials. Understanding sources of heterogeneity and their influence on effect size is critical to successfully translating preclinical findings to human diseases [51].

No animal model mimics perfectly the pathophysiology of human lymphedema [52], mainly because animals present higher regenerative capacity and it is difficult to classify the severity of edema [49]. There are also significant differences between models [52]. Although the tail model yields more consistent results than the hindlimb model, lymphedema resolves naturally over time, thus confounding results of additional interventions [53]. Of note, the current lack of standardization in study design and outcome measures make it hard to compare preclinical results. Despite the experimental heterogeneity of available studies, insight from animal models has shed light on the molecular mechanisms underlying lymphedema, e.g., lymphangiogenesis [54], fibrosis [55] and inflammation [56, 57].

Regarding the human studies, only six studies were identified and included for the analysis, and since only one of them is a randomized clinical trial, it was not possible to perform the meta-analysis. Furthermore, the difference in the follow-up period between the

Fibrotic Tissue:

Study	Experimental Group			Control Group			Hedges'g (SMD)	SEg	95%CI-Lower	95%CI-Upper	weight(%)
	N1	Mean1	Sd1	N2	Mean2	Sd2					
Kawai et al.	5	60.75	4.45	5	114.66	19.60	3.426	0.956	1.553	5.299	36.464
Gousopoulos et al.	5	55.59	9.00	5	74.78	6.38	2.222	0.757	0.738	3.706	38.568
Ogino et al.	6	2.91	5.23	6.00	40.76	1.30	9.164	1.945	5.352	12.977	24.969

	Hedges'g (SMD)	SEg	95%CI	z score	p value	Heterogeneity
Random Effect Model	4.39	1.508	[1.439,7.35]	2.915	3.56E-03	I^2=82.0%, Tau2=5.32

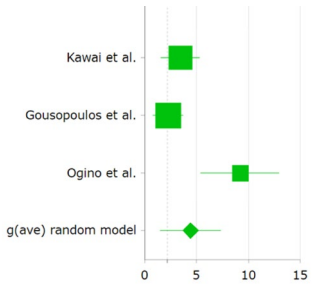
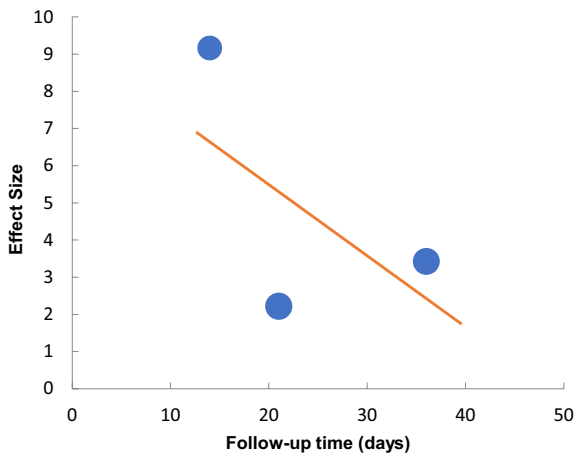


Fig. 10 Forest plot of the effects of cell therapy on the fibrosis reduction



	B	SE	CI LL	CI UL	β	Z-value	p-value
Intercept	9.32	6.71	-19.56	38.19		1.39	0.165
Slope	-0.19	0.26	-1.31	0.93	-0.61	-0.74	0.461

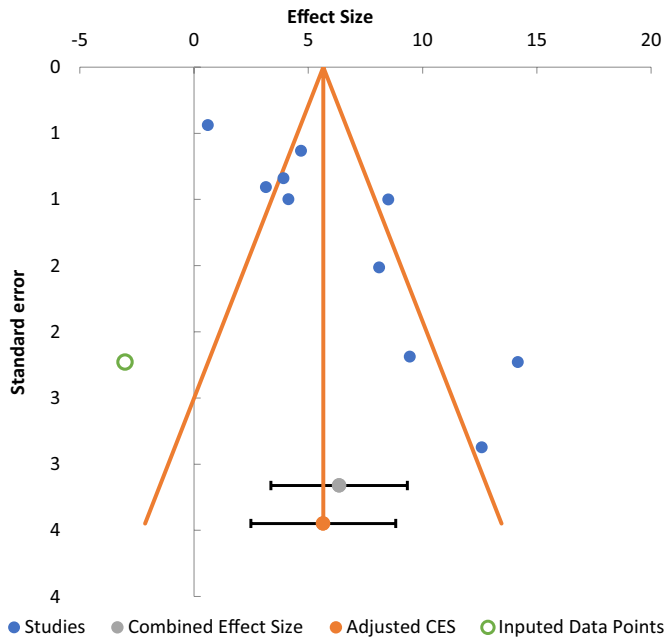
Analysis of variance	Sum of squares (Q*)	df	p	Mean square	F-Value	p-value
Model	0.54	1	0.461	0.54	0.54	0.596
Residual	1.00	1	0.317	1.00		
Total	1.54	2	0.462			

Combined effect size	4.69
T^2 (method of moments estimation)	15.17
R^2	35.18%

Fig. 11 Meta-regression analysis of follow-up time on effect of the cell therapy on the fibrosis reduction

studies did not allow us to confirm the observed effect of cell therapy on secondary lymphedema. However, it should be noted that the current human studies showed promising results of BM-MSCs [29, 30, 33] and ADSCs [31, 32, 34] in terms of reduction in edema, relief of symptoms, and an improved quality-of-life. Although no adverse effects related to cancer have been observed, the potential risk of cancer recurrence of using stem cells in the treatment of secondary lymphedema should be studied. A recently published Phase I study has found no evidence of breast cancer recurrence at 4-year follow up [58]. However, to further substantiate this relevant safety concern, a greater number of patients must be followed up longer-term in randomized clinical studies to formally rule out any contribution of stem cell transplants to cancer recurrence.

In the preclinical studies included in the review, different cell types have been tested for secondary lymphedema. In all cases, stem/progenitor cells have shown promise in halting lymphedema progression, sometimes even reverse the pathological process. However, the underlying mechanisms are not clear. It is speculated that stem cells may differentiate into lymphatic endothelial progenitor cells that in turn generate new lymphatics, or secrete cytokines to induce lymphangiogenesis [59]. Several studies have combined cell therapy with growth factors, such as VEGF-C [36] and PRP [41] which are thought to costimulate lymphangiogenesis. Co-transplantation with lymphatic endothelial cells (LECs) [40] may guide differentiation of stem cells to LEPCs. Combination of cell therapy with lymph node transfer [44] improved both lymphangiogenesis and lymphatic flow. Of course, immune modulation could be another cell-based approach to tackle this



Combined effect size	Observed
Effect Size	6.35
SE	1.32
CI Lower limit	3.36
CI Upper limit	9.34

Combined effect size	Adjusted
Effect Size	5.65
SE	1.42
CI Lower limit	2.48
CI Upper limit	8.83

Trim and Fill	On
Estimator for missing studies	Leftmost Run/Rightmost Run
Search from mean	Left
Number of imputed studies	1

Fig. 12 Funnel plot (with trim-and-fill) analysis of the cell therapy on the lymphatic regeneration

disease. Gousopoulos et al. have shown that treatment with T_{reg} cells reversed major hallmarks of lymphedema, such as edema, inflammation, and fibrosis [43]. Cell-based therapies seem thus to improve lymphedema's outcomes (edema reduction, lymphatic regeneration, lymphatic perfusion restoration, and fibrosis reduction), and the effect is seen across multiple species (mouse, rat, and rabbit), so that translation of these novel therapies to humans seems to be warranted.

The main limitations of this study are (i) the significant methodological differences between studies, especially the animal model used, the number of infused cells and timing of follow-up; (ii) small sample sizes and small study dataset for the meta-analysis, with most studies having no pre-published protocols or sample size estimations; (iii) the included studies had moderate or unknown bias risks, mainly due to poor reporting detail, and (iv) lack of operator blindness and randomization. These limitations emphasize the importance of applying more rigor to reporting standards and publishing in vivo experimental protocols [60].

Conclusions

Cell-based therapies have the potential to improve secondary lymphedema through their effects on the edema, lymphangiogenesis and fibrosis. The underlying mechanisms remain unclear. Due to relevant heterogeneity between studies, further randomized controlled and blinded studies are required to substantiate the use of these novel therapies in clinical practice.

Abbreviations

ADSCs: Adipose-derived stem cells; BM-MSCs: Bone marrow-derived mesenchymal stem cells; ESCs: Embryonic stem cells; iPSCs: Induced pluripotent stem cells; I^2: Effect of heterogeneity; LECs: Lymphatic endothelial cells; LEPCs: Lymphatic endothelial progenitor cells; MSCs: Mesenchymal stromal cells; SMD: Standardized mean differences; 95% CI: 95% Confidence intervals.

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Not applicable.

Authors' contributions

EA and AL developed a search strategy for each database and collected the citations; HL and IJ performed the assessment of study quality and risk of bias, the data extraction and statistical analysis; HL and AI were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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