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Review

Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: a systematic literature review

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SUMMARY

Background: An important goal of stem cell research in orthopaedics is to develop clinically relevant techniques that could be applied to heal cartilage or joint pathology. Stem cell treatment in orthopaedics for joint pathology is promising since these cells have the ability to modulate different processes in the various tissues of the joint simultaneously. The non life-threatening nature of musculoskeletal system disorders makes safety of stem cell therapy a necessary prerequisite.
Objective: To systematically review the literature and provide an overview of reported adverse events (AEs) of intra-articular treatment with culture-expanded stem cells in humans.
Design: A systematic literature search was performed in Pubmed, EMBASE, Web of Science and CINAHL in February 2013. AEs were reported into three categories: local/systemic, serious adverse event or AE (SAE/AE), related/unrelated.
Results: 3039 Potentially eligible articles were identified of which eventually eight fulfilled our inclusion criteria. In total, 844 procedures with a mean follow-up of 21 months were analysed. Autologous bone marrow-derived mesenchymal stem cells (BM-MSCs) were used for cartilage repair and osteoarthritis treatment in all included studies. Four SAEs were reported by the authors. One infection following bone marrow aspiration (BMA) was reported as probably related and resolved with antibiotics. One pulmonary embolism occurred 2 weeks after BMA and was reported as possibly related. Two tumours, both not at the site of injection, were reported as unrelated. Twenty-two other cases of possible procedure-related and seven of possible stem cell-product related adverse events (AEs) were documented. The main AEs related to the procedure were increased pain/swelling and dehydration after BMA. Increased pain and swelling was the only AE reported as related to the stem cell-product.
Conclusions: Based on current literature review we conclude that application of cultured stem cells in joints appears to be safe. We believe that with continuous caution for potential side effects, it is reasonable to continue with the development of articular stem cell therapies.
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Introduction

Stem cell therapies are rapidly emerging as a potential strategy for tissue repair and regeneration in many fields of medicine[1]. The use of autologous or allogenic stem cells is very promising for biological modulation and repair of various disease processes of the musculoskeletal system. In the field of orthopaedics, cartilage repair has played a pioneering role in the translational application of cell therapy. Autologous chondrocyte implantation (ACI) and derivative techniques such as matrix-induced chondrocyte implantation (MACI) have been employed and evaluated in the last two decades. Generally good to excellent results have been reported for these cell transplantation techniques, without significant safety problems for this intra-articular use of differentiated cells[2,3]. The Use of differentiated cells leads to several limitations in number of cells available, choice of cell and donor-site morbidity[4–6]. Stem cells, on the contrary, are multipotent, can be harvested from many different cell sources and have a high proliferation potential[7]. Stem cells have already been used in orthopaedic applications, although experimentally, in the treatment of avascular bone necrosis, osteochondral defects, pseudoarthrosis and traumatic cartilage defects[8–11]. Recently, Pastides *et al.* provided an overview of the effectivity of the clinical application of stem cells in cartilage defects[12].

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Table I
Search strategy in Medline

(stem cells[mesh] OR stemcell*[tw] OR stem cell*[tw] OR progenitor cell*[tw] OR nucleated cell*[tw] OR bone marrow cell*[tw])

AND

(joints[mesh] OR joint*[tw] OR articul*[tw] OR intraarticul*[tw] OR cartilag*[tw] OR chondrocyt*[tw])

AND

(inject*[tw] OR admin*[tw] OR treat[tw] OR treated[tw] OR treatment*[tw] OR therapy[tw] OR therapies[tw] OR therapeut*[tw] OR implant*[tw] OR transplant*[tw] OR repair*[tw] OR reconstruct*[tw])

NOT

((animals[mesh] OR animal*[tw]) NOT (humans[mesh] OR human*[tw] OR patient[tw] OR patients[tw] OR people*[tw] OR men[tw]))

AND

(dut[la] OR eng[la] OR ger[la] OR spa[la] OR ita[la] OR fre[la])

Safety is an important prerequisite for translational application of stem cell therapies. Unlike for life-threatening diseases where stem cell therapy is used for heart failure following myocardial infarction[13], severe graft vs host disease (GVHD)[14], Crohn's disease[15] or leukaemia, diseases in the orthopaedic field eligible for stem cell therapy are generally not life-threatening. For this reason, intensive monitoring of the safety of intra-articular use of culture-expanded stem cells in musculoskeletal diseases is even more important. This systematic literature review provides an overview of reported AEs based on all published studies with human cases of intra-articular treatment with culture-expanded stem cells.

Methods

Search strategy

A comprehensive search of the literature was carried out in February 2013. Electronic databases – PubMed, EMBASE, Web of Science and CINAHL – were used to identify relevant studies since their inception up to February 2013. An overview of the complete search strategy is shown in Table I.

Inclusion and exclusion criteria

The articles retrieved from the search were included in this systematic review according to the following inclusion and exclusion criteria:

(1) Subjects were treated with culture-expanded stem cells in joints. To obtain an extensive perspective on safety, studies with the use of all sorts of stem cells were included, as long as the cells were culture-expanded and applied in joints. No restriction was made on joint disease.
(2) Human subjects.

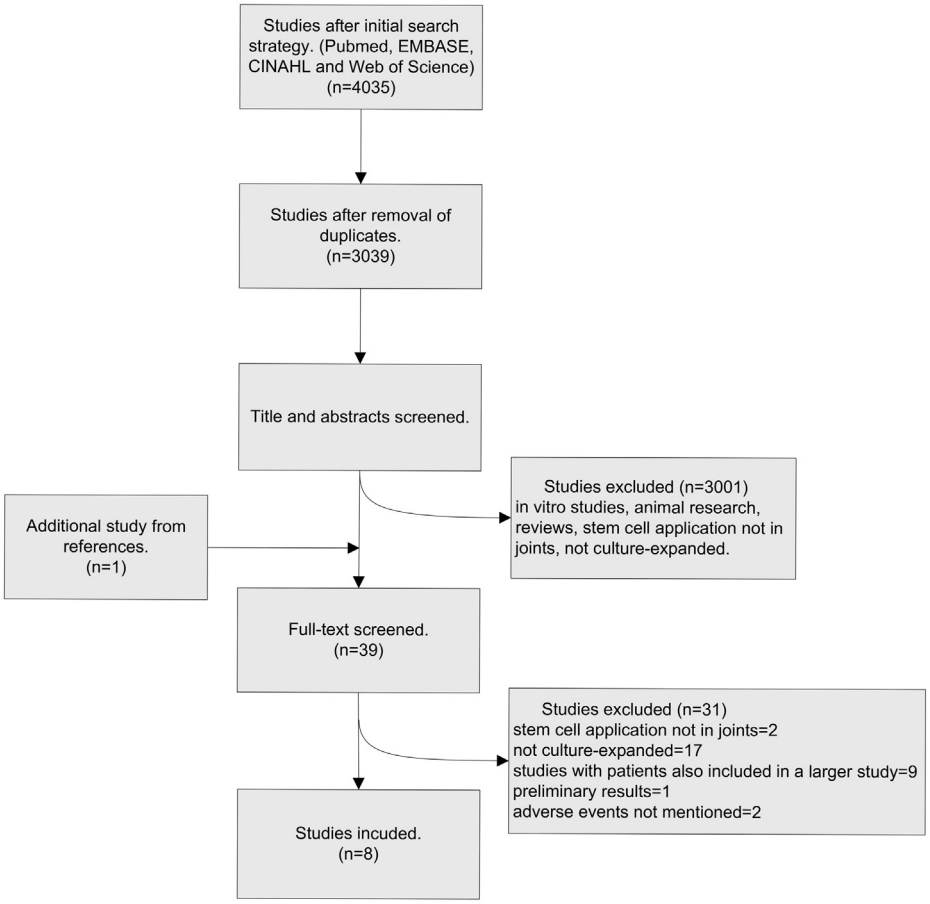

Fig. 1. Study selection.

(3) Full text of the article available.
(4) Study published in English, Dutch, German, Italian, French or Spanish.
(5) Study had to report information about AEs.

In vitro studies and animal studies were considered ineligible for inclusion. Comments, editorials, reviews, letters, guidelines and protocols were also excluded.

In case of potential duplicate studies or studies with overlap (i.e., dynamic cohorts) we contacted the senior authors. In those cases the study with the longest follow-up time and/or more detailed presentation of relevant outcomes was included in the review.

Of the included studies, only intra-articular procedures with culture-expanded stem cells were considered eligible for analysis, procedures in vertebral discs and other treatment sites were excluded.

Study selection

Two reviewers (PB and CP) independently examined article titles and abstracts for eligibility. Subsequently, full-text reports of potential studies were screened to determine final eligibility for inclusion in this review. Disagreements concerning the inclusion of the studies were solved by consensus. A third reviewer (MR) was consulted when disagreement persisted. Disagreements were solved in a single consensus meeting without the help of the third reviewer. In addition, the reference lists of the selected papers were screened with the intention to add eligible studies that were not found with the search. The selection of articles is shown schematically in Fig. 1.

Data extraction and presentation

One author (CP) extracted the data of the finally included studies. Information was collected on study design, study population, origin stem cells, procedure, outcome measures, duration of follow-up and results. All reported AEs are listed in the results. The AEs are subdivided in three tables: complications reported as possibly related to the procedure, stem cell product complications reported as possibly related and AEs reported as unrelated to the procedure or stem cell product. Reported AEs are once more subdivided in local or systemic and serious adverse events (SAE) or other AE. Since no definition of SAE for this application was available we defined SAE as death, neoplasms, infections, pulmonary embolisms, anaphylactic shock and haematological neoplasms.

Quality assessment

Two authors (CP and ML) independently assessed the methodological quality of AEs collection of each included study, using questions from the McHarm quality assessment scale for AEs[16] (the eight quality criteria are listed in Table II). Each item was scored as a 'yes, 'no' or 'unable to determine'. Disagreements were resolved by consensus. Consultation of a third reviewer (GO) when disagreement persisted, appeared unnecessary.

Table II
Methodological quality criteria

Item	Judgement
1. Did the authors state a prospective evaluation of AEs? (=active mode of AEs collection)	Yes/No/Unable to determine
2. Were adequate methods for monitoring AEs reported?	
A) MRI, arthroscopy	Yes/No/Unable to determine
B) Prospective checklist and/or patient questionnaire or patient diary	Yes/No/Unable to determine
3.	
A) Was the number of participants that withdraw or were lost to follow-up specified?	Yes/No/Unable to determine
B) Were patients excluded from the AEs analysis because of an AE?	Yes/No/Unable to determine
4.	
A) Were all categories of AEs reported on (all AEs)?	Yes/No/Unable to determine
B) Did the study specify whether AEs related to harvesting, application procedure or cell product?	Yes/No/Unable to determine
5.	
A) Were SAE precisely defined?	Yes/No/Unable to determine
B) For SAE were all categories of AEs reported on?	Yes/No/Unable to determine
6. Did the study specify who collected the AEs?	Yes/No/Unable to determine
7. Did the study specify the timing and frequency of collection of the AEs?	Yes/No/Unable to determine
8. Was the follow-up of AEs evaluation at least 1 year after the last administration of stem cells?	Yes/No/Unable to determine

Item 4 was scored yes when the following 2/3 categories were reported: local/systemic, serious/nonserious, related/unrelated. Our definitions: local AE, AE limited to the joint; systemic AE, AE unrelated to the joint; SAEs includes death, neoplasms, infections, pulmonary embolisms, anaphylactic shock and leukaemia.

Results

Study inclusion and characteristics

A total of 4035 records were found after the electronic search (Fig. 1). After the removal of duplicates, 3039 potentially eligible articles were identified. Finally, eight articles fulfilled our inclusion criteria and are included in this systematic review[9,17–23] (Table III).

Wakitani and Centeno were contacted for potential duplicate or near duplicate studies. Wakitani reported that all subjects reported in his six papers were included in Wakitani *et al.*, 2011[22,24–28]. Centeno reported that his last safety article[9] included all subjects reported in his five papers[9,29–32].

The prospective cohort study of Centeno *et al.*, 2010 has been updated in 2011[9,32]. The study of 2011 reports changes and AEs since the last reporting in 2010, which made it difficult to obtain a complete overview of the AEs in numbers and details. Therefore Centeno was requested for a complete overview of their reported AEs, which made enumeration and classification possible. We report the AEs based on the acquired list.

From a total of 904 procedures in 470 individuals, 844 were intra-articular procedures (789 injections and 55 cell constructs or sheets) and were analysed with a mean follow-up of 21 months. All included studies used autologous bone marrow-derived mesenchymal stem cells (BM-MSCs). The MSCs were implanted in the knee joint (503 procedures), hip joint (219), foot/ankle joint (55), shoulder joint (48), hand/wrist joint (15) and elbow joint (4). According to our inclusion criteria, we have excluded 34 vertebral disc procedures and 26 procedures in various other treatment sites reported in the study of Centeno *et al.*[9].

Quality assessment

According to the quality assessment (Table IV), the study of Centeno *et al.*, 2011 described an adequate method regarding the AEs collection[9]. All other included studies reported information on AEs, but did not use a standardised method for AE monitoring.

SAEs

Four SAEs have been reported: one infection, one pulmonary embolism and two tumours[9]. The infection was at the bone marrow

Table III
Characteristics of the included studies

First author, year of publication	Design	Study population	Number of procedures (joints) [individuals]*	Origin stem cells	Serum used for expansion in culture	Number of passages in culture	Number of intra-articular injected cells (mean)	Adjuvant of injected/implanted MSCs	Different joint types (%)	Mean age at surgery in years ± SD (range)	Male (%)	Mean follow-up period ± SD (range)
Centeno *et al.*, 2011[9]	Prospective cohort study	Chronic or degenerative joint disease	709, (NA), [>279]†	Autologous iliac crest BM-MSCs	PL	2–7	NA	MSCs in autologous PL10–20% diluted in PBS or conditioned serum of PRP	Knee (52.8%) Hip (30.7%) Foot/ankle (7.6%) Shoulder (6.8%) Hand/ wrist (2.1%)	±53 ± 13.85	±63.1	±435 days ± 261 days
Davatchi *et al.*, 2011[17]	Pilot study	OA	4, (4), [4]	Autologous iliac crest BM-MSCs	FBS	1	$\pm 8–9 \times 10^6$	Normal saline supplemented with 2% human serum albumin	Knee (100%)	57.6 (54–65)	50	1 year
Emadedin *et al.*, 2012[18]	Clinical trial	OA	6, (6), [6]	Autologous iliac crest BM-MSCs	HBS	2	$\pm 20–24 \times 10^6$	Physiological serum	Knee (100%)	54.6	0	1 year
Haleem *et al.*, 2010[19]	Pilot study	OD, OCD	5, (5), [5]	Autologous iliac crest BM-MSCs	FBS	1	$\pm 2 \times 10^6/cm^2$	MSC mixed with allogenous PR-FG and left to gelate	Knee (100%)	25.7 (21–37)	80	14.2 months
Kasemkijwattana *et al.*, 2011[20]	Case serie	CD	2, (2), [2]	Autologous iliac crest BM-MSCs	FBS	2	NA	MSCs seeded in collagen scaffolds fixed, with FG	Knee (100%)	24.5 (24–25)	100	30.5 months (30–31)
Lee *et al.*, 2012[23]	Prospective cohort study	CD	70, (70), [70]	Autologous iliac crest BM-MSCs	FBS	1	$\pm 10 \times l0^6$	In autologous serum followed by 2 ml HA injection	Knee (100%)	44	51.4	24.5 months
Teo *et al.*, 2012[21]	Case serie	OD	3, (3), [3]	Autologous iliac crest BM-MSCs	FBS	NA	$\pm 10\text{-}15 \times 10^6$	Cell sheets in autologous serum fixed with FG	Knee (100%)	NA	NA	NA
Wakitani *et al.*, 2011[22]	Prospective cohort study	OA, CD	45, (45), [41]‡	Autologous iliac crest BM-MSCs	FCS (until 2003) AS (since 2003)	2	NA	Until 2003: embedded in 0.25% acid-soluble type I collagen from porcine tendon and gelated or placed on a collagen sheet and gelated in DMEM + 15% autologous serum. Since 2003: embedded in 1% acid-soluble type I collagen from bovine skin, gelated on porcine tendon collagen sheet in a MEM + 15% autologous serum	Knee (86.7%) Ankle (2.2%) Hip (2.2%) Elbow (8.9%)	50	NA	75 months (5–137)

OA: osteoarthritis, OD: Osteochondritis dissecans, OCD: osteochondral defect, CD: chondral defect, PL: platelet lysate, FBS: fetal bovine serum, HBS: hyclone bovine serum, FCS: fetal calf serum, AS: autologous serum, NA: not available, PR-FG: platelet-rich fibrin glue, FG: fibrin glue, HA: hyaluronic acid.

* Joints: total number of joints injected, procedures: total joint procedures.

† Including three patients of three Case reports of Centeno *et al.*, 2008 and 222 procedures of Centeno *et al.*, 2010. The exact number of patients is not determinable after exclusion of disc procedures and other site treatments.

‡ Including 12 patients of Wakitani *et al.*, 2002, two patients of Wakitani *et al.*, 2004, three patients of Wakitani *et al.*, 2006, one patient of Kuroda *et al.*, 2007 and three patients of Wakitani *et al.*, 2007.

Table IV
Quality assessment

Item	Centeno *et al.*, 2011[9]	Davatchi *et al.*, 2011[17]	Emadedin *et al.*, 2012[18]	Haleem *et al.*, 2010[19]	Kasemkij wattana *et al.*, 2011[20]	Lee *et al.*, 2012[23]	Teo *et al.*, 2012[21]	Wakitani *et al.*, 2011[22]
1	Yes	No	No	No	No	No	No	Yes
2. A)	Yes	No	No	No	No	No	No	No
B)	Yes	No	No	No	No	No	No	No
3. A)	No	No	No	No	No	No	Yes	Yes
B)	Un	No	No	No	No	No	No	No
4. A)	Yes	No	No	No	No	No	No	No
B)	Yes	No	Un	Un	Un	Un	Un	Un
5. A)	Yes	No	No	No	No	No	No	Yes
B)	Yes	No	No	No	No	No	No	No
6.	Yes	No	No	No	No	No	No	No
7.	Yes	No	No	No	No	No	No	Yes
8.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

For the description of items 1–8 see Table II. Un: unable to determine.

aspiration (BMA) site. It was listed by the authors as a probable procedural related complication and was successfully treated with oral antibiotics[9]. The onset of the pulmonary embolism was 2 weeks after the BMA before the initiation of any stem cell therapy and was documented as a possible procedural related complication. The patient was successfully treated in hospital[9]. Two tumours were detected in the period after the stem cell procedure in two separate patients. One patient was diagnosed with a benign schwannoma from T12-L2 and another patient was diagnosed with prostate carcinoma. The stem cell implantations were in the hip and knee joint respectively. Both tumours were not at the site of injection and were reported by the authors as unrelated[9].

AEs

All complications related to the harvesting procedure of stem cells or to the administration procedure were self-limited or were remedied with simple therapeutic therapies[9,17].

Seven complications were reported as related to the stem cell-product. All these patients reported increased pain and swelling. In four cases, drainage *via* arthrocentesis was required to resolve swelling and pain and in one case, an injection of corticosteroids was administered besides drainage. One stem cell treatment was cancelled due to an insidious onset of knee swelling, 2 weeks after procedure. This patient was eventually treated with a total knee arthroplasty (Tables V–VII).

Discussion

The aim of this systematic review is to provide an overview of AEs of the application of culture-expanded stem cells in joints. All published studies with human cases were included to provide an extensive overview of reported AEs. Of the 844 intra-articular implantations with MSCs, four individuals were presented with a serious complication. Two of the four serious complications were probably related to the procedure: an infection at the BMA site and a pulmonary embolism 2 weeks after BMA. Two tumours reported were regarded unrelated[9].

The eight included studies showed no safety issues regarding the MSC-product. The only reported stem cell-product related AEs

Table V
Procedure-related complications

First author, year of publication	Number of procedures [subjects]	Mean follow-up period ± SD (range)	SAE/AE	Procedure location	Procedure complications reported as probably/possibly related	Loc	Sys
Centeno *et al.*, 2011[9]	709, [>279]†	±435days ± 261 days	SAE	Crista iliaca	Infection at bone marrow draw site: 1	X	
				Crista iliaca	Pulmonary embolism 2 weeks after BMA: 1		X
			AE	Crista iliaca (2)	Increased pain at site of BMA: 2	X	
				Crista iliaca (2)	Dehydration after BMA: 2		X
				Crista iliaca	Recurrence of herpes zoster after BMA: 1	X	
				Ankle	Urticaria after procedure: 1		X
				Knee (8), hip (4), facet (l)	Increased pain/swelling at implantation site: 13	X	
				Knee	Laboratory abnormalities: Transient elevation of LFT: 1.		X
				Hand	Transient numbness and tingling in the arm used for blood draw: 1		X
Davatchi *et al.*, 2011[17]	4, [4]	14.2 months	SAE		None		
			AE	Knee	Mild swelling: 1	X	
Emadedin *et al.*, 2012[18]	6, [6]	1 year			None		
Haleem *et al.*, 2010[19]	5, [5]	1 year			None		
Kasemkijwattana *et al.*, 2011[20]	2, [2]	30.5 months (30–31)			None		
Lee *et al.*, 2012[23]	70, [70]	24.5 months			None		
Teo *et al.*, 2012[21]	3, [3]*	NA			None		
Wakitani *et al.*, 2011[22]	45, [41]	75 months (5–137)			None		

Our definitions: SAE included death, neoplasms, infections, pulmonary embolisms, anaphylactic shock and leukaemia. Loc: local AE, Sys: systemic AE, NA: Not available.

* These three patients are part of a larger group, of which 20 received ACI. Therefore follow-up cannot be reduced.

† Including three patients of three Case reports of Centeno *et al.*, 2008 and 222 procedures of Centeno *et al.*, 2010. The exact number of patients is not determinable after exclusion of disc procedures and other site treatments.

Table VI
Stem cell-product related complications

First author, year of publication	Number of procedures [subjects]	Mean follow-up period ± SD (range)	SAE/AE	Procedure location	Stem cell product complications reported as probably/possibly related	Loc	Sys
Centeno *et al.*, 2011[9]	709, [>279]†	±435 days ± 261 days	SAE		None		
			AE	Knee (6), Ankle (1)	Increased pain/swelling: 7	X	
Davatchi *et al.*, 2011[17]	4, [4]	1 year			None		
Emadedin *et al.*, 2012[18]	6, [6]	1 year			None		
Haleem *et al.*, 2010[19]	5, [5]	14.2 months			None		
Kasemkijwattana *et al.*, 2011[20]	2, [2]	30.5 months (30-31)			None		
Lee *et al.*, 2012[23]	70, [70]	24.5 months			None		
Teo *et al.*, 2012[21]	3, [3]*	NA			None		
Wakitani *et al.*, 2011[22]	45, [41]	75 months (5–137)			None		

Our definitions: SAE included death, neoplasms, infections, pulmonary embolisms, anaphylactic shock and leukaemia. Loc: local AE, Sys: systemic AE, NA: Not available.
* These three patients are part of a larger group, of which 20 received ACI. Therefore follow-up cannot be reduced.
† Including three patients of three Case reports of Centeno *et al.*, 2008 and 222 procedures of Centeno *et al.*, 2010. The exact number of patients is not determinable after exclusion of disc procedures and other site treatments.

were increased pain and swelling. These were mild and transient. It is difficult to attribute these stem cell-product related AE to one cause. Prerequirements for stem cell therapy are suitable cell counts and culture passages and applicable compositions of MSC solutions or constructs for injection and implantation. Different cell counts, passages and compositions of MSC solutions or constructs are used in the included studies. These factors can all potentially affect the occurrence of AE. For each individual AE information regarding these factors would be of great interest. However, our included studies did not provide this AE information specifically per patient.

Other clinical studies using culture-expanded MSCs for other applications also did not show any safety problems[15,33,34]. In Duijvestein *et al.* administration of autologous MSCs in nine

Table VII
Reported unrelated AEs

First author, year of publication	Number of procedures [subjects]	Mean follow-up period ± SD (range)	SAE/AE	Procedure location	AEs reported as unrelated
Centeno *et al.*, 2011[9]	709, [>279]†	±435days ± 261 days	SAE	Knee (l), Hip (1)	Tumour: 2
			AE	Hip	TIA: 1
				Hip	MRSA infection: 1
				Knee (7), Hip (ll), Ankle (2), Hand (l), Shoulder (l)	Increased pain and swelling, in most cases: most likely progression of underlying disease: 22
				Knee (4), Hip (5)	Increased pain outside re-implant area: 9
				Hip	Cardiac problems: 1
				Knee (l), Hip (3), Hand (l)	Laboratory abnormalities: 5
				Knee, hip	Low grade fever: 1
				Knee	Adrenal gland on unrelated lumbar MRI: 1
				Hip	Popping sensation in mouth with numbness and drooling: 1
				Hip	Involuntary tremors in treated leg: 1
				Knee	Arm swelling after peripheral blood collection: 1
				Knee	Kidney stone pain: 1
				Hip	Sore throat and congestion: 1
				Knee	Tightness in hamstrings and gluteus: 1
				Knee	Polymyalgia rheumatica: 1
				Knee	Bronchitis: 1
				Knee	Excema and Barrack's disease: 1
				Hip	Dermatomyositis: 1
				Knee (2)	Osteoporosis: 2
				Hip	Shingles after BMA: 1
Davatchi *et al.*, 2011[17]	4, [4]	1 year			None
Emadedin *et al.*, 2012[18]	6, [6]	1 year			None
Haleem *et al.*, 2010[19]	5, [5]	14.2 months			None
Kasemkijwattana *et al.*, 2011[20]	2, [2]	30.5 months (30-31)			None
Lee *et al.*, 2012[23]	70, [70]	24.5 months			None
Teo *et al.*, 2012[21]	3, [3]*	NA			None
Wakitani *et al.*, 2011[22]	45, [41]	75 months (5–137)			None

Our definitions: SAE included death, neoplasms, infections, pulmonary embolisms, anaphylactic shock and leukaemia. NA: Not available.
* These three patients are part of a larger group, of which 20 received ACI. Therefore follow-up cannot be reduced.
† Including three patients of three Case reports of Centeno *et al.*, 2008 and 222 procedures of Centeno *et al.*, 2010. The exact number of patients is not determinable after exclusion of disc procedures and other site treatments.

patients appeared to be safe in the treatment of refractory Crohn's disease[15]. Likewise, Karamouzian *et al.* concluded that transplantation of culture-expanded MSCs *via* lumbal puncture in 11 complete spinal cord injured patients at thoracic level is a safe technique[33].

To provide an impression of the number of AE in relation to other intra-articular treatments, we have compared stem cell injections with hyaluronic acid and high molecular hylan injections. For this comparison we have used the data on stem cell injections from the study of Centeno *et al.*, because this is the only study with an adequate method for AEs collection. In this study 23 (3.2%) AE related to the intra-articular injection with stem cells or the stem cell product were reported[9]. The systematic review and meta-analysis of Reichenbach *et al.* with a total of 890 hyaluronic acid and 650 hylan treatments reported 42 (4.7%) local AEs in the hyaluronic acid group and 50 (7.7%) in the high molecular hylan injection group[35]. This would mean that intra-articular treatments with culture-expanded stem cells have at least a comparable number of AE with hyaluronic acid and hylan treatments.

The follow-up period differed greatly among the included studies. Six studies reported a mean follow-up period between 12 and 31 months[9,17–20,23]. The group of Wakitani reported a follow-up range from 2 to 11 years, with a mean follow-up of 6 years[22]. One year of follow-up will probably not be sufficiently long enough to detect all SAEs such as neoplasms. However, many animal studies showed no evidence of neoplasms at stem cell re-implantation sites[36–39]. Of two studies that did show spontaneous malignant transformation of human tissue-derived culture-expanded MSCs, following extended culture and implantation in mice, one was retracted[40,41] and the other discussed[42,43]by the authors in a later paper based on suspected cross-contamination with human fibrosarcoma or osteosarcoma cell lines.

All included studies used autologous bone marrow-derived MSCs. Companies such as Mesoblast are developing off-the-shelf adult stem cell products that are obtained from a single donor, commercially expanded and frozen, and subsequently used in allogeneic recipients. However, knowledge about the safety of the use of allogeneic MSCs is limited. Of the studies included, seven used fetal calf or bovine serum for cell culturing/expansion (Table I)[17–23]. The use of animal-based serum during the expansion of the stem cells could increase the risks of possible disease transmission and reactions of the immune system[44–47]. To assure maximal safety during the period of culture, contact of MSCs with animal-derived supplementary products must be minimal. Therefore the use of alternative methods of cell culturing such as autologous serum and platelet lysate increases. Each change or difference in the culture procedure can influence cell population, cell phenotype and consequently cell behaviour. Therefore this review is only the beginning of exploring the safety of intra-articular treatment with culture-expanded stem cells.

A limitation of our review is that most studies did not classify the observed AEs. Well-described AE collections lacked in all included studies except in the study of Centeno *et al.* 2011[9]. Furthermore, unpublished studies with detrimental results and studies which did not mention AEs, may have caused publication bias. In this review there were two studies with a total of 37 patients excluded because they did not give information on presence or absence of AEs[48,49].

We have extracted the data and subdivided the AEs into three categories: local/systemic, SAE/AE, related/unrelated. Centeno *et al.*, 2011 reported two of the three categories[9], the other studies did not categorise the AEs. Clear classification of AEs for orthopaedic applications of stem cells is warranted in future study reports.

Furthermore, it is not unlikely that uncommon side effects are not reported yet or which may arise after a longer and accurate follow-up. Future studies should include adequate methods regarding the AEs collection using prospective checklists or patient questionnaires/patient diaries for symptoms, (non)invasive techniques for evaluation for structural changes such as enhanced MRI or arthroscopy, and laboratory controls.

In conclusion, intra-articular cell-therapy with culture-expanded MSCs appears to be safe based on 844 treatments in eight studies. Based on the reported AEs and their classification in this systematic literature review we conclude that there are no compelling arguments against proceeding with intra-articular stem cell application in human cases.

Author contributions

CP, ML, MR, GO and PB contributed to conception and design of this study; Study selection was done by CP and PB independently. CP extracted the data of the finally included studies. CP and ML independently assessed the methodological quality of AEs collection of each included study. CP, ML, MR, GO and PB contributed to preparation of the manuscript. The final version of the article was approved by all the authors. CP takes responsibility for the integrity of the work as a whole.

Conflict of interests

The authors have no conflicts of interest to disclose.

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