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Injection of biologic agents for treating severe knee osteoarthritis: is there a chance for a good outcome? A systematic review of clinical evidence

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Abstract. – OBJECTIVE: Osteoarthritis (OA) is the most common degenerative joint disease and the leading cause of disability in the adult population worldwide. The knee is the most prevalent site of symptomatic arthritis. Treatment options for OA include drugs, surgery and, more recently, biological treatments. Injectable ortho-biological treatments include autologous and more rarely heterologous preparations employed inside and outside the operating room to assist bone and soft tissue regeneration. Our aim was to analyze the rationale for use of injectable ortho-biological treatments such as platelet-rich plasma (PRP) and mesenchymal cells from bone marrow, adipose tissue, and placenta/umbilical cord, in patients with severe OA of the knee (Kellgren-Lawrence grade 4).

MATERIALS AND METHODS: A search in PubMed, ScienceDirect and Google Scholar databases was performed using the following keywords: 'knee osteoarthritis' and 'biological treatment' or 'PRP' or 'adipose' or 'mesenchymal' or 'staminal' or 'stem cells'. Manual research throughout the reference lists of all retrieved articles was further conducted.

RESULTS: A total of 16 articles was selected for this systematic review. The rationale for use of each ortho-biological treatment was discussed. The clinical application showed different therapeutic protocols, different follow-up periods, different outcomes analyzed and small sample size.

CONCLUSIONS: Our study did not demonstrate uniform beneficial effects for the use of injectable ortho-biological. This prevents any advice for routine application in the treatment of severe knee OA (K-L IV). Further prospective clinical trials with randomization, larger sample size, and preliminary power calculation are needed to justify the use of injectable biologic agents in grade IV knee OA in everyday practice.

Key Words:
Biologic agents, Orthobiologics, Osteoarthritis, Knee.

Introduction

Osteoarthritis (OA) is a chronic degenerative disease involving joint tissue homeostasis, which very commonly affects the knee joint. With over 260 million people worldwide affected by the disease, OA is thought to be the most prevalent chronic joint disease and the leading cause of disability in older adults[1]. Its vast prevalence in the general population is reflected by dramatic both direct and indirect annual costs, estimated to be in most countries up to 2,5% of the gross domestic product[1,2].

Cartilage damage but also Hoffa's fat pad alteration, ligaments' degeneration, subchondral bone widening, and osteophytes represent the main changes which legitimate to define OA a whole joint disease[3,4]. In early stages of OA, approaches such as microfractures, autologous chondrocyte implantation, and osteochondral grafts have been proven effective with variable results, but a consensus has not been reached yet[5-8]. Conversely, the treatment for advanced OA is well defined and consisting of total joint replacement. Nevertheless, 5.6% of patients experience complications and the revision rate due to the finite lifespan of prosthetic material advises against surgery in young or active patients[9,10].

In this scenario of scarce reliable treatment options for OA arises ortho-biologics, an innovative

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therapeutic approach aimed at reducing symptoms and disease progression, particularly for mild and moderate disease. In the last 10 years, novel regenerative treatment options have gained popularity offering a cost-effective valid alternative to surgery, moving towards a patient-tailored approach. Injectable ortho-biological treatments exploit the use of autologous growth factors and bioactive agents to prevent further cartilage loss and its anti-inflammatory effect fully endorses the new paradigm which considers OA an inflammatory disease. Platelets-rich plasma and mesenchymal cells, extracted from bone marrow, adipose or placental tissue, now represent feasible options to conservatively treat mild symptoms. However, even if injectable biological treatments are now widely used in all stages of osteoarthritis, with no unanimous agreement, they are mostly indicated and investigated in early stages of OA. The validity and reliability of these therapies has not been fully ascertained in patients with advanced OA, therefore with no clear justification for their use. Furthermore, to date there is no systematic review available focused on the effectiveness of injectable biological treatments restricted to radiologically advanced OA of the knee. The aim of the present systematic review is to investigate the rationale for use of injectable biological treatments currently available and to evaluate their efficacy in radiologically advanced knee OA (Kellgren-Lawrence grade 4).

Materials and Methods

The present systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[11]. On December 2021, two independent reviewers (G.A. and P.C.) performed an extensive search in Pubmed, ScienceDirect and Google Scholar databases from January 2011 to December 2021. The research was performed combining the words 'knee osteoarthritis' and 'biological treatment' or 'PRP' or 'adipose' or 'mesenchymal' or 'staminal' or 'placental' or 'umbilical cord' or 'stem cells'. Only research on humans and full-text articles in English were included. The initial screening was carefully performed analyzing title and abstract. Duplicated articles were removed. Eligible articles were downloaded and only research including the results of injectable biological treatments in severe knee OA (K-L IV) were included. Conversely, articles with no clear evaluation of results obtained in K-L IV OA patients were excluded. Patients who underwent concomitant surgical or non-biological injectable treatments were excluded. A flow-chart of the selected articles according to PRISMA[11] is presented in Figure 1.

A manual check in the citation list of the individual selected articles was also performed.

Controversies were analyzed by a third senior reviewer (E.K.) who gave the final approval to the list of papers included.

Results

The present systematic review focused on results retrieved from January 2011 to December 2021. The inclusion of the papers was done with the consensus of all the authors of the present review. A total of 16 papers were considered suitable, and they have been discussed in separate sections below.

Platelet-Rich Plasma

By the 1980's the popularity of autologous blood derived concentrates rapidly started to increase. The idea of using autologous concentrates to treat damaged structures hides the biology of wound healing, primarily constituted of inflammation, proliferation, and remodeling[12]. The role of platelets is fundamental in clot formation such as much as in progenitor cells recruiting. Alpha granules release factors with both anabolic and angiogenic properties, among which somatomedines (IGF-1) platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), hepatocyte growth factor (HGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)[13]. Furthermore, their high concentration in PRP modulates the Wnt/β-catenin pathway, involved in inflammation cascade activation, type II collagen disruption, chondrocytes apoptosis and finally OA progression[14].

The absolute leukocytes count in autologous concentrates is a notable aspect to be discussed as well. Although white blood cells can secrete important growth cytokines,[15] the net effect of their presence in the final product is the increased oxidative stress and inflammation, therefore they can potentially diminish the clinical benefit of the preparation itself[16-19]. Likewise, the iron-based structure of hemoglobin in red blood cells is a possible source of free-radical species, highly damaging for synovial cells and responsible for cartilage impairment[20,21].

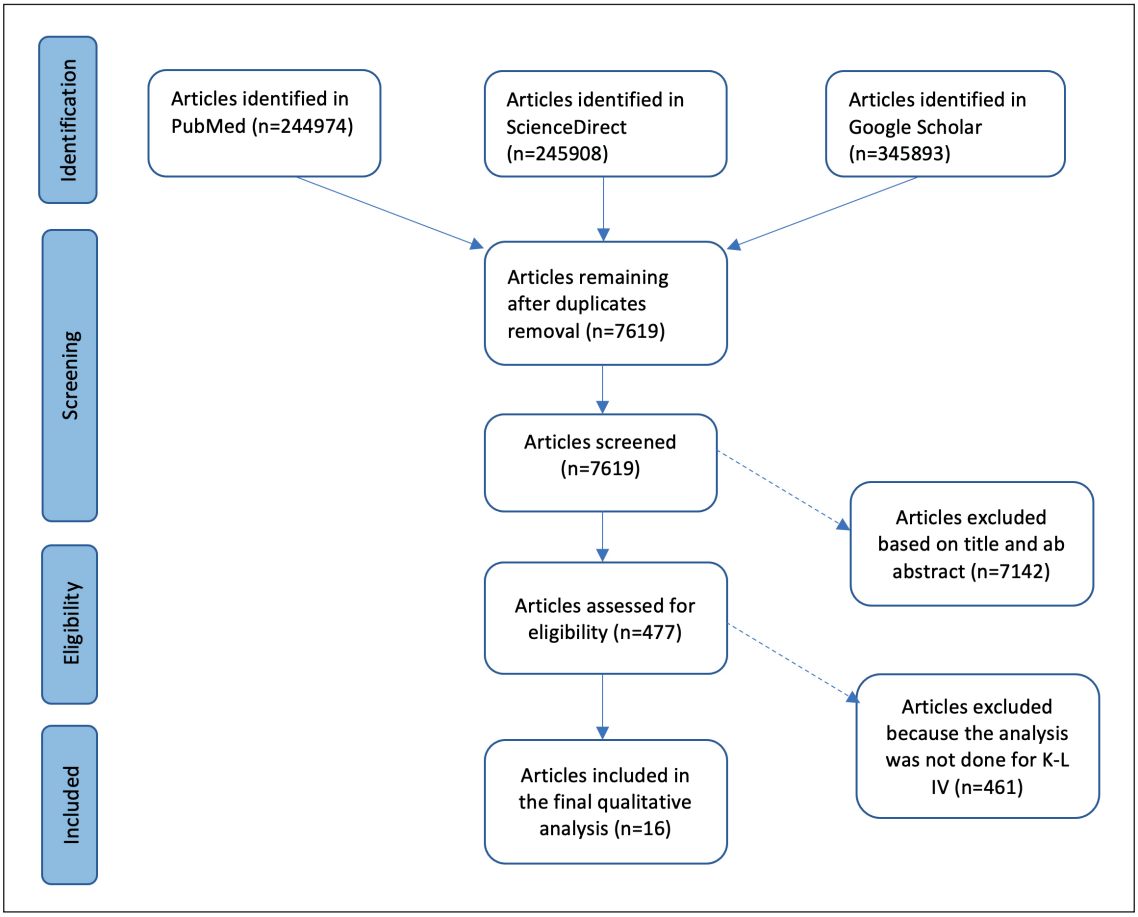

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis) PRISMA flow-chart of the systematic literature review.

The addition of a mixture of calcium and thrombin to activate the PRP is reasonable, but results are scarce and controversial[22-24].

All these aspects justify the variability of PRP characteristics, preventing a universal consensus on PRP and therefore corroborating the commonly accepted idea of considering PRP as a "procedure", not a mere product[25].

Selected studies on the application of PRP in severe knee OA are listed in Table I.

Görmeli et al[26] conducted a RCT on 162 patients comparing the efficacy of PRP (one or three injections), hyaluronic acid and saline (control). 54 patients were affected by grade 4 knee OA. After 6 months, a statistically significant improvement was observed in the IKDC and EQ-VAS scores in all the groups, except for patients injected with saline. Comparing the results among the treatment group, patients treated with three PRP injections achieved significantly better outcomes. The subgroup analysis showed how in the advanced OA group no differences regarding the treatment used (single, multiple PRP or HA injections) was evidenced.

The RCT conducted by Rayegani et al[27] compared PRP to conservative treatment in patients with different stages of knee OA (KL I-IV), evaluating the results up to 6 months. In their study, PRP had better short-term results in pain relief (p-value = 0.006) compared to the control group with no correlation between OA grade with the amount of response to treatment suggesting that PRP could be effective also in severe degrees of OA. Despite these results, the authors suggested the possible bias due to small sample of patients with grades I and IV included in their study compared to the ones with grade II and III.

The last and most recent RCT on PRP in grade IV knee OA was conducted by Ismaiel et al[28]. Sixty patients with grade III and IV knee OA, who already scheduled a total joint replacement, were included and then randomized to either single PRP injection or corticosteroid injection and followed

Table I. Results on PRP.

Study	Study Design	Therapeutic Protocol	Outcomes	K-L IV knees	F.U.	Main Findings
Görmeli et al[26]	Double-blind RCT	3 x PRP *vs.* 1 x PRP *vs.* 3 x HA *vs.* saline	EQ-VAS and IKDC subjective scores	13 (3 x PRP) *vs.* 14 (1 x PRP) *vs.* 14 (3 x HA) *vs.* 13 (saline)	6 months	PRP was superior to control group. However, in advanced OA a single PRP or HA injection was as much effective as multiple PRP injections
Rayegani et al[27]	RCT	2 x LR-PRP injections 4 weeks apart + acetaminophen 500 mg and exercise *vs.* acetaminophen 500 mg and exercise alone	SF-36, QOL, WOMAC	Tibiofemoral (4 *vs.* 0) Patellofemoral (6 *vs.* 1)	6 months	PRP and exercise is superior to exercise alone, but no differences among grades of OA.
Ismaiel et al[28]	Comparative	1 x LR-PRP *vs.* 1 x Corticosteroids	VAS	22 LR-PRP *vs.* 16 C	6 months	VAS score had a statistically significant decrease at 3 and 6 months after the procedure with no differences between the two groups.
Hegaze et al[29]	Prospective Cohort	Maximum 4 PRP injections one months apart	NRS, Range of motion	2 x PRP (32) 3 x PRP (32) 4 x PRP (16)	9 months	Pain and range of flexion improved. Multiple injections gave better results
Kon et al[30]	Prospective	3 x PRP *vs.* 1 x LWHA *vs.* 1 x HWHA	IKDC, EQ-VAS	27 (8 PRP, 9 LWHA, 10 HWHA)	6 months	None of this procedure seems to be highly effective in advanced OA.
Filardo et al[31]	Prospective	3 x PRP injections 21 days apart	IKDC, EQ-VAS	24 Age: range 36-82 Sex: M-F=13:11	24 months	Worsening of all the scores after 24 months in all OA subgroups, after an amelioration at 12 months, however younger patients and early OA had better results.
Sanchez et al[32]	Retrospective	Variable number of PRP cycles (3 injections) (including intraosseous PRP)	Patient Survival (TKA delay of at least 1,5years)	91 Age: median 67	>60 months	Among 91 patients, age <65, three or more PRP cycles and intraosseous PRP achieved in postponing TKA at five years. Patients' survival remains higher in less severe OA

up for 6 months after treatment. In the K-L IV group, VAS score notably improved in both treatment groups at 3 and 6 months follow up (p=0.05 and p=0.025 respectively), although the variations were more significant in the PRP group.

Recently, Hegaze et al[29] conducted a prospective cohort study evaluating the efficacy of repeated PRP injection on pain and articular function in 252 patients, 80 of whom had grade 4 OA. Patients were followed up for 9 months. In their study, PRP injections improved symptoms in grade IV knee OA as much as they did in grade II and III whilst an overall greater improvement in flexion was observed in grade IV knee OA when compared to healthier knees.

The abovementioned satisfactory results on PRP injections in severe knee OA do not reflect the results of a prospective comparative study conducted in 2011 by Kon et al[30].

150 patients affected by cartilage degenerative lesions (Kellgren grade 0), early OA (Kellgren grade I to III), and severe OA (Kellgren grade IV) were enrolled and treated with either 3 injections of PRP alone or one injection of high-molecular weight HA or one injection of low-molecular weight HA. Patients were evaluated at 2 and 6 months and variations of IKDC and EQ-VAS were registered. The PRP group recorded an immediate improvement in IKDC scores and a slight worsening at 6 months follow-up, whereas the group receiving LW HA injections had more stable results in patients with knee affected by severe OA (grade 4), probably due to small amelioration achieved 2 months after the injection. Furthermore, neither PRP nor HA showed statistically significant differences, suggesting that none of them is an effective solution for severe OA.

In the same year Filardo et al[31] published the results of a prospective study on 91 patients (114 knees) treated with three intra-articular injections of PRP and monitored for 24 months. Among the knees treated, 24 were K-L IV. An initial analysis performed at 12 months showed a significant improvement in IKDC objective and subjective score and in EQ-VAS for all the subgroups, although with a greater effect in K-L 0 than K-L IV. All subgroups presented a marked reduction in all the scores at 12 months and 24 months follow-up, suggesting a mean duration of beneficial effects of 11 ± 8 months. Further analysis showed better results in younger patients (p=0.0001) with less extensive cartilage degeneration (p <0.0005), suggesting that PRP could be effective in K-L IV knees, even though not as much as in KL 0-III.

A different approach was adopted by Sanchez et al[32] who evaluated the potential of PRP in postponing and even avoiding total knee replacement in patients with advanced knee OA. The first analysis focused on 186 patients who underwent TKA after PRP injections. Arthroplasty procedure was postponed for more than 1.5 years in 74.1% of patients, with a median of 5.3 years. Secondly, a survival analysis was conducted on 481 patients with grade III and IV OA receiving PRP injections. The analysis showed that 85.7% of the patients treated with PRP did not undergo TKA during the five-year follow-up. A further analysis showed how the severity of knee osteoarthritis did influence the delay to surgical intervention, also considering that survival rates were significantly higher in K-L III patients compared to KL I-V. Statistical analysis showed how among the 91 patients who presented with severe OA, only patients younger than 65 years did benefit from the treatment and were able to postpone the TKA for five years.

Mesenchymal Stem Cells

The biological potential of MSCs, widely investigated in orthopedics, holds the key to cartilage regrowth and anti-inflammatory cytokines release.

Oxidative stress, favored by pro-inflammatory cytokines, drives the osteoarthritic changes of the articular environment, facilitating cartilage degeneration. Mitochondrial dysfunction seems to play a role in the ability of cells to defend themselves from oxidative stress. Electron transport chain impairment in chondrocytes leads to a reduction in ATP formation[33-35]. The adenosine derived from ATP is a fundamental nucleotide of the extracellular space where it inhibits apoptosis and promotes cell survival. When adenosine concentration is reduced, the expression of metalloproteinases (MMPs) raises, as well as cartilage degeneration[36-38]. MSCs are able to secrete exosomes, nanometric extracellular vesicles, containing ATP-producing enzymes, switching off the inflammation[39,40].

After being transplanted inside the joint, the interaction between CXCR-4 and stromal-derived factor-1, drives the MSCs to the injured site, the so called ‘homing effect’, though the ability to engraft in the cartilage tissue and differentiate in chondroblasts and chondrocytes is not clearly demonstrated[41-44]. Therefore, the “secretome” effect [45] could be the leading factor in influencing cartilage repair through anti-inflammatory (i.e.

HIF, bFGF, TNF-a, IGFs, VEGF) and pro-inflammatory (IL-1b, MMP-3 among others) cytokines with a net effect of reduction of inflammation in the articular space. Moreover, few studies reported that hypoxia-inducible factor (HIF) and insulin-like growth factor-1 (IGF-1) stimulate chondrogenesis and drive MSCs proliferation itself[46,47].

The use of the term 'pure MSCs' should be avoided since mesenchymal cells are not separated from the other cells of the graft's micro-environment, and consequently the procedures performed are straightforwardly addressed at concentrating tissues with minimal manipulation, originating bone marrow aspirate concentrates (BMAC) and stromal vascular fraction (SVF). Minimally manipulated tissues containing MSCs can be obtained from a variety of harvest sites such as bone marrow, umbilical cord, placenta, or adipose tissue.

Bone Marrow Aspirate Stem Cell Concentrate (BMAC)

List of the studies focused on results of BMAC treatment for severe knee OA (K-L IV) is presented in Table II. Hernigou et al[48] conducted a study on 30 patients with bilateral severe OA secondary to corticosteroids-related osteonecrosis. All the patients were eligible for bilateral joint replacement surgery and during the same session one knee received TKA whilst the other knee was treated with a subchondral injection of bone marrow concentrate, in both the femur and tibia. At a mean follow-up of 12 years, subchondral BMAC injection demonstrated a lower complication rate, quicker recovery and a higher patient satisfaction when compared to TKA. Interestingly, only six of the TKA knees needed following surgery compared to only one knee treated with subchondral BMAC. The Knee Society Score improved in both groups. Notably, 21 patients declared to prefer the knee subjected to BMAC injection whilst only 9 preferred the knee underwent to surgery. Furthermore, authors declare that cartilage impairment and bone marrow lesions improved in the site of BMAC injection.

Vad et al[49] evaluated the use of BMAC injection in four patients affected by grade IV OA. At an average follow-up of 14 months both the WOMAC and the NRS scores improved significantly, and MRI showed an average increase of the matrix thickness of 14.1%. The K-L IV group showed poorer results compared to K-L III knees. The average NRS score improved by 3.8 points ($p < 0.01$) and WOMAC score improved with a mean of 15.5 points ($p < 0.01$). The intra-articular matrix increased of 5% in one patient out of four (25%), $p < 0.01$.

Mautner et al[50] recently conducted a retrospective study comparing the injections of BMAC *vs.* micro-fragmented adipose tissue (MFAT) in the treatment of 106 knees with all grades of OA progression. All the pain and functional clinical parameters, evaluated by VAS, KOOS and EQOL scores, significantly improved, independently of the autologous tissue sources. 25% of improvement in the VAS score was the cut-off to define the patient as 'responding'. For K-L IV patients, a 55.6% responding rate was found (10 out of 18 patients), with no specifications on which treatment offered the most valuable results.

Kim et al[51] evaluated the efficacy of a combined injection of BMAC mixed with adipose tissue-derived mesenchymal cells. Results in the K-L IV knees were not as satisfactory as observed in less compromised knees in terms of VAS, IKDC, SF-36, Lysholm and KOOS scores. Results at 12 months showed a decreased VAS score compared to baseline from 8.2 to 5.7. The IKDC score incremented from 35.5 preoperatively to 52.4. The SF-36 raised from 25.1 preoperatively to 34.9 at 12 months. The KOOS score increased from 34.3 to 63.1. The Lysholm score increased from 37.7 preoperatively to 62.1. Finally, a modest amelioration was appreciable in K-L IV knees, but it was most significant in K-L I-II and K-L III ($p = 0.002$).

Adipose Mesenchymal Cells

Mesenchymal cells of the adipose tissue were first studied in 2002[52] and owing to their abundance, the safety and the ease of harvesting, Adipose Derived Stem-Cells (ADSCs) are becoming an attractive approach to regenerative medicine.

The stromal vascular fraction (SVF) containing endothelial cells, pericytes, leukocytes and ADSCs is separated from adipocytes through mechanical or enzymatic digestion with collagenase. Even though the literature available on the effectiveness of ADSCs on osteoarthritis has become abundant, only few papers differentiated their results based on OA radiological grades (Table III).

Lapuente et al[53] performed a retrospective study including 50 patients who underwent SVF bilateral knee injection and completed a 1-year follow-up. 50% of the knees suffered from severe OA (K-L IV). In order to evaluate the clinical outcomes, VAS, WOMAC and Lequesne Index were administered to patients before the procedure and at 3, 6 months and 1 year of follow-up. Ultrasound

Table II. Results on bone marrow aspirate concentrate.

Study	Study Design	Therapeutic Protocol	Outcomes	K-L IV knees	F.U.	Main Findings
Hernigou et al[48]	RCT	BMAC subchondral in one knee *vs.* TKA in contralateral knee, in patients with bilateral grade IV OA secondary to osteonecrosis from corticosteroids	Knee Society score, X-Rays, MRI	60 (30 patients) Sex:M-F=12-18 Age: mean 28 (range 18-41)	Mean 12 years, range 8-16 years	Subchondral autologous bone marrow concentrate was effective and had a more rapid recovery compared to TKA.
Vad et al[49]	Prospective Observational	BMAC subchondral	NRS, WOMAC, MRI	4 Age: Mean 62,8	Mean 14 months, range 13-15 months	Good results in terms of pain and function but less severe OA patients achieved better improvement.
Mautner et al[50]	Retrospective	BMAC *vs.* MFAT	VAS, KOOS, EQOL	18	Mean 1.80 ±0.88 years for BMAC and 1.09±0.49 years for MFAT.	Responders were 55,6% (10 out of 18). Among all treatment groups, BMAC and MFAT groups had significantly improved pain and function compared with their baseline without a significant difference in improvements between the two groups. Results in KL IV were not statistically significant.
Kim et al[51]	Clinical Trial	BMAC + SVF	VAS, IKDC, KOOS, SF-36, Lysholm Knee Questionnaire	6	Mean 8,7 months (range 6-19)	Good results were shown including the decrease in pain and the improvement of clinical and functional results of knees treated. 4 out of 6 knees showed poorer results compared to less severe OA knees.

evaluation was performed to establish possible variations in the cartilage characteristics. All the clinical assessment scores showed a statistically significant improvement 12 months after the procedure, regardless of sex and age of the patients. The mean value of Laquesne index in the K-L IV group was 13,77 before treatment, after one year the mean decreased to 5,05. Baseline mean of the VAS score for pain was at 7,70 before the procedure and 3,96 after 12 months. WOMAC score mean values decreased as well from 52,8 before the implantation to 23,8 after one year. Also, the ultrasound revealed a notable, even if minimal, improvement in the soft tissue-cartilage interface characteristics.

Tsubosaka et al[54] prospectively evaluated the clinical results of 10 patients with K-L IV osteoarthritic knees treated with intra-articular SVF injection. VAS, WOMAC, Japanese Knee Osteoarthritis Measure (JKOM) and KOOS scores were administered prior to surgery and 12 months after the procedure. Although there was no statistically significant improvement, probably due to the small sample of patients, the mean improvement rate was 28.8 ± 22.1 for WOMAC, 13.0 ± 18.3 for VAS score, 5.7 ± 27.8 for JKOM score and 17.7 ± 21.2 for KOOS score. Authors stated that clinical improvement was higher in mild-to-moderate OA rather than severe OA (K-L IV).

Simunec et al[55] enrolled 6 patients affected by radiologically confirmed advanced OA (K-L grade 4), equally divided into two treatment groups (SVF and SVF+PRP). Clinical outcome was assessed by KOOS score before the procedure and after 1, 3, 6, and 12 months. In the group treated with SVF only, mean KOOS value dropped by 7.7% at 1-year follow-up, whilst for patients treated with SVF+PRP the mean KOOS decreased by 28.8%, at the same time point. Moreover, a subjective patient evaluation was performed 8 months after the procedure, showing that all the patients treated with SVF alone would not recommend the procedure, meanwhile all patients treated with both SVF and PRP were very satisfied.

Bakowski et al[56] included 37 patients in their study aimed at evaluating the efficacy of SVF in knee OA, that comprised a total number of 7 patients with OA grade IV. At baseline and after a mean of 27 ± 6.5 months after the implantation, patients were administered with questionnaires as the KOOS, the International Knee Documentation Committee 2000 (IKDC 2000), the WOMAC, the Numeric Rating Scale (NRS) and the Health Questionnaire EQ-5D-5L. In this case series all the patients affected by OA in grade 4 stage worsened in each score, except EQ-5D-5L.

Amniotic Derived Products

Since the first preclinical studies, cryopreserved umbilical cord and amniotic membrane (AMUC) demonstrated their ability in attenuating cartilage loss in OA[57].

We selected one study (Table IV) describing the safety and efficacy of placental-derived stem cells for the treatment of severe knee OA, with no concomitant procedure performed. Mead et al[58] treated 42 patients with KL III (36%) and KL IV (64%) with an AMUC obtained from donated human placenta tissue. In KL grade 4 patients, clinically significant improvement in knee pain and function was reported in 67% (18/27) of patients, measured through the Patient Global Impression of Change (PIGC score). Furthermore, pain and function, monitored with the Global Perceived Improvement score (PGI) improved by a mean of 11.5 ± 4.9 months, and the OMERACT–OARSI treatment response rate was 74% (20/27), 12 months after the procedure.

Discussion

The main result we can discern from the present systematic review is the extreme variability of the studies in the field of ortho-biologics. PRP is one of the most commonly viable biological treatment currently available; however, its characteristics legitimate to define it as a procedure more than a product, therefore underpowering any possible comparison. The variability of platelets blood count among people, along with the technical impossibility of analyzing platelets concentration in everyday practice are the main responsible for the discrete quality of methodology in most studies. Moreover, even if well conducted, the paper we processed are biased by different administration protocol, different outcomes analyzed and variable follow-up periods. Furthermore, our research was focused on K-L IV OA patients, which often represented a very small study sample. Evenly, although authors reported separated outcomes for severe OA patients, the epidemiological characteristics of the included patients are not always clearly divided among the OA subgroups, therefore undermining our ability to significantly compare the age and the sex of the treated patients, two well-known factors involved in OA response to treatments.

Table III. Results on Adipose-Derived Stromal Vascular Fraction.

Study	Study Design	Therapeutic Protocol	Outcomes	K-L IV knees	F.U.	Main Findings
Lapuente et al[53]	Retrospective	Bilateral SVF injection	Lequesne, WOMAC, VAS scales, ultrasound control, quantification of the biochemical profiles of synovial fluid.	50 Age: Range 50-89	12 months	Patients manifested satisfaction at 12 months follow-up. Minor clinical improvement was evidenced in grade 4 when compared to grade 3 OA.
Tsubosaka et al[54]	Prospective Case Series	SVF injection	WOMAC, VAS, KOOS	10	12 months	The improvement rate of WOMAC and JKOM scores from baseline was worse for grade IV. No statistically significant difference in improvement for VAS, JKOM, and KOOS among the OA grades.
Simunec et al[55]	Comparative Case Series	SVF alone *vs.* SVF+ PRP injection	KOOS, MRI, Subjective Evaluation	6 (3 SVF, 3 SVF+PRP)	12 months	Patients treated with SVF+PRP showed better results in terms of symptoms than patients treated with SVF alone.
Bąkowski et al[56]	Retrospective	SVF injection	KOOS, IKDC 2000, WOMAC, EQ-5D-5L, NRS.	7	27±6.5 months	6 out of 7 patients were unsatisfied

Table IV. Results on Placental/Umbilical cord stem cells.

Study	Study Design	Therapeutic Protocol	Outcomes	K-L IV knees	F.U.	Main Findings
Mead et al[58]	Retrospective	AM/UC single injection	PGIC, GPI, OMERACT–OARSI responder criteria	27	12 months	Intra-articular injection of AM/UC particulate may be effective in alleviating pain and improving function in patients with severe knee OA

Lastly, regulatory shortcuts such as the 501(k) exemption[59] allowed to introduce in the market a variety of commercial kits if almost equivalent to those already commercialized, increasing variability of the technical procedure adopted to obtain the concentrates.

Despite such notable biases, the RCTs we analyzed reported superior outcomes of PRP when compared to other treatments, whereas the other studies we reported, clearly demonstrated a higher variability in results, albeit conferring to PRP the possibility to often relieve symptoms in short term.

The same variability of results is observed when analyzing the current medical use of mesenchymal cells extracted from bone marrow and adipose tissue. Unlike PRP, BMAC and SVF are the results of surgical operations. Even if the surgical technique of tissue harvesting delineates a simple procedure, they can be followed by complications, usually temporary, such as pain and, for lipoaspirate, hematoma centered in the donor site.

Only one randomized controlled trial was included in the present systematic review, which shows effectiveness of BMAC. Conversely, the other studies included evidenced how the clinical efficacy of this procedure did not fully satisfy the outcomes expected.

However, the aspects concerning the variability in methodology analyzed before in the discussion should be evenly considered when considering BMAC and SVF injection. Besides, the variability increases in operator-dependent procedure, possibly a further confounding factor when analyzing clinical results.

The lack of RCTs for the use of SVF among the studies included is the possible natural consequence of the aforementioned biases. The largest study evaluating the clinical impact of SVF injection for severe knee OA demonstrated good outcomes at 12 months, although the data is undermined by the methodology of the study itself. Indeed, it should be avoided the inclusion of patients who underwent bilateral interventions since the impossibility to objectively evaluate one side completely separated from the contralateral. The other studies included did not show significant clinical benefits after at least one year of follow-up, even if consisting of too small sample size to satisfy adequate power calculation.

Lastly, the use of mesenchymal cells obtained from cryopreserved umbilical cord and amniotic membrane (AMUC) appears to be promising in the treatment of degenerative pathologies, although arising questions in terms of ethical aspects and costs.

Currently, injectable ortho-biological treatments represent an expensive alternative to other non-surgical or non-biological available approaches when treating advanced knee OA. The rationale for their use is proven by robust preclinical and clinical studies, but their current application as a 'standard of care' should not be recommended in advanced knee OA, due to the current lack of solid evidence. Nonetheless, their application in earlier stage of the disease seems to offer greater results compared to later stages, suggesting a research direction for the near future.

Conclusions

The available literature on injectable ortho-biologics in the form of PRP, bone marrow, adipose and placental mesenchymal cells preparations did not demonstrate uniform beneficial effects. This prevents any advice for routine application in the treatment of severe knee OA (K-L IV). Further prospective clinical trials with randomization, larger sample size, and preliminary power calculation are needed to justify their use in everyday clinical practice.

Conflict of Interests

EK reports consultancy for Cartiheal Ltd, Fidia Farmaceutici Spa and Greenbone Ortho Srl, payment for lectures including service on speakers' bureaus from Zimmer Biomet, and stock/stock options in Cartiheal Ltd, all outside the present article. BDM reports consultancy for Cartiheal Ltd, outside the submitted work. The other authors declare no conflict of interests.

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Authors' Contribution

GA wrote the paper and searched for articles, PC contributed to writing and searching for the papers, EMB and MM critically revised the manuscript, BDM apported revisions and critically analyzed the manuscript, EK provided the work project, coordinated the writing, and revised the last version of the paper. All the authors gave their approval to the final version of the present manuscript.

Patient consent for publication

Not applicable.

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