Ortho-Biologics for Osteoarthritis

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KEYWORDS

• PRP • Autologous conditioned serum • Stem cell • Bone marrow • Osteoarthritis

KEY POINTS

- This review seeks to shed light on the current literature in the use of key ortho-biologics and their potential use in the treatment of osteoarthritis.
- The literature supports the safety of viscosupplementation, platelet-rich plasma, autologous conditioned serum, bone marrow aspirate concentrate and adipose derived stromal cell therapy in the treatment of OA.
- The clinical efficacy of these treatments continues to be investigated in comparative studies and meta-analyses.
- Further work is still required to fully understand the clinical role for orthobiologics in OA treatment.

INTRODUCTION

Osteoarthritis (OA) is a debilitating disease affecting approximately 27 million Americans.[1] The most common symptoms of OA are pain and physical limitations that have a significant effect on people's quality of life and their social and economic activities.[2,3] Because of the increasing life expectancy, increasing numbers of elderly, and increasing prevalence of obesity in North America, the prevalence of OA will continue to increase. There are currently limited options for treatment and prevention of OA, with joint replacement often the ultimate outcome. The cost of joint replacements is around $55,000 per person with complication rates of approximately 1% to 10% and mortality rates of 0.25%.[4] In order to reduce costs to the medical system and the risks and costs to patients, we need a better understanding of the disease pathophysiology, improved early detection, and strategies for disease prevention and early disease management. Ortho-biologics may be one such option for the treatment of OA.

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Ortho-biologics as defined by the American Academy of Orthopaedic Surgeons (AAOS) are biological substances found naturally in the body that help injuries heal more quickly.[5] These substances include any biologically derived conductive material that aids in repair and regeneration of bone, muscle, tendons, ligaments and cartilage. There are many treatments that now fit under this overarching term. These treatments include platelet-rich plasma (PRP), prolotherapy, ozone therapy, autologous conditioned serum (ACS), bone marrow aspirate concentrates (BMACs), adipocyte-derived stem cells, mesenchymal-derived concentrates, amniotic-derived cell concentrates, cord blood–derived cell concentrates, interleukin therapies, and alpha-2 macrophages. For the purpose of this review, the authors focus on viscosupplementation, PRP, ACS, BMACs, and other cell-derived therapies, as these are currently in clinical use.

VISCOSUPPLEMENTATION

Viscosupplementation consists of hyaluronic acid (HA) treatments injected into the joint for pain relief and possible antiinflammatory effect.[6] HA is an anionic, nonsulfated glycosaminoglycan found in connective tissues, epithelium, and neural tissue. It is formed in the plasma membrane and is one of the main components of the extracellular matrix, contributing to cell proliferation and migration. HA is found within joints providing viscoelastic properties to the synovial fluid. In OA, there is a reduction in HA synthesis with increased HA degradation, in turn, leading to a lower molecular weight in the synovium, synovial fluid, and cartilage.[7] HA therapy provides relief via various pathways, including suppression of proinflammatory cytokines and chemokines through the synthesis of antiinflammatory mediators.[8] In a systematic review by Altman and colleagues, 48 articles were analyzed to evaluate the antiinflammatory effect of HA in OA.[9] They found that proinflammatory cytokines (interleukin 1β [IL-1β]), tumor necrosis factor α (TNFα), and interferon γ can regulate HA synthase expression. HA binds to cell surface receptors, such as CD44, toll-like receptor (TLR) 2 and 4, layilin, and intracellular adhesion molecule-1 (ICAM-1). In binding to CD44, it suppresses proinflammatory cytokines, matrix metalloproteinases (MMPs), proteoglycans, and prostaglandin E_2 synthesis via CD44 through the downregulation of nuclear factor (NF)-κB. HA also activates the innate immune response via TLR-2. HA treatment was shown to bind to TLR-2 and TLR-4 and decrease TNFα, IL-1β, IL-17, MMP13, and inducible nitric oxide. Layilin is expressed in human articular chondrocytes and synoviocytes; by binding to layilin HA suppressed the expression of IL-1β and MMP1 and 13. ICAM-1 activates the NF-κB regulatory system activating proinflammatory cytokines; HA binds to ICAM-1 and inhibits its action thereby preventing inflammation.[9,10]

Early studies of HA treatments in OA had mixed results. In a large meta-analysis of 89 trials containing 12,667 participants, 71 studies showed a modest effect in decreasing pain, whereas the remainder showed no effect. Fourteen studies had significant adverse effects related to HA injections. Rutjes and colleagues[11] concluded based on these early studies that HA therapy had a clinically irrelevant benefit with significant adverse reactions.

Miller and Block[12] did 2 meta-analyses evaluating 26 articles with a total of 4866 subjects for the safety and efficacy of HA. They found that there was a large treatment effect for up to 26 weeks for pain relief and improved Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores. There were no significant adverse effects reported in this series of studies.[13] In another meta-analysis of high-quality level 1 randomized controlled trials (RCTs), 12 studies consisting of 1794 participants were analyzed. Early on, between 1 and 3 months, corticosteroid injections had improved outcomes in the WOMAC score and lower visual analog scale (VAS) scores. However, at 6 months, the effect of HA was better than corticosteroids in OA.[14] In

another study of 13 articles, HA was shown to have greater effects up to 1 year compared with nonsteroidal antiinflammatories and corticosteroids.[15] Bhandari and colleagues[16] reviewed 8 meta-analyses and found that by 26 weeks there were significant improvements in pain, functional scores, and stiffness after HA injections in patients with mild to moderate OA. In addition, they found HA to be well tolerated and safe. Importantly, they observed that HAs with a molecular weight greater than 6000 kDA or greater had the greatest treatment effect on pain at 13 weeks and *3000 kDA or greater has the greatest treatment effect on pain at 26 weeks*. In addition to one-time injections, patients often require multiple treatments. A meta-analysis of 7404 patients showed that repeat HA injections were safe in patients with OA. In 95% of patients who had an adverse event, it was at the time of the first treatment; there was no increase in frequency or severity of adverse events with repeat treatments. The adverse event rate was 0.008 with repeat injections.[17]

In light of the mixed results in the literature and the changes in AAOS guidelines, a US and a European consensus were formed to help guide the use of HA in OA. The European Viscosupplementation Consensus Group determined that, based on an extensive review of the literature, if HA injections were successful previously, a repeat attempt at treatment should be undertaken. They also recommended the use of HA injections in young patients at high risk of progression of OA and competitive athletes in a possible attempt to slow the progression of OA.[18] A similar US task force of rheumatologists, orthopedic surgeons, physiatrists, sports medicine physicians, and nurses was formed to study HA injections in OA. They reviewed 100 studies that suggested HA was superior to placebo treatments. Based on these studies, they came up with 8 various clinical scenarios by which to use HA injections (3 appropriate uses and 5 unclear uses)[19] (**Table 1**).

Table 1
Clinical scenarios for the use of HA listed by Bhadra and colleagues

1. Symptomatic adults with mild or moderate OA of the knee who have clinically and radiologically confirmed disease who have not received other therapies for the knee	Appropriate
2. Symptomatic adults with severe mild or moderate OA of the knee who have clinically and radiologically confirmed disease and have failed other nonpharmacologic or pharmacologic therapies for the knee	Appropriate
3. Symptomatic adults with mild or moderate OA of the knee who have clinically and radiologically confirmed disease who have incomplete response to other therapies for the knee	Appropriate
4. Symptomatic adults with mild or moderate OA of the knee who are intolerant of, have a high-risk of adverse reaction to, or who are contraindicated for pharmacologic agents for the knee (oral, topical, or intra-articular)	Unclear
5. Symptomatic adults who have mechanical meniscus pathology with underlying OA of the knee	Unclear
6. Symptomatic adults with OA of the knee who have had a significant adverse reaction to an intra-articular HA product	Unclear
7. Symptomatic adults with OA of the knee who have active inflammatory arthritis (rheumatoid arthritis, gout, and so forth)	Unclear
8. Symptomatic adults with OA of the knee who have synovitis of the knee with significant effusion	Unclear

From Bhadra AK, Altman R, Dasa V, et al. Appropriate use criteria for hyaluronic acid in the treatment of knee osteoarthritis in the United States. Cartilage 2017;8(3):239; with permission.

In practice, HA is widely used as a part of the treatment algorithm for mild to moderate OA despite the lack of consensus and the current US and Canadian treatment guidelines. It likely has some benefit in certain patients and is worth a trial of treatment in those who are candidates.

PLATELET-RICH PLASMA

As cartilage is nonvascular, its nourishment is based on diffusion. Therefore, intra-articular injections at high concentrations are often the preferred method to aid in cartilage regeneration. PRP, which has a higher concentration of platelets than whole blood, has been an interesting option for use in OA. PRP is a natural concentrate of autologous factors obtained by centrifugation or filtration of the patients' blood. It is obtained at a low cost, simple to obtain, and minimally invasive. PRP is thought to work via biologically active proteins (including platelet-derived growth factor [PDGF], transforming growth factor [TGF], insulinlike growth factor, fibroblast growth factor, and vascular endothelial growth factor [VEGF][20]) expressed by platelets leading to gene expression by binding to transmembrane receptors in target cells. PDGF is a chemoattractant and stimulator of cell proliferation. TGF is a polypeptide that is abundant in platelets and bone and plays an important role in wound healing; it may negatively influence angiogenesis and promotes matrix production by fibroblasts and stimulates the production of VEGF. VEGF is a family of proteins that act through the kinase family expressed on endothelial cells, which stimulate blood vessel formation and exert a trophic effect on endothelial cells. VEGF is also proinflammatory and stimulates leukocyte adhesion to endothelial cells. As a result of these growth hormones, cellular recruitment, migration, growth, and morphogenesis are triggered and inflammation is decreased.[21] Therefore, it has been widely used and studied as a noninvasive treatment of cartilage regeneration in OA.

As PRP is an autologous product, there is a lot of variability within individual patients. Differences in patients' daily platelet levels, procurement methods, concentration mechanisms, and exogenous factors to enhance platelet activation can all contribute to varied PRP preparations. Platelet concentration varies significantly between procurement method and time of draw.[22,23] Platelet concentrates have been recorded as between 200×10^3 and 1000×10^3 platelets per microliter, with no consensus existing as to which concentration has the best outcomes. However, concentrations greater than this have been demonstrated to be biologically unfavorable.[23,24] In addition to the variation in draw times and platelet concentration, there can be variability in leukocytes within the RPR formulation. It is debatable whether leukocytes are beneficial or detrimental, as they have the potential to aid in healing; however, they can also be the cause of increased injury and adverse reactions.[24] Leukocytes adversely increase local inflammation, beneficially produce VEGF, have antimicrobial effects and are restorative to tissues.[25–27] The addition of leukocytes to PRP has also been shown to enhance the concentration of growth factors in PRP.[27] There are 2 different types of commercially available system for PRP: one producing a leukocyte-rich PRP (LR-PRP) and the other producing a leukocyte-poor PRP (LP-PRP). A buffy coat system, which uses a high centrifugation rate for a longer time, produces LR-PRP.[28] Plasma-based systems produce LP-PRP; it uses slower centrifugation or filtration for a shorter time.[28] The literature is still split on the benefit of LR-PRP versus LP-PRP for a given for a given clinical indication. Exogenous factors can also be added to PRP formulations, the most common being thrombin. Thrombin activates platelets and is often used in combination with

calcium chloride.[22] Thrombin plus calcium chloride was shown to increase the release of growth factors in PRP, releasing 100% of growth factors by 1 hour.[29]

Preclinical studies have been supportive of the use of PRP for the regeneration of joint tissue in OA. PRP increases chondrocyte proliferation and increases the production of proteoglycans and type II collagen in vitro.[30–33] In animal models PRP leads to improved cartilage regeneration,[34] and enhances meniscal cells[35] and synoviocytes.[36] PRP has also been shown to have an antiinflammatory effect.[37,38] Based on these studies of the basic biology involved in PRP, there is evidence to support that PRP enhances cartilage repair and slows degradation.

The initial investigation into the use of PRP injections to treat OA was published in 2008. It was a retrospective observational study of 60 patients, which showed favorable outcomes after intra-articular PRP injections.[39] It was not until 2012 that the first RCT was published. To the authors' knowledge since then, 7 systematic reviews/meta-analyses have been published. This section summarizes the current clinic evidence for PRP in OA focusing on meta-analyses. **Table 2** shows a summary of these articles.

Chang and colleagues[40] in 2014 performed a systematic review and meta-analysis analyzing the effectiveness of PRP in treating chondral lesions in the knee. The investigators included 8 single-arm studies, 3 quasi-experimental studies, and 5 RCTs consisting of 1543 subjects. PRP showed efficacy for 12 months after injection and its effectiveness was better and more prolonged than HA injections in patients with mild-moderate OA.[40] A level 1 systematic review and meta-analysis performed by Laudy and colleagues[41] in 2014 compared PRP with HA and placebo. Six RCTs and 4 non-RCTs were included. They found improved functional outcomes of WOMAC, the VAS, and Lequesne index after PRP injections compared with HA and placebo.[41]

In another meta-analysis of PRP in OA, the use of LR-PRP and LP-PRP was investigated and clinical outcomes (WOMAC and International Knee Documentation Committee [IKDC]) and adverse effects were compared. They included 6 RCTs and 3 retrospective studies containing 1055 participants. LP-PRP had better WOMAC and IKDC scores than HA or controls, whereas there was no difference in LR-PRP scores. Both LP-PRP and LR-PRP had higher adverse reactions compared with HA and controls, being primarily swelling and pain.[42]

Meheux and colleagues[43] performed a systematic review of level 1 RCTs to determine whether PRP improves patient-reported outcomes at 6 and 12 months and to determine any differences between PRP or HA or placebo treatment at 6 and 12 months. After a quality assessment using the modified Coleman methodology score, 6 articles were analyzed. All but one study showed significant differences in clinical outcomes between groups for pain and function. Posttreatment PRP scores were significantly better than for HA at 3 and 6 months. In addition, PRP injections resulted in significant clinical improvements up to 12 months.[43] In another systematic review by Sadabad and colleagues[44] in 2016 evaluating 7 studies consisting of 722 participants, they found that PRP led to significantly improved WOMAC scores compared with HA.

In the most recent meta-analysis by Dai and colleagues,[45] 10 RCTs consisting of 1069 participants were used to compare PRP injections with HA at 6 and 12 months. At 6 months there was no difference in clinical outcomes between HA and PRP treatments; however, by 12 months PRP treatment resulted in significantly improved WOMAC, IKDC, and Lequesne scores.[45]

Overall the body of literature suggests that PRP is a promising therapy for symptom relief and improved functional outcomes in patients with OA for at least 12 months.

Table 2
Summary of meta-analyses looking at PRP

Study	Studies Included	Databases	Dates	Comparison	Sample Size	Average Follow- up	Outcome Measures	Results
Chang et al,[40] 2014	16 Studies • 8 single arm • 3 quasi-experimental • 5 RCTs	MEDLINE	2010–2013	PRP vs HA	1543	12 mo	IKDC KOOS WOMAC	PRP significantly improved scores more than HA. PRP was more effective in less severe OA.
Laudy et al,[41] 2014	10 Studies • 6 RCTs • 6 non-RCTs	MEDLINE Embase CINHAL Web of Science Cochrane database	2011–2013	PRP vs HA PRP vs placebo	1110	6 mo	WOMAC VAS Lequesne	PRP significantly improved scores than HA. PRP significantly improved scores more than placebo.
Riboh et al,[42] 2015	9 Studies • 6 RCTs • 3 prospective	MEDLINE Embase Cochrane database	2011–2013	LP PRP vs LR PRP	1055	Not reported	IKDC WOMAC Adverse reactions VAS Lequesne Tegner Marx KOOS SF-36 MRI	LP-PRP improved WOMAC scores compared with placebo. There were similar adverse events between LP-PRP and LR-PRP.

Meheux et al,[43] 2016	6 Studies	PubMed Cochrane database Central register of controlled trials Scopus Sport discus	2011–2015	PRP vs HA	739	6–12 mo	WOMAC IKDC KOOS VAS Lequesne	PRP had improved outcomes compared with baseline greater than HA.
Sadabad et al,[44] 2016	6 Studies	PubMed Cochrane database Scopus Void database	2005–2015	PRP vs HA	722	5–48 wk	WOMAC	PRP significantly improved WOMAC scores than HA.
Dai et al,[45] 2017	10 RCTs	PubMed Embase Scopus Cochrane database	2011–2016	PRP vs HA PRP vs saline	1069	3–12 mo	WOMAC IKDC Lequesne	At 6 mo, there was no difference between treatments. At 12 mo, PRP had improved outcomes compared with both HA and saline.

Abbreviations: CINHAL, Cumulative Index to Nursing and Allied Health Literature; IDKC, International Knee Documentation Committee; KOOS, Knee Injury and OA Outcome Score; SF-36, 36-Item Short-Form Health Survey.

LP-PRP provided better functional outcomes compared with placebo versus LR-PRP, whereas both have increased adverse events compared with HA or placebo. Further work needs to be done to determine if it has any disease-modifying effects.

AUTOLOGOUS CONDITIONED SERUM

Inflammation has been shown to play a key role in the pathophysiology of OA. Proinflammatory cytokines and MMPs are upregulated in the synovial fluid and tissue of patients with OA,[46] including significantly increased levels of IL-1 receptors on chondrocytes[47] and synovial fibroblasts.[48] IL-1 receptor antagonist (IL-1Ra) is a competitive receptor antagonist and natural inhibitor of IL-1, which blocks IL-1's signaling activity.[49] It was proposed as a therapeutic agent in the early 1980s.[50] Meijer and colleagues[51] created an ortho-biologic based on this known as ACS, marketed as Orthokine. ACS is a process by which venous blood is collected and rapid synthesis of IL-1Ra, IL-4, IL-10, and growth factors are stimulated with glass beads. Orthokine has been on the market since 1998 and has been used in both animal models and orthopedic patients. One proposed application is in patients with OA.

In a level 1 RCT by Baltzer and colleagues[52] in 2008, 376 participants were treated with ACS, HA, or placebo. Participants were followed for 26 weeks using an intention-to-treat analysis. Outcome measures including VAS, WOMAC, Short-Form 8, and the global patient assessment, were assessed at baseline, 7, 13, and 26 weeks. The ACS group had improved WOMAC, VAS, and Short-Form 8 scores compared with baseline and a larger improvement compared with the HA-treated group. At 2 years after treatment, outcomes persisted in the ACS group over the HA and placebo group.

Auw Yang and colleagues,[53] in a 30-month multicenter RCT, compared ACS with a saline control in decreasing symptoms of OA. One hundred sixty-seven participants were treated with either saline or ACS over 3 weeks. Participants completed the VAS, Knee Injury and OA Outcome Score (KOOS), the Knee Society Clinical Rating System, and the WOMAC scores at baseline, 3, 6, 9, and 12 months. Adverse events were similar between groups. The primary outcome measure of this study was not met. Both ACS and placebo-treated patients had a significant improvement in all measures. ACS resulted in a significant improvement in the KOOS score compared with placebo.

In observational studies by Baselga Garcia-Escudero and Miguel Hernandez Trillos[54] and Rutgers and colleagues,[55] ACS treatment was compared with placebo in patients with grade I to IV OA. Baselga Garcia-Escudero and Miguel Hernandez Trillos[54] found that of 118 patients who had ACS injections, there was a significant improvement at 24 months compared with baseline in pain and function scores. Whereas in Rutgers and colleagues'[55] smaller study of patients who self-selected their treatment, there was no difference between placebo and ACS.

In a more recent study looking at 100 patients treated with ACS and followed for a year, there was an 84% improvement in pain and satisfaction at 6 months and a 91% improvement at 12 months after treatment.[56] In a level 1 RCT published by Smith[57] in 2016, ACS proved to be effective for the treatment of OA in 30 patients. The study was designed as a feasibility study in which patients were randomized to receive either ACS or placebo. WOMAC scores were the primary outcome, and patients were followed for 1 year. There were no adverse effects from the ACS treatments. Furthermore, there was a significant increase in WOMAC scores at 1 year from baseline in the ACS-treated group (78% increase), whereas the placebo group had only a 7% increase from baseline. In a subsequent small trial by Zarringam and

colleagues[58] examining the role of ACS to prevent surgery in the long-term, there was no difference in rates of surgery between patients treated with ACS versus those who were not.

There is some preliminary evidence supporting the use of ACS in the treatment of OA. Unfortunately, studies have yet to reproduce the cytokine changes seen in vitro in human studies[59]; clinical outcomes are varied across the literature.

BONE MARROW ASPIRATE CONCENTRATE

Cell-based therapies have emerged as a new potential therapeutic approach in musculoskeletal disease. OA is one of the prominent targets for these therapies. However, most are still in the proof-of-concept phase. BMACs are collected from bone marrow aspirates and processed immediately for use and have been one of the most popular sources for cell therapy. Bone aspiration is typically performed in a percutaneous fashion and is fast, safe, and associated with low donor site morbidity. Once collected, it is in a single-cell suspension that can be immediately processed and used with minimal manipulation,[60,61] therefore, not requiring significant clinical trials to gain regulatory approval. These preparations are classified through the US Food and Drug Administration (FDA) as a 361 product and, hence, are not subject to premarket review and approval, making it easy to access as a treatment. It is most commonly collected from the anterior iliac crest, but yields are higher from the posterior iliac crest.[62] Other areas for harvest include, but are not limited to, the proximal tibia, the proximal humerus, and intercondylar notch. The techniques by which bone marrow aspirates are collected and processed have a large effect on the number of nucleated cells. It is key to maintain low aspiration volumes, because bone marrow–derived cells are collected in the first 2 mL of the aspirate and after that are diluted by the blood volume.[63]

BMAC is rich in mesenchymal stem cells (MSCs), which play a key role in cartilage regeneration. MSCs have a potential for self-renewal and multipotency toward cells of the mesodermal lineage. They have reparative, homing, and trophic properties causing them to migrate to areas of damage; once at the site of injury, they release numerous factors, including many that help in healing.[64] In addition to MSCs, BMAC has recently been shown to have an increased concentration of IL-1Ra protein, which, in combination with the other constituents, may provide antiinflammatory and immunomodulatory effects.[65]

In a prospective case series by Wakitani and colleagues,[66] 24 patients underwent a high tibial osteotomy along with BMAC cell transplantation. Their knees were evaluated arthroscopically at 42 weeks after treatment, and all regions of cartilage defects were found to be covered in a white metachromatic tissue. Further histologic and arthroscopic grades showed a significant improvement compared with baseline. However, there were no differences in clinical outcomes. Further studies by Koh and colleagues[67] were less successful at demonstrating normal coverage with a second-look arthroscopy. In a retrospective case series of 37 patients who had BMAC treatment, patients were found to have higher IKDC and Tegner activity scale scores at 2 years and a 94% satisfaction rate. However, they demonstrated at 2 years that 76% of cartilage defects were still abnormal or severely abnormal. Jo and colleagues[68] in 2014 were able to demonstrate in a small pilot phase I and II study that BMAC was safe and improved WOMAC scores at 6 months in patients treated with high-dose cell numbers (1×10^8). On arthroscopic evaluation there was a hyline like cap and histologic and arthroscopic scores were higher than pretreatment and compared with the low-dose cell treatment.

Multiple small studies have demonstrated improved clinical outcomes after BMAC treatment. In a 6-patient series there were no adverse events by 1 year; by 6 months participants had improved pain and were able to walk further. In addition, T2 relaxation MRIs demonstrated increased cartilage thickness at 6 months compared with pre-treatment MRIs.[69] Similarly, Orozco and colleagues[70] found increased cartilage on MRI over areas of previous poor cartilage coverage at 1 year (n = 12). In a further study, 75 patients also had improved VAS, WOMAC, and Lequesne scores. BMAC therapy improved VAS, IKDC, Short-Form 36, KOOS, and Lysholm in mild to moderate (grade I–III) OA, whereas there was no change in participants with severe grade IV OA.[71] BMAC treatment was also found to be safe in a single blinded pilot RCT after 6 months of treatment, with VAS scores improved from baseline but no different compared with saline controls.[72] Sampson and colleagues[73] found when BMAC was given in conjunction with PRP in a case series of 125 participants followed for 8 weeks that there was an absolute reduction in pain and a 91.7% satisfaction rate. Furthermore, in a comparison of BMAC with placebo to PRP injections, there were low rates of adverse events and improved lower extremity functional scale (LEFS) and pain scores compared with baseline and placebo and PRP in 615 patients.[74]

Lastly, in 2015, Centeno and Bashir[75] examined registry data of 373 patients treated with a low-cell-count ($\leq 4 \times 10^8$) or high-cell-count ($>4 \times 10^8$) BMAC. At 12 months, both low- and high-cell-count treatment groups had better outcomes (IKDC, LEFS, and pain scores) compared with baseline. The higher-cell-count treated group also had significantly lower pain scores than the low-cell-count group.[75]

Despite the high volume of BMAC used clinically, there is a very low level of evidence to support its use. Further and more methodologically stringent studies need to be done in order to evaluate the benefit of BMAC for the treatment of OA.

ADIPOSE-DERIVED STROMAL CELL THERAPY

Adipose-derived stromal cell therapy, also known as adipose stromal vascular (ASC) fraction, has gained recent popularity as a treatment that falls under the 361 product as a minimally manipulated product. ASC is collected and isolated in a closed disposable system. It is most commonly collected from lipo-aspiration of the abdomen but can also be collected from the fat pad in the knee. Once collected, the ASC is processed in cylinders with beads and is filtered and injected into the patients' joints.[76] This process can be done in a single outpatient procedure making it desirable from a patient perspective. ASC contains a high frequency of adipose-derived stem cells; however, the frequency of stem cells relative to mononuclear cells varies significantly.[77]

Initial basic science studies have been performed in vitro. For example, in one study, chondrocytes from OA patient donors were cocultured with ASC. Maumus and colleagues[78] found no effect on chondrocyte proliferation but did note a decrease in apoptosis. ASC treatment decreased TGFβ secretion by chondrocytes and led to the induction of human growth factor (HGF), which was reversed with anti-HGF treatment. IL-1, TNFα, tissue inhibitor of metalloproteinase 1 and 2, and MMP1 and 9 were not changed by ASC treatment.[78] Further studies compared chondrocytes with synoviocytes cocultured with abdominal fat, Hoffa fat pad, or subcutaneous hip fat.[67,79–81] There was no difference between the sources of ASC; all decreased levels of IL-1, TNFα, IL-6, CXCL1, CXCL8, CCL3, and CCL5. This reduction was conditional on the chondrocytes and synoviocytes producing high levels of inflammatory factors. Furthermore, they demonstrated that these decreases were due to alterations in the prostaglandin E_2 and cyclooxygenase 2 pathways.[82] Jin and colleagues,[79] in 2017,

harvested chondrocytes from patients with and without OA undergoing abdominal surgery and treated the chondrocytes with ASC from lipoaspiration. Chondrocytes from OA donors had decreased miR-373, which mediated an increase in P2X76, both involved in inflammation. When chondrocytes were stimulated with IL-1β, secretion of inflammatory factors increased; this was suppressed by the addition of ASC.

Preclinical animal studies have shown some promising results following ASC therapy. New Zealand white rabbits induced with OA were treated with either saline or ASC injection collected from the infrapatellar fat pad 12 weeks after induction.[83] By 20 weeks, radiographic images showed that rabbits had developed OA and that ASC decreased the amount of joint space narrowing, subchondral sclerosis, and osteophytes. The cartilage also showed less signs of degeneration by gross and histologic examination after ASC injection.[83] When ASC was injected into rabbits with OA and healthy rabbits, there were no adverse effects; both the OA rabbits and healthy rabbits had preserved cartilage on MRI, radiograph, and histopathology.[84] Parrilli and colleagues[85] compared dosages of ASC (2×10^6 vs 6×10^6) injected into the rabbit knee joint with OA. They found increased bone turnover and cartilage repair in both groups.

Adipose stem cells harvested from rats maintained fibroblast morphology and differentiated into chondrocytes and stimulated cartilage regeneration when injected into the knees of OA rats.[86] Mei and colleagues[87] demonstrated that ASC therapy versus placebo in a rat model of OA decreased cartilage degeneration seen grossly and histologically by 8 to 12 weeks after treatment. When xanthan gum was added to the ASC injection, there were improved results compared with ASC alone as well as a decrease in IL-1β, TNFα, and MMP3 and 13.[88] In culture, chondrocytes exposed to subcutaneous ASC had increased levels of IL-10[87] and improved chondrogenesis and immunosuppression.[89] ACS was also shown to increase proteoglycan production in mice.[90]

In phase I clinical trials of ASC therapy in knee OA, dose-escalation treatments were all found to be safe, with adverse effects consisting of swelling and pain that were limited to 24 hours after injection. At the low dose, ASC therapy improved WOMAC scores as well.[91] Similarly, Russo and colleagues[92] found ASC therapy was safe in a trial of 30 participants and had a greater than 10-point improvement in all clinical outcomes (KOOS, IKDC, Lysholm, Tegner, and VAS) by 12 months. In a small study of 6 patients, there were no infections after treatment, C-reactive protein remained at baseline levels, and patients had improved range of motion and timed up-and-go at 3 months after treatment, and improved WOMAC and VAS scores for up to a year after treatment.[93] Bansal and colleagues[81] showed favorable results of ASC treatment in mild grade I to II OA. Ten patients with OA undergoing liposuction were treated with ASC and had improvements in WOMAC and 6-minute walk distance up to 2 years after treatment. Six patients also had a 0.2-mm increase in cartilage thickness on MRI. In a prospective non-RCT open-label trial, 32 patients with severe grade III to IV OA were treated with lipoaspirate ASC. VAS, gadolinium MRI, and glycan content were assessed at baseline and 3, 6, and 12 months. There was a significant improvement in VAS sores at all time points compared with the baseline. MRI studies demonstrated an increase in glycan content.[94] In patients with severe OA, stem cells were collected from the Hoffa fat pad and injected into their knees.[95] The synovial fluid was then collected and analyzed with real-time polymerase chain reaction. After exposure to ASC, there was an increase in the expression of OPG, PTH1R, and MMP13.[95]

Koh and colleagues,[80] in 2015, published a small case trial of 30 patients who had ASC therapy from lipoaspirate. They followed up on these patients at 2 years assessing KOOS, VAS, and Lysholm scores as well as by performing a repeat diagnostic

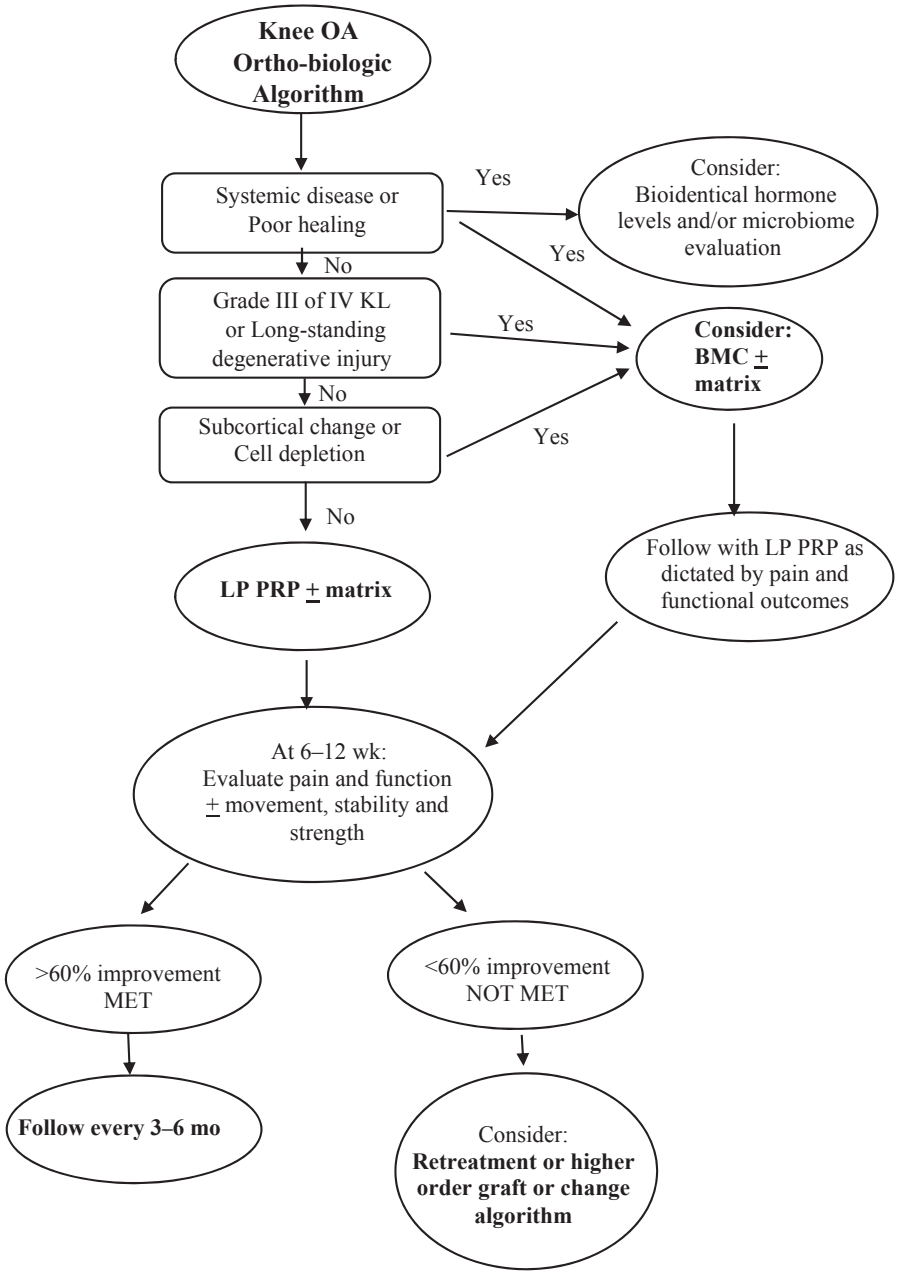

Fig. 1. Proposed algorithm for considering the use of ortho-biologics in OA as per Crane and colleagues. KL, kellgren lawrence. (*Data from* Crane DM, Oliver KS, Bayes MC. Orthobiologics and knee osteoarthritis: a recent literature review, treatment algorithm, and pathophysiology discussion. Phys Med Rehabil Clin N Am 2016;27(4):985–1002).

arthroscopic evaluation. Patients had a significant improvement in clinical outcomes. A total of 87.5% of patients had improved or maintained cartilage on arthroscopic evaluation, and most importantly none required a joint replacement over the study period.[67]

Although promising, these studies have been insufficient to draw conclusions about the efficacy of ASC therapy to adopt it into standard practices. These trials universally lack adequate controls and use a wide variety of approaches, injection regimes, and concentrations making it challenging to determine what would be the most efficacious and safest treatment going forward. In order to use evidence-based applications of ASC in OA, these gaps in knowledge must be studied and evaluated further.

DISCUSSION

In this article, the authors summarize what is known about the treatment of OA with regenerative medicine using 5 ortho-biologics: viscosupplementation, PRP, ACS, bone marrow aspirate concentrate and adipose-derived stromal cell therapy. All of these treatments have shown some promise in the literature; however, there are still substantial gaps in our knowledge. Guidelines for HA treatments have been less than enthusiastic; however, much of the data shows it to be safe and efficacious in patients with OA. Multiple meta-analyses of PRP treatments suggests that PRP is a promising therapy for symptom relief and improved functional outcomes in patients with OA for at least 12 months after treatment. Results of ACS therapy have been less conclusive than the use of PRP. Although there is some preliminary promise in the use of ACS in the treatment of OA, they have yet to reproduce the cytokine changes seen in vitro in humans. Cell therapies, including BMAC and ASC, are at the forefront of tissue engineering with lots of potential benefits in OA. These therapies are stem cell treatments, which are minimally manipulated allowing them to be used without further FDA regulations. With more studies, cell-based therapy may have the most promise when used appropriately in patients with OA.

Rapid advances in tissue engineering will make ortho-biologic therapies, particularly stem cell therapies, more feasible in changing the landscape of OA treatment. Crane and colleagues[96] have suggested that 15 factors will need to be considered going forward for both tissue engineering and treatment: tissue, neurohormonal status, vascular supply, growth factors, progenitor cells, matrix, cartilage, synovium, capsule, movement, stability, strength, tissue inflammation, hormones, and microbiome. Based on these criteria, they have proposed an algorithm for considering various ortho-biologic therapies (**Fig. 1**). Although this is an interesting algorithm, the lack of level 1 evidence to support these treatments makes it impossible at this stage to use this algorithm into daily practice.

In order to move forward with using these treatments, it is critical that we develop standardized study regimes that can be compared in large level 1 RCTs, meta-analyses, and systematic reviews.

SUMMARY

There have been large advancements in regenerative medicine in health care since the initial introduction of bone marrow therapies and PRP in the 1980s.[51,96] As regenerative medicine progresses, clinicians must make decisions on how best to optimize their use and when to use them based on the disease process and patients' treatment plan. This review demonstrates that the studies reviewed support that ortho-biologics are safe and seem to support their use in the treatment of OA for up to 2 years. These treatments are easy to obtain and relatively inexpensive. Ortho-biologics may yield

superior results in the treatment of OA relative to more conventional approaches, because of their ability to target repair and regeneration of the underlying cartilage damage and dampen inflammation leading to this degradation.

Future work should be targeting the factors that are most beneficial and effective in treating OA, determining dosages and timing, in addition to administration methods. It is of the utmost importance that the medical community comes up with treatment algorithms and further trials studying long-term effectiveness.

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