ORIGINAL ARTICLE

Amniotic Suspension Allograft and Bone Marrow Aspirate Concentrate Results in Highly Variable Proinflammatory Cytokines in a Coculture Model of Osteoarthritis

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Purpose: To evaluate the anti-inflammatory and disease-modifying potential of bone marrow aspirate concentrate (BMAC) and amniotic suspension allograft (ASA) in a chondrocyte and synovium coculture model of osteoarthritis.
Methods: Seventeen patients were enrolled for cartilage, synovium, and bone marrow aspirate tissue donation prior to a total knee arthroplasty. Cartilage and synovium explants were cocultured into four different groups: one baseline group, one control group (96-hour coculture), BMAC group, and ASA group. Interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were measured at 96 hours in the media with enzyme-linked immunosorbent assay. Collagen type 1 α 1, Collagen type 2 α 2, Collagen type 3 α 1, aggrecan, and Cartilage Oligomeric Matrix Protein were measured in the cartilage and synovium by reverse transcription polymerase chain reaction. Safranin-O staining were performed on all groups and scored by the modified Mankin scoring system.
Results: Samples treated with ASA showed a significantly lower concentration of IL-1β when compared to control samples (11.2 ± 13.9 vs 20.2 ± 39.5 pg/mL, $P = .04$). Treatment with BMAC was associated with a significantly lower concentrations of IL-6 when compared to control samples (607.32 ± 271.07 vs 767.11 ± 30.84, $P = .02$). No significant differences were observed in gene expression within chondrocytes and synoviocytes or for Mankin scoring between treatment and control groups.
Conclusions: Osteoarthritic chondrocytes and synovial tissue may respond to BMAC and ASA by a reduction in IL-1β and IL-6 concentrations, but short-term cytokine responses are variable. The mechanism of response remains unknown.
Clinical Relevance: This study aims to reveal the mechanism of BMAC and ASA by which these biologics function in a model of an arthritic joint.

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Osteoarthritis is a chronic, costly, and debilitating condition that historically has been attributed to mechanical degeneration. However, this understanding has evolved to incorporate the roles of inflammation and alterations in cartilage metabolism in the pathogenesis of the disease.[1,2] Increased catabolism and decreased anabolism lead to a breakdown of cartilage extracellular matrix and increased inflammation in synovial fluid

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and tissue.[3] The goal of nonsurgical treatment is to slow the physiologic process, reduce pain, and increase function. However, to date, there are no treatments that have been proven to modify these metabolic and inflammatory processes and affect progression of the arthritic process.

The use of intra-articular cellular-based therapies as sources of growth factors, anti-inflammatory mediators, and potentially mesenchymal stem cells (MSCs) for osteoarthritis is rapidly evolving. MSCs have garnered significant interest due to their chondrogenic potential and promise for tissue regeneration.[4] In addition, MSCs may also have significant trophic and immunomodulatory effects on osteoarthritis.[5] Bone marrow aspirate concentrate (BMAC) is one of the safest and most feasible sources of MSCs currently available.[6] While MSCs represent only a small fraction of the total population of nuclear cells (0.001%-0.01%), when concentrated, bone marrow aspirate also contains a rich supply of growth factors.[7-9] Many of these growth factors such as vascular endothelial growth factor and transforming growth factor-ß have chondrogenic effects on MSCs that may be beneficial in osteoarthritis.[10,11] As a result, many translational and clinical studies have investigated the therapeutic potential of BMAC for osteoarthritis, with promising findings of histologic improvement and symptom relief up to 12 months post-injection.[12-14] Despite the limitations of cost and the necessity of another procedure to extract bone marrow aspirate, enthusiasm remains for BMAC for treatment of osteoarthritis.

Separately, amniotic membrane is a metabolically active tissue that has also garnered recent interest in cartilage research for its anti-inflammatory and chondroregenerative potential.[15,16] Amniotic membranes both contain anti-inflammatory factors and upregulate several anti-inflammatory pathways, such as IL-10, which may positively affect osteoarthritis inflammation.[17] In addition, amniotic membranes are rich sources of hyaluronic acid and some proteoglycans, which may play a role in therapeutic relief of osteoarthritis.[18] Amniotic membranes are most attractive for their source of pluripotent stem cells with chondrogenic potential.[15,19] Cryopreserved cellular human amniotic suspension allograft (ASA) has showed symptomatic relief in murine models of OA[20] as well as superior outcomes to control saline and hyaluronic acid injections in randomized controlled clinical trials.[21] There are limitations, however, with regard to cost and the amount of growth factors after treatment of the amniotic membrane tissue.

While early clinical efficacy results are promising, the exact underlying mechanisms of both orthobiologics remain a significant question. Therefore, the purpose of this study was to evaluate the anti-inflammatory and disease-modifying potential of BMAC and ASA in a chondrocyte and synovium coculture model of osteoarthritis. We hypothesized that there would be histologic and biochemical changes associated with the presence of BMAC and ASA in a chondrocyte and synovium coculture model of osteoarthritis.

METHODS

Study Population and Study Design

A prospective series of 17 patients were enrolled at one clinical center by three treating surgeons prior to undergoing elective primary total knee arthroplasty between August 2018 and June 2019 (Figure 1). A total of 18 patients were enrolled in the study prior to their scheduled TKA. One patient was excluded due to failure of bone marrow aspiration intraoperatively, leaving a final cohort of 17 patients with sufficient tissue collection. Sixty five percent (n = 11) were female and the mean age was 65 years of age ±7.43. All patients had a Kellgren-Lawrence grade 3 (n = 10) or grade 4 (n = 7) on preoperative radiographs. All three surgeons were experienced fellowship trained total joint arthroplasty surgeons. Sample size was determined based on prior studies by Cook et al. and adapted by Sundman et al. and Osterman et al all of which utilized similar study designs.[22-24] Institutional review board approval was obtained prior to the initiation of the study (*ORA#* 17013001), and all subjects provided informed consent before any study-related procedures were conducted. The inclusion criteria for the study were as follows: (1) symptomatic osteoarthritis of the knee involving the medial femoral condyle prompting election of total knee arthroplasty and (2) radiographic Kellgren-Lawrence grade 3 or 4.[25] Exclusion criteria included revision cases, bone marrow aspiration failure, concern for septic arthritis, distal femur intra-articular fracture, and a steroid or PRP injection within 3 months of surgery. Enrolled patients consented to donation of cartilage, synovium, and bone marrow aspirate tissue samples for histologic and biochemical analyses.

Tissue Harvest Technique

Synovial tissue was harvested from the anterior aspect of the distal femur utilizing a knife or electrocautery. Cartilage and subchondral bone samples were preferentially harvested from the medial femoral condyle from the femoral cuts routinely performed in a total knee arthroplasty. Areas with intact full-thickness cartilage, with gross normal appearance, and subchondral bone

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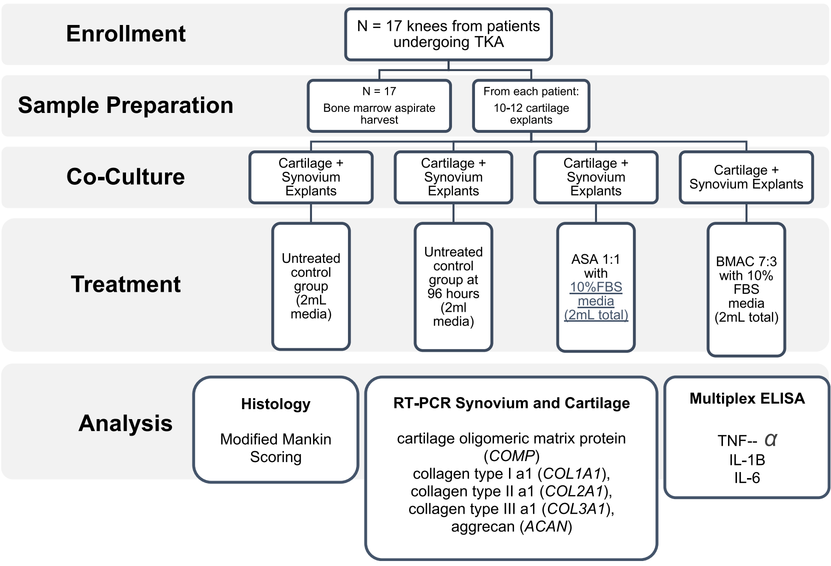

FIGURE 1 Schematic Representation of Study Design. (ACAN, Aggrecan; ASA, Amniotic Suspension Allograft; BMAC, Bone Marrow Aspirate Concentrate; COL1A1, Collagen Type I α 1; COL2A1, Collagen Type II α 1; COL3A1, Collagen Type III α 1; COMP, Cartilage Oligomeric Matrix Protein; ELISA, Enzyme-Linked Immunosorbent Assay.)

were selected for explants. Cartilage tissue was dissected into 10 to 12 explants (3 × 5 × 5 mm).

Bone marrow aspirate was obtained as previously described from the iliac crest from patients undergoing total knee arthroplasty who consented to the additional procedure for the purpose of this study.[26] Using a sharp trocar with a hollow aspiration sleeve, approximately 60 mL of bone marrow was extracted from cancellous bone just posterior to the anterosuperior iliac spine. The aspirate was then prepared and centrifuged using a standard commercially available BMAC centrifuge system (Arthrex Angel System (Arthrex, Inc.; Naples, FL)), typically yielding up to 3 mL of BMAC.[27]

Incubation and Coculture Model

Following dissection, the subchondral bone was discarded. The cartilage and synovium explants were incubated overnight (24 hours total) separately in 6-well plates within medium (complete Dulbecco's Modified Eagle Medium [DMEM] 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (PS), and 50 mg/mL of ascorbic acid).

Following incubation, medium was removed, and synovium and cartilage explant coculture systems were created using 12-well culture plates containing 0.4 mm filtered inserts, as previously described by Sundman et al.[28] One synovial explant was placed at the base of the well with one cartilage explant from the same patient on top of the filtered insert in the same well. Four coculture systems were established per patient to accommodate the 2 biologic treatment groups, one control group at 96 hours, and one baseline (untreated) control cartilage group with no incubation. The four groups analyzed included the following:

1. Untreated control group (2 mL media) with no incubation
2. Untreated control group at 96 hours (2 mL media)
3. Cryopreserved human amniotic suspension allografts [ASA] 1:1 ratio (ReNu, Organogenesis, Birmingham, AL)[20] (final total volume 2 mL) at 96 hours
4. Bone marrow aspirate concentrate [BMAC] 7:3 ratio (Arthrex Angel System (Arthrex, Inc.; Naples, FL)) (final total volume 2 mL) at 96 hours

Cocultures were then incubated at 5% CO_2, 37°C, and 90% humidity for 96 hours. Subsequently, treatment media were stored at −80°C. Synovium and cartilage explants were snap frozen with liquid nitrogen and stored at −80°C until further use for RNA isolation. All incubation and cocultures were created by one investigator highly trained in this area (A.H.).

Multiplex Enzyme-Linked Immunoassay (Luminex)

The instrument that was used is a Luminex FlexMAP3D. The software is xPonent version 4.3.229. The culture media was stored at −80°C before analysis. Multiplex enzyme-linked immunoassay (Enzyme-Linked

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Immunosorbent Assay) (Luminex) was used to measure concentrations of proinflammatory mediators interleukin-1b (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) at 96 hours. Each sample was measured in duplicate to ensure accuracy, and the mean value was obtained.

Polymerase Chain Reaction (PCR)

Frozen cartilage samples were pulverized using a Bio-Pulverizer. Total RNA was then isolated from cartilage and synovial explants using the TRIzol Reagent (Ambion, Life Technologies; Cat# 15596018; 5791 Van Allen Way, Carlsbad, CA 92008, USA).

Endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as control. Each sample was run in duplicate to ensure accuracy.

Both chondrocyte and synovium RNA was isolated followed by cDNA synthesis and quantitative reverse-transcription PCR assays (qRT-PCR) using the ViiA7 Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA). Gene expression was carried out for the following genes: Collagen type I a1 (COL1A1), Collagen type II a1 (COL2A1), Collagen type III a1 (COL3A1), Aggrecan (ACAN), and Cartilage Oligomeric Matrix Protein (COMP).

Histology

Before culture, only one of the 10 to 12 cartilage explants was used as a baseline control and fixed in 4% paraformaldehyde for 24 hours and processed for histological assessment immediately upon collection to preserve the native state of the tissue. The remaining explants were allocated to various experimental groups and subjected to culture conditions. After 96 hours of culture, these samples were also fixed in PF and processed for histological analysis to evaluate treatment-related changes relative to the baseline. Paraffin-embedded samples were sectioned (6 μm) and stained with hematoxylin and eosin (H&E) and Safranin O staining. One graduate level grader (C.H.) scored the samples using a modified Mankin score.[29]

Statistical Analysis

All statistical analyses were performed on STATA v16.1 (Statacorp, Houston TX). Treatment groups were compared to control cartilage cocultures utilizing paired Wilcoxon signed-rank tests. Statistical outliers were determined as those exceeding 1.5 times the interquartile range from the first or third quartile of each group. Measurement errors occurred during assay processing, resulting in data points that were markedly inconsistent with the rest of the dataset. These extreme values were excluded to reduce the standard deviation and minimize distortion of the overall statistical results. Data are presented as mean ± standard deviation. All testing was two-sided, and significance was set at $P < .05$.

RESULTS

Enzyme-Linked Immunosorbent Assay

Measured concentrations of each analyte showed significant variability (range) between patients (Figure 2). There was a significantly lower concentration of IL-1β in the ASA group compared to the control group (ASA: 11.2 ± 13.9 pg/mL vs control: 20.2 ± 39.5 pg/mL, $P = .04$) (Figure 2). There was no difference in IL-1β concentration between the ASA group and the BMAC group ($P = .31$).

After removal of statistical outliers, the concentration of IL-6 in the BMAC coculture was significantly lower than the control coculture (Control: 767.11 ± 30.84 vs BMAC: 607.32 ± 271.07, $P = .03$). There was no difference in IL-6 concentration between the ASA group and the BMAC group ($P = .24$). Lastly, although there was a downward trend with the BMAC and ASA treatments, there were no significant differences in concentration of TNF-α between groups (Figure 2).

PCR

No significant differences in expression were observed for COL1A1, COL2A1, COL3A1, COMP, or ACAN in either cartilage or synovium samples between the treatment and control groups (Tables 1 and 2). However, COL1A1 expression in cartilage was significantly greater in the ASA compared to BMAC groups ($P = .002$), and COL2A1 expression in synovial tissue was significantly reduced in the ASA group compared to BMAC groups ($P = .004$).

Histology

Five samples were excluded from grading due to poor slide preparation, leaving a total of 14/17 and 15/17 slides available for grading in the BMAC and ASA groups, respectively (Figure 3). An increase in Mankin score signified a higher degree of cartilage degeneration. Mean Mankin score increased from the baseline to the 96-hour control samples (3.35 ± 1.80 to 5.12 ± 3.10, $P = .026$). When compared to the control samples, the Mankin scores of the BMAC (4.29 ± 2.64, $P = .24$) and ASA (4.33 ± 2.29,

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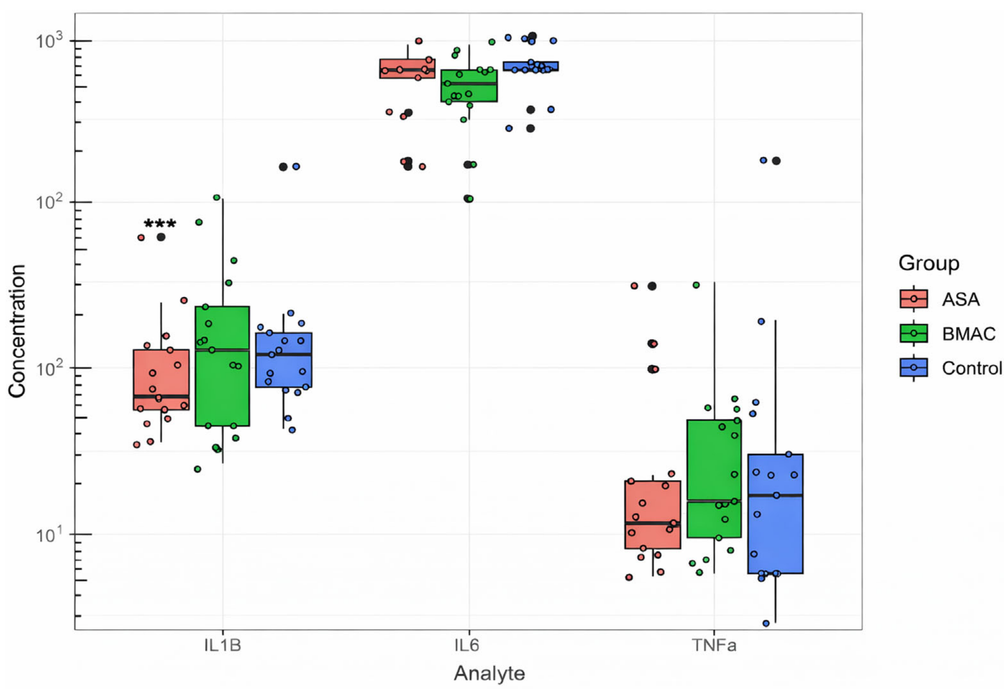

FIGURE 2 Boxplot With Overlaid Points Showing Concentrations of Each Analyte (IL-1β, IL-6, TNFα) From Sample Media From The Three Treatment Groups. **IL-1β *P*-Values:** Control vs BMAC: $P = .407$, Control vs ASA: $\mathbf{P = .044}$***, BMAC vs ASA: $P = .309$. **IL-6 *P*-Values:** Control vs BMAC: $P = .080$, Control vs ASA: $P = .636$, BMAC vs ASA: $P = .266$. **TNFα *P*-Values:** Control vs BMAC: $P = .463$, Control vs ASA: $P = .218$, BMAC vs ASA: $P = .421$. The Box Shows the IQR, With the Bottom and Top of the Box Marking the 25th and 75th Percentiles, Respectively. The Horizontal Line Represents The Median. Whiskers Extend to Values Within 1.5 Times the IQR. Overlaid points indicate individual sample measurements. (ASA, Amniotic Suspension Allograft; BMAC, Bone Marrow Aspirate Concentration; IQR, Interquartile Range.)

TABLE 1 PCR Cartilage Samples (*Ratio to Baseline Sample*)

	Control	ASA	*P*-Value	BMAC	*P*-Value	BMAC versus ASA (*P*-Value)
COL1A1	1.45 [0.55-2.92]	2.28 [1.49-2.96]	.16	1.02 [0.53-1.55]	.13	**.002**
COL2A1	1.18 [0.56-3.07]	1.15 [0.65-1.70]	.93	1.36 [0.36-2.97]	.35	.28
COL3A1	1.13 [0.52-5.30]	1.50 [0.23-3.42]	.58	0.75 [0.25-5.96]	.75	.75
ACAN	3.83 [1.07-10.73]	2.43 [1.36-17.32]	.50	1.08 [0.34-23.34]	.10	.33
COMP	2.18 [1.35-12.73]	6.55 [0.50-13.54]	.25	2.14 [1.33-18.89]	.38	.76

Note: Presented as median [interquartile range]. Bold = Statistically significant ($P < .05$).
ASA, Amniotic Suspension Allograft; ACAN, aggrecan; BMAC, Bone Marrow Aspirate Concentrate; COL1A1, Collagen type 1 α 1; COL2A1, Collagen type 2 α 2; COL3A1, Collagen type 3 α 1; COMP, Cartilage Oligomeric Matrix Protein.

$P = .55$) groups decreased, although these differences were not statistically significant.

DISCUSSION

The findings of this study suggest that BMAC and ASA may induce an anti-inflammatory response on osteoarthritic cartilage and synovial tissue via a reduction in IL-6 and IL-1β concentrations within the media, respectively. BMAC and ASA did not result in changes in cartilage matrix metabolism compared to the control groups during the culture period, although some differences were observed between treatment groups in collagen production.

The observed decrease in IL-6 with BMAC treatment is in parallel to a prior study by Zhang et al.,[30] which highlighted the anti-inflammatory effect of bone-marrow derived MSCs on cultured osteoarthritic chondrocytes. Similarly, a prior translational study by Wang et al.[31]

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TABLE 2 PCR Synovium Samples (*Ratio to Baseline Sample*)

	Control	ASA	*P*-Value	BMAC	*P*-Value	BMAC versus ASA (*P*-Value)
COL1A1	1.05 [0.79-1.75]	1.02 [0.18-1.69]	.27	0.76 [0.32-3.74]	.98	.22
COL2A1	0.30 [0.19-1.01]	0.12 [0.09-0.70]	.53	0.77 [0.22-2.53]	.13	**.004**
COL3A1	1.63 [0.53-3.25]	1.13 [0.47-4.20]	.55	1.88 [0.48-3.39]	.33	.94
COMP	1.43 [0.25-2.93]	1.71 [0.70-2.89]	.16	1.05 [0.44-2.61]	.19	.07

Note: Presented as median [interquartile range]. Bold = Statistically significant ($P < .05$).
ASA, Amniotic Suspension Allograft; BMAC, Bone Marrow Aspirate Concentrate; COL1A1, Collagen type 1 α 1; COL2A1, Collagen type 2 α 2; COL3A1, Collagen type 3 α 1; COMP, Cartilage Oligomeric Matrix Protein; PCR, polymerase chain reaction.

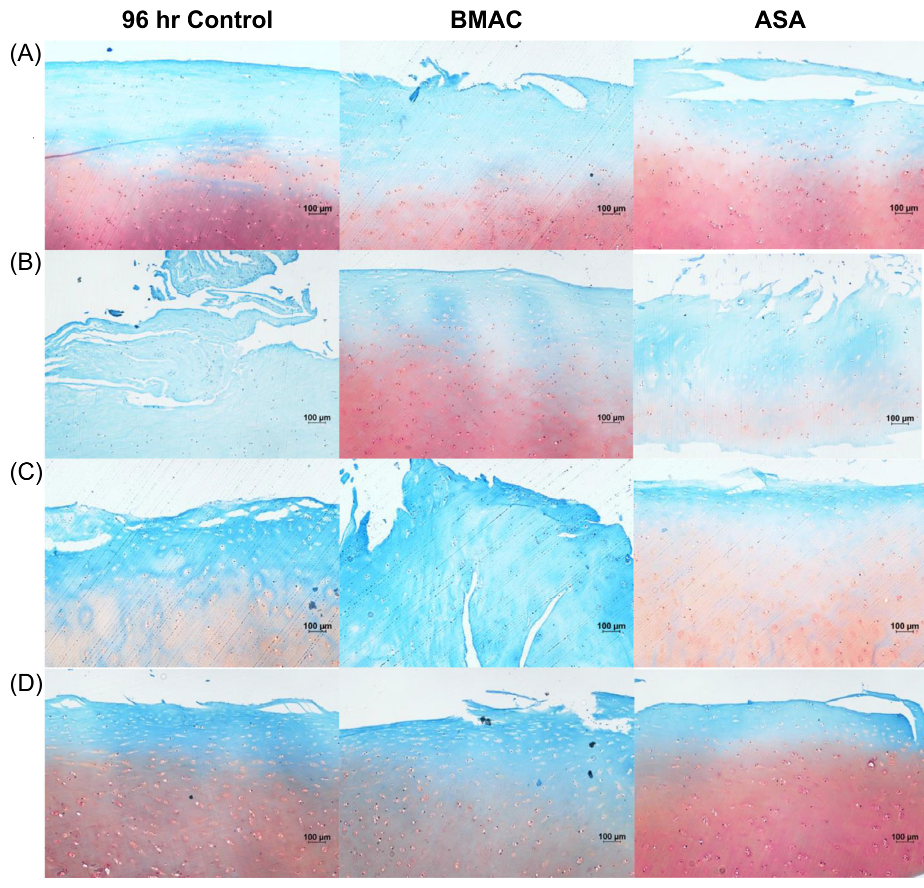

FIGURE 3 Sample Histology From Select Patients (A-D) Highlighting the Variability in Response to Treatment With BMAC or ASA. Tissue (A) Showed a Slight Worsening of Surface Fibrillation in Response to Either Treatment. Tissue (B) Showed Improvement with BMAC, But a Minimal Response to ASA, Whereas Tissue (C) and Tissue (D) Showed Greater Response to ASA. Pink Signifies Proteoglycan is Present, and Blue Signifies the Loss Of Proteoglycans and Is an Indicator of Tissue Damage or Altered Cell Function. Modified Mankin Scores: (A) 3, (B) 14, (C) 13, and (D) 16. (ASA, Amniotic Suspension Allograft; BMAC, Bone Marrow Aspirate Concentrate.)

showed significant decreases of IL-6 in the synovial fluid of goats with osteoarthritis (represented by the media surrounding this coculture model) after a series of intra-articular BMAC injections. The effects of IL-6 in maintenance of articular cartilage are two-fold.[32] While higher levels of circulating IL-6 have been associated with worsening radiographic progression[33] and cartilage loss in the setting of OA,[34] the cytokine has also been associated with some chondroprotective properties, especially in the setting of acute pathology.[35] The findings of the present study

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suggest that the observed clinical benefits of BMAC injection[12,13,36] may be attributed then to a potential reduction in MMP-1 and MMP-13, which are typically upregulated by a combined effect of IL-1β and IL-6.[32]

In contrast to the present study, however, Zhang et al.[30] and Wang et al.[31] observed concurrent decreases in gene expression or protein concentrations of IL-1β and TNFα, respectively. The differential response observed in this study compared to prior literature could be explained by the composition of BMAC. Ziegler et al.[37] showed the relatively high concentrations of interleukin-1 receptor antagonist (IL-1Ra) in BMAC compared to bone marrow aspirate (BMA) and leukocyte-rich and -poor platelet rich plasma (PRP) preparations. The anti-inflammatory mechanisms of IL-1Ra have been explored previously in a study by van Vulpen et al.,[38] which studied the damaging effects of hemarthrosis in an in vitro chondrocyte model. Within their study, the presence of blood was associated with increased concentrations of IL-1β, TNFα, and IL-6.[38] After addition of IL-1Ra, concentrations of IL-1β and IL-6 decreased, but TNFα was unaffected, which suggests separate inflammatory cascades.[38] The results of the current study then validate these findings of IL-6, supporting the potential IL-1Ra driven mechanisms of BMAC. However, no effect of BMAC was observed on IL-1β concentrations, which is in contrast to several prior studies.[30,31,38] This could be due to baseline variation in IL-1β concentration, location of cartilage harvest, or heterogeneity in BMAC content.[39,40] Similarly, the range of composition and effects of processing on BMAC promote heterogeneity in the product.[39] In the broader context of literature, the precise role of IL-1β in the pathogenesis of OA has been reconsidered.[41] Several findings have raised question to the significance of the marker due to (1) similar concentrations in early and late OA,[42] (2) physiologic concentrations of IL-1ra vastly exceeding that of IL-1B,[43] and (3) conflicting clinical results with blockade of its signaling pathway.[44-46] Therefore, the lack of showed effect within the present study does not necessarily preclude a lack of effect on the OA phenotype of tissue. Future investigation is needed to better characterize the response of chondrocytes in relation to various BMAC contents as well as clarify the association between inflammatory markers and OA pathology.

In contrast to the effects of BMAC in the present study, ASA treatment was associated with reduced concentrations of IL-1β without an effect on IL-6 and TNFα. Although the role of IL-1β in clinical benefit is unclear, the observed symptomatic benefits of ASA injections in clinical studies of osteoarthritis[21] could be explained by a reduction of IL-1β, which would reduce nuclear factor – kappa b (NF-κB) pathway activation and its associated downstream proinflammatory effects.[47-49] However, these findings differ from the findings of Kimmerling et al., which utilized the same ASA product as the current study in a murine model and showed decreases primarily in IL-6 (nonsignificant), without any significant trends in IL-1.[20] Similarly, intra-articular injections with human amniotic MSCs with hyaluronic acid were associated with decreased concentrations of IL-1β, IL-6, and TNFα in the synovial fluid of rats with osteoarthritis.[50] Therefore, there is considerable heterogeneity in the responses of osteoarthritic tissue in current literature, which is potentially reflective of the variation in formulations and content of amniotic products. Prior characterization of nine samples of amniotic-derived products showed a broad range of detected proteins (919 total), including IL-1Ra and TIMPs.[51] However, unlike BMAC, there were no live cells detected in these products.

The lack of a statistically significant reduction in TNF-α following BMAC may be attributable to β error. Baseline TNF-α concentrations exhibited considerable variability across samples (mean 12.9 ± 43.2 pg/mL; range 0.13-179.91), suggesting biological heterogeneity. This variability likely contributed to reduced statistical power, despite a directional trend.

Therefore, although this study was able to identify a response from osteoarthritic tissues to the biologics, further investigation is required to determine the specific mediators driving the reduction in proinflammatory markers. Prior studies of intra-articular BMSCs and amniotic membrane have been associated with improvements in histologic scoring[14,52,53] and changes in gene expression.[14,30,53,54] However, the majority of these studies utilized in vivo models which allowed for longer incubation periods prior to analysis, in comparison to the 96 hours in the present study. Since the coculture study by Sundman et al.[28] identified changes in COL1 and aggrecan gene expression within 96 hours in response to PRP, this may suggest a relatively delayed effect of BMAC and ASA on cartilage matrix metabolism and resultant histology, if there is a true effect.

Recent clinical data suggest limited efficacy of MSCs in osteoarthritis management. A recent prepublication meta-analysis by Sadeghirad et al.[54] of randomized trials concluded that MSCs for chronic knee pain associated with osteoarthritis likely provide little to no improvement in pain or physical function. In addition, several studies support the use of MSCs and umbilical derived products for osteoarthritis treatment by highlighting their proposed mechanisms of action, including modulation of inflammation and promotion of tissue repair via secretion of growth factors and cytokines.[54-57] Future research should aim to integrate both mechanistic endpoints and clinical outcomes to better contextualize biological findings within real-world, patient-centered frameworks. Additionally,

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studies with extended culture durations and more refined analysis of product composition will be essential to elucidate the temporal and mechanistic effects of BMAC and ASA on osteoarthritic tissues.

Limitations

There were several limitations to this study. First, there is tremendous heterogeneity in baseline characteristics of osteoarthritic chondrocytes and synovium. This variability may be due to method of harvest, degree that the tissues were affected by osteoarthritis, or Variability in the location of the sampling. This likely contributed to the wide range of proinflammatory cytokine concentrations observed in the study. In addition, the coculture model may not have truly represented osteoarthritis if the cartilage and synovial samples were not osteoarthritic. Similarly, the BMAC and ASA products were not formally analyzed due to cost limitations of the study, and therefore consistency in treatments between samples could not be ensured. Combined, these factors limited the ability to draw conclusions regarding the effect of biologic composition on overall response, which is a question of increasing interest. Variability in biologic composition can further compound the effects of a relatively small sample size ($n = 17$). The study may be underpowered to detect differences between groups. There also may have been heterogeneity between the three surgeons in the samples provided, albeit all were from the medial femoral condyle and anterior distal femur. Although no changes were observed in gene expression and histology compared to control samples, this may be due to an inadequate sample size or timeframe or due to the inherent limitations of the coculture model. The genes investigated are not early response genes, and therefore the 96-hour time frame may have been insufficient. There may have been measurement errors inherent in the testing methods. Disaggregation by sex and/or gender was not performed, as the study was neither designed nor sufficiently powered to detect sex- or gender-based differences. The primary objective and methodology did not include stratification these variables, and doing so would risk overinterpretation of underpowered subgroup analyses. Lastly, this is an in vitro study and may not represent what happens in vivo or clinically.

CONCLUSIONS

Osteoarthritic chondrocytes and synovial tissue may respond to BMAC and ASA by a reduction in IL-1β and IL-6 concentrations, but short-term cytokine responses are variable. The mechanism of response remains unknown.

DISCLOSURES

The authors (A.B.Y., B.J.C.) declare the following financial interests/personal relationships which may be considered as potential competing interests: A.B.Y. reports equipment, drugs, or supplies were provided by Arthrex Inc.; reports financial support was provided by Foundation for Orthopaedic Research and Education; reports equipment, drugs, or supplies were provided by Organogenesis Inc.; reports a relationship with Organogenesis Inc. that includes nonfinancial support. B.J.C. reports a relationship with Organogenesis Inc. that includes nonfinancial support. The other authors (C.P.H., N.D., H.P.H., D.S., N.B.N., D.F., W.M., A.H., S.S.) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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REFERENCES

1. Berenbaum F, van den Berg W. Inflammation in osteoarthritis: Changing views. *Osteoarthr Cartil.* 2015;23:1823-1824.
2. Berenbaum F, Griffin TM, Liu-Bryan R. Metabolic regulation of inflammation in osteoarthritis. *Arthritis Rheumatol.* 2017; 69:9-21.
3. Mueller MB, Tuan RS. Anabolic/catabolic balance in pathogenesis of osteoarthritis: Identifying molecular targets. *PM&R.* 2011;3:S3-S11.
4. Coleman CM, Curtin C, Barry FP, O'Flatharta C, Murphy JM. Mesenchymal stem cells and osteoarthritis: Remedy or accomplice? *Hum Gene Ther.* 2010;21:1239-1250.

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5. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol.* 2007;213:341-347.
6. Hendrich C, Franz E, Waertel G, Krebs R, Jäger M. Safety of autologous bone marrow aspiration concentrate transplantation: Initial experiences in 101 patients. *Orthop Rev (Pavia).* 2009;1:e32-e32.
7. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284: 143-147.
8. Hegde V, Shonuga O, Ellis S, et al. A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. *J Orthop Trauma.* 2014;28:591-598.
9. Cotter EJ, Wang KC, Yanke AB, Chubinskaya S. Bone marrow aspirate concentrate for cartilage defects of the knee: From bench to bedside evidence. *Cartilage.* 2017;9:161-170.
10. Indrawattana N, Chen G, Tadokoro M, et al. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun.* 2004;320:914-919.
11. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. *Clin Orthop Relat Res.* 2011;469:2706-2715.
12. Kim J-D, Lee GW, Jung GH, et al. Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in degenerative arthritis of the knee. *Eur J Orthop Surg Traumatol.* 2014;24:1505-1511.
13. Anz AW, Hubbard R, Rendos NK, Everts PA, Andrews JR, Hackel JG. Bone marrow aspirate concentrate is equivalent to platelet-rich plasma for the treatment of knee osteoarthritis at 1 year: A prospective, randomized trial. *Ortho J Sports Med.* 2020;8:2325967119900958.
14. Fortier LA, Potter HG, Rickey EJ, et al. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg Am.* 2010;92:1927-1937.
15. Riboh JC, Saltzman BM, Yanke AB, Cole BJ. Human amniotic membrane-derived products in sports medicine: Basic science, early results, and potential clinical applications. *Am J Sports Med.* 2016;44:2425-2434.
16. Vines JB, Aliprantis AO, Gomoll AH, Farr J. Cryopreserved amniotic suspension for the treatment of knee osteoarthritis. *J Knee Surg.* 2016;29:443-450.
17. Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea.* 2000;19:348-352.
18. Meinert M, Eriksen GV, Petersen AC, et al. Proteoglycans and hyaluronan in human fetal membranes. *Am J Obstet Gynecol.* 2001;184:679-685.
19. Kolambkar YM, Peister A, Soker S, Atala A, Guldberg RE. Chondrogenic differentiation of amniotic fluid-derived stem cells. *J Mol Histol.* 2007;38:405-413.
20. Kimmerling KA, Gomoll AH, Farr J, Mowry KC. Amniotic suspension allograft modulates inflammation in a rat pain model of osteoarthritis. *J Orthop Res.* 2020;38:1141-1149.
21. Gomoll AH, Farr J, Cole BJ, et al. Safety and efficacy of an amniotic suspension allograft injection over 12 months in a single-blinded, randomized controlled trial for symptomatic osteoarthritis of the knee. *Arthroscopy.* 2021;37:2246-2257.
22. Cook JL, Marberry KM, Kuroki K, Kenter K. Assessment of cellular, biochemical, and histologic effects of bipolar radiofrequency treatment of canine articular cartilage. *Am J Vet Res.* 2004;65:604-609.
23. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med.* 2011;39: 2135-2140.
24. Osterman C, McCarthy MB, Cote MP, et al. Platelet-rich plasma increases anti-inflammatory markers in a human coculture model for osteoarthritis. *Am J Sports Med.* 2015;43:1474-1484.
25. Kohn MD, Sassoon AA, Fernando ND. Classifications in brief: Kellgren-Lawrence classification of osteoarthritis. *Clin Orthop Relat Res.* 2016;474:1886-1893.
26. Chahla J, Mannava S, Cinque ME, Geeslin AG, Codina D, LaPrade RF. Bone marrow aspirate concentrate harvesting and processing technique. *Arthrosc Tech.* 2017;6:e441-e445.
27. Dragoo JL, Guzman RA. Evaluation of the consistency and composition of commercially available bone marrow aspirate concentrate systems. *Ortho J Sports Med.* 2020;8:2325967119893634.
28. Sundman EA, Cole BJ, Karas V, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. *Am J Sports Med.* 2014;42:35-41
29. Mankin H, Lippiello L. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. *J Bone Joint Surg.* 1970;52:424-434.
30. Zhang Q, Chen Y, Wang Q, et al. Effect of bone marrow-derived stem cells on chondrocytes from patients with osteoarthritis. *Mol Med Rep.* 2016;13:1795-1800.
31. Wang Z, Zhai C, Fei H, et al. Intraarticular injection autologous platelet-rich plasma and bone marrow concentrate in a goat osteoarthritis model. *J Orthop Res.* 2018;36:2140-2146.
32. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier J-P, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol.* 2011;7:33-42.
33. Livshits G, Zhai G, Hart DJ, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The chingford study. *Arthritis Rheum.* 2009;60:2037-2045.
34. Pelletier JP, Raynauld JP, Caron J, et al. Decrease in serum level of matrix metalloproteinases is predictive of the disease-modifying effect of osteoarthritis drugs assessed by quantitative MRI in patients with knee osteoarthritis. *Ann Rheum Dis.* 2010;69:2095-2101.
35. Van de Loo FA, Kuiper S, van Enckevort FH, Arntz OJ, van den Berg WB. Interleukin-6 reduces cartilage destruction during experimental arthritis. A study in interleukin-6-deficient mice. *Am J Pathol.* 1997;151:177-191.
36. El-Kadiry AE-H, Lumbao C, Salame N, Rafei M, Shammaa R. Bone marrow aspirate concentrate versus platelet-rich plasma for treating knee osteoarthritis: A one-year non-randomized retrospective comparative study. *BMC Musculoskelet Disord.* 2022;23:23.
37. Ziegler CG, Van Sloun R, Gonzalez S, et al. Characterization of growth factors, cytokines, and chemokines in bone marrow concentrate and platelet-rich plasma: A prospective analysis. *Am J Sports Med.* 2019;47:2174-2187.
38. van Vulpen LF, Schutgens RE, Coeleveld K, et al. IL-1β, in contrast to TNFα, is pivotal in blood-induced cartilage damage and is a potential target for therapy. *Blood.* 2015;126:2239-2246.
39. Piuzzi NS, Hussain ZB, Chahla J, et al. Variability in the preparation, reporting, and use of bone marrow aspirate concentrate

15263231, 2026, 1, Downloaded from https://arthroscopyjournals.onlinelibrary.wiley.com/doi/10.1002/arj.70021 by Rush University Library, Wiley Online Library on [26/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

in musculoskeletal disorders: A systematic review of the clinical orthopaedic literature. *J Bone Joint Surg Am.* 2018;100:517-525.
40. Brozovich A, Sinicrope BJ, Bauza G, et al. High variability of mesenchymal stem cells obtained via bone marrow aspirate concentrate compared with traditional bone marrow aspiration technique. *Orthop J Sports Med.* 2021;9:23259671211058459.
41. Sandy JD, Chan DD, Trevino RL, Wimmer MA, Plaas A. Human genome-wide expression analysis reorients the study of inflammatory mediators and biomechanics in osteoarthritis. *Osteoarthr Cartil.* 2015;23:1939-1945.
42. Kokebie R, Aggarwal R, Lidder S, et al. The role of synovial fluid markers of catabolism and anabolism in osteoarthritis, rheumatoid arthritis and asymptomatic organ donors. *Arthritis Res Therapy* 2011;13:1-10.
43. Richette P, Francois M, Vicaut E, et al. A high interleukin 1 receptor antagonist/IL-1β ratio occurs naturally in knee osteoarthritis. *J Rheumatol* 2008;35:1650-1654.
44. Cohen SB, Proudman S, Kivitz AJ, et al. A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to IL-1R1) in patients with osteoarthritis of the knee. *Arthritis Res Therapy.* 2011;13:1-12.
45. Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P. Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: A multicenter study. *J Rheumatol.* 2005;32:1317-1323.
46. Kraus V, Birmingham J, Stabler T, et al. Effects of intraarticular IL1-Ra for acute anterior cruciate ligament knee injury: A randomized controlled pilot trial (NCT00332254). *Osteoarthr Cartil.* 2012;20:271-278.
47. Henderson B, Pettipher ER. Arthritogenic actions of recombinant IL-1 and tumour necrosis factor alpha in the rabbit: Evidence for synergistic interactions between cytokines in vivo. *Clin Exp Immunol.* 1989;75:306-310.
48. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm.* 2014;2014:561459.
49. Rigoglou S, Papavassiliou AG. The NF-κB signalling pathway in osteoarthritis. *Int J Biochem Cell Biol.* 2013;45:2580-2584.
50. Wang A-T, Zhang Q-F, Wang N-X, et al. Cocktail of hyaluronic acid and human amniotic mesenchymal cells effectively repairs cartilage injuries in sodium iodoacetate-induced osteoarthritis rats. *Front Bioeng Biotechnol.* 2020;8:87.
51. Becktell L, Matuska AM, Hon S, et al. Proteomic analysis and cell viability of nine amnion, chorion, umbilical cord, and amniotic fluid–derived products. *Cartilage.* 2020;13:495S-507S.
52. Saw KY, Hussin P, Loke SC, et al. Articular cartilage regeneration with autologous marrow aspirate and hyaluronic Acid: An experimental study in a goat model. *Arthroscopy.* 2009;25:1391-1400.
53. Prasadam I, Akuien A, Friis TE, et al. Mixed cell therapy of bone marrow-derived mesenchymal stem cells and articular cartilage chondrocytes ameliorates osteoarthritis development. *Lab Invest.* 2018;98:106-116.
54. Sadeghirad B, Rehman Y, Khosravirad A, et al. Mesenchymal stem cells for chronic knee pain secondary to osteoarthritis: A systematic review and meta-analysis of randomized trials. *Osteoarthr Cartil.* May 20, 2024:S1063-4584(24)01200-7. [Online ahead of print.]
55. Woodell-May J, Steckbeck K, King W. Potential mechanism of action of current point-of-care autologous therapy treatments for osteoarthritis of the knee-a narrative review. *Int J Mol Sci.* 2021;22:2726.
56. Fortier LA, Cercone M, Keller LE, Delco ML, Becktell L, Wells KV. Amnion and umbilical cord-derived products in sports medicine: From basic science to clinical application. *Am J Sports Med.* 2021;49:1954-1961.
57. Panero AJ, Hirahara AM, Andersen WJ, Rothenberg J, Fierro F. Are amniotic fluid products stem cell therapies? A study of amniotic fluid preparations for mesenchymal stem cells with bone marrow comparison. *Am J Sports Med.* 2019;47:1230-1235.

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