How Should We Evaluate Outcomes for Use of Biologics in the Knee?

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Abstract

In recent years, the use of biologics for the primary treatment and augmentation of treatment in patients with knee pathology has increased substantially. Techniques and applications for biologic preparations such as platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) have been developed and refined to increase the healing response in bone, ligaments, cartilage, meniscal tissue, and other areas of the knee. Beginning with basic science and animal models, and finally proceeding to clinical human trials, the effect of biologics on clinical outcomes has been widely studied; however, many results have been inconclusive on their true effectiveness. The purpose of this article is to review current strategies for evaluating outcomes after biologic treatment and to propose new recommendations for assessing outcomes following the use of biologics in the knee. In addition, the importance of study design, current challenges, and future directions will be reviewed to describe the current standards for future studies to follow.

Keywords
- outcomes
- biologics
- knee
- platelet-rich plasma
- mesenchymal stem cells

The use of biologic treatments in the knee is among the most controversial topics in orthopedics. The term biologics encompasses numerous autologous preparations and is generally defined as "natural products that are harvested and used to augment a medical process and/or the biology of healing."[1] In orthopedic applications, biologics include platelet-rich plasma (PRP) (►**Fig. 1**) and mesenchymal stem cell (MSCs) preparations, such as bone marrow aspirate concentrate.[1] In addition, another method for intra-articular stimulation of the release of MSCs is "picking the notch." In this technique, small penetrations into the subchondral bone are created to allow MSCs to enter the joint (►**Fig. 2**). PRP is an autologous processed blood product containing high concentrations of platelets, which provide local release of growth factors from α and dense granules.[1–4] MSCs are undifferentiated progenitor cells, which are most commonly isolated from either bone marrow aspirate or adipose tissue.[1] MSCs possess the ability to differentiate into specialized cell lines such as chondrocytes and osteocytes, recruit other cell lineages, and release growth factors that augment healing.[1,5–7] Preliminary evidence suggests that PRP and MSCs may be helpful in stimulating the healing of numerous knee injuries, including patellar tendinopathy, osteoarthritis and/or chondral injuries, acute ligamentous injuries, and meniscal tears.

While many advances have been made in recent years, the use of biologics in orthopedics remains tenuous in large part due to lack of standardization in measuring outcomes following treatment, rendering comparisons across studies difficult. This has led to controversy in the literature about the true effectiveness of biologics. Therefore, the purpose of this article is to review current strategies for evaluating outcomes after biologic treatment and to propose new recommendations for assessing outcomes following the use of biologics in the knee.

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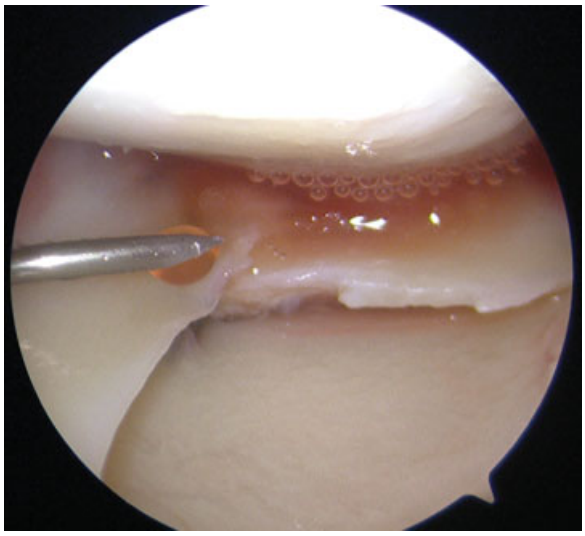

Fig. 1 Biologic augmentation using platelet-rich plasma following repair of a meniscus radial tear.

Outcome Metrics: Lessons from Preclinical Basic Science and Animal Model Studies

To obtain useful outcomes data regarding biologic treatments, it is first necessary to begin with a foundational understanding of the cellular and biochemical mechanisms in play. Many of the ways in which outcomes following biologic treatments are currently measured in human clinical studies have emerged from data obtained in preclinical basic science and animal model studies. As approaches for measuring outcomes following biologic treatment are refined, the lessons from these preclinical studies will continue to influence decisions in human clinical trials. Therefore, we begin with an overview of relevant findings in basic science and animal model studies that have shaped how outcomes are currently measured in human clinical studies following biologic treatments.

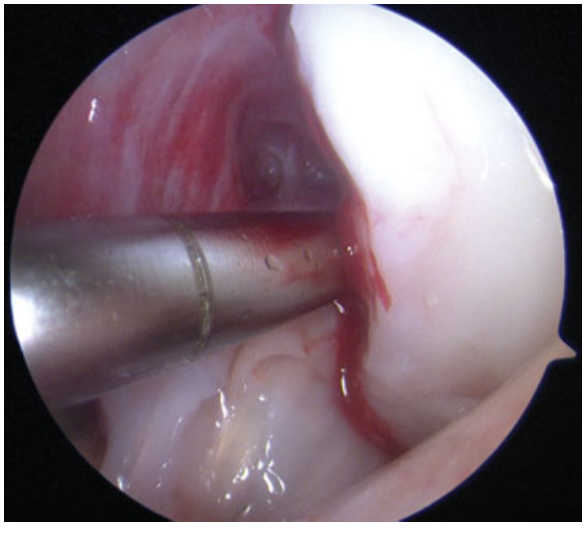

Fig. 2 So-called picking the notch is a method commonly used to access mesenchymal stem cells for biologic augmentation.

Preclinical Evidence

Preclinical basic science studies have elucidated many of the cellular and biochemical mechanisms with functional significance in biologic substances. These findings in turn have laid the groundwork for assessing the concentrations of important cytokines in biologic products and for evaluating the systemic effects of treatment. The α and dense granules in platelets store growth factors and other bioactive factors important for healing. When activated, platelets release growth factors contained in these α granules in a localized, site-specific manner. Growth factors that have demonstrated positive effects include bone morphogenetic protein, fibroblast growth factor, hepatocyte growth factor, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF).[8] However, other cytokines have demonstrated deleterious effects such as including interleukin 1 (IL-1), matrix metalloproteinases, tumor necrosis factor α. Dense granules release other bioactive molecules, such as serotonin, histamine, dopamine, calcium, and adenosine. Together, α and dense granules provide localized delivery of compounds that function in a complementary fashion during wound healing.[2,3] Finally, MSCs provide numerous benefits including the ability to differentiate into one of multiple cell lineages, release growth factors, and mobilize the movement of stem cells during angiogenesis.[1]

To progress toward eventual human clinical studies, preclinical studies have been performed first to evaluate the safety and effectiveness of biological treatment strategies. For the most part, these models typically involve using in vitro basic science human or animal cell models or in vivo animal models. In addition, preclinical studies have facilitated the development and refinement of outcome measures that are later used in human clinical trials, including serum biomarkers and structural assessment tools such as imaging and histology. A general description of findings is presented in the following section.

Basic Science Evidence

Numerous basic science studies have been performed to evaluate the efficacy of biological treatments in promoting healing responses in the knee. These studies have used cellular models, either derived from humans or animals, to evaluate the cellular mechanisms that may have positive therapeutic effects following biologic treatment. De Mos et al[9] reported that PRP resulted in increased collagen and total cell proliferation, as well as matrix-degrading enzymes, in human tenocyte cultured cells, which they hypothesized may help the healing response of tendons.[9] In addition, Schnabel et al[10] cultured equine flexor digitorum superficialis tendon explants in PRP and reported increased gene expression of collagen I, collagen III, and collagen oligomeric protein, without the increase of catabolic matrix-degrading proteins.[10] These authors also promoted the use of PRP to stimulate tendon healing.[10] With regard to cartilage repair, Fukumoto et al[11] reported that TGF-β1 and IGF-1, commonly found in PRP, synergistically promoted the chondrogenesis of MSCs, which was measured by quantifying the amount of

cartilage in the explants, in an in vitro rabbit model. Finally, Ishida et al[12] reported that rabbit meniscal cells exhibited upregulation of meniscal cell viability, including increased synthesis of DNA and sulfated glycosaminoglycans, in vitro after treatment with PRP.

Animal Model Evidence

Animal model studies have been used to demonstrate the proof of concept for new biologic treatment approaches. Given the promising results that have been described by in vitro basic science models, many studies have moved toward the evaluation of biologic knee treatments in animal models. Oftentimes, outcome metrics utilized in animal model studies mirror approach those later used in human clinical trials.

For patellar tendinopathy, Taylor et al[13] reported that an autologous blood product injection was safe in an in vivo rabbit model based on histological analysis, which demonstrated an angiogenic response without abnormal histological markers. Kajikawa et al[14] reported increased collagen I and collagen III and macrophage production on histological and immunohistological evaluation in rats after injecting PRP, indicative of the mobilization of tendon healing. In addition, Wilke et al[15] created 15 mm articular cartilage defects in the patellofemoral joint of horses and reported that injection of autogenous fibrin with MSCs increased the early cartilage healing response in comparison to a cartilage-only model after biopsy. Therefore, biopsy with histological analysis may likewise prove useful in human clinical trials following biologics treatment.

To evaluate the effect of biologics on meniscal healing, Ishida et al[12] created a 1.5 mm defect in the avascular zone of meniscal tissue, and treated the meniscal tissue with PRP, enclosed in a gelatin hydrogel scaffold designed to gradually time release PRP. After 12 weeks in vivo, the PRP-treated group demonstrated more fibrochondrocytes on histology, which led the authors to propose that PRP may be able to stimulate healing of the avascular meniscal tears.[12] However, Zellner et al[16] did not report improved tissue fill in rabbits with the application of MSCs and PRP, with a hyaluronan-collagen scaffold, implanted into 2 mm defects in the avascular meniscal zone, at 6 or 12 weeks in comparison to a cell-free scaffold.

With regard to soft tissue usage, biologics have also been evaluated in preclinical animal models. Unlike human models, in which biomechanical or histological analysis of tissue is typically very difficult, animal models allow for the direct comparison of healing after in vivo biological treatment. One group has reported enhanced ACL primary repair in a porcine model with significantly improved yield and stiffness at 3 months after implantation of a collagen-scaffold complex, intended to provide structural support for a healing clot, in comparison to a suture only repair.[17] The same group also reported similar results in a canine model with increased biomechanical strength after use of a collagen-PRP scaffold to treat a surgically sectioned ACL in comparison to controls at 6 weeks.[18] In addition, two studies reported similar increased biomechanical strengths of the medial collateral ligament (MCL), and increased neovascularization after the addition of growth factors that are commonly found in PRP after 6 weeks of healing in rabbit models.[19–21]

Future animal models should also evaluate soft tissue healing at multiple time points using magnetic resonance imaging (MRI), such as those described by Biercevicz et al,[22] that can assess the signal intensity at numerous time points.[22] Increased and/or decreased signal intensity on MRI imaging can indicate the progression of healing in ligamentous healing and should be further investigated. As shown by Biercevicz et al,[22] decreased signal intensity is significantly correlated with increased structural properties of anterior cruciate ligaments (ACL) of grafts. Therefore, assessing outcomes following biologic treatment using MRI obtained at multiple time points can further enhance understanding of outcomes in animal models.[22] In summary, the outcome measures used in basic science and animal model studies represent excellent techniques to emulate in various settings across human clinical trials.

Measuring Outcomes in Human Clinical Studies

Against the backdrop of advances made in preclinical basic science and animal model studies, biologic approaches for primary treatment or augmentation of treatment have been developed for a host of knee pathologies. The success of these approaches is measured using outcomes data. Outcome metrics for human clinical studies following biological treatment can be broadly divided into subjective and objective measures (►**Table 1**). Subjective measures are patient reported outcomes, and they generally require a follow-up period of a minimum of 2 years. These include clinical outcome scores, which have been extensively used to evaluate how patients fare following a vast array of procedures from cartilage restoration to ligament reconstructions. Objective outcome measures include biomarkers to measure the biochemical consequences of treatment, imaging to measure the structural properties of tissue, and direct visualization to measure gross morphologic or histological appearance following treatment. Follow-ups for these objective studies are variable and should be individually tailored for the purpose of the study.

The use of preclinical studies has helped to show the potential for biologics in stimulating increased or faster healing. However, clinical studies have not always shown the same benefits in human clinical trials. In addition, comparison to a control group is not always possible and/or performed. Therefore, while the authors stress that future studies should strive to be level I randomized controlled trials, there are many different outcomes that can be evaluated in any study evaluating biologics. These outcome metrics will be discussed in depth in the later sections, with the potential advantages and disadvantages of each, as well as the potential ways to standardize each based on the current literature and future directions.

Clinical Outcome Scores

Subjective clinical outcomes consist of patient reported outcomes that are quantified using outcome scores. Examples of

Table 1 Outcome markers for biologics

Type of measure	Examples
Subjective outcomes	
Clinical outcomes scores	Lysholm score
	Tegner activity level
	Short form 36
	International Knee Documentation Committee form
	Western Ontario and McMaster Universities Osteoarthritis Index
	Knee Injury and Osteoarthritis Outcomes Score
	Victorian Institute of Sports Assessment
	Visual analog scale
	Patient satisfaction with outcome
Objective outcomes	
Direct visualization	Second-look arthroscopy
	Histological outcomes
Imaging	Radiographs
	Magnetic resonance imaging
	Ultrasound
Biomarkers	Bone morphogenetic protein
	Fibroblast growth factor
	Hepatocyte growth factor
	Insulin-like growth factor
	Platelet-derived growth factor
	Transforming growth factor-β
	Vascular endothelial growth factor
	Interleukin 1
	Matrix metalloproteinases
	Tumor necrosis factor-α

outcome scores include the Lysholm score to report patient function after knee surgery,[23] the Tegner scale to document activity level,[24] and the short form 36 (SF-36) health questionnaire.[25] Some metrics, such as the International Knee Documentation Committee (IKDC) form, include both a subjective questionnaire and objective exam components.[26] Other metrics are more specific. For example, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[27] and Knee injury and Osteoarthritis Outcome Score (KOOS)[28] were developed to assess important patient-centric outcomes in osteoarthritis. A second example is the Victorian Institute of Sports Assessment (VISA) to document outcomes for patellar tendinopathy.[29,30] Still other measures are symptom specific, for example, the visual analog scale (VAS) to document the intensity of a patient's pain.[31] Finally, a patient satisfaction with outcome question is often used to report overall satisfaction following treatment. Together, these outcome scores represent important tools for assessing patient-reported outcomes following biologic treatment. In the future, these scores will need to be evaluated for reliability, validity, and responsiveness for specific conditions.

There are many examples in the literature on effective use of clinical outcome scores following biologic treatment in the knee. To begin, the effects of biologics on patellar tendinopathy have been examined in numerous clinical studies. Preliminary studies reported significantly improved clinical outcomes after a combination of dry needling and PRP or autologous blood injections using the SF-36, Tegner, VAS, and VISA outcome scores.[24,32,33] These studies involved numerous injections spaced at least 2 weeks apart; however, no control groups were compared.[24,32,33] Vetrano et al[34] performed a randomized controlled trial comparing the effect of PRP injections of extracorporeal shock wave therapy. Both treatments significantly improved VAS and VISA clinical outcome scores at 2-, 6-, and 12-month follow-ups, with PRP resulting in significantly better outcomes than shock wave therapy at 6 and 12 months.[34] In addition, Dragoo et al[29] performed a randomized controlled trial comparing the effects of PRP and dry needling to dry needling alone.[29] They reported significantly better results at 12 weeks with the PRP and dry needling in comparison to dry needling alone on the VISA outcomes, but these reported differences were not present at 26 weeks of follow-up.[29]

With regard to treating osteoarthritis, a level II prospective cohort study reported that intra-articular PRP injections resulted in improved outcomes on IKDC and VAS evaluations, in comparison to high- or low-molecular-weight hyaluronic acid at 6-month follow-up in a prospective comparative study.[35] A recent randomized controlled trial described better WOMAC and VAS outcomes from single or double injections of PRP to treat osteoarthritis in comparison to a saline control. Results revealed adverse effects in 17% of patients receiving PRP treatments.[36] In addition, a recent randomized controlled trial reported that patients who received injections of peripheral blood progenitor cells and hyaluronic acid did not result in significant differences in IKDC outcome scores in comparison to a hyaluronic acid control group.[37]

Finally, clinical outcome scores have been evaluated for soft tissue injuries. Three studies have evaluated clinical outcomes after ACL reconstruction.[38–40] All the three studies reported no significant difference between the control groups and groups treated with PRP using variable clinical outcome scores at follow-up. At 6 months follow-up, Orrego et al[38] reported no difference on IKDC or Lysholm clinical outcome scores,[38] whereas Ventura et al[39] reported no difference in the KOOS or Tegner scores.[39] At 2-year follow-up, Nin et al[40] reported no difference in VAS or IKDC scores between the control and PRP-treated groups. However, the lack of significant difference is not all that surprising due to the transtibial tunnel drilling for the control groups that would act as a source of platelets and MSCs functioning in effect as incidental biological treatments.

As shown earlier, there are currently numerous strategies for incorporating subjective clinical outcome measures in

human clinical trials for the evaluation of biological treatments. The authors recommend using outcome metrics that are pathology-specific to achieve a more specific evaluation of outcome. However, at the same time, caution is necessary because these outcome metrics do not measure structural outcomes of the cartilage, tendon, or ligaments; therefore, supplementation of other metrics that specifically look at structural outcomes or other objective measures is advised.

Direct Visualization Metrics

Second-look arthroscopy and biopsy are considered the gold standard for assessing the presence of gross morphology of tissue following treatment or augmentation of treatment with a biologic agent. While these studies are often commonly performed in preclinical animal studies, the ability to perform these invasive procedures at any or multiple clinical follow-ups is very difficult, and therefore, any studies using these measures are oftentimes limited to level IV case studies or series. Moreover, this practice is also outside the standard of care and its use would be limited to patients enrolled in clinical studies approved by an institutional review board. However, certain situations, such as when patients return for hardware removal,[41,42] may warrant second-look arthroscopy.

Second-Look Arthroscopy

Second-look arthroscopy can be an excellent tool for assessing the gross morphology of a specific structure in the knee. Saw et al[42] used second-look arthroscopy as a means to assess the effects of autologous peripheral blood progenitor cells on articular cartilage regeneration.[42] In this study, second-look arthroscopy was possible due to the clinical course of five patients from a larger level II, randomized clinical trial. The ability to perform second-look arthroscopy allowed for a direct visualization of the articular cartilage, and a biopsy of chondral core tissue. On second-look arthroscopy, the articular cartilage appeared regenerated with excellent integration into the surrounding tissue. The authors also reported the absence of delamination or hypertrophy of the cartilage.[42] A follow-up study by the same group was also able to recruit patients for second-look arthroscopy and biopsy at 18-month follow-up in a level II study.[37]

In addition, Sánchez et al[41] performed a level III case–control study in which they enrolled 37 patients who underwent ACL reconstructions and presented for hardware removal or other knee pathologies within 6 to 24 months after initial ACL reconstruction with or without PRP supplementation. Second-look arthroscopy allowed for the gross arthroscopic evaluation, which was evaluated using graft thickness and tension and synovial coverage of the graft as metrics. The authors reported that the group treated with PRP and the control group were not significantly different (though the results may nevertheless be of marginal significance, $p = 0.051$).[41] In addition, the PRP groups demonstrated significantly more remodeling and connective tissue coverage compared with the control group.[41]

Histological Outcomes

Studies that evaluate the direct appearance of the tissue via biopsy and histological analysis provide a gold standard for future imaging studies strive toward. Unlike many other methods of assessing outcomes, these invasive procedures allow for assessment of the tissue directly in the area of treatment. However, these studies are often limited to special situations, in which patients require additional surgery.[37,42] In the same studies, biopsies on second-look arthroscopy were taken to evaluate the effect of autologous peripheral blood progenitor cells to regenerate articular cartilage. In the first study,[42] a case series of five patients was used to histologically assess collagen I and collagen II content, proteoglycan content, and tissue morphology in patients who underwent subchondral drilling to regenerate grade III or grade IV articular cartilage lesions was examined. These patients also were augmented with injections of peripheral blood progenitor cells and hyaluronic acid, and histology revealed the formation of hyaline cartilage.[42] In a follow-up randomized controlled trial, 24 patients in the treated group, using the same biologic treatment, reported significantly higher scores on the International Cartilage Repair Society Visual Assessment Scale II than the 25 patients in the hyaluronic acid control group.[37]

Structural outcome metrics that involve direct visualization of structures in the knee usually require proactive planning and identification of ideal candidates by the researchers. However, as described earlier, these assessments are certainly possible with adequate planning by the researchers. Therefore, while level I double blind, randomized clinical trials will likely be difficult to pursue with these invasive outcome assessments, these studies can help to validate the findings of other level I or level II studies that evaluate other outcome metrics. Finally, the authors propose that future studies can be undertaken to evaluate the effect of biologics using these direct visualization outcome metrics whenever feasible.

Imaging

Radiographs

Plain radiographs can be used to evaluate changes in joint space for patients treated with biologic agents for early or advanced osteoarthritis. Grading is typically conducted using the Kellgren–Lawrence grading scale,[43] which is presented in ►**Table 2**. However, there are questions regarding the reproducibility of grading because the interrater reliability has been reported to be as low as 0.36.[44] In addition, radiographs can be used to detect signs of bone healing such as the degree of incorporation of bone grafts with biologic augmentation.[45] While radiographs are an inexpensive, widely available, and noninvasive tool for outcomes assessment, they do not provide a detailed assessment of soft tissue changes and thus likely have a limited role in assessing outcomes following biologic treatments. Plain radiographs may be most useful for long-term outcome studies of the effect of biologic treatment as a modifier of osteoarthritis disease progression.

Table 2 The Kellgren–Lawrence grading scale for classifying changes on plain radiographs indicative of early or advanced osteoarthritis[43]

Grade 1	Questionable joint space narrowing
	Possible osteophyte formation
Grade 2	Possible joint space narrowing
	Osteophyte formation
Grade 3	Joint space narrowing
	Multiple osteophytes
	Sclerosis
	Possible boney deformity
Grade 4	Joint space narrowing present
	Multiple large osteophytes
	Marked sclerosis
	Definitive boney deformity

Magnetic Resonance Imaging

MRI provides the advantage of a noninvasive and widely available outcome assessment following treatment with biologics. Gross morphology is readily assessed using standard MRI techniques in the axial, coronal, and sagittal planes. The structural appearances of graft incorporation or fill in an articular cartilage defect are commonly reported outcomes on MRI. Human clinical studies have evaluated structural outcomes on T1- and T2-weighted MRI images, particularly after ACL reconstruction with PRP augmentation.[38,40,45–48]

Clinical studies evaluating bone-graft integration into the reconstruction tunnels and ligament maturation after ACL reconstruction are numerous.[2] A systematic review[49] evaluated four studies (one level I randomized controlled trial,[50] two level II studies,[38,48] and one case–control study[46]) to see if PRP or autologous platelet concentrations had any effect on graft integration into reconstruction tunnels, as determined on T1- or T2-weighted MRI scans. Two studies reported no difference between the control groups and PRP groups in terms of tunnel widening at 3 or 6 months using T1- or T2-weighted MRI images,[38,48] while two reported no difference in graft integration at 3 and 6 months between PRP or autologous platelet concentrate-treated and control groups on T2-weighted sequences.[38,46] In contrast, a level I study reported that the use of a platelet gel significantly increased early revascularization in the bone–graft interface at 4 to 6 weeks, although not at 10 to 12 weeks.[50] In regard to graft vascularization, the same systematic review reviewed four studies (two level III studies[46,47] and two level I studies[40,50]) that evaluated the effects of PRP, as determined by T1- or T2-weighted MRI scans. One of these studies, as well as another level II study,[38] reported increased speed of graft maturation (48% of time in comparison to control),[47] and lower intensity signal on MRI on T1- and T2-weighted MRIs.[38] However, two studies did not report differences in MRI signal intensity between PRP or autologous platelet-treated and control groups at 6 months or 2 years on T1- and T2-weighted MRIs,[40,46] whereas another noted no graft vascularization in control or PRP groups at up to 12-week follow-up.[50] Finally, special MRI sequences will likely have an increased role in assessing outcome in the future. A recent study described increased and/or decreased signal intensity on MRI imaging can indicate the extent of ligamentous healing.[22] This study, used a relatively new MRI modality, T2-star–weighted (T2*) imaging, which provides higher spatial resolution to obtain signal from the short T2 signal in cartilage.[22,51] In addition, T1 rho (T1ρ) to measure to provide more accurate quantification of subtle tissue changes, oftentimes present with early development of osteoarthritis.[51] Therefore, further studies should aim to evaluate structural outcomes using new MRI modalities that may be able to more effectively visualize certain regions or types of tissue or cartilage.

Evaluating structural outcomes on MRI will likely be the most realistic and valuable outcome metric for clinicians. However, current evaluations of biological treatments on MRI are very limited and mostly related to the reconstruction of the ACL. Future studies should use variable MRI modalities and assess the many different possible applications of biological treatments. However, just as with second-look arthroscopy, second-look MRIs are not always indicated for patients, and therefore, their use as an outcome measure may require proactive planning on the part of clinicians. However, unlike other means of structural outcome metrics, measuring outcomes via MRI in large randomized controlled trials should be feasible for clinicians as MRI is widely available to most research institutions. Therefore, the authors propose increased MRI evaluation of biologics as essential for determining the effect of biologics on structural outcomes.

Biomarkers

Outcomes following treatment with biologic agents can also be documented using biomarkers in serum assays. While the use of these measures in human clinical studies is currently limited, there is great interest in developing outcome parameters that document the systemic effects of biologic augmentation. Wasterlain et al[52] examined the systemic effects following intratendinous injection of leukocyte-rich PRP. Six growth factors were measured in human subjects using enzyme-linked immunosorbent assays at 0.25, 3, 24, 48, 72, and 96 hours following injection including human growth hormone, IGF-1, IGF binding protein 3, basic fibroblast growth factor (bFGF or FGF-2), VEGF, and PDGF-BB. The results demonstrated an increase in serum IGF-1, VEGF, and bFGF levels, suggesting that systemic response to biologic augmentation may represent a viable outcome measure in other human clinical trials. Results also suggest that biologic augmentation may stimulate the upregulation of systemic signaling pathways for growth factor production, rather than localized delivery of growth factors. While the evaluation of outcomes using biomarkers may be indirect measures of structural outcomes, they should be a focus of future studies to determine their effectiveness in assessing outcomes after biological treatments.

Influence of Study Design on Outcomes

Study design influences both the outcome measure chosen and the quality of the outcomes data obtained. In studies with small patient cohorts, outcomes such as biopsy with detailed histological evaluation or extensive biomarker assays may be preferred given the small sample size. These strategies may be beneficial for early clinical studies to demonstrate proof of concept, but they may not be practical in large, multicenter trials. In studies with large patient cohorts, such as large comparative studies or multicenter randomized controlled trials, factors such as cost of implementing the outcome measure and ease of use may outweigh other considerations.

In addition, a power analysis is another important factor. For studies that aim to detect a small effect size, hundreds or thousands of patients may be required to demonstrate a given therapeutic effect. A power analysis is essential for determining the power of a certain study, which is defined as the probability of not making a type II β error.[53] A type II β error is defined to be a false-negative error rate, and is typically set at a maximum level of 20% for clinical trials.[53] Therefore, for clinical trials, power, defined as (1-β), is generally required to be at a level of 80% during a power analysis.[53] Another important concern during a power analysis is that the analysis is performed a priori, or before testing, to determine the required sample size.[53] This requires an estimate of the minimum clinically important difference (MCID) for treatments before testing, which helps to prevent potential bias that may be present if this level is determined after testing. One way to determine this MCID is to use values that may be similar to the literature.[29] As described by Walters[53] a post hoc power analysis is not recommended because it really does not provide an accurate description of the sample size. After testing has been completed, the observed data will determine the size and direction of the treatment effect, and it does not add any new information to the study.[53] As shown by many recent high-quality level I or level II comparative studies evaluating the use of biologics, this emphasis performing a priori calculations using a previously determined MCID seems to have become the standard in the literature.[29,35,36]

Finally, study design influences the quality of the data obtained, with randomized trials that incorporate appropriate blinding and random treatment allocation[54] preferred over case series and case–control designs. High-quality level I clinical trials provide the benefit of eliminating potential bias, especially when they are performed double blinded to both the patient and physician. In addition, when possible, compared to a placebo group, as done by Patel et al,[36] can provide additional strength to the study. The addition of a placebo control ensures that the treatments performed are actually impacting the patient in comparison to a control group that believes that a treatment is being performed on them as well.

Current Challenges

At present, there are numerous unresolved technical challenges that call into question the reproducibility of biologic treatment methods, which renders interpretation of clinical outcomes data difficult. For example, the concentration of platelets and growth factors in various preparations of PRP has been reported to vary considerably even when using the same preparation technique.[55] Therefore, if it is presently unclear whether biologic preparations can be considered sufficiently similar across treatment groups in experimental studies, it is likewise unclear whether it is safe to attribute differences in outcomes to a true therapeutic effect or to error due to inconsistent biologic preparations. In the future, techniques must be refined to improve reproducibility across iterations.

Furthermore, indications for biologic treatment have not been well established. This represents a major hurdle to the advancement of biologic therapeutic approaches. As with any treatment, proper patient selection is essential to maximize the benefit experienced by patients following biologic approaches. However, it is presently unclear whether demographic factors, genetic makeup, environmental conditions, or other presently unidentified variables are the largest determinants of patient outcomes following biologic therapy. Future studies must be undertaken to identify the most important predictors of outcomes after treatment, leading to improved patient selection and evidenced-based recommendations for the most important outcome measures to document in clinical trials.

Future Directions

With the advent of biologic treatments, questions have arisen regarding the efficacy of current approaches. As the use of biologic treatments expands, reliable and valid outcome metrics to judge success and failure will be essential. The authors recommend a combination of subjective and objective outcome measures including clinical outcome scores, direct visualization, imaging, and serum or urinary assays to assess treatment efficacy from multiple perspectives. Noninvasive outcome measures, such as imaging, should be emphasized to best mirror what is feasible in standard clinical practice, and cost should also be considered. New MRI modalities, such as $T2^*$ and T1ρ, may help to more accurately describe the revascularization or subtle changes of tissue, which are typically indicative of healing, for different structures in the knee. Using these imaging modalities would then theoretically help determine the contribution of biological treatments in improving knee function. Inexpensive serum or urinary analysis test will be key to expand outcomes assessment to centers with limited financial resources. Moreover, lack of standardized reporting regarding outcome measures has led to the inability to compare treatment efficacy across techniques. Standardized outcome metrics will be needed to allow for better conclusions to be drawn. Finally, improved experimental design using sufficiently powered comparative studies and randomized controlled trials will be necessary to demonstrate therapeutic benefit with high-quality outcomes evidence. This will help elucidate both the short- and long-term therapeutic effects of biologic agents.

In addition, while numerous outcome metrics are currently available to clinicians, the authors are unaware of any

human clinical studies evaluating the effect of biologics for meniscal injuries or ACL primary repairs. Therefore, future studies should also investigate these avenues in future research to see if biological treatments may augment the healing after injuries to these structures.

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