Systematic Review

Limitations and Sources of Bias in Clinical Knee Cartilage Research

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Purpose: The purpose of this study was to systematically review the limitations and biases inherent to surgical trials on the management of knee chondral defects. **Methods:** A literature search of PubMed/Medline, CINAHL (Cumulative Index to Nursing and Allied Health Literature), EMBASE, and the Cochrane Central Register of Controlled Trials was conducted in September 2010 and updated in August 2011 to identify all English-language, Level I evidence, prospective, randomized controlled trials published from 1996 to present. The keyword search included the following: "autologous chondrocyte," "cartilage graft," "cartilage repair," "chondroplasty," "microfracture," "mosaicplasty," and/or "osteochondral." Nonoperative studies, nonhuman studies, ex vivo studies, non-knee studies, and/or studies with follow-up of less than 1 year were excluded. A systematic review was performed on all included studies, and limitations and/or biases were identified and quantitated. **Results:** Of 15,311 citations, 33 abstracts were reviewed and 11 prospective, randomized controlled trials were included. We identified 9 major limitations (subject age, subject prior surgery, subject duration of symptoms, lesion location, lesion size, lesion number, procedure selection, procedure standardization, and limited histologic analysis) and 7 common biases (selection, performance, transfer, nonresponder, detection, publication, and study design). **Conclusions:** Level I therapeutic studies investigating the surgical management of human knee cartilage defects have substantial identified biases and limitations. This review has limitations because other classifications of bias or limitation exist. Optimal management of cartilage defects is controversial, and future rigorous research methods could minimize common biases through strict study design and patient selection criteria, larger patient enrollment, more extended follow-up, and standardization of clinical treatment pathways. **Level of Evidence:** Level I, systematic review of Level I studies.

The current orthopaedic research in the field of cartilage restoration is extensive. However, evidence-based articular cartilage research conducted in randomized, controlled prospective trials has been limited by difficulties in enrollment, conflicting evidence for evaluation, diverse methodology, inadequate follow-up, strict government guidelines, varying regulatory environments, and the numerous inherent potential biases faced by investigators. Furthermore, the presence of an isolated articular cartilage lesion of

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a given size with a surrounding rim of healthy cartilage and the absence of confounding or exclusionary comorbidities is relatively rare. Despite reports of the high incidence of articular cartilage pathology identified during knee arthroscopy,[1] enrollment of patients who qualify for inclusion in articular cartilage randomized controlled trials (RCTs) with those lesions is actually quite low.

Biases in articular cartilage research arise from both patient-specific and study design factors. Patients' comorbidities, both within and outside of the joint, variably affect outcomes. Similarly, many studies have failed to control for the wide array of surgical techniques and disparate rehabilitation protocols. Likewise, no consensus exists on the optimal primary and secondary outcome endpoints, not to mention the appropriate instruments used to evaluate clinical results. These multivariant parameters introduce many forms of bias, including selection bias, performance bias, transfer bias, detection bias, reporting bias, and confounding. In addition, there can potentially be bias on the basis of corporate involvement or conflict of interest, whether disclosed or not, and economic and practical constraints can also confound or introduce other forms of bias. Lastly, systemic bias from a given practice location or setting may also impair the extrapolation of certain research because of unique institutional or governmental constraints.

This investigation serves as a systematic review of Level I articular cartilage studies with the purpose of identifying obstacles and sources of bias in available published RCTs. The Level I studies that were reviewed represent the most structured and reproducible research in this subject matter but are not amenable to meta-analysis given the differences in their methodology and varying surgical procedures. Ultimately, this systematic review seeks to identify shortcomings in the existing body of literature on cartilage research and offer guidance to investigators on future prospective trial design.

METHODS

A literature search of PubMed/Medline, the Cochrane Central Register or Controlled Trials, CINAHL (Cumulative Index to Nursing and Allied Health Literature), and EMBASE was performed in August 2011 to identify all randomized prospective comparative studies evaluating operative treatment of articular cartilage defects of the knee in vivo. The search identified articles published from January 1, 1966, to September 1, 2010, containing at least 1 of the following words: "osteochondral," "autologous chondrocyte," "mosaicplasty," "microfracture," "chondroplasty," "cartilage graft," and "cartilage repair." The search was limited to English-language articles in all available journals on human subjects classified by PubMed as RCTs or clinical trials. Inclusion criteria consisted of studies of Level I evidence reporting on surgical treatment of full-thickness osteochondral defects in the knee. All studies were included independent of date of publication if published on or after January 1, 1966. Follow-up studies reporting on clinical outcomes of their respective Level I RCTs were included. Exclusion criteria consisted of studies pertaining to nonsurgical interventions, Level II to V evidence studies, nonhuman studies, surgical interventions on body parts other than the knee, and studies with follow-up of less than 1 year.

Data extracted from each study included the level of evidence, number of subjects, patient demographics, location of cartilage lesion, surgical techniques used for treatment and control, method of randomization and blinding, presence of co-interventions, rehabilitation protocols, overall methodologic quality, and outcome measures. Analysis of these data was performed in an attempt to highlight the challenges faced by these investigators and the limitations of current Level I cartilage research, as well as to identify the potential bias present in these studies (Table 1).

RESULTS

In total our literature search yielded 15,311 cited manuscripts. The abstracts of 33 studies were reviewed,[2-34] and 14 Level I evidence–based RCTs were isolated for review.[2,3,5,11-15,20,21,25,26,28,33] Three additional studies were excluded from further analysis: 1 study was excluded because of the topic,[11] 1 study was excluded because of its isolated comparison of first- and second-generation autologous chondrocyte implantation (ACI) techniques,[12] and 1 study that presented a subset of the multicenter trial previously described by Saris et al.[25,26] was also excluded.[28] Three prospective, randomized comparative studies were not considered because of a Level II evidence rating.[7,8,17]

The presence or risk of bias can be difficult to elucidate from any study, given that each clinical research situation is inherently unique, with political, societal, and economic pressures specific to the individual investigators and their locale. These 11 Level I evidence–based studies represent the most rigorous scientific research available on articular

TABLE 1. *Identified Forms of Bias in Cartilage Research*

Form of Bias	Definition	Strategy
Selection	Error in patient selection resulting in nonrepresentative or fundamentally different treatment groups; may include sampling bias, volunteer bias, and nonresponder bias	Randomization protocol; strict inclusion/exclusion criteria; control of patient- and surgery-specific factors
Nonresponder	Form of selection bias that fails to account for the outcomes of patients who do not respond or complete follow-up	Comprehensive patient follow-up (>80%) Minimal losses to follow-up
Performance	Systematic differences in the care provided to the patients in the comparison groups other than the intervention under investigation	Standardized surgical technique and postoperative protocol; similar surgical experience; surgeon facile in both surgical treatments
Transfer	Bias resulting from differential losses to follow-up	Comprehensive patient follow-up (>80%) Minimal losses to follow-up
Detection	Artifactual findings resulting from errors in diagnostic or outcome measurement	Double-blinded protocol; third-party/independent observers at follow-up; objective outcome measures
Publication	Form of reporting bias leading to over-representation of significant or positive studies in systematic reviews	Publication of well-designed RCTs irrespective of clinical results; limitations of multiple publications
Study design	Error resulting from failure to identify issues with internal or external validity	Designation of an appropriate control group Control for open surgical technique or staged surgical interventions
Measurement	Systematic error in data collection	Use of validated patient outcome measures
Confounding	Interference from a third variable that distorts the association between treatment and clinical outcomes	Strict inclusion/exclusion criteria; isolated chondral lesions; limited prior or concomitant procedures; short preoperative symptom duration

cartilage. However, given the intrinsic challenges of this particular subject matter, the limitations of patient parameters and study design consideration can be difficult to avoid. Similarly, comparative evaluations can be difficult to assess based on the differences highlighted later. Table 2 defines the biases present in each study, and Table 3 defines specific limitations and challenges highlighted by the authors of each study.

Bartlett et al.[2] (2005)

Clinical Study: Bartlett et al.[2] compared the results of ACI with a porcine-derived type I/type III collagen cover versus matrix-induced autologous chon-

TABLE 2. *Presence of Bias in Selected Cartilage Studies*

Study	Bartlett et al.[2] ACI *v* MACI	Basad et al.[3] MACI *v* MFX	Bentley et al.[5] ACI *v* MOATS	Gudas et al.[13,15] MOATS *v* MFX	Gudas et al.[14] MOATS *v* MFX	Knutsen et al.[20,21] ACI *v* MFX	Saris et al.[25,26] CCI *v* MFX	Visna et al.[33] OATS *v* CP
Selection bias	+	−	++	++	++	++	−	+
Performance bias	NP	−	NP	−	−	−	+	+
Transfer bias	−	+	−	−	+	−	+	−
Nonresponder bias	+	++	+	+	+	−	−	+
Detection bias	+	NP	+	−	+	+	−	+
Publication bias	−	−	−	−	−	−	+	−
Study design bias	−	+	+	−	−	+	−	−

NOTE. One plus sign denotes the presence of a particular form of bias within the study, 2 plus signs indicate the presence of a more extensive form of bias, and minus sign denotes the absence or marginal presence of bias. ACI and CCI denote second-generation ACI.

Abbreviations: CP, chondroplasty; MACI, third-generation autologous chondrocyte implantation; MFX, microfracture; MOATS, mosaicplasty-type osteochondral autograft transfer surgery; NP, information required to make determination of bias is not provided; OATS, osteochondral autograft transfer surgery.

TABLE 3. *Design Limitations and Confounding Variables in Selected Cartilage Studies*

Study	Bartlett et al.[2] ACI *v* MACI	Basad et al.[3] MACI *v* MFX	Bentley et al.[5] ACI *v* MOATS	Gudas et al.[13,15] MOATS *v* MFX	Gudas et al.[14] MOATS *v* MFX	Knutsen et al.[20,21] ACI *v* MFX	Saris et al.[25,26] CCI *v* MFX	Visna et al.[33] OATS *v* CP
Population (yr)	15-50	18-50	16-49	<40	0-18	18-50	18-50	NP
Anatomic location	All	All	All	FC	FC	FC/T	FC	All
Size (cm^2)	>1	4-10	>1	1-4	2-4	2-10	1-4	NP
Multiple lesions	Yes	No	Yes	No	No	No	No	Yes
Prior surgery	Yes	NP	Yes	Yes	No	Yes	Yes	NP
Concurrent procedures	Yes	Yes	No	Yes	No	No	Yes	Yes
Prolonged preoperative symptoms	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NP
Standardized technique	Yes	Yes	No	No	No	Yes	Yes	NP
Limited histologic analysis	Yes	Yes	Yes	Yes	Yes	No	No	Yes

NOTE. Anatomic location may include all locations (i.e., femoral condyles, trochlea, and patella), femoral condyle, or trochlea. ACI and CCI denote second-generation ACI.

Abbreviations: CP, chondroplasty; FC, femoral condyle; MACI, third-generation autologous chondrocyte implantation; MFX, microfracture; MOATS, mosaicplasty-type osteochondral autograft transfer surgery; NP, information required to make determination of bias is not provided; OATS, osteochondral autograft transfer surgery; T, trochlea.

drocyte implantation (MACI) for osteochondral lesions of the knee (>1 cm^2) at 1-year follow-up.

Presence of Bias: Inclusion and exclusion criteria were explicitly defined, although several patient-specific factors contribute to significant selection bias. However, despite detailed methodology, it was unclear how many different surgeons were involved. No patients were lost to follow-up, limiting transfer bias. The study also did not include independent observers, which can lead to detection bias. In addition, nonresponder bias may be of concern, given the incomplete secondary histologic analysis (46%).

Limitations and Challenges: Multiple locations and variable sizes of chondral lesions were included in this study. Previous or concomitant treatments for joint instability, malalignment, and/or other "corrective surgery" at the time of cartilage implantation were also noted as limitations. Within this study, government recommendations specified that ACI treatment was reserved only for those individuals in whom earlier treatments had failed, thus predisposing this patient population to prolonged duration of symptoms and poorer preoperative function, as well as variable index procedures. Furthermore, not all patients were available for second-look arthroscopy, thus precluding adequate histologic and clinical evaluation. Although the merits of ACI treatment are debated, it still represents a useful tool in the treatment of chondral defects, particularly with advancements to second-generation (e.g., ACI with a porcine-derived type I/type III collagen cover) and third-generation (e.g., MACI) techniques. However, this study does not offer insight as to the comparative efficacy of this treatment versus other potentially more cost-effective, single-stage operations. As a result, the scope and impact of this investigation are somewhat limited.

Basad et al.[3] (2010)

Clinical Study: Basad et al.[3] evaluated MACI versus microfracture for symptomatic, post-traumatic cartilage defects (>4 cm^2) of the knee at 36-month follow-up.

Presence of Bias: Precise enrollment criteria were elucidated, thus obviating a large degree of selection bias. A single surgeon was allocated for both treatment arms, thereby limiting performance bias. Reasonable follow-up was achieved (>80%), although the use of third-party observers is unclear in this study. Limited histologic evaluation contributes to nonresponder bias.

Limitations and Challenges: Both condylar and patellar lesions were collectively randomized without subgroup analysis, and concomitant treatment of smaller meniscal pathology was permitted. Lesion size was fairly well controlled for, with consideration of only those lesions between 4 and 10 cm^2, and

osteochondral lesions were excluded. Symptom duration remained significant (mean, 2.3 years), and the extent of prior surgical management was unspecified. The microfracture technique was not described, although this was presumably performed arthroscopically versus an open approach for MACI. In addition, separate rehabilitation pathways were involved according to treatment arm, which may stratify patient recovery. Although magnetic resonance imaging (MRI) was performed in the early postoperative period to exclude delamination and/or hypertrophy in the MACI treatment arm, second-look arthroscopy with histologic analysis at 12 months postoperatively was limited because of the patients' reluctance.

Two separate but identical matrices were used as chondrocyte-seeded scaffolds in the MACI cohort and subsequently combined for statistical analysis. Although the manufacturers validated the similarity of these matrices in this study, the authors note that the variability in processing and material composition of commercially available scaffolds confounds the ability to objectively evaluate comparative studies using alternate products.

Bentley et al.[5] (2003)

Clinical Study: Bentley et al.[5] evaluated the results of ACI versus mosaicplasty for osteochondral defects in the knee (>1 cm^2) at a mean follow-up of 19 months.

Presence of Bias: This study had clearly described enrollment criteria and methods for randomization; however, patient-specific factors again present issues in terms of selection bias. Although complete clinical follow-up was described, there was insufficient postoperative histologic evaluation, which contributes to nonresponder bias. Performance bias cannot be assessed because the number of surgeons was unspecified. Detection bias was present because the study lacked independent observers and no description of a blinding method was found in the study. Finally, the utilization of mosaicplasty as a control for ACI may represent a study design bias, because these techniques are typically used for differentially sized lesions, in terms of both depth and diameter.

Limitations and Challenges: Even though the selection criteria were clearly defined in the study, patient-specific factors were challenges in themselves. These factors included varied size and location of the chondral lesion, differing underlying etiologies, wide age distribution (14 to 49 years), prolonged duration of preoperative symptoms (mean, 7.2 years), and high prevalence of previous nonarthroscopic surgery (mean, 1.5 operations). In the context of a nationalized health care system, the specific government restrictions may have contributed to the long duration of preoperative symptoms and high reoperation rate in this study. Differences in surgical techniques and intrinsic limitations inherent to each surgery also complicate comparative evaluation. The mosaicplasty technique used by the authors often resulted in incomplete filling of the cartilage defect with variable cartilage orientation and donor-site morbidity. Similarly, in the ACI group, both porcine collagen membrane and periosteal coverage were used, which confounds outcome interpretation because of the discreet differences in the complication profile depending on the type of patch used. Furthermore, the difficulty in establishing a control group was notable, particularly with the previously mentioned treatment arms, and this challenge was present in most of the included studies.

Gudas et al.[13,15] (2005, 2006)

Clinical Study: Gudas et al.[13,15] compared mosaic-type osteochondral autograft transplantation (OAT) versus microfracture for osteochondritis dissecans (OCD) or articular cartilage defects (1 to 4 cm^2) of the knee joint in young athletes at up to 36-month follow-up.

Presence of Bias: This study used very strict exclusion criteria with highly active selected patients, which can also result in selection bias given the physical demands in this population. Performance bias was limited because of a clear method and the presence of only 2 surgeons. Follow-up was adequate, and detection bias was also limited with 2 independent observers present at all follow-up visits.

Limitations and Challenges: A notable limitation is the difficulty in comparing the narrow, active patient demographic featured in this study with other broader patient populations. All patients were competitive or well-trained athletes with a mean age of 24 years, no prior surgeries on the affected knee, and only grade 3 or 4 condylar lesions. Similarly, the techniques used differed from those described in other studies of the same level of evidence. Of note, no arthrotomies were used in either procedure, and an arthroscopic assisted technique was used for the OAT procedure using a mosaicplasty-type technique. A common postoperative rehabilitation protocol was developed to facilitate comparison. Clinical, arthroscopic, and radiographic follow-up with MRI was sufficient, but histologic evaluation was incomplete despite a relatively high rate of subsequent biopsy (43%). The

authors noted the limitations of short-term follow-up and recommended future long-term studies to clarify long-term viability and indications for microfracture and osteochondral autologous transplantation, particularly in more generalizable populations. In addition, regenerate fibrocartilage and scar tissue associated with microfracture may be biomechanically insufficient to withstand the repetitive stresses encountered in this patient cohort.

Gudas et al.[14] (2009)

Clinical Study: Gudas et al.[14] compared arthroscopic mosaicplasty versus microfracture for treatment of OCD (2 to 4 cm^2) involving the femoral condyles of children aged under 18 years at 3- to 6-year follow-up.

Presence of Bias: Specific inclusion criteria were stipulated, although selection bias was still present, given the predominately adolescent patient composition and prolonged history of loose osteochondral fragments in some patients. An experienced, single surgeon performed all surgeries and subsequent follow-up without independent reviewers, exposing this study to detection bias. Transfer and nonresponder bias may also present an issue in the microfracture group because 3 patients were lost to follow-up and subsequently excluded from further analysis.

Limitations: The variability in skeletal maturity and Tanner staging, both within and between treatment arms, can introduce some degree of confounding. Moreover, although the area of the lesion was well defined, the depth of the lesion was not explicitly addressed and may have hindered success in the microfracture group vis-à-vis an osteochondral autograft transfer surgery technique because of lack of restoration of subchondral bone stock. The interval duration of symptoms before surgery was also considerable (mean, 23.54 months) and may complicate subsequent analysis. Second-look arthroscopy was performed in symptomatic individuals and those identified as treatment failures, which over-represents the patients receiving microfracture. However, whereas radiographic evaluation with MRI was obtained before second-look arthroscopy, no histologic analysis was performed to evaluate prior surgical intervention. As with the previous study, it is difficult to extrapolate outcomes from the treatment of OCD lesions in a pediatric population to a broader population with more traumatic chondral lesions, because these represent distinct and separate entities.

Knutsen et al.[20,21] (2004, 2007)

Clinical Study: Knutsen et al.[20,21] compared ACI versus microfracture for isolated condylar cartilage defects (2 to 10 cm^2) of the knee at 2-year follow-up and then 5-year follow-up.

Presence of Bias: This study involved 4 surgeons at 4 different hospitals who performed both procedures equally to limit performance bias. In contrast with other studies, this study presented mitigated transfer and nonresponder bias with excellent clinical, arthroscopic, and histologic follow-up at 2 and 5 years. Detection bias was also limited, with blinded independent observers present at all follow-up visits up to 2 years, but not at 5 years.

Limitations and Challenges: Numerous patient-specific factors served as limitations in this study. Chronic chondral lesions of widely variable size (2 to 10 cm^2), severity, and etiology involving the femoral condyle were considered, and in most patients a long period of conservative treatment (mean, 36 months) had failed and they had undergone previous surgery (93%). Although patients with malalignment, instability, or early evidence of arthritic change at the time of enrollment were excluded, the inconsistent use of other treatment modalities was another potentially confounding factor cited by the authors. When compared with an all-arthroscopic microfracture procedure, ACI surgery was considerably more invasive, necessitating an open arthrotomy for periosteal flap coverage, and more prone to complication with symptomatic graft hypertrophy, given the first-generation technique. In contrast to other studies, histologic evaluation during second-look arthroscopy was performed in most patients (83%). In addition, a high percentage of osteoarthritis was noted at 5 years after treatment. As such, the initial clinical and radiographic screening may have lacked the appropriate sensitivity to detect early degenerative changes. The unknown long-term natural history of cartilage injury and subsequent repair was also discussed as a limitation. Lastly, the notable lack of a control group was also a problem acknowledged in this and other studies.

Saris et al.[25,26] (2008, 2009)

Clinical Study: Saris et al.[25,26] compared characterized chondrocyte implantation (CCI) versus microfracture for treatment of symptomatic cartilage lesions of the femoral condyles (1 to 4 cm^2) at a follow-up of up to 36 months.

Presence of Bias: There were clearly described enrollment criteria, method of randomization, and detailed descriptions of methodology available in this

study, precluding significant selection bias. However, a total of 13 surgeons at 13 hospitals certainly contributes to possible performance bias. A multinational patient cohort was enrolled across multiple different health care settings, which could contribute to a form of reporting bias based on inherent cultural differences. Excellent follow-up was ensured (>80%), and detection bias was limited because of the presence of blinded independent observers present at all clinical follow-up visits, as well as histologic analysis. Duplicate publication bias was also encountered, with a subset of these patients separately described by Van Assche et al.[28,29]

Limitations and Challenges: The interval between the onset of preoperative symptoms and surgical treatment was less in this patient population when compared with other studies. However, patients were of a wide age range, and several had undergone previous or concomitant knee surgery to address intra-articular pathology. Again, limitations of the 2 techniques were noted, because the CCI group required initial arthroscopy with secondary lateral arthrotomy and periosteal flap coverage whereas the microfracture group entailed arthroscopy alone. A broader portion of the CCI group had previous surgery and a comparatively longer history of symptomatology than those patients in the microfracture group. After exclusion of tissue samples for poor processing or patient refusal, histologic analysis was still available for a large portion of patients (79%). Despite its conclusion, this study still does not elucidate whether characterized chondrocytes contribute to improved structural repair vis-à-vis microfracture, because both methods showed improved clinical outcome at 36 months. Results suggested that the most suitable candidates for cartilage implantation procedures are those patients with a shorter duration of symptoms (i.e., 2 to 3 years). However, the authors recommend further research to establish the role of pertinent patient- and surgery-based variables to better predict which individuals are likely to respond to such treatment.

Visna et al.[33] (2004)

Clinical Study: Visna et al.[33] compared the treatment of deep cartilage defects of the knee with OAT versus abrasive techniques at 12-month follow-up.

Presence of Bias: Selection and performance biases were limited except for the presence of co-interventions. Transfer bias was limited with 100% clinical follow-up, although the authors were unable to obtain adequate second-look arthroscopies and histologic follow-up. This study also lacked independent observers, thereby introducing detection bias.

Limitations and Challenges: Approximately 86% of cartilage lesions were post-traumatic, which is discontinuous with the breakdown by mechanism of injury identified in other studies. In addition, this study included not only lesions from various locations about the knee but also those patients presenting with multiple chondral defects. The length of pre-existing symptoms was not reported, and concurrent ligamentous reconstruction was permitted along with the study procedure. Overall, few patients in this study were evaluated with second-look arthroscopy and histologic analysis (8%), including no patients from the abrasion treatment group. The variability in articular involvement and extent of concomitant procedures preclude any meaningful comparison with other studies.

DISCUSSION

Articular cartilage research is intrinsically difficult for a variety of reasons. In addition to its limited capacity for healing, a wide assortment of treatments have been described to repair or replace this specialized joint surface. Biologic and novel prosthetic products designed to treat damaged articular cartilage are being introduced into the clinical realm at an ever-increasing pace. Unfortunately, very little Level I scientific evidence exists on these treatments to critically analyze and direct clinical care of this common problem. There are quality-of-care, socioeconomic, and commercial interests vested in cartilage research, yet there is a paucity of published information. The existent literature on this topic is replete with design flaws, and this investigation seeks to systematically highlight these challenges and potential biases within this area of clinical research to advance future studies and guide clinical management of this complex problem.

The dearth of well-designed and unbiased cartilage research in the contemporary literature has been previously discussed. Jakobsen et al.[19] had previously evaluated the body of literature using a modified Coleman Methodological Score (CMS) with 10 established criteria to assess the quality of study on a scale from 0 to 100. Among 61 reviewed studies evaluating the outcomes of microfracture, ACI, and/or osteochondral autograft transfer surgery procedures, the mean CMS was 43.5, connoting a generally poor methodologic quality. Significant methodologic concerns were highlighted in the areas of study design, clarification of postoperative rehabilitation protocol, outcome mea-

sures or means of patient assessment, and patient selection. Whereas a positive correlation was noted between CMS rating and level of evidence, significant variations existed between studies at each level and the ratings of Level I studies were still suboptimal. Limited patient enrollments (median, 30; range, 14 to 58) predisposed studies to underpowered statistical evaluations, and the use of 27 separate outcome scales impedes further meta-analysis. More recently, Benthien et al.[35] further investigated the evidence-based methods for cartilage restoration and reported similar conclusions, with a mean CMS of 58 among 133 studies. They similarly emphasize the importance of uniform validated outcome measures and standardized surgical techniques to improve collaboration and facilitate medical decision making.

Cartilage studies around the world have had, and continue to have, challenges with patient enrollment. A primary reason is that most cartilage research is seeking to principally assess the safety and efficacy of treatment directed at isolated, focal defects of the distal femur. Unfortunately, these chondral defects most frequently present to investigators as compound knee problems, often with variable levels of chronicity and lesion progression. This particular injury pattern is fairly ubiquitous throughout the health care system and does not necessarily warrant referral to a secondary or tertiary treatment center conducting relevant cartilage research. Many patients with cartilage lesions, perhaps the vast majority, are treated (or in many cases undertreated) by community-based orthopaedists and do not have access to the studied interventions or study enrollment. As evidence of the difficulty of conducting this type of articular cartilage research, 1 of the authors (P.A.D.) was the principal investigator for an RCT to evaluate an orthobiologic scaffold for repair of isolated lesions of the distal femur. The study was designed in cooperation with the US Food and Drug Administration (FDA) and in nearly full accordance with International Cartilage Repair Society guidelines articulated by Mithoefer et al.[24] With 9 dedicated cartilage centers enrolling patients, the study was canceled for lack of enrollment, with only 4 patients meeting the inclusion/exclusion criteria over a 9-month period. Despite methodologic challenges among the Level I studies reported in our review, the prevalence of cartilage lesions that qualify a patient for this type of research is obviously very low in light of typical inclusion/exclusion criteria and study design.

The biases present in these studies are not entirely unique to cartilage research, although inherent limitations make certain forms of bias unavoidable even in well-designed clinical trials. Selection bias is particularly difficult to avoid given that cartilaginous defects most frequently occur vis-à-vis other intra-articular injury and with varying degrees of chronicity. Performance bias may also be problematic within the context of cartilage restoration, particularly with multiple treating surgeons across numerous hospital settings in selected studies. Detection bias was clearly present in studies lacking adequate description of blinding protocols or designated independent observers for histologic analysis and follow-up.[25,26] Transfer and nonresponder bias may be minimized with adequate follow-up or histologic analysis (e.g., $>80\%$). Clinical follow-up was appropriate in most of these studies; however, second-look arthroscopy, as well as histologic evaluation, was extremely limited because of the necessity for an additional secondary procedure, the limited gains from the patient's perspective, increased medical cost, and the inherent risks associated with subsequent surgery.

There are 2 types of comorbidities that confound cartilage research studies. First, there are patient-specific parameters. These include overall patient health, lifestyle, and compliance issues. Patients with negative comorbid parameters such as chronic illness, smoking, and obesity are more likely to have impaired outcomes when compared with healthier cohorts. Some studies attempt to control for these conditions, although inclusion and exclusion criteria may not be consistent from study to study. Furthermore, some comorbidities such as alcohol or substance abuse are markedly under-reported by patients, further impairing the ability to control for patient-specific risk factors contributing to poor outcomes. The second type of comorbidity involves concurrent knee pathology. Malalignment, meniscal absence or damage, instability (gross or subtle), and/or progressive degeneration within the knee can all confound the capacity of investigators to accurately assess the effect of cartilage treatments. Lastly, concurrent surgical procedures intended to address coexisting knee pathology may obscure the investigator's capacity to discern the patient outcome relative to the cartilage interventions, in terms of both subjective and objective outcome measures.

The history of antecedent pathology, as well as prior treatment of cartilage lesions, is another confounding variable in analyzing the outcome of cartilage treatments. Ideally, treated lesions would be primary and without prior directed interventions. In fact, many lesions treated for cartilage repair have under-

gone prior interventions before more directed surgeries. For example, many cartilage lesions are identified at the time of ligament reconstruction, and marrow stimulation techniques are readily available at the time of the index procedure in most settings. This will, by definition, violate the subchondral bone and may ultimately confound or bias research involving subsequent cartilage surgery. Given the predisposition of patients with cruciate injury toward accelerated degenerative change irrespective of later reconstruction, the presence of a prior ligament surgery in the same knee will also potentially confound subsequent outcomes of isolated cartilage research.

The interventions analyzed in cartilage research have been widely varied, in terms of both surgical technique or available technology and anatomic location. It is difficult and sometimes frankly impossible to compare studies that use different index procedures or address chondral lesions in different geographic distributions about the knee. For example, studies looking at ACI may use a variety of covering membranes, from autologous periosteum to xenograft tissue or biosynthetic scaffolds according to first-, second-, and third-generation techniques. The confounding potential of these different membranes results from varying complication profiles and surgical approaches, and this complicates the comparison from one study to another. Furthermore, surgically treated lesions involving the patellofemoral articulation, or so-called kissing bipolar lesions, are associated with more modest clinical results when compared with those of isolated condylar lesions, and these lesions are frequently enrolled collectively without being appropriately controlled for or undergoing further subgroup analysis. In addition, frequent secondary surgical procedures (e.g., second-look arthroscopy) may also contribute to these confounding variables because of their additional inherent risk.

Cartilage research, particularly in the United States, has been guided by the policies and restrictions of the Department of Health and Human Services' FDA. This agency has, for the most part, insisted that all new modalities to treat articular cartilage defects undergo a 2-part evaluation, with an initial pilot trial to assess safety and subsequent studies to ascertain efficacy. These studies, even the preliminary pilot trials, may require up to 3 or more years in duration to discern differences between treatments. Furthermore, the FDA has been relatively insistent that the therapeutic modality being tested be compared with a microfracture control. This choice of a control has several limitations and shortcomings, because microfracture represents a minimally invasive modality, whereas many of the cartilage treatments being used and tested require an arthrotomy and therefore cannot be blinded. Thus far, the FDA has not allowed comparison among cartilage treatment modalities that could effectively be blinded. Definitive studies, or Level I data, require follow-up of more than 3 years, and these studies cannot be initiated until conclusion of the pilot. In addition, the FDA has been insistent that studies establish superiority, rather than non-inferiority, of a given treatment, thereby increasing the number of patients needed to show statistically significant differences in primary and secondary outcomes. Furthermore, the FDA has been reluctant to allow the utilization of indirect biomarkers (e.g., joint aspirate) to assess efficacy of therapeutic modalities on articular cartilage. Lastly, the application of guidelines under the 510(k) pathway, whereby products of substantial equivalence might be afforded a relatively expedited pathway to approval, remains in a state of flux at this time, adding further uncertainty to academic and commercial sponsors of clinical research. In the context of this intense regulatory environment, most articular cartilage research is presently being performed outside the United States, primarily in the European Union.

CONCLUSIONS

Systematic review of randomized articular cartilage studies shows the prevalence of forms of bias in even the highest level of research, particularly selection bias, nonresponder bias, and study design bias. Identified design limitations include variability in anatomic location, lesion size and depth, presence of coexisting chondral lesions, prior or concomitant procedures, prolonged preoperative symptoms, and/or limited histologic follow-up. Whereas current studies have attempted to control and mitigate these factors, the referral of patients to dedicated cartilage research centers, adherence to strict study design criteria, larger study enrollment, and extended follow-up will enhance the quality and quantity of valid Level I articular cartilage studies and serve to inform appropriate clinical guidance. In addition, the heterogeneity in primary and secondary endpoints, the variations in surgical technique, and the disparate rehabilitation protocols are among the factors that continue to challenge advancements in cartilage research and must be addressed for further studies.

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