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Johan Vanlauwe, Daniel B.F. Saris, Jan Victor, Karl Fredrik Almqvist, Johan Bellemans, Frank P. Luyten and TIG/ACT/01/2000&EXT Study Group


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What is This?
Five-Year Outcome of Characterized Chondrocyte Implantation Versus Microfracture for Symptomatic Cartilage Defects of the Knee

Early Treatment Matters

Johan Vanlauwe, MD, Daniel B.F. Saris, § MD, PhD, Jan Victor, || MD, PhD, Karl Fredrik Almqvist, || MD, PhD, Johan Bellemans, † MD, PhD, and Frank P. Luyten, † MD, PhD, for the TIG/ACT/01/2000&EXT Study Group

Background: Characterized chondrocyte implantation (CCI) results in significantly better early structural tissue regeneration than microfracture (MF), and CCI has a midterm clinical benefit over microfracture.

Purpose: This study was undertaken to evaluate the 5-year clinical outcome of CCI in a randomized comparison with MF for the treatment of symptomatic cartilage defects of the femoral condyles of the knee.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: Participants aged 18 to 50 years with a symptomatic isolated International Cartilage Repair Society (ICRS) grade III or IV cartilage lesion of the femoral condyles between 1 and 5 cm² were randomized to either CCI or MF. Clinical outcomes were measured up to 60 months after surgery using the Knee Injury and Osteoarthritis Outcome Score (KOOS). The main outcome parameter was change from baseline in overall KOOS (oKOOS). Adverse events were monitored.

Results: Fifty-one participants were treated with CCI and 61 with MF. On average, clinical benefit was maintained through the 60-month follow-up period. The average change from baseline in oKOOS was not different between both groups (least squares [LS] mean ± standard error [SE] 18.84 ± 3.58 for CCI vs 13.21 ± 5.63 for MF; P = .116). Treatment failures were comparable (n = 7 in CCI vs n = 10 in MF), although MF failures tended to occur earlier. Subgroup analysis revealed that CCI resulted in better outcome in participants with time since symptom onset of less than 3 years, which was statistically significant and clinically relevant (change in oKOOS >3 years mean ± SE 25.96 ± 3.45 for CCI vs 15.28 ± 3.17 for MF; P = .026 vs oKOOS >3 years mean ± SE 13.09 ± 4.78 for CCI vs 17.02 ± 4.50 for MF, P = .554). Other subgroup analyses such as age (cutoff 35 years) did not show a difference. Female patients showed more failures irrespective of treatment.

Conclusion: At 5 years after treatment, clinical outcomes for CCI and MF were comparable. In the early treatment group, CCI obtained statistically significant and clinically relevant better results than MF. Delayed treatment resulted in less predictable outcomes for CCI. These results provide strong evidence that time since onset of symptoms is an essential variable that should be taken into account in future treatment algorithms for cartilage repair of the knee.

Keywords: autologous chondrocyte implantation; chondrocyte; chondral; regenerative medicine; Knee Injury and Osteoarthritis Outcome Score (KOOS); microfracture; cartilage repair; randomized controlled trial; long term

Articular cartilage lesions of the knee are known for their limited potential to heal spontaneously. Persistent defects in the condyle or patella will frequently become symptomatic and many progress toward secondary osteoarthritis (OA), affecting daily living and quality of life.8–10,19 The understanding of the relationship of structural changes in an affected joint and the subsequent development of OA could lead to new treatment strategies to prevent and treat this debilitating condition.16 Treatment modalities of joint surface lesions aim to restore pain-free joint function by promoting the formation of repair tissue that has the structure and durability of natural hyaline-like articular cartilage.11,23,42 Interventions intended to reestablish the cartilage surface by tissue repair include marrow stimulation techniques such as microfracture (MF),52 mosaicplasty22 or regenerative approaches such as autologous chondrocyte implantation (ACI),7 and other variations on...
chondrocyte-based therapies. While MF consists of a single-step arthroscopic procedure, ACI requires an arthroscopic intervention to obtain a good-quality cartilage sample for expansion and a subsequent mini-arthrotomy to implant the expanded chondrocytes.

At the time of the design of this study (2001), ACI had been in use for about a decade, but no randomized controlled trials were published comparing ACI and MF in the treatment of deep isolated cartilage defects of the knee. Microfracture was believed to result in fibrocartilaginous repair with good short-term clinical outcome but limited durability, while ACI was claimed to result in more hyaline-like cartilage with better biomechanical characteristics. Recently it was reported that the use of ACI as a second-line procedure after MF results in less favorable and less predictable results and that it might be better to be cautious with the use of marrow stimulation techniques in larger lesions. This raises the issue of positioning ACI as first-line treatment in lesions without preexisting damage to the subchondral bone plate.

ChondroCelect (characterized viable autologous cartilage cells expanded ex vivo; TiGenix, Leuven, Belgium) is an ATMP (Advanced Therapy Medicinal Product) recently approved as the first cell-based therapy by the European Medicines Agency for the treatment of symptomatic isolated full-thickness cartilage defects of the femoral condyles. The expansion process was fine-tuned to yield highly chondrogenic cells by minimizing chondrocyte dedifferentiation, thereby preserving the articular cartilage phenotype. In the design of a prospective randomized trial with as primary end point structural superiority at 12 months using histomorphometry and the International Cartilage Repair Society (ICRS) II score and clinical noninferiority at 12 to 18 months (http://clintrials.gov: NCT00414700), we also included an extension protocol with a 3-year evaluation and a long-term follow-up (ie, up to 5 years after surgery). MATERIALS AND METHODS

Full details of the study inclusion/exclusion criteria, methodology, statistical analysis, surgical procedures, rehabilitation program, histologic assessment, and safety assessment up to 3 years of follow-up have been reported previously.

Clinical assessments (Knee Injury and Osteoarthritis Outcome Score [KOOS], visual analog scale [VAS], physical examination) were continued up to 60 months after the repair intervention. Assessments were performed by an independent evaluator before visiting the treating surgeon.

Study Population

The study was conducted in accordance with current Good Clinical Practice Guidelines at 13 orthopaedic centers in Belgium, the Netherlands, Germany, and Croatia. The first participant was enrolled in the study on February 21, 2002, and the last participant completed the 60-month assessments on December 1, 2009.

Eligible men and women, aged between 18 and 50 years, were required to have a single symptomatic cartilage lesion (ICRS grade III or IV) of the femoral condyle of between 1 and 5 cm² and agree to actively participate in a strict rehabilitation protocol and follow-up program. The inclusion and exclusion criteria were published previously.

Study Design

Eligible participants were randomized using an interactive voice response system to either characterized chondrocyte implantation (CCI) with ChondroCelect or MF at the time of the initial arthroscopy. Randomized participants were treated according to previously described techniques of ACI using periosteum and a full arthrotomy and MF. Both CCI and MF groups participated in an identical standardized, strict rehabilitation program that started on the day of surgery, and attended regular follow-up visits at the clinic. All patients who participated in the initial 12-month study were invited to participate in the extension phase. After surgery, outpatient visits were scheduled at 8 weeks and at 3, 6, 9, 12, 18, 24, 30, 36, 48, and 60 months.

Outcome Assessments

Clinical outcome was primarily assessed using the KOOS questionnaire. The primary outcome variable for statistical purposes was the change from baseline of the averaged, “overall KOOS” (oKOOS). Treatment failure was defined as a reintervention affecting more than 20% of the index lesion. Time to treatment failure was the time between the end of the surgical procedure and the date of failure or reintervention. Adverse events (AEs) were monitored throughout the 60-month follow-up. Adverse events were reviewed considering 2 time periods: events occurring...
Clinical Outcomes

Both treatment groups experienced statistically significant oKoos improvements, with maximal scores at approximately 12 months for MF and at approximately 24 months for CCI. On average, clinical benefit gained at 24 months was maintained throughout the follow-up period (Figure 1, Table 1, and Appendix 2 [available online]). There were comparable clinically relevant improvements at 60 months in oKoos in both the CCI and MF groups (least squares [LS] means ± standard error [SE], 18.84 ± 3.58 and 13.21 ± 3.40; Δ = 5.63, P = .116).

Appendix 3 (available online) shows the baseline demographic characteristics of the planned subgroups with symptom onset of less than or equal to 3 years. Except for a slight preponderance of female patients in the CCI group with symptom onset greater than 3 years, no differences were apparent.

For patients with symptom onset less than 3 years, the oKoos showed a statistically significant and clinically relevant difference in improvement in the CCI group over the MF group at 60 months (means ± SE, 25.96 ± 3.45 vs 15.28 ± 3.17; Δ = 10.69; 95% confidence interval [CI] 1.30, 20.07; P = .026). Significant differences versus MF were also observed in the pain and quality of life domains (Figure 1). Participants with symptom onset of 3 years or more clearly have less benefit from CCI at 60 months (oKoos means ± SE, 13.09 ± 4.78 for CCI vs 17.02 ± 4.50 for MF; P = .554). Figure 2 shows improvements in oKoos means over time for both groups.

There was no appreciable difference at 60 months between younger patients (age <35 years) (means ± SE, CCI 22.41 ± 3.70 and MF 16.59 ± 3.55; P = .262) and patients aged 35 years or more (means ± SE, CCI 19.61 ± 4.51 and MF 15.16 ± 4.01; P = .465).

Statistical Analysis

The primary population for efficacy and safety analyses included all treated participants. Sample size determination was previously reported.49 The Koos values were imputed using a last observation carried forward approach for patients who left the study after a documented treatment failure. Other missing data points were not imputed.

Preplanned efficacy analyses included calculation of the change from baseline in oKoos, adjusted for the baseline covariates oKoos, age, associated lesions, and lesion location. The changes from baseline in the different Koos domains were considered exploratory and hence no adjustments were made for multiple testing.

The Kaplan-Meier product limit estimator was used to display time to treatment failure for each treatment group, which was compared using the log-rank test. Any AEs that occurred with a frequency of 5% or more in either treatment group were compared using the Fisher exact test.

Planned exploratory analyses included time since onset of symptoms (<3 years or ≥3 years) and age (<35 years vs ≥35 years).

RESULTS

Patient Demographics

A total of 118 patients were randomized to treatment: 57 to CCI and 61 to MF. Six patients assigned to CCI could not be treated and were not included in the efficacy analysis. Patient disposition is shown in the Appendix 1 (see Appendix 1, available in the online version of this article at http://ajs.sagepub.com supplemental/). Baseline participant characteristics have been reported in full elsewhere47,49.
Seven (13.7%) patients in the CCI group and 10 (16.4%) patients in the MF group underwent a reintervention on the index lesion during the study (Table 2). Reinterventions were nearly exclusively prompted by clinical deterioration (ie, pain). Survival analysis did not show statistically significant difference between groups (log rank $P = .561$), although MF failures occurred mostly in the first 3 years while 4 of 7 CCI failures occurred in or beyond the fourth year of follow-up, 3 of which happened at the same center (Figure 3).

Male patients had fewer interventions as compared with female patients, as 6 of 19 females versus 1 of 32 males failed CCI treatment (Fisher exact test, $P = .007$, relative risk [RR] 4.21; 95% CI 1.03, 17.57). The same trend was observed in the MF group, where 7 of 20 females versus 3 of 41 males underwent reinterventions on the index lesion ($P = .010$, RR 4.78; 95% CI 1.49, 15.62).

Radiographic Outcomes

In 49 patients, radiographic data were available at baseline and 60 months. Four of 49 patients (8%) had a Kellgren grade 2 score; there was no significant difference in the

### Radiographic Outcomes

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>CCI</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall KOOS</td>
<td>0.048</td>
<td>0.116</td>
</tr>
<tr>
<td>Pain</td>
<td>0.044</td>
<td>0.509</td>
</tr>
<tr>
<td>Symptoms &amp; Stiffness</td>
<td>0.123</td>
<td>0.095</td>
</tr>
<tr>
<td>ADL</td>
<td>0.064</td>
<td>0.178</td>
</tr>
<tr>
<td>Sports</td>
<td>0.123</td>
<td>0.660</td>
</tr>
<tr>
<td>QoL</td>
<td>0.036</td>
<td>0.129</td>
</tr>
</tbody>
</table>

*average of all KOOS domains except Sports
frequency of radiographic changes between ChondroCelect and MF-treated patients at 60 months.

Safety Assessment

Forty-three CCI patients (84%) and 45 MF patients (74%) provided data for the 60-month study period. A total of 98% of patients in the CCI group and 84% in the MF group experienced at least 1 treatment-emergent AE during the 60 month study period; 84% and 69% of participants reported AEs more than 36 months after randomization (Appendix 4, available online). The majority of AEs were of mild or moderate intensity. The AEs related to the study procedure occurred in 82% of patients in the CCI group and 62% (Fisher exact test, \(p = .022\)) in the MF group. Serious AEs leading to prolonged or renewed hospitalization occurred in 20% of patients in both groups. In the CCI group, 5 serious AEs occurred. Two were considered as unlikely to be related (hypersensitivity and ligament rupture), 2 as possibly related (deep vein thrombosis and arthralgia), and 1 as probably related (tendinitis) to the procedure. In the MF group, 5 serious AEs were considered as unlikely to be related (syncope, intervertebral disc disorder, spinal column stenosis and sciatica, and synovial cyst). All other serious AEs (10 CCI and 18 MF) were considered not to be related to the procedure.

The most common treatment-emergent AE reported for patients in the CCI and MF groups was arthralgia, 75% versus 62%, and after 36 months, 14% versus 4%. Joint swelling was reported by 22% of participants in the CCI group versus 7% in the MF group (\(p = .026\)); beyond 36 months, this event was only reported in 0% versus 2% of participants. We have already reported that joint crepitation was significantly more common in the CCI group than in the MF group both in the short term (12% vs 2% \(p = .028\)) and in the period between 18 and 36 months after surgery (11% vs 0% \(p = .011\)). In the period from 36 months onward, joint crepitation was reported more in the CCI group (9.3% vs 0% \(p = .053\)). Additionally, slightly more patients in the CCI group versus the MF group reported joint effusion between 36 and 60 months after surgery (12% vs 2% \(p = .106\)). None of the cases of joint effusion and joint crepitation in the CCI group were rated severe. At 60 months, most of the AEs had resolved (ongoing at 60 months: effusion, 3 of 37 CCI and 1 of 40 MF; joint crepitation, 1 of 37 CCI and 1 of 40 MF).

<table>
<thead>
<tr>
<th>Reintervention</th>
<th>No Reintervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCI (n = 7)</td>
</tr>
<tr>
<td>Mean age, y (range)</td>
<td>33.7 (29-40)</td>
</tr>
<tr>
<td>Age, % patients &lt;35 y</td>
<td>57%</td>
</tr>
<tr>
<td>Mean height, cm (range)</td>
<td>169 (158-190)</td>
</tr>
<tr>
<td>Mean weight, kg (range)</td>
<td>66 (50-87)</td>
</tr>
<tr>
<td>BMI, % of patients</td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>86%</td>
</tr>
<tr>
<td>&gt;25 and &lt;30</td>
<td>14%</td>
</tr>
<tr>
<td>&gt;30</td>
<td>0%</td>
</tr>
<tr>
<td>% of male patients</td>
<td>14%</td>
</tr>
<tr>
<td>Mean symptom duration since onset, y (range)</td>
<td>6.81 (0-17)</td>
</tr>
<tr>
<td>Mean lesion size, cm² (range)</td>
<td>3.5 (2-5)</td>
</tr>
<tr>
<td>Acute symptom onset, %</td>
<td>43%</td>
</tr>
<tr>
<td>Abnormal opposite knee condition, %</td>
<td>71%</td>
</tr>
<tr>
<td>Previous knee surgery, %</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14%</td>
</tr>
<tr>
<td>1</td>
<td>43%</td>
</tr>
<tr>
<td>≥2</td>
<td>43%</td>
</tr>
<tr>
<td>Concomitant lesions (ACL, menisci, other), %</td>
<td>86%</td>
</tr>
</tbody>
</table>

CCI, characterized chondrocyte implantation; MF, microfracture; BMI, body mass index; ACL, anterior cruciate ligament.

![Figure 3. Time to failure (Kaplan Meier graph).](image)
DISCUSSION

We have previously reported that 12 months after treatment, CCI resulted in significantly better structural tissue regeneration as compared with MF, measured using quantitative histomorphometry and overall histology assessment score of biopsy specimens from the repair zone. At 36 months, the CCI-treated patients experienced significantly greater improvement in change from baseline in oKOOS and the domains of pain and quality of life as compared with those treated with MF, as measured by a mixed linear model with time as a categorical variable. In addition, it was also demonstrated that patients with a shorter time between symptom onset and treatment with CCI experienced a more robust response.

These 5-year data provide strong support for the benefit of both cartilage repair methods as similar improvements were recorded in both groups. There is a clear difference in the biologic response between “old” and “younger” defects to CCI, which is a trigger for us to reconsider treatment algorithms and use better-defined indications to further improve the outcome of 2 such different treatment strategies as cell therapy and microfracture. This is essential since both treatments can be of clinical benefit for the patient if applied to the correct patient category in the correct timing and biologic context.

Regardless of treatment, clinical response obtained at 2 years was durable and predictive of long-term clinical outcomes. This confirms the observations made by other authors. It appears, however, that the therapeutic effect in the overall study population was greatly diluted by the poor clinical response seen in those who had been symptomatic for over 3 years. The 3-year cutoff point was maintained as the 3-year data already indicated that early treatment with CCI does significantly better than MF (the mean time since onset was 0.95 for CCI and 1.1 years for MF). The majority of the population of this trial had a larger gap between onset of symptoms and treatment because of the referral system via a general practitioner or another orthopaedic surgeon. The time from onset of symptoms was affecting the treatment response, as could be demonstrated by applying the method for subgroup analysis proposed in the ICH E9 (International Conference on Harmonisation topic E9 [statistical principles for clinical trials]) guideline, which yielded significant interaction. Also, the analysis at 3-year cutoff had adequate numbers of patients for relevant statistical analysis. Patients who were treated with CCI within 3 years of symptom onset showed a statistically significantly better and, more importantly, a clinically relevant difference versus MF. This is reflected in the clinically relevant difference of 10.69 percentage points, which is more than the 9 percentage points, not only accepted as a clinically relevant improvement in literature, but also by the regulatory bodies/Committee for Medicinal Products for Human Use (CHMP).

Characterized chondrocyte implantation performed better in early lesions. The average response of patients treated longer than 3 years past onset was less impressive and not discriminative between the 2 treatments. A recent review on learning on OA by studying the healthy pointed to the fact that knee structural changes may be reversible. Overall, the combined findings do suggest that early intervention could potentially prevent or even reverse some structural changes that may evolve into knee OA as tissue regeneration appears to be better in fresh defects. The mechanism of action of MF relies most likely on the availability of the progenitor cells in the underlying bone marrow to generate a repair response, resembling endochondral bone formation, which is probably independent of the chronicity of the lesion. In CCI, however, care is taken not to violate the underlying bone to avoid this process, but in chronic lesions, the lack of homeostasis in the joint leads to a worse response of the implanted chondrocytes, which may have to rely on a favorable environment for tissue regeneration.

Although subgroup analysis has its limitations, and should be interpreted with caution, other studies have previously reported this variable to be a predictor for good clinical outcome in ACI. In addition, in vivo evidence indicates that cartilage regeneration is impaired in a setting of disrupted joint homeostasis because of treatment delay, suggesting that the microenvironment has changed, that different pathophysiologic processes are at work in these subpopulations, and that joint homeostasis is even permanently lost after a certain time point. This influences both the biopsy tissue entering the expansion phase in vitro as well as the vitality and biologic viability after reimplantation in vivo.

Importantly, time since symptom onset was not correlated with other potentially confounding baseline characteristics such as age, gradual versus acute onset, or lesion size, but patients who had been symptomatic for 6 years or more were more likely to have had multiple interventions on the index knee, whether on the index lesion or elsewhere. This study is to our knowledge the first randomized prospective study to confirm these observations, and show a beneficial long-term effect of cartilage cell therapy over marrow stimulation in patients treated early.

In our study, age did not seem to matter for the clinical outcome of the CCI-treated patients, whereas in the MF group the difference between the age groups below and above 35 years was significant, which corroborated with the findings of Kreuz et al and Mithoefer et al, who found the same results in athletes younger than 40 who were treated early. A recent publication from Niemeyer et al comparing a matched group of ACI-treated patients younger than 40 (mean age, 31 years) versus a patient group older then 40 (mean age, 47.8 years) could not show an inferior clinical result for the older age group up to 24 months after treatment. The 35-year cutoff age was chosen in the initial study set-up in 2000 because there were unpublished data at that time mentioning that MF above 35 years of age results in less good clinical outcome. Steadman et al published this later in 2003. In our study, it yields a more equal number of patients than the 40-year cutoff, which again makes statistical comparison more relevant. This is indirectly supported by the findings that in an in vivo implantation of expanded chondrocytes in nude mice, chondrocytes isolated from old donors performed equally well as those from younger donors, and...
expressed the same marker profile predictive of the capacity to generate cartilage tissue independent of age.\textsuperscript{15} Taken together, it is hard to predict what the age limitations will be, but the joint microenvironment is probably of more importance than the age of the patient.

In this study, the median time to failure among those who failed in the CCI group was 50 months versus 27 months for the MF group. The fact that the median failure time for CCI is 50 months warrants a further follow-up of these patient groups beyond 5 years. The 14% failure rate observed in the CCI group is comparable with previous reports (16% failures in MF).\textsuperscript{26,35,39} Moseley et al\textsuperscript{35} reported that the ACI failures occurred at a mean follow-up of 2.5 years (16% failure rate, 6-10 years’ follow-up). Peterson et al\textsuperscript{39} reported that most ACI failures occurred within 2 years after operation (16% failure rate, mean of 7.4 years’ follow-up) and concluded that survival beyond these 2 years is likely to result in a durable repair. Recently, a follow-up of these patients to an interval of 20 years after surgery was described with positive outcomes.\textsuperscript{40} Seventy-four percent of the patients (out of 224 of 341 who replied) reported their status as better or the same as the previous years. There were 92% who were satisfied and would have ACI again. The failure rate in the early onset group is 9% for CCI versus 18% for MF, further supporting the message of improved clinical benefit in the early onset CCI group, besides the KOOS superiority.

Treatment failure after ACI is a multifactorial phenomenon. In this study, patients experiencing a CCI treatment failure were more likely to be female; 4 of 7 had symptoms after more than 5 years and up to 17 years. Loosening of the periosteal flap was involved in 5 of 7 failure cases, 2 of which occurred in the first year, while 1 occurred as late as 57 months after the implantation. Whether it is the periosteal flap or the “implant” that loosened is not easy to discern macroscopically during the arthroscopy, because in all cases the piece of tissue that was retrieved was definitely thicker than the periosteal flap. According to experiments with rabbit periosteum that were already done in 1982 by Rubak,\textsuperscript{44,45} it was seen that as soon as 4 weeks after implantation of a periosteal flap cartilaginous tissue formation that was derived from the periosteal flap. This can also be a possible explanation for the more frequent occurrence of hypertrophy as there are 2 sources of cartilage tissue: the implanted cells and the mesenchymal stem cells that reside in the periosteal cartilaginous layer. We also know that the periosteal flap can vary in composition and cell populations depending on age or the patient and not in the least on surgical skill in harvesting. Hence, in Europe, bioabsorbable porcine collagen type I/III membranes have become very popular as standard cover for an ACI implant.\textsuperscript{1} Collagen membranes by themselves have no chondrogenic potential and are populated by the implanted chondrocytes, leading to a much lower frequency of hypertrophy and thus may diminish the need for surgical interventions to correct this and be less liable to contribute to subsequent graft delamination.\textsuperscript{17,37} A prospective randomized study showed that at 12 months of follow-up, use of periosteum resulted in a 36% rate of shaving reinterventions because of cartilage hypertrophy versus none in the collagen membrane group, while clinical outcomes were equivalent after 24 months.\textsuperscript{21}

It is not clear why women in our study were more likely to fail either treatment. In the full study population, women reported baseline KOOS pain scores that were on average 11 points lower than men. In the CCI group, women had been symptomatic for a median duration of 30 months, versus 8 months in men. It is possible that the random clustering of certain risk factors in the female segment of this study population may have contributed to worse outcomes, since in the early intervention group there was a preponderance of male patients. A subgroup analysis for gender was done at 36 months and showed no statistical difference; this analysis was not repeated at 60 months, because it would probably not add to the knowledge why this occurred. Some authors have suggested that prior surgery likely affects the integrity of the subchondral bone and was predictive of subsequent ACI treatment failure.\textsuperscript{29} When looking at histologic outcomes in the failure versus the nonfailure group of all patients (CCI and MF), item 5 of the IKDC II score (cartilage surface) was near significant (P = .059), probably indicating that disruption of the tidemark is clinically relevant for predictability of a good result and should be taken into account in the treatment algorithm. Since the CCI group included only 7 patients who had prior subchondral surgery, we were not able to confirm this hypothesis for CCI alone. However, in this small subset, a long time since symptom onset once again seemed associated with poor outcomes. Two patients with symptom onset of 22 and 32 months had excellent responses. The 2 most chronic patients had been symptomatic for 14 and 17 years, and failed. Another patient with 11 years’ duration of symptoms responded poorly to treatment. In this small group of patients, treatment failure or poor response was also associated with low baseline KOOS scores (<50).

Autologous chondrocyte implantation failures have been linked to insufficient debridement of the index lesion, resulting in suboptimal vertical or lateral integration, and to premature exposure to shear forces by noncompliance with the prescribed rehabilitation regimen.\textsuperscript{30} In our study, a great effort was put into standardization of the surgical procedures and the rehabilitation program. These factors may explain that the relatively high success rate of MF in this trial and CCI failure rates are very close to those observed in ACI case series of experienced surgeons, although in our trial the surgeons were far less experienced in the ACI procedure despite training with animal models.

Large lesions are believed to be more prone to treatment failure, especially in MF.\textsuperscript{12,29} A recently published single-center randomized controlled study by Basad et al\textsuperscript{1} studied the treatment of large (4-10 cm\textsuperscript{3}) deep cartilage lesions of the knee in a randomized controlled setting. At 2 years of follow-up, MF was clinically inferior to matrix-induced ACI. It must be noted that few recent treatment algorithms advocate the use of MF in lesions exceeding 4 cm\textsuperscript{2}.\textsuperscript{2,9} Therefore, the design of our study seems more
relevant to answer today’s questions regarding the relative therapeutic position of MF versus ACI in the treatment algorithm of cartilage lesions between 2 and 5 cm². We also might have to reconsider the position of ACI as a second-line procedure in the treatment of cartilage lesions without involvement of the subchondral plate as literature points to worse results of ACI after marrow-stimulation techniques that per definition penetrate this layer of bone.

This study is one of the only 3 published randomized, prospective studies comparing ACI with MF in the treatment of deep symptomatic cartilage defects of the knee.²⁵,²⁶ The study reported by Knutsen et al had a design rather similar to ours, and the somewhat different outcomes deserve consideration.²⁵,²⁶ Our studies were of comparable size, cartilage end-point biopsy specimens were taken in a similar number of patients (although at 24 months in the Knutsen study instead of 12 months after surgery in this study), and clinical follow-up extended to 5 years. Histology results were analyzed using different methodologies, and trended positively in the Knutsen study (P = .08), while they were clearly significant in our study (P = .01). Knutsen et al observed a 23% failure rate in the ACI group with a mean time to failure of 26.2 months as compared with 50 months for CCI in our study. Although neither of the studies revealed statistically significant clinical differences at 5 years in the overall study population, there was a clear trend toward higher clinical scores for the cell-treated group in our study whereas Knutsen et al found numerically higher scores in the MF group. Both studies saw better clinical response in younger patients, regardless of treatment, although not statistically significant in this study. The participants in Knutsen’s study had mainly chronic lesions (median symptom duration 36 months vs 22 months in our study). Although both studies excluded patients with radiologic evidence of OA, substantially more patients in the Knutsen study showed radiologic progression toward OA (Kellgren-Lawrence grade 2 or more): 34% (21 of 62 patients where the procedure did not fail) versus only 8% (4 of 49 patients) in our study (Fishers exact test: P = .001). Judging from these observations, it appears that Knutsen et al may have recruited a study population with a more chronic symptomatology, early OA, and/or a predisposition to OA. Another variable possibly responsible for our better results compared with the Knutsen data could be the use of more robust cell technology with quality control, and in-depth characterization of the cell population, with cell culture technology, developed to preserve the articular cartilage phenotype, as described previously.¹⁵ Contrary to Knutsen, who found a possible relation between the likelihood of failure and the quality of the repair tissue, we could not establish this connection, but found a connection between the absence of a calcification front at 12 months and later failures.

In conclusion, the clinical benefit of both CCI and MF was proven to be durable for at least 5 years after surgery. In the overall group, no significant difference was found in the KOOS between the 2 treatments at 60 months. In patients with less than 3 years since onset of symptoms, clinical response to CCI was more consistent, significantly superior to MF, and clinically relevant. Based on these results, current treatment algorithms should be carefully reconsidered and include time of symptom onset as a key factor.

CONTRIBUTING AUTHORS
Michael Bohnsack, MD, PhD; Toon Claes, MD; Yves Fortems, MD; Frank Handelberg, MD; Miroslav Haspl, MD, PhD; Mislav Jelic, MD, PhD; Koen Lagae, MD; Bruno Vandenekkerhove, MD; Hilde Vandenheuvel, MD; Jan van der Bauwhede, MD; and Rene Verdonk, MD, PhD.

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REFERENCES


