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# Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis

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## Summary

*Objective*: Osteoarthritis (OA) is prevalent and difficult to treat. Autologous conditioned serum (ACS), marketed under the trade name Orthokine, is a novel, injectable antiarthritic derived from the patient's own blood. The present study is the first time ACS has undergone a controlled clinical trial.

*Method*: We investigated 376 patients with knee OA in a prospective, randomized, patient- and observer-blinded, placebo-controlled trial using an intention-to-treat analysis (ITT). The clinical effects of ACS were compared to hyaluronan (HA) and saline (placebo) as assessed by patient-administered outcome instruments (Western Ontario and McMaster Universities osteoarthritis index, global patient assessment, visual analog scale, Short-Form 8) after 7, 13 and 26 weeks. After 104 weeks an observer-blinded follow-up was carried out. Frequency and severity of adverse events were used as safety parameters.

*Results*: In all treatment groups, intra-articular injections produced a reduction in symptoms as well as an improvement in quality of life. However, the effects of ACS were significantly superior to those of HA and saline for all outcome measures and time points, and improvements were clinically relevant; there were no differences between the effects of HA and saline. The frequency of adverse events was comparable in the ACS and saline groups, but higher in the HA group.

*Conclusion*: The data demonstrate that ACS injection considerably improves clinical signs and symptoms of OA. It remains to be determined whether ACS is disease-modifying, chondroprotective, or chondroregenerative.


*Key words*: Autologous conditioned serum (ACS), Orthokine, Orthokin, Osteoarthritis, Interleukin-1 receptor antagonist (IL-1Ra), Intra-articular injections, Hyaluronan.

## Introduction

Osteoarthritis (OA) is the single most important cause of locomotor disability in Western societies and a major burden on their healthcare systems[1,2]. It is a progressive, chronic condition leading to pain and loss of function that dramatically reduces patients' quality of life and ability to work. Pharmacologic treatment options for OA are very limited[3–7]. They include analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and the intra-articular injection of steroids or hyaluronan (HA). Intra-articular HA is commonly used as a safe, off-the-shelf, treatment for OA of the knee, but its efficacy is controversial. There is a pressing need for novel, improved, mechanism-based agents for treating OA.

OA is accompanied by a number of mechanical and biologic dysfunctions within the joint, the central pathologic feature being the destruction of hyaline cartilage. Of the catabolic cytokines identified in osteoarthritic joints, interleukin-1 (IL-1), the most potent known mediator of cartilage loss[8–17], appears pivotal. The naturally occurring inhibitor of IL-1, the IL-1 receptor antagonist (IL-1Ra), could potentially limit the intra-articular actions of IL-1[18–20] and thereby control the disease process. Several investigators have reported effectiveness of IL-1Ra when delivered by intra-articular injection in a canine model of OA and in a pilot human study[21], or when delivered by intra-articular gene transfer[22,23] in dogs[24], rabbits[25], and horses[26].

Autologous conditioned serum (ACS) was developed in the mid-1990s in an attempt to generate an injectable material enriched in endogenous IL-1Ra as a novel therapeutic for OA. Meijer *et al.*[27] noted that exposure of blood to glass beads elicits a vigorous, rapid increase in the synthesis of several anti-inflammatory cytokines, including IL-1Ra. This observation is the basis for producing ACS, which is injected into the affected joint in a series of six intra-articular injections given twice a week for 3 weeks. This therapy is currently available for humans in several European countries and its use is even more widespread for equine OA, where ACS considerably improves clinical lameness in horses and may protect cartilage from degradation[28]. Preliminary data from a large non-blinded patient observational study in humans[29] provided encouragement for the present trial, which was designed to test the hypothesis that ACS is superior to saline and HA as an intra-articular therapy for reducing the signs and symptoms of knee OA.

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## Method

This study was based on a 26-week prospective, randomized, controlled, parallel-group design trial. During the first 6 months of the initial randomized controlled trial (RCT), both patients and observer were blinded (masked observer).

Patients with primary OA of the knee were recruited from five orthopedic centers between October 2003 and July 2004 (consolidated statement of reporting trials (CONSORT) flow chart, Fig. 1). Participants had to be older than 30 and willing to discontinue all analgesics and NSAIDs for at least 6 months. Participants had to have had OA for at least 3 months at the time of inclusion, as assessed by American College of Rheumatology criteria[30], Kellgren–Lawrence grade 2–3 radiographic evidence of knee OA[31], and a visual analog scale (VAS) pain rating of at least 50 on a 100-mm scale at the time of inclusion. Previous surgery of the studied knee was acceptable, provided it preceded the start of injections by at least 3 months. Exclusion criteria were grade IV OA, systemic or inflammatory joint diseases, a history of crystalline arthropathy or neuropathic arthropathy, clinically relevant hematologic or abnormal clinical chemistry values, bone cancer, and metastasis or tumor-like lesions in immediate proximity to the treated joint. Patients were also excluded if they were pregnant or lactating, abused drugs (alcohol, analgesics, and opiates), had received an intra-articular injection of any of the trial substances within the previous 6 months, or had a known allergy or hypersensitivity to any of the trial substances. Kellgren–Lawrence scores were assessed on the basis of X-rays (conventional standing anteroposterior radiograph) up to 1 year old. Patients with a score of 2 or 3 on entry were eligible. Joint space width was not measured, as literature indicates that changes may only be apparent radiographically in longer studies of 18–24 months duration. The 6-month follow-up period of the current trial was therefore too short to reasonably expect protective effects to be detected in knee radiographs[32–34].

Subsequently, all volunteers were seen by one specially trained study physician (blinded observer) at the study center, who provided information concerning the trial, products, alternatives and risks, and obtained written informed consent. Next, the volunteers completed all baseline questionnaires. After application of inclusion and exclusion criteria, a total of 376 patients (safety population analysis) were enrolled. Participants were allocated to one of the three groups (ACS, HA or saline) on the basis of a randomization list, by individuals at the blood-processing site who were not otherwise involved in the trial.

All patients had 50 mL of whole blood taken using a special syringe with increased inner surface area (Orthogen, Düsseldorf, Germany). Samples from patients receiving saline or HA injections were discarded. Medical-grade glass beads in the special syringes increase the nonpyrogenic surface area. These glass spheres induce the dose-dependent production of IL-1Ra by white blood cells in whole blood incubated at 37°C. After incubation, the blood-filled syringes were centrifuged, and the serum supernatant was filtered (0.22 μm; Millipore, Carrigtwohill, Co. Cork, Ireland) and aliquoted into 6–8 2 mL portions. The aliquots were frozen at −20°C and tested for human immunedeficiency virus (HIV), syphilis, hepatitis B and C before being released for injection.

All participants had two appointments with a physician per week for three consecutive weeks. Subjects in the placebo group received one injection per week of saline. Subjects in the HA group received one injection per week of a 1% solution of HA with a molecular weight of $1.4 \times 10^6$ D (HYA-Ject®, Ormed, Freiburg, Germany). The injections consisted of 2 mL of HA

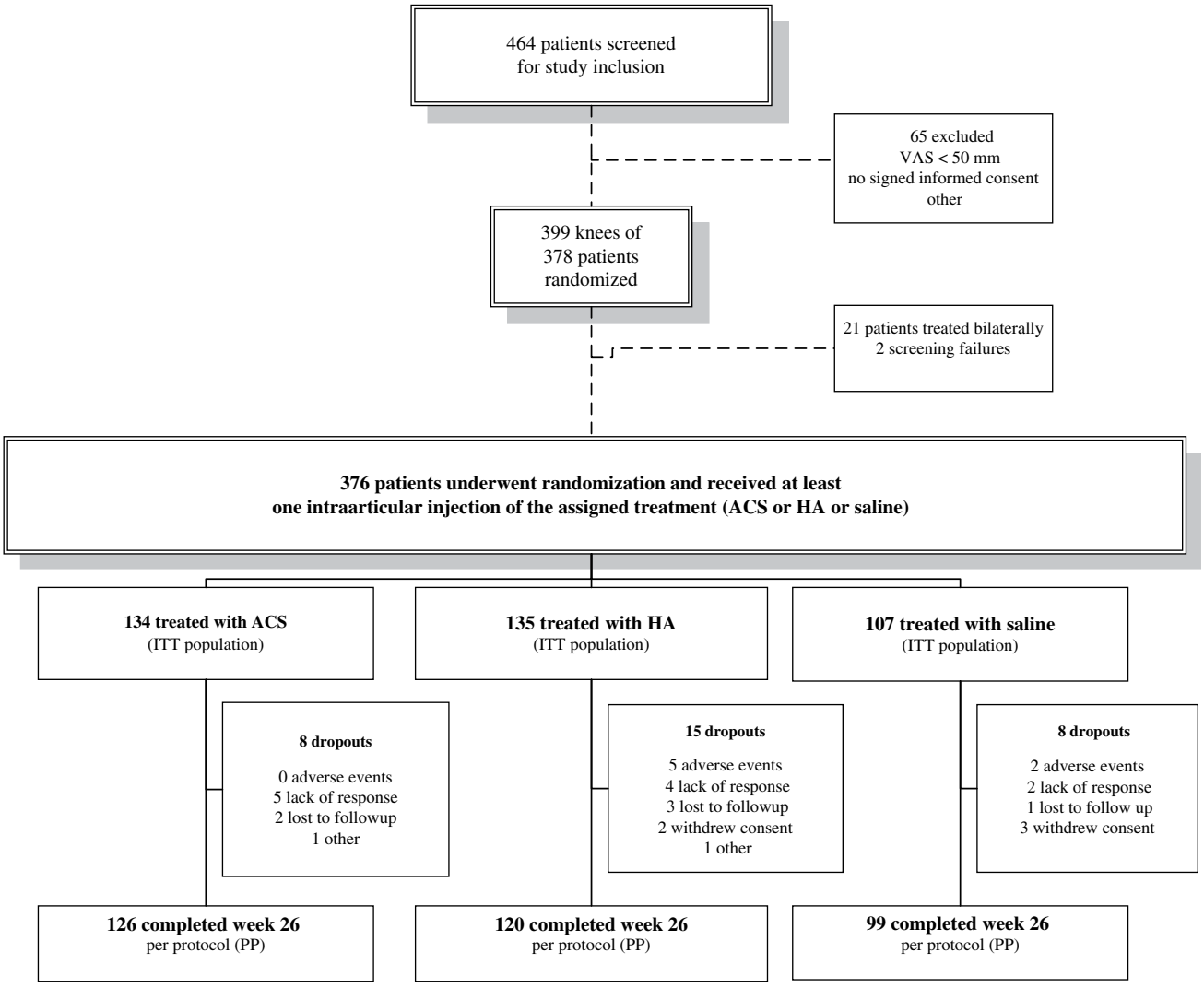


Fig. 1. Patient disposition. Flow chart showing screening and inclusion of patients.

administered in accordance with the manufacturer's recommendations. Subjects in the saline and HA groups received an application of topical heparin-natrium cream at the second appointment every week. This was done so that all patients would have six appointments with a treating physician. Subjects receiving ACS also had six appointments but received an injection of ACS at each appointment. In all cases, a sterile 21-gauge needle was inserted antero-laterally into the joint under aseptic conditions and synovial fluid present was aspirated to minimize drug dilution. The needle was left in place and 2 mL of the respective study medication was injected. The procedures were identical for each group and were performed without any concurrent medication or use of local anesthesia. For technical reasons (e.g., differences in viscosity, color) blinding of the doctor administering the medication was not possible.

To evaluate pain severity, analgesic and anti-inflammatory medications were discontinued before the start of treatment. The washout period was 3 weeks, starting from the day of inclusion until the first injection. ACS was produced for patients in the ACS group, and all patients were scheduled for therapy. Patients were permitted to use paracetamol (acetaminophen)[35,36], up to 4 g/day, as a rescue medication. NSAIDs were not permitted throughout the trial.

In all, 376 patients underwent randomization and received at least one intra-articular injection of the assigned treatment. Total study duration was 32 weeks for patients receiving all injections, or 29 weeks for patients who received only one injection. Six weeks, 3 months and 6 months after the last trial injection, patients were recalled to the study center and completed the same questionnaires as they had at baseline prior to being seen by the blinded observer.

Twenty-one patients also received injections of the study medications in the contralateral knee 3–6 months after the first knee. The second knees were evaluated separately and the data were not included in our analyses. In each of these cases the more painful knee was treated first, and only data from the first knee were included in the study. Two patients (one ACS and one HA) were excluded from statistical analysis after exclusion criteria became apparent during the injection period. Treatment failures due to aggravation of symptoms and dropouts (lost to follow-up) were equally distributed between the three treatment groups (Fig. 1).

Patients requiring additional treatment during the trial were documented and were regarded as clinically failing or withdrawing from treatment. Clinical failure was defined as the use of concurrent treatment for the study knee, e.g., analgesics (more than 4 g acetaminophen per day), surgery, NSAIDs (on prescription), or additional injections. Patients who elected to withdraw from the trial were considered treatment failures but were still followed for 6 months to monitor possible late adverse reactions.

Baseline characteristics were recorded before the first injection. Patients rated their subjective status using a 100-mm VAS. The patient-administered Western Ontario and McMaster Universities (WOMAC) osteoarthritis instrument, the Short-Form 8 health-related quality of life (SF-8 HRQL) survey, and the global patient assessment (GPA) of treatment efficacy were performed at baseline and at weeks 7, 13 and 26.

The WOMAC index is a disease-specific quality-of-life instrument developed for assessing patients with OA. It comprises three dimensions: pain, stiffness, and physical function. It is both reliable and valid[37,38]. Scores were calculated as an average for each subscale (0–10 scale, with higher scores indicating worse condition).

The 100-mm VAS (0 = no pain; 100 = worst possible pain) has been validated and is comparable to other methods. It has adequate sensitivity and statistical power for data collection[39,40]. The overall question was "How much pain did you have due to your knee joint in the past week?" A VAS > 50 mm is a stricter inclusion criteria than has been used in some other studies[41–44], but our objective was to treat painful OA cases.

It may be that including only patients with VAS > 50 mm resulted in a remarkably low standard deviation (SD) seen for VAS at baseline (perhaps in part as a result of primary care physicians knowing the inclusion criteria thresholds) and in bias toward more aggressive and more inflammatory manifestations of OA.

The SF-8 HRQL is a self-administered questionnaire comprising eight dimensions. Single-item scores, physical component score (PCS) and mental component score (MCS) were calculated, with higher scores representing better HRQL[45–47].

In addition to the treatment outcome measures, patients' satisfaction with treatment efficacy was assessed by GPA[48]. Each patient was asked "How would you rate the effectiveness of your osteoarthritis treatment right now?" Clinically meaningful satisfaction was defined as a grade of 1–3 on a six-point scale with lower grades representing better outcomes.

Baseline demographics and baseline disease characteristics (Table I) were statistically identical in the three groups, except for age differences between ACS and HA and between ACS and saline ($P < 0.05$). Subsequent stratification and correlation analysis showed no significant correlations between baseline demographic characteristics (gender, previous knee surgery or laboratory test results) and treatment outcome. In addition, sub-analysis for age (age groups determined using quartiles and median of age distribution were 30–48, 49–59, 60–66 and 67–84 years) did not show significant differences in responsiveness between these subgroups (e.g., WOMAC: pain $P = 0.9827$, stiffness $P = 0.7207$, function $P = 0.8995$, global $P = 0.9011$, VAS: $P = 0.8364$).

Table I
*Baseline demographics and baseline disease characteristics of study subjects*

| Parameter | ACS | HA | Saline |
|---|---|---|---|
| Number of patient knees | 134 | 135 | 107 |
| Average age (year) | 53.8 ± 12.2 | 57.4 ± 12.0 | 60.3 ± 10.7 |
| Gender (f/m) | 65/69 | 74/61 | 68/39 |
| Previous knee surgery (%) | 59.4 | 58.7 | 60.2 |
| WOMAC pain (mean) | 5.2 ± 2.4 | 4.9 ± 2.1 | 4.9 ± 2.0 |
| WOMAC stiffness (mean) | 5.6 ± 2.8 | 6.0 ± 2.7 | 5.8 ± 2.8 |
| WOMAC function (mean) | 5.2 ± 2.4 | 5.2 ± 2.1 | 5.2 ± 2.2 |
| Global WOMAC (mean) | 5.2 ± 2.3 | 5.2 ± 2.0 | 5.2 ± 2.1 |
| VAS (mm) | 69.6 ± 13.1 | 68.3 ± 12.8 | 66.3 ± 14.5 |
| SF-8 PCS | 29.4 ± 6.7 | 28.7 ± 5.7 | 29.5 ± 5.8 |
| SF-8 MCS | 42.6 ± 11.7 | 43.3 ± 12.1 | 43.8 ± 12.4 |

WOMAC osteoarthritis index (mean scores on each subscale and global WOMAC; range 0–10). VAS – weight-bearing pain (range 0–100 mm). SF-8 = Medical Outcomes Study Short-Form 8 Health Survey (range 0–100). Plus–minus values are mean ± SD.

The trial protocol was designed to detect a change of 20% in the WOMAC score between ACS and HA, and between HA and saline, with a two-sided level of significance of 5%[49]. The calculation of the number of patients was performed with nQuery Advisor version 4.0 (Statistical Solutions, Saugus, MA, USA). With an expected SD of 40% (WOMAC score), as frequently found in other OA treatment trials, and taking into account that there would be a dropout rate of approximately 25%, 108 patients per group were required for a power of >80%. Descriptive statistics were calculated by treatment group and time point, using the last observation carried forward (LOCF) method of imputation. At baseline, homogeneity of demographic and disease characteristics was tested with an analysis of variance one-way method for continued parameter and a chisquare test for categorical parameter. A general linear model for repeated measurement was used to compare the longitudinal profile of the groups, and a multiple comparison between the groups was performed. Changes from baseline and comparisons of groups for all scores were calculated using an analysis of variance one-way test. Results are presented graphically, using the mean profile per group, and analytically, reporting the *P* values for each contrast. Statistical analysis was performed using SAS for Windows (SAS Institute, Cary, NC, USA) on a personal computer.

Intention-to-treat analysis was performed for the outcome of primary and secondary variables, and a safety analysis was performed including all patients who received at least one intra-articular injection.

Adverse events were used to compare the safety profile of the three groups. At each study visit, adverse events experienced since the previous visit were evaluated. Each event was recorded, along with whether the event was localized to the injected knee or general (systemic) in nature, its duration, and what, if any, measures or treatment were required. Adverse events localized to the injected knee were defined as pain, swelling, or effusion and were reported as such, even though these can also be symptoms of OA.

### TWO-YEAR FOLLOW-UP

Patients who completed the 6 months of the trial without any major protocol violations (per-protocol population) were recalled to the study center and followed up prospectively 2 years after the last trial injection in an observational, prospective, cohort study with a new blinded observer. This follow-up evaluation was conducted to determine whether therapeutic effects were still present after 2 years and which patients subsequently needed concomitant medication or additional therapy.

## Results

ACS (Orthokine) resulted in significantly greater improvement over time than did the control treatments. Furthermore, patients treated with ACS consistently showed significantly higher relative improvements compared to the control groups for all outcome parameters (Tables II and IV).

WOMAC subscale scores were reduced in all treatment groups, with the largest reduction occurring in the ACS group (Table II). The ACS group scored significantly better

Table II
*Outcome scores per treatment group over time*

| | $WOMAC_{Global}$ | | $WOMAC_{Pain}$ | | $WOMAC_{Stiffness}$ | | $WOMAC_{Function}$ | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| *ACS (N = 134)* | | | | | | | | |
| Baseline | 5.24 | 2.32 | 5.18 | 2.39 | 5.59 | 2.7 | 5.21 | 2.41 |
| Week 7 | 2.80 | 2.30 | 2.71 | 2.37 | 3.07 | 2.49 | 2.80 | 2.34 |
| Week 13 | 2.42 | 2.06 | 2.33 | 2.14 | 2.80 | 2.33 | 2.40 | 2.08 |
| Week 26 | 2.42 | 2.19 | 2.42 | 2.25 | 2.78 | 2.45 | 2.37 | 2.21 |
| *HA (N = 135)* | | | | | | | | |
| Baseline | 5.19 | 2.04 | 4.89 | 2.12 | 6.04 | 2.65 | 5.17 | 2.11 |
| Week 7 | 4.02 | 2.09 | 3.63 | 2.09 | 4.82 | 2.65 | 4.04 | 2.14 |
| Week 13 | 4.00 | 2.17 | 3.73 | 2.22 | 4.75 | 2.68 | 4.00 | 2.19 |
| Week 26 | 3.75 | 2.42 | 3.59 | 2.47 | 4.32 | 2.78 | 3.74 | 2.44 |
| *Saline (N = 107)* | | | | | | | | |
| Baseline | 5.16 | 2.12 | 4.86 | 2.01 | 5.78 | 2.77 | 5.18 | 2.24 |
| Week 7 | 3.81 | 2.33 | 3.49 | 2.23 | 4.45 | 2.89 | 3.83 | 2.42 |
| Week 13 | 3.99 | 2.13 | 3.61 | 2.11 | 4.69 | 2.78 | 4.01 | 2.20 |
| Week 26 | 3.93 | 2.38 | 3.68 | 2.24 | 4.51 | 2.82 | 3.94 | 2.48 |

| | VAS | | PCS (SF-8) | | MCS (SF-8) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| *ACS (N = 134)* | | | | | | |
| Baseline | 69.6 | 13.10 | 29.39 | 6.65 | 42.60 | 11.74 |
| Week 7 | 33.8 | 23.92 | 40.21 | 9.96 | 49.96 | 10.48 |
| Week 13 | 29.6 | 23.14 | 42.87 | 9.46 | 51.09 | 9.22 |
| Week 26 | 29.5 | 22.58 | 41.62 | 10.12 | 50.66 | 10.68 |
| *HA (N=135)* | | | | | | |
| Baseline | 68.3 | 12.81 | 28.65 | 5.65 | 43.25 | 12.10 |
| Week 7 | 52.6 | 23.15 | 34.57 | 9.07 | 46.50 | 12.46 |
| Week 13 | 52.1 | 22.97 | 34.66 | 9.42 | 46.43 | 11.19 |
| Week 26 | 49.3 | 25.90 | 35.62 | 10.10 | 46.51 | 11.48 |
| *Saline (N=107)* | | | | | | |
| Baseline | 66.3 | 14.49 | 29.45 | 5.84 | 43.79 | 12.38 |
| Week 7 | 46.7 | 23.52 | 35.60 | 9.12 | 47.26 | 11.08 |
| Week 13 | 48.8 | 22.51 | 34.52 | 8.59 | 45.69 | 10.61 |
| Week 26 | 48.2 | 25.59 | 34.99 | 8.96 | 46.22 | 11.07 |

Note that ACS-treated patients scored significantly better than those treated with HA and saline at all data points, whereas there are no significant differences at any data point between HA and saline.

than either HA or saline on all WOMAC subscales at all time points after the injections ($P < 0.001$ for each comparison). No differences were observed between HA and saline in any of the WOMAC scores across time (each $P > 0.05$ for the comparison HA vs saline).

VAS ratings at weeks 7, 13, and 26 were lowest in the ACS group ($P < 0.001$ for all comparisons with either HA or saline at all time points). After ACS injections, the mean pain rating decreased from 69.6 mm to 29.6 mm at week 13 and to 29.5 mm at week 26 (Table II). ACS treatment produced a higher number of patients who experienced more than 50% improvement as assessed by VAS at all time points (VAS response [>50% improvement] at week 26: ACS 67%, HA 32%, saline 33%; each $P < 0.001$). GPA scores at all of the follow-up visits were higher (each $P < 0.001$) with ACS than with either HA or saline (Table III).

In all SF-8 HRQL dimensions and component scores, ACS treatment was associated with the largest improvement ($P < 0.001$ for each comparison). In particular, the SF-8 PCS and MCS were higher ($P < 0.001$ in both cases) after ACS treatment than in either control group (Table II).

Only local adverse events (Table V) occurred in all groups, with one exception (in the HA group). We observed no infections. Five patients in the HA group (3.7%) and two patients in the saline group (2%) withdrew because acute local reactions developed after an injection. Of the five patients in the HA group, four required treatment with synovial aspiration and intra-articular administration of a corticosteroid (triamcinolone). Clear yellow fluids were aspirated. Cell counts revealed inflamed states. There were no crystals. Gram stain and microbial cultures were negative. These patients received NSAIDs, ice packs, and rest. Their symptoms improved within a couple of days. One patient receiving HA had a generalized skin reaction (allergy) after the second injection and discontinued the trial medication. In the ACS group, mild and moderate symptoms were observed directly after the intra-articular injections. These symptoms (pain or pressure sensation) improved within minutes or within 24 h after injection at most. None of the reactions in the ACS group needed further intervention. There were no differences in the three treatment groups with respect to use of concomitant medication or number of medications used, and no correlation between use of medication and treatment outcome. Numbers with bilateral therapy were too small to provide useful information. However, we cannot exclude completely that there may have been a contralateral effect, too.

Table III
*GPA of treatment efficacy*

| Treatment group | | GPA values | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| ACS | Week 13 | 29 (22%) | 45 (34%) | 26 (20%) | 12 (9%) | 16 (12%) | 3 (2%) |
| | Week 26 | 32 (24%) | 36 (27%) | 24 (18%) | 20 (15%) | 15 (11%) | 4 (3%) |
| HA | Week 13 | 3 (2%) | 22 (18%) | 27 (22%) | 25 (20%) | 38 (31%) | 9 (7%) |
| | Week 26 | 9 (7%) | 21 (17%) | 20 (16%) | 26 (21%) | 33 (27%) | 15 (12%) |
| Saline | Week 13 | 9 (9%) | 13 (13%) | 14 (14%) | 20 (20%) | 26 (26%) | 19 (19%) |
| | Week 26 | 7 (7%) | 15 (15%) | 20 (20%) | 13 (13%) | 27 (27%) | 19 (19%) |

Overall assessment of treatment efficacy as a function of time and treatment group. The patient was asked: "How would you rate the effectiveness of your osteoarthritis treatment right now?" Very good (1), good (2), satisfactory (3), adequate (4), poor (5), and unsatisfactory (6). Data are for visits at weeks 13 and 26: ACS: $N = 131$; HA: $N = 124$; and NaCl: $N = 101$ (ITT population). The percentage of patients who were at least satisfied with the treatment efficacy was significantly higher in the ACS group.

TWO-YEAR FOLLOW-UP

Of the 345 patients who participated in and completed the initial study, 310 were traceable after 2 years (mean follow-up time: 2.14 years, follow-up rate: ~90%). Of these, 122 had received additional therapy (e.g., surgery, acupuncture, subsequent medication on prescription, or another series of intra-articular injections) for their study knee after the 6-month period and were thus re-evaluated separately using the LOCF method of imputation (Fig. 2a and b).

In both evaluations, all three groups still demonstrated significant improvements in OA symptoms as measured by the WOMAC index and VAS. At the 2-year follow-up evaluations, there were still statistically significant differences between the ACS group and both control groups with regard to WOMAC, VAS and GPA. The results demonstrate that treatment with ACS results in a significantly better therapeutic effect compared to HA and saline not only at 6 months (double-blinded design), but also at 2 years (observer-blinded design). Remarkably, the effects seen in patients who received HA or saline also persisted for the additional 18 months.

## Discussion

Our data show that ACS (Orthokine) is safe and has a therapeutic effect on the major clinical parameters of painful knee OA. Remarkably, the therapeutic effect persists for at least 2 years. The ACS production process has been shown to reproducibly elevate IL-1Ra and other factors[27,50,51], although the mechanisms by which the effects are mediated are not fully understood. The multitude of synergistic, active therapeutic molecules may explain the observed clinical effect, but its long-term persistence is more difficult to explain. One possibility may be that the therapeutic molecules help to re-establish a healthy joint homeostasis[52].

The authors conclude that ACS is effective for treatment of patients with low- to medium-grade, painful knee OA. We treated only patients with a VAS pain score > 50/100 mm (severe pain), so the results cannot necessarily be generalized to all OA patients.

Responder rates (patients with >50% pain reduction in VAS pain score) were in the range of 71% after 3 months and declined to 67% after 6 months. The mean improvement for patients treated with HA or placebo was less than half that in the ACS group (VAS). The differences compared to the ACS group were statistically significant from week 7 through week 26 ($P < 0.001$). There were no significant differences between the HA treatment and placebo injections throughout the 26 weeks of the study ($P > 0.05$).

These observations are in line with the well-recognized significant placebo response in patients with OA after either aspiration of the knee synovial fluid or injection with pharmacologically non-active viscoelastic substances[53–65]. However, the placebo group showed a longer beneficial response than expected based on the results of previous studies with a similar design[54,55,57,60].

The present study demonstrated highly similar treatment effects for both HA and saline, despite an over 80% statistical power to detect clinically relevant differences. It was not the aim of this work to evaluate the effectiveness of HA in OA. However, in the light of these clinical results, we wish to comment that a number of publications and meta-analyses have seriously questioned the effectiveness

Table IV
*"Last visit minus baseline" statistical effects for efficacy variables at weeks 7, 13 and 26 (ITT population)*

| Variable | *P*-value | Comparison | Significance |
|---|---|---|---|
| WOMAC global | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| WOMAC pain | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| WOMAC stiffness | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| WOMAC function | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| VAS, response (50% improvement) ACS: 67%, HA: 32%, saline: 33% | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| GPA, response (score ≤ 3) ACS: 70%, HA: 40%, saline: 42%) | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| SF-8 PCS | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |
| SF-8 MCS | <0.001 | ACS–saline | Yes |
| | | ACS–HA | Yes |
| | | HA–saline | No |

Table V
*Percentage (and number) of patients with adverse events and concomitant medication*

| | ACS | HA | Saline |
|---|---|---|---|
| Overall incidence | 23% (31)‡ | 38% (51)† | 28% (30)‡ |
| Mild | 19% (25) | 27% (37) | 24% (26) |
| Moderate | 4% (6) | 7% (9) | 2% (2) |
| Severe | 0% (0) | 5% (5) | 2% (2) |
| Paracetamol/acetaminophen | 17% (23) | 26% (35) | 31% (33) |

Local adverse events as a function of treatment group. Anticipated adverse events were defined as local reactions such as pressure, transient knee pain, swelling, tenderness and heat at the injection site. Patients with severe adverse events were treated as withdrawals. Note that there is a statistically significant difference in adverse effect occurrence between the HA group and the ACS and saline groups (‡$P < 0.001$ for the comparisons HA−ACS and HA−saline) and no differences between the ACS and the saline group (†$P > 0.05$). Paracetamol, up to 4 g/day, was allowed as rescue medication during the trial.

of HA in OA[5,57,66–70] as well as the dependency of efficacy on the molecular mass of HA[67,68,71,72].

NSAIDs give only modest control over the signs and symptoms of OA. Scholes *et al.*[73] found that only 15% of patients with OA of the knee for whom a NSAID was prescribed were still taking the same drug 12 months later. Certain cyclooxygenase-2 (COX-2) inhibitors have recently been withdrawn as a result of cardiovascular complications[7]. The future of this class of drugs is now unclear. Their claimed qualification as OA medication may possibly be due to what has been until now a lack of effective treatment alternatives.

Although this study supports the use of ACS in mid-stage painful OA of the knee, we are aware of its shortcomings. The lower number of patients in the saline group may be explained by an initially asymmetric randomization process at the external blood-processing site favoring the ACS and HA groups. This problem was identified and corrected after inclusion of the first 80 patients. The authors believe that, given the strength of the clinical results, the informative

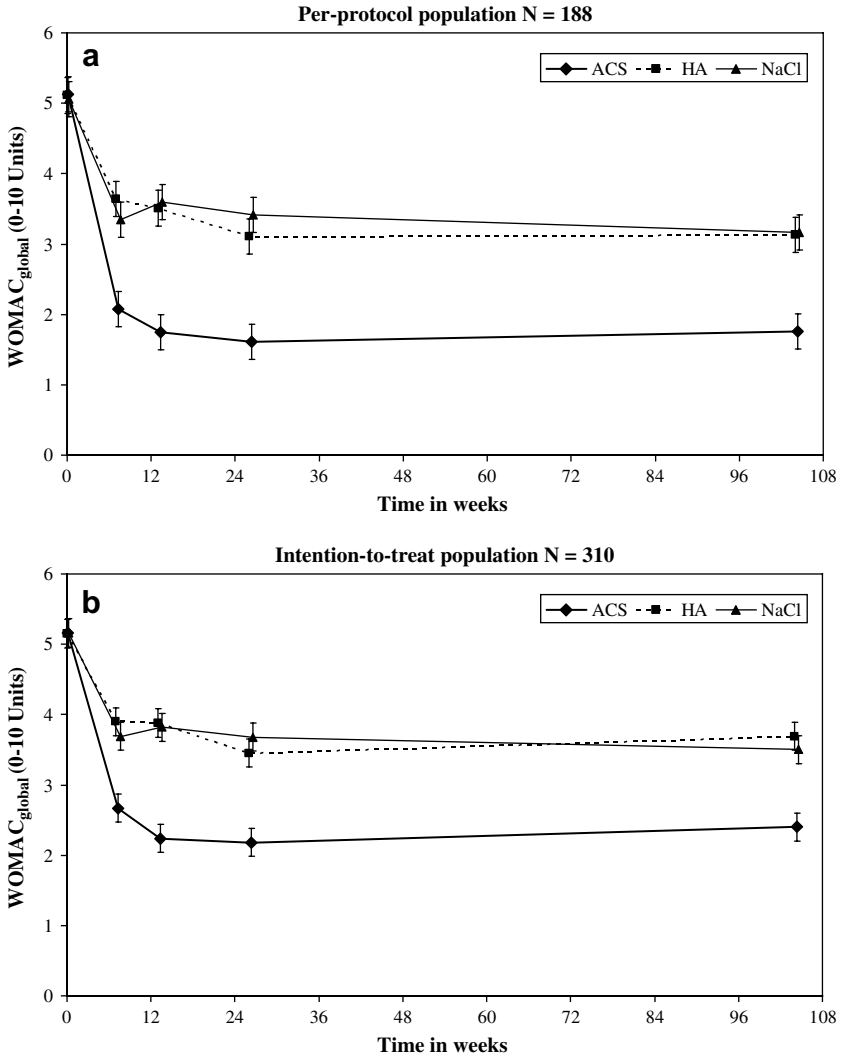


Fig. 2. WOMAC scores (0−10 scale) as a function of time and treatment group 2 years after the last trial injections. (a) Valid-for-efficacy-population ($N = 188$): patients who did not receive subsequent pharmacological or surgical treatment for OA (ACS: $N = 76$; HA: $N = 56$; saline: $N = 56$). Figure shows mean ± SE. *$P < 0.05$ for the comparison ACS vs HA and saline; ‡$P > 0.05$ for the comparison HA vs saline. (b) Intention-to-treat (LOCF) population ($N = 310$): overall cohort with last value carried forward in patients who received subsequent therapies. Figure shows mean ± SE. *$P < 0.05$ for the comparison ACS vs HA and saline; ‡$P > 0.05$ for the comparison HA vs saline.

value of the clinical results is not reduced by this finding. Approximately 60% of subjects had undergone previous knee surgery. The exclusion criteria eliminated subjects who had undergone knee surgery within the previous 3 months, but earlier surgery could be a confounder in this trial. However, there was no correlation between previous surgery and treatment outcome. Interestingly, both cohorts benefited from the procedure. Analysis of the treatment results showed that the non-surgically treated group experienced the same statistically significant changes as the total study cohort. Another issue is the use of the Kellgren–Lawrence score as an entry criterion. This score is quite insensitive and prone to inter-observer error. Another problem is that knees receiving HA and saline had three intra-articular injections, while those receiving ACS had six. This could not be avoided, because the manufacturer's instructions for HA (Hyaject®) require it to be given as a series of three injections, and the ethics committee would not permit six injections of saline. We do not know whether a higher number of HA or saline injections would have altered the outcome of the study. However, there is no unequivocal evidence that a higher number of HA injections coincides with better clinical results[68,69,74–77]. The number of ACS injections was deduced from clinical experience gathered since 2000. However, there is no conclusive data suggesting that a lower number of ACS injections is clinically less effective. Treatment in the aforementioned horse study was limited to four injections of ACS or saline, and showed considerable efficacy in the ACS group[28].

In a recently published ACS-Orthokine knee OA trial, Yang *et al.*[52] showed that ''Autologous interleukin-1 receptor antagonist improves function and symptoms in OA when compared to placebo in a prospective randomized controlled trial''. Although their WOMAC data did not show an effect as strong as that observed in the German Orthokine Osteoarthritis Trial (GOAT), knee injury and osteoarthritis outcome score (KOOS) and knee society clinical rating scale (KSCRS) indicated significant clinical superiority of ACS over placebo. However, the two studies are only partially comparable since the inclusion criteria (lower pain scores at time of inclusion [VAS $>$ 40 mm]), outcome instruments, statistical methods and duration of observation were different.

The demonstrated clinical improvement in a large number of patients with painful OA treated with intra-articular ACS containing elevated levels of autologous factors such as IL-1Ra suggests that further investigation of the role of cytokines in the pathogenesis of OA is merited. To this end, it would have been instructive to measure the concentrations of key cytokines in synovial fluids aspirated from the study subjects. Synovial IL-1Ra measurements in the horse study showed qualitatively increasing IL-1Ra levels until day 71. However, these assays were not performed in this study because the protocol did not allow serial synovial fluid aspiration.

In summary, intra-articular ACS (Orthokine) reduces pain and increases function and mobility for up to 2 years, based on double-blind 6-month results and observer-blinded 2-year results. It can be considered as clinically very safe because of its autologous origin. No clinically serious side effects were observed in the ACS group during the observation period.

## Conclusion

Intra-articular injection of ACS (Orthokine) in patients with painful knee OA has an excellent safety profile and results in a strong clinical response. The data show that ACS (Orthokine) represents an effective and well-tolerated alternative to currently predominant treatments of OA. Further investigation is necessary to determine whether these effects are symptom-modifying or structure-modifying.

## Conflict of interest

Each author certifies that he has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article. Prof. Dr. Krauspe received a consultant fee in the initiating period prior to the start of the knee OA study from Orthogen AG, Germany.

Each author certifies that his institution has approved the human protocol for this investigation that all investigations were conducted in conformity with ethical principles of research, and that informed consent was obtained.

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