Autologous conditioned serum for the treatment of osteoarthritis and other possible applications in musculoskeletal disorders

Antonio Frizziero†, Erika Giannotti†, Francesco Oliva‡, Stefano Masiero†, and Nicola Maffulli§*

†Department of Orthopaedic Rehabilitation, University of Padova, Padova, Italy; ‡Department of Trauma and Orthopaedic Surgery, University of Rome ‘Tor Vergata’, School of Medicine, Rome, Italy, and §Centre for Sports and Exercise Medicine, Queen Mary University of London, Barts and the London School of Medicine and Dentistry, Mile End Hospital, London, UK

Introduction: The therapeutic use of interleukin 1 (IL-1) cytokine receptor antagonists (IL-1RA) has promoted the development of new biological therapies for osteoarthritis (OA). Autologous conditioned serum (ACS) is an alternative, safe and well-tolerated treatment in OA.

Sources of data: We performed a comprehensive search of PubMed, Medline, Cochrane, CINAHL, Embase, SportDiscus, Pedro and Google scholar databases using keywords such as ‘interleukin 1’, ‘osteoarthritis’ and ‘autologous conditioned serum’.

Areas of agreement: ACS, containing endogenous anti-inflammatory cytokines including IL-1RA and several growth factors, could reduce pain and increase function and mobility in mild to moderate knee OA.

Area of controversy: Given the limited data available on the composition of ACS, the mechanisms through which ACS produces clinical improvement, the duration of its effect and the changes in cytokine levels after repeated injections are still unknown.

Growing points: Although previous clinical data are encouraging and confirm the safety of this modality, given the limitations of current studies, there should be additional, controlled trials to further confirm efficacy for the use of ACS in OA treatment.

Area timely for developing research: ACS can lead to enhancement of tissue regeneration and to reduction of degenerative mechanisms.

Keywords: autologous conditioned serum (ACS)/interleukin 1 (IL-1)/knee osteoarthritis/Orthokine

Accepted: June 5, 2012
Introduction

Osteoarthritis (OA) is a slowly progressive, disabling and degenerative joint disease characterized by destruction of articular cartilage, remodeling of the subchondral bone, joint marginal osteophyte formation and synovitis.\(^1\)

OA most often occurs in the elderly, but can also affect active and younger patients, especially in association with high-impact sports and injuries.\(^2,3\) Inflammation plays a key role in the pathogenesis of OA, and synovial and cartilage tissues are involved, through the production of catabolic inflammatory cytokines such as tumor necrosis factor alpha (TNF-\(\alpha\)) and interleukin-1\(\beta\) (IL-1\(\beta\)). These promote synovial inflammation and activate chondrocytes to produce matrix metalloproteinases (MMPs) that are responsible for degradation of the cartilage matrix.\(^4\)

Pro-inflammatory cytokines and MMPs are present in the synovial fluid (SF) and synovial tissue of OA patients,\(^2\) and the amount of type-1 IL-1 receptors is significantly increased in OA chondrocytes\(^5\) and synovial fibroblasts,\(^6\) promoting susceptibility to IL-1\(\alpha\) and IL-1\(\beta\)-mediated effects.

Moreover, some growth factors (GFs) such as platelet-derived GF (PDGF), transforming GF beta (TGF-\(\beta\)), insulin-like GF-I (IGF-I), basic fibroblast GF (bFGF) and vascular endothelial GF (VEGF) take part in regulating articular cartilage metabolism and modulating chondrogenic expression. These GFs are all present in the \(\alpha\)-granules of platelets.\(^7\)

Considering the multifactorial pathophysiology of OA in which imbalance between anabolic and catabolic processes is important, and the poor self-healing capacity of chondrocytes and cartilage defect, current research is dominated by innovative biochemical therapies, including intra-articular administration of IL-1 receptor antagonist (IL-1RA) and specific GFs, aimed at reducing pain as well as limiting the destruction of articular cartilage and stimulating healing processes.\(^8,9\)

A potential treatment option to improve joint function and enhance the repair process is administration of platelet-rich plasma (PRP) derived from autologous blood containing platelet-derived cytokines and GFs.

Although in some studies injections of PRP have been shown to reduce pain and improve quality of life scores for patients with degenerative conditions of the knee,\(^10-12\) a recent systematic review and meta-analysis suggests considerable uncertainty about the evidence regarding a clinical benefit of the use of PRP for a variety of musculoskeletal disorders.\(^13\)

Other areas of interest include the use of IL-1RA, the natural inhibitor of IL-1\(\beta\). This competitive receptor antagonist of IL-1, with affinity...
for type I and II IL-1 receptors, can block the signaling activity of IL-1. The therapeutic use of IL-1 inhibitors in such conditions was proposed in the early 1980s, and in early experimental studies, IL-1RA was administered to animals, using gene transfer.

Intra-articular gene expression of IL-1Ra was successfully obtained with synovial cells injection into the joint of normal rabbit. In two studies, intra-articular injection of equine IL-1Ra into horses knee affected by osteochondral defects and intra-articular injection of canine IL-1Ra in rabbit knees after menisectomies produced reduction of OA modifications, and IL-1Ra levels increased for 4 weeks after treatment.

In their study in horses, Frisbee et al. administered IL-1RA gene sequence into a joint that had previously developed experimentally produced OA. The injection caused the horse’s own synoviocytes to produce equine IL-1RA within the affected joint, and this evidence led to hypothesize a potential anti-arthritic or chondroprotective effect of IL-1RA. In another two studies conducted in animal OA models, gene expression of IL-1RA persisted 2 weeks after implantation with reduction of cartilage severity degradation.

Later on, early applications of IL-1RA in patients with painful knees produced no significant clinical improvement in OA symptoms, with a half-life of IL-1RA of ~4 h after intra-articular injection. Moreover, Arend et al. demonstrated the induction of IL-1RA synthesis in purified human monocytes after immunoglobulin G stimulation. Even though these previous techniques for induction of IL-1RA were too laborious, expensive and time-consuming to be used therapeutically, the potential benefits of IL-1RA to modulate OA progression has encouraged the search for novel methods to stimulate the synthesis of endogenous source of IL-1RA by blood cells.

Meijer et al. devised a biologic therapeutic preparation known as autologous conditioned serum (ACS) marketed as ‘Orthokine’, (Orthogen, Düsseldorf, Germany), a medical device (a syringe) used to produce ACS enriched with anti-inflammatory cytokines.

The production of ACS rich in IL-1RA using the Orthokine technique is characterized by incubation into a syringe of 50–60 ml of venous blood. After incubation for 24 h at 37°C, the blood is recovered and centrifuged. This process stimulates a rapid synthesis of IL-1RA, the concentration of which increased 140-fold, of other anti-inflammatory cytokines (IL-4, IL-10) and GFs (IGF-I, PDGF and TGF-β) that are slightly induced by treatment with glass beads. In addition, other authors observed that, during incubation, the IL-1RA concentration steadily increases for 24 h, possibly also through de novo synthesis, stimulated by the interaction between the glass surface and the blood cells. This was confirmed by the action of cycloheximide to
inhibit the accumulation of IL-1RA. No significant alterations in protein composition and no significant increase of pro-inflammatory mediators IL-1β and TNF-α were noticed. The autologous serum, now enriched in IL-1RA, IL-4, IL-10 and several GFs, is returned to the patient. Since 1998, ACS, marketed under the trade name Orthokine®, has been used in orthopedic patients and in experimental animal models.

**Methods**

**Aim of the study**

We review the current knowledge in the field of effects and results of treatment with ACS in knee OA.

**Literature search and data extraction**

We performed a comprehensive search of PubMed, Medline, Cochrane, CINAHL, Embase, SportDiscus, Pedro and Google scholar databases using various combinations of the commercial names of each preparation and the keywords ‘osteoarthritis’, ‘Autologous conditioned serum’, ‘IL-1RA’, ‘Intra-articular injections’ and ‘Orthokine®’. We excluded from our investigation case reports and letters to editors. Eligible studies had to show clinical effectiveness and safety of ACS in animal OA models and in human clinical studies for the treatments of knee OA. Abstracts were read and screened. Relevant articles from peer-reviewed journals were retrieved. Bibliographies were hand searched for further relevant articles. Given the linguistic capabilities of the research team, we considered publications in English, Italian, French, Spanish and Portuguese.

Articles reporting employment of ACS in other conditions, including musculoskeletal injuries, were excluded from the study (Fig. 1).

**Results**

**ACS in animal OA model**

The equine model of OA has been widely used to study the pathophysiologic processes of OA and the effectiveness of new therapies. Frisbie et al. in a controlled randomized block design study, evaluated the effects of intra-articular administration of ACS compared with placebo treatment in equine OA. At Day 0, OA was induced...
unilaterally in the mid-carpal joint of 16 horses. At Day 14, the horses were divided into two treatment groups: placebo control and ACS-treated horses. In eight placebo control and eight ACS-treated horses, saline or ACS was injected into the OA-affected joint at Days 14, 21, 28 and 35. Moreover, at Day 14, the horses began a strenuous exercise regimen 5 days/week for the remaining 8 weeks of the study. SF and serum were assessed every other week. The horses were assessed for lameness, using the American Association of Equine Practitioners grading scale every 2 weeks. At the end of the study, operated joints were evaluated grossly, and tissues were harvested for biochemical and routine histologic examinations. Compared with placebo, the horses treated with ACS had significantly improved lameness in OA joints, even 5 weeks after the last treatment, and had a significant reduction in synovial membrane hyperplasia at Day 70. Trends for improvement in cartilage immunohistochemistry and gross necropsy were noted for OA ACS-treated joints compared with placebo treatment. No measurable levels of IL-1RA were found in ACS assessed by human IL-1RA antibody.

A similar study used mouse anti-IL-1RA antibody to estimate IL-1RA concentration: there was a significant increase in IL-1RA in SF
of the ACS-treated joints compared with placebo. Probably, mouse anti-IL-1RA antibody is more appropriate than the human anti-IL-1RA antibody for estimating IL-1RA concentration in those samples. Also, the concentration of IL-1RA in the SF of joints of all the horses in the ACS-treated group increased significantly with time.\textsuperscript{28} This evidence became apparent at Day 35 and was still evident at Day 70, and similar findings were also apparent after gene transfer of equine IL-1RA.\textsuperscript{18} These data suggest that the administration of IL-1RA may stimulate endogenous production of IL-1RA. These two studies recorded no adverse events. ACS was safe and well-tolerated with significant clinical and histologic improvements in the OA-affected joints of the horses following treatment with four injections of ACS compared with placebo. Although further controlled clinical trials should be performed to confirm these results, these favorable data in the management of equine OA led to trials in humans.\textsuperscript{29}

\textit{ACS in clinical human studies}

Two human trials evaluated the efficacy of ACS in symptomatic OA of the knee, using the Orthokine\textsuperscript{\textregistered} technique. The method for administration of ACS was the same in each trial and as recommended by the manufacturer. A 21-gauge needle was inserted in the supra-patellar couch through an antero-lateral approach, and 2 ml of ACS was injected.\textsuperscript{30,31} After encouraging preliminary data from a large non-blinded patient observational study in humans,\textsuperscript{32} Baltzer \textit{et al.}\textsuperscript{30} tested whether ACS was superior to saline and hyaluronan (HA) as an intra-articular therapy to reduce the signs and symptoms of knee OA. This study was based on a 26-week prospective, randomized, patient- and observer-blinded, placebo-controlled trial using an intention-to-treat analysis (ITT). Inclusion and exclusion criteria are reported in Table 1.

From 464 patients, 376 were eligible for randomization into one of three treatment arms, receiving ACS or HA or saline. The washout period of analgesic and anti-inflammatory medications was 3 weeks, starting from the day of inclusion until the first injection, and only acetaminophen was permitted during the study. Subjects in the placebo and in the HA group received, respectively, one injection per week of saline and of HA and an application of topical heparin sodium cream at the second appointment every week. The ACS group received two injections per week for 3 consecutive weeks. Outcome measures, visual analogic scale (VAS), Western Ontario and McMaster (WOMAC) OA instrument, the Short-Form 8 health-related quality of life (SF-8 HRQL) survey and the global patient assessment (GPA), were assessed
Table 1 ACS marketed as Orthokine® in clinical human studies for the treatment of knee OA

<table>
<thead>
<tr>
<th>Author/years</th>
<th>No. of patients</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
<th>No. of injection</th>
<th>Follow-up</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltzer et al.30</td>
<td>376</td>
<td>Older than 30; Willing to discontinue all analgesics and NSAIDs for at least 6 months; A clinical diagnosis of OA by at least 3 months; Kellgrene–Lawrence grade II–III; VAS: 50 on a 100 mm; at least 3 months after studied knee’s surgery</td>
<td>Grade IV OA; Systemic or inflammatory joint diseases; Hematological diseases; cancer; Pregnancy or breastfeeding; intra-articular injection of any of the trial substances within the previous 6 months</td>
<td>One/week of saline or of HA or 2 injections/week of ACS for 3 consecutive weeks</td>
<td>First: 6 months; Second: 2 years</td>
<td>VAS, WOMAC SF-8 HRQL GPA</td>
</tr>
<tr>
<td>Yang et al.31</td>
<td>167</td>
<td>Aged &gt; 18 years; Clinical evidence of OA; Kellgren-Lawrence grades I–III; Maximal 60 points out of 100 points on the WOMAC; At least two of these questionnaires: the pain sub-item of KOOS or KSCRS, and minimal 40 mm for the 100-mm VAS for pain</td>
<td>Painful spine, hips or lower limbs; Hip prosthesis or ipsilateral coxarthrosis; Vascular, neurological disorder; Inflammatory and infectious arthropathies; Alcohol/drug abuse; OA grade IV; surgical and intra-articular treatment within 6 months of inclusion; Immunodeficiency; Coagulopathy; morbid obesity</td>
<td>Six of saline or of ACS, were given over 3 weeks at Days 0, 3, 7, 10, 14 and 21</td>
<td>12 months</td>
<td>VAS KOOS KSCRS WOMAC</td>
</tr>
</tbody>
</table>

Nr, number; VAS, visual analog scale; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities; SF-8 HRQL, osteoarthritis instrument, the Short-Form 8 health-related quality of life; GPA, survey, and the global patient assessment; ACS, autologous conditioned serum; HA, hyaluronan; KOOS, Knee injury and Osteoarthritis Outcome Score; KSCRS, Knee Society Clinical Rating Scale.
at baseline and at Weeks 7, 13 and 26. The 26-week trial was completed per protocol in 345 patients. The patients in the ACS group performed better in all WOMAC subscales ($P < 0.001$) than those in the HA or saline group. The VAS scores were lowest in the ACS group, with at least a 50% improvement at Week 26 ($P < 0.001$). In all SF8-HRQL scores, ACS treatment was associated with the largest improvement ($P < 0.001$). GPA scores at all follow-up visits were higher (each $P < 0.001$) with ACS than with either HA or saline. Only some local adverse events occurred in all the groups. 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.

At 2 years from the entry in the study, 310 of the 345 patients were re-evaluated by a blinded observer. Of these 310 patients, 122 had received additional surgery, medications or injections. Statistically significant differences for the ACS group over the HA and saline group persisted past the initial 6 months in all outcome parameters, and the therapeutic effect of ACS persisted for at least 2 years. The effects seen in patients who received HA or saline also persisted for an additional 18 months. However, the ACS group received two injections per week while the HA and saline groups received only one. Also, any disease or structure-modifying effect of ACS was not evaluated, and IL-1RA levels in the SF were not determined.

Yang et al.,$^{31}$ in a 30-month prospective double-blinded placebo-controlled randomized multi-center trial, compared ACS with a saline control in reducing symptoms of OA in the knee (Table 1). In 167 patients, six intra-articular injections of saline or of ACS were given over 3 weeks at Days 0, 3, 7, 10, 14 and 21. Pharmacological therapy was limited to acetaminophen up to 4 g/day. However, 13 patients were placed into the non-steroidal anti-inflammatory drugs (NSAIDs) group in which these additional rescue medications were permitted. All rescue medications were to be discontinued 1 week before the scheduled follow-up evaluation. At 3, 6, 9 and 12 months after the first injection, the patients completed the same questionnaires as at baseline, namely the VAS, the Knee Injury and OA Outcome Score (KOOS) and the Knee Society Clinical Rating System (KSCRS). The WOMAC scores were deducted from the separate KOOS items. The primary end point of this trial, a 30% improvement in the WOMAC index, was not reached. Both ACS and placebo-treated patients showed a significant improvement on all outcome measures ($P < 0.001$). ACS resulted in significant improvement in the KOOS symptomatology ($P = 0.002$) and KOOS sport ($P = 0.042$) subscales, compared with placebo treatment. The superior improvement resulting from Orthokine® treatment appeared even more pronounced on subgroup analysis for the patients
who continued using NSAIDs during the trial, especially in the KOOS sport parameters \((P = 0.011)\) and the physician section of the KSCRS \((P = 0.005)\). Adverse events were similar in the two groups. These results are questionable, as an ITT was not undertaken, and the primary outcome measure was not met. Moreover, the use of NSAIDs in a small group of participants and the influence of NSAIDs on the results noted in the association of ACS with NSAIDs group were not explained, and the type and the amount of NSAIDs were not specified. Finally, the chondroprotective effects of ACS were not evaluated, and the follow-up period was too short to reasonably expect a detectable protective effect on knee radiographs.

**ACS effects on cartilage metabolism and on SF**

Rutgers *et al.*\(^{33}\) evaluated the *in vitro* effect of ACS, marketed as Orthokine\(^{®}\), on cartilage proteoglycan metabolism to examine to what extent intra-articular injection of ACS is reflected in cytokine level changes in human osteoarthritic SF. The effects of ACS on proteoglycan metabolism were measured using 48 full-thickness osteoarthritic cartilage explants, taken from the femoral condyles of OA patients undergoing a total knee arthroplasty (Kellgren–Lawrence grade III). These explants were divided into two groups and were cultured for 16 days, one group in the presence of ACS \((n = 24)\) and the other of non-conditioned control serum \((n = 24)\) of healthy serum donors. The experiment was repeated with two other OA cartilage and serum donor combinations.

The analysis of cytokines in SF after ACS injection was performed in 22 OA patients meeting the American College of Rheumatology criteria for OA (mean age 52 years, range 35–72). ACS for intra-articular treatment was prepared by whole blood incubation in the presence of ACS-specific glass beads. Unconditioned serum was taken as control. Twenty-two patients were treated with six consecutive injections of ACS at Days 0, 3, 7, 10, 14 and 21, according to the ACS treatment schedule. To this end, a 21-gauge needle was inserted into the knee joint through a lateral supra-patellar approach. After aspiration of the SF, 2 ml of ACS were injected into the joint through a 0.22-mm sterile nitrocellulose filter (Millex\(^{®}\), Millipore Express, Carrigtwohill, Co. Cork, Ireland). The knee was flexed and extended manually to ensure thorough distribution of the serum throughout the joint. *In vitro*, ACS does not seem to have a direct effect on cartilage metabolism compared with unstimulated serum, possibly because of the fast disappearance of cytokines from the SF after injection. In fact, ACS had a short intra-articular half-life with fast clearance of injected cytokines from
the joint. Moreover, in addition to the increase in anti-inflammatory cytokines, pro-inflammatory cytokines, in particular IL-1β and TNF-α, were significantly upregulated, in contrast to previous investigations. In the end, during the course of treatment, no significant changes in cytokine levels in SF occurred despite repeated ACS injection: IL-1RA levels did not increase during the course of treatment. Although these last results are in contrast to those of former study in horses, it is uncertain whether these IL-1RA levels were associated with OA symptoms or disease progression, as the ratio of IL-1RA to IL-1β in the SF of human OA subjects was not associated with pain or with the Lequesne OA index.

The authors, according to their in vitro data, did not recommend the use of ACS in the management of OA. This study presents some shortcomings that could explain the controversial results compared with previous studies. The Kellgren–Lawrence grade of knee OA in patients treated with intra-articular injection of ACS was not described. Meijer et al. proposed this novel therapy in mild to moderate OA. Despite the elevated concentrations of IL-1β and TNF-α, there was no adverse effect of ACS on proteoglycan turnover in the cartilage explant cultures. ACS should be used only when knee effusion has been removed effectively. The described possible aspiration of SF after treatment with ACS raises questions about the composition of the injected ACS.

Other possible applications of ACS in muscle, tendon, ligament and spinal injuries

ACS in muscle injuries

Wright-Carpenter et al. investigated the effects of local administration of ACS in animals and humans in the management of muscle injuries. In an experimental muscle injury model, mice were subjected to a blunt injury to their gastrocnemius muscle. At 2, 24, and 48 h after the injury, one group received local injections of ACS, and a control group received saline injections. The ACS group showed accelerated satellite cell activation and increased diameter of regenerating myofibers compared with the controls. The high concentrations of fibroblast GF (FGF-2) and TGF-β1 noticed in ACS compared with a control group could be partly responsible for the accelerating effects on regeneration from proliferative and chemotactic properties.

A pilot study compared the return with play time of 18 professional sportsmen with a variety of muscle strains treated with ACS injection into the lesion and a similar group of 11 athletes treated
with Actovegin® and Traumeel® injection. Both groups received a standard rehabilitation program, an oral anti-inflammatory drug, and underwent magnetic resonance imaging (MRI) scanning between Day 14 and 16 after the start of treatment. The results show a significant reduction in recovery time for the ACS group (16 vs 22 days) compared with standard care. The large number of methodological concerns, including choice of control, lack of randomization, lack of blinding and potential bias of the MRI, limits the interpretation of these positive results.36

This novel therapy could be used in professional athletes to reduce injury ‘down’ time and may also be interesting in postoperative situations. Further randomized controlled trials should be performed in laboratory animals and in human athletes to confirm these preliminary results.

ACS in tendon injuries

Studies were conducted in the rat Achilles tendon. Majewski et al.37 analyzed with biomechanical and histologic tests the effect of administration of ACS on the healing of transected and sutured rat Achilles tendons (ACS group = 40) compared with a control group (=40). The ACS-treated tendons were thicker and had more type I collagen, exhibiting an accelerated recovery of tendon stiffness and histologic maturity of the repair tissue, but both groups showed no increase in strength. In another study,38 rat tendons were transected and resutured and the role of GFs was investigated, in particular bFGF, bone morphogenetic protein (BMP-12), VEGF and TGF-β1 during tendon healing and their reaction to single and multiple GF treatment. The effects of TGF-β1 or BMP-12 added by adenoviral transfer and of ACS on GF expression were analyzed. Additional BMP-12 or TGF-β1 had no significant effects, whereas ACS increased expression of all the factors after 8 weeks, except VEGF.

The use of ACS in tendon injuries may be a promising biomolecular treatment, but the lack of increase of strength after ACS incubation limits the potential application in human trials. Investigation of the mechanisms of action of ACS in tendon regeneration should be pursued in animal models to evaluate the potential effects of this treatment.

ACS in ligament injuries

IL-1β plays an important role in the pathogenesis of bone tunnel enlargement following anterior cruciate ligament (ACL) reconstruction.39,40 Darabos et al.41 hypothesized that, even though during the
postoperative ACL healing process IL-1β is mostly localized in the area of its activation in the SF of the knee joint, a certain amount of this cytokine reaches the systemic circulation via capillary blood flow. For this reason, elevated SF IL-1β levels may induce an increased concentration of IL-1β in the peripheral circulation. This could suggest a strong osteoclastic influence and a possible less-than-optimal result after surgery.

In a prospective, randomized, double-blinded, placebo-controlled group study, investigators treated the group A patients \((n = 10)\) with intra-articular application of 2 ml of ACS and the control group B patients \((n = 10)\) with placebo (2 ml of physiological solution) on the day of the surgery and at postoperative days 1, 6 and 10. The measurements of IL-1β concentration in the peripheral circulation and in the SF were performed on the day of operation and at postoperative days 1, 6 and 10. The levels of IL-1β concentration in the SF after operative ACL reconstruction were higher than normal in both groups, but a decrease in IL-1β SF concentration appeared to be more pronounced in absolute terms in group A. After 10 days, in group A, these values were equal to or below the concentration in a normal knee and statistically significantly lower than in group B. Correlation between serum and SF IL-1β appearance persists only in patients after ACL surgery and ACS application. Later the same authors confirmed these results in a prospective, randomized, double-blinded, placebo-controlled trial. In 62 patients, (group A = receiving ACS; group B = receiving placebo), they estimated the IL-1β concentrations in the SF and investigated the correlation between the levels of IL-1β at three different postoperative points following the ACL reconstruction. In addition, bone tunnel width was measured by computed tomography (CT) scans, and the IKDC 2000 (Subjective International Knee Documentation Committee) and WOMAC scales were used for clinical evaluation up to 1 year following the ACL reconstruction. The decrease in IL-1β SF concentration was more evident in the ACS group, and values were lower, to a statistically significant degree, in the ACS group at Day 10. Bone tunnel enlargement was significantly less in Group A than in Group B and clinical outcomes were consistently better in patients treated with ACS with statistically significant differences in the WOMAC stiffness subscale after 1 year. These previous data had shown positive ACS influence on the ACL post-traumatic healing process after an acute rupture and operative reconstruction.42

**ACS in spinal disorder**

Becker *et al.*, in a prospective, randomized, patient- and observer-blind, reference-controlled, single-center study, evaluated the effects of ACS
compared with triamcinolone (5 or 10 mg) in 84 patients with lumbar radicular compression. Participants were randomized as follows: 32 to the ACS group, 27 to receive triamcinolone 5 mg and 25 to receive triamcinolone 10 mg. Each patient received three injections (one injection a week for 3 consecutive weeks) performed under radiographic control in the close vicinity of the nerve root involved.

Only the ACS group reported 6 months after the first injection further pain reduction, already underlined at 6- and 12-week follow-up using objective and subjective assessment score VAS. On the contrary, patients treated with steroid injections showed a tendency to experience increased pain at the 6-month follow-up. This study shows the potential therapeutic efficacy and safety of ACS in patients with lumbar radicular compression. Further research should be directed into this area.43

Discussion

OA is an active process involving the entire synovial joint with both degenerative and repair processes.44,45 A history of knee injury is a major risk factor for the development of knee OA.46 Although the causes of OA are not completely understood, biomechanical stresses affecting the articular cartilage and subchondral bone, biochemical changes in the articular cartilage and synovial membrane, imbalance between anabolic and catabolic mechanisms, GFs and inflammatory mediator are all important in its pathogenesis.47

Inflammation in the synovial membrane has been observed during the early phases of idiopathic OA. Pro-inflammatory cytokines identified in OA joints, IL-1 and TNF-α, produced by the inflamed tissue, activated chondrocytes and infiltrated inflammatory cells seem to be important mediators in the development of OA.2 As the factors responsible for the development of OA become better understood, and with the emerging interest in regenerative medicine and tissue engineering, new treatment modalities are being developed for articular cartilage defects. The last two decades have seen an increasing interest for drugs that may alter the course of OA development. Such areas of interest include the inhibition of IL-1β, which seems to be the principal mediator responsible for the inflammatory changes in OA.

ACS seems, on the basis of prospective randomized controlled trials,48 an interesting, well-tolerated and possibly effective option in human knee OA. This modality of management is convenient. Produced from the blood of the recipient, ACS has demonstrated an excellent safety profile, with no serious side effects in the published studies.
Orthokine®-derived ACS reduces pain and increases function and mobility for up to 2 years in early knee OA.\textsuperscript{30} No clinically relevant improvements have been highlighted in advanced OA.

Although one of the actions may be enhancement of cartilage integrity through the inhibition of inflammatory activity, in particular with respect to IL-1 signaling, the mechanisms through which ACS produces clinical improvement are incompletely understood, and should be the object of additional research.

ACS is based on human serum, and the composition of the product may therefore vary in the concentration of cytokines and GFs among different patients, and even in the same patient depending on a variety of yet-unidentified factors. Moreover, the direct effect of the entire blend of known and unknown factors present in ACS on the metabolism of articular cartilage in human OA has not been described. Only limited data are available on the actual composition of the conditioned serum.

The production of ACS using the Orthokine® method reproducibly elevates IL-1RA, but new investigations are necessary to determine the mechanisms by which the effects of ACS are mediated and the quality of the product, by analysis of knee radiographs after a more extended follow-up period, and by determining cartilage breakdown products in SF.

Finally, large and carefully designed randomized clinical trials are needed to draw definitive conclusions on the potential risks and benefits of ACS and to evaluate the duration of its effect, to better select appropriate patients and optimize the procedure. Referring to this last aim, recently Luyten \textit{et al}.\textsuperscript{49} have proposed a classification criteria of ‘Early Osteoarthritis’ to better define patients a risk of OA development, potentially responders to certain novel biological treatments such as ACS intra-articular injections.\textsuperscript{50,51}

References

2 Wassilew GI, Lehnigk U, Duda GN \textit{et al}. The expression of proinflammatory cytokines and matrix metalloproteinases in the synovial membranes of patients with osteoarthritis compared with traumatic knee disorders.\textit{Arthroscopy} 2010;26:1096–104.
6 Sadouk MB, Pelletier JP, Tardif G et al. Human synovial fibroblasts coexpress IL-1 receptor type I and type II mRNA. The increased level of the IL-1 receptor in osteoarthritic cells is related to an increased level of the type I receptor. Lab Invest 1995;73:347–55.