Autologous Conditioned Serum

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KEYWORDS

• Interleukin-1 receptor antagonist • Osteoarthritis • Radicular compression
• Intra-articular therapy • Anterior cruciate ligament • Tendinopathy • Muscle injury
• Pain

KEY POINTS

- Autologous conditioned serum is prepared by the incubation of whole blood with surface-treated glass beads within a special syringe.
- During incubation, the serum is enriched in products synthesized and released by peripheral blood platelets and leukocytes including, but not limited to, the interleukin-1 receptor antagonist.
- Randomized, controlled trials find that locally injected autologous conditioned serum is effective in treating osteoarthritis, radicular compression, and tunnel widening after reconstruction of the anterior cruciate ligament.
- Additional studies suggest utility in treating tendinopathies and muscle injuries.
- Further studies are required to confirm clinical effectiveness in specific indications, to determine the composition of autologous conditioned serum, to determine its mode of action, to understand individual responses to therapy, and to explore potential synergies with other therapeutic agents.

INTRODUCTION

Autologous conditioned serum (ACS) is an autologous blood product enriched in the interleukin-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of interleukin-1 (IL-1).[1–4] ACS is administered locally to treat conditions in which IL-1 is thought to be an important agent of pathologic conditions. Several reviews have been written on this topic.[5–8]

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IL-1Ra has been produced in *Escherichia coli* as the recombinant molecule anakinra, marketed as Kineret. Anakinra, in combination with methotrexate, is approved by the US Food and Drug Administration for the treatment of rheumatoid arthritis (RA), self-administered subcutaneously at a daily dose of 100 mg. However, the therapeutic efficacy of anakinra in RA has generally been disappointing, and it is not widely used in this context. Clinical responses in sepsis have also been weak. However, systemic anakinra is effective in systemic juvenile idiopathic arthritis and a variety of rare autoinflammatory disorders; it is also of benefit in gout and pseudogout.[9]

There is considerable interest in using anakinra intra-articularly in the treatment of osteoarthritis (OA) and injured joints. An initial, open-label clinical trial in patients with OA of the knee provided highly encouraging results with sustained clinical improvement after intra-articular injection of 100 mg of anakinra.[10] However, a subsequent multicenter, randomized controlled trial (RCT) showed no sustained benefit of intra-articular Anakinra.[11] Nevertheless, there was transient improvement, observed at day 4, in certain parameters, notably pain. The temporary nature of the beneficial effects probably reflects the rapidity with which proteins are removed from joints.[12] An additional clinical trial administered anakinra intra-articularly to patients after rupture of the anterior cruciate ligament (ACL) and again found improvement in certain parameters during the 2-week study period.[13] In a further small, uncontrolled, unblinded study of 6 patients with persistent postsurgical knee effusions, a single 200-mg injection of anakinra decreased pain and swelling, improved range of motion, and permitted return to sporting activities.[14]

There is, thus, optimism that IL-1Ra could prove efficacious in injured and arthritic joints if there were a way to maintain therapeutic concentrations intra-articularly. Gene delivery provides one technology for achieving this, and proof of principle has been established in animal models and human clinical trials for RA.[15–17] Genetic delivery of IL-1Ra into human knee joints with OA is at an advanced preclinical stage of development.[18]

AUTOLOGOUS CONDITIONED SERUM

Background

Wehling and colleagues developed ACS in the mid-1990s as an expeditious, practical, and relatively inexpensive means of generating IL-1Ra for local, therapeutic application in musculoskeletal diseases. ACS is based on studies that found that macrophages and monocytes are major endogenous sources of IL-1Ra.[19,20] Production of IL-1Ra can be enhanced by a variety of stimuli, including adhesion to certain surfaces. Based on this information, Meijer and colleagues[21] developed a method for stimulating IL-1Ra synthesis by whole human blood. According to their method, peripheral blood is drawn into a syringe containing treated glass beads to which blood monocytes and other adherent cells have the opportunity to attach. The syringe and its contents are then incubated at 37° for several hours, during which time platelets degranulate and mononuclear cells synthesize and secrete IL-1Ra along with a variety of additional anti-inflammatory products. During this period, synthesis of the inflammatory cytokines IL-1β and tumor necrosis factor-α (TNF-α) does not increase greatly. After incubation, the ACS is recovered and sterilized by filtration. ACS is then injected locally into sites of injury or disease.

Stimulation of blood cells by the glass beads is not specific to IL-1Ra, and ACS contains a variety of growth factors and cytokines (**Table 1**). Indeed, it has not been formally demonstrated that IL-1Ra is responsible for the therapeutic properties of ACS. The composition of ACS shown in **Table 1** is in rough agreement with that reported by Darabos and colleagues[22] and Rutgers and colleagues[23]; the only major discrepancy is the

Table 1
Cytokines and growth factors present in autologous conditioned serum

Cytokine	N	Basal Concentration	Concentration in ACS
IL-1Ra	224	236	2015
IL-1β	224	UD	7.9
IL-6	200	UD	28.7
TNF-α	92	UD	10.1
IL-10	92	UD	33.4
FGF-2	92	14.6	26.6
VEGF	92	61	508.6
HGF	92	431	1339
IGF-1	92	86,000	117,209
PDGF AB	92	205	39,026
TGF-β	80	1165	97,939

Concentrations are averages, given in picograms per milliliter before (basal) and after (ACS) incubation. N indicates the number of different samples tested.

Abbreviations: FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; UD, undetectable; VEGF, vascular endothelial growth factor.

Modified from Wehling P, Moser C, Frisbie D, et al. Autologous conditioned serum in the treatment of orthopedic diseases: the Orthokine therapy. BioDrugs 2007;21:326; with permission.

approximately 10-fold higher TNF-α concentrations noted by the latter investigators. These authors also reported the presence of osteoprotegerin, interferon-γ, and oncostatin M. The data in **Table 1**, including the TNF-α levels, are in agreement with the data of Wright-Carpenter and colleagues[24] who also noted the presence of IL-7. Darabos and colleagues[22] detected epidermal growth factor in ACS.

Clinical Development

ACS was first used clinically in 1997. Beginning in 2001, ACS was manufactured as Orthokine in a Good Manufacturing Process (GMP) facility. Participating physicians were provided with syringes, known as *Orthokine syringes*, containing treated glass beads. The beads are 3.5 mm in diameter, comprise borosilicate glass of maximum hydrolytic resistance, and are polished according to a proprietary process. Blood was drawn into Orthokine syringes and shipped to the GMP facility for the production of Orthokine. Typically, 6 injections of Orthokine, each 2 mL, were injected into knee joints over a period of 3 to 6 weeks. For epidural use, 3 injections, each 1 mL, were injected over a 3-week period. The number of injections and their timing were determined empirically.

This means of distribution proved cumbersome, time consuming, and geographically limiting and could not be performed in all markets because of regulatory hurdles. For these reasons, the system was changed in 2004 to one in which the physician was provided with a smaller, disposable Orthokine syringe and an incubator so that ACS could be produced locally in the individual clinic or physician's office. Although the original 50-mL syringe required a 24-hour incubation at 37° for optimal production of ACS, the newer 10-mL syringe allows a shorter incubation time of 6 to 9 hours.

Clinical Experience with Autologous Conditioned Serum —Intra-Articular Delivery

Baltzer and colleagues[25] published the first clinical use of ACS, describing treatment of 1000 patients with OA of the knee and Kellgren Lawrence (KL) scores of 1 to 3 (**Table 2**). In this prospective, nonrandomized, uncontrolled study, Western Ontario

Table 2
Clinical studies of intra-articular autologous conditioned serum

Indication	No of Patients	Study Design; Entry Criteria	Outcome Measures	Main Findings	Reference
Human knee OA	1000	Retrospective, uncontrolled, unblinded Established OA KL grades 1–3	WOMAC	WOMAC scores improved ≥50% in ≥70% of patients Improvement sustained for 3.5 y in ≥35% patients	[25]
Human knee OA	376	Randomized, placebo controlled ACS compared with saline and HA Established OA KL grades 2–3 Follow-up: 7 wk, 13 wk, 6 mo, and 2 y	WOMAC VAS SF-8 HRQL GPA	Sustained, statistically significant improvement in all outcome measures compared with saline and HA No severe adverse events	[26]
Human knee OA	167	Randomized, multicenter, placebo controlled ACS compared with saline KL grades 1–3 Follow-up: 3, 6, 9, 12 mo	WOMAC KOOS VAS KSCRS	Statistically significant improvements in KOOS symptoms and KOOS sport compared with saline No difference in WOMAC Trend toward improvement in other parameters Two severe adverse events, one of which attributed to ACS	[27]
Human knee OA	20	Patients who received placebo in the above study[27] were given ACS	WOMAC KOOS VAS KSCRS	Improvement to the same degree as previously[27] seen when receiving placebo	[28]
Human knee OA	118	Uncontrolled study; subjects received ACS (2 mL/wk for 4 wk) with physiotherapy Follow-up: 2 y Painful OA of knee, KL grades 1–4	WOMAC Pain	Rapid and sustained improvement in pain and WOMAC scores Only 1 patient progressed to total knee joint replacement	[29]
Human knee OA	30	Uncontrolled study; low-dose ACS (1 mL/wk for 3 wk) Follow-up: 3 mo Painful OA of knee, KL grades 1–3	WOMAC	Rapid and progressive improvement in WOMAC scores Maintained for at least 3 mo	[30]

Human hip OA	119 (150 hips)	Retrospective, nonrandomized, no placebo KL grades 2–4 ACS ± cortisone ± anakinra Follow-up: 14 mo	VAS	Statistically significant improvement in VAS in all groups No adverse events	[31]
Tunnel widening after single bundle ACL reconstruction (human)	62	Randomized, double blind, placebo controlled Isolated ACL rupture Outerbridge up to grade 2 Surgical reconstruction of ACL Follow-up: 6 and 12 mo	CT WOMAC IKDC 2000	Statistically significant reduction in tunnel widening Lower effusion at 6 mo Improved range of motion Improved WOMAC stiffness No serious adverse events	[32]
Tunnel widening after double bundle ACL reconstruction (human)	62	Randomized, double blind, placebo controlled Isolated ACL rupture Outerbridge up to grade 2 Double-bundle surgical reconstruction of ACL Follow-up: 6 and 12 mo	CT Lysholm IKDC 2000	Statistically significant reduction in tunnel widening Better Lysholm and IKDC 2000 scores No serious adverse events	[22]
Equine OA	262	Nonrandomized; no control Lameness unresponsive to intra-articular glucocorticoid or HA Follow-up: 6 and 12 wk	Lameness	Elimination or improvement in lameness in 221 horses at 6 wk No lameness in 178 horses at 12 wk No adverse events	[41]
Equine OA	20	Nonrandomized Lameness unresponsive to intra-articular PSGAG or HA Follow-up: 3 mo	Lameness	Full activity restored in 10/10 PSGAG failures and 7/10 HA failures	[43]
Equine OA	54	27 lame horses in each group received either ACS or a mixture of HA and betamethasone Follow-up: 6 mo	Lameness	ACS produced a stronger reduction in lameness	[42] (abstract)
Canine OA	11 dogs 15 joints	OA confirmed by clinical examination and radiology 2–4 injections of 1.4 mL ACS Observational study; no control group	Lameness	Clinical signs improved within 1 wk of the first or second injection Decrease in lameness in all dogs	[39] (abstract)

Abbreviations: CT, computed tomography; GPA, global patient assessment; KSCRS, Knee Society Clinical Rating System; SF-HRQL, Short-form 8 Health-related Quality-of-Life Survey.

MacMaster Universities (WOMAC) arthritis scores improved by ≥50% after 3 months in ≥70% of patients receiving intra-articular ACS. Each subcategory of the WOMAC scoring system improved, and improvements were maintained for 3.5 years in ≥35% of patients. There were no infections or allergic reactions to injection of ACS; 3.5% of patients reported joint swelling or pain immediately after injection, but these symptoms subsided spontaneously over the course of a few hours. Subsequently, there have been 2 large, double-blind RCTs evaluating the efficacy of ACS for the treatment of OA of the knee.

The first of these, also by Baltzer and colleagues,[26] compared ACS (6 injections, twice per week, 2 mL per injection) with standard of care (hyaluronic acid; HA) and placebo (saline). Subjects in the HA group received 1 injection (2 mL) per week for 3 weeks of a 1% solution of HA with a molecular weight of 1.4×10^6 Da (HYA-Ject Ormed, Freiburg, Germany). Entry criteria included age greater than 30 years, established OA of the knee, a KL score of 2 to 3, a pain score of at least 50 mm on a 100-mm visual analog scale (VAS), and a willingness to discontinue nonsteroidal anti-inflammatory drugs (NSAIDs) and other analgesics for 6 months. Outcome measures included VAS, WOMAC scores, Short-Form 8 Health-Related Quality-of-Life (SF-8 HRQL) survey, and the global patient assessment of treatment efficacy. Outcomes were measured at 7 weeks, 13 weeks, and 6 months; traceable patients were recalled after 2 years and reassessed in an observational, prospective, cohort study with a new, blinded observer.

A total of 376 patients were randomly assigned to 1 of the 3 study groups; 345 completed the initial, 6-month study, and 310 were traceable after 2 years. Patients receiving ACS experienced considerable, statistically significant improvement, beyond that obtained with placebo, in all outcome measures. Remarkably, these improvements were maintained for at least 2 years (**Fig. 1**). HA, in contrast, was no more effective than placebo. ACS produced no severe adverse events. At 3 months, 71% of subjects had greater than 50% reduction in VAS pain score; at 6 months, this response was 67%.

In the multicenter RCT of Yang and colleagues,[27] 176 patients with OA of the knee, KL scores 1 to 3, and VAS greater than 40 mm were randomly assigned to receive 6 injections of saline or ACS. Injections (2 mL) were given twice per week. Unlike the Baltzer and colleagues[26] study, continued use of NSAIDs and acetaminophen was permitted. Patients were assessed quarterly for 1 year. Efficacy was determined by VAS scores, the Knee Injury and Osteoarthritis Outcome Score (KOOS) and the Knee Society Clinical Rating System. WOMAC scores were calculated from components of the KOOS scores.

In this study, ACS did not improve the deduced WOMAC scores beyond placebo. However, ACS produced a statistically significant improvement in the KOOS symptom and KOOS sport scores. Moreover, use of ACS was associated with improvement in most other outcome measures, although these did not achieve statistical significance. Subgroup analysis found that participants who continued to take NSAIDs throughout the study had a superior response to ACS. This finding suggests that combination therapy including ACS could be an interesting option. Of note, the patients treated in this study had milder disease, as assessed by KL and VAS, than those recruited to the Baltzer study.[26] One serious adverse event, severe inflammation of the knee, was ascribed to ACS. In a follow-up study by the same group, ACS was administered to a subset of previous placebo patients without improvement beyond the previous placebo effect.[28]

A recent, nonblinded, 2-year prospective study by Baselga and Hernandez[29] showed promising results for highly symptomatic knee OA. Patients had mean

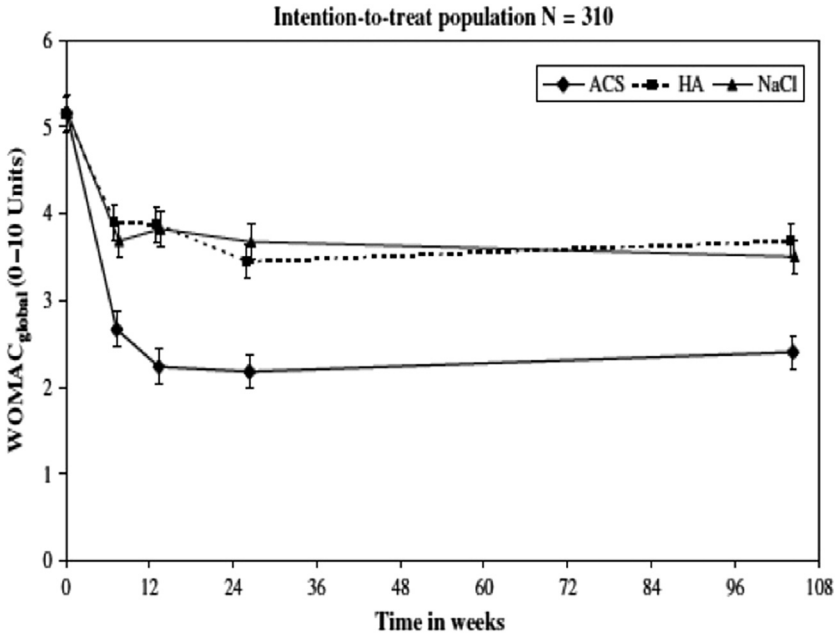

Fig. 1. WOMAC scores (0–10 scale) as a function of time after the last intra-articular injections of ACS into the knee joints of patients with OA. Figure shows mean ± standard error; $P<.05$ for the comparison ACS versus HA and saline; $P>.05$ for the comparison HA versus saline. (*From* Baltzer AW, Moser C, Jansen SA, et al. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. Osteoarthritis and Cartilage 2009;17:157; with permission.)

VAS scores of 8.1 and mean WOMAC global scores of 81; they entered the clinic expecting surgery. One injection per week of ACS (2 mL) was administered intra-articularly to 118 patients over a 4-week period followed by 30 physiotherapy sessions spread over 10 weeks. Follow-up at 24 months found a 62% decrease in VAS scores and 56% decrease in WOMAC scores. Joint stiffness was the only WOMAC subcategory that did not improve. Of interest, there was no significant difference in response between patients with different KL scores. Only one patient progressed to joint replacement surgery during this study. This study suffered from the lack of a control group receiving physiotherapy alone. According to the authors, however, in their experience, physiotherapy alone produces a pain improvement of about 25%.

The optimum dose and frequency of ACS administration remains to be determined. A recent small, uncontrolled study[30] noted a strong clinical response in symptomatic knee joints with OA after the intra-articular injection of just 1 mL ACS once per week for 3 weeks. Clinical improvement was already noted 1 week after the first injection of ACS. However, follow-up was curtailed at 3 months.

Although nearly all interventional studies have been performed with OA of the knee, Baltzer and colleagues[31] recently reported efficacy when using ACS to treat OA of the hip. This retrospective, uncontrolled study involving 119 patients and 150 hips was based on just 1 outcome measure, pain, measured by VAS. Nevertheless, it suggested sustained improvement over a 14-month period with no adverse events. Co-administration of glucocorticoid or anakinra provided no additional clinical benefit.

As described in a later section, ACS has also proved of clinical value in treating OA in horses and dogs.

ACS has been administered intra-articularly to prevent tunnel widening after single bundle reconstruction of the ACL. A prospective, double-blind, RCT by Darabos and colleagues[32] randomly assigned 62 patients to receive 4 injections of ACS or saline (placebo) after surgical reconstruction of a traumatically ruptured ACL using autograft of the semitendinosus or gracilis tendon, or the patellar ligament. ACS considerably reduced tunnel enlargement measured at 6 and 12 months by computed tomography. WOMAC stiffness scores were also improved to a statistically significant degree. Other components of the WOMAC scale and the International Knee Documentation Committee (IKDC 2000) score, trended toward improvement but did not reach statistical significance. Fewer patients receiving ACS had effusions at 6 months. There were no severe adverse events. A follow-up study using double-bundle ACL reconstruction surgery showed similar results.[22]

Clinical Experience with Autologous Conditioned Serum—Other Indications

Becker and colleagues[33] conducted an RCT to determine the effectiveness of ACS versus nonsurgical standard of care in treating lumbar radicular compression (**Table 3**). A total of 84 patients were randomly assigned to receive ACS, 5 mg of triamcinolone, or 10 mg of triamcinolone; the local ethics committee did not allow a placebo group. Each patient received 1 epidural perineural injection per week of test material for 3 weeks. Patients were followed for 6 months and evaluated for pain (VAS) and by the Oswestry Disability Index (ODI).

Initially, all 3 treatments were equally effective, but the improvements in VAS produced by the glucocorticoid proved less durable and by the 6-month time point were separating from those produced by ACS, which remained high (**Fig. 2**). ODI improved to an equal degree in all 3 groups. There were no adverse events.

These findings were confirmed by Goni and colleagues,[34] who administered ACS or methylprednisone epidurally to patients with unilateral lumbar radiculopathy. ACS produced progressive improvements in pain and function scores during a 6-month follow-up. Although methylprednisone also improved these parameters initially, the scores were deteriorating by the 6-month time point.

Based on promising data from a muscle contusion model in mice,[35] Wright-Carpenter and colleagues[24] conducted a small pilot study in human athletes. Professional athletes with muscle injuries were treated with standard rehabilitation therapy involving the use of oral anti-inflammatories. One group of 18 individuals was also administered ACS, whereas a second group received Actovegin and Traumeel, a combination very commonly used in European sports medicine. In this study, 2.5 mL of ACS was diluted with 2.5 mL of serum, and 5 injections, each of 1 mL, were delivered into the damaged area. Injections were initiated 2 days after injury and repeated according to the clinical changes. There was a significant reduction in the recovery time for athletes treated with ACS.

Preclinical data in a rat model suggest a role for the local application of ACS in treating tendon injuries.[36,37] These studies used a model in which the Achilles tendon was transected and sutured. Rat ACS (170 μL) was injected percutaneously into the sutured area 24, 48, and 72 hours after surgery. Animals were euthanized after 1 week, 2 weeks, 4 weeks, and 8 weeks. The ACS-treated tendons were thicker, had more type I collagen, and displayed an accelerated recovery of tendon stiffness and histologic maturity of the repair tissue. Data consistent with these findings have been reported for horses with naturally occurring tendinopathies.[38]

Table 3
Clinical use of autologous conditioned serum in other musculoskeletal settings

Indication	No of Patients	Study Design; Entry Criteria	Outcome Measures	Main Findings	Reference
Lumbar radicular compression	84	Prospective, randomized, placebo-controlled study comparing ACS with triamcinolone Unilateral lumbar radicular compression ACS or steroid injected epidurally, perineurally Follow-up: 6 mo	VAS ODI	Reduction in pain and disability, equal to glucocorticoid but more sustained	[33]
Unilateral cervical Radiculopathy	40	Prospective, randomized trial comparing ACS with methylprednisolone Patients 30–60 y with neck pain >6 wk duration, pain VAS >7 ACS or steroid injected epidurally Follow up: 6 mo	VAS Neck Disability Index SF-12	Improved outcome scores Improvement more gradual with ACS, but sustained	[34]
Muscle injury	29	Unblinded, no control ACS or Actovegin or Traumeel injected into lesion	MRI Recovery time	Reduction in recovery time in ACS group	[24]
Tendon healing (equine)	15 horses; 17 tendons	Intra-lesional ACS (n = 10) vs untreated or saline (n = 7). Follow up: 190 d	Swelling, ultrasonography, histology	More rapid recovery with ACS	[38]

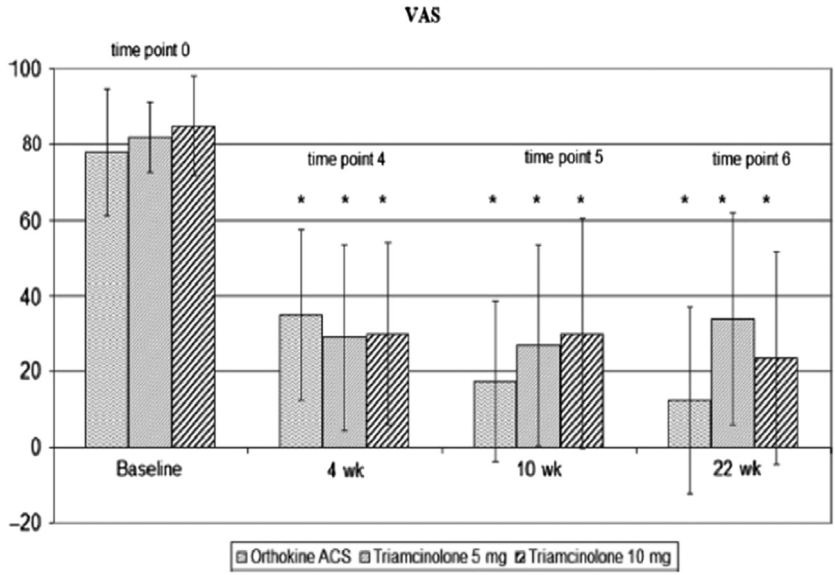

Fig. 2. Reduction in pain (VAS) after epidural injection of ACS or triamcinolone in patients with radicular compression. Mean, standard deviation, and median are given. Asterisks indicate significant difference from baseline. Time schedule is given in weeks after the first injection. (*From* Becker C, Heidersdorf S, Drewlo S, et al. Efficacy of epidural perineural injections with autologous conditioned serum for lumbar radicular compression: an investigator-initiated, prospective, double-blind, reference-controlled study. Spine (Phila Pa 1976) 2007;32:1805; with permission.)

Veterinary Applications of Autologous Conditioned Serum

ACS is widely used as an intra-articular injection for the treatment of OA in horses, whereas preliminary data suggest effectiveness in dogs.[39] Experimental studies conducted in an equine model of OA determined that ACS reduced lameness and synovial hyperplasia to a greater degree than saline.[40] There was also a trend toward improved cartilage morphology.

Although there have been no RCTs for the veterinary use of ACS, Weinberger[41] studied a large series of horses with OA whose lameness did not respond to either intra-articular glucocorticoids or HA. Remarkably, of these 262 horses treated with ACS, 178 were free from lameness at 12 weeks. These data are consistent with those of Jöstingmeier and colleagues[42] who found superiority of ACS over a combination of HA and betamethasone in treating aseptic arthritis of the coffin joint. A smaller study by Österdahl[43] injected ACS into the joints of horses that did not respond to intra-articular therapy with HA or polysulfated glycosaminoglycans (PSGAG). By 3 months, full activity was restored in 10 of 10 PSGAG failures and 7 of 10 HA failures. As noted earlier, ACS also shows promise as a treatment for equine tendinopathies.[38]

DISCUSSION

Safety and Efficacy of Autologous Conditioned Serum

From the combined experience of administering ACS to more than 100,000 human and 40,000 equine patients during a 15-year period it can be concluded that ACS is safe. Side effects are rare, the most common being a transient, local inflammatory

response. The literature suggests that ACS can be effective in treating certain aspects of OA and radicular compression and in the postsurgical management of ACL reconstruction and certain other conditions.

Nevertheless, efficacy trials involving intra-articular therapies face certain challenges. Double blinding can be an issue, and, ideally, the injection syringes should be masked. When HA is used, its high viscosity reveals its identity. This obstacle can be addressed by separating the role of injection from that of evaluation. Because the patients' expectations of a new, safe treatment may be high, there is a marked placebo effect, the effect size of which is around 0.5 for OA in general and 0.7 with the intra-articular route of administration.[44] Thus, a placebo group is advised for such studies. When this is not possible, the test drug can be compared with that of standard of care. Finally, the interval between 2 injections, the volume injected, and the optimal number of injections are still empirical in intra-articular therapy studies.

Response rates of OA to ACS seem to be around 70%. However, there is disagreement about the specifics. For instance, Baltzer and colleagues[26] reported improvements in WOMAC scores for patients with OA receiving ACS, whereas Yang and colleagues[27] did not, although they did find improved KOOS scores. In a follow-up study delivering ACSs to a subset of previous placebo patients from this trial, Rutgers and colleagues[28] found no improvement beyond the previous placebo effect. Based on initial KL and VAS scores, patients entering the latter 2 studies had milder disease. This is interesting in light of the data of Baselga and Hernandez[29] reporting dramatic improvements in patients with highly symptomatic disease. However, these patients also received intensive physiotherapy. Although physiotherapy alone had only a small effect, there was no group that received only ACS. The concept of using ACS in synergy with other modalities merits further consideration. Patient selection may also be important, and ways to predict good responders would be helpful. Resolution of these issues and determination of the place of ACS in the musculoskeletal armamentarium should be accomplished with additional, well-powered, well-controlled studies.

In this context, it will be important to determine whether ACS has a disease-modifying effect in OA. The evidence so far suggests that ACS reduces pain and inflammation; in many patients symptomatic improvement is rapid and sustained. However, no clinical data address its effects on cartilage loss. In an in vitro study, Rutgers and colleagues[23] found no effect of ACS on the turnover of proteoglycans in fragments of cartilage recovered from human joints with end-stage OA. On the other hand, Frisbie and colleagues[40] noted a trend toward reduced cartilage fibrillation in an equine model of OA. This remains an unresolved issue.

Efficacy in Comparison with Alternative Intra-articular Treatments for Osteoarthritis

With the exception of the Baltzer study, which compared ACS with HA, there have been no direct comparisons between ACS and other intra-articular treatments for OA.[26] However, it is possible to calculate effect sizes from the published data (**Table 4**). This analysis suggests that ACS is superior to corticosteroids, HA, and platelet-rich plasma (PRP) in terms of pain and function.

Relationship to Other Autologous Blood Products

Since ACS was developed, there has been a rapid expansion in the popularity of autologous blood products, and variety of such products has become available for treating orthopedic conditions. There is now a bewildering array of choices including PRP,[45]

Table 4
Effect sizes of various intra-articular treatments for osteoarthritis

	Effect Size	
Treatment	**Pain**	**Function**
Corticosteroids (1–4 wk)	<0.50	0.06
HA (24 wk)	<0.46	0.33
PRP (24–52 wk)	<0.40	0.40
ACS (24–104 wk)	<0.73	0.54

Effect sizes were calculated from RCT data published before January 2016.

Effect sizes range from 0 to 1 and can be considered as follows: less than 0.1, no effect; 0.1–0.29, small effect; 0.3–0.49, moderate effect; greater than 0.5, large effect.

platelet-poor plasma,[46] autologous conditioned plasma,[47] autologous protein solution,[48] and plasma rich in growth factors.[49]

The incubation step and the absence of anticoagulants, activators such as Ca^{2+}, or cells, provide the main differences distinguishing ACS from these other products. Although they should all contain the contents of platelets, only ACS captures the products of blood mononuclear cells that are synthesized during prolonged in vitro incubation. The data of Meijer and colleagues,[21] for instance, show that a major portion of the IL-1Ra in ACS results from de novo synthesis. Other important products are also likely to be synthesized during this period.

Composition and Mode of Action

The sustained effectiveness of ACS when injected intra-articularly for OA is remarkable, given the rapid egress of molecules from the joint space[12] and the transient responses to intra-articular anakinra noted clinically.[11] Glycosylated, native IL-1Ra, as presumably present in ACS, has approximately the same potency as the recombinant form,[50] but it may have superior retention properties. However, it is more likely that other components within ACS are responsible for sustaining any effects of IL-1Ra, providing additional therapeutic signals, or both. Indeed, although the development of ACS was predicated on the induction of IL-1Ra, it remains to be shown that IL-1Ra is a key or important ingredient. It is clear that many other substances are induced, some of which may have therapeutic properties of their own. In this regard, attention has understandably focused on the growth factors contained within ACS, but the list shown in **Table 1** is almost certainly incomplete and does not even begin to address nonproteinaceous components that might be important therapeutically. Further analysis of the composition of ACS and its biological mode of action are required. Such information could lead to improving the qualities of ACS by addition or subtraction.[51,52]

Regulatory Status

The Orthokine syringe for producing ACS is an approved medical device in the European Union, Australia, and several other markets, but not in the United States. However, the Orthokine syringe is permitted for veterinary use in all markets, including the United States.

The World Anti-Doping Agency confirmed that the local application of ACS (Orthokine) is not considered doping.

SUMMARY

ACS is a safe and, based on the preponderance of evidence from a limited number of trials, effective treatment of a variety of orthopedic conditions including OA, radicular compression, tunnel widening after ACL reconstruction, and, possibly, soft tissue injuries. Further RCTs and laboratory studies are needed to refine and optimize its use and to provide more information concerning its composition, mode of action, and possible synergies with other treatments.

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