

# Chondroprotective Effects of Intra-Articular Autologous Conditioned Serum (ACS) Injection: A Human Literature Review.

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## Abstract

Background and purpose

Autologous conditioned serum (ACS) is a disease-modifying drug for treatment of osteoarthritis (OA). To establish that the autologous serum has protective effect on chondropathy. The aim of this study was evaluate the efficacy of ACS as osteoarthritis therapy.

Methods

Literature review for intraarticular ACS somministration in human study.

Results and interpretation

We analyzed five major work to evaluate the effectiveness of the ACS.

The use of ACS, in articulation pathologies, has been reported as a new treatment for chondropathy. The ACS have biochemical and biomechanical properties able to alleviating suffering cartilage. They contain components such as growth factors that have an reparative effect on cartilage.

Conclusions

Through different clinical trials has been demonstrated variable efficacy. Among several studies there were differences also in the process of preparation and use of autologous serum.

## 1. Introduction

The chondropathies are the most common joint diseases and their natural evolution is the osteoarthritis (OA) [1]. The OA is a slow progression disease, characterized by articular cartilage destruction, with subchondral bone and synovial involvement. The OA mainly affects the older people, but can affect young patient too, when they are active sport players or accidents victims. The Inflammation plays a key role in the pathogenesis of OA, through the production of catabolic inflammatory cytokines such as  $TNF\alpha$  and  $IL1\beta$ , which promote the synovial inflammation and trigger the production of metallo- proteases by chondrocytes (MMPS) [2].

In developing of chondropathies both local and systemic factors could be involved. In literature there are several studies [3,4] that describing the chondropathies development and their evolution in OA as a result of the metabolic changes in the chondrocytes, due to the release of proinflammatory cytokines. These factors can induce the production of free radicals, chemokines, and metallo-proteases that can cause enzymatic degradation of the cartilage. The Reactive Oxygen Species (ROS) play a major role in the suffering chondrocyte. They are defined as chemical components containing oxygen having reactive chemical properties; they include free radicals, such as superoxide ( $O_2^-$ ) and the hydroxyl radical ( $OH\cdot$ ) and non-radical molecules such as hydrogen peroxide  $H_2O_2$ . ROS are commonly produced in several cellular processes and especially during oxidative phosphorylation. The human articular chondrocytes produce actively ROS such as  $O_2^-$ ,  $HO\cdot$  and  $H_2O_2$ , which are able to induce apoptosis in chondrocytes themselves [5,6].

## 2. ROS and joint damage

Several studies have revealed that ROS have a major role in the onset and progression of OA, causing the death of chondrocytes and matrix degradation [7].

The most important component of articular cartilage is the avascular matrix, that is synthesized by chondrocytes. It is mainly composed by type II collagen and proteoglycans. Although the exactly pathogenic mechanism has yet to be fully clarified. An hypothesis is that the excessive mechanical stimulation can increase the ROS production by chondrocytes, which is sufficient to depolymerise the hyaluronic acid or even to kill the chondrocytes [8]. In recent years it was discovered that the ROS also have a physiological role within the cell by activating proteins such as receptor tyrosine kinases, MAP kinases and transcription factors. Furthermore, it was shown that low levels of ROS stimulate the progression of the cell cycle, while higher levels determine cell cycle arrest and death by apoptosis or necrosis (9).

Because OA is a disease mainly age-related, and the presence of ROS increases with the age, the chondrocyte death oxidative-stress induced is a mechanism by which the aging process may contribute directly to the disease progression. The ability of ROS to induce cell death suggests the importance of the cellular antioxidant mechanisms in the prevention of progression of some age-related diseases .

### 3. Anti-inflammatory effect of blood serum

As we have seen above, in OA the central pathologic feature being the destruction of hyaline cartilage. Of the catabolic cytokines identified in OA joints, interleukin-1 (IL-1), is the most powerful inflammatory mediator responsible for cartilage loss [10,11]. The natural inhibitor of IL-1, the IL-1 receptor antagonist (IL-1Ra), could potentially limit the intra-articular actions of IL-1 and the disease evolution. 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, or when it was delivered by intra-articular gene transfer in dogs, rabbits, and horses [12]

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. 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 was the base to producing ACS, which can be injected into an affected joint [13].

### 4. Literature review

We carried out a review of the current literature of the in vivo human OA treatment with ACS. After an exhaustive search on the major medical search engines we got a small number of items with such unique characteristics, after excluding in vitro and in animal models works and individual clinical cases. We therefore evaluated the efficacy of ACS in symptomatic OA in four papers (tab. 1).

**Tab. 1**

Work	Population	Material and Methods	Resultati	Conclusion
<b>Yang KG, 2008 [14].</b>	167 patients in 2 groups ACS vs NaCl	Orthogen system Follow-up max: 1 year Target: 30% improvement of WOMAC	Similar improvements on WOMAC(16,8% vs 16,5%) KOOS scores improvement for ACS (p<0.004).	Primary objective not met.
<b>Baltzer WA, 2009 [15].</b>	376 patients in 3 groups (ACS: 134 pts; HA: 135; NaCl: 107). Inclusion criteria: over 30 y.o.; VAS >50; OA sec. K-L: I-III.	Orthogen system; 6 intra-articular injection of 2ml were performed within 3 weeks Follow-up max: 32 weeks	<u>ACS:</u> VAS from 69.6±13.1 to 29,5 ±22.6 Womac pain from 5.2±2.4 to 2.42±2.3 Womac stiffness from 5.6±2.8 to 2.4±2.3	Statistical significance between ACS and NaCl for womac pain and stiffness results. No statistical
Double blinded RCT study. Clinical effects of ACS compared to				

HA and placebo.			<b>HA:</b> VAS from 68.3±12.8 to 49.3 ± 25.9 Womac pain from 4.9 ±2.1 to 3.6±2.5 Womac stiffness from 6.0±2.7 to 4.3±2.8 <b>NaCl:</b> VAS from 66.3±14.5 to 48.2±25.6 Womac pain from 4.9±2.0 to 3.7±2.2 Womac stiffness from 5.8±2.8 to 4.5±2.8	significance difference between HA and NaCl for VAS score
<b>Baltzer WA, 2013 [16].</b> Effects of intra-articular injection of ACS on human hip OA	46 patients (62 hips) with hip OA Inclusion criteria: over 30 y.o.; K-L: II-IV	Orthogen system; 6 intra-articular injection of 2ml were performed Follow-up max: 14 months	VAS score improved from 5.5±0.3 to 2.6±0.3 at max F-U.	ACS successfully reduces pain in hip OA
<b>Motaal F, 2014 [17].</b> To Evaluate the effect of intra-articular injection of low-dose ACS on knee OA	30 patients OA kl 1-3 eta media 54,21 aa (42-67)	Orthogen system; 3 intra-articular injection of 1ml were performed within 3 weeks	Womac da 45,63 ± 9.9 a 8.27 ± 5.45 con p<di 0,01 Womac stiffness 2,61± (1,04) a 0,11 ± (0,32) con p<0,01 Womac pain 9.87± 2.11 a 3.22 ± 2,11 p<0,01 Max follow up 3 mesi	The IL1-ra from ACS established a promising strategy in the treatment of OA
<b>Rutgers M, 2015 [18].</b> Placebo-treated patients in previous RCT now treated with ACS.	20 patients 50 mean y.o. (range 34-70) OA sec. K-L: I-III	Orthogen system; 6 intra-articular injection of 2ml of ACS performed within 3 weeks VAS e WOMAC evaluations.	VAS score light improvement from 5 to 4.5 at 1 year. Only significative improvement was VAS at 3 months vs PreOp; p=0,02 Not WOMAC improvement.	ACS therapy not improve OA symptoms

In all the studies to collect ACS was used Orthogen System (Orthogen, Düsseldorf, Germany), a special syringe with increased inner surface area used to process 50 mL of whole periferical blood. 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 at 0.22 µm and aliquoted into 6–8.2 mL portions. The aliquots could be stored at -20°C until use.

Our review contains the first randomized controlled trial [15] that was conducted by Baltzer et al. to study the clinical efficacy of ACS enriched with high-dose IL-1Ra in patients with OA. They investigated 376 patients with knee OA randomized in three group comparing ACS effects to HA and saline (placebo). ACS (Orthokine) resulted in significantly greater improvement over time than did the control treatments. It consistently showed significantly higher relative improvements for all outcome parameters. WOMAC subscale scores were reduced in all treatment groups with the largest reduction occurring in the ACS one. VAS was lowest in ACS group, with the more than 50% improvement at all time points. Only local adverse events occurred with mild and moderate symptoms improved within 24h after injection at most. At a 2-years follow-up evaluation, there were still statistically significant differences between the ACS group and both control group with regard to WOMAC and VAS.

In addition, Yang et al. [14] studied 167 patients with OA of the knee after receiving the same high dose of ACS intra-articular (twice a week for 3 weeks later), and the result was evaluated by observing the knee at 3, 6, 9 and 12 months post -injection. They didn't find statistically improvement of KOOS symptom and sports parameters so they can't recommended this therapy.

Baltzer et al. published a recent study to investigate the effect of ACS in human hip OA [16]. They evaluated ACS-only treatment results and ACS with IL-1ra protein anakinra (rIRAP) or cortisone. After a 14 months follow-up they reported a large statistically significant reduction in pain in all intervention group (ACS, ACS+C, ACS+C+rIRAP). Subgroup analyses showed that these effects were valid not only for mild OA but also for severe OA. Second finding is that additional injections of steroids or steroids and rIRAP did not enhance the beneficial effect of ACS. Study's limits were the non-blinded and non-randomized method and that VAS score was used only.

To try to set the best ACS dose to fight knee OA, Motaal et al. used a low-dose ACS injections protocol, with only 1ml ACS once weekly for 3 weeks on 30 patients [17]. The outcomes showed similar results to that of Baltzer, where there was significant reduction in WOMAC score after 13 weeks follow-up. So Motaal suggested that low-dose ACS gives a similar results to high-dose ACS, with reduce adverse-events rate.

In the most recent study, Rutgers et al. treated, with ACS, 20 patients recently treated with placebo in a previous trial. VAS score improved after three months, but not after 12 months; clinical results were similar to those after placebo treatment. No improvement was seen after ACS treatment at 3 or 12 months for KOOS and WOMAC scores.

Findings from the present review demonstrate that an increase of oxidative stress induces cellular death in chondrocytes, mediated by oxidative processes through deregulation of antioxidative systems. This may represent an important contributing factor for arthritis onset in elderlies, due to the higher ROS quantity in this age group. ACS can be an effective biological therapy with intra-articular ROS, and chondrocytes suffering [5,6] reduction, with a consequent reduction of pain. Yang and Baltzer, with randomized trials too, demonstrated that ACS can slightly to improve functional ability and pain reduction, in knee and hip both, however with statistical significance. There is not agreement on therapy protocol yet. Motaal demonstrated that a low-dose ACS injections scheme is as effective as high-dose once. The pain, however, is the main evaluated ACS target, and it is an inherently subjective phenomenon that can be influenced by many factors. Rutgers evidenced only a light improvement in ACS patient previous treated with placebo, where subjective results were referred similar.

## 5. Conclusion

The intra-articular injection of ACS (Orthokine) in patients with painful knee OA has an excellent safety profile and results in a significant clinical response in randomized trials. The data show that ACS can be an effective and well tolerated alternative to the treatment of OA. Further investigations are necessary to determine if the effects of the ACS only modify the *symptoms or even the structure*.

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