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Autologous conditioned serum (ACS) for intra-articular treatment in Osteoarthritis: Retrospective report of 28 cases

Michela Tassara^{a,*}, Alessandro De Ponti^b, Lorena Barzizza^a, Matilde Zambelli^a, Cristina Parisi^a, Raffaella Milani^a, Luca Santoleri^a

^aImmunohematology and Transfusion Medicine, IRCCS Ospedale San Raffaele, Milan, Italy

^bIRCCS Galeazzi Orthopedic Institute, Milan, Italy

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ABSTRACT

Introduction: Autologous conditioned serum (ACS) is a novel blood product developed for intra-articular injection as a novel therapy for Osteoarthritis (OA).

This study is a retrospective evaluation of 28 cases (25 Knee-OA and 3 hip-OA) treated with ACS between November 2013 and February 2016.

Materials and methods: ACS was prepared according to standards in an accredited Cell Manipulation Lab, and applied by an expert clinician (2 ml injection once weekly over 4 weeks). At any injection visit (Timepoints 1–4), and after a follow-up of 1 (Timepoint 5) and 6 months (Timepoint 6), patients were asked to describe the intensity of their pain with the VAS (visual analog scale) psychometric scale, and the objective parameter ROM (Range Of Motion) was recorded in case of injection in the knee.

Results: Pain (VAS) reduced in all cases since the first injection up to Timepoint 5.

A significant improvement was observed in VAS between Timepoint 1 and 6 (primary objective), with a median VAS decrease of 60 mm (range 20–100, $p < 0.01$).

A significant difference was also recorded in ROM between Timepoint 1 and 6 (secondary objective), with a median increase of 25° (range 5–40, $p < 0.01$).

Ten out of 14 patients (71%) who were undergoing a chronic therapy to relieve pain were able to interrupt it. No serious adverse events were recorded.

Conclusions: Treatment with ACS produced a rapid decline in pain, accompanied by a large improvement in ROM. These results suggest that ACS is a valid option for the treatment of OA.

1. Introduction

Over the last decades, major evolutions have taken place in the production of blood biomaterials and components for not-transfusion use, with novel spectra of clinical indications, especially in the field of reparative and regenerative medicine [1].

Autologous conditioned serum (ACS) is a blood product developed in the 1990s in an attempt to generate an injectable material as a novel therapeutic for Osteoarthritis (OA) [2–4].

Osteoarthritis 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 [5].

Among the cytokines identified in the development of OA, IL-1 appears to be of special importance [6]. Many attempts have been done

to exploit the therapeutic use of IL-1 inhibitors in such disease; this led to the development of new biological treatments such as IL-1 receptor antagonist (Ra), soluble forms of IL-1 receptors, and type 1 cytokines (IL-4, IL-10, IL-13) that inhibit the synthesis of IL-1 and increase the synthesis of IL-1Ra.

The history of ACS began when Meijer and colleagues firstly developed a method for stimulating IL-1Ra synthesis in human blood [4]. According to their method, peripheral blood was drawn into a syringe containing glass beads treated with chromium sulfate, to which blood monocytes and other adherent cells had the opportunity to attach. The syringe and its contents were then incubated at 37° for several hours, during which platelets degranulated and mononuclear cells synthesized and secreted IL-1Ra (100–1000 times more than after a standard exposure to glass) along with a variety of additional anti-inflammatory products [6,7], without significant increase of IL-1 β and Tumor

* Corresponding author.

E-mail address: tassara.michela@hsr.it (M. Tassara).

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Necrosis Factor- α (TNF- α) [8,9].

ACS was firstly used clinically in 1997. Beginning from 2001, ACS was manufactured as Orthokine in a Good Manufacturing Process (GMP) facility.

In current times, physicians are directly provided with syringes, known as EOTII syringes (Orthogen Lab Services GmbH), containing glass beads treaded on their surface, for ACS preparation in the local GMP [10].

Baltzer et al. [11] published the first clinical uses of ACS for the treatment of OA of the knee, firstly in a nonrandomized study on 1000 patients, and subsequently [12] in a randomized study in which ACS was compared to standard of care (hyaluronic acid) and placebo on 376 patients. Results were in both cases in favor of ACS, being responses superior and longer-lasting.

The same results were confirmed in a randomized study by Yang et al. [13] on 176 patients with OA of the knee, and by Baselga and Hernandez in a non-blinded 2-year prospective study [10].

In more recent times, the use of ACS was successfully extended to OA of the hip [14], and other orthopedic disorders in which inflammation plays a major pathogenic role, such as lumbar radicular compression [15,16] and stress lesions of muscles/tendons [17].

Following this encouraging results, a collaboration was stated with the Immunohematology and Transfusion Medicine Service (SIMT) in San Raffaele Hospital (OSR) for the production of ACS-Ortokine.

This work is a preliminary retrospective report of the first 28 patients with OA of the knee and the hip, treated from November 2013 to February 2016.

According to a Legal Decree of the Italian Estate, from November 2015 blood products for non-transfusion use can be administered only in the context of clinical trials; since then, therefore, patients have been enrolled in a prospective trial.

2. Materials and methods

All patients were treated between November 2013 and February 2016.

X-ray based grading for OA was performed before treatment, according to Kellgren-Lawrence Classification System [18].

All patients were proposed to receive intra-articular injection of ACS in case of symptomatic AO of the knee or hip with clinical indication for treatment: (a) in case they preferred conservative treatment to surgery or (b) were not candidate to surgery due to medical problems. They were adequately informed about possible alternative therapies and agreed for treatment with ACS.

Written informed consent was obtained for blood uptake and ACS preparation, and for intra-articular injection.

2.1. ACS preparation

ACS was prepared at the Immunohematology and Transfusion Medicine Unit of OSR, according to the product instruction manual (Orthogen Lab Services GmbH).

A total amount of 40 ml full-blood was taken from a venous puncture with a standard winged pick-up needle and drawn into 2 EOT II syringes (Orthogen Lab Services GmbH), adequately labeled with name and Unique Patient Number (UPN) barcode.

In the Cell Manipulation Laboratory (CML), the EOT II were incubated for 6 h at 37° in a controlled incubator (Forma Scientific 3165 S/N), and subsequently centrifuged at 5000 rpm for 10 min. The resulting supernatant (ACS) was therefore divided in 4 aliquots of 2 ml, each in a 5 ml-syringe under a sterile laminar-flow cabinet and conserved at the controlled temperature of -20° until use.

2.2. ACS administration

The adopted schedule was 4 consecutive weekly intra-articular

injections of 2 ml of ACS [13].

An expert orthopedic performed the injection with sterile instruments and materials, in an adequate outpatient environment according to the standards of good clinical practice. A barrier filter 0.22 μ M (Millex GP, Merck Millipore) was applied to the injection syringe.

In case of hip OA the injection was performed under echo guide.

2.3. Clinical evaluations and follow-up

According to standard practice, the same expert orthopedic evaluated patients before any ACS infusion (Timepoints 1–4), one month (Timepoint 5) and 6 months (Timepoint 6) after the last infusion.

At any visit, all patients were asked to describe the intensity of their pain using the universally recognized VAS (visual analog scale) psychometric scale [19,20] as a measurement of their subjective symptoms.

The objective parameter ROM (Range Of Motion) -expressed in degrees- was recorded in case of injection in the knee.

Any adverse event (persistent pain in the site of injection, bleeding or intra-articular hematoma, local infection etc.) was reported.

2.4. Data collection

This study was approved by the local Ethical Committee (CE).

Clinical data were retrospectively collected from the patients' files and reported on a Case Report Form (CRF) before analysis.

The CRF reported VAS and ROM for all the Timepoints.

In some cases, patients had been asked to indicate the intensity of their pain on a VAS template, and the clinician had reported only the numeric values. As these data were no longer reproducible, and some of the values could have been approximated to the nearest multiple of 10 mm, it was decided approximate all VAS values in the same way for the retrospective data collection.

The chronic use non-steroidal anti-inflammatory drugs (NSAIDs), steroids and other painkillers was recorded in the patient's file, and reported in the CRF.

For some patients the on-demand use of drugs had not been prospectively recorded, therefore this information was omitted on the CRF.

2.5. Inclusion and exclusion criteria

All patients who agreed for the treatment with ACS before November 2015 could be included, given that the therapy had been completed (4 total injections) and the VAS before treatment was 50 mm or more.

Of note, all patients should have been aged 18 or more and should not have been pregnant or childbearing women for receiving ACS treatment. Moreover, were initially excluded from treatment with ACS patients with serious neurologic/psychiatric diseases, peripheral vascular diseases, positive serology for Hepatitis B or C or HIV, or documented infection of the joint.

2.6. Primary and secondary endpoints

Primary endpoint was VAS reduction from Timepoint 1 to Timepoint 6

Secondary endpoints were:

- VAS reduction at one months after the end of treatment (Timepoint 5).
- Increase of ROM from Timepoint 1 to Timepoint 6
- Treatment safety, measured as number of adverse events connected to ACS therapy in the period of treatment.

2.7. Statistical analysis

For the comparison of VAS and ROM a Mann Whitney test was used. Due to the small numbers, the adverse events were only described. Data were tabulated using MS Excel, which was also used to produce basic statistics and graphs. The statistical analyses were performed with the software R, version 2.9.0 (<http://www.r-project.org>)

3. Results

3.1. Patients' characteristics

A total of 39 patients were treated, but only 23 had a complete data set available and could be enrolled in the study. The median age was 68 years (range 34–87).

Twenty patients suffered from knee OA (87%) and 3 from hip OA (13%).

Five patients with bilateral knee OA received a complete ACS treatment for each knee; their data were analyzed separately for each ACS cycle; the data set, therefore, consisted of 28 cases overall.

Patients were highly symptomatic, with average VAS score of 80 mm (range 100–60). For patients with knee OA median VAS was 80 mm (range 100–60) and median ROM in flex-extension 95° (range 90–105).

Baseline demographics and patients characteristics are given in Table 1.

At the time of the first injection, 11 patients were undergoing a chronic therapy for pain: 4 NSAIDs, 2 COX-2 inhibitors, 2 steroids, 2 Paracetamol and 1 Paracetamol + Tramadol. Three had a chronic therapy with effect on pain due to concomitant medical conditions (2 steroids + Methotrexate, 1 COX-2 inhibitors).

Fourteen (50%) used to take drugs only in case of clinical need.

3.2. Efficacy: pain reduction

Pain (measured with VAS) reduced in all cases since the first injection, reducing further or remaining stable until Timepoint 5. During the follow-up period before Timepoint 6 VAS increased in 7 cases (25%, median increase 10 mm), remained stable in 19 (68%) and improved further in 2 cases.

A significant improvement was observed between Timepoint 1 and 6 (primary objective), with a median VAS decrease of 60 mm (range 20–100, $p < 0.01$) overall and 60 mm (range 20–80, $p < 0.01$) for knee OA only.

Between Timepoint 1 and 5 (secondary objective), median VAS decrease was 60 mm overall (range 40–100, $p < 0.01$) and 60 mm (range 40–80, $p < 0.01$) for knee OA only.

Full details are given in Table 2 and summarized in Fig. 1.

No significant difference was observed in VAS reduction according

Table 1
Patients-cases characteristics (N = 28).

Parameter	Median	Range
Age (years)	70	34–87
Gender (F/M)	21/7	–
VAS (mm)	80	60–100
ROM flex knee (degrees)	95	90–105
Chronic therapy	Number	%
NSAIDs	4	14
COX-2 inhibitors	3	10
Steroids	2	7
Paracetamol	2	7
Combi Paracetamol + Tramadol	1	4
Combi Steroids + Methotrexate	2	7

Table 2
Median pain (VAS) scores at the scheduled Timepoints.

Timepoint	All cases			Only knee OA		
	Median	range	p-value	Median	range	p-value
Baseline	80	70–100	–	80	70–100	–
Timepoint 2	50	30–80	–	50	30–80	–
Timepoint 3	30	10–60	–	30	10–60	–
Timepoint 4	20	0–60	–	20	0–60	–
Timepoint 5	20	0–60	< 0.01	20	0–60	< 0.01
Timepoint 6	20	0–60	< 0.01	20	0–60	< 0.01

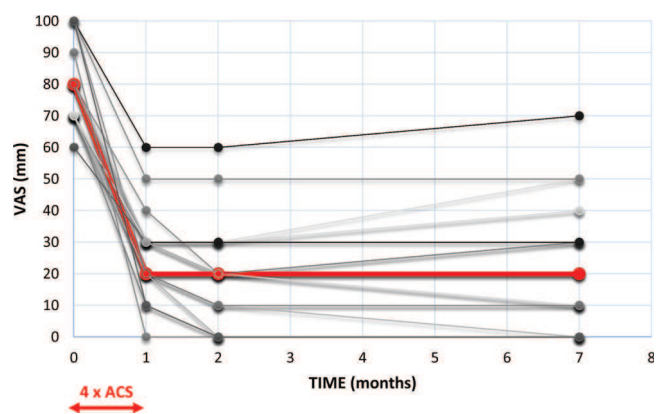


Fig. 1. VAS during and after treatment (red line = median). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to gender.

Some difference was observed in favor of younger patients, being the patients divided in over vs. under the median age (70 vs. 50 mm), but this did not reach statistical significance.

3.3. Efficacy: ROM improvement

The parameter ROM (knee OA only) improved in all cases from the first injection to Timepoint 5. In the majority of cases, the best result was obtained after 2 injections. Between Timepoint 5 and 6, ROM improved further in one case (10°) and worsened in another (5°).

A significant difference was observed in ROM between Timepoint 1 and Timepoint 6 (secondary objective of the study), with a median increase of 25° overall (range 5–40, $p < 0.01$).

Full details are given in Table 3 and summarized in Fig. 2.

3.4. Efficacy: concomitant medications

Ten out of 14 patients (71%) who were undergoing a chronic therapy to relieve pain at the beginning of the treatment were able to reduce and subsequently interrupt the therapy, after 1–3 infusions.

No correlation was found between the use of drugs and VAS or ROM reduction.

Table 3
Median ROM at the scheduled Timepoints.

Timepoint	Median	range	p-value
Baseline	95	90–105	–
Timepoint 2	105	90–120	–
Timepoint 3	120	95–140	–
Timepoint 4	120	95–140	–
Timepoint 5	120	95–140	$p < 0.01$
Timepoint 6	120	95–140	$p < 0.01$

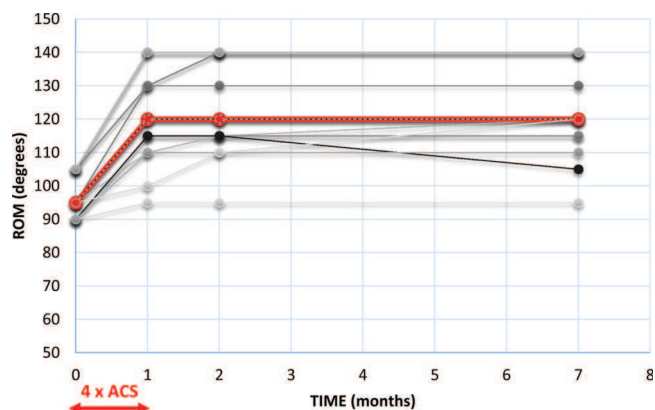


Fig. 2. ROM during and after treatment (red line = median). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Toxicity

No unexpected adverse events were reported, in particular no persistent pain, no hemorrhages and no infections. None of the patients asked to stop the serial infusions at any stage.

Only short-term pain in the site of injection was reported. Of note, hip injections were performed using an echo guide.

4. Conclusions

This retrospective study demonstrates the efficacy of ACS for the treatment of symptomatic OA of the knee. Due to the small number, the efficacy for hip OA cannot be demonstrated in this set, but the 3 treated patients experienced a consistent release of their pain.

Symptomatic improvement extended across all grades of OA, including those with the most severe disease (documented with ROM). This observation agrees with findings by Baltzer et al. who reported excellent improvement in pain in OA of the hip with little correlation to radiologic staging of the disease [14].

Even if some superior benefit was seen in favor of younger patients, age did not correlate significantly with outcome.

Even if the subjective perception of pain may be influenced by age (as well as by external factors such as culture, race and gender), the analysis of the objective parameter ROM was not in contrast with VAS at any measurement.

The inclusion of younger patients may be considered a certain limitation of this study. In younger patients, in fact, pain is probably be not only to osteochondral degeneration, but also to inflammation of muscles, tendons and capsule due to intense activity and/or mechanic stress. Also in this condition, anyway, the ACS has recently shown promising results as demonstrated by Wright-Carpenter and colleagues [17].

ACS, therefore, may be suggested in younger adults with mild-moderate OA to delay surgery and to cure sub-acute or chronic inflammation of the joint and capsule, whereas in older patients with moderate-severe OA it could be a valid option when certain drugs are contraindicated, and, above all, when there's no surgical indication due to concomitant medical conditions.

Of note, the treatment with ACS was uncomplicated and very safe, at least in the optimal condition offered to those patients, such as ACS production in an accredited Cell Manipulation Laboratory and injection administered by an experienced clinician in an adequate outpatient setting.

In this study, a durable effect of the therapy with ACS was obtained in all cases.

Of the 7 patients who experienced a worsening of their VAS between 1 and 6 months of follow-up, only one had also a worsening of

the ROM.

One could speculate that a longer follow-up period may have changed the subjective perception of pain; independently from this aspect, anyway, given the minimal toxicity of the treatment, ACS could theoretically be repeated several times in patients who relapse after a significant benefit.

Some authors have recently reported median time to surgery of more than 7 years for patients who underwent ACS therapy [21], whereas other have described a worsening of the pain of 50% at 24 months [10].

Further studies are needed to test the clinical variable related to duration of response an relapse, also in patients non candidate to surgery.

This work has two main limitations.

The first is that data were collected retrospectively months or years after treatment. Some patients could not be included due a lack of a complete file, and the final number is quite small. Also in cases a complete file was available, VAS could not be precisely collected on a 1-to-100 scale, and it was possible to report only the chronic therapy the patients were taking, but not the use of on-demand painkillers.

The second limitation is the lack of control group, undergoing other intra-articular injection therapy. It has been demonstrated that the placebo effect for intra-articular injection may be quite significant (up to 30% of improvement in trials for OA non involving ACS [22]). Placebo effects for intra-articular treatment are usually higher than for oral placebo, probably due to the most impressive effect such a therapy has. On the other end, other studies have clearly shown superiority of ACS when compared to other therapies, in spite to placebo effect [12,23].

Despite these limitations, the presented data support the previously reported results of several trials [24], providing further confirmation of the efficacy and safety of ACS as a valid option in case of symptomatic OA when surgery is contraindicated or refused by the patient.

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