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Autologous conditioned serum for chronic pain in patients with osteoarthritis: A feasibility observational study

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Abstract

Background: Autologous conditioned serum is a product of blood origin, with fragmented evidence of therapeutic properties in osteoarthritis chronic pain. This pilot observational prospective study aimed to evaluate the feasibility of a treatment with conditional autologous serum (ACS) in patients with severe chronic pain and grade I-III osteoarthritis and to describe its cytokine content.
Methods: We prospectively collected data on consecutive patients affected by osteoarthritis grade I to III and treated with four weekly injections of ACS at our outpatient pain service. The primary outcome was pain intensity, measured with the visual analogic scale (VAS). Additional outcomes were symptoms evaluated using joint district-specific scales. The study also evaluated concentrations of 48 cytokines and chemokines involved in the balance pro-inflammation/anti-inflammation and tissue repair in the ACS.
Results: We included 26 patients, mostly female (65.4%), with a median age of 63.5 years [IQR 58.25–73]. A median reduction of VAS of −3 cm [−5; −1.25] was observed 6 months after the first injection of ACS. The analysis showed a statistically significant difference between the values of VAS ($p < .01$; $X^2 = 69.6$; df = 6, $N = 26$) at the different time points. No adverse events were observed or reported by patients during the entire study period.
Conclusions: Conditional autologous serum may be a feasible option for patients with chronic pain due to grade I-III osteoarthritis refractory to other treatments. These preliminary findings should be confirmed in studies with adequate design.

Keywords

osteoarthritis, autologous conditioned serum, visual analogic scale, short form health survey-36

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Introduction

Chronic pain has a strong impact on quality of life, it is related to complex medical issues, such as anxiety and depression, leading to a reduction in the quality of life of patients.[1] About 20% of chronic pain in Europe is attributable to osteoarthritis.[2] Chronic osteoarthritis pain is mainly nociceptive, with not negligible neuropathic and inflammatory components perpetuated by elevated levels of pro-inflammatory cytokines, especially IL-1.[2] To date, the gold standard treatment is the combined use of analgesics (e.g., non-steroidal anti-inflammatory drugs and opioids) and infiltrative therapy, mainly with steroids or hyaluronic acid or platelet-rich plasma.[3–5] The use of NSAIDs or steroids may be contraindicated in some patients with comorbidities or may determine side effects.[3] In addition, patients with osteoarthritis may suffer from inadequate pain relief despite treatment with the available therapies.[6] On such basis, an effective, well-tolerated and safe treatment for chronic pain in patients with osteoarthritis would be of great benefit.

Autologous conditioned serum (ACS-ORTHOKINE) is a product of blood origin, whose therapeutic properties are mainly due to the presence of high concentrations of IL1-Ra (antagonist of the IL-1 receptor).[3,7] The use of ACS for the treatment of pain due to osteoarthritis has been described in literature, with fragmented evidence.[8–10]

The aim of this pilot observational prospective study was to evaluate the feasibility of a treatment with conditional autologous serum (ACS) for patients with severe chronic pain and grade I-III osteoarthritis not responsive to conventional treatments, and to describe its cytokines and chemokines content.

Methods

The protocol was approved by the Ethical Committee Palermo I (ID 9/2020, date of approval 19/10/2020, Chair Leone, S.), the study was conducted according to the Helsinki Declaration and all the participants signed a written informed consent. The design was a single center observational study, and data were prospectively collected. The reporting of this study followed the STROBE statement, and the STROBE checklist is available at Supplementary Material 1.

For the purpose of this pilot study, we screened for eligibility all the patients affected by osteoarthritis grade I to III (Kellgreen–Lawrence grading system) and treated with injections of autologous conditioned serum at the outpatient pain service of a single tertiary hospital in Italy. A convenient sample of consecutive eligible patients was enrolled in the study from 5th November 2020 to 15th June 2021 and the last follow-up was 6 months after study inclusion. Patients were included if they were affected by osteoarthritis grade I to III unresponsive to previous treatments (e.g., platelet-rich plasma or other infiltrative treatments) or without indications for other treatments, and thus treated with autologous conditioned serum. Patients were excluded if they were under 18 years of age, pregnant, immunocompromised, affected by cancer pain, coagulopathies or rheumatoid arthritis or had active infections. Patients were also excluded if they had clear indication for surgical joint replacement. Written informed consent was collected and an individual alphanumerical code was assigned to each included patient, to ensure anonymity during data analysis.

Outcome measurements and data collection

The primary outcome of the study was pain intensity, measured with the self-reported visual analogic scale (VAS),[11] expressed in centimeters (0, "no pain"–10, "pain as bad as it could possibly be"), and rounded to the nearest integer. Additional outcomes were symptoms and joint function evaluated using joint district specific scales (Western Ontario and McMaster Universities Osteoarthritis Index, WOMAC;[12–14] Disability of the Arm, Shoulder and Hand, quickDASH;[15,16] and Oswestry Disability Index, ODI),[17,18] the quality of life, evaluated using the Short Form Health Survey 36 (SF-36),[19] the functional impairment in daily life, evaluated using the Karnofsky performance status,[20,21] and the occurrence of adverse events (e.g., clinical signs of inflammation, redness or swelling of the injection point, hypotension, nausea, vomiting, fever, joint infections, hemarthrosis, persistent pain and anaphylactic shock).

In detail, the WOMAC Index is a self-report multidimensional questionnaire comprising 24 items assessing pain, stiffness, and physical functional disability in patients with knee osteoarthritis (Minimum score 0, i.e., best articular function–Maximum score 96, i.e., worst articular function. The total score is then expressed as percentage using 96 as denominator.); the quickDASH is a self-report questionnaire comprising 11 items assessing physical function and symptoms of musculoskeletal disorder affecting the upper limbs (Minimum score 0, i.e., no disability–Maximum score 100, i.e., most severe disability); the ODI is a self-report questionnaire comprising 10 sections assessing disability in patients with low back pain (Minimum score 0, i.e., no disability–Maximum score 50, i.e., most severe disability. The total score is then expressed as percentage using 50 as denominator.); the Karnofsky

Table 1. Characteristics of included patients.

	Entire cohort (n = 26)	Hip, knee, ankle (n = 9)	Upper limb (n = 5)	Low back pain (n = 12)
Age, years	63.5 [58.2; 73]	64 [61; 79]	58 [58; 68]	63.5 [59; 71.5]
BMI, kg/m^2	26.4 [25.2; 28.6]	26.3 [22.5; 28.7]	25.2 [23.4; 25.3]	27 [25.5; 28.9]
Male	34.6%	44.4%	60%	16.6%
Comorbidities, at least one	84.6%	100%	80%	83.3%
Time from symptoms onset, years	10 [7; 14]	9 [7; 12]	8 [5; 10]	12.5 [9.5; 19.25]

Data are presented as median [IQR] or percentages (%).
IQR: interquartile range.

performance status is a guided tool which helps the physician assessment of patients' functional impairment (Minimum score 0, i.e., dead–Maximum score 100, i.e., no evidence of disease). SF-36 is a set of 36 self-report quality-of-life measures, belonging to eight dimensions. The SF-36 recommended scoring system is a weighted Likert system for each item, then transformed onto a scale from 0 (negative health) to 100 (positive health). It is also possible to calculate two aggregate summaries of the domains, that is, the Physical Component Summary and the Mental Component Summary. We used an online free version of the questionnaire (http://lsi.marionegri.it/qdv/questionari/sf36/sf36v1ita.htm) to automatically calculate the score.

All the outcome measures were evaluated weekly, for the first 4 weeks, then at one, three and 6 months, except for SF-36, that was administered at baseline and then at one, three and 6 months from the beginning of the treatment. At each follow-up timepoint, the patients were clinically examined at our outpatient pain service and the data were collected in person by one investigator of the study.

The study also had laboratory outcomes, that is, the median concentrations of forty-eight cytokines in the ACS, [IL1α, IL1β, IL1R antagonist, IL2, IL2Rα, IL3, IL4, IL5, IL6, IL7, IL9, IL10, IL12, IL12 (p40), IL13, IL15, IL16, IL17, IL18, TNF-α, TNF-β, IFNα2, IFNγ, G-CSF, GM-CSF, M-CSF, FGF-β, VEGF, PDGF, MIF, MIG, HGF, LIF, β-NGF, SCF, SCGF-β, SDF-1α, TRAIL, eotaxin, IP-10, IL8, MIP-1α, MIP-1β, MCP-1, RANTES, CTACK, GRO-α, and MCP-3], to be dosed right after the conditioning of patients' serum.

We also collected patients' anthropometric characteristics (age at symptom onset, age, weight, and height at the enrollment time and gender), data on comorbidities and clinical history. All the data were collected by AA and GS and stored using an electronic database.

Treatment procedures

The treatment was administered via intra-articular injections to the target sites (i.e., knees, facets, and upper limbs joints), identified by the treating physician basing on clinical presentation and imaging. Injections of 2 mL of autologous serum were carried out weekly, for 4 weeks.

For knee infiltrations, having the patient in Fowler's position, with knee flexion at 90–100°, a 22G needle was introduced between the lateral femoral condyle and the tibial plateau, with ultrasound assistance if needed. For ankle joint infiltrations, having the patient in Fowler's position, a 27G needle was introduced in-plane under ultrasound guide, using a linear probe (5–10 MHz). For shoulder infiltrations, having the patient in sitting position and following the adduction of the arm, the injection was performed with an anterior or a posterior approach, in-plane, ultrasound guide using a linear probe (5–10 MHz). For metacarpophalangeal joint infiltrations, the injection was performed with a 27 G needle, out-of-plane under ultrasound guide, using a linear probe (5–10 MHz). For facet infiltrations, having the patient in prone position, a 22G needle was introduced in-plane under ultrasound guide, using a curvilinear probe (2.5–5 MHz). For sacroileal joint infiltrations, having the patient in prone position, a 22G needle was introduced in-plane under ultrasound guide, using a curvilinear probe (2.5–5 MHz).

After each injection, the patients remained 20 min under clinical observation, to assess and treat any immediate adverse event. The patients were also invited to contact the physician in case of occurrence of any adverse event at any time after the treatment. All the treatments were performed by the same physician, a certified anesthesiologist expert in pain infiltrative therapy (AA), following all the standards of good clinical practice. Other therapies used for chronic pain were eventually optimized (e.g., kept without variations, reduced, or suspended)

Table 2. Outcomes measures at different timepoints.

	T0	T1	T2	T3	T4	T5	T6	*p* value	T6-T0 difference
VAS	8 [7; 9]	6 [4.25; 7]	5 [4; 6]	3 [3; 4]	4 [2; 5.75]	5 [2; 7]	5.5 [3; 6]	<0.01	−3 [-5;-1.25]
Karnofsky	90 [80; 90]	90 [80; 90]	90 [80; 90]	90 [90; 90]	90 [90; 90]	90 [90; 90]	90 [82.5; 100]	<0.01	5 [0; 10]
SF-36 PCS	27.5 [22.5; 32.5]	NA	NA	NA	37.5 [27.5; 41.75]	31.5 [27.25; 43.5]	33 [29; 41.5]	<0.01	4.5 [0; 12.5]
SF-36 MCS	35.5 [32; 39]	NA	NA	NA	41.5 [35; 45]	41 [35; 47.75]	41 [35.75; 48.75]	<0.01	7.5 [1.25–11]
quickDASH	65.9 [65.9; 70.45]	47.7 [47.7; 56.8]	40.9 [34.1; 45.45]	31 [21.8; 40.1]	34.1 [25; 40.9]	27.3 [25; 27.3]	29.5 [20.45; 31.8]	<0.01	−40.91 [-50;-29.55]
ODI	41 [34.9–60]	35.5 [29.8–55.5]	31.1 [24.4–44.4]	23.33 [16–33.3]	14.44 [7.65–28.82]	22.2 [13.3–38.3]	25.5 [12.3; 34]	<0.01	−17.9 [-36.55;-1.67]
WOMAC	62.6 [58.3–66.6]	54.2 [39.5–61.45]	44.8 [36.4–52.1]	33.3 [30.2–37.5]	41.6 [29.2–53.1]	40.6 [37.5–46.9]	40.6 [39.6–56.25]	<0.01	−17.7 [-33.34; 0]

NA, Not assessed at this timepoint.
PCS: Physical Component Summary; MCS: Mental Component Summary; ODI: Oswestry Disability Index; Quick-Dash: Disability of the Arm, Shoulder and Hand; SF-36: Short Form Health Survey 36, VAS: visual analogic scale; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.
T0, baseline assessment; T1, 1-week; T2, 2-weeks; T3, 3-weeks; T4, 30-days; T5, 3-months; T6, 6-months.

during the period of study, according to the treating physician's clinical judgment.

ACS preparation

The autologous conditioned serum was prepared at the Immunohematology and Transfusion Medicine Unit of the Paolo Giaccone University Hospital in Palermo. A total of 40 mL of venous blood was taken by patients, after screening for infectious diseases, using a standard needle syringe and then transferred to 2 EOT II syringes (as indicated by protocol Orthogen Lab Services Gmbh, Düsseldorf, Germany). In the laboratory, EOT II, was incubated for 6 h at 37 C° and then centrifuged at 5000 rpm for 10 min. The supernatant, represented by the autologous conditioned serum, was divided into 4 aliquots of 2 mL each into a 5 mL syringe under a sterile laminar flow hood. The aliquots of autologous conditioned serum were stored in the freezer at a controlled temperature of −20 C° until its use. At the time of administration each aliquot was defrosted separately at room temperature and used.

Analysis of cytokines and chemokines content in ACS

Forty-eight cytokines and chemokines [IL1α, IL1β, IL1R antagonist, IL2, IL2Rα, IL3, IL4, IL5, IL6, IL7, IL9, IL10, IL12, IL12 (p40), IL13, IL15, IL16, IL17, IL18, TNF-α, TNF-β, IFNα2, IFNγ, G-CSF, GM-CSF, M-CSF, FGF-β, VEGF, PDGF, MIF, MIG, HGF, LIF, β-NGF, SCF, SCGF-β, SDF-1α, TRAIL, eotaxin, IP-10, IL8, MIP-1α, MIP-1β, MCP-1, RANTES, CTACK, GRO-α, and MCP-3] were analyzed in Orthokine sera by xMAP multiplex technology on the Luminex platform (Luminex), using Bio-Rad reagents (Bio-Plex Pro Human Cytokine 27-plex Assay #M500KCAF0Y and Bio-Plex Pro Human Cytokine 21-plex Assay #MF0005KMII, Bio-Rad) acquired and analyzed with the Bioplex Manager Software (Bio-Rad). Briefly, 50 μL bead solution (containing assay buffer and 5000 beads) was added to the appropriate wells in a 96-well Millipore filter plate (Millipore). Fifty microliters assay buffer was added to each background well: 50 μL diluted standard serum pool, diluted 2-fold from 1:25 to 1:3,200 to each standard well and 50 μL diluted positive serum control, diluted 1:25 to each positive control well. Fifty microliters sample diluted 1:4 was added to each sample well. Standard and positive controls were diluted in assay buffer, and samples were diluted in assay buffer with 10% sample blocking buffer. After 30 min of incubation at room temperature on a plate shaker and two washes, 25 μL biotinylated detection Ab, diluted 1:10 in

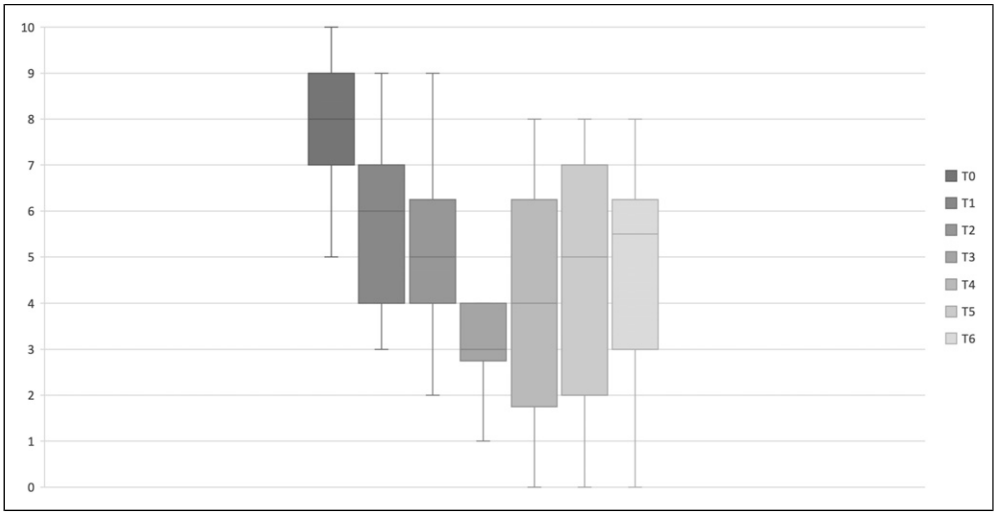

Figure 1. Box whisker plots of visual analogic scale at study timepoints.

assay buffer, was added to each well. After further 30 min of incubation at room temperature on a plate shaker and two washes, 50 µL diluted streptavidin–R-PE, diluted 1: 100 in assay buffer, was added to each well. After further 30 min of incubation at room temperature on a plate shaker and two washes, the samples were analyzed on the Luminex machinery (Luminex).

Statistical analysis

Data were analyzed using descriptive statistics and reported as percentages for categorical or dichotomous variables and as median and interquartile range for continuous variables, as appropriate for non-normal distribution. The outcomes measures at the different timepoints were analyzed using the Friedman test, assuming significance with $p < .05$. The pairwise comparisons between the different timepoints were assessed using the Durbin–Conover test for the primary outcome (VAS). The median difference was also calculated between values at baseline and at the longest follow-up for all the outcomes. No imputation of data was needed due to the absence of missing data. Data analysis was conducted by MI, with input from AC, using Jamovi version 2.2.5.

Results

A total of 26 patients were consecutively included in the study from 5th November 2020 to 15th June 2021. The characteristics of the included patients are presented in Table 1. Most of the included patients (46%) were affected by low back pain, with facets identified as the main osteoarthritis site; the 34.6% of the included patients had hip, knee, or ankle osteoarthritis and 19% had upper limbs osteoarthritis (e.g., metacarpophalangeal joint, shoulder). The site of injection per patient is showed at Table S2, Supplementary Material 1.

The patients were mostly female (65.4%), with a median age of 63.5 years [IQR 58.25–73]. Most of the patients had at least one comorbidity (84.6%), and the median body mass index (BMI) of the population was 26.4 kg/m^2 [IQR 25.2–28.6]. The median time from symptom onset to the first treatment with ACS was 10 years [IQR 7–14].

At baseline, the intensity of pain was registered, with a median value of 8 cm [IQR 7–9] at VAS scale, despite a median Karnofsky performance status of 90 [IQR 80–90], defined as "minor signs of disease." All the measures performed at baseline are reported in Table 2.

Outcomes

The full results of outcome measures at each timepoint are reported in Table 2. The analysis showed a statistically significant difference between the values of VAS ($p < .01$; $X^2 = 69.6$; df = 6, $N = 26$), Karnofsky performance status ($p < .01$; $X^2 = 25.7$; df = 6, $N = 26$), SF-36 MCS ($p < .01$; $X^2 = 18.1$; df = 3, $N = 26$), and SF-36 PCS ($p < .01$; $X^2 = 13.3$; df = 3, $N = 26$) at different timepoints (see Table 2). In addition, a statistically significant difference was found between the values of the WOMAC Index ($p < .01$; $X^2 = 22$; df = 6, $N = 9$), ODI ($p < .01$; $X^2 = 33$; df = 6, $N = 12$), and quick-DASH ($p < .01$; $X^2 = 18.2$; df = 6, $N = 5$) at different timepoints (see Table 2).

A T0-T6 median reduction of VAS of −3 cm [−5; −1.25] was observed (see Table 2 and Figure 1).

Table 3. Characteristics of autologous conditioned serum.

Anti-inflammatory cytokines	
IL-1RA, pg/mL	471.6 [404.7–747.1]
IL-2Ralfa, pg/mL	20.8 [1–76.7]
IL-4, pg/mL	4.2 [3.1–6.8]
IL-10, pg/mL	<0.53[a]
IL-13, pg/mL	1.24 [0.61–2]
IL-9, pg/ml	6343.9 [3062.1–9511.7]
Proinflammatory cytokines	
IL-1a, pg/mL	<0.77[a]
IL-1b, pg/mL	10 [5.1–27.2]
IL-2, pg/mL	<1.7[a]
IL-6, pg/ml	0 [0–113.8]
IL-12p70, pg/mL	<3.18[a]
IL-12p40, pg/mL	<19.64[a]
IL-17, pg/mL	13.2 [4.7–20.4]
IL-18, pg/mL	11.7 [0.6–44.1]
IFN-G, pg/mL	<132.56[a]
TNF-a, pg/mL	123.9 [102.9–222]
Cytokines involved in tissue repair	
PDGFbb, pg/mL	11592.5 [5703.1–15461.1]
Basic FGF, pg/mL	<30.15[a]
HGF, pg/mL	605.9 [290–1178.1]
TNF-b, pg/ml	9497.7 [6569.6–13892.1]
Grow factors	
G CSF, pg/mL	182 [40.2–371.7]
GM-CSF, pg/mL	<0.34[a]
LIF, pg/mL	88.2 [55.3–136.3]
M-CSF, pg/mL	19.6 [1.58–59.8]
MIF, pg/mL	Level above evaluable range[b]
bNGF, pg/mL	<12.6[a]
SCF, pg/mL	137 [68.2–228.2]
SCGFb, pg/mL	3166734.4 [1898352.6–5855471.8]
VEGF, pg/mL	<539.45[a]
Other cytokines and chemokines	
IL-3, pg/mL	<0.62[a]
IL-5, pg/mL	<0.33[a]
IL-7, pg/mL	<23.16[a]
IL-8, pg/mL	5285.8 [2047.6–7559.2]
IL-15, pg/mL	Level below evalauble range[b]
IL-16, pg/mL	116.3 [71.6–140]
IFNalfa2, pg/mL	2.3 [1.6–4.7]
TRAIL, pg/mL	58.5 [35.3–76]
IP10, pg/mL	569 [133.3–1306.3]
MCP-1, pg/mL	127.8 [76.9–256.6]
MCP-3, pg/mL	<0.08[a]
MIG, pg/mL	673.1 [304.8–1166.8]
MIP-1a, pg/mL	80.5 [27.6–151.8]
MIP-1b, pg/mL	3495.3 [2519.2–4956.7]
CTACK, pg/mL	3473.4 [2181.5–7920]
Eotaxin, pg/mL	181.7 [67.1–295.7]
RANTES, pg/mL	123739.5 [102369.4–154891.5]
Gro alfa, pg/mL	312.3 [17–628.2]
SDF1a, pg/mL	16912.5 [12806.8–21587.4]

(continued)

Table 3. (continued)

The table shows the concentration of cytokines and chemokines dosed in the autologous conditioned serum.
Data are presented as median [IQR] unless otherwise specified.
[a]More than 80% of samples had level below the reported automated cutoff, with median [IQR] not calculable.
[b]Automated result given by the analyzer.

The pairwise comparison between these two timepoints was statistically significant ($p < .01$; please see Figure S1, Supplementary Material 1). An improvement was also observed from baseline to last follow-up in all the outcome measures (see Table 2, "T6-T0 difference").

No adverse events were observed or reported by patients during the entire study period.

Characteristics of autologous conditioned serum

The level of cytokines measured in the autologous conditioned serum of each patient are reported in Table 3. Notably, IL-1RA median concentration was 471.6 [404.7–747.1] pg/ml and factors involved in tissue repair were detected.

Discussion

The most relevant finding of this study was that the use of autologous conditioned serum was feasible in this cohort of patients. Our data may be used to design a trial, assuming that the observed reduction in severe to moderate pain[22] at the longest follow-up may be due to the use of autologous conditioned serum, but due to the nature of the study, we could not confirm it. We observed a statistically significant difference between the values of VAS measured at all the timepoints. Moreover, all the additional outcome measures showed a statistically significant difference at all the timepoints evaluated. Despite statistical significance, the improvement registered with Karnofsky performance status may not be clinically relevant (median of differences of 5), and the PCS may have registered a subclinical improvement (median of differences of 4.5) in comparison with the most used cut-offs for clinical relevance.[23,24] Importantly, joints functional improvements were clinically relevant in all the district

evaluated with specific scales.[25–27] Notably, no side effects were reported from included patients after any injection and during the entire period of follow-up, supporting the safety of this treatment. Our findings were in line with the available literature, ACS resulted as a feasible option but still not supported by high quality evidence. Indeed, a recent meta-analysis had showed that the ACS can reduce pain and improve function in patients with knee osteoarthritis,[10] with similar effect size. A RCT including 83 patients with low back pain showed a statistically significant difference between ACS treatment and triamcinolone with regard to VAS, but no statistically significant difference in ODI measurements.[8]

Interestingly, our findings were in line with the proposed role of IL1Ra levels in ACS efficacy, with a higher median concentration observed in ACS, in comparison with normal values.[28] Furthermore, the presence of growth factors, with potential role in tissue regeneration, gives space for speculations on a possible disease-modifier role of ACS in osteoarthritis. All these findings should be further assessed and confirmed in studies with adequate design (e.g., control group) and repeated dosages at different timepoints (e.g., before and after the conditioning of serum), for adequate interpretation.

Our study had limitations. The design was observational, the study was conducted in a single center, the sample size was small, the protocol was not prospectively registered in clinicaltrials.gov or other public databases and a priori sample size calculation was not performed. We did not collect data on dosage variations on concomitant medications and patients were aware of being receiving ACS, with an unmeasured psychogenic effect on chronic pain. Thus, the findings should be considered as exploratory and hypothesis-generating for future studies with control group (e.g., placebo control group). Furthermore, we did not collect data on radiological imaging at follow-up timepoints and the study did not provide any data on the potential role of ACS as disease-modifying (e.g., chondroregenerative) treatment.

However, the study also has strengths, for example, the prospective design, the assessment of quality-of-life outcomes, the inclusion of multidistrict osteoarthritis cases and the length of follow-up without loss to follow-up. We also measured the concentration of cytokines and chemokines in the ACS used for the treatment, thus providing a clinical and laboratory description of the included patients. Thus, our study may contribute as background for future well-designed studies to evaluate the efficacy of the treatment in comparison with the gold standard. It also remains to be evaluated if the treatment can be considered at earlier stages, that is, as alternative to other therapies, and not only after their failure.

Conclusions

Conditional autologous serum may be a feasible option for patients with chronic pain due to grade I–III osteoarthritis refractory to other treatments. No adverse events were registered. These preliminary findings should be confirmed in studies with adequate design.

Authors' contribution

MI, AA, and AC conceived the content. GS, VC, AR, and AA collected the clinical data. MDS, AMC, SM, and GM collected the laboratory data. MI and AC revised and validated the databases. MI performed the analysis with inputs and supervision by AC. MI and AC drafted the manuscript. GS, VC, AR, GM, SM, MDS, AMC, AG, and AA revised it critically for important intellectual content. All the authors approved the final version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Data availability

The data are available upon reasonable request to the corresponding author.

Ethical approval

Ethical approval to report this case was obtained from Ethical Committee I of Palermo ID 9/2020, date 19/10/2020.

Informed consent

Written informed consent was obtained from all subjects before the study.

Trial registration

This clinical trial was not registered because it is a pilot observational study.

Guarantor

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Supplemental material

Supplemental material for this article is available online.

References

1. Hadi MA, McHugh GA and Closs SJ. Impact of chronic pain on patients' quality of life: a comparative mixed-methods study. *J Patient Exp* 2019; 6(2): 133–141.
2. Perrot S. Osteoarthritis pain. *Best Pract Res Clin Rheumatol* 2015; 29(1): 90–97.
3. Wehling P, Moser C, Frisbie D, et al. Autologous conditioned serum in the treatment of orthopedic diseases the orthokine therapy. *Nov Ther Strateg* 2007; 21(5): 323–332.
4. Desai MJ, Mansfield JT, Robinson DM, et al. Regenerative medicine for axial and radicular spine-related pain: a narrative review. *Pain Pract* 2020; 20(4): 437–453.
5. Goldring MB and Berenbaum F. Emerging targets in osteoarthritis therapy. *Curr Opin Pharmacol* 2015; 22: 51–63.
6. Conaghan PG, Peloso PM, Everett SV, et al. Inadequate pain relief and large functional loss among patients with knee osteoarthritis: evidence from a prospective multinational longitudinal study of osteoarthritis real-world therapies. *Rheumatol (United Kingdom)* 2015; 54(2): 270–277.
7. Evans CH, Chevalier X and Wehling P. Autologous conditioned serum. *Phys Med Rehabil Clin N Am* 2016; 27(4): 893–908.
8. Becker C, Heidersdorf S, Drewlo S, et al. Efficacy of epidural perineural injections with autologous conditioned serum for lumbar radicular compression. *Spine* 2007; 32(17): 1803–1808.
9. Godek P, Szajkowski S and Golicki D. Evaluation of the effectiveness of orthokine therapy – Retrospective analysis of 1000 cases. *Ortop Traumatol Rehabil* 2020; 22(2): 107–119.
10. Raeissadat SA, Rayegani SM, Sohrabi MR, et al. Effectiveness of intra-articular autologous-conditioned serum injection in knee osteoarthritis: a meta-analysis study. *Futur Sci OA* 2021; 7(9): FSO759.
11. Price DD, McGrath PA, Rafii A, et al. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. *Pain* 1983; 17: 45–56.
12. Ponkilainen VT, Häkkinen AH, Uimonen MM, et al. Validation of the western ontario and mcmaster universities osteoarthritis index in patients having undergone ankle fracture surgery. *J Foot Ankle Surg* 2019; 58(6): 1100–1107.
13. Bellamy N, Buchanan W, Goldsmith CH, et al. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol* 1988; 15(12): 1833–1840.
14. Salaffi F, Leardini G, Canesi B, et al. Reliability and validity of the western Ontario and McMaster universities (WOMAC) Osteoarthritis index in Italian patients with osteoarthritis of the knee. *Osteoarthr Cartil* 2003; 11(8): 551–560.
15. Gummesson C, Ward MM and Atroshi I. The shortened disabilities of the arm, shoulder and hand questionnaire (QuickDASH): Validity and reliability based on responses within the full-length DASH. *BMC Musculoskelet Disord* 2006; 7: 44.
16. Padua R, Padua E, Ceccarelli E, et al. Italian version of the disability of the arm, shoulder and hand (DASH) questionnaire. Cross-cultural adaptation and validation. *J Hand Surg Am* 2003; 28: 179–186.
17. Monticone M, Baiardi P, Ferrari S, et al. Development of the Italian version of the oswestry disability index (ODI-I): A cross-cultural adaptation, reliability, and validity study. *Spine* 2009; 34(19): 2090–2095.
18. Vianin M. Psychometric properties and clinical usefulness of the oswestry disability index. *J Chiropr Med* 2008; 7(4): 161–163.
19. Brazier JE, Harper R, Jones NMB, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *Br Med J* 1992; 305(6846): 160–164.
20. Schag CC, Heinrich RL and Ganz PA. Karnofsky performance status revisited: Reliability, validity, and guidelines. *J Clin Oncol* 1984; 2(3): 187–193.
21. Mor V, Laliberte L, Morris JN, et al. The karnofsky performance status scale: an examination of its reliability and validity in a research setting. *Cancer* 1984; 53(9): 2002–2007.
22. Boonstra AM, Preuper HRS, Balk GA, et al. Cut-off points for mild, moderate, and severe pain on the visual analogue scale for pain in patients with chronic musculoskeletal pain. *Pain* 2014; 155(12): 2545–2550.
23. Carreon LY, Glassman SD, Campbell MJ, et al. Neck disability index, short form-36 physical component

summary, and pain scales for neck and arm pain: the minimum clinically important difference and substantial clinical benefit after cervical spine fusion. *Spine J* 2010; 10(6): 469–474.
24. Ogura K, Yakoub MA, Christ AB, et al. What are the minimum clinically important differences in sf-36 scores in patients with orthopaedic oncologic conditions? *Clin Orthop Relat Res* 2020; 478(9): 2148–2158.
25. Clement ND, Bardgett M, Weir D, et al. What is the minimum clinically important difference for the womac index after TKA? *Clin Orthop Relat Res* 2018; 476(10): 2005–2014.
26. Vanti C, Ferrari S, Villafañe JH, et al. Responsiveness and minimum important change of the oswestry disability Index in Italian subjects with symptomatic lumbar spondylolisthesis. *J Orthop Traumatol* 2017; 18(2): 145–150.
27. Franchignoni F, Vercelli S, Giordano A, et al. Minimal clinically important difference of the disabilities of the arm, shoulder and hand outcome measure (DASH) and its shortened version (quickDASH). *J Orthop Sports Phys Ther* 2014; 44(1): 30–39.
28. Chapman P, Reyes C and Gupta V. Normal physiological levels of human cytokines using bio-plex pro™ cytokine assays. *Bull Bio-rad Lab* 2010: 6029: ■■■.