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Pain control and functional improvement in patients treated by autologous conditioned serum after failure of platelet rich plasma treatments in knee osteoarthritis

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Abstract

Objective: To assess the efficacy of autologous conditioned serum (ACS) for the treatment of patients with knee osteoarthritis after failure of medical treatments and platelet rich plasma (PRP) injections.

Background: Knee osteoarthritis is the most common form of arthritis. Prior to prosthetic surgery these patients might benefit from medical treatments, physiotherapy, and in case of their ineffectiveness, from autologous blood component injections.

Methodology: We have treated 30 patients with Kellgren-Lawrence I-III knee osteoarthritis with ACS after failure of standard medical treatments/physiotherapy and platelet rich plasma (PRP) injections for a full cycle, within the previous year from enrollment. *Results:* ACS administration was performed in all patients with mild side effects and produced prompt (1 month) improvements of VAS and Lequesne scales in 67% of patients and this result persisted at 6 and 12 months. No relationship between the rate of response and Kellgren-Lawrence scale at enrollment was observed whilst responders had a significantly higher amount of interleukin-1 receptor antagonist (IL1-RA) in ACS as compared to nonresponders.

Conclusion: The present study confirms the efficacy of ACS in pain control and functional recovery of patients with knee osteoarthritis resistant to medical and PRP treatment. These results were obtained in a well-defined cohort of resistant patients and seem to be related with IL1-RA content in injected ACS.

KEYWORDS
autologous conditioned serum, knee osteoarthritis, pain control

1 | BACKGROUND

Osteoarthritis (OA) is the most common form of arthritis which causes joint pain and stiffness with consequent functional impairment associated with a significant worsening of quality of life.[1,2] In general, OA is the prevalent form of pain in the older subjects and seems to be related to an increased risk of all-cause mortality.[2,3] OA occurs in subjects with a wide range of age, with a peak of incidence in subjects with more than 50 years, and it may be related to several conditions causing joint inflammation and different level of cartilage damage. The incidence of OA is increasing and this fact is in part related to the aging population and to the increased occurrence of obesity in a large numbers of people.[4] OA mainly affects the knees so that >200 millions of people in the world suffer from joint impairment due to symptomatic knee OA,[5] which progresses invariably as a disabling disease, showing different degrees of severity and requiring treatments

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that encompass nonsurgical approaches such as systemic treatments by oral paracetamol and nonsteroidal anti-inflammatory drugs, visco-supplementation by ialuronic acid (IA) intra-articular infiltration, steroid and nonsteroidal local injections and, in cases of pain persistence, opioid administration. At last, end-stage cases of knee OA may benefit from surgery represented by partial or total knee replacement. In these last years an increasing application of intra-articular blood products, such as platelet rich plasma (PRP) and autologous conditioned serum (ACS), has been reported in subjects requiring pain control for knee OA, after failure of pharmacological treatments or visco-supplemetation. In these patients favourable results have been observed by using PRP or ACS even though a clear superiority of these autologous preparations over other medical treatments is still lacking in large controlled studies or it is based on preliminary data.[6] In general, the principle by which products based on autologous blood components should be effective in pain and inflammation control is the presence of anti-inflammatory cytokines and proregenerative factors, released by blood cells, in plasma or serum administered through intra-articular injections.[7] Moreover, differences in techniques for collection and preparation of these blood-derived products confound the data resulting from studies in small cohort of patients treated using different and customised methodologies. Recently, a specific medical device for the production of ACS in amounts that may be administered in single or multiple injections has been produced and proposed as a commercial CE-marked product.[8–10] The availability of specific device for ACS production by overnight activation of blood cells in well-defined conditions and starting from preset autologous blood amounts may serve to standardise the production process and to optimise the bio-availability of relevant autologous factors to be injected to these patients. Here, we report the results obtained with ACS in 30 patients with knee OA who were resistant to medical treatments, visco-supplementation and PRP injections; ACS was produced through the use of a CE-marked dedicated device and administered by three intra-articular injections, in the site of knee OA at weekly intervals. A wide set of cytokines was also evaluated in patients sera and in each ACS product to ascertain whether a possible association exists between autologous cytokine levels and pain/functional response to ACS treatment.

2 | METHODS

2.1 | Patients and study characteristics

The present study is a prospective-interventional study to assess the safety and efficacy of ACS treatment in 30 patients with knee OA who received previous intra-articular treatments by visco-supplementation, autologous PRP and had no chance to obtain pain-control through the application of other nonsurgical approaches. The study was approved by the Lazio 1 Ethical Committee (authorization Prot. N. 2486-12-05-2017). All patients gave their informed written consent to participate in the study. Characteristics of enrolled patients are described in Table 1. Enrollment criteria were as follows: male or female patients with age ≥18 years, with mono-lateral or bilateral knee osteoarthritis, resistant to previous treatments by IA visco-supplementation and subsequent PRP injections (resistance to PRP was defined as the lack of 20 mm reduction of the VAS scale and at least a class in Lequesne scale 1 month after the end of PRP treatment), ranging in the Kellgren-Lawrence scale from 1 to 3 at enrollment (Figures1 and 2). Exclusion criteria included pregnancy, the presence of major neurological diseases, of any active thrombovascular diseases, of solid or haematological neoplasms, of joint infections. Also patients with a body mass index >30 and with serologically documented HIV, HCV and HBV infections were excluded from the study. Briefly, between 05-03-2018 and 18-10-2018 30 symptomatic consecutive patients (with a visual analogical scale, VAS, ≥30 mm) suffering from resistant mono-lateral (no patients with bilateral knee osteoarthritis had been diagnosed and enrolled in this study in spite of enrollment criteria) knee OA after a full treatment with nonsurgical approaches including visco-supplementation and PRP injections (at least one cycle including a minimum of three PRP injections in the involved knee joint), ranging in age from 35 to 77 years, 17 females and 13 males, 15 with grade 3 Kellgren-Lawrence scale, 13 with grade 2 and 2 with grade 1, had been enrolled in the present study consisting in the collection of autologous serum by a CE marked device and production of at least four aliquots of 2 ml of ACS for subsequent injections in the involved knee joints. Primary objectives of the study were:

a. The safety of ACS treatment in terms of adverse events including infections at the site of injections, systemic infections, pain/edema at the injection site for more than 7 days from treatment, local bleeding episodes and allergic reactions to the injected product; adverse events were monitored from the time of first ACS injection.
b. The efficacy of ACS treatment in terms of VAS and Lequesne scales improvements (response was defined as a reduction of 20 mm of the VAS scale and at least a class in Lequesne) from the beginning of treatment (T0), to 1 month (T1) and 6 months (T2) from treatment.

Secondary objectives of the study were:

a. The efficacy of ACS treatment at 12 months (T3) from the beginning of treatment in terms of VAS and Lequesne scales improvements, as compared to T0 and T2.
b. Patients' satisfaction about the ACS treatment by a multidimensional evaluation.

At the start of treatment and at different time points of follow up patients' response to ACS treatment was evaluated in the following way:

a. By a clinical evaluation of the treated knee joint with recording of pain by VAS and Lequesne scales.
b. By recording possible adverse events during and after treatments.
c. Through the administration of a questionnaire to collect patient's satisfaction about the efficacy and tolerability of treatment.

TABLE 1 Patients' characteristics and outcome after ACS treatment

				VAS scale				Lequesne scale			
	Age (years)	Gender	Kellgren-Lawrence	T0	T1	T2	T3	T0	T1	T2	T3
UPN001	77	F	2	50	20	20	20	4	2	2	3
UPN002	64	M	2	50	10	10	10	2	0	1	1
UPN003	72	F	2	80	10	10	10	4	2	2	1
UPN004	47	F	2	70	20	30	50	3	2	2	2
UPN005	41	M	2	70	40	40	50	3	2	2	2
UPN006	66	F	2	80	10	10	10	4	3	3	1
UPN007	55	M	2	70	30	40	30	4	2	2	1
UPN008	57	M	2	50	20	10	10	2	2	1	1
UPN009	**66**	**F**	**2**	**50**	**40**	**50**	**50**	**2**	**2**	**2**	**2**
UPN010	**72**	**M**	**2**	**50**	**50**	**50**	**50**	**2**	**2**	**2**	**2**
UPN011	**69**	**F**	**2**	**30**	**10**	**30**	**30**	**4**	**1**	**4**	**4**
UPN012	**52**	**M**	**2**	**70**	**50**	**70**	**70**	**2**	**1**	**2**	**2**
UPN013	**52**	**F**	**1**	**90**	**80**	**90**	**90**	**3**	**3**	**3**	**3**
UPN014	**53**	**M**	**2**	**60**	**60**	**60**	**60**	**0**	**0**	**0**	**0**
UPN015	**35**	**M**	**1**	**40**	**10**	**10**	**10**	**1**	**0**	**1**	**1**
UPN016	72	F	3	50	20	30	30	2	2	1	1
UPN017	73	F	3	70	10	40	40	4	2	2	1
UPN018	54	F	3	70	30	20	30	3	2	1	1
UPN019	69	F	3	50	10	0	0	2	0	0	0
UPN020	69	F	3	60	20	20	20	3	2	2	1
UPN021	73	F	3	70	20	20	20	4	1	2	2
UPN022	75	M	3	60	20	20	30	3	2	1	1
UPN023	62	M	3	80	10	10	10	3	1	1	1
UPN024	61	F	3	90	60	50	50	5	3	3	3
UPN025	61	F	3	90	30	20	20	4	3	3	3
UPN026	53	F	3	90	70	70	70	3	2	2	2
UPN027	**70**	**M**	**3**	**70**	**70**	**70**	**70**	**3**	**3**	**3**	**3**
UPN028	**67**	**M**	**3**	**50**	**10**	**40**	**50**	**3**	**3**	**3**	**3**
UPN029	**73**	**M**	**3**	**70**	**50**	**60**	**60**	**2**	**2**	**2**	**2**
UPN030	74	F	3	40	10	20	80	4	4	3	4
Median (range)	66(35–77)	17F/13M	3(1–3)	70(30–90)	20(10–80)	30(0–90)	30(0–90)	3(0–5)	2(0–4)	2(0–4)	2(0–4)

Note: Bold values identify nonresponder patients; response was defined as a reduction of 20 mm of the VAS scale and at least a class in Lequesne scale; Kruskal Wallis nonparametric test confirmed in all patients' series a significant decrease of VAS and Lequesne scale during follow up (T1,T2, primary objective; $p < 0.00001$ and $p = 0.00032$, respectively). Posthoc Dunn for Kruskall Wallis test showed that a significant decrease in VAS and Lequesne scales occurred early at T1 and this result was maintained at later time points (T0 vs. T1 $p = 0.000001$, T0 vs. T2 $p = 0.000015$, T0 vs. T3 $p = 0.00013$, whilst $p > 0.05$ for T1 vs. T2, T1 vs. T3 and T2 vs. T3).

Abbreviations: T0, at enrollment, T1, 1-month follow up; T2, 6-month follow up; T3, 12-month follow up; UPN, unique patient number.

d. By recording patient's need of analgesics and anti-inflammatory drugs.

2.2 | Autologous blood collection, ACS preparation, storage and administration

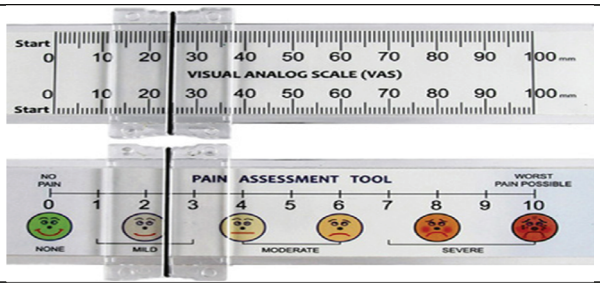
Sixty millilitres of autologous venous blood had been collected in each enrolled patient by a 60 ml dedicated syringe containing glass beads (Orthokine II, Orthogen Lab Services GmbH, Dusseldorf) in the absence of anticoagulation. Concomitant samples of 8 ml of venous blood had been collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) to perform serological tests for HIV, HBV and HCV, to confirm the absence of current or previous viral infections, and blood counts with leukocyte differentials. Syringes containing whole blood and beads were directly incubated at 37°C in a 95% air–5% CO_2 incubator for 24 h and then centrifuged at 3000 g for 10 min to separate and collect ACS from the rest of whole blood.

Kellgren-Lawrence Scale

Grade	Radiologic findings
0	No radiologic findings
I	Doubtful narrowing of joint space and possible ostephytic lipping
II	Definite osteophytes and possible narrowing of joint space
III	Moderate multiple ostepohytes-definite narrowing of joint space-small pseudocystic areas with sclerotic walls and possible deformity of bone contour
IV	Large ostephytes-marked narrowing of joint space-severe sclerosis and definite deformity of bone contour

FIGURE 1 Kellgren-Lawrence scale to stage initial severity of patients' knee osteoarthritis

VAS scale



Lequesne scale

Parameter	Finding	Points
Pain of discomfort during nocturnal bedrest	None Only on movement or in certain positions Without movement	0 1 2
Duration of morning stiffness or pain after getting up	None < 15 min >= 15 min	0 1 2
Remaining standing for 30 min increases pain	No yes	0 1
Pain on walking	None Only after walking some distance Early after starting	0 1 2
Pain or discomfort after getting up from sitting without use of arms	No yes	0 1

FIGURE 2 VAS and Lequesne scale used to assess the degree of patients' response to ACS [Color figure can be viewed at wileyonlinelibrary.com]

ACS were aliquoted in five sterile syringes (Texium® Syringe, Becton Dickinson) with a nominal capacity of 5 ml, containing a volume of 2 ml of ACS each. An additional aliquot of ACS of 5 ml was subjected to sterility tests for aerobics, anaerobics and fungi. The ACS aliquots of 2 ml were then stored at −20°C until their use for intra-articular administration. Prior to use, ACS aliquots were thawed at room temperature. Each patient was treated by a single 2 ml aliquot injection in the involved knee joint per week, for a total period of treatment of four consecutive weeks. Each aliquot of 2 ml of ACS was injected by the Texium Syringes connected with a filter of 0.22 um (Millex GP, Merck Millipore, Burlington, MA, USA) and a proper needle after that a sterile field had been obtained on the site of injection following standard disinfection procedures.

2.3 | Cytokine concentration assay in patients' serum and ACS

Orthokine system allows the production of ACS enriched in anti-inflammatory cytokines and in particular in the Interleukin-1 Receptor Antagonist (IL-1RA), a glycoprotein produced by monocytes which has an inhibitory action against interleukin-1, a proinflammatory cytokine involved in inflammatory processes of arthritic diseases. Therefore aliquots of patient serum before treatment and all ACS productions were collected and kept frozen at −80°C until testing the cytokine panel. The following set of soluble factors were quantified by Luminex ProcartaPlex immunoassays (ThermoFisher Scientific, Waltham, MA, USA): interleukin-10 (IL-10), IL1-RA, tumour necrosis factor alfa (TNF-a), interleukin-6 (IL-6) and interleukin-1 beta (IL1-b).

2.4 | Statistical analysis

Result analysis has been carried out by SPSS 15.0 software for Windows (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA). Shapiro–Wilk normality test was initially used to confirm that our data were not normally distributed, so that non parametric tests were subsequently used for data comparison . The whole results observed for Lequesne and VAS scales were compared at T0, T1, T2 (primary objectives) and T3 (secondary objective) by Kruskal Wallis

non parametric test. This test was also applied after patients' stratification for Kellgren-Lawrence scale, grouping patients in 1–2 and 3 series, to assess if clinical improvement was related to initial disease severity. Then, Wilcoxon test for paired data was applied to compare VAS and Lequesne data on responder patients by comparing both T0 and T3 and T2 and T3 to verify the persistence of response at 12-month follow up. Fisher exact test was used to evaluate the occurrence of adverse events related to ACS treatment in all patients. Mann–Whitney and Wilcoxon tests were used to compare cytokine concentrations in ACS of responder and nonresponder patients and between basal serum and ACS of all patients, respectively. A $p < 0.05$ was considered as significant in all statistical analysis.

3 | RESULTS

3.1 | Adverse events

All patients completed the treatment receiving four subsequent ACS injections in four consecutive weeks (a single injection per week). Adverse events were monitored from the time of first ACS injections and prior and after subsequent ACS injections. In nine patients out of 30 (30%; $p = 0.001$ at Fischer exact test) we observed mild adverse reactions after the first ACS, consisting in pain and knee edema in seven cases and knee edema only in two cases. In six cases these conditions required treatment by ice application at the site of pain/edema (two cases), analgesics alone (one case) or analgesics and ice application (three cases). After second ACS injection seven patients (these patients were part of those who experienced adverse events after the first ACS injection; 23% $p = 0.010$) experienced mild adverse reactions which were edema in two cases, pain only in one case and pain and edema in four cases. In five cases these clinical conditions required treatments which consisted in ice application in four cases and analgesics in one case. After the third and fourth ACS injections we observed five and one adverse reactions (these patients were part of those who experienced adverse events after the first and second ACS injections), respectively, ($p > 0.05$) which did not require any treatment except ice application in two and one cases, respectively.

3.2 | Rate of response

The rate of response at T1 and T2 of whole patients' series were 67% for both time points with 20 patients in the responder group and ten patients in nonresponders (Table 1). This result was also confirmed at T3, after a follow up of 12 months. The median value of VAS scale decreased from 70 to 30 and of Lequesne from 3 to 2 for all patients at any follow up time point. The median value of VAS scale decreased from 70 to 20 and of Lequense from 3 to 1 in responder patients at any time point, including at last follow up of T3 (1 year; Figure 3). Kruskal Wallis non parametric test confirmed in all patients' series a significant decrease of VAS and Lequesne scale during follow up (T1,T2, primary objective; $p < 0.000$ and $p < 0.000$, respectively). Post-hoc Dunn for Kruskall Wallis test showed that a significant decrease in VAS and Lequesne scales occurred early at T1 and this result was maintained at any later follow up time point (T0 vs. T1 $p < 0.000$, T0 vs. T2 $p < 0.000$, T0 vs. T3 $p < 0.000$, whilst $p > 0.05$ for T1 vs. T2, T1 vs. T3 and T2 vs. T3). In responder patients Wilcoxon test for paired data demonstrated that also at 1 year follow up (T3, secondary objective) VAS and Lequesne scales decreased significantly ($p < 0.000$ for both scale). In responders, comparison of VAS and Lequesne scales between T2 and T3 showed no significant differences, indicating a persistence of the therapeutic effect at the latest follow up ($p > 0.05$). When patients were stratified for Kellgren-Lawrence scale at enrollment, we observed a significant decrease in Kellgren-Lawrence 1–2 and 3 groups for both VAS and Lequesne scale ($p = 0.004$ and $p < 0.000$ for VAS scale in Kellgren-Lawrence 1–2 and 3, respectively; $p = 0.044$ and $p = 0.004$ for Lequesne scale in Kellgren-Lawrence 1–2 and 3, respectively; Figure 4). These results indicated no significant

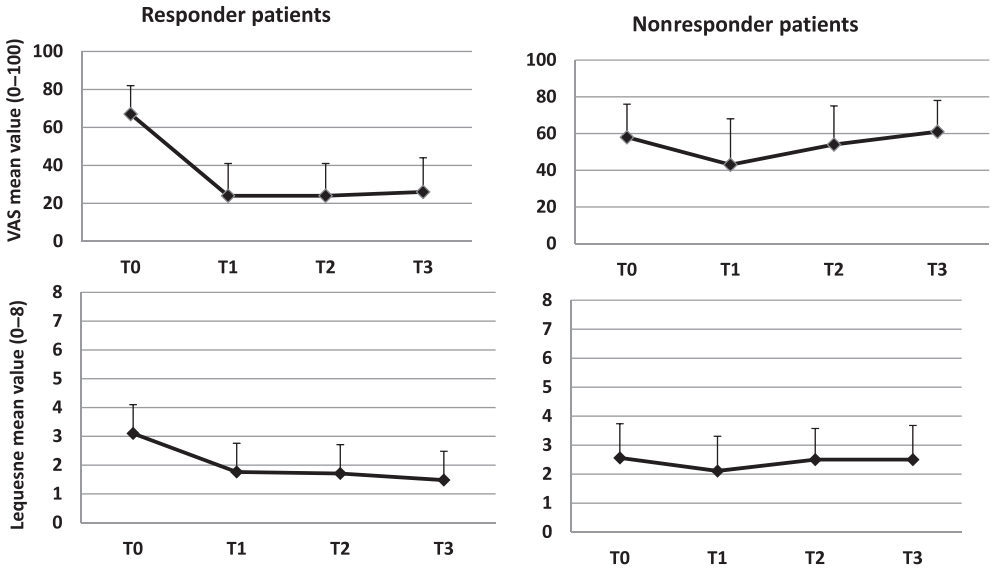


FIGURE 3 VAS and Lequesne scale values in responder and nonresponder patients' at different time points. Results are presented as the mean value (+*SD*). T0, enrollment; T1, 1 month, T2, 6 months, T3, 12 months from treatment. ($p = 0.00014$ between T0 and T3 for both VAS and Lequesne scales at Wilcoxon test in responder patients; $p > 0.05$ between T0 and T3 for both VAS and Lequesne scales at Wilcoxon test in nonresponder patients)

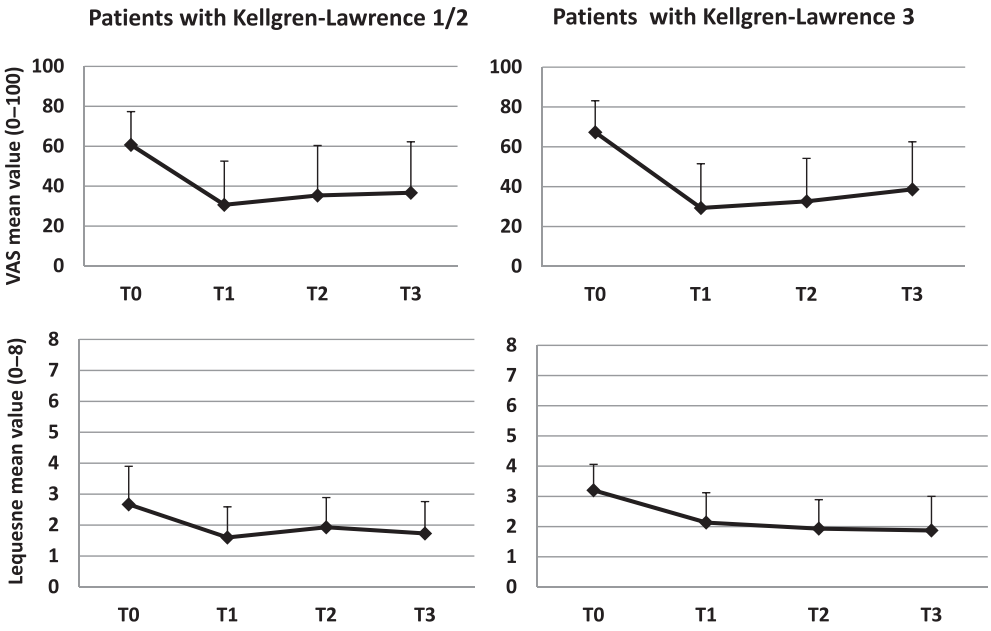


FIGURE 4 VAS and Lequesne scale values in Kellgren-Lawrence 1–2 and 3 at different time points for all patients' series (responders and nonresponders). Results are presented as the mean value (+*SD*). T0, enrollment; T1, 1 month, T2, 6 months, T3, 12 months from treatment. ($p = 0.00446$ and $p = 0.00023$ for VAS scale in Kellgren-Lawrence 1–2 and 3, respectively, at Kruskall Wallis test; $p = 0.04491$ and $p = 0.00498$ for Lequesne scale in Kellgren-Lawrence 1–2 and 3, respectively, at Kruskall Wallis test)

relationship between the severity status of knee OA and response to ACS treatment at any time point. All responders patients discontinued medical treatments and all avoided surgery and maintained response at a median follow up of 24 months. No significant relationship was found between response and patients' gender, age, weight, duration of disease prior ACS injection, degree of edema at enrollment or the traumatic or nontraumatic nature of the disease (indeed, the rate of response in nontraumatic OA was 60% vs. 80% in traumatic, a trend that, in any case, did not reach statistical significance; $p = 0.246$ at Fischer exact test). Finally, we administered a questionnaire to all treated subjects to assess the level of patients' satisfaction with respect to ACS treatment after 6 months of follow up. A six-dimensional evaluation with a score ranging from 1 (total dissatisfaction/clear worsening) to 5 (full satisfaction/great improvement) for the following questions gave the following results: (a) pain control, median score 4; (b) autonomy in routine daily activities, median score 4; (c) treatment efficacy, median score 4; (d) treatment tolerability, median score 5; (e) treatment expectations, median score 4; (f) treatment reliability, median score 4.

3.3 | Cytokine concentration in ACS and patients' serum at baseline

Several cytokines were assayed in patients' basal serum and in aliquots of their ACS by Luminex High Performance assay. The concentration of the following biological factors were quantified: IL-10, IL1-RA, TNF-a, IL-6 and IL1-b. Relevant differences in concentrations were observed between basal serum and the corresponding ACS for all cytokine tested at Wilcoxon paired test (Figure 5(A)). Notably, IL1-RA showed a higher concentration in ACS of responder patients, as compared to nonresponders, with an average concentration of 930 and 200 pg/ml, respectively. However, no significant differences

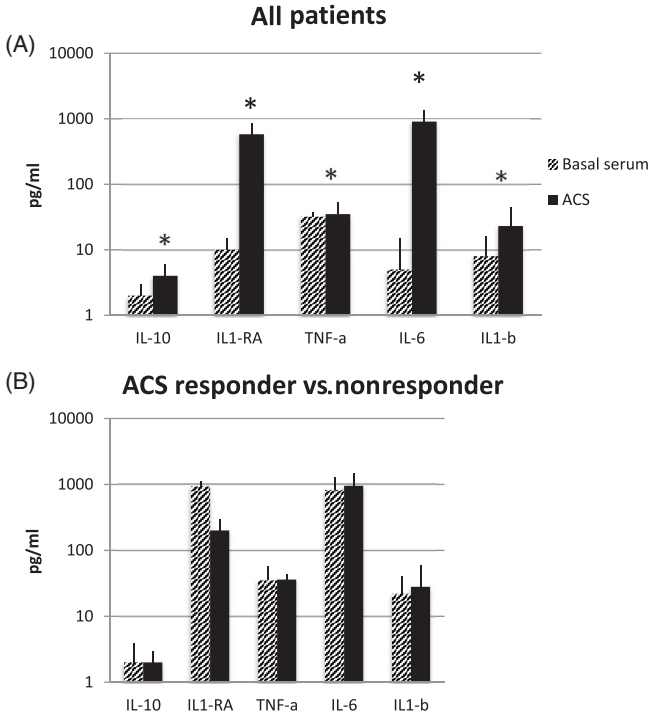


FIGURE 5 (A) Cytokine level in basal serum and ACS by Luminex ProcartaPlex immunoassays. Results are presented as the mean values +/-*SD*. *Statistically significant at Wilcoxon paired test ($p = 0.0009$ for IL-10, $p < 0.00001$ for IL1-RA, $p = 0.00138$ for TNF-a, $p = 0.00096$ for IL-6 and $p = 0.00018$ for IL-1b). (B) Cytokine level in ACS of responders (20 patients) and nonresponders (10 patients). Results are presented as the mean values +/-*SD*. $p > 0.05$ between any cytokine concentration of responders and nonresponders at Mann–Whitney U test

were observed between cytokine concentrations of ACS in responders and nonresponders, including IL1-RA at Mann–Whitney U test (Figure 5(B)).

4 | DISCUSSION

Knee OA is a frequent and disabling disease of joints that occurs in subjects with a wide age range, being related to several pre-existing conditions, including the increasing occurrence of obesity in several populations of developed countries.[1–5] This disease is progressive and when medical treatments are ineffective it may require surgical intervention consisting in the placement of knee prosthesis. In these last years, injection of ialuronic acid or autologous blood products has contributed to delay surgery in those patients who did not respond to standard medical treatments for pain control.[6,8–11] In particular, the injection of PRP in knee joints of patients with OA resistant to standard medical treatments produces long-lasting pain control in more than half of treated patients, as reported by several studies.[6] On the other hand, patients who show resistance to PRP injection for pain control seems to have no chance of cure other than prosthetic surgery. The principle by which products based on autologous blood components should be effective in pain and inflammation control is the presence of anti-inflammatory cytokines and proregenerative factors, released by autologous blood cells, which are administered through intra-articular injections.[7] Failure of these blood-based treatments may reside in an ineffective release of these factors in autologous blood components due to an insufficient individual capacity of patients to transfer relevant factors from the intracellular compartments to plasma, associated or not with particular local joint conditions. Local treatments by blood-components for knee OA may be also attempted using innovative technologies aimed to potentiate factor release through ex vivo stimulation of patients' blood cells.[12] The application of this approach produces a patient serum which is reported to be enriched in anti-inflammatory factors, especially in IL1-RA. Local injection of this form of ACS seems to have potent anti-inflammatory activity, leading to pain control in several forms of OA.[8–10] Here we have investigated the effect of the Orthokine ACS production technology to treat 30 patients who failed pain control by standard medical treatments and a full cycle of PRP injection and who had as the sole chance of cure the placement of knee prosthesis. This study showed several important results:

a. All patients completed the planned scheme of therapy and around 70% of treated patients had prompt response to ACS treatment with few side effects.
b. The response was long-lasting since all responder patients maintained pain control and a recovered functional activity at 6 months and 1 year follow up.
c. Response was not related to Kellgren-Lawrence scale at enrollment so that both one–two and three grade patients had a similar proportion and duration of response to ACS.

All responder patients (20 patients out 30 treated) have postponed any surgery over the time with discontinuation of all medical treatments in all of them. This study is the first report of ACS treatment in a very well-defined group of patients with knee OA, refractory to non-surgical treatments, including recent PRP injections. A recent publication reported the use of ACS, compared to PRP, in an earlier stage of treatment of patients with knee OA, but it did not include patients who failed a blood component-based treatment, as in the present study.[13] Our cohort of patients represents the worse series that can be treated by a nonsurgical approach and this fact gives a preliminary demonstration of some relevant circumstances:

a. ACS can rescue patients who failed medical treatments and PRP injections.
b. ACS may control pain in critical patients with knee OA and is able to postpone knee prosthesis placement in these patients.

Furthermore, this study indicates that a higher amount of IL-RA may be found in ACS of responders, as compared to nonresponders. The average IL-RA level of responders was 930 pg/ml (200 pg/ml in nonresponders). However, this difference was not statistically significant due to the small size of comparison and to the very high individual variability of all cytokine concentrations in ACS. On the other hand, it may assumed, as preliminary observation, that an increased IL1-RA level in ACS might predict response. Indeed, also nonresponders increased IL1-RA level in their ACS as compared to baseline and experienced an initial mild response. Likely, suboptimal IL1-RA increase combined with a higher, albeit not statistically significant, concentration of IL-6 (951 pg/ml vs. 823 pg/ml, on average) in nonresponders ACS affected the persistence of the anti-inflammatory effect during time. In reference to this, an increased IL-6 concentration in synovial fluids has been related to worse symptoms and outcome in knee OA.[14,15] It is not so trivial to hypothesize that the balance between anti−/proinflammatory cytokines in synovial fluids makes the difference in long-lasting pain control and to support this view we found an IL1-RA/IL-6 average ratio of 1.13 and 0.21 in responders and nonresponders, respectively. In other words, a proper cytokine profile of ACS may turn off inflammation the first weeks after injection then allowing a local response of synovia which regulates production of inflammatory molecules during time. The characteristics of the cytokine profile of nonresponders support the hypothesis that these patients are unable to produce a prevalent amount of anti-inflammatory molecules at any time of their disease, with no chance to control pain with the planned four or more ACS injections.

5 | CONCLUSIONS

Overall, the present study indicates and confirms that ACS is a safe and effective treatment for knee OA;[9,16,17] particularly, it is able to rescue a good proportion of patients from orthopaedic surgery, once medical and PRP treatments have failed. On the other hand, to widen these results to the general population of patients with knee OA, as well as to replace PRP treatment with ACS after failure of standard medical interventions, larger controlled studies are required to confirm our encouraging data and to determine whether ACS may be a standard of treatment when current medical treatments fail.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

RL, AD and LP planned and designed the study; RL and AD enrolled patients' by clinical and laboratory evaluation, collected and produced autologous conditioned serum and evaluated patients' response during follow up; LP and MAI performed statistical analyses and LP wrote the manuscript; GC and FR made orthopaedic examinations and administered autologous conditioned serum by intra-articular injections; PI and DF performed cytokine assays and quality controls. All authors read and approved the final manuscript.

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