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Randomised Clinical Trial

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Intra-articular autologous conditioned serum and triamcinolone injections in patients with knee osteoarthritis: a controlled, randomized, double-blind study

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Abstract

Objective: This study was performed to assess the impact of autologous conditioned serum (ACS) when added to preceding intra-articular glucocorticoid therapy on pain, function, and quality of life outcomes over 24 weeks.

Methods: In this single-center, randomized controlled trial involving 40 patients with advanced knee osteoarthritis (Kellgren–Lawrence grades III and IV), ACS or saline placebo was injected after 40 mg triamcinolone acetonide (TA) intra-articular injection. Numerical rating scale (NRS) pain scores and Knee Injury and Osteoarthritis Outcome Score (KOOS) assessments were conducted at baseline and at weeks 3, 6, 12, and 24. The primary endpoint was the change in KOOS Pain at 24 weeks. Patient safety events were also monitored.

Results: At week 24, TA + ACS significantly improved KOOS Pain, Symptoms, Activities of Daily Living, Quality of Life, and KOOS Sport scores. TA + ACS also outperformed TA + placebo in NRS pain scores (average and maximum intensity) at week 24 and NRS pain score (at rest) at weeks 12 and 24. The TA injection followed by ACS or placebo was well-tolerated.

Conclusion: ACS adds long-term pain relief and functional improvement to the short-term pain relief provided by glucocorticoids.

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Keywords
Knee osteoarthritis, intra-articular injection, autologous conditioned serum, blood cell secretome, triamcinolone acetonide, pain, function, quality of life

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Introduction

Osteoarthritis (OA) is a chronic disease characterized by progressive accumulation of damage to the joint cartilage and underlying bone with repeated inflammatory flares. The knee is one of the most commonly affected joints in human patients with OA. Complex interactions between genetic, metabolic, biochemical, and biomechanical factors are thought to be important in disease progression. Patients with knee OA experience joint pain, swelling, and stiffness. OA is associated with a combination of risk factors, including age, obesity, sex, genetic predisposition, and joint injury.[1] OA is a common disease that mainly affects people over 50 years of age. It is estimated that approximately one-third of the population in European countries has some form of OA,[2,3] and the incidence has been rising as the population ages. OA largely contributes to functional impairment and decreased quality of life of affected patients.[4] This has severely detrimental effects on the costs of health care and sick leave from work. Therefore, effective and safe treatment options for knee OA are needed.

The pathogenesis of OA involves an inflammatory component; the production of inflammatory cytokines, chemokines, and other inflammatory mediators by the synovium and chondrocytes is well documented.[5] Tissue debris, cellular debris, and cellular stress signals are damage-associated molecular patterns (DAMPS) that additionally irritate the intra-articular (i.a.) innate immune system, increasing the risk of synovitis in early and advanced OA. DAMPs are products released in response to stress or derived from host cells, including dead or dying cells. DAMPs (e.g., proteins, extracellular nucleic acids and nucleotides, extracellular matrix components, and escaped intracellular organelle fragments) are often present in environments of trauma and ischemia. They can, for example, activate Toll-like receptors.[6,7] The synovia of OA-affected joints is infiltrated by inflammatory cells and inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor-α, and IL-6, which are believed to mediate cartilage degradation or bone resorption.[8,9] To achieve long-term improvement of chronic degenerative tissue destruction, it is necessary to resolve both the inflammation and dysregulated metabolism, both of which have been correlated with cartilage destruction.

OA is, at present, an incurable disease largely treated through pain management. Such management includes the use of analgesics (i.e., acetaminophen, oxycodone, and propoxyphene) and nonsteroidal anti-inflammatory drugs (NSAIDs) (i.e., ibuprofen, diclofenac, and celecoxib), alone or in combination. These therapies have shown limited effectiveness, both in clinical studies and in real life. Moreover, these drugs are known to have significant and sometimes serious adverse effects, particularly in patients with chronic use.[10–13] Most surgical treatment of OA is recommended for disease that has progressed to an advanced stage[14]; joint replacement is only advisable for patients with end-stage disease. Patients with mid-stage disease wishing to maintain an active lifestyle often prefer less invasive treatments.[15] Intra-articular drug delivery has several advantages over systemic delivery, such as increased local bioavailability, lower systemic exposure, fewer adverse events, and reduced cost. However, the efficacy of i.a. therapies remains controversial, and evidence of long-lasting efficacy is unclear. Current clinical guidelines show inconsistency regarding their use.[16,17] Intra-articular treatment options for the management of knee OA include analgesics, glucocorticoids (GCs), hyaluronic acid, biologics, and combinations of drugs.[18] Intra-articular analgesics and GCs have been attributed with cytoinhibitory or even cytotoxic properties, but they are highly effective against pain and therefore useful in acute synovitis associated with OA.[19]

Intra-articular biologics are a class of drugs with high potential to stabilize the biological metabolism of OA-affected joints. The goal of regenerative medicine is to treat underlying pathomechanisms in an attempt to shift treatment from symptomatic to causal. This is a fundamental difference from symptomatic therapies using agents such as nonsteroidal anti-inflammatory drugs or GCs.

Autologous conditioned serum (ACS) is a biological cell-free treatment option for various musculoskeletal diseases. ACS is effective for various stages of knee OA in prospective, double-blinded, randomized controlled trials.[20,21] The efficacy of ACS has also been shown in clinical studies of other musculoskeletal disorders, such as hip OA, as well as radiculopathies with varying effect sizes (ESs).[22–30] The therapeutic effects of ACS on pain reduction and functional improvement are primarily ascribed to elevated physiological levels of growth factors and cytokines in the serum.[31]

ACS is obtained through extended blood coagulation under physiological conditions using a specific medical device for blood-taking. Generally, under conditions of injury, coagulation serves to close a wound and exclude pathogens. It also serves to initiate wound healing and restore tissue function. Thus, ACS contains a secretome of cell-derived factors that contribute to the healing response, including the resolution of inflammation and promotion of regeneration. Factors that play a role in resolving inflammation and promoting in-tissue homeostasis include growth factors (e.g., hepatocyte growth factor, transforming growth factor β, and insulin-like growth factor)[31,32] and cytokines (e.g., IL-1 receptor antagonist, IL-4, and IL-10). The process of obtaining ACS is simple and safe.[20]

Treatment with i.a. GC injections provides short-term pain relief by turning off acute inflammation. It is a commonly applied treatment option that is strongly[33] or conditionally[34] recommended by guidelines. Various publications on i.a. GC injections in patients with OA have shown a beneficial effect between 1 and 6 weeks.[34–37] Intra-articular GC injections have been studied and recommended in combination with other non-pharmacological and pharmacological therapies.[38–40] However, recent publications question the frequent use of GC in OA. A double-blind clinical study of patients with knee OA treated with i.a. GC injections every 3 months for 2 years showed significant cartilage volume loss versus placebo.[41] GCs are an effective treatment option, but they are often used too frequently because of their relatively short-duration effect on chronic disease with little to no long-term treatment alternatives. The addition of a durable treatment such as ACS with a fast-acting GC may help to reduce the frequency of GC injections, thus keeping GC use within label guidance and guidelines. This will lead to safer and more effective management of OA with respect to both pain and function.

The present study was performed to examine efficacy and safety of ACS in patients with knee OA. The aim of the study was to statistically verify whether an additional therapy to preceding triamcinolone acetonide (TA) injections can result in extended efficacy with respect to pain, function, and quality of life in patients with knee OA.

Patients and methods

Study design

This therapeutic, investigator-initiated trial was designed as a prospective, placebo-controlled, randomized, double-blind, two-armed, single-center, interventional, add-on clinical study without a statistical interim analysis and with a calculated sample size of 38 patients (2 × 19). The reporting of this study conforms to the CONSORT statement.[42] A CONSORT flow diagram of the study is shown in Figure 1.

Participants

All eligible male and female patients aged 40 to 75 years with knee OA who presented to the study center were offered participation in the study. Only patients with Kellgren–Lawrence grade III and IV knee OA were included. A further inclusion criterion for participation in the study was persistent OA-associated knee pain for at least 4 weeks with a baseline score of ≥5 for arthritis pain on a numeric rating scale (NRS) ranging from 0 to 10.

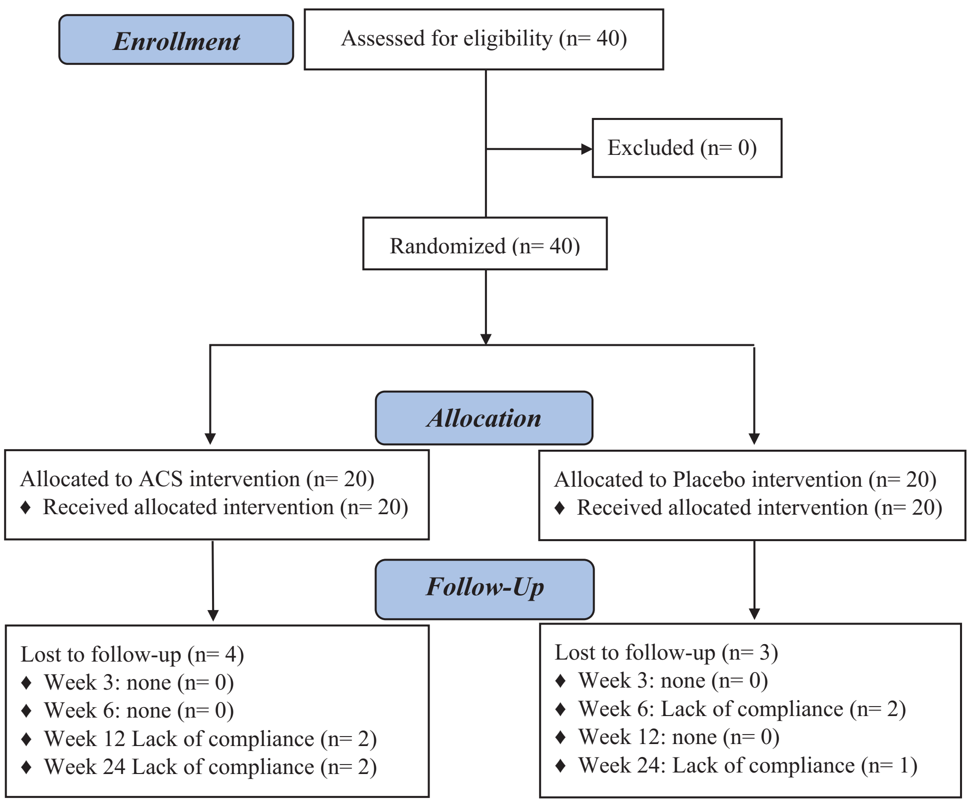

Figure 1. CONSORT flow diagram showing enrollment, allocation, follow-up, and participants included in the statistical analysis. The reason for dropout was lack of compliance.

The exclusion criteria for study enrollment were surgical interventions involving the target joint during the previous 6 months; i.a. injections with GCs, hyaluronan compounds, platelet-rich plasma (PRP), or ACS in the target joint during the previous 3 months; a diagnosis of rheumatoid arthritis or other autoimmune cause of arthritis; systemic bone or joint illnesses; active inflammation of predominant patellofemoral disease; a body mass index of $>30\,kg/m^2$; malignancy; infection; pregnancy; and any known condition that represents a contraindication for the use of GCs or ACS (e.g., use of GCs in patients with diabetes mellitus).

The study was performed at the Institute of Rheumatology, Belgrade, Serbia in accordance with the Declaration of Helsinki to ensure protection of human subjects, and it was approved by the medical ethics committee at the study site on 28 September 2016 (Number: 21/1-78) (UTN: U1111-1272-7333; German Clinical Trials Register: DRKS00009969). All enrolled patients were appropriately informed about the study and provided written informed consent for blood sample collection, ACS preparation, and i.a. injections.

Interventions

Two 10-milliliter blood samples were taken from every patient included in the study. The processing of cell-free ACS involved the drawing of two 10-mL whole blood samples with a CE-labeled device (Orthogen Lab Services GmbH, Düsseldorf, Germany).[20,32] The device was adequately labeled with the patient's data. The blood-filled device was then left for extended coagulation (3 hours at 37°C) in a controlled incubator and subsequently centrifuged for 10 minutes at $3000 \times g$. The extracted serum (3–4 mL per device) was ACS, a cell-free autologous serum containing growth factors and cytokines released during extended coagulation. ACS may be injected immediately or after frozen storage at −18°C or colder. The ACS was passed through a sterile syringe tip filter (0.22 µm, Millex GP; MilliporeSigma, Burlington, MA, USA) and injected into the affected knee joint of the same patient. An expert rheumatologist performed the ultrasound-guided injection in the assigned patients with sterile instruments and materials in an adequate outpatient environment.

All study patients received a single i.a. injection of 1 mL TA (40 mg) into the index knee joint. The syringe was then removed from the needle and replaced with a syringe containing either 5 mL ACS (active treatment) or 5 mL saline (placebo). This procedure was chosen so that all components could be injected in a single session, avoiding more than one i.a. puncture.

Outcomes

All outcomes were measured at baseline and after weeks 3, 6, 12, and 24. An independent rheumatologist who was blinded to the treatments examined the patients at each visit.

Outcomes were measured with the Knee Injury and Osteoarthritis Outcome Score (KOOS), developed by Roos et al.[43,44] to assess patients' opinions about their knees and associated problems. The KOOS instrument is composed of 42 questions divided into 5 subscales: Pain (9 questions), Symptoms (7 questions), Activities of Daily Living (ADL) (17 questions), Sport/Recreation Function (5 questions), and Knee-related Quality of Life (QoL) (4 questions).

The primary outcome was assessment of the KOOS Pain at 24 weeks between the ACS group and placebo group. In addition to the primary outcome, the following secondary outcomes were analyzed: assessment of all other above-mentioned KOOS subdomains[43] and assessment of pain in the last week (average pain, maximum pain, and pain at rest) according to the NRS pain score (0–10).[45] Higher NRS pain scores were associated with more severe symptoms. For the KOOS, lower scores were associated with more severe symptoms and higher scores were associated with less severe symptoms.

Sample size

The sample size calculation was based on the primary outcome. The calculation was not planned on specifications of published trials; instead, it was based on the assumption that a clinically relevant ES (Cohen's d) should be greater than large (0.8) according to the classification suggested by Cohen.[46,47] For this reason, therapeutic effectiveness was conservatively considered clinically relevant with a mean ES (change in outcome/standard deviation) in the primary endpoint of at least 0.95. A minimum sample size of 38 (2×19) evaluable patients was needed to provide 80% power to show a significant therapeutic effect in the primary statistical analysis with a two-sided t-test for unpaired data. A two-sided global significance level of $\alpha = 0.05$ was chosen. To compensate for a dropout rate of 5%, we recruited 40 (2×20) patients.

Randomization

Structural equality of the two trial arms was achieved by randomization using a random number generator. The allocation took the form of permutated blocks of variable length in an allocation ratio of 1:1.

Blinding

Observational equality of the trial arms was achieved by blinding, which occurred at the patient and observer levels. ACS and saline were available in blinded separate syringes. Application of TA, ACS, or saline was performed consecutively, without mixing in a syringe. The unblinded physician who prepared the syringes handed the blinded syringes to the blinded physician who administered the i.a. injections.

Statistical methods

Statistical analyses were performed with descriptive methods (e.g., frequency tables, mean, standard deviation, and ES) and inferential analyses using appropriate significance tests and confidence intervals. No imputation of missing values was performed. Probabilities were calculated with a two-sided t-test for unpaired data, with a global significance level of $\alpha = 0.05$ and a null hypothesis of mean effect equal to 0.

The statistical analysis of the primary and secondary endpoints was performed according to intention-to-treat principles. The results were interpreted on a confirmatory basis. Safety was evaluated exploratively. The statistical analysis was performed with SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Upon enrollment in the study, all pain medications for any painful conditions (including conditions apart from the target knee) were discontinued.

Adverse events

Adverse events were assessed and documented throughout the whole study period. The patients' self-assessment records were reviewed. Adverse events were assessed by the clinical investigator at each study visit and summarized according to intensity and causality. Patients were allowed to withdraw their participation in the study at any time without explanation.

Results

Patients' characteristics

Patients' baseline characteristics. Forty patients were screened from October 2016 to March 2017. All patients were allocated for treatment and enrolled in the study, and all of them finished the treatment schedule.

All patients received a single i.a. injection of TA 40 mg. Twenty patients were randomized to receive a single add-on i.a. injection of placebo (saline), and 20 patients were randomized to receive a single add-on i.a. injection of ACS. The patients' demographic and clinical characteristics are presented in Table 1. All patients enrolled in the study had Kellgren–Lawrence grade III and IV knee OA.

Overall treatment effects. At baseline, the placebo and ACS groups exhibited similar levels of pain intensity; the KOOS Pain and NRS knee pain score in the last week (average, maximum, and at-rest intensities) showed no significant difference at baseline between the groups. The placebo and ACS groups also did not differ in KOOS Symptoms, KOOS ADL, or KOOS Sport. KOOS QoL was significantly better in the placebo group than in the ACS group at baseline ($p = 0.02$). The patients' baseline characteristics are shown in Table 2.

Statistical analysis between the placebo and ACS groups demonstrated no significant difference in the NRS pain score (average) or NRS pain score (maximum intensity) at weeks 3, 6, and 12 after treatment. A significant difference in favor of adjunct ACS was observed at 24 weeks (NRS pain score (average): $p = 0.02$, NRS pain score (maximum): $p < 0.01$). The NRS pain score (at rest) was significantly lower in the ACS than placebo group at weeks 12 and 24 (Figure 2(a)–(c)) ($p = 0.03$ and $p = 0.02$, respectively).

For the primary endpoint (change in KOOS Pain at 24 weeks), a statistically significant improvement was found in the ACS group over the placebo group ($p < 0.01$) (Figure 3(a)). Furthermore, at week 24, a statistically significant improvement in the ACS group over placebo was observed in KOOS Symptoms ($p = 0.04$), KOOS ADL ($p < 0.01$), and KOOS QoL ($p = 0.01$) (Figure 3(b), (c), and (e)). The ACS group exhibited a significantly higher KOOS Sport at weeks 3, 12, and 24 than the placebo group ($p < 0.01$, $p = 0.02$, and $p < 0.01$, respectively) (Figure 3(d)).

The ES analysis at weeks 12 and 24 following i.a. treatment revealed a large improvement in the NRS pain score; this improvement was even more pronounced at week 24 than at week 12 in the ACS group. Similarly, the ES analysis of the KOOS indicated improvements in KOOS Pain (ES = 1.00), KOOS Symptoms (ES = 0.74), KOOS ADL (ES = 1.05), KOOS Sport (ES = 1.32), and KOOS QoL (ES = 0.92) at 24 weeks in the ACS group compared with the placebo group. The results of the significance level and ES analysis are presented in Tables 2 and 3.

Safety data. No serious adverse events were reported in either treatment group during the 24-week study period. Both treatments were well tolerated.

Adverse events by number of patients in the ACS versus placebo groups are as follows: transient sensation of redness/heat (1 vs. 4), increase in blood pressure (4 vs. 3), transient increase in pain (4 vs. 3), and numbness in knee/leg/toes (2 vs. 1). One patient reported a nosebleed, and one patient reported thigh skin friction (both in placebo group). One patient reported knee swelling after physical activity (ACS group). No adverse events were reported after week 6 (Table 4).

Discussion

Components of the described treatment regimen

Because of their potent anti-inflammatory effect, i.a. injections with GCs, such as TA, are part of standard care and are generally effective for acute and short-term

Table 1. Patients' demographic and baseline clinical characteristics.

Table 1a. Demographic characteristics

	ACS		Placebo	
	n	Mean ± SD	n	Mean ± SD
Age (years)	20	62.6 ± 9.9	20	64.9 ± 9.0
Weight (kg)	20	79.3 ± 14.4	20	80.6 ± 10.4
Height (cm)	20	170.6 ± 8.8	20	170.2 ± 9.3
Body mass index (kg/m^2)	20	27.0 ± 3.5	20	27.9 ± 3.1

Table 1b. Baseline clinical characteristics

	ACS	Placebo
	n (%)	n (%)
Sex		
Female	17 (85)	17 (85)
Male	3 (15)	3 (15)
Smoking		
Yes	5 (25)	1 (5)
No	15 (75)	18 (90)
Physical activity		
None	2 (10)	3 (15)
Regularly, 1 to 2 times a week	3 (15)	2 (10)
Regularly, 3 to 4 times a week	2 (10)	3 (15)
Almost daily	13 (65)	12 (60)
Sports		
None	14 (70)	11 (55)
Regularly, 1 to 2 times a week	3 (15)	3 (15)
Regularly, 3 to 4 times a week	0 (0)	3 (15)
Almost daily	3 (15)	3 (15)
Kellgren–Lawrence classification of knee osteoarthritis		
III	16 (80)	13 (65)
IV	4 (20)	7 (35)
Affected knee (side)		
Right knee	9 (45)	10 (50)
Left knee	7 (35)	7 (35)
Both[1]	3 (15)	3 (15)
Pain duration		
4 to 6 weeks	1 (5)	0 (0)
6 weeks to 3 months	1 (5)	0 (0)
3 months to 6 months	2 (10)	2 (10)
>6 months	16 (80)	18 (90)
Pain relief by medication		
Insufficient	3 (15)	4 (20)
Moderate	17 (85)	16 (80)

(continued)

Table 1. Continued.

Table 1b. Baseline clinical characteristics

	ACS	Placebo
	n (%)	n (%)
Already treated because of existing pain in the target knee		
Yes	18 (90)	11 (55)
No	2 (10)	8 (40)
Previous treatment of current pain in the target knee*		
Physiotherapy	6 (30)	8 (40)
Cartilage-building preparations	5 (25)	10 (50)
Insoles	3 (15)	1 (5)
Bandage/orthosis	0 (0)	1 (5)
Physical therapy	9 (45)	6 (30)
Cortisone injections within last 3 months	10 (50)	2 (10)
Hyaluronic acid injections within last 3 months	1 (5)	1 (5)
ACS injections within last 3 months	0 (0)	0 (0)
Surgery within last 6 months (e.g., arthroscopy)	1 (5)	0 (0)
Further complaints regarding the musculoskeletal system (except knees)*		
Hip, same side	3 (15)	6 (30)
Hip, opposite side	2 (10)	1 (5)
Ankle, same side	1 (5)	2 (10)
Ankle, opposite side	0 (0)	1 (5)
Spine	12 (60)	13 (65)

*multiple answers possible.
ACS, autologous conditioned serum; SD, standard deviation.

pain relief (1–6 weeks).[34–37] GCs take very rapid effect by receptor-mediated regulation of numerous genes involved in inflammatory pathways.[48] Short-term use of GCs may protect cartilage by downregulating acute, post-traumatic inflammation.[49–51] However, repeated administration and long-term use of GCs may potentially damage articular tissue, including cartilage.[41,52–54] Adjunct ACS injections may contribute to reduced frequency of GC injections, thereby staying within label guidance and current guidelines.

ACS is a physiological blood cell secretome. Both terms are used synonymously. This secretome is released during extended coagulation under standardized conditions. ACS contains the totality of the mediators released by cells during coagulation, including growth factors. Extended coagulation leads to the release of insulin-like growth factor 1, transforming growth factor β, platelet-derived growth factor, and vascular endothelial growth factor.[31,55] These (mostly platelet-derived) growth factors and their effects on tissues have recently been reviewed, albeit in the context of PRP therapy for OA.[32] In ACS, growth factors constitute the majority of mediators (with concentrations typically exceeding those of cytokines by a factor of 100×).[31,56] These growth factors originate from the natural quantity and composition of cells, thus contributing to a physiological blood cell secretome. Profiles of ACS content vary inter-individually and have been published.[24,31,56,57] Given this complexity of blood serum, more components with

Table 2. Therapeutic effect as shown by NRS scores between treatment groups at baseline and at weeks 3, 6, 12, and 24 after therapy.

	ACS		Placebo		ACS/Placebo			
	within group		within group		Difference between groups			
	n	Mean ± SD, ES (p-value)	n	Mean ± SD, ES (p-value)	n	Mean of difference (95% CI)	p-value	ES
NRS score: Knee pain in the last week (average)								
Baseline	20	6.85 ± 1.73	20	6.75 ± 1.52	20	0.10	0.85	
						(−0.94 to 1.14)		
Week 3	20	3.70 ± 2.37	20	3.50 ± 2.50	20	0.10	0.91	−0.04
ES of diff. to BL		1.13 ($p < 0.01$)		1.25 ($p < 0.01$)		(−1.63 to 1.83)		
Week 6	20	4.15 ± 1.84	18	4.50 ± 1.86	18	−0.48	0.49	0.23
ES of diff. to BL		1.14 ($p < 0.01$)		1.26 ($p < 0.01$)		(−1.86 to 0.91)		
Week 12	18	4.17 ± 2.38	18	4.94 ± 1.95	18	−1.00	0.15	0.50
ES of diff. to BL		1.16 ($p < 0.01$)		1.14 ($p < 0.01$)		(−2.37 to 0.37)		
Week 24	16	3.19 ± 2.34	17	5.24 ± 1.89	16	−2.03	0.02	0.85
ES of diff. to BL		1.22 ($p < 0.01$)		0.85 ($p < 0.01$)		(−3.70 to 0.35)		
NRS score: Knee pain in the last week (maximum)								
Baseline	20	7.30 ± 1.81	20	6.75 ± 1.65	20	0.55	0.32	
						(−0.56 to 1.66)		
Week 3	20	4.25 ± 2.77	20	4.25 ± 2.81	20	−0.55	0.58	0.17
ES of diff. to BL		1.01 ($p < 0.01$)		0.76 ($p < 0.01$)		(−2.56 to 1.46)		
Week 6	20	4.95 ± 1.88	18	4.94 ± 2.15	18	−0.52	0.52	0.21
ES of diff. to BL		0.80 ($p < 0.01$)		1.02 ($p < 0.01$)		(−2.14 to 1.11)		
Week 12	18	5.17 ± 2.43	18	5.61 ± 2.00	18	−1.06	0.16	0.48
ES of diff. to BL		0.80 ($p < 0.01$)		0.80 ($p < 0.01$)		(−2.56 to 0.45)		
Week 24	16	4.00 ± 2.78	17	6.35 ± 1.80	16	−2.71	<0.01	1.13
ES of diff. to BL		1.01 ($p < 0.01$)		0.24 ($p = 0.35$)		(−4.40 to −1.02)		
NRS score: Knee pain in the last week (at rest)								
Baseline	20	4.55 ± 2.86	20	4.25 ± 2.51	20	0.30	0.73	
						(−0.42 to 2.02)		
Week 3	20	2.20 ± 2.19	20	2.60 ± 2.66	20	−0.70	0.53	0.20
ES of diff. to BL		0.76 ($p < 0.01$)		0.43 ($p < 0.07$)		(−2.94 to 1.54)		

(continued)

Table 2. Continued.

	ACS		Placebo		ACS/Placebo			
	within group		within group		Difference between groups			
	n	Mean ± SD, ES (p-value)	n	Mean ± SD, ES (p-value)	n	Mean of difference (95% CI)	p-value	ES
Week 6	20	2.45 ± 2.06	18	3.22 ± 2.24	18	−0.93	0.34	0.32
ES of diff. to BL		0.62 (p = 0.01)		0.49 (p = 0.05)		(−2.87 to 1.01)		
Week 12	18	1.83 ± 1.50	18	3.56 ± 2.28	18	−2.00	0.03	0.78
ES of diff. to BL		1.03 (p < 0.01)		0.35 (p = 0.16)		(−3.74 to −0.26)		
Week 24	16	1.56 ± 1.75	17	3.94 ± 2.08	16	−2.53	0.02	0.82
ES of diff. to BL		0.92 (p < 0.01)		0.16 (p = 0.52)		(−4.72 to −0.34)		

NRS, numerical rating scale; ACS, autologous conditioned serum; SD, standard deviation; CI, confidence interval; ES, effect size (Cohen's d, standardized mean difference), defined as very small (0.01), small (0.2), medium (0.5), large (0.8), very large (1.2), or huge (2.0)[44]; BL, baseline; diff., difference.

potential mediator effects will likely be identified in the future.

In contrast to GCs, ACS produces a gradual and continuous improvement in OA-related symptoms that has been demonstrated in both randomized and open-label clinical studies. Most first follow-ups were performed at $\geq$3 weeks after the end of treatment. Two uncontrolled studies, however, examined the onset of improvement after ACS injections.[58,59] Abd-EL Motaal et al.[58] administered a 1-mL ACS injection at 0, 1, 2, and 3 weeks (total of 4 injections) in 30 patients. Western Ontario and McMaster Universities Osteoarthritis (WOMAC) score was gathered at baseline, weeks 0 to 3, and months 1 to 3. The maximum improvement in the WOMAC score was apparent at 8 weeks. Tassara et al.[59] administered a 2-mL ACS injection at 0, 1, 2, and 3 weeks (total of 4 injections) in 28 patients. The visual analog scale score and range of motion were assessed at weeks 0 to 3 and months 2 and 7. Again, the maximum improvement was apparent at 8 weeks, and this persisted until 6 months.

No data are available regarding potential differences in the rapid onset of clinical effects between the groups of this study. However, at week 3, the effect of TA + ACS was very similar to that of TA + saline. ACS as an adjunct to TA does not appear to impair the rapid short-term pain relief mediated by the background TA.

The pathologic mechanisms of OA include cell damage, which leads to both chondral and osteochondral defects. The therapeutic goal is to exploit the intrinsic bioactive molecules liberated during blood coagulation. In the wound healing process, blood coagulation first closes the wound and then facilitates tissue healing mediated through the secretome released during blood coagulation. This may serve as one model of the mechanism of action of ACS.

The mode of action of the adjunct treatment centers on the fact that the onset of

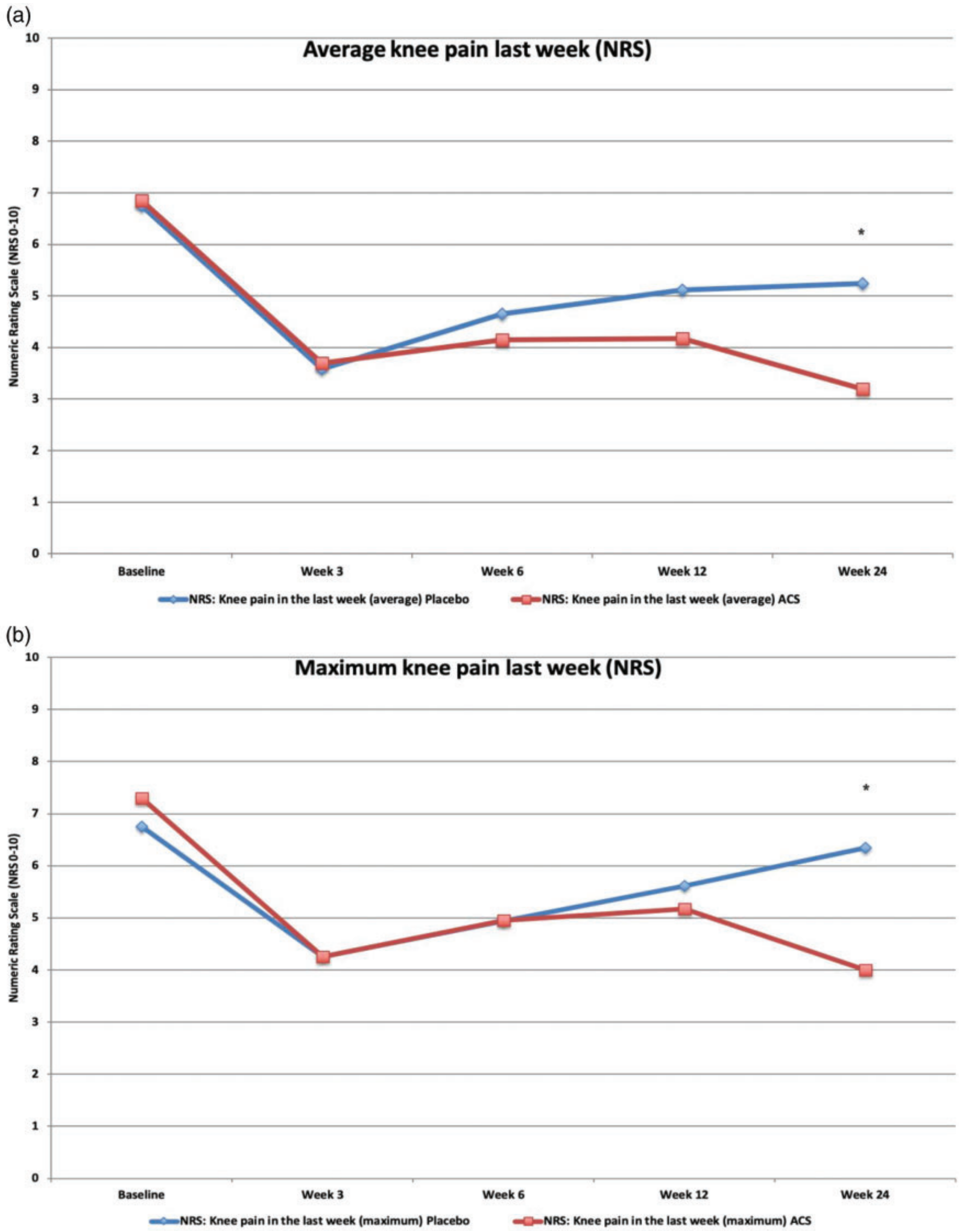

Figure 2. Patients with advanced knee OA were treated with a single injection of ACS or saline, both adjunct to TA. Follow-up was performed at week 3, 6, 12, and 24. The figures show the mean numeric rating scale scores at measured time points. (a) Statistically significant superiority of ACS adjunct to TA over placebo adjunct to TA at week 24. (b) Statistically significant superiority of ACS adjunct to TA over placebo adjunct to TA at week 24 and (c) Statistically significant superiority of ACS adjunct to TA over placebo adjunct to TA at weeks 12 and 24.

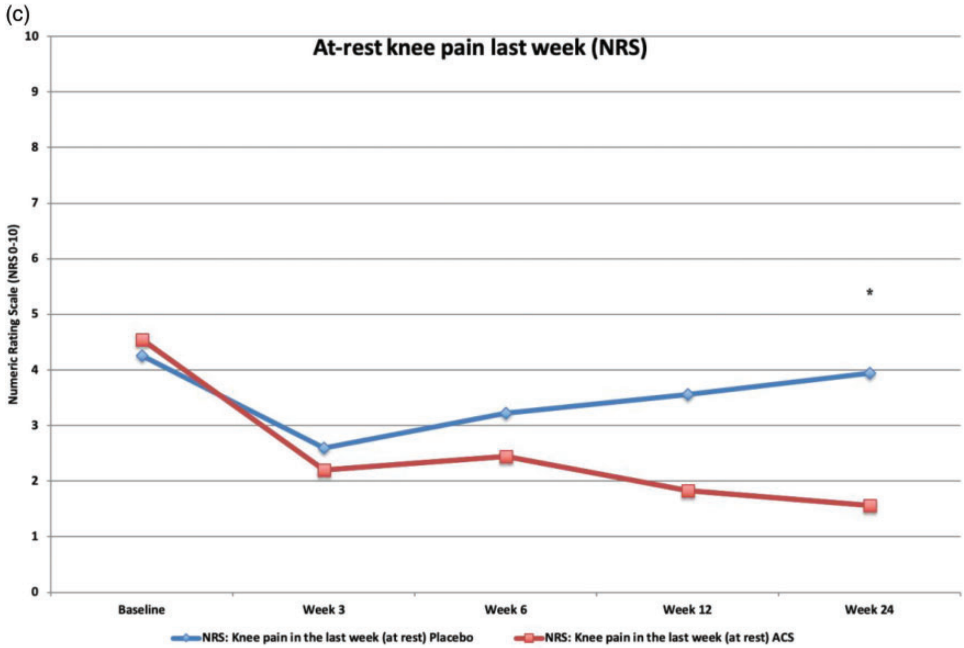

Figure 2. Continued.

TA is very rapid, whereas the onset of ACS can take up to 6 weeks. Patients benefit from the long-term efficacy provided by ACS and the immediate onset of symptom relief provided by TA. TA acts by shutting down multiple inflammatory pathways. ACS is less "mono-directed." The multitude of growth factors and cytokines each have their own receptor(s) that modulate a complex network of biochemical and metabolic pathways. Interestingly, ACS normalizes reactive oxygen species/nitric oxide* radicals and synovial fluid viscosity while also increasing intra-joint IL-1 receptor antagonist expression, which has been demonstrated in an equine OA model[31] and in human OA. This suggests that the mode of action is directed to regain tissue homeostasis[60] instead of immediate anti-inflammation. ACS compensates for the known cytostatic effects of GCs. These are also used in the *in vitro* differentiation of mesenchymal stem cells to chondrocytes by adding dexamethasone. Near-arrest of cell division is the price for the resulting differentiation and production of extracellular matrix components *in vitro*. However, cell cycle arrest is not beneficial for tissue regeneration, which in part is dependent on cell division.

In contrast to PRP processing, ACS processing does not involve the selection and alteration of specific blood cell numbers. It rather involves the comprehensive mechanisms that are activated in whole blood during coagulation inside a purpose-built medical device. This results in individual, physiological concentrations and proportions of biomolecules ready to be injected. For PRP, coagulation or degranulation of platelets takes place inside the joint, with a less predictable outcome and uncertain concentrations of released biomolecules. The therapeutic efficacy of PRP with respect to pain and function is attributed to *non*-physiological elevations of growth factor concentrations. By contrast, the therapeutic efficacy

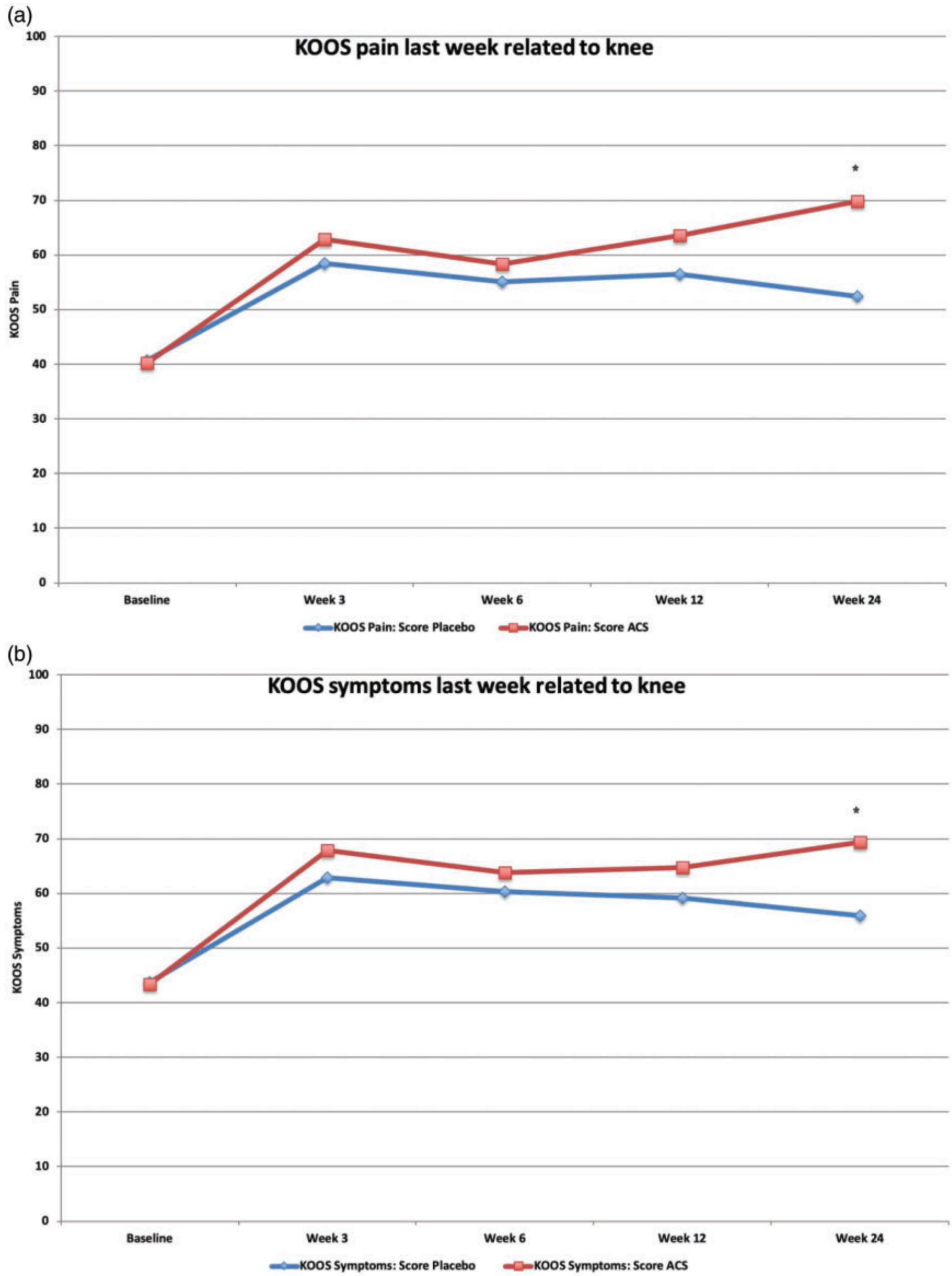

Figure 3. Patients with advanced knee OA were treated with a single injection of ACS or saline, both adjunct to TA. Follow-up was performed at week 3, 6, 12, and 24. The figures show the mean KOOS subscales at measured time points. (a) Treatment effect on KOOS Pain. Both treatment groups showed similar behavior until week 12. ACS adjunct to TA displayed statistically significant superiority over placebo adjunct to TA at week 24. (b) Treatment effect on KOOS Symptoms. Both treatment groups showed similar behavior until week 12. ACS adjunct to TA displayed statistically significant superiority over placebo adjunct

Continued.

of ACS is attributed to *physiological* elevations of growth factor concentrations.

Growth factors reportedly have an ameliorating effect on inflammation (e.g., through inhibition of NFκB signaling).[32] Based on this mechanism, growth factors are directly relevant to pain management in patients with OA. It is possible that growth factors contribute to long-term therapeutic effects in patients with knee OA by acting through their cognate receptors. The biological complexity of OA and of blood products, such as ACS and PRP, makes validation of postulated modes of action (in the sense of "which factor is the active agent") difficult.[61,62]

The safety of the active components of TA and ACS is well established when these agents are administered alone and according to the manufacturers' instructions.[20–29,63] In the randomized controlled German Orthokine Osteoarthritis Trial (GOAT), which compared ACS, hyaluronan, and placebo in 376 patients with knee OA, no serious side effects were observed over 2 years.[20]

In agreement with other studies,[20,22] no serious drug-related adverse events occurred in either group of the present study. To the best of our knowledge, the only known ACS-associated joint infection was reported by Auw Yang et al.[21] They evaluated this as an injection procedure-related event.

Discussion of clinical results

This study is the first randomized clinical trial to investigate the adjunct approach of ACS added to GC in patients with OA. Very few studies have evaluated the additional use of autologous blood products with GC in patients with OA or other orthopedic conditions (e.g., rotator cuff injuries). In one randomized study, Camurcu et al.[64] demonstrated that i.a. injection of a single dose of methylprednisolone (MP) prior to injection of PRP resulted in better clinical outcomes in patients with knee OA ($n = 115$). WOMAC scores were significantly lower after 1 month in the MP + PRP group than in the PRP group and significantly lower after 6 months in the MP + PRP group than in the MP group.[64] Their study design was based on principles similar to those of the treatment regimen in the present study.

In this clinical study, we evaluated the safety and efficacy on pain and function of an adjunct injection of ACS following TA in patients with advanced knee OA for 24 weeks of follow-up in comparison to adjunct placebo (saline). The primary endpoint, the change in KOOS Pain at 24 weeks, was met with statistically significant improvement.

The most prominent effect of ACS (with respect to NRS endpoints) was evident in the NRS pain score at rest, which was markedly lower at 12 and 24 weeks post-intervention compared with placebo. Specifically, the improvement in pain at rest (a typical inflammation-dependent parameter) suggests that ACS boosts the clinical effects of TA while simultaneously

Figure 3. Continued.
to TA at week 24. (c) Treatment effect on KOOS Activity of Daily Living (ADL). Both treatment groups showed similar behavior until week 12. ACS adjunct to TA displayed statistically significant superiority over placebo adjunct to TA at week 24. (d) Treatment effect on KOOS Sports. The results of KOOS Sports showed significant superiority of ACS adjunct to TA over placebo adjunct to TA at week 3. At week 6 and week 12, patients in both groups had similar scores. The ACS group showed statistically significant superiority over placebo adjunct to TA at week 24. (e) Treatment effect on KOOS Quality of Life (QoL). Both treatment groups were statistically different at baseline ($p = 0.02$) and showed similar behavior until week 12. ACS adjunct to TA displayed statistically significant superiority over placebo adjunct to TA at week 24.

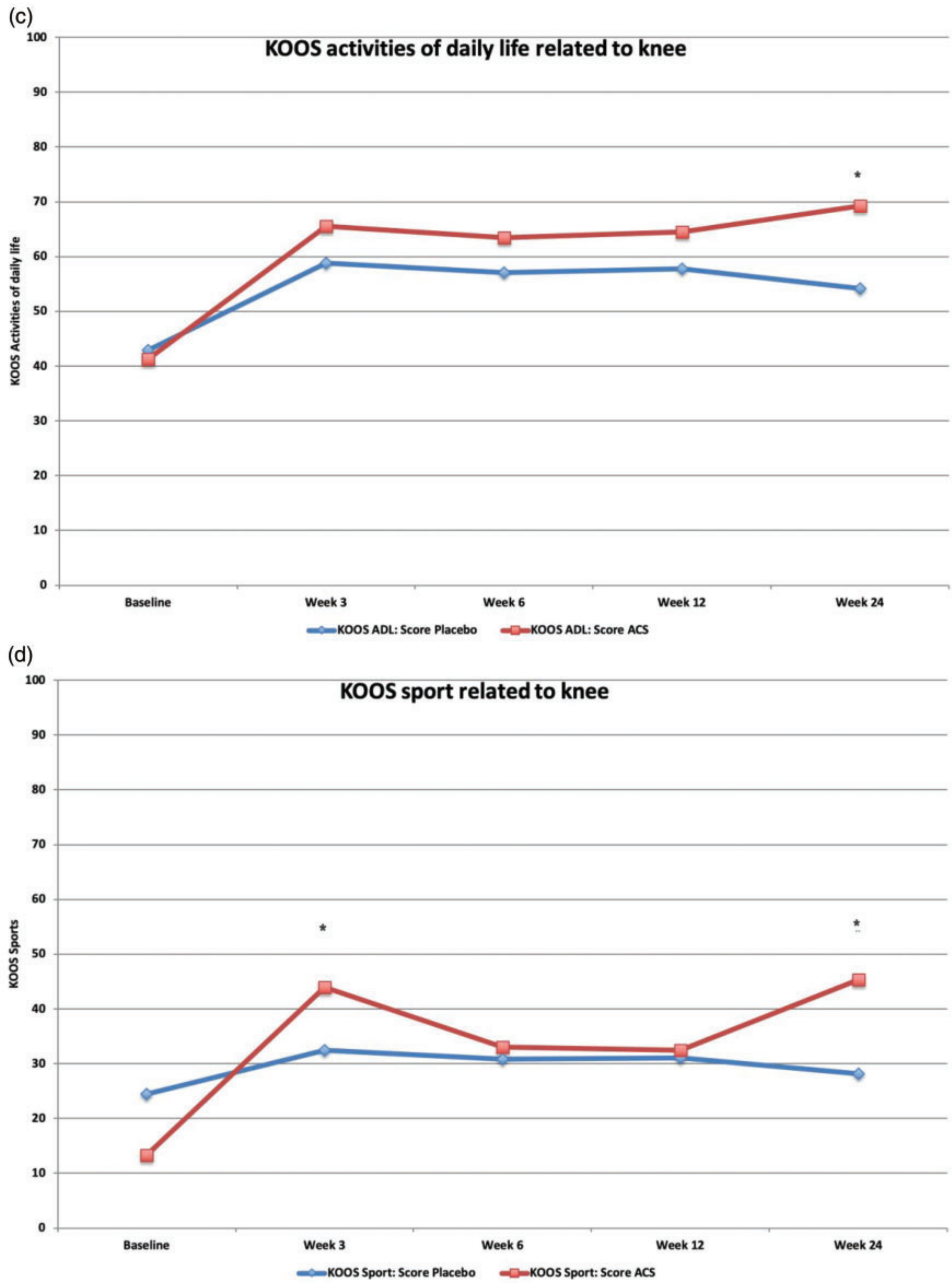

Figure 3. Continued.

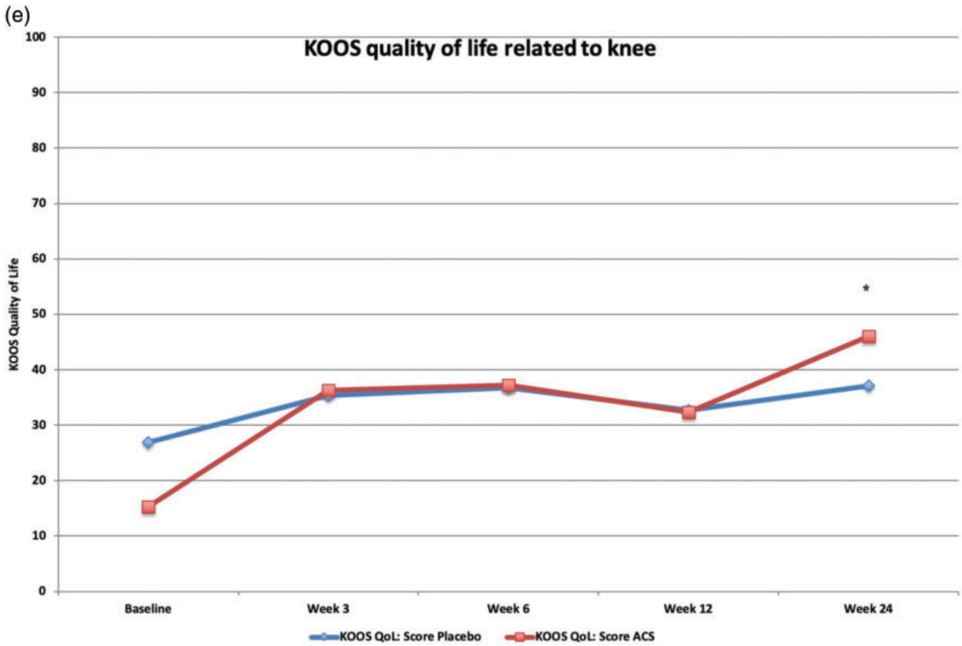

Figure 3. Continued.

reducing the GC-associated cytostatic effects (data to be published elsewhere).

Statistically significant superiority in all endpoints of adjunct ACS was reached at 24 weeks. A medium ES (>0.5) between the groups was also observed at 12 weeks in most of the variables (NRS pain score (average), NRS pain score (at rest), and KOOS Pain, ADL, Sport, and QoL).

Interestingly, the baseline KOOS QoL was significantly worse in the ACS than placebo group. Nonetheless, KOOS QoL at 24 weeks was significantly superior in the ACS group.

In a study by Sawilowsky,[47] ES analysis revealed a large improvement across all NRS pain scales and KOOS subcategories in the ACS treatment group compared with placebo. At 24 weeks, ES versus baseline in the ACS group was consequently better than 0.9 (0.92–1.52) in all parameters. The placebo group did not reach an ES of 0.9 (0.16–0.85) at any endpoints at 24 weeks. In fact, placebo did not achieve superiority over ACS in any endpoint. According to Roos and Lohmander,[44] the minimal perceptible clinical improvement for the KOOS is at 10 points per domain. In the present study, all KOOS domains for both groups at week 24 versus baseline were above this cut-off except for KOOS Sport in the placebo group. KOOS Sport displayed an interesting feature: it improved dramatically in the ACS group between baseline and week 3, only to deteriorate at week 6 and week 12. This subscore recovered at week 24 to its 3-week value. One possible interpretation might be that, having experienced an improvement of 30 points in KOOS Sport, the patients misjudged the robustness of this clinical improvement. They may have excessively engaged in physical activities, potentially leading to the 6- and 12-week decrease in

Table 3. Therapeutic effect as shown by KOOS between treatment groups at baseline and at weeks 3, 6, 1,2 and 24 after therapy.

	ACS		Placebo		ACS/Placebo			
	Within group		Within group		Difference between groups			
	n	Mean ± SD, ES (p-value)	n	Mean ± SD, ES (p-value)	n	Mean of difference (95% CI)	p-value	ES
KOOS Pain: Score								
Baseline	20	40.28 ± 14.32	20	40.69 ± 17.39	20	−0.42 (−10.61 to 9.78)	0.94	
Week 3	20	62.92 ± 21.79	20	58.47 ± 22.01	20	4.86	0.48	0.23
ES of diff. to BL		0.97 ($p < 0.01$)		0.91 ($p < 0.01$)		(−8.89 to 18.61)		
Week 6	20	58.33 ± 18.93	18	55.09 ± 19.44	18	4.63	0.50	0.22
ES of diff. to BL		0.69 ($p < 0.01$)		1.02 ($p < 0.01$)		(−9.16 to 18.42)		
Week 12	18	63.58 ± 19.59	18	56.48 ± 18.94	18	10.03	0.10	0.56
ES of diff. to BL		1.18 ($p < 0.01$)		1.06 ($p < 0.01$)		(−2.04 to 22.10)		
Week 24	16	69.79 ± 22.29	17	52.45 ± 22.65	17	19.73	<0.01	1.00
ES of diff. to BL		1.33 ($p < 0.01$)		0.61 ($p < 0.02$)		(5.77 to 33.69)		
KOOS Symptoms: Score								
Baseline	20	43.39 ± 19.29	20	43.75 ± 21.49	20	−0.36 (−13.43 to 12.72)	0.96	
Week 3	20	67.86 ± 21.93	20	62.86 ± 19.30	20	5.36	0.46	0.24
ES of diff. to BL		0.91 ($p < 0.01$)		1.14 ($p < 0.01$)		(−9.04 to 19.76)		
Week 6	20	63.75 ± 17.62	18	60.32 ± 19.17	18	3.69	0.56	0.19
ES of diff. to BL		0.87 ($p < 0.01$)		1.22 ($p < 0.01$)		(−9.10 to 16.49)		
Week 12	18	64.68 ± 19.36	18	59.13 ± 24.01	18	8.73	0.16	0.47
ES of diff. to BL		1.25 ($p < 0.01$)		0.89 ($p < 0.01$)		(−3.77 to 21.23)		
Week 24	16	69.42 ± 23.58	17	55.88 ± 22.41	16	15.02	0.04	0.74
ES of diff. to BL		1.15 ($p < 0.01$)		0.70 ($p = 0.01$)		(0.69 to 29.36)		
KOOS ADL: Score								
Baseline	20	41.32 ± 17.38	20	42.87 ± 17.06	20	−1.55 (−12.57 to 9.48)	0.78	
	20		20		20		0.25	0.37

(continued)

Table 3. Continued.

	ACS		Placebo		ACS/Placebo			
	Within group		Within group		Difference between groups			
	n	Mean ± SD, ES (p-value)	n	Mean ± SD, ES (p-value)	n	Mean of difference (95% CI)	p-value	ES
Week 3		65.52 ± 22.23		58.82 ± 22.00		8.23		
ES of diff. to BL		0.95 (p < 0.01)		0.84 (p < 0.01)		(−6.16 to 22.63)		
Week 6	20	63.46 ± 17.86	18	57.11 ± 16.61	18	8.49	0.22	0.42
ES of diff. to BL		0.87 (p < 0.01)		0.99 (p < 0.01)		(−5.15 to 22.13)		
Week 12	18	64.46 ± 18.58	18	57.76 ± 17.82	18	11.93	0.05	0.67
ES of diff. to BL		1.48 (p < 0.01)		0.81 (p < 0.01)		(−0.08 to 22.93)		
Week 24	16	69.30 ± 21.22	17	54.15 ± 21.97	16	18.39	<0.01	1.05
ES of diff. to BL		1.52 (p < 0.01)		0.65 (p = 0.02)		(5.99 to 30.79)		
KOOS Sport: Score								
Baseline	20	13.25 ± 14.44	20	24.50 ± 21.82	20	−11.25 (−23.09 to 0.59)	0.06	
Week 3	20	44.00 ± 23.87	20	32.50 ± 26.43	20	22.75	<0.01	1.05
ES of diff. to BL		1.26 (p < 0.01)		0.43 (p = 0.07)		(8.82 to 36.68)		
Week 6	20	33.00 ± 26.38	18	30.83 ± 27.93	18	15.58	0.07	0.62
ES of diff. to BL		0.69 (p < 0.01)		0.20 (p = 0.41)		(−1.16 to 32.32)		
Week 12	18	32.50 ± 27.40	18	31.11 ± 26.10	18	17.77	0.02	0.80
ES of diff. to BL		0.84 (p < 0.01)		0.26 (p = 0.28)		(2.76 to 32.79)		
Week 24	16	45.31 ± 30.74	17	30.88 ± 25.87	16	30.24	<0.01	1.32
ES of diff. to BL		1.21 (p < 0.01)		0.24 (p = 0.34)		(14.16 to 46.32)		
KOOS QoL: Score								
Baseline	20	15.31 ± 12.25	20	26.88 ± 17.69	20	−11.56 (−21.30 to −1.82)	0.02	
Week 3	20	36.25 ± 24.04	20	35.31 ± 19.16	20	12.50	0.09	0.55
ES of diff. to BL		0.80 (p < 0.01)		0.46 (p = 0.06)		(−1.97 to 26.97)		
Week 6	20	37.19 ± 23.78	18	36.81 ± 17.00	18	11.81	0.10	0.55
ES of diff. to BL		0.85 (p < 0.01)		0.63 (p = 0.02)		(−2.48 to 26.09)		

(continued)

Table 3. Continued.

	ACS		Placebo		ACS/Placebo			
	Within group		Within group		Difference between groups			
	n	Mean ± SD, ES (p-value)	n	Mean ± SD, ES (p-value)	n	Mean of difference (95% CI)	p-value	ES
Week 12	18	32.29 ± 23.99	18	32.64 ± 15.83	18	12.15	0.07	0.64
ES of diff. to BL		0.76 ($p < 0.01$)		0.46 ($p = 0.07$)		(−0.81 to 25.12)		
Week 24	16	46.09 ± 30.35	17	37.13 ± 21.02	16	20.54	0.01	0.92
ES of diff. to BL		1.10 ($p < 0.01$)		0.65 ($p = 0.02$)		(4.80 to 36.29)		
KOOS Overall: Score								
Baseline	20	30.71 ± 13.25	20	35.74 ± 17.55	20	−5.03	0.31	
						(−14.98 to 4.93)		
Week 3	20	55.31 ± 20.75	20	49.59 ± 19.26	20	10.74	0.08	0.57
ES of diff. to BL		1.09 ($p < 0.01$)		0.97 ($p < 0.01$)		(−1.37 to 22.85)		
Week 6	20	51.15 ± 19.05	18	48.03 ± 17.48	18	8.84	0.15	0.49
ES of diff. to BL		0.90 ($p < 0.01$)		0.99 ($p < 0.01$)		(−3.25 to 20.94)		
Week 12	18	51.50 ± 20.24	18	47.42 ± 17.90	18	12.12	0.03	0.77
ES of diff. to BL		1.22 ($p < 0.01$)		0.96 ($p < 0.01$)		(1.52 to 22.73)		
Week 24	16	59.98 ± 24.65	17	46.10 ± 21.57	16	20.78	<0.01	1.17
ES of diff. to BL		1.41 ($p < 0.01$)		0.70 ($p = 0.01$)		(8.30 to 33.27)		

KOOS, Knee Injury and Osteoarthritis Outcome Score; ACS, autologous conditioned serum; SD, standard deviation; CI, confidence interval; ES, effect size (Cohen's d, standardized mean difference), defined as very small (0.01), small (0.2), medium (0.5), large (0.8), very large (1.2), or huge (2.0)[44]; BL, baseline; diff., difference.

Table 4. Reported adverse events.

Event	ACS group	Placebo group
Serious adverse events	0	0
Adverse events		
Transient sensation of redness/heat	1	4
Transient increase in blood pressure	4	3
Transient increase in pain	4	3
Numbness in knee/leg/toes	2	1
Knee swelling after physical activity	1	0
Nosebleed	0	1
Thigh skin friction	0	1

Data are presented as number of patients.

KOOS Sport. However, the individual increase in physical activity was not documented.

After ACS injection, all KOOS domains were >20 points better than baseline. It is generally accepted that GCs in patients with OA provide only short-term improvement, usually for 1 to 6 weeks.[34,36] Unexpectedly, the placebo group showed a clinical benefit until week 12 in our study. This may have been a result of pure coincidence, or placebo (saline) might have an additive effect to TA.

The results obtained in this study are consistent with previously reported studies in that ACS was superior to placebo. Although the small cohort of 20 patients receiving add-on ACS to TA 40 mg suggests the safety of the adjunct treatment regimen, replication in a larger cohort should be performed to validate this observation.

Potential benefits

Currently, standard care options in the management of OA are limited in terms of adequate long-term efficacy and safety, potentially resulting in major health issues with severe individual and socioeconomic consequences. The rationale for using adjunct ACS versus placebo in patients treated with TA in our study was to achieve rapid pain relief (aided by TA) as well as a longer-term effect induced by the adjunct ACS injection. This approach aims to narrow the gap of the critical unmet medical need that exists for OA.

OA therapy is largely based on chronic pain medication. Because of the risk of serious and potentially life-threatening side effects, it is crucial to reduce the frequency of interventions and avoid permanent pain medication. Published data question the efficacy of standard i.a. treatments, and the appropriateness of such treatments has not been defined.[18] McAlindon et al.[41] showed that in patients with knee OA, 2 years of repetitive i.a. TA compared with i.a. saline resulted in significantly greater cartilage volume loss and no significant difference in knee pain. The adjunct use of ACS injections may help to reduce the frequency of GC injections and enable doctors and patients to administer these agents according to label guidance and guidelines, thereby reducing intervention to a minimum.

This clinical trial required immediate sequential dosing to minimize the patients' risk and burden. However, this does not exclude the possibility of separating sequential treatments by several days in clinical practice, should routine procedures require such an approach.

A limitation of this study is the dropout of four patients in the ACS group and three patients in the placebo group, decreasing these groups to less than the established sample size (38 patients). Nevertheless, the results are compelling. Further investigation should include the verification of safety and efficacy in a larger cohort and the examination of possible reductions of additional OA pain medication and other therapeutic interventions. We believe that ultimately, OA should become a disease that requires injection treatment and not one that terminally requires surgical interventions.

Conclusion

The addition of i.a. ACS versus placebo to an initial injection of TA 40 mg significantly reduced pain and improved symptoms (function), sports activity, ADL, and QoL at 24 weeks in patients with knee OA. The data of this cohort show that adjunct ACS therapy has a safety profile similar to that of placebo as an adjunct to TA. Superior pain reduction (average, maximum, and at rest) was demonstrated in the ACS group over placebo at 24 weeks.

Add-on ACS treatment to GC injections represents an efficient local pain management therapy that results in rapid pain relief mediated by the GC and durable pain relief and functional improvement mediated by the adjunct ACS. Improved pain and function enable patients with OA to better pursue their daily life activities, hence increasing fitness and resilience against common comorbidities associated with OA.

Declaration of conflicting interest

The authors declare no conflicts of interest.

Ethics approval and consent to participate

This study was approved by the medical ethics committee of the Institute of Rheumatology, Belgrade, Serbia on 28 September 2016 (Number: 21/1-78) and performed in accordance with the Declaration of Helsinki to ensure protection of human subjects. (UTN: U1111-1272-7333; German Clinical Trials Register: DRKS00009969). All patients enrolled in the study were appropriately informed about the study and provided written informed consent for participation.

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Note

1. If both knees were affected, the patients received the study treatment only in the more severely affected knee.

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