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REVIEW ARTICLE

Orthogen Autologous Conditioned Serum: An Update of the currently published Clinical Studies

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ABSTRACT

Orthogen Autologous Conditioned Serum is a proven non-recombinant *auto*biological therapeutic for local treatment of pain, inflammation and tissue injury, functional both in humans and animals. Unlike other autobiologics, Orthogen-ACS stands out wielding the full spectrum of mediators released from all cells present in whole blood, the blood cell secretome. Through a process of extended coagulation of whole blood, Orthogen-ACS contains a unique composition of mediators including but not limited to growth factors, cytokines, lipid mediators, mitochondrial derived peptides and extracellular vesicles. Release of these mediators is triggered by the "tissue" blood when artificially confronted with the tasks *Stop bleeding, Fend off pathogens and Initiate healing*. The clinical effectiveness of local injection treatment with Orthogen-ACS has been shown in a multitude of clinical indications incl. osteoarthritis, radiculopathy, tendinopathy, muscle strain, ligament, meniscal tear, wound healing and others. This review reports on the 44 published human clinical studies dating from early 2000 until recent. Several of these publications report small case number; however, strong clinical evidence exists for knee osteoarthritis, followed by lower back pain and radiculopathy. This broad spectrum of indications suggests that ACS mode of action has profoundly regenerative and immune-modulating properties. In conclusion, ACS therapy is efficacious, safe, simple to process. Orthogen-ACS appears to activate a wide variety of pathways that converge on the attempt of tissue repair and regeneration.

Introduction

Orthogen-ACS is a treatment class pioneered in the 1990s by the Orthogen group in Germany. Unlike other *auto*biologics, Orthogen-ACS exploits the complete spectrum of mediators derived from all cell types present in whole blood, forming a comprehensive blood cell secretome. Orthogen-ACS stands out in relation to e.g. PRP and stem cell treatments by commanding a distinct composition of immune modulatory and regenerative mediators, relevant for inflammation resolution and tissue regeneration.

ACS aims to stimulate natural healing processes and tissue homeostasis, enabling regeneration. In the joint, for example, ACS leads to upregulated IL-1Ra synthesis *in situ,* normalization of synovial fluid viscosity and ROS footprints up to the end of follow up in Shirokova et al.[10] Indications for this therapy are defined by criteria including but not limited to: the presence of a tissue lesion, a disturbance of local tissue homeostasis, chronic inflammation, lack of local regenerative potency. In these indications homeostasis needs to be restituted to avoid/revert chronification. Last but not least, *in vitro* data show a significant support of stem cell vitality and immunomodulatory potency[11] and *in vivo* data show that mouse muscle stem cells are recruited to the site of ACS injection[12].

Novel therapeutic approaches back down from the concept of suppression / blocking of inflammatory pathways. Also, surgical interventions are increasingly seen as a last resort since biomolecular aspects of healing are not sufficiently addressed by operative procedures alone. Novel therapies intend to focus on immune modulation as the relevant therapeutic action. Orthogen-ACS addresses principles of immune modulation.

How is Orthogen-ACS characterized?

Orthogen-ACS is generated by extended coagulation of whole blood in dedicated medical devices at physiologic temperature[1,15,16]. Extended coagulation has been identified as an essential step for its full therapeutic potency to unfold[17]. ACS is separated from the cellular part of blood by centrifugation and can then be reinjected into diseased tissues[18,23-65].

The resolution of pain and inflammation has been shown to be an active process involving the up -as well as down-regulation of multiple genes and pathways. Parisien et al. showed that resolution of chronic lower back pain is linked to a transient neutrophil-driven inflammatory response through S100A8/A9 proteins and a plethora of transcriptome-wide changes[13]. Patients whose pain persisted over three months were unable to unleash dynamic transcriptional changes. In a mouse model anti-inflammatory drugs including dexamethasone and diclofenac –drugs with immune-suppressing action– lead to a delayed resolution of pain despite normal pain relief during drug administration[13]. Similar effects have been observed in the analysis of UK Biobank. NSAIDs and Glucocorticoid (GC) treatment in lower back pain are associated with an increased risk of pain chronification in patients[13].

A new, protected combination therapy allows for safer use of GC, compensating GC-associated immunosuppressive and cytotoxic effects[14]. The combination therapy consists of an adjunct regimen utilizing the benefits of both GC and Orthogen-ACS. GC delivers rapid but short-lived effect with no impact on function and a critical cytotoxic side effect. Orthogen-ACS delivers a long-lived effect on pain as well as function, with slower onset. It appears to compensate the unwanted effects by GC (pain chronification and cytotoxic effect on cells) improving patient safety[14]. This may possibly be effected in part by the S100A8/A9 content in Orthogen-ACS.(Table 1)

Why may Orthogen-ACS improve various clinical indications?

Conceived in the 1990's Orthogen-ACS was first used to treat osteoarthritis (OA) and spinal radiculopathy[18,23-65]. Unaltered whole blood is incubated in purpose built and approved medical devices at physiological temperature. This process triggers the accumulation of an abundant content

of regenerative components in the serum (Table 1) that are -by evolution- geared to *Stop bleeding, Fend off pathogens and Initiate healing*. Confronted by unmet medical need, the ACS technology was applied to a rapidly expanding variety of treated pathologies incl. tendon, muscle, ligament, meniscus, bone, wounds, nerves[18,23-65]. Surprisingly, but importantly, the effects do not only trigger these 3 outcomes above, but they also reach into the resolution of the symptom "pain". The authors suggest that related underlying pathomechanisms exist for various clinical diagnoses. Lack of regeneration and the continuation down a path of degeneration is often at the core of the well-known clinical problem "pain and loss of function", independently of affected tissue. Multiple studies have observed clinical efficacy of ACS, by far exceeding what classical pharmacology has to offer. This implies a profound mechanism of action, much in excess of the half-life of any of ACS' individual components which in sum turn metabolic pathways from "chronic and dysfunctional" to "resolve". This suggest a possible acceleration of healing after surgery and acute injury; such paracrine effects have been described for other biological treatments.

This article looks at the role of Orthogen-ACS in chronic degenerative joint disease, chronic spinal pathologies, and miscellaneous clinical pictures illustrating a wide array of clinical indications and clinical studies accessible to this technology.

Table 1: Components in Orthogen-ACS are involved in triggering a plethora of genes and biochemical pathways converging on targets required to promote healing and resolve chronic pain. ACS resolves run-away inflammation by modes unlike the anti-inflammatory action of NSAID, GC et al., but by "resolution of inflammation" as introduced by Serhan[3,4].

Mediator class	Effects	Exemplary mediators present in Orthogen-ACS
Cytokines[1,2]	Cell proliferation / differentiation immune modulation	IL-1Ra, IL-10, IL-4, TNF, Rantes et al.
Growth factors[1,2]	Cell proliferation / differentiation immune modulation	PDGF, VEGF, TGFb1, IGF et al.
Ω-3 derived specific pro-resolving mediators (SPM)[3,4]	SPM resolve inflammation, are released during incubation and e.g. while Macrophages switch from Type M1 to Type M2	Resolvin, Protectin et al.
Mitochondrial-derived-Peptides (MDP)[5]	modulate adaptative mechanisms and metabolic stress	e.g. Humanin
Neutrophil-Mediators[6, 13]	Alarmins, e.g. prevent pain chronification	e.g. S100A8/A9 elevated 10-fold vs baseline.
other[1,19,20]	Cytoskeleton components, stem cell modulation, modulation of gene expression, antioxidant	Gelsolin, Thy-1, SDF-1a, SCGFb, Bilirubin et al.
Extracellular Vesicles (incl. Exosomes)[8,9,17,21,22]	Immune modulating, resolve inflammation, ATP↑	Concentration ≈10^{10} per ml
EV cargo in Serum[8,22]	Modulate immune- and metabolic functions	RNA/DNA, Proteins, Lipids, Mitochondria

Methods:

The authors performed an analysis of the 44 published papers of clinical studies performed with Orthogen-ACS. Only publications were selected that report human clinical studies. This report is partitioned into three chapters: Osteoarthritis, Spinal and Nerve Tissue, and Other Pathologies. Each chapter consist of a table listing the published studies and the basic facts plus a more detailed summary of each study by year of publication in the same order as in the table.

The subject of this manuscript are the clinical studies. These studies have shown successful clinical effects in numerous indications. The authors intend as overall aim to substantiate the versatility of the *auto*biological action of Orthogen-ACS

rather than the efficacy in one specific clinical entity. Thus, the clinical studies are presented both in tables and also in more detail.

Osteoarthritis.

Cytokines are thought to be among the agents involved in progression of OA[66,67]. Among these, interleukin-1 (IL-1) is considered a key mediator of cartilage degeneration, promoting the production of proteolytic enzymes, inflammatory cytokines, prostaglandins and chemotactically attracting inflammatory immune cells to the joint. These mechanisms can lead to cartilage deterioration if left unchecked[68]. Interleukin-1 receptor antagonist (IL-1Ra), a naturally occurring competitive inhibitor of IL-1, is found in high concentrations in Orthogen -ACS and can block the effects of IL-1[68]. However, recombinant human IL-1Ra (Anakinra, rhIL-1Ra) failed to show clinical efficacy versus placebo in a study in 2009[69]. One conclusion from this may be that IL-1Ra as sole substance cannot be effective in knee OA. OA is a complex pathology, a single-molecule approach may simply not be sufficient for treatment. Biomolecular comprehensiveness may be required. Studies have been published using Orthogen-ACS; most of which found clinically significant efficacy in osteoarthritis.

Baltzer et al.[23] conducted a large, retrospective, unblinded analysis on 1000 patients with Kellgren & Lawrence (K&L) I-III OA receiving ACS therapy. After 3 months the Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores significantly improved 50 - 100% in ≈70% of patients. Improvement lasted for up to 3.5 years in more than ≈35% of patients. After 6000 injections, no serious adverse events were reported. This is the first long-term clinical report demonstrating significant results for intra-articular (IA) ACS injections in OA. ACS is an alternative therapy for treatment of OA.

Yang et al.[37] performed a multicentric knee OA RCT with IA ACS (n=80) versus IA Saline (n=74) in patients with K&L grade I-III. Six injections were administered with ACS or Saline. The (very ambitious) primary efficacy objective was to demonstrate 30% superiority of the Orthogen-ACS treatment on the WOMAC OA indexes at 3-, 6-, 9-, and 12-months post-treatment, compared to placebo treatment. This ambitious objective was not met. However, values of WOMAC and most other outcome measures showed that Orthogen-ACS treated patients versus placebo-treated patients scored consistently better. Orthogen-ACS superiority was found to be statistically significant by KOOS symptom score and KOOS sport score. Altogether, these findings suggest a beneficial biological effect of ACS on clinical symptoms arising from knee OA.

Baltzer et al.[38] performed an RCT with 376 patients with knee OA (K&L II and III). 134 patients were injected with IA ACS, 135 with HA, 107 with saline. ACS patients received 6 injections (2 per week), while HA and saline groups received 3 injections (1 per week) of 2 ml HA (HYA-Ject®, Ormed, Freiburg, Germany) or 2 ml of saline and alternating 3 weekly topical heparin-natrium cream applications. All patients had the same number of visits. Followed up was performed with VAS, WOMAC, PGA, SF-8 at 7, 13, and 26 weeks. IA injections reduced symptoms and improved quality of life in all treatment groups. At 26 weeks, VAS and WOMAC for ACS versus baseline was significantly better than for HA or the saline group (67%, 32%, 33%, and 53.8%, 38.4%, 31.3%, respectively). For all outcome measures and time points, ACS was significantly superior to HA and saline, no differences were detected between the effects of HA and saline. The frequency of adverse events with ACS was comparable with the saline group but higher in the HA group. ACS injection significantly improves clinical signs and symptoms of OA. A telephone questionnaire 2 years after therapy confirmed stable clinical results in those patients available for questioning.

Noskov et al.[43] prospectively evaluated clinical efficiency of IA ACS (activated serum n=30) versus hyaluronic acid (HA n=24) in Hip OA K&L I-III. For 3 weeks, Groups 1 and 2 received IA therapy with 6x ACS or 3x Sinochrome forte (40mg, Croma Pharma, Sotex), respectively. At 1, 3, 6 and 12-months, follow-up was evaluated by VAS, WOMAC

and general health score (EQ-VAS). HA was superior by WOMAC (–21.8%; t=2.56) only at 1-month follow-up. After 3 months ACS group had VAS reduction 76.5% over (t=4.31) the HA group. ACS was also superior over HA at 6 months. The authors summarize: Autologous activated serum is practically the same as the method of HA treatment but is characterized by a longer duration of the positive results.

Baltzer et al.[44] retrospectively reported effects of IA ACS injections in OA hips (K&L II-IV) in a retrospective, non-blinded, non-randomized study. Aim was to evaluate whether glucocorticoids (GC) and recombinant IL-1Ra (Anakinra, rIL-1Ra) boost ACS treatment efficacy. Forty-six patients received ACS, 56 patients received up to 6x 2 ml ACS plus GC (10 mg Triamcinolone, up to 2x), and 17 patients received ACS plus GC (up to 3x) plus rIL-1Ra (up to 4x 0.2mg). VAS data were available for baseline and at an average of 14.35 months post treatment. Patients in all three groups had statistically significant improvements with large effect sizes versus baseline, independent of the severity of the osteoarthritis. VAS scores of ACS patients were significantly lower than for the ACS+GC patients. GC or GC+rIL-1Ra did not increase the beneficial treatment effect over and above the effect of ACS alone. ACS successfully reduces pain in patients with hip OA.

Abd-El Motaal et al.[24] prospectively treated 30 patients with knee OA (K&L I-III) with low dose IA ACS. For three weeks, the knee joints received weekly injections of 1 ml of ACS (half of the recommended dosage). Knee scores were assessed at weeks 1, 2, 3, then on months 1, 2, 3. All WOMAC scores improved significantly versus baseline at each study point, and scores changed significantly after each injection. WOMAC total improved by 82% at 3 months follow-up. In contrast to other studies, WOMAC stiffness score increased by 96%. ACS is easily prepared with no risk for disease transmission (autologous serum) and has good functional and psychological satisfaction and is a promising strategy for treatment of OA.

Rutgers et al.[25] conducted a prospective follow-up study on a group of 74 knee OA patients who previously received IA saline (placebo) in an RCT[37]. 20 of the saline patients chose to have ACS treatment. IA ACS treatment did not improve OA symptoms more than previous placebo treatment. Due to the study's limited power and potential selection bias, advice on whether to use ACS was not given.

Baselga and Hernandez[18] reported a 2-year prospective observational study of surgery candidates with highly symptomatic knee OA (K&L I-IV). Patients received IA ACS (4 injections, 1 per week) in conjunction with physiotherapy (three times per week for 10 weeks, starting 4 weeks post treatment). Patients were followed up with NRS pain at 3, 6, 12, and 24 months and WOMAC at 24 months. For 118 patients with unilateral knee OA (NRS > 6), ACS produced a statistically significant reduction in pain, lasting 2 years (at 3 months NRS decreased by 63% and remained the same at 2 years). At two years, there was also a statistically significant improvement in WOMAC scores (all K&L grades). One patient received total knee replacement in the duration of this study. ACS, when used in conjunction with physiotherapy, resulted in a rapid pain reduction and an increase in function lasting throughout the study. Gender, age, K&L grade, or BMI had no statistically significant effect on clinical efficacy. These findings provide essential information on the efficacy of ACS combined with physiotherapy in a real-world setting.

Öç et al.[26] prospectively treated 33 patients with bilateral knee OA (K&L II-III and VAS >4) with VAS, KOOS, KSS at 12 months after 6 weekly IA injections of ACS. 1 year after end-of-treatment, all scores (VAS, KOOS, KSS) had improved statistically significant. VAS improved by 53.5%, KOOS by 53.5%, KSS by 68.3%. IA ACS for painful knee OA has an excellent safety profile that results in a strong clinical efficacy. ACS is a well-tolerated alternative to currently available OA treatments.

Tassara et al.[28] retrospectively reported on 28 patients with knee and hip OA (hip OA n=3, knee

OA n=25). Patients received 4 weekly IA injections of ACS and were assessed with VAS and range-of-motion (ROM) at week 4 (immediately after the 4th injection), at 2 and at 7 months. VAS improved significantly from the first injection to 7 months, median VAS reduction was 60 (75% reduction). The median knee ROM increased by 25° between week 1 and month 6. 10 of 14 patients (71%) on chronic medication (NSAIDs, COX-2, steroid, Paracetamol, Paracetamol plus Tramadol, steroids plus Methotrexate) stopped medication after ACS therapy. ACS produced rapid decline in pain and large improvement in ROM. In younger adults with mild-moderate OA, ACS was better for treating subacute or chronic inflammation of the joint and capsule and for delaying surgery. In patients who relapse after experiencing a significant benefit, ACS can be repeated several times. ACS is a viable treatment option for OA when surgery is contraindicated or refused by the patient.

Zarringam et al.[30] conducted a retrospective follow-up study to investigate the long-term effect of IA ACS injection from subjects in their previous RCT (Auw Yang et al. 2008). Seventy-two patients (K&L I-III) who were in a previous RCT had received ACS versus 54 patients who had received saline in their osteoarthritic knees. These patients were contacted to determine whether any IA surgical intervention or osteotomy of the study knee had taken place during the past decade. After 10 years, 46.3% of the placebo and 40.3% of the ACS group had been treated surgically. Authors concluded ACS injection did not significantly delay the need for knee arthroplasty over a 10-year period versus saline. Only incidence of knee arthroplasty in patients was investigated in this study. A survival plot shows advantages of ACS therapy particularly from 20 months. The Orthogen-ACS group showed better survival after 7.5 ± 3.9 years of follow-up. 54 events occurred with an estimated mean time of 102.66 months (standard error [SE] = 4.56, 95% confidence interval [CI] = 93.73-111.60) since the initial Orthogen-ACS study. 29 events in the Orthokin group had an estimated mean occurrence time of 109.27 months (SE = 5.52, 95%CI = 98.45-120.09), while the 25 events in the placebo group had an estimated mean occurrence time of 93.76 months (SE = 7.56, 95% CI = 78.94-108.58). This is 1 year earlier in the placebo group than the ACS group.

Hashemi et al.[39] conducted an RCT in which 60 patients with knee OA (30 in each group with K&L II-III) received three weekly injections of either ACS or HA. These patients were evaluated with NRS at 1, 3, and 6 months and with WOMAC, KOOS at 6 months. ACS group was statistically significant better by NRS at 1 and 6 months, by KOOS symptom, ADL, and sport at 6 months. Authors conclude: ACS has beneficial biological effects in patients with knee OA, is minimally invasive, safe, and effective for patients suffering from chronic knee pain.

Kilinç and Öc[30] treated 33 patients (66 knees) with K&L II-III knee OA. Patients received 6 weekly IA injections and follow up by NRS, KOOS, KSS at baseline and at 12 months. Retrospectively at 12 months, all 33 patients with bilateral knee OA reported statistically significant improvement for all scores versus baseline. Mean NRS improved from 7.36 to 3.27, KOOS total improved from 42.4 to 72.4, and KSS improved from 42.8 to 70.6 at 12 months. IA ACS injections in patients with painful OA led to statistically and medically significant improvements in pain, KOOS and KSS scores. ACS is an effective treatment for patients with medium grade OA.

Vitali et al.[31] published a prospective study. 15 subjects with K&L I-III knee OA, were followed for 6 months after 4 weekly ACS injections. NRS, WOMAC, KSS were used to assess the patients at 1, 2, 3, 4 weeks and 6 months. At 6 months post IA ACS treatment, VAS, KSS function, KSS clinical, and WOMAC scores were improved significantly (NRS decreased 35.8%, KSS function improved 38.2%, KSS clinical improved 28.9%, and WOMAC improved 19.8%). Most common complaint was pain and swelling in the days following the IA injection, with one patient reporting rigidity. This study, in conjunction with preexisting studies supports the viability of this therapy for the

treatment of knee OA. ACS represents the new direction of disease-modifying osteoarthritis drugs (DMOADs) for treatment of knee OA by targeting the specific compounds.

Shirokova et al.[10] published a prospective study with 123 female patients with K&L II-III knee OA with 6x ACS (2.5mL) (65 patients) versus 6x PRP (5mL) (58 patients) in an open setting. By VAS after 3 months ACS was superior to PRP in patients with subclinical synovitis ($p=0.03$) and moderate synovitis ($p<0.001$). By WOMAC after 3 months ACS was superior to PRP in patients with subclinical synovitis ($p=0.044$) and moderate synovitis ($p<0.001$). Importantly this study evaluated IA biochemistry also. After 1 month IL-1b in the ACS group was statistically significant lower versus PRP ($p=0.008$) while IL-1Ra was statistically significant higher ($p=0.016$). Synovial fluid viscosity was better in ACS, ROS and NO* loads were statistically significant lower in ACS at 3 months. ACS and PRP were safe and exerted a differential therapeutic effect on knee OA. PRP was not effective in cases of moderate synovitis/effusion, ACS was. ACS was significantly superior to PRP in all groups at the 3-month follow-up and met the MCII criteria for VAS in knee OA. Biochemical data revealed rejuvenation-associated properties of ACS from reduced reactive oxygen species footprint CDs to improved synovial fluid. Strength of this open clinical study is the combination of clinical and biochemical data, all showing that ACS is superior to PRP. Peripheral blood biomarkers for OA are unreliable; therefore, routine inclusion of SF analysis in OA therapy studies is advocated. This is easy to implement when multiple injections are performed anyway.

An RCT by Hashemi et al.[40] compared ACS to ozone (common outsider therapy in orthopedic clinics) in patients with knee OA. This study included 60 patients with K&L I-II knee OA (30 in each group). ACS group received 4 injections of ACS (2 ml) over 4 weeks, whereas the ozone group received 10 ml of ozone (30 g/ml) plus 5 ml of lidocaine (1%) on day 1, 1 and 2 months. NRS, WOMAC, and KOOS were used to assess the patients at 6 months after end of treatment. NRS were statistically significant different between the two groups at 6 months (57% versus 28%). ACS group had statistically significant better KOOS scores for symptoms, daily activities, and sports functions. Physical function, stiffness, and overall score for the WOMAC score were statistically significant better in the ACS group. Clinical improvements and responses to ACS are better and last longer in patients with knee OA than ozone. ACS is low invasive, safe, effective, and long acting in patients with knee OA.

An RCT by Pishgahi et al.[41] compared dextrose prolotherapy (n=30, 2 ml of 50% dextrose weekly injection for 3 weeks), PRP (n=30, weekly for 2 weeks), and ACS (n=32, weekly x 2 weeks) in patients with K&L II-IV knee OA. Follow ups were evaluated by NRS and WOMAC at 1 and 6 months. ACS patients had the highest percentage of radiographic K&L IV findings (53.1%) compared to dextrose prolotherapy (36.7%) and PRP (30.0%). Obese classification also was the highest in the ACS group. ACS and PRP patients improved statistically significant by pain and function after 1 and 6 months. At 6 months after treatment, NRS reduction for ACS patients was 42%, versus 10% for PRP versus 5.5% for prolotherapy. ACS WOMAC scores improved by 38% versus PRP by 24%, versus prolotherapy by 9.6%. No significant changes in pain or function were observed in the dextrose prolotherapy group. OA therapy with ACS and PRP is associated with improvement of pain and function. In this study ACS therapy is statistically and medically significant more effective than PRP, which additionally has more variability in processing and more reported side effects. ACS has both inflammation resolving and regenerative properties, which PRP does not have.

Godek et al.[41] retrospectively evaluated 1000 cases of ACS treatment for miscellaneous indications in detail. 4-6 ml of ACS were injected in these patients which were pooled cases from 3 centers. Results were classified after 2 and 6 months by A through D. A: Excellent, B: Good, C: Fair or D:

Poor. The conclusion was: Orthogen-ACS is highly effective in tendinopathy, enthesopathy, small joints OA of the hand and in early stages of knee OA. Satisfactory results are seen in cervical and lumbar discopathy, unsatisfactory results in severe degenerative changes in knee and hip joints and in spinal canal stenosis.

Khurana et al.[33] retrospectively compared PRP, HA, GC, and a matched cohort of ACS in patients with knee OA (K&L I-III). ACS (n=21), PRP (n=27), GC (n=28) Depot-Methylprednisolone 40 mg, HA (n=20, Synvisc) were followed at 2 weeks, 3 and 6 months with VAS and WOMAC. PRP and ACS were better than control groups HA and GC. ACS had better WOMAC and VAS mean outcome scores (from baseline to 6 months) versus PRP, the difference not statistically significant (VAS reduction of 61% versus 53% and WOMAC improvement of 70% versus 60%). The HA group was statistically significant better by VAS than GC. Both PRP and ACS are judged effective for OA pain at 6 months. IA GC and HA give initial pain relief, HA provides more significant pain relief until 6 months than GC. Comparison of the PRP, HA and GC groups to a historical cohort of ACS patients possibly complicates the conclusions.

Hussein et al.[34] retrospectively compared IA HA (n=171, 2 ml, 20 mg), ACS (n=222), BMAC (n=112) for the treatment of knee OA. These patients received 6 ACS injections, 3 weekly HA injections, and one injection of 8 ml of BMAC. They were assessed with VAS and WOMAC at 3 and 12 months after injection. All three treatments improved significantly in NRS and WOMAC after three months. At 12 months, NRS improved significantly in all three groups. ACS versus HA versus BMAC (40% versus 33% versus 35%). BMAC group showed sustained improvement in WOMAC score at 12 months. BMAC is more effective than HA and ACS knee OA, particularly in patients with more severe degenerative changes. A significant number of patients were lost to follow-up between three and twelve months after treatment. Of interest is that the reduction in WOMAC score for the ACS group was unusually small (16%) in this study. The difference to BMAC is small.

Leone et al.[28] prospectively followed 30 subjects (K&L grade I-III) who received ACS injections after failed standard medical treatments (physiotherapy, Hyaluronic acid (HA) and Platelet-Rich Plasma (PRP)) for knee OA. Patients received 4 weekly injections of ACS and were evaluated at 1, 6, and 12 months after with VAS and Lequesne scales. Immediate (1 month) improvements in VAS and Lequesne scales were demonstrated in 67% of patients, and the effect persisted at 6 and 12 months in responders (the 20 responders are subjects who improved by more than 33% on VAS). Their results showed a significant improvement in VAS from 70 to 30 at 12 months after injection. At a median follow-up of 24 months, all responder patients had stopped receiving medical treatments, avoided surgery, and remained in response. Furthermore, responders had significantly more IL-1Ra in ACS than non-responders. This study confirmed the efficacy of ACS in pain control and functional recovery of patients with knee OA resistant to medical and PRP treatment; moreover, it may help postpone orthopedic surgery after medical and/or PRP treatments have failed.

Simon et al.[46] performed a prospective longitudinal observational study in 34 patients (40 shoulders) with glenohumeral OA (K&L II-III) to see if ACS (up to 6 weekly injections) improved symptoms and delayed the need for a shoulder replacement. ASES, SPADI, CSS, ROM, and NRS were used to assess the patients at baseline and at 3 months. NRS, SPADI, and need for total shoulder replacement were used to assess these patients at 2 years. There was a statistically significant improvement 3 months after injection (NRS, CSS, ROM, SPADI). Six patients received a second ACS injection series (at 1.5 ± 0.7 years) after the first series, two of these six patients had total shoulder replacement one year later. Sixteen patients (47%) had shoulder replacement after an average of 1.8 years, while the remaining patients had no shoulder replacement after 3.6 years. ACS

injections in OA shoulders may reduce pain and disability while delaying the need for a shoulder replacement.

Cortegiani et al.[45] prospectively recorded 26 patients with K&L I-III treated with 4 weekly ACS injections. 13 patients for facet syndrome (ODI), 9 patients in the lower limb (WOMAC) and 5 patients in upper limb (Quick Dash). NRS, SF-36 and Karnofsky performance status were used to evaluate all patients at 0-3 weeks and 1, 3, and 6 months. They reported a significant reduction of pain from the first injection (median VAS value of 7.5) to 6 months (median VAS value of 2). There was significant difference between the VAS, Karnofsky performance status, SF-36 sub-scale for mental health, ODI, Quick-Dash at different timepoints. ACS injections produced promising results, reducing painful symptoms, improving joint function and improving quality of life for up to 6 months without significant adverse effects.

Vitali et al.[31] published a prospective, controlled study in patients with knee OA (K&L I-III). Patients (n=12) were treated with a single injection of Bone Marrow Aspirate Concentrate (BMAC) versus ACS group (n=12) treated with four weekly injections. At 1 and 6 months, patients were evaluated by NRS and WOMAC. Both had improvements in NRS and WOMAC at 1 and 6 months. BMAC versus ACS was statistically significant in NRS at 1 and 6 months and at WOMAC 6 months. WOMAC BMAC improved 37.9% at 1 month and 66.1% at 6 months, ACS improved 17.2% and 16%. NRS BMAC group was 43.3% and 59.3% at 1 and 6 months respectively, NRS ACS was 35.9% and 33.5%. No significant difference between groups at ROM improvement, significant reduction of NSAIDs consumption in the BMAC group versus ACS group at 6 months (58.3% versus 8.3%). Both BMAC and ACS are effective and safe treatment for osteoarthritis, but BMAC is more efficacious than ACS.

Of interest: ACS subjects had less improved WOMAC (16%) and VAS (33.5%) scores at 6 months, compared to previous studies. Hang et al. reported VAS improvement of 56.3% and WOMAC improvement of 59.4% at 6 months. Baselga & Hernandez reported a WOMAC improvement of 56% and VAS improvement of 65.9% at 24 months, based on patients with KL grade I-IV knee OA. The lower-than-usual scores may have resulted from limitations of this study as stated by the author (small sample, lack of randomization and short follow up). Another potential reason for the better performance of the BMAC group might be the much more invasive nature of the intervention.

Ippolito et al.[2] reported on 26 patients with K&L I-III OA (hip, knee, ankle, low back, and upper extremities) which were followed 1, 2, 3, 4 weeks and 3 and 6 months after 4 weekly injections. Patients were assessed with NRS, WOMAC, Quick DASH, ODI, SF 36 and Karnofsky performance status. All outcome measurements showed statistically significant difference versus baseline at 3 and 6 months. NRS improvement of 31% at 6 months (from 8 to 5.5) and WOMAC improvement of 35.14% (from 62.6 to 40.6) were reported. This is the first report that identified the presence of cytokines CTACK, Eotaxin, RANTES, Gro alfa, SDF1a in Orthogen ACS, which may have potential roles as disease-modifier for osteoarthritis. ACS may be a feasible option for patients with chronic pain due to K&L I-III osteoarthritis refractory to other treatments.

Coşkun et al.[35] retrospectively compared long-term (five-year) clinical outcomes of PRP (n=42) versus ACS (n=40) patients after receiving 3 weekly injections of either PRP or ACS. At 1, 6, 12, 24, and 60 months after injection, clinical evaluations with KOOS and NRS were performed. Both groups experienced statistically significant decrease in NRS at 6 and 12 months versus baseline. Compared to PRP, there was a statistically significant change in NRS, KOOS symptoms, KOOS pain, and KOOS ADL in the ACS group at 12 and 24 months. Prevalence of side effects was significantly higher in the PRP subjects (38% versus 5%). Effectiveness of ACS and PRP treatments was maintained for one year, decreased after two years. Future reports should include biological analysis of joint fluid to objectively evaluate both IA therapies.

Hang et al.[36] published a retrospective, comparative observational clinical study on IA ACS injections versus Dexamethasone GC in 34 patients with bilateral knee OA (subjects randomly received ACS in one knee and GC in the contra-lateral knee). All patients received 6 IA injections of ACS into one knee over three weeks, while the contralateral knee received a single 5 mg Dexamethasone injection. Intra-group improvement in clinical symptoms post treatment in both groups was statistically significant at all follow-up time points (3, 6, and 12 months). Improvements of VAS, WOMAC global, WOMAC pain, WOMAC stiffness, and WOMAC function at 3, 6, and 12 months were statistically significant better in ACS. PGA also confirmed that knees injected with ACS benefited more than knees injected with GC. IA injections of either ACS

Spine (Radiculopathy) and Nerve Tissue

As in OA, spinal pathologies also involve degenerative changes of various tissues. These include the facet joints, intervertebral discs, vertebrae and neural components of the spine. One common denominator is often the inability to resolve pain and regenerate tissue. Degenerative spinal pathologies often comprise a considerable neuropathic component, further complicating the therapeutic approach. Peripheral and central sensitization play a substantial role in pain chronification associated with spine pathologies involving a malfunctioning immune-system[49]. Current standard of care follows similar principles as treatment of OA, amongst others: analgesic medication with immune-suppressing modes of action, anti-neuropathic medication with unclear effects and unwelcome side effects or surgery (such as neural decompression, disc replacement or fusion). Spinal surgeries are commonly associated with chronic postoperative pain. Persistent pain after surgery is reported in up to 40% of cases[47,48]. Enormous clinical problems associated with prolonged rehabilitation, reduced quality of life, long-term intake of pain medication, reduced mobility and thus risk for additional comorbidities arise from such[49].

The high prevalence associated with postoperative pain raise concern on our current therapeutic approaches that exclude molecular aspects and immune processes.

Effective pain therapy with Orthogen-ACS has been published for spinal diseases[49-54,65]. A newer publication demonstrates Orthogen-ACS efficacy also in trigeminal neuropathic pain[55].

Becker et al.[49] performed an RCT comparing ACS to two different triamcinolone dosages (5 and 10 mg) in lumbar radicular compression. ACS group (n=32) received three 2 ml weekly epidural injections, 27 patients received 5 mg triamcinolone and 25 received 10 mg triamcinolone by the same technique. VAS and ODI were used to assess the patients at 6, 10, and 22 weeks. The results showed that all 3 groups experienced significant reductions in pain and disability. From week 12 to week 22, ACS outperformed both triamcinolone groups in terms of VAS score. ACS showed statistical significance at week 22 versus the triamcinolone 5 mg group. At no timepoint there was a statistically significant difference between the two triamcinolone dosages. ACS is a viable treatment option for patients suffering from unilateral lumbar radicular compression. The reduction in pain was significant, clinically remarkable, and potentially superior to steroid injections. Later follow-ups were not performed.

Goni et al.[53] in an RCT compared the efficacy of perineural injection (1 injection) of 2-3ml ACS versus similar volume methylprednisolone (GC) in 40 patients with unilateral cervical radiculopathy (20 subjects in each group). VAS, NPDS, NDI, SF-12 were used to evaluate the patients at 3 weeks, 3 and 6 months. At 6 months, patients in both groups showed significant improvement in pain intensity (VAS and NPDS) and disability (NDI). VAS improvement in ACS patients was 73.24% from baseline to 6 months, versus 58.54% in GC patients. Similarly, at 6 months, NPDS improved 73.76% in the ACS group versus 55.60% in the GC group. The ACS group experienced gradual

improvement continuously progressing throughout the follow-up period, whereas the GC group did not improve after follow-up 1 (3 weeks). No major complications were noted. In patients with unilateral cervical radiculopathy, ACS is an equally good or better non-operative management modality than GC. ACS may be offered to affected patients prior to surgery due to its acceptable safety profile.

Ravi Kumar et al.[51] treated 20 patients with unilateral lumbar radiculopathy. They performed epidural injections of ACS at 7 days intervals (average of 2 injections, ranging from 1 to 3) under bi-planar fluoroscopy. NRS, Straight Leg Raise test (SLR), revised ODI (RODI), and SF-12 were used to assess patients at 3 weeks, 3 and 6 months. All parameters changed significantly from baseline to 3 weeks, 3 months, and 6 months after injection. The improvement was immediate after 3 weeks (VAS, RODI, and SF-12) and continued from 3 weeks to 3 months, to 6 months. The NRS improved significantly from baseline to all follow-ups (baseline 6.95 to 3.65 to 2.55 to 2.00 at 6 months), with a 71.2% reduction compared to baseline. The RODI scores had significant changes from baseline to first, second, and third follow-ups. There was also a significant difference from the first follow-up to the second follow-ups (from 12.95 to 17.4) and between the second to the third follow-ups (from 17.4 to 19.4). The SF-12 revealed similar significant results. ACS may modify disease progression to reduce pain and disability and improve general health.

Godek[52] reported 15 patients with MRI-confirmed single-level nucleus pulposus herniation and signs of radicular compression without paresis. Godek administered 6 doses of ACS to the area of the intervertebral foramen. VAS, OLS test, SLR test, and ODI were assessed at 1 and 3 months. Thirteen of fifteen patients had a statistically significant reduction of VAS pain (from baseline of 48 to 30 to 25 at 3 months), OLS test and SLR test, and ODI at 3 months after injection. ODI significantly improved from a baseline of 39.2% to 20.9% after 1 month and to 14.7% after 3 months. There was no radicular damage or serious complications from the treatment. Two patients chose surgery because of increasing pain and signs of paresis. The hernias in both cases were massive (>8 mm) and filled the vertebral canal and lateral recess. ACS therapy is an alternative to local and systemic anti-inflammatory treatment.

Godek et al.[65] Ninety patients with cervical neuropathy were assigned to six treatment groups. Biological treatment: 4 ultrasound-guided peri radicular injections of ACS or PRP (1 per week); Mechanical treatment: manual therapy (MT) or traction therapy (TT) - 8 sessions (two per week); Physical treatment: laser therapy (LT) or collagen magnetophoresis (CM) - 8 sessions (two per week). Assessment with NRS (0-10), NDI (0-50) cross section root area (in mm^2) in ultrasound examination (CRA) and hand sensorimotor function test (DPT) at baseline (W0), after treatment (W1), 2 months (W2). Biological treatments were more effective than mechanical and physical therapies for pain reduction, ODI and proprioception of the hand improved immediately after completion of therapy and after follow-up, which may suggest regenerative properties. ACS therapy produced the highest percentage of patients achieving minimal important difference in NRS and NDI.

Aghamohammadi et al.[55] published a case series regarding the efficacy and safety of ACS for the treatment of trigeminal neuralgia (TGN) in 11 patients by injecting ACS (4 weekly injections) into the foramen oval (FO). The inclusion criteria were >3 pain attacks per day, pain intensity >4 on VAS at least 7 days before treatment, and a history of more than 3 months of carbamazepine, gabapentin, pregabalin, or tramadol use with poor clinical response. At 1, 2, 3, 4 weeks, and 2 months VAS was used for assessment. At 2 months after ACS injections patients had a statistically significant reduction in NRS pain intensity (NRS decreased from baseline 8.18 to 3.36). Carbamazepine consumption was statistically significant lower at 3 weeks. This was maintained at 4-week and 2-month follow-ups (from 636 to

200mg). No serious adverse events were observed. The results of ACS injection into the FO provide solid preliminary evidence that ACS is an effective and safe pain treatment for refractory TGN.

In an RCT, Godek et al.[54] evaluated the safety and analgesic efficacy of two different ACS delivery methods (perineural versus epidural). Godek had established solid clinical evidence for ACS therapy in back pain. Now he wanted to find out if treatment of low back pain secondary to lumbar disc herniation, intervertebral disc disease, and lumbar spinal stenosis can be achieved with a less demanding injection technique. Patients were randomly assigned to 2 groups (n=50 each) to receive 2 weekly spinal injection of ACS. Efficacy was evaluated with NRS, ODI, RMQ, EQ-5D-5L at week 4, 12, and 24. Both groups improved statistically significant in pain and disability NRS 5.7/6.2 to 2.8/2.4 at endpoint; no statistically significant difference between the 2 groups at any timepoint. There was significant improvement at week 4 and continued improvement until 6 months. Both methods are equally effective for treatment of low back pain secondary to lumbar degenerative disc disease. The perineural route should be preferred because of potential risks associated with the epidural interlaminar approach.

Other Pathologies

Orthogen-ACS is rich in regenerative and immune-modulatory mediators including cytokines, extracellular vesicles (incl. exosomes) and growth factors including insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), hepatocyte growth factor (HGF), transforming growth factor-beta 1 (TGF-1), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). The therapeutic use of ACS, a saturated secretome, has been validated in human studies also for soft-tissue disorders. Since the Orthogen-ACS composition addresses a wide variety of biochemical pathways it has been utilized in further clinical indications beyond those treated initially. This approach has been attractive since the identified mediators have a general invigorating effect on dysfunctional and injured tissues.

Muscle and Tendon

Wright-Carpenter et al.[12] treated athletes with muscle injuries by local injection of ACS in the injured area versus the standard therapy Actovegin /Traumeel injections. All patients had moderate muscle tears (second degree) with bleeding visible on MRI and moderate loss of strength. Eighteen athletes received an average of 5.4 injections of ACS starting 2 days after injury and repeated injections every other day. 11 athletes received an average of 8.3 injections by the same roster. Assessment method was time until return to 100% training and isokinetic testing with >90% strength recovery versus contralateral limb. ACS subjects statistically significant recovered in 16.6 days versus 22.3 days in control group. This is significant (p=0.0004, effect size Cohen's d: 1.45). MRI analysis revealed that all ACS subjects had complete regression of bleeding/edema versus baseline and regeneration/reconstitution of muscle fiber at 16 days after injection. At that time, it was thought that FGF-2, HGF, and TGF-β1 may be responsible for the significant effect on muscle regeneration.

Von Wehren et al.[56] treated chronic Achilles tendinopathy patients with ACS versus patients performing eccentric exercise in a retrospective study. ACS subjects (n=25) were given 2 ml ACS *in punctum dolens* and three weekly peritendinous injections. The eccentric training group (n=25) followed eccentric training according to Alfredson et al.[55] for 3 months. MRI were taken before and six months after the ACS or eccentric training. VISA-A-G was used for clinical evaluation and showed statistically significant improvement versus baseline in both groups. Change versus baseline comparison between both groups shows statistical significance in favor of ACS with an effect size d of 0.62 which is above medium (0.5). Tendon thickness and quality improved from baseline to 6 months. There was no statistically significant difference in MRI findings between groups. ACS injections provide

greater clinical long-term benefits than eccentric training and are a viable alternative.

Damjanov et al.[58] In a 24-week RCT Damjanov et al. looked at the effects of ACS injection on supraspinatus tendinopathy versus GC. The ACS group (n=15) received 4 weekly ACS injections, the GC group (n=16) received 3 weekly Betamethasone injections plus one saline injection at week 4 into the supraspinatus tendon enthesis and paratenon. VAS and CSS were used to evaluate patients at 4 and 24 weeks. Pain intensity improved in both groups after 4 weeks. ACS subjects had improved statistically significant versus GC at 24 weeks. At 24 weeks, VAS pain decreased in the ACS group from 70 to 15, GC subjects improved from 65 to 40. At 4 weeks, both groups' CSS had improved equally. At 24 weeks, ACS patients' CSS scores had improved statistically significant versus GC ("excellent results" changed from 13.3% to 80% in the ACS group versus 12.5% to 13.3% in the GC group). ACS therapy improved joint function and reduced shoulder pain more effectively and consistently until week 24. ACS may improve quality of life in patients with chronic rotator cuff tendinopathy.

Anterior Cruciate Ligament

Darabos et al.[60] (RCT). Bone tunnel enlargement is a potential cause of failure after anterior cruciate ligament (ACL) reconstruction. IL-1, through its bone resorption and osteolytic functions, may play a role in the pathogenesis of bone tunnel enlargement. During and post ACL reconstruction patients received either ACS IA or saline IA injections (31 subjects in each group) on day of the surgery and day 1, 6, 10 post-op. Follow up was conducted by WOMAC, IKDC 2000 at 6 and 10 months, CT was conducted on post-op day 1, 6 months, and 12 months, serum and synovial fluid IL-1 concentration was determined for post-op day 1, 6, 10. The ACS group had statistically significant less bone tunnel enlargement versus saline at 12 months (3% versus 13%). Bone tunnel enlargement was more pronounced in the hamstring tendon group versus the Bone-Tendon-Bone group. At all data points and for all outcome parameters, clinical outcomes (WOMAC, IKDC 2000) were consistently better in the ACS group. At 1 year there were statistically significant differences in the WOMAC stiffness subscale. Synovial fluid IL-1 concentration was statistically significant lower in ACS group versus saline. IA ACS injection reduces bone tunnel widening after ACL plasty. ACS has beneficial biological effects on patient-documented symptoms and structure preserving effects after ACL bone tunnel plasty.

Comment by the current authors: This study has received too little attention. Here it was shown that Orthogen-ACS has the potency to inhibit bone resorption or bone lysis. In other medical fields this would have attracted more attention since bone deterioration is a serious problem, also in conjunction with medication that must be taken constantly over many years. Orthogen-ACS might be wielding tools that deserve pursuing. Could surgery possibly profit from ACS to address a widespread phenomenon in ACL plasty and other bone surgeries?[70-72]

Meniscus

Strümper et al.[61] In this retrospective case analysis (n=47), patients with meniscal lesions received weekly ACS injections (average 5.2 injections). OKS and BLOKS were used to evaluate the patients at baseline and at 6 months after treatment. Surgery was avoided during the 6-month observation period in 83% of the patients. At 6 months OKS had changed statistically significant from 29.1 to 44.3, and BLOKS improved statistically significant from 0.81 to 0.71. OKS pain improvement was stronger than OKS functional improvement, consistent with previous knee pain WOMAC results. OKS improvement is safely within the minimally detectable difference (MID). IA ACS injection is an effective treatment option for knee pain associated with meniscal lesions. Post-surgical ACS may improve the healing of complex meniscal tears following arthroscopic meniscal repair.

Strümper et al.[62] Retrospective study of IA meniscal fibrin glue fixation combined with Orthogen-ACS injections in subjects with MRI-confirmed meniscal defects. In this retrospective out-patient case

study, 170 subjects with meniscal lesions of the knee were treated with MRI-guided fibrin glue injection into the lesion followed by weekly IA ACS injections (4-6 weekly injections). WOMAC scores were collected at baseline, 6 weeks, and yearly intervals for up to 4 years. During this time 8 patients chose meniscal surgery. 162 patients avoided surgery. The average WOMAC global score fell from 33.83±18.99 to 13.18±9.52 in 4 years. This archive study of a heterogeneous real-life cohort showed that IA fixation of meniscus injuries with fibrin glue followed by IA ACS injections improved knee symptoms associated with meniscal injury.

Wound

Gholian et al.[63] in a single-blinded RCT investigated efficacy and tolerability of ACS as wound dressing in local management of difficult-to-heal wounds. 30 patients with chronic wounds of miscellaneous genesis (2.8-3.0 months duration) were randomly assigned to either ACS dressing applied once a week for three weeks or normal saline-moistened dressings. ACS versus Saline produced statistically significant differences in wound surface area and PUSH score. At 3 weeks after treatment statistically significant differences of ACS versus Saline were evident in wound surface area (-6.4 cm^2 versus +0.4 cm^2), area score (-2.2 cm^2 versus +0.2 cm^2), exudate (-1.3 versus -0.1), tissue (-1.8 versus -0.1), and PUSH total score (-5.3 versus 0.0). ACS therapy resulted in a statistically significant decrease in wound surface area and improved wound healing. Application of ACS dressing for three weeks can provide an effective and safe treatment for hard-to-heal wounds. Adverse events were described in either treatment group.

Tennis Elbow

Ipek et al.[59] published a prospective study on efficacy of ACS for lateral epicondylitis (LE, Tennis elbow) in n=42 patients. Patients received four ACS intra- and peri-tendinous injections over two weeks. Clinical and functional outcomes of injections were assessed 1 month and 1 year after therapy using NRS, MEPS, and OES. NRS score improved statistically significant from 7.13 before injection to 3.52 after 1 month and 1.71 after 1 year. MEPS (56.43±7.51 to 94.29±4.07) and OES (84.17±6.07 to 7.44±4.32) improved statistically significant between baseline and 1 year. Six patients (14.2%) experienced mild ecchymosis and swelling around the injection site, which resolved spontaneously. ACS is a promising treatment option for lateral epicondylitis due to its rapid onset of pain relief and long-lasting functional effects.

COVID 19

Shakouri et al.[64] published a study of 5 versus 5 COVID-19 patients in intensive care under mechanical ventilation. They were treated with standard of care or standard of care plus intratracheal application of ACS (x3). 3 of 5 patients died in the standard of care group; 2 of 5 patients died in the standard of care plus ACS group. Most blood test analytes improved significantly in the ACS group vs control between baseline and 3rd application. Shakouri et al. conclude: The most striking clinical change observed in association with the administration of ACS in this study was the rapid decline in body temperature (from 38.2 to 37.7) and serum CRP levels which can be explained by the decrease in the level of inflammation. The most significant change was seen for driving pressure and oxygenation index.

Discussion:

44 clinical studies with Orthogen-ACS, including randomized, controlled studies have been published in peer-reviewed journals. Most have demonstrated efficacy; all have demonstrated safety of ACS therapy. Injection treatment with ACS demonstrated significant superiority in multiple studies over standard of care in a wide spectrum of clinical entities. All these pathologies are linked to degeneration, tissue senescence, inflammation, chronic pain and do not respond well to classical pharmaceutical and surgical treatments. The apparent immune modulatory and regenerative properties of ACS may support application in other areas of medicine, such as peripheral nerve

pathologies, or immunological diseases addressing universal pathomechanisms.

Nonetheless, a paradigm shift in therapeutic approaches for degenerative, chronic pain pathologies appears to be emerging, transitioning from mere suppression of symptoms to approaches focused on immune modulation. Orthogen-ACS stands as an example for such principles. This evolving philosophy may also extend to surgery, reflecting a broader trend towards less invasive techniques, reminiscent of the trend from open to minimally invasive surgery. Since operative procedures often fail to adequately address biomolecular processes, perioperative application of ACS may present a unique opportunity to integrate the strengths of both domains – combining mechanical precision of surgical interventions with biomolecular efficacy of ACS[60].

Conclusion:

44 clinical studies with Orthogen-ACS, including randomized, controlled studies have been published in peer-reviewed journals. All demonstrated safety of ACS therapy. Injection treatment with ACS demonstrated significant superiority in multiple indications over standard of care.

An extended coagulation phase and leverages the whole blood secretome. Thus, Orthogen-ACS achieves a unique composition of mediators aimed at immune modulation, inflammation resolution and tissue repair.

This and filterability underline the distinction between Orthogen-ACS and PRP or stem-cell therapy. The unique composition renders autologous ACS not only an effective but also exceedingly safe treatment option.

Conflict of Interest:

Nicole N. Hang: Has no conflicts of interest to declare.
Glyn Hamed: Has no conflicts of interest to declare.
Jana Wehling is employed at Private Practice Dr. Wehling & Partner and Orthogen AG, Dusseldorf, Germany.
Julio Reinecke is employed at Orthogen AG, Germany and is co-inventor.
Minna Yia Hang: Has no conflicts of interest to declare.

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Supplement Section:

Table 2a: Osteoarthritis studies (in total n=29, RCT: n=6).

Clinical Studies Osteoarthritis	Study design	Sample Size	Intervention/ Comparison	Outcome Measure/Follow-up	Results	Conclusion
Baltzer (2003) Knee OA (K&L I-III)	Retrospective 1-arm	n=1000	ACS	WOMAC 3 months	- WOMAC scores improved >50% in 70-75% of patients - After 3.5 yrs.: improvement sustained in 35-40% patients	- First long-term clinical report, significant results for treating OA with IA ACS-injections - ACS is an alternative therapy to the standard conservative for OA
Auw Yang (2008) Knee OA (K&L I-III)	RCT 2-arm	n=80 (ACS)	ACS (2 ml x 6 over 3 weeks)	VAS, WOMAC, KOOS, KSCRS 3-, 6-, 9-, and 12 months	ACS and placebo: similar improvements by WOMAC (16.8% versus 16.5%, respectively) ACS stat. signif. for KOOS symptom and KOOS sport parameters versus placebo	Stat. signif. improvement of KOOS symptom and sport. Consistently higher, though stat. non-signif. improvement of most other parameters. ACS induces biological response different from placebo treatment
		n=74 (saline)	Saline (2 ml x 6 over 3 weeks)			
Baltzer (2009) Knee OA (K&L II-III)	RCT 3-arm	n=134 (ACS)	ACS (2 ml 2x weekly, 6 x times total)	VAS, WOMAC, GPA, SF-8 7-, 13- and 26 weeks	WOMAC and VAS improvement at all time points	ACS injection improves clinical signs and symptoms of knee OA
		n=135 (HA)	HA (2 ml x 3 weekly + 3 weekly topical heparin-natrium cream)			
		n=107 (saline)	Saline (2 ml x 3 weekly + 3 weekly topical heparin-natrium cream)			
Noskov (2012) Hip OA (K&L I-III)	Prospective comparative 2-arm	n=30 (ACS)	ACS (2 ml weekly x 3) 6 x times total	VAS, WOMAC, EQ-VAS 1-, 3-, 6- and 12 months	At 3 m, VAS in ACS group was stat. signif. superior versus HA group (76.5%) At 6 months ACS was stat. sign. Superior with all scores.	There was a long-term preservation of positive results after ACS injection
		n=24 (HA)	HA (40 mg weekly x 3)			
Baltzer (2013) Hip OA (K&L II-IV)	Retrospective comparative (post hoc) 3-arm	n=46 (ACS)	ACS (2 ml x 5.9)	VAS 14.35 months	Over 14 months, all treatments resulted in large, stat. signif. improvement (independent of OA grade)	- ACS successfully reduces pain in hip OA - GC or GC+rIL-1Ra did not increase the beneficial treatment effect over and above the effect of ACS alone
		n=56 (ACS + GC)	ACS (2 ml x 5.7) plus GC (TA 10 mg x 1.94)			
		n=17 (ACS + GC + rIL-1Ra)	ACS (2 ml x 5.88) GC (TA 10 mg x 2.88) rIL-1Ra (0.2 mg x 3.53)			
Motaal (2014) Knee OA (K&L I-III)	Prospective 1-arm	n=30	ACS (1 ml weekly x 3)	WOMAC 1-, 2-, 3 weeks, then 1-, 2-, 3 months	All WOMAC ratings improved stat. signif. versus baseline for all follow-up points	ACS is easily prepared and has good functional and psychological satisfaction in OA knee
Rutgers (2015) Knee OA (K&L I-III)	Prospective 1-arm	n=20 Previously treated with saline	ACS	VAS, KOOS, WOMAC 3-, 12 months	VAS scores improved stat. signif. versus baseline at 3 months	
Baselga & Hernandez (2015) Knee OA (K&L I-IV)	Prospective 1-arm	n=118	ACS (2 ml weekly x 4) PT (4 weeks post injection): 3x per week for 10 weeks	NRS (3-, 6-, 12- and 24 months) WOMAC (24 months)	- NRS improved by 63% at 3 months, continued to 24 months - WOMAC global improved 56.9% (even in K&L IV)/WO MACa improved (86%) / WOMACc improved (51.3%)	- ACS + physio produced rapid decline in pain, sustained for 2 years - ACS + physio. is an effective treatment for OA of the knee, independent of K&L grade

Table 2b Osteoarthritis studies.

Clinical Studies Osteoarthritis	Study design	Sample Size	Intervention/ Comparison	Outcome Measure/Follow-up	Results	Conclusion
Öç (2017) Knee OA (K&L II-III)	Prospective 1-arm	n=33 (66 knees) bilateral knees	ACS (2 ml weekly x 6)	VAS, KOOS, KSS 12 months	12 monthsafter treatment: VAS improved 53.5%, KOOS improved 53.3%, and KSS improved 68.3%	ACS injection has excellent safety profile, results in a strong clinical response, effective and well-tolerated alternative
Tassara (2018) Knee OA and Hip OA	Retrospective 1-arm	n=23 (25 knees) n=3 (hips)	ACS (2 ml weekly x 4)	VAS, ROM week 4 (immediately after 4th injection), then 2 and 7 months	VAS 80 to 20 for all OA and subsets of knee OA (1 and 6 months) ROM: median increase of 25° (1 and 6 months)	ACS causes rapid improvement of pain and ROM. ACS is a valid option for the treatment of OA when surgery is contraindicated or refused by the patient
Zarringam (2018) Knee OA (K&L I-III)	Prospective comparative (from Yang RCT) 1-arm	n=72 (ACS) n=54 (saline)	ACS: 11 years, 2 months (mean follow-up) Saline: 11 years, 3 months (mean follow-up)	Orthokin group showed better survival after 7.5 ± 3.9 years of follow-up	After 7.5 years (± 3.9): 46.3% of the placebo and 40.3% of the Orthokin group had been treated surgically	Orthokin for knee OA did not result in a delay regarding surgical treatment for OA, compared with placebo.
Hashemi (2019) Knee OA (K&L II-III)	RCT 2-arm	n=30 (ACS) n=30 (HA)	ACS (2 ml weekly x 3) HA (2 ml weekly x 3)	WOMAC, KOOS 6 months NRS 1-, 3- and 6 months	ACS group was stat. signif. better by NRS at 1 and 6 months, by KOOS symptom, ADL, and sport at 6 months	ACS has beneficial biological effects in patients with knee OA, is minimally invasive, safe, and effective for patients suffering from chronic knee pain
Kilinç (2019) Knee OA (K&L II - III)	Retrospective 1-arm	n=33 (66 knees)	ACS (2 ml twice a week x 6)	VAS, KOOS, KSS 12 months	Baseline versus 1 y: VAS (7.36 versus 3.27), KOOS (42.4 versus 72.4), KSS (42.8 versus 70.6) all stat. signif.	IA ACS in painful OA significant improvements in pain severity, KOOS and KSS scores. ACS is effective for patients with low to medium grade OA
Vitali (2020) Knee OA (K&L I-III)	Prospective 1-arm	n=15	ACS (2 ml weekly x 4)	VAS, WOMAC, KSS 1-, 2-, 3-, 4 weeks and 6 months	Stat. signif.: VAS (35.8%), WOMAC (19.8%), KSS functional (38.2%), KSS clinical (28.9%)	ACS represents the new direction of DMOADs for knee OA treatment targeting specific compounds responsible for the pathogenesis of disease
Shirokova (2020) Knee OA (K&L II-III)	Female only Prospective 2-arm	n=65 (ACS) Subclinical: n=26 Moderate: n=39 n=58 (PRP) Subclinical: n=30 Moderate: n=28	ACS (2.5 ml bi-weekly x 6) PRP (5 ml bi-weekly x 6)	VAS, WOMAC 1 and 3 months Synovial fluid viscosity (ACS - 180 days and PRP - 90 days) IL-1Ra, IL-1, IGF1, TGFb Nitrate: NO-footprint conjugated dienes (CDs): ROS footprint signif. better in ACS versus PRP	- 3-month: clinical efficacy ACS versus PRP signif. in all groups (VAS and WOMAC) - VAS (subclinical synovitis): ACS versus PRP (46% versus 29%) VAS (moderate synovitis): ACS versus PRP (47% versus 8%) - PRP signif. versus baseline in subclinical only - PRP was signif. versus baseline in subclinical synovitis cases only - Both groups improved SF viscosity, IL-1 Ra >> IL-1 but signif. higher in ACS - NO-footprint and conjugated dienes (ROS footprint) signif. better in ACS	- ACS was clinically and biochemically superior to PRP and met the MCII criteria for VAS in knee OA. - ACS displayed significant efficacy in all groups, was clinically and biochemically superior to PRP PRP was not effective in cases of moderate synovitis - Data show potential rejuvenation-associated properties, reducing ROS and NO footprint - Routine inclusion of SF analysis in OA therapy studies is advocated
Hashemi (2020) Knee OA (K&L I-II)	RCT 2-arm	n=30 (ACS) n=30 (ozone)	ACS (2 ml weekly x 4) Ozone (10 ml monthly x 3)	VAS, WOMAC, KOOS 1-, 3- and 6 months	KOOS and VAS scores of pain, symptoms, daily activities, and athletic and recreational functions were significantly higher in ACS	- IA injection of ACS is low invasive, safe, effective, and long-acting in patients with knee OA - Clinical improvements and responses to ACS injection are better and longer than ozone injection
Pishgahi (2020) Knee OA (K&L II-IV)	RCT 3-arm	n=32 (ACS) n=30 (PRP) n=30 (Dextrose)	ACS (2 ml weekly x 2) PRP (weekly x 2) Dextrose Prolotherapy (2 ml 50% dextrose weekly x 3) and water (2 mL), and 2% lidocaine (1 mL)	VAS, WOMAC 1- and 6 months	- ACS- and PRP-treatment improved pain and knee function at 1 and 6 months. Stat. signif. stronger in ACS group - 6 months VAS in ACS was 42%, versus 10% for PRP and 5.5% for prolotherapy - ACS improved WOMAC by 38%, PRP by 24%, prolotherapy by 9.6%	Treating knee OA with ACS or PRP is associated with pain reduction and improved knee function. ACS therapy is more effective than PRP

Table 2c Osteoarthritis studies.

Clinical Studies Osteoarthritis	Study design	Sample Size	Intervention/ Comparison	Outcome Measure/ Follow-up	Results	Conclusion
Godek (2020) 1000 cases miscellaneous indications	Retrospective 1-arm	n=1000	ACS 4-6 ml			Orthokine is highly effective in tendinopathy, enthesopathy, small joints OA of the hand and in early stages of knee OA. Satisfactory in cervical and lumbar discopathy, unsatisfactory results in severe degenerative changes in knee and hip joints and in spinal canal stenosis.
Khurana (2020) Knee OA (K&L I-III)	Retrospective 4-arm	n=21 (ACS) n=27 (PRP) n=28 (GC) n=20 (HA)	ACS (? ml) PRP (5 ml x 1) GC (Depot-Methylprednisolone) 40 mg x1) HA (Synvisc-one) 6 ml	VAS, WOMAC 2 weeks, 3-, 6 months	- VAS, WOMAC scores better for ACS versus PRP (baseline to 6 months), no stat. signif. Difference - 6 months ACS versus PRP: VAS (61% versus 53%) and WOMAC (70% versus 60%) - ACS better versus PRP and HA and GC at 6 months. No stat. signif. difference ACS versus PRP at 6 months.	PRP and ACS are effective in relieving OA pain at 6 months. HA and GC are not effective at 6 months.
Leone (2021) Knee OA (K&L I-III)	Prospective 1-arm after failed PRP treatment	n=30	ACS (2 ml weekly x 4)	VAS, Lequesne scales 1-, 6-, 12 months	1 month: VAS >33% in 67% of patient (effect until 6 and 12 months (70 to 30)) 24 months: ACS responders had stopped medical treatments, avoided surgery, remained in response.	Confirmed efficacy of ACS in pain resolve and functional recovery of patients with knee OA resistant to other medical and PRP treatment
Simon (2021) Shoulder OA (K&L II-III)	Prospective 1-arm	n=36 (40) n=20 (HA)	ACS (2 ml weekly x 6) HA (6 ml Synvisc x 1)	ASES, SPADI, CSS, ROM, VAS Total shoulder Replacement (2 years)	- 3 months after ACS, all scores stat. signif. improved. 6 patients had second series of ACS at 6 months - 47% had shoulder replacement (at av of 1.8 year), 53% had signif. pain relief in SPADI scores (av of 3.6 years)	ACS injections in the shoulder joint for OA can reduce pain and disability, and postpone the need for a shoulder replacement
Hussein (2021) Knee OA (K&L I-III)	Retrospective 3-arm	n=222 (ACS) n=171 (HA) n=112 (BMAC)	ACS (2 ml bi-weekly for 3 weeks) HA (weekly x 3) BMAC (8 ml x 1)	VAS, WOMAC, N at baseline, 3- and 12 months	- All groups improved stat. signif. at 3 months versus baseline in VAS and WOMAC - 12 months: VAS improved stat. signif. versus baseline in all groups (ACS 40%, BMAC 36%, HA 33%) - 12 months: WOMAC improved best for BMAC (BMAC 33.3%, ACS 16.1%, HA 14.9%)	BMAC is more effective than HA and ACS in knee OA (mainly in patients with more severe degenerative changes). Potential bias may result from significant loss of follow up at 12 months. ACS n=222 down to n=50, HA n=171 down to n=30, BMAC n=112 down to n=25
Coşkun (2022) Knee OA (K&L II-III)	Retrospective 2-arm	n=42 (ACS) n=40 (PRP)	ACS (3 ml weekly x 3) PRP (3 ml weekly x 3)	VAS, KOOS 1-, 6-, 12-, 24-, 60 months	- Stat. signif. decrease of VAS in both groups at both 6 and 12 months versus baseline - Improvement in VAS, KOOS.S, KOOS.P, KOOS.ADL more significant in ACS versus PRP (at 12 and 24 months) - Adverse events: ACS: 2 patients (5%) PRP: 16 patients (38.1%)	- ACS shows better results on VAS and KOOS scores versus PRP 6-24 months -This study confirms that ACS is a safe and effective treatment for knee OA. It can be used as an alternative treatment method, especially when standard OA treatments fail.
Vitali (2022) Knee OA (K&L I-III)	Prospective 2-arm	n=12 (ACS) n=12 (BMAC)	ACS (3 ml weekly x 4) BMAC (7-10 ml x 1)	VAS, WOMAC 1- and 6 months	- WOMAC and VAS significantly lower in BMAC versus ACS at 6 months - No significant differences in ROM	Both approaches are safe and effective in knee OA, major efficacy of BMAC
Cortegiani (2022) Spinal Facet Joint OA (K&L I-III)	Prospective 1-arm	n=13	ACS (2 ml weekly x 4)	1-, 3- and 6 months, VAS, WOMAC, Quick-DASH, ODI, SF-36, Karnofsky Index	- Stat. signif. pain relief versus baseline (median VAS of 7.5) to 6 months (median VAS of 2) - Stat. signif. relief versus T0 in VAS, Karnofsky, SF-36, ODI, Quick-Dash	Reduction of pain, improved joint function and quality of life for up to 6 m. no significant adverse events

Table 2d Osteoarthritis studies.

Clinical Studies Osteoarthritis	Study design	Sample Size	Intervention/ Comparison	Outcome Measure/Follow-up	Results	Conclusion
Ippolito (2023) Multiple OA locations (K&L I-III)	Prospective 1-arm	n=9 (hip, knee, ankle) n=5 (upper limb) n=12 (low back pain)	ACS (2 ml weekly x 4)	NRS, WOMAC, Quick DASH, SF 36, Karnofsky performance status, ODI	- NRS: stat. signif. relief at 3 and 6 months versus T0 (6 months reduction of -3) - WOMAC 6 months: stat. signif. improvement of 35.14% - Other outcome measures stat. signif. versus baseline	ACS a feasible option for chronic pain due to grade 1 to 3 OA refractory to other treatments
Hang (2023) Knee OA (K&L I-III)	Retrospective 2-arm (bilateral injections)	n=37 knees (ACS) n=37 knees (GC)	ACS (2 ml weekly x 6) GC (5 mg Dexa x1)	VAS, WOMAC, GPA 3-, 6- and 12 months	- Stat. signif. improvements VAS, WOMAC global, WOMAC pain, stiffness, function at 3, 6, 12 months in favor of the ACS - 2 GPA (Global Patient Assessment) scores show doubling of good/very good and 60-80% better evaluations in ACS versus GC group.	ACS is stat. signif. superior to Dexamethasone, both treatments are safe. Clinical efficacy of Dexa was surprising long
Damjanov (2023) Knee OA (K&L III-IV)	RCT 2-arm	n=20 (GC+ACS) n=20 (GC+Saline)	ACS+GC (40 mg TA + 5 ml ACS) Saline+GC (40 mg TA + 5 ml Saline)	NRS, KOOS 3-, 6-, 12- and 24 weeks	- Stat. signif. improvement at 24 weeks: KOOS pain, symptoms, activities of daily living, quality of life, and KOOS sport scores - Stat. signif. pain reduction (NRS average pain) over control at 24 weeks	- Both groups signif. reduced pain improved symptoms for knee OA at 24 weeks - ACS therapy had signif. less pain versus placebo group at 24 weeks extending the GC improvement

Tables 2a-2d Abbreviations:

OA (Osteoarthritis), ACS (Autologous Conditioned Serum), HA (Hyaluronic acid), BMAC (Bone marrow aspirate concentrate), PRP (Platelet-rich-plasma), GC (Glucocorticoid), TA (Triamcinolone), Dexa (Dexamethasone), Depot (Depot-Methylprednisolone), MP (Methylprednisolone), rIL-1Ra (recombinant interleukin 1 receptor antagonist), ROS (reactive oxygen species), NO (Nitric Oxide), WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index), VAS (Visual analog scale 0-100), NRS (numeric rating scale 0-10), ROM (range of motion), KOOS (Knee injury and Osteoarthritis Outcome Score), KSS (Knee Society Score), IL-1 (Interleukin 1), IL-1Ra (Interleukin 1 receptor antagonist), IGF (Insulin-like growth factor), TGF-B (Transforming growth factor-beta), KSCRS (Knee Society Clinical Rating System), GPA (Global Patient Assessment), SF-8 (Short Form 8 Health Survey), ODI (Oswestry Disability Index), Quick DASH (Disability of the Arm, Shoulder and Hand), SF-36 (Short Form 36 Health Survey), SF-8 (Short Form Health Survey 8), ASES (American Shoulder and Elbow Surgeons), SPADI (Shoulder Pain and Disability Index), CSS (Constant Shoulder Score), MCII (Minimal Clinically Important Improvement)

Table 3 Spine and nerve tissue studies (n=7, RCT: n=2).

Clinical studies backpain and nerves	Study design	Sample Size	Intervention/Comparison	Outcome Measure/Follow-up	Results	Conclusion
Becker (2007) Lumbar radiculopathy	RCT 3-arm	n=32 (ACS)	2ml ACS (weekly x 3)	VAS, ODI 6-, 10-, 22 weeks	- All groups stat. signif. improved pain and disability. No stat. signif. difference between TA dosages - week 12 - week 22, ACS showed consistent pattern of superiority (VAS score) versus both GC groups - ACS stat. signif. at week 22 versus GC 5 mg	Pain relief was pronounced, clinically remarkable, and potentially superior to corticosteroid injection.
		n=27 (GC)	2ml 5 mg TA (weekly x 3)			
		n=27 (GC)	2 ml 10 mg TA (weekly x 3)			
Ravi Kumar (2015) Lumbar radiculopathy	Prospective 1-arm	n=20	ACS (weekly x average of 2)	NRS, SLR test, RODI, SF-12 PCS/MCS 3 weeks, 3, 6 months	Stat. signif. change in all parameters from baseline to 3 weeks, 3, 6 months)	ACS can modify disease course in addition to reducing pain, disability and improving general health.
Goni (2015) Cervical radiculopathy	RCT Pilot Trial 2-arm	n=20 (ACS)	ACS (2-3 ml x 1)	NRS, NPDS, NDI, SF-12 3 weeks, 3-, 6 months	- ACS and GC groups had stat. signif. improvements. ACS, sustained improvement during follow up. GC group had deterioration over time. - At 6 months: stat. signif. NRS ACS versus GC (73.2% versus 58.5%) NPDS (73.8% versus 55.6%) NDI (74.5% versus 52.8%)	In unilateral cervical radiculopathy, ACS is thought to be equally good or better than GC over longer periods of time
		n=20 (GC)	GC (3 ml Methyl-Prednisolone x 1)			
Godek (2016) Lumbar radiculopathy	Retrospective 1-arm	n=15	ACS	VAS, OLS, SLR test, ODI 1-, 3 months	- Stat. signif. reduction of pain and clinical signs of compression, improved ODI - Two out of 15 patients chose surgery due to increasing pain	ACS injection under ultrasound guidance may be an option for treatment of radiculopathy due to herniated disc.
Godek (2020) Cervical Radiculopathy	Randomized Prospective n=90	ACS n=15	ACS 3-4 ml x 4 weekly	NRS, NDI, CRA (mm^2), Minimal important difference (MID) at 2 months	ACS, PRP at 2 months: NRS: 71.7%, 70.6% NDI: 61.5%, 56.6% CRA: 23.6%, 25% MID NRS: 60%, 35.7% MID NDI: 40%, 35.7%	- Biologics more effective than mechanical and physical, improving pain, disability index and proprioception of the hand at completion of therapy and after follow-up, - ACS > PRP were superior to other therapies. - Biologics may have regenerative properties
		PRP n=15	PRP 2 ml x 4 weekly			
		Manual Therapy n=15	12-15 min 2x per week			
		Traction Therapy n=15	12-15 min 2x per week			
		High intensity Laser n=15	1064 nm, 30W			
		Collagen magnetophoresis n=15	2ml collagen x 8			
Aghamohammadi (2022) Trigeminus neuralgia	Prospective 1-arm	n=11	ACS (2 ml weekly x 4)	NRS 1-, 2-, 3-, 4 weeks, 2 months	- NRS stat. signif. reduced at 3 weeks versus baseline, retained at 4 weeks, 2 months (8.18 versus 3.36) - Carbamazepine was stat. signif. reduced in 3 weeks versus baseline, retained at 4 weeks, 2 months	- ACS injection into the Foramen Oval significantly reduced NRS and signif. lowered need for carbamazepine - Solid preliminary evidence that ACS is an impressive pain relief technique for refractory TGN
Godek (2023) Lumbar degenerative disc disease (LDDD)	RCT 2-arm	n=50 (ACS Perineural)	ACS (8 ml weekly x 2) 4, 12, 24 weeks	NRS, ODI, RMQ, EQ-5D-5L	- Both routes of ACS application stat. signif. similar improvement in primary clinical parameters, pain and disability - Stat. signif. improvement versus baseline at 4 weeks, continued at 6 months	Both methods can be considered equally effective in managing LBP due to LDDD. For safety the perineural route should be favored.
		n=50 (ACS Epidural)				
Godek (2025) Cervical Fascial versus periarticular	RCT 2-arm	n=50 ACS fascial	ACS 4 ml x 4 every 3 days	NRS, NDI, DPT	To be published	
		n=50 ACS periarticular	ACS 4 ml x 4 every 3 days			

Table 3 Abbreviations: ODI (Oswestry Disability Index), SLR (Straight leg raise test), SF-12 (12-Item Short Form Survey), OLS (One-leg stance test), NPDS (Neck Pain Disability Scale), NDI (Neck Disability Index), CRA (cross-section root area by ultrasound), MID (minimal important difference), PCS-12 (Physical Health Component Score SF-12), MCS-12 (Mental Health Component Score SF-12), FO (Foramen Oval), RMQ (Roland Morris Question), TGN (trigeminal neuralgia)

Table 4 Other Pathologies studies (n=9, RCT: n=2).

Clinical study	Study design	Sample Size	Intervention/ Comparison	Outcome Measure/ Follow-up	Results	Conclusion
Wright-Carpenter (2004) Muscle strain	Prospective 2-arm	n=18 (ACS)	ACS (1 ml every 2nd day x 5.4)	- Time to 100% sports participation and >90% strength recovery - MRI (baseline and 14-16 days after injection)	- Stat. Sign. difference in recovery time was 16.6 versus 22.3 days - MRI analysis: ACS induced complete regression of bleeding and edema and regeneration of muscle fiber 16 days after injection	- ACS injection is promising, reduced the time to recovery - Allows return to full activity and full muscular function
		n=11 (Actovegin /Traumeel)	Actovegin/Traumeel (2 ml + 3 ml every 2nd day x 8.3)			
Strümper (2017) Meniscus	Retrospective 1-arm	n=47	ACS (2 ml weekly x 5.2 injections)	OKS, BLOKS 6 months	BLOKS (0.81 to 0.71) and OKS (29.1–44.3%) Stat. Sign. improved at 6 months. 83% of patients avoided surgery during the 6 months observation period	IA ACS injection may be an effective treatment option for knee pain associated with meniscal lesions
Darabos (2014) ACL plasty	RCT 2-arm	n=31 (ACS)	ACS (2 ml on day 0, 1, 6, 10)	- WOMAC, IKDC 2000 6, 10 months - CT 0, 1, 6, 10 months - Serum and synovial fluid IL-1 concentration on days 0, 1, 6, 10	- WOMAC, IKDC 2000 were better in ACS at all timepoints and all outcome parameters. Stat. Sign. differences in the WOMAC stiffness subscale after 1 year - Decrease in IL-1 synovial fluid conc. in ACS, stat. Sign. lower in ACS group at day 10. Bone tunnel was stat. sign. less large versus saline (6 months: 8%, versus 31% 12 months: 13% versus 38%)	- IA administration of ACS results in decreased bone tunnel widening after ACL reconstructive surgery - ACS appears to have a beneficial biological effect on patient-documented symptoms and structure modifying effects arising after ACL reconstructive surgery
		n=31 (Saline)	Saline (2 ml on days 0, 1, 6, 10)			
Damjanov (2018) Shoulder tendinopathy	RCT 2-arm	n=15 (ACS)	ACS (2 ml weekly x4)	VAS, CSS 4, 24 weeks	ACS versus GC: VAS, CSS improve at 4 weeks and stat. sign. improved at 24 weeks. VAS ACS versus GC: week 4: 22 versus 32, week 24: 15 versus 40	- Versus glucocorticoid, ACS improved joint function and reduced shoulder pain more effectively after 4 and 24 weeks. - ACS for chronic supraspinatus tendinopathy pain has an excellent safety profile and larger improvements in shoulder pain and function versus glucocorticoid over 24 weeks
		n=16 (GC)	GC (Betamethasone x 3 and saline x 1)			
Von Wehren (2019) Achilles tendonitis	Retrospective 2-arm	n=25 (ACS)	ACS	VISA-A-G 6, 12 weeks, 6 months, MRI 6 months	- Stat. sign. better VISA-A-G in both groups at 6 months versus baseline. 6 months ACS has signif. higher changes versus baseline versus eccentric training - Baseline versus 6 months MRI: ACS stat. sign. better changes versus eccentric training (thickness, length, quality)	- Both therapies lead to improvement of MRI findings, including reduction of tendon thickness and tendon quality - ACS shows greater clinical long-term benefit versus eccentric training, offers a favorable alternative to the gold standard of eccentric training
		n=25 (Eccentric training)	Eccentric training			
Strümper (2021) Meniscus	Retrospective 1-arm	n=170	ACS (2 ml weekly x 4, 1 week post fibrin)	WOMAC 6 week, 1, 2, 3, 4 years	- Mean WOMAC global score improved from 34.62 to 13.18 at 4 years - Only 8 of 170 patients chose surgical treatment of the meniscal injury	IA fixation of meniscus injuries with fibrin glue in combination with ACS improves knee symptoms associated with meniscal injury
			Tisseel Fibrin Glue (x 1)			
Gholian (2022) Wound	Single-blinded, RCT 2-arm	n=15 (ACS)	ACS (applied on dressing weekly x 3)	Wound surface area, PUSH 3 weeks	- Significant differences for wound surface area and PUSH scores from baseline to the end of the study in ACS patients, but not in control - Comparison at 3 weeks: wound surface area (-6.4 versus +0.4), area score (-2.2 versus +0.2), exudate (-1.3 versus -0.1), tissue (-1.8 versus -0.1), PUSH total score (-5.3 versus -0.0)	- ACS signif. reduce wound surface area and improve healing process, which is likely due to high concentrations of growth factors and anti-inflammatory cytokines - ACS dressings for three weeks can provide an effective and safe treatment for hard-to-heal wounds
		n=15 (Saline)	Saline (applied on dressing weekly x 3)			
Ipek (2022) Tennis elbow (lateral epicondylitis)	Prospective 1-arm	n=42	ACS (2 ml 2x weekly 2 weeks)	NRS, MEPS, OES 3, 12 months	NRS, MEPS, OES: Stat. signif. improvement at 3 and 12 months	Intra-tendinous ACS is a promising option for rapid pain relief long-lasting functional improvement in patients with LE.
Shakouri (2022) COVID-19	Prospective 2-arm	n=10	Standard care plus ACS (2 ml x3)	Panel of analytes	3/5 survivors	The most striking clinical change was the rapid decline in body temperature (from 38.2 to 37.7) and serum CRP level in ACS treated patients
			Standard care	Panel of analytes	2/5 survivors	

Table 4 Abbreviations: ACL (Anterior cruciate ligament), VAS (Visual analog scale), VISA-A-G score (Victorian Institute of Sport Assessment-Achilles Questionnaire - German), CSS (Constant Shoulder Score), MEPS (Mayo Elbow Performance Score), OES (Oxford Elbow Score), IL-1 (Interleukin 1), IKDC 2000 (International Knee Documentation Committee Rating System 2000), OKS (Oxford Knee Score), BLOKS (Boston Leeds Osteoarthritis Knee Score), PUSH (Pressure Ulcer Scale for Healing