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ORIGINAL ARTICLE

Efficacy of Autologous Conditioned Serum (ACS), Platelet-Rich Plasma (PRP), Hyaluronic Acid (HA) and Steroid for Early Osteoarthritis Knee: A Comparative Analysis

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Abstract

Background Intra-articular injection therapy constituting corticosteroids, viscosupplements and blood-derived products are considered to have a key role in non-operative management of osteoarthritis knee. While corticosteroids and viscosupplements have proven short-term efficacy in early osteoarthritis; orthobiologics are gaining increased attention in osteoarthritis management. The aim of present study was thus to compare two commonly used biologics (platelet-rich plasma/PRP and autologous conditioned serum/ACS) to each other and to established therapies.

Methods After required institutional clearances, all patients presenting with early primary osteoarthritis knee who had failed initial conservative management and received only unilateral knee injection were included. Patients in the PRP group were compared to the other groups (comprising the HA/hyaluronic acid group, steroid group, and a matched cohort who had been administered ACS for the same indication earlier). Clinical outcome was evaluated using the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire and Visual Analogue scale (VAS) pre-injection and at 6 months.

Results ACS and PRP did not have any significant difference in terms of either WOMAC score ($p = 0.154$) or VAS score at 6 months ($p = 0.850$). The scores for both these orthobiologics were better than the control groups (HA group and Steroid group). Between the two control groups, HA group had better VAS scores as compared to the Steroid group ($p = 0.008$).

Conclusion The clinical outcomes following intra-articular injection of ACS and PRP are better than controls (HA and steroid), but a difference between the two orthobiologics could not be demonstrated.

Level of Evidence 3b.

Keywords Autologous conditioned serum (ACS) · Platelet-rich plasma (PRP) · Osteoarthritis knee · Intra-articular injections · Hyaluronan

Introduction

Various conservative treatment modalities are recommended as first line in osteoarthritis management. However, if these are ineffective, then intra-articular (IA) injections (corticosteroids, viscosupplements, blood-derived products) are considered as second line in non-operative management of osteoarthritis. Viscosupplementation has found its own special niche in management of early osteoarthritis based on potentially therapeutic physicochemical properties. Use of viscosupplementation has proven to be effective for stages 1 and 2 osteoarthritis [1]. Similarly, intra-articular corticosteroid injections are widely used in OA [2]. Suppression of local joint inflammation by corticosteroids is pronounced, and may be achieved with only minor systemic effects. Clinically, corticosteroids have shown to decrease tenderness of inflamed joints and they also increase the relative viscosity of joint fluid. Steroid injections are shown to be effective in acute and acute on chronic knee inflammatory states as they decrease acute episodes of pain and increase joint mobility during the flare of knee OA [3, 4].

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With evolving times, the emphasis in chronic disease management is shifting from lifestyle modification and symptom mitigation to tissue healing, regeneration and repair of failing organ systems. Orthobiologics encompass a diverse group of tissue-derived therapeutics that sufficiently fulfills this requirement. The most commonly reported biologics for use in osteoarthritis knee include platelet-rich plasma (PRP), bone marrow aspirate concentrate (BMAC), mesenchymal stem cells (MSCs), micro-fragmented fat, stromal vascular fraction (SVF), and autologous conditioned serum (ACS). These biological therapies have gained increased attention in the past decade due to their healing potential. Another reason for this popularity is that the patient's own body provides the medication for treatment [5].

Platelet-rich plasma (PRP) was the first autologous orthobiologic that has found wide usage in present day orthopedics. It stimulates a supraphysiological response, also referred to as biological augmentation which is responsible for its healing potential. PRP has several growth factors of which most are pro-chondrogenic [6]. Last 10 years have seen several publications supporting the use of PRP in OA knee, but methodology of most of these studies is variable and some of them even deviate from available evidence [7]. Mechanism, efficacy, and indication for use of PRP is controversial due to non-uniform PRP preparations and formulations as well as regimens and lack of large trials [8]. Orthobiologics, especially PRP, provide a safe option with acceptable short to medium term outcomes [9–14].

Autologous conditioned serum (ACS) is another orthobiologic derived from patient's own blood which uses the anti-inflammatory properties of substances found in blood. IL-1 receptor antagonist (IL-1Ra) is a naturally occurring inhibitor of IL-1 and is produced by macrophages. It binds to IL-1 receptor, thereby blocking the biologic actions of IL-1. As IL-1 is thought to be involved in producing the symptoms of OA, the blockade of its action is expected to produce relief of symptoms. A high concentration of IL-1Ra can be obtained by incubation of blood with medical grade glass beads to further obtain ACS [15, 16]. Clinically, ACS has been used in orthopedic conditions like muscle regenerative treatment, [17] lumbar radiculopathy [18, 19], and cervical radiculopathy [20] with encouraging results. Recent literature also supports its role in OA knee with some literature backing [16, 21].

The purpose of this study was to assess the commonly used injectable orthobiologics (PRP and ACS) and to compare them to other injectable therapies (hyaluronic acid and steroid). Additionally, this study reviews the current evidence on the efficacy of ACS and PRP in the treatment of early OA knee.

Methods

Patients and Design

Ethical and Scientific Committee approval was obtained from Institutional Review Board of the hospital. All the patients with symptomatic early OA knee, who had undergone injection therapy were included. The choice of injection therapy was a combined physician and patient decision. Patients needing bilateral knee injections, those with bone-on-bone arthritis, lumbar spondylosis with radiculopathy, systemic disorders such as diabetes, inflammatory arthritis, major knee deformity (varus > 5, valgus > 10), hematological diseases, e.g. coagulopathy, severe cardiovascular diseases, infective foci elsewhere in the body, immunosuppression, cases with history of previous IA injection and those undergoing another form of conservative therapy for osteoarthritis were excluded from the study. Standing radiographs of involved knee was carried out and Kellgren Lawrence (KL) radiographic grading was done and documented. Patients in the PRP group were compared to the other groups (comprising the HA/hyaluronic acid group, steroid group, and a matched cohort who had been administered ACS for the same indication earlier).

Patients included in the HA group were given IA 6 ml HA (Synvisc-one™, Sanofi, Genzyme). Synvisc (Hylan polymer A and B, G-F 20) contains high molecular weight (HMW) elasto-viscous fluid with long chain chemically cross-linked polymer. Patients in the steroid group received depot-Methylprednisolone 40 mg mixed with local anaesthetic (5 mL of 2% lidocaine hydrochloride with 1:80,000 epinephrine). These two groups acted as the control in our study.

Preparation of Orthobiologic

PRP preparation: PRP required for injection was prepared and provided by the Department of Transfusion Medicine of our hospital. 36 ml of blood was harvested and 4.2 ml of citrate phosphate dextrose was added. The blood was then transferred to sterile tubes. Centrifugation was done using a table-top centrifuge machine (PRESVAC DCS-16 rvt plus, Buenos Aires, Argentina) in sterilized tubes. Double centrifugation technique was used with first spin at 1700 rpm for 10 min, and second spin at 3000 rpm for 10 min to concentrate the platelets. The final product used was 5 ml of platelet-rich plasma that was injected into the knee.

ACS preparation: 50 ml of blood was drawn from the antecubital vein of the patients in the ACS group into

an autoclaved centrifuge tube without anticoagulants. Blood was incubated in the centrifuge tube containing medical grade borosilicate glass beads (2.5 mm/21 mm^2). The beads were prewashed with sterile, double-distilled water and their surface was modified by incubation in 50% *v*/*v* chromium sulfate ($CrSO_4$) for 5 min. Incubation was done aseptically at 37 °C with 5% CO_2 for 24 h. After incubation, the tubes were centrifuged at 3500 rpm for 10 min to retrieve the ACS.

Peri-procedure Protocol and Outcome Measurements

There is little consensus in the literature on the appropriate technique of administration. All injections were given with sterile precautions after aspiration of synovial fluid from knee into the suprapatellar pouch through a superolateral approach using an 18G needle. In all the groups, participants were advised for cold compression for 10 min, 6–8 times at 15 min interval and rest for a day at home. Paracetamol and cold compression therapy was allowed for next few days. Patients were advised to avoid massage or hot fomentation. When necessary, oral paracetamol was recommended (maximum 2 g/day) during the follow-up period. All patients were taught quadriceps strengthening exercises to perform 15 repeats per day in three sets. They were instructed to start a week after the intra-articular injection and the same was continued for all patients irrespective of the injection given.

All patients were examined and data collected as per Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score (after applying for permission license), and Visual Analog Scale (VAS) before injection and at subsequent follow-ups at 2 weeks, 3 months and 6 months. WOMAC is a patient-administered, quality-of-life instrument validated for the assessment of patients with OA [22]. Comparison was, however, done between pre-injection and 6 months scores of each group. Comparison was also carried out between baseline variables of each group like age, sex, and BMI.

Statistical Analysis

Data were coded and recorded in MS Excel spreadsheet program (Microsoft Redmond, WA). SPSS v23 (IBM Corp, Chicago, IL) was used for data analysis. Normal distribution of data was assessed using the Shapiro–Wilk test. Descriptive statistics were elaborated in the form of means for continuous variables, and frequencies and percentages for categorical variables. Group comparisons for continuously distributed data were made using independent sample *t* test when comparing two groups, and one-way ANOVA when comparing more than two groups. Post hoc pairwise analysis was performed using Tukey's HSD test in case of one-way ANOVA. If data were found to be non-normally distributed, appropriate non-parametric tests in the form of Kruskal–Wallis test were used for these comparisons. Post hoc analysis for Kruskal–Wallis test was performed using Dunn test with Sidak correction. Chi-squared test was used for group comparisons for categorical data. In case the expected frequency in the contingency tables was found to be < 5 for $> 25\%$ of the cells, Fisher's exact test was used instead. Paired variables which were continuously distributed were compared using paired *t* test when comparing two variables, and repeated measures ANOVA when comparing more than two variables. Statistical significance was kept at $p < 0.05$.

Research Ethics and Patient Consent

The study was conducted according to the World Medical Association Declaration of Helsinki. The study conformed to the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, and institute ethics Committee and scientific committee provided approval (F-5/280/2016/BSAH/DNB/Committees/1973) prior to start of the study. Informed consent was obtained prior to the intra-articular injection for each patient.

Results

Baseline Characteristics

The groups were comparable in terms of gender and KL grade of the knee. Age (years) was normally distributed in four subgroups of the variable group. Thus, parametric tests (one-way ANOVA) were used to make group comparisons. Despite the statistical significance, age (years) in all groups ranged from 45 to 65. There was a significant difference between the four groups in terms of age (years) ($F = 3.620$, $p = 0.016$), with the mean age being slightly higher in the PRP group than the other groups. (56.3 ± 4.4 versus 50.8 ± 8.9 in ACS group, 54.6 ± 5.00 in Steroid group, and 51.85 ± 7.66 in HA group). On using the post hoc pairwise tests for ANOVA performed using Tukey HSD method, the only significant difference that could be found was between the ACS and the Steroid group ($p = 0.022$). But comparisons between all the other subgroups did not reveal any significant difference ($p > 0.05$) (Table 2).

Chi-squared test was used to explore the association between various groups and gender. There was no significant difference between the various groups in terms of distribution of gender ($X^2 = 0.515$, $p = 0.916$). 66.7% of the participants in the PRP group were females which was same as the ACS group. The control groups (steroid group

and HA group) also showed a similar distribution, 71.4% of the participants in the steroid group and 75.0% of the participants in the HA group were females.

Fisher's exact test was used to explore the distribution of KL Grade within the four groups as more than 20% of the total number of cells had an expected count of less than 5. There was no significant difference between the various groups in terms of distribution of KL Grade ($X^2 = 8.689$, $p = 0.275$). Majority patients in all the four groups belonged to KL grade 2 (59.3% in PRP group, 38.1% in ACS group, 53.6% in Steroid group and 55% in HA group) and the remaining to grade 3 and 1 which had almost similar distribution among all the groups.

The variable BMI (Kg/m^2) was normally distributed in the four subgroups of the variable group. Thus, parametric tests (one-way ANOVA) were used to make group comparisons. The BMI (Kg/m^2) in the PRP group ranged from 25.5 to 42.8 (Mean 31.48 ± 3.91); in the ACS group from 23.8 to 34.8 (Mean 30.46 ± 3.57); in the Steroid group from 23.8 to 42.1(Mean 31.19 ± 4.05); and in the HA group from 26.1 to 37.7 (Mean 31.05 ± 3.39). Thus, majority of the patients belonged to overweight–obese category. There was no significant difference between the groups in terms of BMI (Kg/m^2) ($F = 0.298$, $p = 0.827$). Results are summarized in Table 1.

Pre-injection Scores

Pre-injection VAS score was not normally distributed in the four subgroups. Thus, non-parametric tests (Kruskal–Wallis test) were used to make group comparisons. The mean (SD) of VAS (Pre-Injection) in the PRP group was 6.22 (1.34), the ACS group was 6.14 (1.24), the Steroid group was 6.79 (0.96), and the HA group was 6.15 (1.31). The VAS score in most of the patients ranged from 4 to 9. There was no significant difference between the groups in terms of VAS (Pre-Injection) ($X^2 = 6.454$, $p = 0.091$). Pre-injection WOMAC score was not normally distributed in the four subgroups of the variable group. Thus, non-parametric tests (Kruskal–Wallis Test) were used to make group comparisons. The mean (SD) of WOMAC score (Pre-injection) in the PRP group was 48.04 (9.53), the ACS group was 43.29 (16.15), the steroid group was 44.61 (10.38), and the HA group was 41.30 (9.60). There was no significant difference between the groups in terms of pre-injection WOMAC score ($X^2 = 6.162$, $p = 0.104$).

Outcome Scores (at 6 months)

VAS score at 6 months was not normally distributed in the four groups. Thus, non-parametric tests (Kruskal–Wallis test) were used to make group comparisons. The mean (SD) of VAS (6 months) in the PRP group was 3.07 (2.30), the ACS group was 2.29 (1.45), the steroid group was 6.32 (2.06), and the HA group was 3.90 (1.62). There was a significant difference between the four groups in terms of VAS (6 months) ($X^2 = 38.461$, $p \leq 0.001$), with the median VAS (6 months) being highest in the steroid group. Post hoc pairwise tests for Kruskal–Wallis test were performed using Dunn test method with Sidak correction. Significant

Table 1 Association between group and parameters

Parameters	Group				*p* value
	PRP ($n = 27$)	ACS ($n = 21$)	Steroid ($n = 28$)	HA ($n = 20$)	
Age (years)[a]	56.33 ± 4.37	50.81 ± 8.93	54.68 ± 5.00	51.85 ± 7.66	0.016[1]
Gender					0.916[2]
Male	9 (33.3%)	7 (33.3%)	8 (28.6%)	5 (25.0%)	
Female	18 (66.7%)	14 (66.7%)	20 (71.4%)	15 (75.0%)	
KL grade					0.275[3]
Grade 1	2 (7.4%)	7 (33.3%)	2 (7.1%)	3 (15.0%)	
Grade 2	16 (59.3%)	8 (38.1%)	15 (53.6%)	11 (55.0%)	
Grade 3	9 (33.3%)	6 (28.6%)	11 (39.3%)	6 (30.0%)	
BMI (Kg/m^2)	31.48 ± 3.91	30.46 ± 3.57	31.19 ± 4.05	31.05 ± 3.39	0.827[1]
VAS (pre-injection)	6.22 ± 1.34	6.14 ± 1.24	6.79 ± 0.96	6.15 ± 1.31	0.091[4]
VAS (6 months)[a]	3.07 ± 2.30	2.29 ± 1.45	6.32 ± 2.06	3.90 ± 1.62	< 0.001[4]
WOMAC (pre-injection)	48.04 ± 9.53	43.29 ± 16.15	44.61 ± 10.38	41.30 ± 9.60	0.104[4]
WOMAC (6 months)[a]	20.52 ± 14.53	11.33 ± 6.48	42.29 ± 21.13	25.40 ± 9.64	< 0.001[4]
Percent change in VAS[a]	− 53.07 ± 31.16	− 61.24 ± 25.30	− 7.99 ± 30.71	− 35.21 ± 26.89	< 0.001[4]
Percent change in WOMAC[a]	− 59.71 ± 24.07	− 70.10 ± 20.46	0.01 ± 62.08	− 35.51 ± 26.01	< 0.001[4]

[a]Significant at $p < 0.05$, 1: one-way ANOVA, 2: Chi-squared test, 3: Fisher's exact test, 4: Kruskal–Wallis test

differences were found between the ACS and the steroid group; PRP ($p < 0.001$) and steroid group ($p < 0.001$); and HA and steroid group ($p = 0.008$). The remaining pairwise differences were not significant ($p > 0.05$). Post hoc analysis is summarized in Table 2.

WOMAC score at 6 months was not normally distributed in the four subgroups of the variable group. Thus, non-parametric tests (Kruskal–Wallis test) were used to make group comparisons. The mean (SD) of WOMAC score at 6 months in the PRP group was 20.52 (14.53), the ACS group was 11.33 (6.48), the steroid group was 42.29 (21.13), and the HA group was 25.40 (9.64). There was a significant difference between the four groups in terms of WOMAC (6 months) ($X^2 = 36.911$, $p \leq 0.001$), with the median WOMAC (6 months) being highest in the steroid group. Post hoc pairwise tests for Kruskal–Wallis test were performed using Dunn test method with Sidak correction and revealed the ACS group to have significant post procedure WOMAC over the HA and the steroid groups. Similarly, PRP had better WOMAC score at 6 months as compared to the steroid group ($p < 0.001$).

Evaluation of Percent Change in Scores

The authors also performed a comparison of change in VAS and WOMAC scores between pre-injection to 6 months following injection. Percent change in VAS was not normally distributed in the four subgroups and non-parametric tests (Kruskal–Wallis test) were used to make group comparisons. The mean (SD) of percent change in VAS in the groups was: the PRP group − 53.07 (31.16), the ACS group − 61.24 (25.30), the steroid group − 7.99 (30.71), and the HA group − 35.21 (26.89). There was a significant difference between the four groups in terms of percent change in VAS ($X^2 = 36.999$, $p \leq 0.001$), with the median percent change in VAS being highest in the steroid group. Post hoc tests revealed a significant difference between percent change in VAS score between pairwise comparison of the ACS–steroid group and the PRP–steroid group ($p < 0.001$) (Table 2).

Similarly percent change in WOMAC from pre-injection to 6 months was not normally distributed in the four subgroups of the variable group. Thus, non-parametric tests (Kruskal–Wallis test) were used to make group comparisons. The mean (SD) of percent change in WOMAC in the PRP group was -59.71 (24.07), the ACS group was − 70.10 (20.46), the Steroid group was 0.01 (62.08), and the HA group was − 35.51 (26.01). There was a significant difference between the four groups in terms of percent change in WOMAC ($X^2 = 37.503$, $p \leq 0.001$) and post hoc analysis revealed a significant difference for the ACS–HA pair ($p = 0.002$), the ACS–steroid pair ($p < 0.001$) and the PRP–steroid pair ($p < 0.001$) (Table 2).

Discussion

The present day holy grail in orthopedics is finding a solution for quicker healing and faster recovery. The solution should not only be effective, but at the same time should have an acceptable safety profile. Healing potential is inherently present in every tissue and same can be initiated by providing signals with innovative use of either body's own available products or using the same available factors in combination with synthetic products. Orthobiologics are made up of substances found naturally in our body and are capable of promoting faster healing. They have broadly been classified into three categories: matrix, stem cells, and growth factors. Bone graft and scaffolds constitute the matrix. Bone marrow aspirate concentrate (BMAC), adipose-derived stem cells (ADSC) and cultured stem cells form the subgroup stem cells. Platelet-rich plasma (PRP), autologous conditioned serum (ACS) and synthetic peptides constitute the subgroup growth factors [23].

Results of the present study showed that ACS and PRP did not have any significant difference in terms of either WOMAC score at 6 months ($p = 0.154$) or VAS score at 6 months ($p = 0.850$). The scores for both these orthobiologics were better than the control groups (HA group and steroid group). Between the two control groups, HA group had better VAS scores as compared to the steroid group. Our results thus, show superiority of ACS and PRP over both HA and steroid.

Various authors have compared PRP to HA and majority have reported PRP to be superior to HA (Cole et al., Louis et al., Duymus et al., Gormeli et al., Kon et al., Spakova et al., Guler et al., Sanchez et al., Kuan-Yu Lin et al., Yong Huang, Lana et al., Raeissadat et al., and Vaquerizo et al.) [24–34]. Peterson and Filardo have contrarily found HA to give better results than PRP, but in a later RCT, Filardo has found the results of the two groups to be comparable [35–37]. Similar comparisons have been done between steroid and PRP as well and results are similar (Yong Huang et al. and Uslu Guvendi et al.) [32, 38]. Camurcu et al. have found a combination of methylprednisolone and PRP to have better outcomes over isolated use of either of the two agents [39]. In contrast to our methodology Wu et al., Smith et al., and Patel et al. have used saline as the control group [40–42]. Authors have also used acetaminophen, and ozone therapy as control groups [24, 43]. We disagree to use of saline injection as control as we feel that comparison is better done with other second-line treatments that are accepted and serve as a better reference. Use of saline as control eliminates only placebo effect, but use of HA and Steroid group as control provides superior evidence. This allows evaluation of a novel technique by comparison to other established therapeutic interventions (HA and steroid in present study).

Table 2 Post hoc analysis with comparison of the four subgroups in terms of various variables ($n=96$)

Dependent variable	(I) Group	(II) Group	Mean difference (I-II)	Std. error	*p* value
Age (years)	HA	ACS	0.990	0.208	0.956
		PRP	−4.483	0.196	0.096
		STEROID	−2.830	0.194	0.449
	ACS	HA	−1.040	0.208	0.956
		PRP	−5.520	0.193	0.022
		STEROID	−3.870	0.192	0.173
	PRP	HA	4.483	0.196	0.096
		ACS	5.520	0.193	0.022
		STEROID	1.653	0.179	0.781
	STEROID	HA	2.830	0.194	0.449
		ACS	3.870	0.192	0.173
		PRP	−1.653	0.179	0.781
VAS (at 6 months)	HA	ACS	1.614	0.604	0.148
		PRP	0.825	0.570	0.747
		STEROID	−2.421	0.566	0.008
	ACS	HA	−1.614	0.604	0.148
		PRP	−0.788	0.562	0.850
		STEROID	−4.035	0.558	<0.001
	PRP	HA	−0.825	0.570	0.747
		ACS	0.788	0.562	0.850
		STEROID	−3.247	0.521	<0.001
	STEROID	HA	2.421	0.566	0.008
		ACS	4.035	0.558	<0.001
		PRP	3.247	0.521	.<0.001
WOMAC (at 6 months)	HA	ACS	14.066	4.624	0.004
		PRP	4.881	4.366	0.651
		STEROID	−16.885	4.333	0.163
	ACS	HA	−14.066	4.624	0.004
		PRP	−9.185	4.306	0.154
		STEROID	−30.952	4.273	<0.001
	PRP	HA	−4.881	4.366	0.651
		ACS	9.185	4.306	0.154
		STEROID	−21.767	3.992	.<0.001
	STEROID	HA	16.885	4.333	0.163
		ACS	30.952	4.273	<0.001
		PRP	21.767	3.992	.<0.001
Percent change in VAS	HA	ACS	26.03	0.604	0.133
		PRP	17.86	0.562	0.532
		STEROID	−27.22	0.558	0.019
	ACS	HA	−26.03	0.570	0/133
		PRP	−8.17	0.562	0.951
		STEROID	−53.25	0.521	<0.001
	PRP	HA	−17.86	0.566	0.532
		ACS	8.17	0.558	0.951
		STEROID	−45.08	0.521	<0.001
	STEROID	HA	27.22	4.624	0.019
		ACS	53.25	4.366	<0.001
		PRP	45.08	4.333	<0.001

Table 2 (continued)

Dependent variable	(I) Group	(II) Group	Mean difference (I-II)	Std. error	*p* value
Percent change in WOMAC	HA	ACS	34.59	4.624	0.002
		PRP	24.20	4.306	0.061
		STEROID	−35.50	4.273	0.510
	ACS	HA	−34.59	4.366	0.002
		PRP	−10.39	4.306	0.785
		STEROID	−70.11	3.992	<0.001
	PRP	HA	−24.20	4.333	0.061
		ACS	10.39	4.273	0.785
		STEROID	−59.72	3.992	<0.001
	STEROID	HA	35.50	4.273	0.510
		ACS	70.11	4.366	<0.001
		PRP	59.72	4.306	<0.001

Post hoc pairwise tests for ANOVA performed using Tukey HSD method. Post hoc pairwise tests for Kruskal–Wallis test performed using Dunn test method with Sidak correction

The role of ACS in OA knee has not been studied as much as PRP and literature supporting the use of the same is deficient. Evaluation of the role of ACS in OA management began with the in vitro assessment of its effects on cartilage metabolism profile by Rutgers et al. [44]. The authors could not demonstrate an effect on proteoglycan metabolism in the 48 samples they studied, which they postulated was due to short half-life of ACS. Furthermore, no major change in cytokine levels could be demonstrated. Garcia-Escudero group in their prospective observational study demonstrated significant improvement in WOMAC score at 2 years follow-up when using a combination of intra-articular ACS with physiotherapy [45]. Level 1 evidence by Yang et al. and Baltzer et al. has proven superiority of ACS over saline placebo and HA respectively in blinded randomized control trials [16, 46]. However, in the last decade, not much evidence has been seen in favor of ACS. Rutgers et al. also went on to dismiss evidence provided by Yang et al. by re-evaluating patients in their study and concluded that patients in the placebo group were found reluctant to undergo ACS treatment due to its laborious nature. A further analysis revealed that those who were subjected to ACS treatment after placebo did not result in greater clinical improvement than the placebo treatment itself. But, the authors themselves have pointed out to selection bias in their study and its underpowered nature due to its small sample size ($n=20$) [47].

Intra-articular injections of ACS and PRP have been in use for more than a decade for OA knee. However, there is a dearth of studies comparing the two modalities directly. A recent randomized trial comparing ACS to PRP has been published by Shirokova et al., where authors have found ACS to be not just clinically, but also biochemically superior to PRP [48]. This detailed study involved evaluation of IL-1Ra, IL-1b, reactive oxygen species, nitrate levels and synovial fluid viscosity apart from clinical scoring and has found ACS to be significantly superior to PRP at 3 months follow-up. ACS has been recently renamed as blood clot secretome (BCS).

ACS in the present study was processed in a sterile environment with reference to techniques proposed by Meijer and Baltzer [15, 16]. Our results are similar to Shirokova et al. in terms of mean scores, but the difference between PRP and ACS was not found to be significant. However, the mean outcome scores were better with ACS than with PRP. A larger sample size might have contributed to statistically significant differences in terms of our scores.

Authors of the present study have used activated-leucocyte rich-double spin PRP. All PRP preparations are not the same. Arnoczky et al. have stated that various techniques are currently available for preparing PRP and have revealed wide variety of final product which varies in terms of volume of blood harvested, inclusion or exclusion of leucocytes, or whether platelets are activated or not [49]. PRP can thus, be buffy coat versus plasma based, leucocyte rich (LR) versus leucocyte poor (LP) and single spin versus double spin [50]. So, a very elaborate classification has been developed following the work of Mishra et al. further modified by DeLong et al., called the PAW classification to classify various PRP preparations that are being commonly used [51].

Abundance of Leucocytes in LR PRP release Matrix Metalloproteases (MMP) 2, 3 and 9 and other pro-inflammatory mediators which are postulated to have deleterious effects in the knee [6, 52]. These deleterious effects have been proposed to manifest as local adverse effects owing to greater inflammatory response. Contrarily, Cavallo et al. in their in vitro study have noticed that chondrocyte proliferation and hyaluronan secretion were more with leucocyte rich PRP [53]. Patel and Sanchez et al. have used leucocyte filter in their initial RCTs [42, 54]. Filardo and Elizaveta Kon were using leucocyte rich PRP initially [25] and later compared LR versus LP PRP. They were of the consensus that the results of the two groups were comparable, but the group receiving LR PRP suffered more local adverse effects [55]. This was also confirmed by Riboh et al. who also found initial pain and swelling with LR PRP [56]. A recent study has found contradictory data suggesting that LR PRP is better in terms of decreased recurrence of symptoms, but local adverse effects were more common with LR PRP [57]. Hence, there is no final verdict on advantage of LP over LR PRP or vice versa and both have been found to be effective in literature. The authors of present study have used leucocyte rich PRP and our outcomes were acceptable and comparable to the existing literature evaluating both LR and LP PRP.

Similarly, some literature supports use of exogenous agents for PRP activation. These exogenous agents include calcium chloride (presently calcium gluconate) or thrombin or a combination of the two. Another option is activation by contact with the tissue, which is endogenous activation. Activation helps in formation of platelet fibrin clot inside knee which traps the platelets thus, helping in gradual and slow release of growth factors. There is, however, no literature consensus on whether exogenous activation is advantageous [58]. Regimens of PRP use also differ in literature. The number of injections and duration between injections is highly variable between the studies. Initial studies focused on three injections as the use of HA was prevalent with a three injection regimen in these studies and PRP was compared to HA given by the same regimen to maintain uniformity. Patel et al. compared 1 vs 2 injections, but found no difference in the outcome of early OA knee in alleviating pain [42]. Gormeili et al. [59] suggested that multiple injections are better than single injection. Chouhan et al. [60] in their animal model have found that anti-inflammatory effects of three doses of PRP are better than single dose of PRP at 6 months. They have also found chondro-protective effect of three doses to be better than single dose at as early as 3 months. Moreover, Patel et al. have found that outcomes deteriorate after 3 months slightly and this has also been shown by Gobbi et al. where they proposed annual repetition of injections [61]. Since the present study is a short follow-up study, the number of injections was limited to one. With wearing of effects, some patients of the present study were considered for a repeat injection at approximately 1 year after the first injection.

Hylan polymer A and B, G-F 20 (HA) and methylprednisolone (steroid) were used as controls in the present study. The short-term benefits of intra-articular corticosteroid injection are well recognized, but long-term benefits and the value of repetitive injections are still debatable [63]. A previous randomized study comparing three doses of hylan G-F 20 and multiple corticosteroid (Betamethasone) injections showed that there was no difference between the two drugs in relieving pain and improving function during a 6-month follow-up [64]. This is contrary to our findings as we found GF20 to have superior pain scores at 6 months, possibly due to steroid preparation used in the present study (methylprednisolone) or use of a single injection of steroid. The same fact is supported by more recent meta-analyses which suggests that intra-articular injection of hyaluronic acid might have a slower onset, but in some cases, has longer and better effects than corticosteroid [1, 65].

This study did not investigate the effect of these injectables on the cartilage and joint structure. A study with a placebo arm or a basic science study may further clarify role of control group injectables as second-line agent in OA management. Another limitation of this study is its retrospective design, with comparison of the PRP and controls to a historical cohort of patients receiving ACS. Non-randomized nature of the study, lack of synovial fluid analysis, and small sample size per group are limitations of the present study.

Conclusion

The present study demonstrated that both PRP and ACS are effective in relieving OA pain at 6 months; hence, are effective palliative agents in second-line therapy for early OA knee management. Steroid and HA given IA can give initial pain relief, while HA provides more significant pain relief until 6 months after the injection. The results of ACS and PRP are better than controls (HA and steroid), but a difference between the two orthobiologics could not be demonstrated statistically.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical standard statement This article does not contain any studies with human or animal subjects performed by the any of the authors.

Informed consent For this type of study, informed consent is not required.

IRB Information Scientific committee and ethical approval has been obtained for this study.

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