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Original Research

Intralesional Platelet-Rich Plasma Injection in Patients with Recalcitrant Alopecia Areata

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Abstract

Objectives: Alopecia areata (AA) is a common, chronic autoimmune disease that causes non-cicatricial hair loss. Its relapsing and remitting nature leads to the search for new, effective treatment options. The study aimed to evaluate the therapeutic efficacy of intralesional platelet-rich plasma (PRP) and intralesional steroid (ILS) injections in patients with AA.

Methods: A retrospective chart review was carried out from 2020-2021. A total of 75 patients with AA were included in the study. Thirty-six patients were treated with intralesional PRP, and 39 patients were treated with ILS injections for three sessions. The patients were evaluated with a hair pull test and SALT scores at months 0, 3, and 6.

Results: Of 75 patients, the mean age of the PRP group was 34.33±10.61, and the mean age of the ILS group was 33.82±13.31 years. After three PRP or ILS therapy sessions, at 3. and 6. months, SALT 3 and SALT 6 scores were statistically significantly lower in the PRP group than in the ILS group (p=0.038, p<0.001, respectively). When the treatment response was evaluated at the end of the 6th month in the PRP group, there was no response in 2 (5.5%) patients, partial response in 1 (2.7%) patient, good response in 3 (8.4%) patients, and very good response in 30 (83.4%) patients. Only 2 (5.9%) patients had a clinical relapse in a 6-month period. Side effects were seen in 16 (44.4%) patients in the PRP group and 8 (20.5%) patients in the ILS group, and the frequency of side effects in the PRP group was statistically significantly higher than in the ILS group (p=0.026). However, the side effects of both groups were minor, such as itching, pain, burning, ecchymosis, and folliculitis.

Conclusion: PRP seems to be an effective and safe treatment option for limited patchy alopecia areata, but its superiority over ILS has not been fully demonstrated, making ILS still the first-line treatment

Keywords: Alopecia, ILS, platelet-rich plasma, PRP, steroid, triamcinolone

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Alopecia areata (AA) is a common, chronic, autoimmune disease that causes non-cicatricial hair loss due to disruption of immune privilege of the hair follicles.[1] The scalp is the mainly involved area, but the disease can affect the beard, eyebrows, eyelashes, the hair follicles of the whole body, and nails.[2] The course of the disease is variable. The disease may start as a single alopecia patch on the scalp and undergo spontaneous resolution, or it may present with widespread alopecia patches with frequent relapses and even progress to alopecia universalis.[3]

Topical treatments are generally preferred over systemic therapies for the limited patchy forms of AA. Intralesional

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triamcinolone acetonide injection (TA) is the first line of treatment in adult patients with a SALT score of 0-30%.[4] Intralesional steroid injection (ILS) is a convenient, effective, and inexpensive treatment modality.[5] Relapses and treatment failures with ILS lead to the search for new effective treatment options.

Since AA is known to be an acquired autoimmune disorder, treatment strategies are always based on reducing the immune response by providing immunosuppression. However, it can be suggested that achieving hair regeneration by restoring normal hair follicle function in AA requires more than immunosuppression.[4] Platelet-rich plasma (PRP) is known to play a role in tissue regeneration and/or restoration by stimulating cell proliferation and differentiation with its rich growth factor content.[6, 7] In the literature, several studies reported the effectiveness of PRP in AA with varying success rates.

This study aimed to evaluate the therapeutic efficacy of intralesional PRP in patients with chronic alopecia areata and to compare the effectiveness of PRP and intralesional steroid injection in AA.

Methods

A retrospective chart review was conducted over 20 months, from January 2020 to August 2021. The study included 75 patients with alopecia areata who were admitted to a dermatology outpatient clinic. The Ankara Training and Research Hospital Clinical Research Ethics Committee approved the study (20.10.2021 E-93471371-514.01.02). The study was performed in accordance with the latest version of the 'Helsinki Declaration' and 'Guidelines for Good Clinical Practice'.

Inclusion criteria consisted of patients with limited patchy AA on the scalp aged 18-65 years who had not received any topical and/or systemic treatment in the last 1 month. When the SALT score is 0-30 %, and the type of AA is patchy, the disease is considered "limited patchy AA". Patients with systemic diseases that may cause platelet disorders (malignancy, hematological diseases, autoimmune diseases, HIV, hepatitis B-C) were excluded.

Demographic data such as age, gender of the patients, disease duration, family history, comorbidities, and side effects of the treatment were recorded. The study group (75 patients) consisted of patients with a duration of the disease of more than 1 year, which is defined as "chronic AA". Of the 75 patients with AA, 36 were treated with intralesional PRP injections for three sessions at 3-week intervals. PRP group included patients with chronic AA who were treated with topical steroids and/or ILS with failure and/or relapses previously. The ILS group included the remaining 39 patients with chronic AA (the patients who received topical steroids and/or ILS with failure and/or relapses previously) who were treated with ILS injections again for three sessions at 3-week intervals. Clinical photographs before and after treatment were obtained from the patient photographs archive. Clinical response was evaluated by calculating the Severity of Alopecia Tool (SALT) score. All the patients were assessed with a hair pull test at the margins of alopecia patches and SALT scores at months 0, 3, and 6 and were followed up for relapse for the next 6 months. SALT is an assessment method to measure the severity of hair loss in patients with AA. The scalp is divided into four parts to calculate the SALT score: the vertex, the right/left lateral side and the back. The percentage of hair loss in the four areas is multiplied by 0.4, 0.18, 0.18, and 0.24, respectively, and the SALT score is calculated by summing the scores in 4 regions.[3, 8] AA investigational assessment guidelines divided SALT score into subgroups: S0-S5. The S1 subgroup defines hair loss that is less than 25%.[3, 8] In the evaluation of post-treatment responses: < 25% decrease in SALT score="no response", a decrease of 25-49% in SALT score="partial response", a reduction of 50-74% in SALT score="good response", and a decrease of 75% or more in SALT score="very good response".

Treatment Technique and Protocol

PRP Group: A PRP kit (T-lab PRP Kit, T-lab Regenerative Medicine Company, Bursa, Turkiye) with a Class IIb certificate was used for the patients in the PRP group. Sixteen ml of peripheral venous blood was collected into two self-vacuumed tubes and centrifuged for 2 minutes at 2000 rpm. After centrifugation, supernatant plasma with the whole buffy coat was drawn up into 1 ml 30-gauge insulin syringes without adding any activator. The platelet concentration in PRP was increased to 3-7 times the basal serum value. Then, it was injected intradermally into the alopecia patches of the scalp in aliquots of 0.05-0.1 ml/cm^2 at 1-centimetre intervals.

ILS Group: Triamcinolone acetonide was diluted with saline solution to obtain a concentration of 5 mg/ml according to the recommendations of the AA Consensus of Experts study and drawn into 1 ml 30-gauge insulin syringes.[4] The suspension was then injected intradermally into the alopecia patches of the scalp in aliquots of 0.05-0.1 ml/cm^2 at 1-centimetre intervals.

Statistical Analysis

All analyses were carried out using IBM SPSS Statistics for Windows, Version 20.00 (Armonk, New York, USA: IBM Corp), and a p-value less than 0.05 was considered statistically significant. The normality of the data was tested by the Shapiro-

Wilk test, and it was determined that continuous variables did not show normal distribution. Continuous variables were expressed by mean±standard deviation and median, interquartile range. Categorical variables were represented by number and percentage. Mann-Whitney U and Chi-Square tests were used to compare continuous and categorical data between the groups. The Friedman test was used to determine whether the change in the SALT score was significant during the treatment process, and the post hoc Dunn test was used for pairwise comparisons. The Cochran Q test was used to compare the positivity rates according to the hair pull test results in three different periods. The Conover post-hoc test was used for pairwise comparisons.

Results

This comparative study included 75 patients with patchy AA. Thirty-six out of 75 patients were treated with PRP and 39 with ILS. The age, sex distributions, family history, medical history, and disease duration of the PRP and ILS groups are presented in Table 1. Of the 36 patients treated with PRP, 20 (55.6%) were female, 16 (44.4%) were male, and the mean age of the PRP group was 34.33±10.61 years. The median disease duration in the PRP group was 14 (min:12, max:60, IQR:4) months. Characteristics of the two groups in terms of sex, age, and disease duration were similar (p=0.416, p=0.504, p=0.071).

The patients were evaluated with SALT scores and hair pull tests at 0, 3, and 6 months. There was no statistically significant difference in the SALT 0 scores of the PRP and ILS groups at baseline (p=0.067). At 3. and 6. months, SALT 3 and SALT 6 scores were statistically significantly lower in the PRP group than in the ILS group (p=0.038, p<0.001, respectively) (Table 2). At baseline, the number of patients in the PRP group with a positive hair pull test was statistically significantly higher than in the ILS group (p=0.005). There was no statistically significant difference between the groups regarding hair pull test results at 3. and 6. months (p=0.509, p=0.669, respectively) (Table 2).

Table 1. Demographic features of the PRP and ILS groups

	PRP group (n=36)	ILS group (n=39)	p
Sex (n/%)			
Female	20 (55.6)	18 (46.2)	0.416
Male	16 (44.4)	21 (53.8)	
Age (Mean±SD, years)	34.33±10.61	33.82±13.31	0.504
Family history (n/%)			
Present	3 (8.3)	5 (12.8)	0.713
Absent	33 (91.7)	34 (87.2)	
Medical history (n/%)			
Present	6 (16.7) 1. Atopic dermatitis 2. Celiac disease 3. Gastritis 4. Gallstone 5. Depression 6. Hypertension and asthma	13 (33.3) 1. Coronary artery disease, diabetes mellitus 2. Atrial septal defect 3. Hypothyroidism 4. Epilepsy 5. Hypertension and gastritis 6. Asthma 7. Hypothyroidism 8. Hypertension 9. Hypertension 10. Hypothyroidism 11. Atopic dermatitis 12. Hypertension 13. Hypothyroidism	0.097
Absent	30 (83.3%)	26 (66.7%)	
Disease duration (median/minimum, maximum, IQR, months)	14 (min:12, max:60, IQR:4)	15 (min:12, max:72, IQR:5)	0.071

SD: Standard deviation; IQR: interquartile range; Data were expressed as mean±SD, median, minimum, maximum and IQR in continuous variables and n (%) in categorical variables, respectively. Independent samples were compared with Mann–Whitney U test and Chi Square test.

Table 2. SALT scores and hair pull test at 0, 3 and 6 months in the PRP and ILS groups

	PRP group (n=36)	ILS group (n=39)	p
SALT 0			
Median (min, max, IQR)	3.6 (min:1.2, max:22.4, IQR:2.9)	3 (min:1, max:21.5, IQR:2)	0.067
Hair pull test 0 Number/%			
Positive	28 (77.8)	18 (46.2)	0.005*
Negative	8 (22.2)	21 (53.8)	
SALT 3			
Median (min, max, IQR)	1.6 (min:0, max:16.4, IQR:2.83)	2.5 (min:1, max:20, IQR:1.8)	0.038*
Hair pull test 3 Number/%			
Positive	13 (36.1)	17 (43.6)	0.509
Negative	23 (63.9)	22 (56.4)	
SALT 6			
Median (min, max, IQR)	0 (min:0, max:8.4, IQR:1.35)	2 (min:0.8, max:18, IQR:1.7)	<0.001*
Hair pull test 6 Number/%			
Positive	6 (16.7)	8 (20.5)	0.669
Negative	30 (83.3)	31 (79.5)	
New Hair Growth Time (months)			
Median (min, max, IQR)	1 (min:1, max:2, IQR:0)	2 (min:1, max:3, IQR:2)	0.001*
Pigmentation status of the new hairs Number/%			
Pigmented	25 (69.4)	5 (12.8)	0.001*
Depigmented	11 (30.6)	34 (87.2)	

Min: Minimum; Max: Maximum; IQR: interquartile range; Data were expressed as median, minimum, maximum and IQR in continuous variables and n (%) in categorical variables, respectively. Independent samples were compared with Mann–Whitney U test and Chi Square test.

All the patients were included in the S1 (SALT score < 25%) group according to AA investigational assessment guidelines. When treatment responses were compared over three periods (months 0, 3, and 6), there was a statistically significant difference in SALT scores between the three measurement periods. The score decreased significantly until the measurement at the 6th month in the PRP group (p<0.001) (Fig. 1, Table 3). There was a statistically significant difference between the three measurements regarding hair pull test results (p<0.001). Still, there was no statistically significant difference in the PRP group's hair pull test results between the 3rd and 6th months (p=0.068) (Table 3).

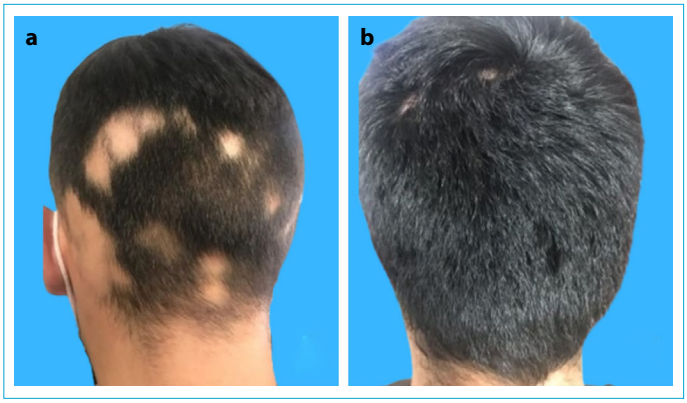

Figure 1. Before treatment **(a)** and after 3 sessions of PRP **(b)**.

When the treatment response was evaluated at the end of the 3rd month, there was no response in 5 (13.8%) patients, partial response in 7 (19.4%) patients, good response in 11 (30.5%) patients, and very good response in 13 (36.3%) patients. However, according to the evaluation at the end of the 6th month, there was no response in 2 (5.5%) patients, partial response in 1 (2.7%) patient, good response in 3 (8.4%) patients, and very good response in 30 (83.4%) patients. Thirty-four (94.5%) patients who responded to treatment were followed up for relapse for a total of 6 months after excluding 2 (5.5%) patients who were unresponsive to PRP treatment. Only 2 (5.9%) patients had a clinical relapse.

Side effects were seen in 16 (44.4%) patients in the PRP group and 8 (20.5%) patients in the ILS group, and the frequency of side effects in the PRP group was statistically significantly higher than in the ILS group (p=0.026). However, the side effects of both groups were minor, such as itching, pain, burning, ecchymosis, and folliculitis, and none of the patients experienced major side effects (Table 4).

Discussion

Alopecia areata is a common autoimmune disease with inflammation-induced patchy hair loss that is easy to diagnose with its typical clinical findings. However, the

Table 3. Change in SALT scores and hair pull test results assessed in three different periods in PRP and ILS groups

PRP group (n=36)		**p**
SALT scores		
SALT 0 Median (min, max, IQR)	3.6 (min:1.2, max:22.4, IQR:2.9)	<0.001*
SALT 3 Median (min, max, IQR)	1.6 (min:0, max:16.4, IQR:2.83)	
SALT 6 Median (min, max, IQR)	0 (min:0, max:8.4, IQR:1.35)	
Pairwise Comparisons		
SALT 0 vs SALT 3		<0.001*
SALT 0 vs SALT 6		<0.001*
SALT 3 vs SALT 6		<0.001*
Hair pull test		
Hair pull test 0 (positive, n/%)	28 (77.8%)	<0.001*
Hair pull test 3 (positive, n/%)	13 (36.1%)	
Hair pull test 6 (positive, n/%)	6 (16.7%)	
Pairwise Comparisons		
Hair pull test 0 vs Hair pull test 3		<0.001*
Hair pull test 0 vs Hair pull test 6		<0.001*
Hair pull test 3 vs Hair pull test 6		0.068
ILS group (n=39)		**p**
SALT scores		
SALT 0 Median (min, max, IQR)	3 (min:1, max:21.5, IQR:2)	<0.001*
SALT 3 Median (min, max, IQR)	2.5 (min:1, max:20, IQR:1.8)	
SALT 6 Median (min, max, IQR)	2 (min:0.8, max:1.8, IQR:1.7)	
Pairwise Comparisons		
SALT 0 vs SALT 3		<0.001*
SALT 0 vs SALT 6		<0.001*
SALT 3 vs SALT 6		0.005*
Hair pull test		
Hair pull test 0 (positive, n/%)	18 (46.2%)	0.002*
Hair pull test 3 (positive, n/%)	17 (43.6%)	
Hair pull test 6 (positive, n/%)	8 (20.5%)	
Pairwise Comparisons		
Hair pull test 0 vs Hair pull test 3		0.743
Hair pull test 0 vs Hair pull test 6		0.001*
Hair pull test 3 vs Hair pull test 6		0.003*

Min: Minimum, Max: Maximum, IQR: interquartile range; Data were expressed as median, minimum, maximum and IQR in continuous variables and n (%) in categorical variables, respectively. Continuous dependent samples were compared with Friedman test and post hoc Dunn test was used for pairwise comparisons. Categorical dependent samples were compared with Cochran Q test and Conover post-hoc test was used for pairwise comparisons.

disease poses a therapeutic challenge because no treatment is either curative or preventive. PRP is a promising treatment modality that has gained importance in managing dermatological diseases with its rich content of growth factors, cytokines, and versatile effects. PRP ensures cell proliferation and differentiation, stimulates angiogenesis, prevents apoptosis, and has a potent anti-inflammatory effect.[6, 7] Also, specifically for hair follicles, PRP was shown to induce cell proliferation in the dermal papilla, improve dermal papilla cell survival via anti-apoptotic effects, prolong the anagen phase of the hair life cycle, prevent transition to the catagen phase resulting in hair regrowth.[9] Last but not least, PRP decreases local tissue inflammation by suppressing cytokine release, which may be beneficial in treating the inflammatory component of AA.[10]

Table 4. Distribution of side effects in the PRP and ILS groups

	PRP group (n=36)	ILS group (n=39)	p
Side effects (n/%)			0.026*
Present	16 (44.4)	8 (20.5)	
Absent	20 (55.6)	31 (79.5)	
Side effect list (n/%)			
Present	16 (44.4)	8 (20.5)	
	3 (8.3) erythema	3 (7.7) erythema	
	3 (8.3) pain	3 (7.7) pain	
	2 (5.6) ecchymosis	1 (2.6) ecchymosis	
	3 (8.3) itching	1 (2.6) folliculitis	
	4 (11.1) burning sensation		
	1 (2.8) folliculitis		

Data were expressed as n (%) in categorical variables. Independent samples were compared with Chi Square test.

This study investigated the therapeutic efficacy of PRP in patchy AA patients. In our study, we evaluated the SALT scores and results of the hair pull test of the patients who received PRP (single spin, 2000 rpm for 2 minutes without activator) or ILS (5 mg/ml TA). SALT scores were similar in PRP and ILS groups at baseline evaluations. We found a statistically significant decrease in SALT scores after each of the three treatment sessions compared to baseline in both ILS and PRP groups. Besides, the SALT scores were significantly lower in the PRP group in the 3rd and 6th months compared to the ILS group.

Multiple studies in the literature have demonstrated encouraging outcomes of PRP in the treatment of AA whereas some studies could not show its superiority over ILS and even found ILS more effective. Kapoor et al.[11] compared the therapeutic efficacy of intralesional TA and PRP prospectively in 40 patients with AA. Twenty patients received ILS (10 mg/ml), and 20 patients received (single spin, 2000 rpm for 3 minutes without activator) PRP every 3 weeks for 12 weeks. They reported that the reduction in the SALT score at each visit was greater in the TA group than the PRP group and concluded that triamcinolone was more effective in AA. Albalat et al.[12] conducted a randomized, double-blind study and evaluated PRP and ILS in treating 80 patients with AA. Forty patients received ILS (5 mg/ml TA), and 40 patients received (double spin, 150g for 10 minutes, 1500-2000 g for 10 minutes with activator) PRP 3-5 sessions every 2 weeks. They found a statistically significant improvement in SALT scores after treatment compared to baseline in both ILS and PRP groups. After 3 months, 65% of the patients in the ILS group and 72.5% of the patients in the PRP group showed >70% improvement. Balakrishnan et al.[13] evaluated therapeutic response to PRP and TA in AA in a comparative study with 40 patients. Sixteen patients who completed the study received (double spin, 1500 rpm for 15 minutes, 2500 rpm for 10 minutes without activator) PRP and 16 who completed the study on the other arm received ILS (10mg/ml TA) 3 sessions every 4 weeks. They reported no statistically significant difference between the two groups at the 4th and 12th weeks. Fawzy et al.[14] used trichoscopy, SALT score, and Alopecia Areata Symptom Impact Scale to compare ILS and PRP in AA. Fourteen patients were treated with ILS (5 mg/ml TA), and 17 patients were treated with PRP (single spin, 3000 rpm for 10 minutes without activator) once monthly for 3 months. They reported a significant improvement in trichoscopy findings and SALT scores compared to baseline levels in both groups. Hegde et al.[15] conducted a randomized, placebo, and active-controlled split scalp study to evaluate the efficacy of PRP in AA. The left side of the scalp of 50 patients with AA received a placebo (intralesional normal saline), the right side of the scalp of 25 patients received intralesional PRP (double-spin 1400 rpm for 10 minutes, 2800 rpm for 10 minutes without activator), and right side of the scalp of 25 patients received ILS (5mg/ml TA) for three treatment sessions at 4 weeks intervals. The SALT score showed significant improvement from baseline in both groups. Additionally, the maximum absolute regrowth was shown by the steroid group, followed by PRP, followed by the placebo group.

On the other hand, a study also reported PRP as a more effective treatment alternative. Trink et al.[16] evaluated the efficacy and safety of PRP for the treatment of AA in a randomized, double-blind, placebo- and active-controlled, half-head, parallel-group study consisting of 45 patients. Fifteen patients received (single spin, 70 g for 8 minutes with activator) PRP, 15 patients received (2.5mg/ml TA) ILS, and 15 patients received placebo (distilled water) for one half their scalp for three sessions 1 month apart. They re-

ported that patients treated with PRP had significantly increased hair regrowth, Ki-67 levels, and decreased hair dystrophy and burning or itching sensation compared with TA or placebo groups.

In summary, Kapoor et al.[11] reported that ILS was more effective than PRP in treating patches of AA. Albalat et al.,[12] Balakrishnan et al.,[13] Hegde et al.[15] and Fawzy et al.[14] found that PRP was an effective treatment option, but they could not demonstrate its superiority over ILS. Trink et al.[16] reported that PRP was more effective than placebo and ILS in terms of increased hair regrowth, which is in line with the findings of our study. The patients in our study were patients with chronic AA who did not benefit from ILS. Given that PRP was effective in this patient group, it can be suggested that sometimes anti-inflammatory treatment is not sufficient in the treatment of AA, and the use of mechanisms effective in prolonging the anagen phase, inducing cell differentiation and promoting hair growth with PRP may provide additional benefits in the treatment AA. Besides, it should be noted that there were some differences in the materials and methods of the studies, such as PRP preparation protocols, treatment schedules, and TA concentration. In a recent meta-analysis, the aforementioned four studies investigating the effectiveness of PRP in alopecia areata were evaluated and found that pooled mean differences from the four studies did not exhibit a significant difference in the mean change in the SALT score between PRP and TA groups. Thus, it was concluded that PRP is a promising steroid-saving treatment option in the management of AA.[5] When we evaluated all the studies together, the dose of TA seemed to affect the results of the studies. While ILS was more successful at 10 mg/ml TA doses, PRP was more successful when TA was used in 2.5 mg/ml doses. Although PRP was more effective in our study, ILS seems to retain its place in the first step of treatment due to its accessibility and cost-effectiveness, and PRP will take its place as the second option in cases where ILS fails.

While the clinicians are more cautious about the cutaneous side effects of TA, interestingly, in our study, side effects related to PRP were more common significantly. In line with the reported side effects of our study, Albalat et al.[12] reported erythema and burning sensation in 20 patients (50%) in both groups after the first session, but no other serious side effects were observed. Consequently, PRP seems to be a safe alternative treatment since no side effects were reported in many studies in the literature.[15, 16]

The present study has several limitations. The main limitation is its retrospective design and small sample size, which might not be enough to determine the true prevalence of side effects and complications. SALT scores were calculated from photographs, and there was not a blind investigator. Lastly, PRP and ILS injections were only performed on different areas of the scalp but not on beards and eyebrows. Therefore, it was not possible to assess different response rates for other areas of involvement.

Conclusion

PRP seems to be an effective and safe treatment option for limited patchy alopecia areata, but its superiority over ILS has not been fully demonstrated, making ILS still the first-line treatment. Further prospective randomized controlled studies with a greater number of patients and with a standardized protocol for the preparation and administration of PRP are needed to fully address the place of PRP in the treatment algorithm of AA.

Disclosures

Ethics Committee Approval: The study was approved by the Ankara Training and Research Hospital Clinical Research Ethics Committee (date: 20.10.2021, number: E-93471371-514.01.02).

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