Article type: Concise Communication

A randomized, double-blind, placebo and active-controlled, half-head study to evaluate the effects of platelet rich plasma on alopecia areata

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Running title: PRP for alopecia areata

What's already known about this topic?

- Platelet-rich plasma (PRP) has emerged as a new treatment modality in dermatology, and preliminary evidence has suggested it might have a beneficial role in hair growth. However, no study has ever evaluated the effect of PRP on hair growth in alopecia areata (AA) patients.

What does this study add?

- PRP was found to increase hair regrowth when compared with triamcinolone acetonide or placebo, and Ki-67 levels were significantly higher. PRP also decreased the percentage of dystrophic hairs and burning/itching sensation.
- This study, which is the first to investigate the effects of PRP on AA, suggests that PRP may serve as a safe and effective treatment option in AA

Conflict of interest: None declared

Funding sources: No funding was received for this research

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Summary

Background Alopecia areata (AA) is a common auto-immune condition, causing inflammation-induced hair loss. This disease has very limited treatment possibilities, and no treatment is either curative or preventive. Platelet-rich plasma (PRP) has emerged as a new treatment modality in dermatology, and preliminary evidence has suggested it might have a beneficial role in hair growth.

Objectives To evaluate the efficacy and safety of PRP for the treatment of AA in a randomized, double-blinded, placebo and active-controlled, half-head, parallel group study.

Methods Forty five AA patients were randomized to receive intralesional injections of PRP, triamcinolone acetonide (TrA) or placebo on one half of their scalp. The other half was not treated. A total of three treatments were given for each patient, with an interval of one month from each other. The endpoints were hair regrowth, hair dystrophy as measured by dermoscopy, burning/itching sensation and cell prolifertation as measured by Ki-67 evaluation. Patients were followed for 1 year.

Results PRP was found to significantly increase hair regrowth and decrease hair dystrophy and burning/itching sensation when compared with TrA or placebo, and Ki-67 levels, which served as markers for cell proliferation, were significantly higher. No side effects were noted during treatment.

Conclusions This pilot study, which is the first to investigate the effects of PRP on AA, suggests that PRP may serve as a safe and effective treatment option in AA, and calls for more extensive controlled studies with this method.

Alopecia areata (AA) is the most common condition to cause inflammation-induced hair loss, having a calculated lifetime risk of 2%.[1] It is characterized by well demarcated patches of hair loss, which can progress to complete loss of hair from the scalp (alopecia totalis) or from the whole body in severe cases (alopecia universalis).[2] Most patients are relatively young, and disease burden is commonly substantial, leading to overwhelming effects on the patient's quality of life and self esteem. AA is considered an organ-specific autoimmune disease, stemming from loss of the hair follicle's (HFs) immune privilege; therefore, therapies are mostly immunosuppressive. Nevertheless, treatment is still a challenge in AA, and no treatment is either curative or preventive.[3] Finding new therapies for this condition, and improving effectiveness of existing conditions, are therefore of utmost important.

Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma.[4] It has been investigated in several disciplines in medicine for its role in wound healing, especially orthopedics and dentistry.[5] Recently, it has also been found to be beneficial in dermatology, for example in acne scarring, wound healing and fat transplantation. It has also been shown to promote hair survival and growth, both *in vitro* and *in vivo*.[4,6] However, no study has ever evaluated the effects of PRP on hair growth in AA patients. It is on this basis that we performed a randomized, double-blinded, placebo and active-controlled, half-head, parallel group study on 45 patients to evaluate the efficacy and safety of PRP in AA patients. This is the first time that such extensive and comprehensive assessment methods are used for evaluating efficacy of AA treatment.

Patients and methods

Study design

This was a randomized, double-blinded, placebo and active-controlled, half-head, parallel group study. All patients provided written informed consent before participating in the study, and the study was performed according to the declaration of Helsinki.

Patients

Subjects were 45 male and female otherwise healthy AA patients with a chronic, recurring disease with at least 2 years duration, and consisting of between 4-6 symmetrically distributed patches of hair loss. For each patient, essential background data were collected at baseline according to the guidelines of the National Alopecia Areata Foundation.[7,8] In accordance with these guidelines, in addition to patient demographics, the following parameters were collected: pattern of hair loss, age of onset, number of relapses, total duration of disease, duration of last relapse and number of alopecia areata patches. Supplementary Table 1 summarizes patient demographics and parameters of disease severity for all patients enrolled in the study. Exclusion criteria included any other medical condition or other scalp or hair diseases. All patients were evaluated and enrolled to the study in Rinaldi Dermatologic Clinic, Milan, Italy. Ki-67 evaluation was performed in the University of Brescia, Brescia, Italy.

Treatment

A statistician who was not involved in the study prepared a randomized allocation table. Patients were randomized to one of three groups: PRP, triamcinolone acetonide (TrA, 2.5 mg/ml, current standard treatment modality) or placebo. For PRP preparation, 36 ml of

peripheral blood was centrifuged for 8 minutes at 580 revolutions per minute. PRP fraction was separated and suspended with calcium gluconate. Platelet count was in average about 3.5 times higher than whole blood. One lesion in each patient was treated with intralesional injections of PRP, TrA or placebo. Patients with lesions localized to the temporal and nuchal areas were injected with the test material only on their right side of the scalp, while patients with lesions in the frontal-occipital parts were injected with the test material only in the occipital part of the scalp. The other side was injected with distilled water. A total of three treatments were given for each patient, with an interval of one month from each other. Since consistency and color of the three different treatments differ from each other, the physicians injecting the test material were not blinded to the treatment modalities. Nevertheless, the injections were concealed from the patients themselves, and the injecting personnel were not involved in the evaluation of efficacy of treatment.

Assessment criteria

All patients were evaluated at 4 time points: T0 = beginning of study, T1 = 2 months, T2 = 6 months and T3 = 12 months. Each patch was digitally macro-photographed, measured, and evaluated with video-dermoscopy for the detection of dystrophic forms and possible skin associated manifestations.

In accordance with the guidelines of the National Alopecia Areata Foundation, for the evaluation of hair regrowth we used the SALT score, which represents hair regrowth as percentage of change from baseline.[8-10] Macrographs were evaluated by three independent evaluators, who were blinded to treatment modalities.

Since AA patches are commonly accompanied by burning or itching sensation, which often appears during the development of the disease,[11] subjective assessment of burning/itching sensations was also performed. Itching/burning sensation was determined on a 4-point scale: 3=strong itching/burning sensation, 2=moderate itching/burning sensation, 1 = mild itching/burning sensation, 0 = no itching/burning sensation.

Dermoscopic evaluation was carried out using dermoscopic photomicrographs, which were evaluated by two independent evaluators. This evaluation intended to evaluate the number of dystrophic hairs in the patch area. Markers for dystrophic hairs included exclamation mark hairs, black dots, yellow dots and pigtail regrowing hair. The percentage of dystrophic hairs was evaluated on a 4-point scale: 3= >50% dystrophic hairs, 2 = 30-50% dystrophic hairs, 1 = 1-29% dystrophic hairs, 0 = no dystrophic hairs.

Levels of Ki-67, a marker for cellular proliferation, were assessed from 20 hairs that were removed from the active margins of patches in each time point. Ki-67 levels were measured by immunohistochemical staining with the immunoperoxidase method using Ki-67 monoclonal antibodies.

A two sample Student's *t* test was used for comparisons at baseline and during the study. The tests were interpreted with the risk α of 5%.

Results

A total of 45 AA patients (20 males, 25 females, mean age 28) were enrolled to the study from June 2009 – January 2010. Patients had between 4-6 symmetrically distributed patches of hair loss (mean 4.85). Duration of last relapse was between 1-3 years (mean 1.6), and no

treatment was given to the patients for at least one year. Comprehensive assessment of blood parameters was normal for all patients, except for two patients with mild elevation in anti-nuclear antibody titers (1:80) and one patient with elevated cholesterol levels. Groups were homogeneous in terms of age, gender and severity. A summary of patient characteristics is listed in Table 1 (full patient characteristics can be found in Supplementary Table 1).

Administration of both TrA and PRP led to a significant hair regrowth in AA lesions compared to placebo as assessed by three different independent dermatologists (Figure 1a). Both treatments also led to increased hair regrowth when compared to the untreated side of the scalp (Figures 1 and 2 and Supplementary figures 1-3). Additionally, patients treated with PRP had significantly increased hair regrowth when compared with those treated with TrA. 26.6% of patients treated with TrA achieved complete remission at T3, compared with 60% of patients treated with PRP, which is significantly higher than TrA and placebo treated patients.

At T2, 38% of the patients in the TrA group had relapse of the disease, while no patients from the PRP group had relapse at this time point. At T3, 71% of the patients in the TrA group experience relapse of disease, while only 31% of the patients in the PRP group had a relapse. While 96% of the patients in the PRP group had regrowth of fully pigmented hair from the beginning of hair growth, only 25% had pigmented hair in the beginning of hair regrowth in the TrA group.

In accordance with these results, both PRP and TrA decreased the number of dystrophic hairs as assessed by dermoscopic photo-micrographs, and also decreased the itching/burning sensation of the patients (Figure 1b,c and Supplementary figure 4). PRP led to significantly better dermoscopy results when compared to TrA treatment (Figure 1b).

Both PRP and TrA significantly increased the levels of Ki-67 in AA patches compared to placebo; and levels were significantly higher after PRP treatment compared to TrA (Figure 1d). PRP effect on Ki-67 levels was evident already after two months (T1), and sustained throughout the study period (1 year, T3). No adverse effects were noted with placebo, TrA or PRP administration.

Discussion

In this randomized, double-blinded, placebo and active-controlled, half-head, parallel group study, we have shown that PRP administration leads to major improvements in AA lesions, with 60% of patients achieving complete remission at study termination. It should be noted that spontaneous remissions have been reported to occur in 34%-50% of patients at 1 year.[12] Nevertheless, in this study cohort, comprising patients suffering from a chronic, recurring disease, these figures are believed to be lower.

Ki-67 analysis revealed that PRP administration led to a significant increase in Ki-67 levels in AA patches. Both Ki-67 and SALT score parameters were significantly better than TrA administration, which is currently considered as treatment of choice for patch-stage AA.[13] This is the first report to establish the efficiency of PRP as treatment modality in AA, and the first time that such extensive and comprehensive assessment methods are used for evaluating efficacy of AA treatment. These comprehensive methods, where each patient served as his own control, helped us eliminate allocation bias. Nevertheless, it should be noted that since AA affects mainly young men and women, the age of the patients enrolled to the study was relatively young. Additionally, our study focused on the more chronic and relapsing form of AA, and not the more common spontaneously remitting type of AA. Therefore, the results may not be applicable to other age groups or to the spontaneously remitting type.

PRP is known to contain more than 20 different growth factors, which are important in promoting cell proliferation and differentiation.[14] These properties are thought to lead to its beneficial effects on acne scarring[15] and wound healing.[16] More recently, the role of PRP in promoting hair growth has also been investigated. Uebel *et al.* have shown that storing hair grafts in PRP can enhance graft survival, improve hair density and stimulate growth of transplanted follicular units.[6] Still, the mechanisms by which PRP exert its effects on HFs are still obscure. A recent study has shown *in vitro* that PRP increases the proliferation of dermal papillae cells, and activates the signaling pathways extracellular signal-regulated-kinase and Akt.[4] Additionally, fibroblast growth factor-7 and beta catenin, which are both stimulators of HF growth, were stimulated after PRP administration. Our study gives further support to the growth-promoting effect of PRP in hair, by providing evidence that levels of Ki-67, a marker for cell proliferation, are increased after PRP administration in humans.

In addition to its proliferation-inducing effects, PRP is also a potent anti-inflammatory agent, which can suppress cytokine release and thereby limit local tissue inflammation.[17] Since AA is characterized by an extensive inflammatory infiltrate, responsible for secretion of a variety of inflammatory cytokines, it is probable that the anti-inflammatory effects of PRP may be of great benefit in this condition.

Taken together, this study suggests PRP as a new treatment modality for AA, being a safe and a more efficient alternative for TrA, the current treatment of choice for AA. However, further controlled and randomised studies are needed to validate our findings in a larger cohort of patients.

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Figure legends:

Figure 1. Evaluation scores of (a) SALT, (b) dermoscopy, (c) burning/itching sensation and Ki-67 levels at 4 time points: T0 = beginning of study, T1 = 2 months, T2 = 6 months, T3 = 1 year. N=15 for each treatment modality. Student t test, * $p<0.05$, ** $p<0.01$, ***$p<0.001$.

Figure 2. Clinical photos of the scalp of a patient who was treated on the occipital part of the scalp with PRP and on the frontal part of the scalp with placebo. The patch treated with PRP had completely resolved after 1 year (T3), while the frontal AA patch increased in size.

Table 1. Patient characteristics at baseline

	Placebo	TrA	PRP
Number of patients	15	15	15
Average age (years)	28.1	27.2	28.8
AA duration (in years)	4.36	4.64	4.57
Duration of last relapse (in years)	1.2	1.5	1.6
Average number of AA patches	4.84	4.93	4.6
SALT score at baseline[a]	32%	36%	35%

AA, alopecia areata; PRP, platelet-rich plasma; SALT, severity alopecia tool; TRA, triamcinolone acetonide

[a]Calculated according to Olsen et al., *J Am Acad Dermatol* 2004