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**Preliminary Report**

# The Effects of Lower vs Higher Cell Number of Platelet-Rich Plasma (PRP) on Hair Density and Diameter in Androgenetic Alopecia (AGA): A Randomized, Double-Blinded, Placebo, Parallel-Group Half-Scalp IRB-Approved Study

Gordon H. Sasaki, MD, FACS

## Abstract

**Background:** Androgenetic alopecia (AGA) is a common disorder in both males and females and may be improved by platelet-rich plasma (PRP) treatment.

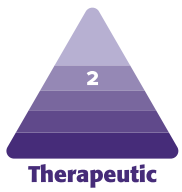
**Objectives:** The aim of this study was to compare safety, efficacy, and satisfaction following treatment with a lower or higher number of platelets over 6 months.

**Methods:** This was a prospective randomized, double-blinded, placebo, parallel-group, half-scalp IRB-approved study involving 8 subjects with moderate AGA. Participants received intradermal PRP injections (baseline and Month 3), according to 2 treatment protocols (high vs low platelet numbers) to the frontal and crown portions of the hemiscalp and normal saline to control sites. Phototrichoscans were recorded at baseline and at 6 months, and global photography and subject and investigator satisfaction questionnaires were obtained at baseline, 3, and 6 months.

**Results:** At the end of 6-month evaluation period, both groups demonstrated absolute increases in total hair density, follicle diameter, and terminal hair density, as well as absolute and percentage changes at the frontal and crown targeted sites compared with baseline. These improvements tended to occur more often in areas treated with higher platelet numbers than with lower numbers. Vellus hair densities did not exhibit any significant changes with either PRP dosages. The investigator and 6 of the subjects were "satisfied" with the results at 3 months and no adverse reactions were associated with the treatments.

**Conclusions:** Intradermal injections with 2 therapeutic quantities of platelets were equally safe and efficacious for treating men and women with AGA. Higher numbers of platelets may have a greater effect than lower numbers of platelets in regard to hair density, follicle diameter, and terminal hair density but exhibited minimal effects on vellus hair densities at the 6-month evaluation point. Further studies are required to determine whether any significant advantages occur when delivering either lower or higher numbers of platelets in AGA treatments as long as therapeutic levels are administered.

**Level of Evidence: 2**

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Androgenetic alopecia (AGA), commonly known as male and female pattern hair loss, affects more than half of men and women in the United States over the age of 50 years.[1,2] Most AGA patients begin to exhibit hair loss at a relatively young age, such that the disease burden is substantial, leading to significant psychosocial effects in

Dr Sasaki is a cosmetic plastic surgeon in private practice in Pasadena, CA, USA.

**Corresponding Author:**
Dr Gordon H. Sasaki, 800 S. Fairmount Ave., #319, Pasadena, CA 91105, USA.
E-mail: ghsasakimd@drsasaki.com

both sexes.[3,4] Although male AGA is believed to be due to a polygenic mode of inheritance and the presence of dihydrotestosterone, the precise biomolecular mechanisms are unclear.[5] Female pattern hair loss remains a poorly understood complex in which a number of factors, such as heredity, androgen dependency and other hormonal effects, inflammation, immunologic diseases, drugs, acute stress, diet, significant weight changes, and partum continue to be investigated.[6-9] Whatever etiologic factors may be in play, the pathologic changes appear nearly identical in men and women and include but are not limited to: (1) shortening the duration of the anagen phase, (2) prolongation of the telogen phase, (3) prolongation of latent empty hair follicles in the kenogen phase between telogen shedding and new anagen growth, (4) miniaturization of terminal and vellus hairs, and (5) follicular deletion, leading to a progressive reduction in the thickness, density, and total number of hairs.[10]

Hair transplantation[11,12] and a number of specific drug therapies have been approved by the US FDA for treating AGA; the latter are primarily limited to minoxidil[13,14] and finasteride,[15-17] given alone or in combination.[18] For most patients the beneficial results of minoxidil and finasteride treatments are less than satisfactory: unpredictable response rates of 20% to 40% are observed after 6 months of daily use.[18,19] Upon drug discontinuation, loss of achieved hair growth and density occurs in a short time.[20] For these reasons, a number of novel approaches, such as stem cell–conditioned media,[21] adipose-derived stem cells,[22] and low-level light therapy,[23] have emerged as options for stimulation and improved maintenance of hair growth. Since the 1970s platelet-rich plasma (PRP) has also received attention as a rich source of growth factors for tissue repair, hemostasis, and hair growth. Peer-reviewed studies on PRP treatment of alopecia reported increased hair densities and diameters.[24-27] Despite the growing interest in PRP for the treatment of alopecia, questions remain about its efficacy after reviewing meta-analyses of current evidence-based protocols.[28,40,52]

This study was designed to compare PRP treatment containing 2 different amounts of cells applied to each half of a subject's scalp as to their relative safety and effectiveness for stimulating hair densities and follicle diameters in the management of male and female noncicatricial alopecia.

# METHODS

## Study Design

This single-center study initially considered over 75 men and women candidates in the author's private practice, from which subjects were selected who met inclusion (Table 1) and exclusion (Table 2) criteria. The investigation was designed as a randomized, double-blinded, placebo, parallel-group half-scalp study that accepted 4 male subjects and 4 female subjects. Upon enrollment, participants reviewed and signed informed consents that complied with the standards of the IRB of the Institute of Regenerative and Cellular Medicine (protocol number SA-AA-401v2, November 28, 2018), the Bill of Rights, and the Health Insurance Portability and Accountability Act, and that permitted standardized digital photography and computerized trichoscan studies, and publication release forms. Each subject received an initialed copy of their consent forms and protocol. Subjects were informed that they would have no financial responsibilities from their participation in the study.

**Table 1.** Inclusion Criteria

| Inclusion Criteria |
|---|
| • Males and females, age 18-65 years and in good health |
| • Males with a diagnosis of androgenetic alopecia (male pattern hair loss) with early to moderate hair loss consistent with |
| ◦ Grades III and IV on the Norwood-Hamilton Scale |
| • Females with a diagnosis of androgenetic alopecia (female pattern hair loss) with early limited or diffuse hair loss consistent with |
| ◦ Advanced Grades I and II on the Ludwig Scale |
| • Absence of physical or psychological conditions unacceptable to primary investigator |
| • Postmenopausal for at least 12 months prior to study; post oophorectomy/hysterectomy, bilateral tubal ligation |
| • Subjects of child-bearing potential had negative urine pregnancy test and were willing to use an acceptable method of birth control (barrier methods with spermicidal agent, hormonal methods, intrauterine device, or abstinence) during the study |
| • Willingness and ability to provide written consent for study-required photography and Health Insurance Portability and Accountability Act authorization prior to performance of any study-related procedure |
| • Provide written informed consent and comply with the study requirements |

## Study Determinants

### *BMI*

Total body fat was analyzed by applying a Futrex Light Wand (Futrex Tech Inc., Hagerstown, MD) at a standardized point on the midpoint of the bicep muscle; all measurements were performed by the same technician. Analysis of the Light Wand's near-infrared light beam yields the overall level of body fat, based on age and gender. The correlation coefficient of percentage body fat as predicted by the Futrex near-infrared system was 0.94 ($P < 0.01$). An average of 3 measurements provided the changes in

**Table 2.** Exclusion Criteria

| Exclusion Criteria |
|---|
| • A diagnosis of telogen effluvium (generalized shedding of hair) or any cicatricial (burn scars) or inflammatory alopecia, or immunogenic-related alopecia (alopecia areata) |
| • A sensitive, irritated, or abraded scalp area |
| • Use of minoxidil, or any oral or topical medication including over-the-counter and herbal medications for the treatment of hair loss within 1 year of study screening, or finasteride or dutasteride within 6 months of study screening |
| • Known allergic reaction to components of study treatment and/or study injection procedure |
| • Previously failed or has been deemed nonresponsive to an experimental hair loss treatment |
| • Women who have blood tests results indicating hair loss etiology different from female pattern hair loss |
| • History of autoimmune disease, diabetes mellitus, or organ transplantation |
| • Use of systemic agents that increase bleeding or clotting, or disorders associated with these effects |
| • Clinically significant medical or psychiatric illness currently or within 30 days of study screening as determined by the investigator |
| • Prior surgery in the treatment area |
| • Any disease or condition (medical or surgical) that, in the opinion of the investigator, might compromise hematologic, cardiovascular, pulmonary, renal, gastrointestinal, hepatic, or central nervous system function; or any condition that would place the subject at increased risk |
| • Pregnant or lactating women or women trying to become pregnant |

**Table 3.** Clinical Documentations

| Clinical Documentations |
|---|
| Determinants at baseline ($V_0$), Month 3 ($V_3$), and Month 6 ($V_6$)<br>• Standardized digital photography |
| • Height (m) |
| • Weight (kg) |
| • BMI (kg/m$^2$) |
| • Pull test at right/left frontal-temporal zones, biparietal zones, crown, vertex, and occiput |
| • IGAIS and SGAIS subjective questionnaires<br>Determinants at baseline ($V_0$) and Month 6 ($V_6$) |
| • Computerized phototrichoscans at tattooed dot in the center of 1-cm$^2$ squares (1 control and 2 PRP) on each hemiscalp |

IGAIS, Investigator Global Aesthetic Improvement Scale; SGAIS, Investigator Global Aesthetic Improvement Scale.

percentage of total body fat and BMI during the course of the study (Table 3).

## Standardized Photography

All photographs were taken in color with a high-quality D70 Nikon digital camera fitted with a macro lens. All photographs were taken by a trained technician at standardized angles, positions, and distances in the same room against a solid backdrop with tangential lighting to highlight features. All photographs were compiled by removal of identifying information prior to their assessment. Each of 3 blinded evaluators was instructed to judge unlabeled pre- and posttreatment photographs of each subject's scalp as an unbiased evaluation of changes in hair density and growth patterns. The various changes in each split scalp were scored as "improvement (mild, modest)," "no change," or a "worsening" of hair density and growth coverage.

## Trichoscans

Trichoscans were obtained with a TrichoSciencePro computerized micrographic digital system (International Enterprises, Boston, MA), which uses an automated module to measure hair density and follicle diameter within a 1.08-cm2 area for close to exact follicle-to-follicle matching. A long-nose cone probe was applied to each tattooed dot with a threshold for terminal and vellus hair set at 40 µm. A trained technician performed 3 measurements from each tattooed site; these were averaged to provide a final number that compared the effects between baseline ($V_0$) and Month 6 ($V_6$), as shown in Figure 1. The trichoscan probe was placed on a permanent mark (Indian ink tattoo), located in the center square within the 9-square "control" paramedian midscalp area, and on the center of 2 "treatment" squares (lateral fontal and lateral midscalp) on the each side of the scalp (ie, sides receiving lower or higher numbers of platelets).

## Pull Test

Gentle traction was exerted on a small lock of hairs (approximately 50) in 8 zones (bitemporal, frontal, biparietal, crown, vertex, and occiput). If 4 to 6 telogen hairs were extracted with each pull, the pull test was suggestive of telogen effluvium. This test has never been critically evaluated, and therefore its predictive value remains arbitrary.

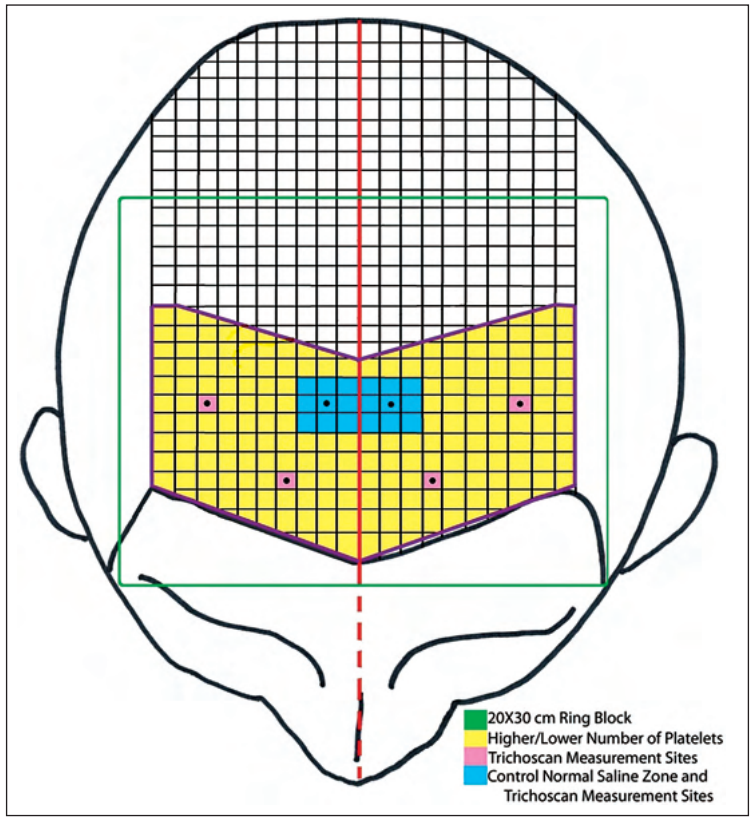


**Figure 1.** Template designates 100-cm$^2$ treatment halves with either low or high numbers of platelets and 2 trichoscan measurement sites in the "active-PRP" zone and 1 trichoscan measurement site in the "placebo-saline" zone on each hemiscalp.

However, the dominant presence of telogen effluvium with male and female pattern hair loss might complicate data interpretation.

## Satisfaction Questionnaires

Secondary endpoints included investigator (Table 4) and subject assessments of global and hemiscalp changes in hair density and thickness at baseline and follow-up visits. For each subject, paper surveys were identified by a unique number (1-8) and evaluation date and distributed as a batch prior to the starting date. Each subject was asked to fill out their surveys in the privacy of their homes prior to their evaluation sessions. The investigator filled out his assessment for each subject in privacy after each evaluation session. The registered nurse collected all subject and investigator surveys, which were calculated at the end of study by a statistician utilizing the designated identification number for each subject on a 6-point satisfaction scale for the entire scalp and hemiscalp.

## PRP System

The Eclipse PRP HC System (EclipseMed, The Colony, TX), an FDA-cleared 510(k) Class II single-spin, horizontal spin centrifugation device (2950 rpm, 1500 *g*, 10-minute cycle), yielded high levels of PRP over baseline values and resulted in consistent product purity (near-absence of problematic red blood cells and inflammatory white blood cells, but presence of plasmatic fibrinogen, vitronectin, fibronectin, growth factors, and cytokines).[29,30,51] The vacuum separator gel PRP tubes came in 22-mL vials.

## Clinical Protocol

Each subject had a history taken and a scalp examination performed by the author, received medical clearance from their internists, and baseline complete blood count and platelet count were measured. Serum levels of iron, thyroid, and testosterone were obtained at the request of their internists. Additional baseline assessments ($V_0$) included initial classification on the male Norwood-Hamilton

**Table 4.** Investigator or Subject Global Aesthetic Improvement Scales

| Global Aesthetic Improvement Scales | | |
|---|---|---|
| 1 | Extremely dissatisfied | Marked worsening in appearance from the initial condition |
| 2 | Very dissatisfied | Appearance worse than the original condition |
| 3 | Dissatisfied | Appearance is essentially the same as the original condition |
| 4 | Satisfied | Obvious improvement in appearance from the initial condition |
| 5 | Very satisfied | Marked improvement from initial appearance |
| 6 | Extremely satisfied | Optimal cosmetic result for the procedure in this subject |

Scale and female Ludwig Scale, pull testing, urine pregnancy test, and BMI measurements. Standardized photographs, computerized trichoscans, and Subject (SGAIS) and Investigator (IGAIS) Global Aesthetic Improvement Scale questionnaires were obtained per protocol, as listed in Table 3.

The primary efficacy endpoints were the safety of treatments and the differences in density and follicle diameter in targeted areas that received either a lower or higher dosage of platelets per hemiscalp. The secondary endpoints were: (1) differences in density of terminal and vellus hairs in targeted areas that received either a lower or higher dosage of platelets per hemiscalp; and (2) changes in subject and investigator self-assessment questionnaires.

Eight eligible subjects were randomly assigned to receive hemiscalp treatments with a lower number of platelets (L-PRP) on one side and a higher number of platelets (H-PRP) on the opposite side to minimize selection bias. From 1 nontransparent jar each of the 4 male subjects selected 1 colored paper strip from a mixture of 2 red strips (L-PRP on right hemiscalp) and 2 black strips (L-PRP on left hemiscalp). From a second nontransparent jar each of the 4 female subjects selected 1 colored paper strip from a mixture of 2 red strips and 2 black strips to determine similar randomization of treatment. This method straightforwardly divided the subjects into 2 sets: Set A was designated to receive L-PRP treatment on the right hemiscalp and H-PRP treatment on the left hemiscalp, whereas Set B was assigned to receive H-PRP treatment on the right hemiscalp and L-PRP treatment on the left hemiscalp. Knowledge of the allocation assignment was blinded with the exception of the surgical registered nurse involved in the study. Each subject received 2 sessions of the 2 PRP dosages and control normal saline injections to opposing hemiscalps as determined by the randomization process. Subjects were advised to adhere to their usual daily hair regimens of shampooing and hair styling.

## PRP Preparation

Utilizing an FDA-cleared 510(k) PRP kit and centrifuge system, whole blood was withdrawn from an antecubital arm vein through an 18G butterfly needle and collected into three 22-mL vacuum separator gel tubes. The PRP tubes were pyrogen-free, had an internal glass coating to prevent platelets from adhering to tube walls, and contained a proprietary sodium citrate–based anticoagulant to prevent platelet clumping. After a single horizontal spin cycle of 1 tube at 2950 rpm (1500 *g*) for 10 minutes, 11 to 12 mL of clear, amber-colored platelet-poor plasma (PPP) was separated from the red-stained gel plug. A sufficient volume of PPP was removed from the upper portion of the PPP volume to retain 6 mL of PPP above the gel. The PPP was gently agitated 5 times for 5-second periods in order to lift platelets off the surface of the gel, producing a slightly pink-tinged PRP preparation, designated as Batch A. A hematologic Coulter Counter analysis (Department of Pathology and Laboratory Medicine, Arcadia Methodist Hospital, Arcadia, CA), performed within 1 hour of collection, determined the number of platelets in a 1.0-mL aliquot from Batch A.[53] The final platelet concentration in the remaining prepared 5 mL PRP in Batch A was calculated to be about 4.5 times the baseline platelet concentration of the patient's whole blood.

Preparation of the H-PRP material was achieved by processing 44 mL of whole blood (two 22-mL separator tubes). After centrifugation, a sufficient volume of PPP was removed from the upper portion of the PPP volume to retain 3.0 mL of PPP above the gel in each tube. The remaining PPP was gently agitated 5 times for 5-second periods, lifting platelets off the gel surface to produce a slightly pink-tinged PRP preparation. The two 3.0 mL PRP preparations were combined to produce a final volume of 6 mL of PRP, designated as Batch B. The number of platelets from a 1.0-mL aliquot of the final PRP Batch B volume was quantitated by Coulter Counter at the same hospital facility. The platelet concentration in the remaining prepared 5 mL PRP in each Batch B was calculated to be approximately about 4.5 times the baseline platelet concentration of a patient's whole blood. Finally, 5 mL containing a lower number of platelets (Batch A) and 5 mL containing a higher number of platelets (Batch B) were loaded in 2 sets of five 1-mL tuberculin syringes from which about 0.05-mL aliquots were

then injected intradermally into the selected 1-cm2 squares in the designated hemiscalp. From the manufacturer's data sheet[29] and third-party validation studies,[30,31] the proprietary gel plug was found to remove 99% of entrapped red blood cells and 92% of granulocytes, but released 86.2% of mononuclear cells and platelets, resulting in an 80% to 85% efficiency platelet recovery.

## Procedure

Each hemiscalp was marked, with a matte lipstick pen, with a grid of one hundred 1-$cm^2$ areas within a 4-sided squared area defined by a straight 10-cm line projected from midsagittal scalp and a 10-cm line along the anterior hairline (Figure 1). A cubicle of nine 1-$cm^2$ squares (7-9 cm posterior from the frontal hairline and 1-3 cm lateral from midline) was designated as the "placebo-saline" zone in the paramedian midscalp on each hemiscalp. The remaining area of 91 squares was determined to be the "active-PRP" zone. Transdermal marks tattooed with Indian ink in 1 centralized square within the "placebo" zone, and 2 squares (3 cm posterior from the frontal hairline and 4 cm from midline in the frontal scalp; 6 cm posterior from the frontal hairline and 8 cm from midline in the lateral midscalp) in the "active" zone in each hemiscalp were designated as representative sites for repeat measurements of hair density, follicle diameter, and terminal/vellus counts.

The scalp was prepped with Hibiclens antiseptic solution, rinsed with sterile water, and toweled dry. At the request of a subject, a 20 cm × 30 cm ring block (8 mL 0.5% lidocaine solution/2 mL sodium bicarbonate) provided sufficient anesthesia for the 200 injection sites on both halves of the treated scalp. Five PRP-filled tuberculin syringes attached to 30G needles were used to inject about 0.05 mL of Batch A PRP into each of 91 squares intradermally on 1 hemiscalp. On the opposite side, 5 PRP-filled tuberculin syringes injected about 0.05 mL of Batch B PRP intradermally into each of 91 squares. Each hemiscalp received a total of approximately 5.0 mL Batch A or 5.0 mL Batch B. Each of the 9 squares within the "control" space was intradermally injected in a similar manner with 0.05 mL 0.9% of sterile normal saline. No massaging was performed on each treated hemiscalp to reduce unintentional spreading of PRP into the control zones. As the consistency and color of the 2 different PRP preparations were similar, the injecting physician and subjects were blinded to: (1) the preparation and loading of the 2 identically colored PRP batches (A or B) within tuberculin syringes; and (2) the treatments delivered to each respective hemiscalp. As previously mentioned, the surgical registered nurse was aware of the identity of tested PRP solutions, and instructed the physician as to which batch and which side of the scalp were to be injected at baseline ($V_0$) and at the Month 3 ($V_3$) session. Subjects resumed their normal scalp shampoo without any active ingredients the night of the procedure.

## Trichoscan Measurements and Safety Assessments

Trichoscan measurements were obtained from all subjects at baseline ($V_0$) and Month 6 ($V_6$). A trained technician performed 3 measurements at each tattooed site. The presence of permanent tattoo dots in 2 treatment squares (frontal and lateral midscalp) and 1 control square (paramedian midscalp) on each hemiscalp permitted greater accuracy and reproducibility of measurements. The occurrence of any safety issues or side-effect data were assessed at the $V_0$, $V_3$, and $V_6$ appointments.

## Statistical Analyses

The technician responsible for the collection of data remained blinded with regard to treatment and control areas and was not present during treatment sessions. At the end of the clinical trial, the technician was unblinded in order to calculate and analyze each data set.

The final data points were calculated by, first, averaging the 3 separate measurements at each of 6 tattooed sites of each subject at baseline ($V_0$) and Month 6 ($V_6$), and, second, obtaining the mean, or average, at each data set by calculating the sum of all sorted values at each of the individual 6 sites from 8 subjects divided by the number values in the data set. Subjects' density, hair diameter, terminal hair density, and vellus hair density at Month 6 were compared with baseline values. Statistical analyses were performed with the mixed procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). Values of $P < 0.05$ were regarded as statistically significant. Data are presented as mean [standard deviation].

# RESULTS

The duration of the study extended from November 2018 to March 2021 because of varying individual enrollment and closing dates. The average follow-up time for all 8 subjects was estimated to be 6 to 8 months, while the range varied between 6 and 12 months because of scheduling issues. The baseline demographics of the 4 males and 4 females (comprising 3 Caucasians, 2 Asians, 2 Hispanics, and 1 Middle Eastern) are summarized in Table 5. The 4 male subjects were assessed with Hamilton-Norwood Patterns Class III to IV, and 4 female subjects were evaluated with Ludwig Patterns Stages I-4 to II-2. The mean ages of males and females were comparable: 51.8 years (range, 34-65 years) and 51.3 years (range, 39-65 years), respectively. The weight, height, and BMI of the subjects did not change significantly

**Table 5.** Characteristics of Subjects Treated with 2 Dosages of PRP

| Demographics | Male | Female |
|---|---|---|
| Age range (years) | 34-65 | 39-65 |
| Age (years) | 51.8 [12.7] | 51.3 [13.8] |
| Ethnicity | | |
| Caucasian | 2 | 1 |
| Hispanic | 1 | 1 |
| Asian | 0 | 2 |
| Middle Eastern | 1 | 0 |
| Weight (kg) | 88.8 [35.7] | 59.5 [22.5] |
| Height (m) | 1.7 [0.9] | 1.6 [1.0] |
| BMI | 32.5 [11.6] | 22.6 [7.4] |
| Body fat | 35.9 [13.0] | 37.8 [13.8] |
| Baseline platelets/μL (whole blood) | 276,750 [113,546] | 245,000 [99,948] |
| Mean PRP/μL (4.5-fold over baseline) | 1,245,375 [510,962] | 1,102,500 [449,773] |
| Mean number of platelets in Batch A PRP (5 mL) | 136,991,250 [56,206,022] | 121,275,000 [49,475,242] |
| Mean number of platelets in Batch B PRP (5 mL) | 273,982,500 [112,412,046] | 242,550,000 [98,950,486] |
| Duration of hair loss | | |
| Less than 6 years | 1 | 2 |
| More than 6 years | 3 | 2 |
| Hair loss therapy | | |
| Finasteride | 2 | 0 |
| Minoxidil | 1 | 1 |
| Finasteride and minoxidil | 0 | 0 |
| No therapy | 1 | 3 |
| Hair pattern loss | Norwood-Hamilton | Ludwig |
| | Class III (frontal-temporal loss) | Stage I-4 (midline widening) |
| | Class III (early crown loss) | Stage II-1 (increased widening with early diffused scalp thinning) |
| | Class IV (band of hair separating frontal and crown loss) | Stage II-2 (more widening and thinning) |

Values are n or mean [standard deviation].

during the study. Male and female subjects had a mean platelet count of 276,750 [113,546] and 245,000 [99,948] platelets/μL, respectively. The mean platelet concentration of PRP preparation, calculated based on 4.5-fold increases over baseline peripheral blood, in male and female subjects was 1,245,375 [510,962] and 1,102,500 [449,773] platelets/μL, respectively. The average platelet percentage recovery in each 22-mL tube was found to be approximately 82% [10%] to 87% [10%]. From Coulter Counter analyses, the total mean number of platelets in 5 mL of PRP Batch A was 136,991,250 [56,206,022] and 121,275,000 [49,475,242] in male and female subjects,

**Table 6.** Relevant Hair-Growth Parameters by Trichoscan Analysis

| Hair-growth parameters | Session | Control 1-cm² square | | Frontal 1-cm² square | | Midscalp 1-cm² square | |
|---|---|---|---|---|---|---|---|
| | | Low PRP | High PRP | Low PRP | High PRP | Low PRP | High PRP |
| Hair density (no/cm²) | $V_0$ | 114.3 [34.8] | 117.5 [45.6] | 110.9 [44.8] | 110.7 [29.7] | 116.0 [53.2] | 103.1 [45.6] |
| | $V_6$ | 116.6 [31.0] | 118.6 [44.2] | 120.2 [37.2] | 123.3 [52.8] | 141.6 [51.4] | 131.3 [57.7] |
| Change | Absolute number | 2.3 | 1.1 | 9.3 | 12.6 | 25.6 | 28.2 |
| | % change | 2.0 | 0.94 | 8.4 | 11.4 | 22.1 | 27.4 |
| Follicle diameter (µm) | $V_0$ | 65.3 [28.8] | 67.8 [24.2] | 67.5 [29.2] | 65.6 [19.4] | 67.5 [27.2] | 63.5 [29.2] |
| | $V_6$ | 66.8 [28.1] | 69.2 [28.8] | 74.9 [28.1] | 75.6 [28.8] | 78.6 [30.2] | 80.5 [39.2] |
| Change | Absolute number | 1.5 | 1.4 | 7.4 | 10.0 | 11.1 | 17.0 |
| | % change | 2.3 | 2.1 | 11.0 | 15.2 | 16.4 | 26.8 |
| Terminal hair density (no/cm²) | $V_0$ | 88.2 [39.2] | 86.5 [43.2] | 86.9 [37.0] | 87.5 [30.2] | 90.3 [43.6] | 94.0 [40.9] |
| | $V_6$ | 89.4 [23.0] | 93.1 [33.4] | 93.6 [30.1] | 95.6 [38.2] | 112.8 [31.5] | 119.3 [45.6] |
| Change | Absolute number | 1.2 | 6.6 | 6.7 | 8.1 | 22.5 | 25.3 |
| | % change | 1.4 | 7.6 | 7.7 | 9.3 | 24.9 | 26.9 |
| Vellus hair density (no/cm²) | $V_0$ | 25.5 [16.6] | 23.5 [17.9] | 27.5 [18.8] | 25.5 [20.6] | 22.7 [16.1] | 25.5 [12.0] |
| | $V_6$ | 27.5 [18.4] | 26.5 [17.8] | 32.6 [18.7] | 27.8 [9.9] | 23.5 [17.2] | 27.8 [7.5] |
| Change | Absolute number | 2.0 | 3.0 | 5.1 | 2.3 | 0.8 | 2.3 |
| | % change | 7.8 | 12.8 | 18.5 | 9.0 | 3.5 | 9.0 |

Values are n or mean [standard deviation]. $V_0$, baseline; $V_6$, 6 months.

respectively, in contrast to the calculated higher total mean total number of platelets in 5 mL of PRP Batch B of 273,982,500 [112,412,046] and 242,550,000 [98,950,486] in male and female subjects, respectively. Three of 4 male subjects experienced alopecia more than 6 years before PRP treatment, whereas 2 of 4 female subjects observed alopecia less than 6 years before treatment. Male subjects who were treated with either minoxidil or finasteride, and women subjects who were treated with minoxidil, expressed minimal satisfaction with their overall results after using these drugs for 6 to 12 months. These candidates were not on minoxidil or finasteride for at least 1 year before entry to this PRP study.

## Hair Density Counts

No significant differences were observed in baseline hair densities, follicular diameters, and terminal or vellus hair densities at the frontal or midscalp measurement sites in either hemiscalp or their "control zones," as summarized in Table 6. After Month 6 ($V_6$), all sites treated with either H- or L-PRP demonstrated increased mean hair densities compared with their respective baseline ($V_0$) values at the frontal and midscalp sites. When comparing differences in densities, however, H-PRP-treated sites yielded greater densities than L-PRP-treated sites (frontal, 12.6 hairs/cm² vs 9.3 hairs/cm²; midscalp, 28.2 hairs/cm² vs 25.6 hairs/cm²). The advantage of administering greater numbers of platelets became more apparent when assessing differences in absolute number values and their percentage changes from baseline values at H-PRP-treated sites (frontal, 12.6 hairs/11.4%; midscalp, 28.2 hairs/27.4%) compared with L-PRP-treated sites (frontal, 9.3 hairs/8.4%; midscalp, 25.6 hairs/22.1%), and saline control midscalp sites (low, 2.3 hairs/2.0%; high, 1.1 hairs/0.94%).

## Follicle Diameter Measurements

After Month 6 ($V_6$), all treated sites demonstrated numerical increases in mean follicle diameter over their baseline ($V_0$) values. When comparing differences in follicle diameters, however, H-PRP-treated sites yielded greater diameter changes than L-PRP-treated sites (frontal, 10.0 µm vs

7.4 µm; midscalp, 17.0 µm vs 11.1 µm). The advantage of administering greater numbers of platelets became more apparent by assessing differences in absolute diameter values and their percentage changes from baseline values for H-PRP-treated sites (frontal, 10.0 µm/15.2%; midscalp, 17.0 µm/26.8%) compared with L-PRP-treated sites (frontal: 7.4 µm/11.0%; midscalp, 11.1 µm/16.4%) and saline control midscalp sites (low, 1.5 µm/2.3%; high, 1.4 µm/2.1%).

## Terminal Hair Density

Doubling the number of platelets from lower to higher numbers produced absolute increases in terminal hair densities at Month 6 ($V_6$) at both target sites over their baseline ($V_0$) values. When comparing differences in densities, H-PRP-treated sites yielded greater densities than L-PRP-treated sites (frontal, 95.6 hairs/cm$^2$ vs 93.6 hairs/cm$^2$; midscalp, 119.3 hairs/cm$^2$ vs 112.8 hairs/cm$^2$). The advantage of administering greater numbers of platelets became more apparent when assessing differences in absolute number values and their percentage changes from baseline values for H-PRP-treated sites (frontal, 8.1 hairs/9.3%; midscalp, 25.3 hairs/26.9%) compared with L-PRP-treated sites (frontal, 6.7 hairs/7.7%; midscalp, 22.5 hairs/24.9%) and saline control midscalp sites (low, 1.2 hairs/1.4%; high, 6.6 hairs/7.6%).

## Vellus Hair Density

After Month 6 ($V_6$), both H- and L-PRP groups demonstrated minimal increases in mean hair density over their baseline ($V_0$) values. When comparing differences in densities, however, H-PRP-treated sites yielded inconsistent changes from those observed at L-PRP-treated sites (frontal, 27.8 hairs/cm$^2$ vs 32.6 hairs/cm$^2$; midscalp, 27.8 hairs/cm$^2$ vs 23.5 hairs/cm$^2$). The advantage of administering greater numbers of platelets was not apparent when assessing differences in absolute number values and their percentage changes from baseline values at H-PRP-treated sites (frontal, 2.3 hairs/9.0%; midscalp, 2.3 hairs/9.0%) compared with L-PRP-treated sites (frontal, 5.1 hairs/18.5%; midscalp, 0.8 hairs/3.5%), and saline control midscalp sites (low, 2.0 hairs/7.8%; high, 3.0 hairs/12.8%).

## Pull Test

None of the subjects exhibited a positive pull test in 8 scalp zones either at baseline or at the Month 6 evaluation period with less than 0 to 1 hairs/pull site.

## Global Questionnaire Assessments

All subjects and the investigator completed satisfaction and outcome questionnaires at baseline, Month 3, and Month 6 on a 6-point scale (Figure 2). At Month 3, there were no discernible changes from baseline assessment. At Month 6, 6 subjects (3 males; 3 females) and the investigator reported they were "satisfied" with overall improvement in hair density, texture, thickness, growth rate, and uniformity. However, 1 male subject (65 years old; 30+ years of alopecia; moderate results with combined prior treatments with finasteride and minoxidil) and 1 female subject (63 years old; 35+ years of alopecia; minimal results from prior minoxidil treatments) observed a global reduction in hair density and thickness at Month 6, while the investigator assessed a continuation of "satisfied" improvements in these 2 subjects' parameters. There was no consensus from either the subjects or the investigator that the H-PRP-treated hemiscalp exhibited a more favorable outcome than the L-PRP-treated side.

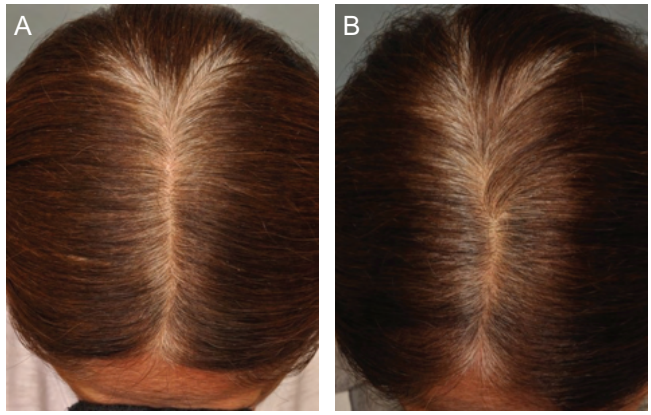


**Figure 2.** (A) A 61-year-old Hispanic female patient with sagittal alopecia extending from the frontal to vertex scalp in a Christmas tree configuration at baseline evaluation. (B) Evaluation at 6 months after 2 administrations (baseline, $V_1$; 3 months, $V_3$) of low numbers of PRP to the right hemiscalp and high numbers of PRP to the left hemiscalp demonstrating increased hair density and follicular diameters at both treatment sides. PRP, platelet-rich plasma.

## Tolerability and Safety

No adverse events (side effects or complications) were observed throughout the study period of 6 months. No subject reported associated pain or discomfort, other than pain from needle insertions during the ring block. There were no observed infections or hair loss associated with the minimal trauma of intradermal injections or local swellings.

# DISCUSSION

In this randomized, double-blind, placebo- and active-controlled half-head, parallel-controlled study, the author demonstrated stimulatory effects of PRP on AGA in 4 men and 4 women by phototrichoscan measurements.

A method to attain discernibly improved hair density, follicular diameter, terminal hair density, and vellus hair density was to vary the starting whole blood volumes, each of which contained different total numbers of isolated platelet particles for their stimulatory effects. In contrast, the use of different platelet concentrations, obtained by manipulating the volumes of PPP with the platelet layer or plug from the same starting volume of whole blood, would not be expected to affect clinical results because the same number of platelets is administered regardless of the PRP concentration.

In this study, the administration of 2 doses of a lower number of nonactivated of platelets on one side of the scalp was tested against 2 doses of a higher number of nonactivated platelets on the opposite half of the scalp in mirror-imaged zones (91/100 $cm^2$) in frontal and midscalp portions of the hemiscalp. Treatment zones (91-$cm^2$ squares) in each respective hemiscalp received 5 mL of 1 of 2 batches of platelets injected intradermally with 0.05 mL PRP/$cm^2$. In contrast, the 2 placebo zones of nine 1-$cm^2$ squares, adjacent to the midsagittal plane on each hemiscalp, received 2 sessions of intradermal injections of 0.05 mL saline/$cm^2$.

The study design implemented internal randomized controls, where the subject's contralateral side served as its own control, thereby reducing testing variations and biases, such as gender, age, and initial grade classification of hair loss. The targeted tattooed sites in each hemiscalp for trichoscan measurements were sufficiently distant from the sagittal midline to reduce cross-contamination by using slow, nonforceful injections of small PRP volumes confined to the dermis and avoiding the looser subdermal space. The use of a single PRP device, uniform centrifugation and extraction methods, Coulter Counter quantification of platelets, similar injection procedures, and statistical methodology reduced as much as possible variations due to statistical heterogeneity rather than by chance in this small number of subjects.

At the end of the 6-month evaluation period, both higher and lower numbers of platelets resulted in numerical increases in hair densities, follicle diameters, and terminal hair densities, as well as absolute number and percentage changes over their baseline measurements. The values were not statistically significant when compared to determinations at placebo-control sites, however, because of the small number of subjects and wide range of standard deviations. That being said, improvements tended to occur more frequently in areas receiving higher than lower numbers of platelets when equivalent platelet concentrations were delivered. In contrast, change responses to vellus hair densities after either lower or higher numbers of platelets were negligible when comparing values at Month 6 with baseline values. The reasons for the minimal responses of vellus hairs in the frontal and crown regions to PRP stimulation were unclear. Because the transformation of vellus to terminal hairs is complex, in part due to dissimilar cycling processes and structural components, their responses to stimulation are not well-understood. Scalp hair may begin as vellus hair and then be transformed over 6 weeks to 6 months into thicker, colored, terminal hairs by a number of factors—especially hormones and genetics. Vellus hairs have a dissimilar structure to terminal hairs. Each hair follicle contains a sebaceous gland. Unlike terminal hair, however, vellus hair do not typically have a medulla that strengthens it and allows it to grow longer. As a consequence, a vellus hair is short (up to 30 µm), thin, and light-colored, grows no longer than 2 cm, and reaches down only in the upper third of the dermis. The pigmented terminal hairs reach into the lower dermis and subcutaneous fat with diameters typically between 50 and 100 µm and grow longer than 2 cm in length.

Clinical studies[27,32,39] with PRP have observed inconsistent zonal responses to hair growth in the frontal, crown, and vertex regions in male and female alopecia. An explanation for this variable response may be the fact that female alopecia usually involves the frontal-temporal zones along with generalized thinning of the remaining scalp. In males, the majority of hair loss occurs in the frontal, vertex, and crown regions of the scalp. It is unknown whether distinct receptor sensitivities to growth factors within stem cell centers in hair follicles account for the disparities in terminal and vellus hair stimulation observed in different zones of alopecia in male and female patients.

As well as trichoscan measurements, the subjects and the investigator scored the results subjectively to assess clinical efficacy and reported they were "satisfied" with both treated sides in 6 subjects at the 6-month evaluation. However, 1 male and 1 female subject recorded a reduction in their hair density, texture, hair growth rate, and uniformity at 6 months compared with their hair appearance at the start of treatment. These subjects' histories included over 30 years of alopecia, and experience of a poor to no response to prior use of FDA-approved drugs. Although these subjects assessed an overall reduction in hair appearance at Month 6, the investigator judged their appearance as a continuation of "satisfied" improvements. Whether the presence of these factors represents significant negative indicators for unsuccessful PRP treatments requires larger and longer cohort studies. There was no consensus from either the subjects or investigator whether the H-PRP-treated hemiscalp exhibited a more favorable appearance-outcome than the L-PRP side.

Early results from PRP treatments are variable and may be more accurately determined at longer follow-up periods. Meta-analysis studies[26-28,52] suggest that PRP injections directly into the scalp result in various outcomes

and may require up to 3 to 6 treatment sessions per year, including yearly booster sessions. The rationale for administering only 2 treatment sessions in this clinical trial was to determine whether a greater number of injected platelets would result in greater density, follicle diameter, terminal hair density, and vellus hair density, as documented by phototrichoscan measurements, than that observed after delivering a lower number of platelets, as shown in this study. Possible explanations for the absence of progressive hair loss in the areas receiving only normal saline may be ascribed to the relatively short observation period from start of treatments to final evaluation at Month 6 for each subject. The duration of a terminal hair's existence is estimated to be about 2.5 to 3.0 years. Thus, one would expect minimal changes at saline control sites during the 6-month evaluation period. Also, it would be very difficult to know whether PRP injections diffused into the control sites and contributed to salutary effects on these hairs. The observation that hair densities at the saline-control sites remained essentially the same as their baseline values over 6 months supports that limited spreading and contamination effects of platelets into control sites occurred during the treatment periods.

Although both treatment sides demonstrated improved photoscan measurements of hair densities, follicle diameters, and terminal hair densities, final review of unmasked clinical and photographic changes was unable to discern a superior appearance on the side receiving the higher number of platelets. Notwithstanding these outcomes, the majority of subjects considered additional treatments if they were given the opportunity to receive the higher dose of PRP therapy over the entire scalp with added booster sessions. All subjects reported minimal pain, swelling, or side effects throughout the duration of study.

This study's trichoscan determinations, clinical outcomes, and safety profiles agreed with data from other split-scalp trials when therapeutic levels of platelet concentrations were delivered in the treatment of early stages of male or female AGA.[32,38,39] The optimal dose-dependent concentration of platelets for alopecia has yet to be defined; ratios of less than 2-fold to 8.5-fold have been advocated.[27,28,30,40,52] Although optimal platelet concentrations in the PRP preparations have not been established, preparations containing more than 1,000,000 to 1,500,000 platelets/μL were generally believed to be necessary for comparable favorable results.[25,26,35,39,43,50,51] In our study, baseline platelet concentrations of each subject's peripheral blood were within normal physiologic ranges. In addition, quantification of platelets by Coulter Counter in our 8 subjects' PRP Batches A and B were calculated as 4.5-fold increases over baseline values, which were acceptable levels of mean platelet concentrations to obtain favorable therapeutic outcomes. However, because the platelet concentration factor is arbitrarily calculated, based on the PPP diluent volume, the total numbers of functioning platelets that are delivered to a given area remains the same as found in the starting whole blood or PRP concentrate. As a consequence of doubling donor whole-blood volumes, the differences in total mean numbers of platelets—either lower (Batch A) or higher (Batch B)—were achieved in equal volumes of 5 mL, as shown in Table 5. To the author's knowledge, this study represents the first clinical investigation on dosimetry that compares the consequences of using either higher or lower numbers of platelets on hair stimulation and growth.

The anatomic hallmarks of AGA and final molecular pathophysiologic mechanisms that lead to dysregulation of signaling pathways have been well-documented and discussed in numerous studies.[54-59] The exact mechanisms of action of PRP on follicle growth centers in the treatment of alopecia are still not completely understood. Once PRPs are isolated, the platelets must be activated to release their growth factors from primarily α granules. Growth factors, including platelet-derived growth factor, transforming growth factor β, vascular endothelial growth factor, epidermal growth factor, and insulin growth factor 1 are thought to be the most important to promote cellular proliferation, differentiation, angiogenesis, and chemotaxis necessary for renewed hair growth, prolongation of the anagen growth phase, and stimulation of vellus hairs.[25,33-37,41,42,45,46] These growth factors are believed not only to stimulate telogen-to-anagen transition, extend the anagen phase, and promote neovascularization,[45-47] but also to decrease microperifollicular inflammation, and promote anti-apoptotic effects on the dermal papillary growth center.[25,33,35,41-44,47-49] Although the pharmacokinetics of released growth factors and cytokines have half-lives of minutes to hours, their immediate stimulatory effects of dose and spatiotemporal release are believed to initiate series of molecular events that determine the fate of hair growth over a period of months to years. On a cellular level, dermal papilla cells have shown an increased proliferation, increased Bcl-2 and FGF-7 levels, activated ERK and Akt proteins, and upregulation of β-catenin when cultured in an activated PRP-supplemented growth medium.[25] Because each of these factors positively influences hair growth through cellular proliferation to prolong the anagen phase (FGF-7),[56] including cell growth (ERK activation),[60] stimulating hair follicle development (β-catenin),[61] and suppressing-apoptotic cues (Bcl-2 release and Akt activation),[62] human scalp should display increases in cellular activity and delayed positive growth responses. These signaling processes, when activated (eg, at the base of the follicle or bulge), can have a cascade of downstream effects.

General consensus suggests that the greater the quantity and exposure of active growth factors and cytokines interacting with the complex regulating stem niches, the greater the chance for promoting prosignaling and transcriptional factors and downregulating bone morphogenetic protein signals for hair regeneration.[63] However, a few investigations have reported that higher concentrations of platelets may have a minimal effect or be counterproductive not only in the treatment of alopecia,[60] but also in reducing fat graft survival in animal and clinical studies.[61,62] Currently, the optimal numbers of platelets, concentrations, and activation considerations have not been determined in the treatment of varying stages of AGA. Although the use of activated platelets prior to delivery has been shown to increase hair density and follicular diameter, there is no consensus that a substantial release of growth factors and cytokines in a short time provides significant advantage over a slower release with native, nonactivated PRP.[64,65] Although aggregation inhibitors such as thrombin, calcium chloride, or calcium gluconate have been commonly used, the author chose not to use any external activators because the gradual release of growth factors and cytokines from the trauma of processing and collagen contact would confer a more natural and longer exposure.[35]

This study employed the same PRP device and preparation methods as reported by Hausauer and Jones.[51] These authors stated that their final concentrate ranged between 4 and 6 times the baseline platelet concentration of whole blood. Their delivery volumes were between 0.2 and 0.5 mL in the subdermal plane, allowing for greater dispersion. The investigators concluded that superior outcomes could be expected with 3 monthly sessions and a booster 3 months later than anticipated after 2 sessions every 3 months. The current study supports Hausauer and Jones observation that male and female subjects were less apt to respond to PRP when their hair loss was present for more than 6 years, they displayed a more advanced stage of alopecia, or they were greater than 60 years of age. Both trials reported no significant adverse events.

The author is aware of the limitations of this study, which include the need for a larger cohort of subjects for sufficient powered analysis, repeated frequencies for more positive outcomes, and longer follow-ups. Further studies are being prepared to address the shortcomings of this trial. To advance the field it is necessary to undertake high-quality standardized controlled trials that use quantitative measures of delivered platelet numbers and PRP preparation purity, and also consider randomization, blinding, sample size, statistical methods, intervention technique and frequency, study duration, and reliability of evaluation.

## CONCLUSIONS

This clinical study demonstrated that platelets are beneficial in the treatment of AGA. The study further suggests that the application of either a higher or lower number of platelets in the "therapeutic" PRP range was safe and effective for increasing hair density, follicle diameter, and terminal hair density but exhibited minimal effects on vellus hair density in this hemiscalp model. The evidence to date is suggestive rather than definitive: larger controlled, double-blinded, multicentered studies with the inclusion of more precise metrics are needed to confirm these preliminary findings.

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