

Platelet-Rich Plasma for Androgenetic Alopecia: A Pilot Study

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BACKGROUND Androgenetic alopecia is a common condition, with severe attendant psychosocial implications, and for which it is difficult to obtain a satisfactory degree of clinical improvement.

OBJECTIVE To explore the possible clinical benefit of injecting platelet-derived growth factors into the scalp of patients using a specific autologous blood concentrate.

MATERIALS AND METHODS Two injections of a leukocyte platelet-rich plasma (L-PRP) with the addition of concentrated plasmatic proteins were administered at baseline and after 3 months (single spin at baseline and double-spin centrifugation at 3 months). Macrophotographs were taken at baseline and after 6 months, and 2 independent evaluators rated them using Jaeschke rating of clinical change.

RESULTS Sixty-four consecutive patients were enrolled. Some improvement was seen in all patients by 1 evaluator and in 62 by the other. The mean change in clinical rating was 3.2 (95% confidence interval [CI], 2.9–3.5) and 3.9 (95% CI, 3.5–4.3), and the proportion of patients reaching a clinically important difference was 40.6% and 54.7%, according to the 2 evaluators, respectively.

CONCLUSION Our pilot study may provide preliminary evidence that this treatment may induce some degree of clinical advantage for male- and female-pattern baldness. This may warrant the design of randomized controlled clinical trials to formally test this procedure.

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Basic science and research findings have pointed out the role of platelet-derived growth factors (PDGFs) to be biologically active in many key regenerative steps, such as the development of angiogenesis, the formation of the protein matrix, and the enhancement of cell proliferation. Autologous blood concentrates, aimed at both the delivery of biologically active substances such as growth factors, cytokines, fibrin, and at autologous blood cell transplantation (e.g., leukocytes), have recently been involved in a large number of new clinical applications in different medical fields. The use of platelet concentrates has spread worldwide, fueling a large clinical interest in

regenerative medicine, in which it has been directed to many diverse conditions in several specialties, such as orthopedics, sport medicine, plastic and aesthetic surgery, and in the treatment of diabetic ulcers. In fact, it is so widespread that terms such as “platelet-rich plasma” (PRP) and “platelet gel” have rapidly become popular among doctors and patients.

Whereas, androgenetic alopecia causes many male and female patients worldwide to feel uncomfortable with their appearance, and affected individuals feel that alopecia is a serious condition with major implications in their daily life, often leading to high levels of

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anxiety and depression.^{1,2} Currently available treatments are at times perceived as having limited effectiveness. For example, topical minoxidil has to be used twice daily, which can be inconvenient; it can also cause scalp itching, dryness, scaling, flaking, irritation, or burning. Side effects are also a concern for oral finasteride, especially in young males, because it could decrease sexual ability/desire and the amount of semen released during sex. In addition, the safety and effectiveness of oral finasteride should be more thoroughly assessed in women.

The trend in therapies and clinical studies seems now to focus with a new, more “biologically oriented” approach to male- and female-pattern hair loss since new findings pointing out the influence of hair follicle stem cells in the hair growth cycle have been reported.³

We now know that progenitor stem cells in the bulge area play a key role in igniting the hair cycle⁴; furthermore, PDGF may have related functions in the interactions arising between the bulge and associated tissues starting with follicle morphogenesis.^{5,6} We have also considered the hypothesis that PDGFs seem to act on specific populations of progenitor cells that give rise to several different cell types with distinct functions in a variety of developmental processes.⁷

Furthermore, various reports suggest other possible interactions between hair follicles and PDGF. Platelet-derived growth factor signals, in fact, seem to play a role in hair canal formation,⁸ whereas vascular endothelial growth factor (VEGF)-mediated angiogenesis involvement⁹ was demonstrated in the control of hair growth and follicle size.

Recently, activated PRP has been reported to stimulate the proliferation of human dermopapillary cells, thereby increasing the survival of hair follicle cells through its antiapoptotic effects on dermopapillary cells and possibly stimulating hair growth by prolonging the anagen phase of the hair cycle. The *in vitro* findings were confirmed by *in vivo* results, which showed faster growth of hair in mice treated with PRP every 3 days for 3 weeks than that in the untreated controls.¹⁰

Most recently, intradermal injections of PRP at the thinning scalp site in 26 patients were shown to markedly increase hair diameter, whereas histologic findings showed a thickened epithelium, proliferation of collagen fibers and fibroblasts, and increased vessels around follicles.^{11,12}

We conducted this study to verify whether such a cellular and biologically oriented approach could represent an alternative to traditional therapies in male- and female-pattern androgenetic alopecia. In fact, miniaturized hair follicles maintain the same number of epithelial hair follicle stem cells in the bulge region on their outer root sheath as do the large terminal follicles.¹³ The aim of our study was to demonstrate that transplanted autologous PDGFs, cytokines, and leukocytes, when compounded with a plasmatic protein concentrate, may stimulate the activation of the stem cells in the miniaturized hair follicles, thus favoring the conversion of hair follicle stem cells to progenitor cells.^{14,15} This would induce a new anagen phase and, consequently, a clinical improvement due to a consistent and clinically evident increase both in quantity of hair and in hair shaft diameter.

Materials and Methods

After having received approval from the Ethical Committee of IDI-IRCCS, a clinical protocol for the treatment of the male- and female-pattern baldness was started at our hospital, a national reference center for skin conditions. All diagnoses were confirmed by dermatologists, who were with at least 15 years of clinical experience and specialized in conditions that affect the hair and scalp. The approved protocol consisted of:

1. Induction of cutaneous inflammation on the affected scalp to favor the activation of the injected platelets.
2. Intradermal/subdermal delivery in the thinning areas using the same syringe injection of:
 - Autologous platelet and leukocyte concentrate at high concentration levels (L-PRP).
 - Autologous concentrated plasmatic protein solution as a biologically active scaffold.

Only patients whose blood test revealed a platelet count above 140,000 platelets per microliter were

included in the study. Also, in this study we, included only patients who had never been treated with finasteride or minoxidil, or patients who had used finasteride or minoxidil for at least 2 years.

A 2-injection approach was then planned, with a 3-month interval between the 2 interventions. At the time of the first procedure, 60 mL of venous whole blood were drawn, and then processed through the GPS III Platelet Separation System (Biomet, Warsaw, IN), thus obtaining 6 to 8 mL of a solution rich in leukocytes and platelets. On average, when compared with the baseline, a sixfold to sevenfold increase in the platelet concentration was obtained according to quality control tests performed by our blood unit on all patients at the time of the first injection.

Based on the assumption that PRPs with a high platelet concentration could be more effective in promoting a better stem cell proliferation as compared with PRP with lower platelet concentration, we always injected the highest possible concentration. Both the single-spin and double-spin PRP systems that were used in this study (Figure 1) yielded concentrations of approximately 1,000,000 platelets per microliter in each patient.

After the injection of a local anesthetic (xylocaine 1%, with adrenaline 1:100,000), a cutaneous inflammatory response was induced through gentle pressure of a 1.0-mm deep Scalproller (Nanogen Pangaea Laboratories, UK) on the thinned scalp areas to be treated. The liquid platelet-poor plasma layer, harvested from the blood centrifugation, was then filtered using a mini-hemoconcentrator (Glo plasma filter; surface area 0.06 m², priming volume 5 mL; Glofinn OY, Salo, Finland). Then, 3 to 4 mL of this dense plasmatic protein concentrate were then added to the previously obtained “buffy coat” layer, yielding a total estimated concentration of platelets 3.5 to 4 times higher as compared with the baseline, in a total volume of 9 to 12 mL.

This solution was then injected through a 24- to 26-gauge needle in a 10 mL Luer-lock syringe on the superficial cutaneous scalp layers. Injections were 1 cm apart, that is, 4 injections would be on the vertices of a square with sides = 1 cm. The amount injected, per each injection, was approximately 0.2 to 0.3 mL. No new oral or topical treatments, such as anti-dihydrotestosterone or topical minoxidil were instituted, but patients who were already undergoing such treatments were encouraged not to quit.

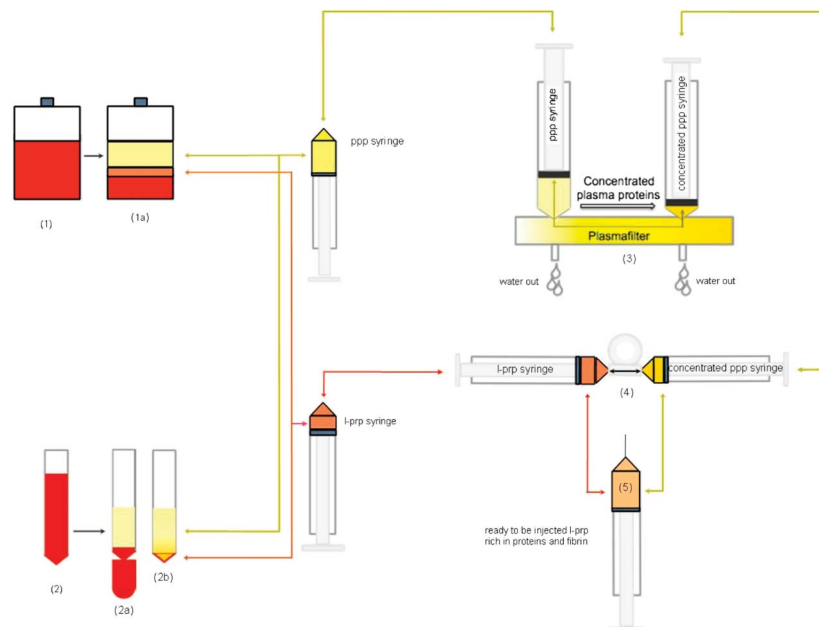


Figure 1. Diagram of the steps to produce I-PRP. Peripheral venous blood was centrifuged using a single-spin (1) or a double-spin (2) system, during which red blood cells were first separated (2a) and then discarded (2b). The obtained platelet-poor plasma (yellow lines) was filtered through a plasma-filter to reach a higher concentration of proteins (3) and then mixed (4) with the ready-to-be-injected I-PRP (red lines) to obtain a PRP richer in fibrin and proteins (5).

Three months after the first injection, a second procedure was carried out, drawing 40 mL of venous blood, this time using a double-spin¹⁶ centrifugation method (Glo PRP; Glofinn Oy, Glotech, Asan City, Chungnam, Korea). The concentrated solution of plasmatic protein was then added to the obtained platelet concentrate, as described for the first procedure. Platelet concentration after this procedure was estimated at approximately 4 times the baseline concentration. We were able to choose this dosing regimen for 2 main reasons: (1) because we delivered a higher concentration of platelets (obtained, of course, from a larger blood sample), as compared with other recent studies on hair restoration; (2) because we used a fibrin matrix: the entrapment of the platelets in the fibrin matrix has been demonstrated to give PDGFs a longer release potential.

To document any possible clinical change in the scalp region and in the hair, we adopted a macrophotographic protocol. We preferred this to the spot and ultraspot images obtained with software-assisted methods, because techniques such as the tricho-scan, although very popular, have been criticized¹⁷⁻¹⁹ over the last decade. In fact, although relying on such images would have stressed the almost-microscopic details of the follicular units emerging on the scalp, this technique would have missed the “wider shot” images that may provide an idea of the “scalp framed as a whole,” which we rated as the most relevant aesthetic parameter for a realistic evaluation of any kind of clinical improvement.

Our photographic protocol required patients to wet their hair by wiping their scalp with a sponge soaked with 5 mL of saline solution. No products were added to the patients' hair. Pictures were taken at the time of the first injection, at the time of the second procedure, and then at a 6-month follow-up (i.e., 3 months after the second procedure).

Pictures were always shot with the same FX reflex digital camera (Nikon d-700, d-800), same lens (Nikkor micro 60 mm f. 2.8), using as far as possible the same patient position (3 angles for the parietal/frontal scalp and 2 angles for the crown) and maintaining approximately the same head angle. Pictures were all taken by the same author (G.S.) even if in different light conditions. A total of 576 pictures were examined.

Because our study was ethically approved as an observational study, our evaluation was necessarily constrained to “before/after” observations, using each patient as his/her own control. The clinical change between the first assessment and the end of the follow-up was rated by 2 independent evaluators according to the 15-point scale proposed by Jaeschke and colleagues.²⁰ Each evaluator had to answer the following question: “Overall, has there been any change in this patient's condition since the first visit? Please indicate if there has been any change by choosing one of the following options. Compared to the previous data-collection visit, the hair condition is [...]”. The answers ranged from -7, corresponding to “A very great deal worse,” to +7, corresponding to “A very great deal better,” and with 0 corresponding to “About the same.”

We report here the whole Jaeschke scale:

- -7: A very great deal worse
- -6: A great deal worse
- -5: A good deal worse
- -4: Moderately worse
- -3: Somewhat worse
- -2: A little worse
- -1: A little worse, hardly any worse at all
- 0: About the same
- +1: A little better, hardly any better at all
- +2: A little better
- +3: Somewhat better
- +4: Moderately better
- +5: A good deal better
- +6: A great deal better
- +7: A very great deal better.

We compared the ratings of clinical change obtained independently from 2 of the authors (G.S., a surgeon, designated as Evaluator 1, and D.A., an epidemiologist, designated as Evaluator 2) using intraclass correlation coefficients (ICC) that were obtained by the R free statistical software, Nonlinear Mixed Effects Models package (www.r-project.org). The ICC is equivalent to the kappa statistic for continuous values. It has the advantage over the Pearson or Spearman correlation coefficient in that it is a true measure of agreement, combining information on both the correlation and the systematic differences between the readings.²¹

The baseline severity of the condition was estimated using the global physician assessment (GPA) score. The author who performed the clinical intervention evaluated each patient answering this question: “In your experience, among all patients you have seen with this condition, how severe is the condition of this patient today?” On a 5-point scale, the possible answers were: very mild, mild, moderate, severe, and very severe. In the analysis, the 2 extreme categories were grouped together.

The ratings of clinical change were dichotomized, using as a cutoff point the score of +4 (i.e., “moderately better”), because it was deemed—by a consensus among the authors—a conservative indication for the minimal clinically important difference.

The differences in clinical change within groups were tested using Fisher exact test. Also, to assess the possible independent role of the variables of interest, while simultaneously controlling for each considered variable, a logistic regression model was implemented including gender, age group, number of platelets before the intervention, and the GPA as predictor variables, and using the dichotomized rating of clinical change as outcome variable.

Results

Forty-two men and 22 women were enrolled in this study, of a total of 64 studied patients. The median age was 28 in the male group and 32 in the female group. Patients were all affected by different degrees of male- or female-pattern baldness, ranging from Hamilton Class 2 to 5 for men, and Ludwig Class 1 and 2 for women. No immediate adverse effects, such as allergic reactions, postoperative pain or fever, prolonged redness, nor delayed side effects such as telogen effluvium were reported.

Only 10 patients (15.6%) were classified as “severe or very severe” according to the GPA at baseline. The characteristics of the study population are displayed in the first columns of Table 1.

An improvement (i.e., positive scores) was observed in 62/64 patients by Evaluator 1 and in all 64 patients by

Evaluator 2. The 2 patients not improved, according to Evaluator 1, were scored as zero, that is, “about the same.” Figure 2A,B show the condition of a 24-year-old female patient at baseline and at the 6-month follow-up, whereas Figure 3A,B show the condition of a 26-year-old male patient, at the same time points. The visible improvement at follow-up should not be attributed to increased hair length, but rather to an increase in the hair thickness.

The crude correlation between the clinical change scores of the 2 evaluators was .932 (using Pearson correlation coefficient) and .803 (using Spearman correlation coefficient). The 2-way mixed-effects model for intra-class correlation coefficient yielded an estimate of .903 (95% confidence interval [CI], .845–.940). All these estimates were statistically significant, with $p < .01$.

Table 1 also shows the changes in rating of clinical change—as evaluated independently by the 2 authors—both in terms of mean change and of proportion of patients reaching an improvement of 4 or more—which was deemed to be clinically important.

The overall mean change in clinical rating was 3.2 (95% CI, 2.9–3.5) and 3.9 (95% CI, 3.5–4.3), and the overall proportion of patients reaching a clinically important difference was 40.6% and 54.7%, according to the 2 evaluators, respectively.

No significant differences were observed in the rating of clinical change for gender, age group, and number of platelets before the intervention, and GPA, although on average, a greater degree of improvement was seen in patients with a higher GPA score at baseline. The proportion of patients reaching a clinically important improvement in the different GPA levels ranged from 21.7% to 60.0% in Evaluator 1 and from 39.1% to 80.0% in Evaluator 2 ($p = .027$, and $= .030$, respectively, using the chi-square test for linear trend).

The logistic regression model confirmed the above findings. No statistically significant results were observed for gender, age group, and number of platelets before the intervention. However, when simultaneously adjusting for the aforementioned variables included in the model, the GPA reached statistical

TABLE 1. Changes in Rating of Clinical Change Scores, by 2 Independent Evaluators: Mean Change at 6-Month Follow-up and Proportion (%) of Patients Reaching a Minimal Clinically Important Difference (MCID, i.e., a Score of At Least +4 on the Rating of Clinical Change)

Variable	Level	Mean Change						Proportion Reaching the MCID							
		Total		Evaluator 1			Evaluator 2			Evaluator 1			Evaluator 2		
		N	%	Mean	SD	p	Mean	SD	p	N	%	p*	N	%	p*
Overall		64	—	3.2	1.4	—	3.9	1.6		26	40.6		35	54.7	—
Gender	Men	43	67.2	3.2	1.4		4.0	1.5		18	41.9		25	58.1	
	Women	21	32.8	3.2	1.2	.707	3.7	1.7	.459	8	38.1	.794	10	47.6	.593
Age, years	20–25	19	29.7	3.2	1.3		3.9	1.7		7	36.8		10	52.6	
	26–30	21	32.8	3.2	1.4		3.6	1.8		9	42.9		10	47.6	
	31+	24	37.5	3.2	1.5	.993	4.1	1.2	.500	10	41.7	.902	15	62.5	.601
Platelets, thousands per microliter	<200	22	35.9	3.5	1.4		4.1	1.4		10	45.5		13	59.1	
	200–224	18	28.2	3.1	1.5		3.9	1.8		8	44.4		10	55.6	
	225+	22	35.9	3.0	1.3	.503	3.6	1.6	.573	7	31.8	.648	11	50.0	.858
GPA	Mild	23	35.9	2.8	1.3		3.5	1.5		5	21.7		9	39.1	
	Moderate	31	48.4	3.3	1.4		3.9	1.7		15	48.4		18	58.1	
	Severe	10	15.7	3.5	1.3	.271	4.5	1.1	.245	6	60.0	.055†	8	80.0	.093†

*Fisher exact test.

†Chi-square for linear trend was .027 and .030 for Evaluator 1 and 2, respectively.

significance. In fact, the “risk” of reaching a clinically important improvement according to Evaluator 1, as estimated through the adjusted odds ratios (OR), was

4.1 (95% CI, 1.04–16.3) for “moderate” patients and 7.0 (95% CI, 1.2–41.1) for “severe and very severe” patients compared with the “very mild and mild”



Figure 2. Baseline (A) and follow-up (B) condition of a 24-year-old patient. Preoperative platelet count 285,000 per microliter. Baseline GPA rated as “very severe,” rating of clinical change rated as +5 (i.e., “a good deal better”) by both evaluators.

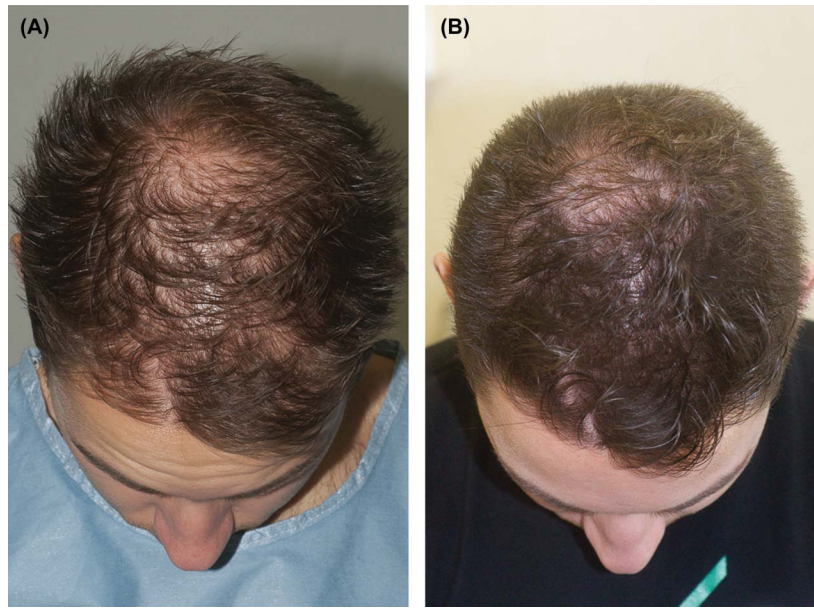


Figure 3. Baseline (A) and follow-up (B) condition of a 26-year-old patient. Preoperative platelet count 210,000 per microliter. Baseline GPA rated as “severe,” rating of clinical change rated as +6 (i.e., “a great deal better”) by both evaluators.

ones. The estimates according to Evaluator 2 were similar, although the first one did not reach statistical significance: OR = 3.0 (95% CI, .8–11.1) for “moderate” patients, and OR = 8.4 (95% CI, 1.2–57.7) for “severe or very severe” patients, compared to the “very mild and mild” ones.

Discussion

In our pilot study, we observed that patients treated with our plasmatic protein-rich I-PRP almost invariably showed some degree of clinical improvement at 6-month follow-up, and that—according to the more conservative of the 2 evaluators in our study, that is, the plastic surgeon—approximately 2 of 5 (i.e., 40.6%) reached at least a moderate level of improvement, which was deemed to be clinically relevant.

It should be noted that because the rate of growth of hair is approximately 0.3 mL per day—or 1 cm (i.e., approximately one-third of an inch) per month—the clinical result visible at a 6-month follow-up is mainly due to increased thickness and/or higher number of hairs, rather than increased length, of the hair.

Of course, in this first phase of the investigation on the possible effectiveness of PRP for androgenetic alopecia, we have to acknowledge that our observational

study has the limitation of having included only treated patients. Although it is accepted that spontaneous recoveries in a 6-month observation period are rare, it is clear that any further study will have to include a control group, possibly within the setting of a randomized controlled clinical trial. Such a study would also help in defining an appropriate timeframe for the possible continuation of the treatment. As of now, our preliminary evidence indicates that a retreatment may be advisable at 10 to 12 months from baseline, but a prolonged more systematic observation on a larger number of patients will be needed to firmly establish a retreatment schedule.

In addition, the follow-up period will have to be extended, to verify whether the improvement observed at an interval of 6 months after the intervention may be maintained over time.

Both measures used to assess the clinical severity and the possible improvement at follow-up, that is, the GPA and the rating of clinical change are subjective, and so are exposed to some extent to the bias of the investigator. However, the GPA is currently accepted and used in many clinical trials—including, for instance, those evaluating the new and expensive “biological” treatments for psoriasis. As for the rating of clinical change, although it is also currently

used in many studies, including those to validate the evaluative properties of instruments such as quality of life questionnaires, we resorted to 2 independent evaluators with different expertise: one was the surgeon who performed the intervention, and the other the epidemiologist who managed and analyzed the data. It is noteworthy that the epidemiologist, who had in no way participated in the clinical intervention and never had any contact with the patients, gave a less conservative evaluation of the clinical improvement. In addition, the very high intraclass correlation coefficient indicates that there was a high level of agreement between the 2 independent evaluators.

Another possible limit of our study is that it included patients with mostly mild or moderate clinical severity of androgenetic alopecia, who may not be representative of the whole population of patients with this condition. But, concerning the baseline level of clinical severity, it is interesting to note that higher levels of clinical improvement were observed by both of the evaluators in those few patients who were deemed to be “severe or very severe” at baseline. Although it could be argued that more severe patients, in general, have a higher probability of showing a larger degree of regression to the mean, it has to be considered—as noted above—that in androgenetic alopecia, a relevant spontaneous clinical improvement in the short-term is quite unlikely.

The generic term “platelet-rich plasma” basically does not allow for a clear distinction anymore between the available products, because it does not take into account some key specifications such as platelet concentration potential, presence or absence of cells such as leukocytes, and of plasmatic proteins such as fibrinogen, vitronectin, fibronectin, and cytokines. Not all platelet concentrates are created equal, and fine-tuning the PRP production methods for our required clinical purpose seemed to be an essential step, because the many different products that are available in an ever-expanding market today, often lead to different types of blood aggregates, with different biology and potential uses.^{22–24}

We first estimated that a significant volume of whole-blood (i.e., 40–120 mL) was necessary to inject the

scalp surface with an adequate volume of 5 to 15 mL (depending on the extent of baldness) of highly platelet-enriched plasma.

Regarding the issue related to the dependence of clinical benefit on platelet concentration, this is still a chapter under investigation, because concentration ratios of less than twofold to 8.5-fold have been reported,^{25–30} and some authors³¹ suggest that different individuals may require different platelet concentration ratios to achieve comparable biological effects. Nevertheless, we have based this study on the vision that Marx and other investigators have suggested: that PRP should achieve a threefold to fivefold increase in platelet concentration over baseline^{32–34} to be effective and successfully targeting in our case, follicular stem cells.

Although researchers have mainly considered only PDGFs to be responsible for all the PRP’s biological effects, we hypothesize that the presence of cells, such as leukocytes, and/or the addition of some plasmatic proteins that could carry a biologically active role, have to be seriously considered; this is why both were included in our plasmatic concentrate. In fact, several studies have pointed out the importance of leukocytes in PRP³⁵ for their anti-infectious action^{36,37} and immune regulation,^{38,39} outlining their capability to produce large amounts of VEGF.⁴⁰

At the same time, the presence of some cytokines and the density and composition of the fibrin matrix (which supports their release) should be considered as another key parameter of any platelet concentrate.⁴¹ In fact, we argue that the “entrapment” of platelet and leukocyte growth factors into the fibrin matrix,^{38,42} as it is hypothesized to occur at the PRP injection site when the fibrinogen-enriched plasmatic concentrate we injected interacts with the local autologous thrombin (produced by the previously induced local tissue trauma-inflammation), promotes a slower and more efficient platelet and leukocyte growth factor release.⁴³

At the same time, we hypothesize that concentrating fibrinogen, plasmatic vitronectin, and fibronectin from the platelet-poor plasma layer obtained after

centrifugation would be another key explanation for the encouraging clinical outcomes of this study. These same proteins in fact have been demonstrated to bind growth factors and to enhance their activity in the extracellular matrix environment,^{44,45} while whole human plasma itself has been shown to be effective in promoting *in vitro* angiogenesis.^{46,47}

All this, taken together, indicates that our pilot study may provide at least preliminary evidence that the biological background and rationale for this treatment may be translated into some degree of clinical advantage for patients with androgenetic alopecia of mild to moderate clinical severity. This may prompt other groups to perform studies on this procedure, possibly using a control group and ideally using the constraints of a well-designed randomized controlled clinical trial.

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