Injectable Platelet-, Leukocyte-, and Fibrin-Rich Plasma (iL-PRF) in the Management of Androgenetic Alopecia

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BACKGROUND The role of enriched autologous plasmas in androgenetic alopecia (AGA) management is emerging in recent literature.

OBJECTIVE In this prospective study, the authors aimed to confirm that the induction of a minor local trauma immediately followed by injections of an enriched plasma made of a strongly concentrated platelet fraction, a robust white cell presence, concentrated fibrinogen, and other plasma proteins (injectable leukocyte platelet-rich fibrin [iL-PRF]) could be able to produce positive clinical results in patients with AGA.

MATERIALS AND METHODS A 2-injection regimen was instituted, with a 3-month interval between the 2 interventions. A treatment group (TG) and a control group (CG) were instituted. Macrophotographs were taken at baseline and after 6 months, and rated by 5-people expert panel (blinded to this assignment) using the 15-point scale proposed by Jaeschke to evaluate the clinical change.

RESULTS Overall, TG showed better scores compared with the CG in all 5 classes of global physician assessment at baseline, all age groups, and in both sexes, and such differences always reached statistical significance. A greater severity at baseline showed a larger improvement after treatment in the TG.

CONCLUSION This study provides preliminary evidence that the biological composition of the iL-PRF is of crucial importance in ensuring a good degree of clinical efficacy in patients with AGA.

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Enriched autologous plasmas have been described as an innovative treatment for androgenetic alopecia (AGA),[1–9] and literature has more recently commented on its possible mechanism of action.[10] However, various enriched plasmas are not created equal,[11] and the unevenness in reported clinical results[12–16] is presumably related to large differences in preparation and delivery methods. The generic term "platelet-rich plasma" (PRP) is used to describe various protocols and harvesting methods,[17] each delivering different mixes and amounts of blood cells and biologically active molecules.[18,19] It follows that the term "injectable leukocyte platelet-rich fibrin" (iL-PRF) was adopted (in part because PRF or L-PRF acronyms have already been applied to define only gel and not injectable blood products[19–21]). This term better matches and explains this study's "PRP" contents.

This larger controlled observational study follows the promising results of the authors' pilot study[5] and represents a further step toward a substantial investment in financial and organizational aspects required by randomized controlled trials, which are the gold standard for evaluating treatment interventions.

The authors used a validated scoring system[22] to demonstrate that the induction of a minimal local trauma on the scalp followed by injections of a filtered

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plasma made of a strongly concentrated platelet fraction, a robust white cell presence, and the inclusion of concentrated fibrinogen and other plasma proteins yields positive clinical results in patients with AGA.

Materials and Methods

Injectable Leukocyte Platelet-Rich Fibrin Preparation

A clinical protocol for the treatment of AGA was approved by the ethics committee at IDI-IRCCS, a national reference hospital for skin conditions.

The iL-PRF preparation is based on a 2-step procedure:

(1) whole blood centrifugation and consequent red blood layer discard;
(2) filtration of the remaining autologous plasma.

Each treated patient had 60 to 120 mL of venous blood harvested. Anticoagulants ("ACD-A") were then added, and the sample was soft-spin centrifuged for 5 minutes at 1,500 rpm. Two European Union–certified systems, the GLO PRP blood separation kit (Glotech, Asan City, Korea) and CPunT (Biomed, Modena, Italy), were used in Step 1.

The remaining fluids after centrifugation (25–50 mL of the intermediate buffy coat layer plus the upper yellowish platelet-poor plasma layer) were filtered in Step 2.

This was made possible using a small dedicated hemofilter manufactured according to the authors' specifications (Protsmart, Medisulfone, Medica, Medolla, Italy, cutoff of 15 kDa). Its filtration and concentration capabilities derive from the structure of the hollow fiber[23] membranes made by an internal skin layer (1-micron thickness) with a very high density of small pores (nanometers scale) (Figure 1).

After the filtration process, which discarded the water and lightest blood proteins, left 6 to 14 mL of concentrated iL-PRF ready to be injected in the very superficial scalp layers through 26-gauge needles on 2.5-mL luer lock syringes. The following concentrations were obtained when comparing injected plasma with the patient's whole blood:

(1) a roughly 4.5-fold average increase in platelets concentration;
(2) a nearly 2-fold increase in granulocyte numbers;
(3) a 4.5-fold increase in lymphocyte number;
(4) a 5-fold increase in fibrinogen values (the authors assume that this figure is similar to be expected for all plasma proteins heavier than 15 kdalton).

A local inflammatory cutaneous reaction (another key step of the authors' procedure) was simultaneously performed by superficially traumatizing the scalp with an oscillating fractional microneedling unit (Dermapen 3; Equipmed, Sydney, Australia). With the needle depth sets between 1 and 2 mm, this procedure produces cutaneous damage with minimal or no bleeding. Therefore, injected platelet activation is physiologically achieved as a response to substantial injury.[24] No oral antidihydrotestosterone or topical minoxidil was prescribed, but patients undergoing such treatments for at least 1 year were encouraged not to quit. A 2-injection regimen was implemented with a 3-month interval between the 2 interventions.

Study Design and Clinical Evaluation

A 5-people expert panel was formed by:

(1) a plastic surgeon with experience in hair transplantation;
(2) an epidemiologist (author, D.A.);
(3) a dermatologist with extensive experience in hair disorders;
(4) a professional hairdresser;
(5) a professional photographer with experience in medical images.

The panel was asked to examine and rate a total of 686 standardized images ordered in 343 pre-op/post-op slides from all enrolled patients.

To provide evaluators with a standardized, high-quality set of comparable, high-definition (HD) images, the following procedures were followed:

(1) The patients were asked to wet their hair by wiping the scalp with a 10-cc water-soaked

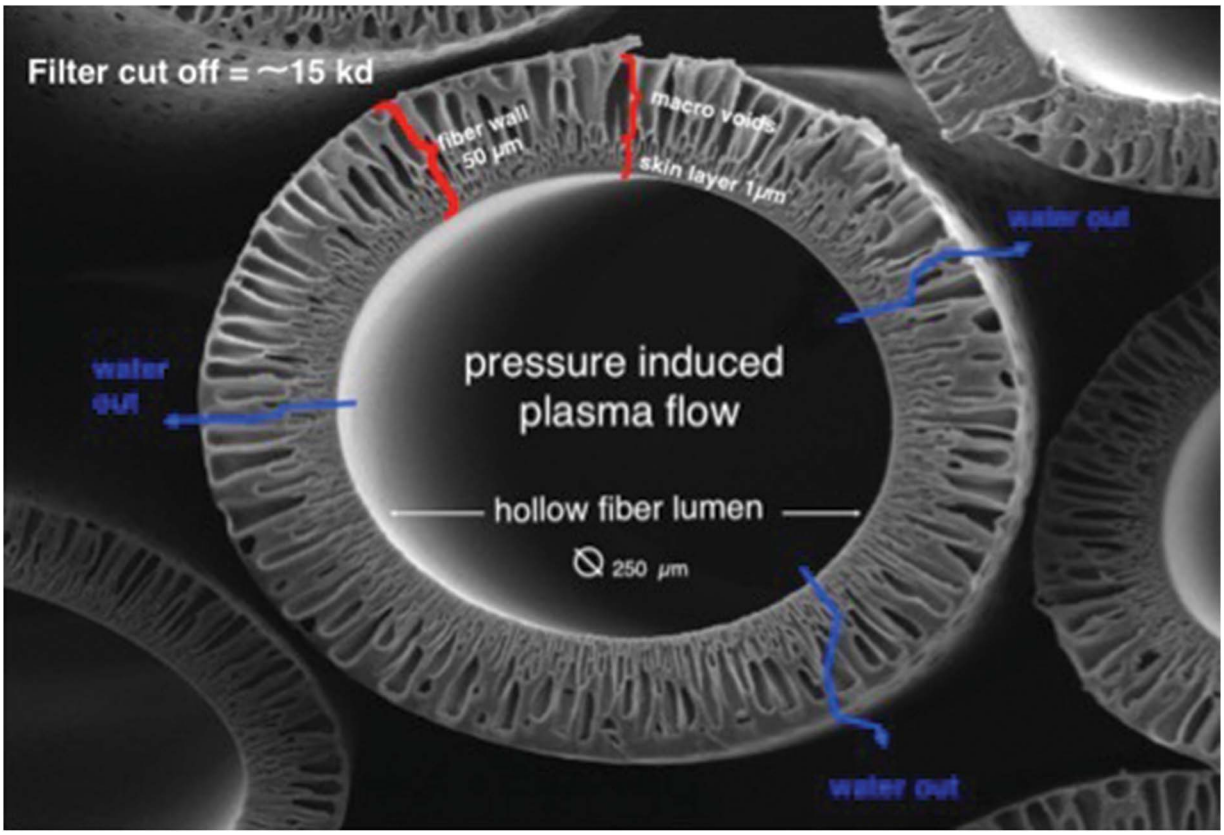

Figure 1. Section of a dedicated Medisulfone hollow fiber: electron microscopic image (×300). Plasma flows inside the fiber lumen, whereas water and the smallest peptides are pushed out through the fiber wall pores. The 1-micron thickness skin layer has the highest density of small pores, whereas macrovoids primarily retain a structural function.

sponge to favor a comparable "see-through" vision of the underlying scalp skin.

(2) Wet hairs were systematically combed laterally off the midline to maintain the same hair style and direction.

(3) Images were shot in RAW mode (full-frame reflex digital cameras Nikon, D800, D750, fixed focal macro lenses), generating only large 24.3- and 36-megapixel images. Only full HD resolution images (1920 × 1080) were shown to the evaluators.

(4) Care was taken to maintain the same head position (3 angles for the parietal/frontal scalp and 2 angles for the crown) and hair length in the pre–post images.

Images were always taken by the same author (G.S.) under direct flash.

For each patient, the images were paired on the same slide, but the pre–post order was scrambled according to a random assignment.

The patients in the control group (CG) came from a waiting list. Their images were taken at the same time intervals as the treatment group (TG).

The evaluators were completely blinded to treatment status and were not involved in any phase of treatment.

Each evaluator had to answer the following question: "Overall, how does the condition seen in the image on the right compare with the condition in the image on the left?" The answers ranged from −7 ("A very great deal worse") to +7 ("A very great deal better") with 0 corresponding to "about the same" according to the 15-point scale proposed by Jaeschke and colleagues.[25] Finally, the author responsible for data analysis (D.A.) was unblinded concerning the pre–post photograph order and TG–CG status.

The authors compared the ratings of the clinical change obtained through intraclass correlation

coefficients. These ratings were dichotomized using the score +4 (i.e., "moderately better") as a cutoff point because it was deemed by consensus among the authors to represent a conservative indication for the minimal clinically important difference. The baseline severity of the condition was estimated using the global physician assessment (GPA) score on a 5-point scale. The possible answers were very mild, mild, moderate, severe, and very severe.

The differences in the clinical change within groups were tested using the Fisher exact test. In addition, to assess the possible independent role of the variables of interest, a logistic regression model was implemented including sex, age group, the number of platelets before the intervention, and the GPA as predictor variables, as well as using the dichotomized rating of the clinical change as the outcome variable.

Results

A total of 168 patients (102 men and 66 women) affected by different degrees of AGA (from Hamilton Class 2 to 5 for men and Ludwig Class 1 and 2 for women) were enrolled in this study. The median age was 28 years in men and 36 in women. A frontal cutaneous bruising occurred after 48 to 72 hours and spontaneously resolved in the fourth to fifth post-op day in 24 patients. This was the only adverse effect reported.

The mean scores for the clinical change scale rating by TG and by sex, age, and GPA at baseline are shown in Table 1.

Overall, the TG (126) showed better scores compared with the CG (28) in all 5 classes of GPA at baseline, all age groups, and in both sexes, and such differences always reached statistical significance. Considering the clinical changes in the TG compared with baseline, it is interesting to note that the differences increased with severity from very mild to very severe, indicating that a greater severity at baseline benefitted from a larger improvement after treatment. Figure 2 shows the clinical results in 2 treated patients.

Table 2 shows the proportions of patients with no clinical improvement (i.e., a score of 0 or less on the Rating of Clinical Change scale) and the proportion of patients with at least a moderate clinical improvement (i.e., a score of +4 or more on the Rating of Clinical Change scale) for each evaluator and overall. Overall, the proportion of patients with no improvement was 2.2 in the TG and 41.4 in the CG ($p < .001$); the proportion of patients with at least a moderate clinical improvement was 51.8 in the TG and 0.0 in the CG

TABLE 1. Mean Scores for the Rating of Clinical Change Scale by Treatment Group and by Sex, Age, and Global Physician Assessment (GPA) at Baseline

	N	*Column %*	*TG (N = 139)*	*CG (N = 29)*	p
Overall	168		—	—	—
Sex					
Female	66	39.3	3.3	0.2	<.001
Male	102	61.7	3.6	0.1	<.001
Age (yr)					
<30	85	50.6	3.6	0.3	<.001
30–44	57	33.9	3.3	0.0	<.001
45+	26	15.5	3.4	0.2	<.001
GPA					
Very mild	12	7.1	1.9	0.2	.038
Mild	56	33.3	3.2	0.4	.001
Moderate	60	35.7	3.6	0.1	<.001
Severe	35	20.8	4.1	0.1	<.001
Very severe	5	3.0	4.5	−0.2	.054

CG, control group; TG, treatment group.

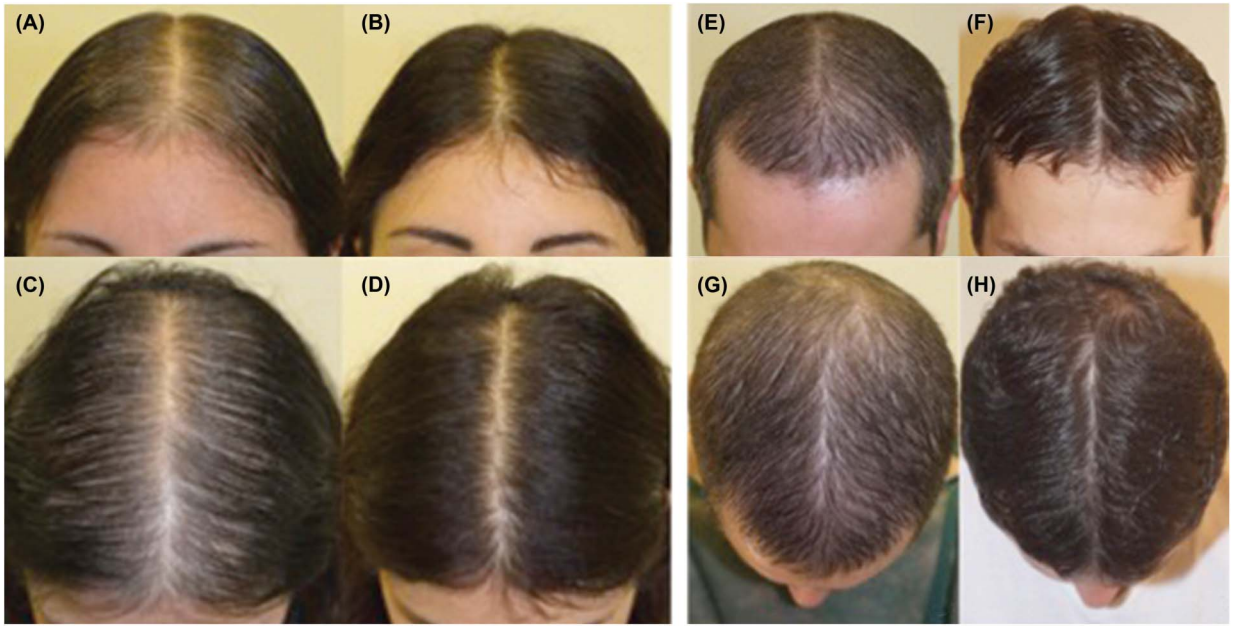

Figure 2. Baseline frontal (A and E) and from above (C and G) and follow-up frontal (B and F) and from above (D and H) condition of a 24-year-old woman (left) and a 39-year-old man (right) affected by androgenetic alopecia. Baseline GPA rated as "severe," rating of clinical change rated as +5 (i.e., "a good deal better") by all evaluators. GPA, global physician assessment.

($p < .001$). Both sets of data confirm a significant difference between the 2 groups.

The mean scores for the clinical change scale were assessed separately for each level of the GPA at baseline and were expressed by each evaluator. The scores confirm that, for each separate observer, the difference in the clinical change was always higher in the TG compared with the CG at each level of clinical severity (data not shown). Furthermore, a more severe AGA led to greater clinical differences. Overall, the differences in the clinical change between the TG and the CG always reached statistical significance for each observer ($p < .001$).

Discussion

The authors observed that patients treated with iL-PRF almost invariably showed some degree of clinical improvement in AGA management. In particular, such improvement was obtained for all levels of clinical severity at baseline and in both sexes. Improvement also tended to be greater for higher levels of AGA severity.

One of the main obstacles in trials evaluating hair growth is the absence of a standard and objective noninvasive method to compare hair numbers and diameter. Global photography and phototrichograms are the commonest evaluation methods.[15] The authors decided to avoid spot and ultraspot images because the strong potential of computer-assisted technology in this field is yet to be maximized.[26,27]

Comparing scalp images taken on a mere 1–2 square mm could be considered helpful only when analyzing the almost microscopic appearance of scalp follicular units, a feature the authors did not consider as "key" in this study for the following reasons:

(1) "Wider-shot" images may better convey the concept of the "scalp framed as a whole," which the authors rate as the most relevant aesthetic parameter.
(2) Doubtful evidence exists that the same degree of improvement occurs in larger, unselected, distant portions of the hairy scalp when demonstrated on a phototrichogram.
(3) To obtain standardized and comparable results, phototrichograms should be performed only on a shaven part of the scalp that needs to be landmarked with a tattoo for future site location, which is not easily applicable in patients, especially women.[28]

TABLE 2. Proportions of Patients With No Clinical Improvement (i.e., Scoring 0 or Less on the Rating of Clinical Change Scale) and the Proportion of Patients With at Least a Moderate Clinical Improvement (i.e., Scoring +4 or More on the Rating of Clinical Change Scale) for Each Evaluator and Overall

	% Not Improved Treatment			*% Improved 4+ Treatment*		
	TG (N = 139)	*CG (N = 29)*	p	*TG (N = 139)*	*CG (N = 29)*	p
Plastic surgeon	6.5	93.1	<.001	33.1	0.0	<.001
Epidemiologist	6.5	69.0	<.001	57.6	3.4	<.001
Dermatologist	5.8	41.4	<.001	27.3	3.4	.003
Hairdresser	8.6	58.6	<.001	51.1	10.3	<.001
Photographer	5.8	65.5	<.001	71.2	10.3	<.001
Overall*	2.2	41.4	<.001	51.8	0.0	<.001

*Proportion of patients unanimously evaluated as "not improved" and unanimously evaluated as "improved 4+."
CG, control group; TG, treatment group.

A distinctive aspect of this study is the prominent role that plasmatic proteins could play. The procedure used to implement peptide concentration was also unique. A new filtration method was introduced for this purpose, and a small dedicated plasma filter was herein described and used for the first time.

Since the first works of Marx and colleagues,[29] platelets and growth factors (GFs) have predominantly been considered "key" components in PRP preparations. Growth factors in general and platelet-derived GF (PDGF) specifically are involved in a wide range of regenerative functions including the proliferation, recruitment, and migration of stem cells; chemotaxis in various cells; the modulation of local inflammatory responses; and the stimulation of new blood vessel formation.[18,30,31] These features, in addition to the inclusion of more concentrated leukocytes, could be considered to positively influence the hair growth cycle.[10,32–34]

Nevertheless, "pure" GF/PDGF translation to clinical efficacy has been modest.[35] One reason for this limited translation could be the lack of extracellular matrix (ECM) binding in clinically used forms of GF. Growth factor interactions with ECM components facilitate localized and spatially regulated signaling,[36,37] whereas the partitioning, availability, and signaling of GFs are orchestrated by their binding to the ECM.[11,38]

The ability to mimic the mechanisms by which the ECM controls GFs is becoming critical in designing successful GF-based therapies.[39,40] Beyond acting as a GF source, blood could also represent a source for affordable protein-based carriers and delivery systems that seem necessary[34] for the clinical effectiveness of PDGF.

The promising results obtained when injecting concentrated alpha-2-macroglobulin[41] in the synovial space of patients with post-traumatic osteoarthritis represents a recent example for the central role that plasmatic proteins could play in delivering blood-derivative therapies. This circulating plasma protein[42] is intended to help bind and regulate the distribution and activity of many cytokines and GF, including transforming GF-beta, tumor necrosis factor–alpha, PDGF, interleukin (IL)-6, nerve GF, fibroblast GF, and IL-1 beta.[43,44] Therefore, filtration methods capable of increasing the concentration of such circulating plasma proteins to the levels that the authors demonstrated for fibrinogen could be beneficial in improving GF clinical effectiveness.

Moreover, fibrous-type blood proteins, such as fibrinogen and fibronectin (one of the constituents of ECM that provides a protective milieu for cells and tissues[45]), together with those of the ECM (e.g., collagen and elastin), are currently regarded as ideal components to prepare scaffolds and biomaterials.

Their normal physiological function is to provide mechanical support, tensile strength, and stability to biological structures as GFs or cytokines. Thus, the authors hypothesize that, when L-PRF is injected, new fibrin constructs[46] are generated that could efficiently mimic the natural ECM microenvironment and display a microporous structure that is useful in cell adhesion, migration, proliferation, and differentiation.[19]

This study provides additional in-depth and controlled preliminary evidence that injecting the scalp with autologous iL-PRF ensures a good degree of clinical efficacy in patients with AGA. Formally designed controlled clinical trials will be conducted to test such intriguing preliminary evidence.

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