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EDITORIAL

Use and Misuse of PRP: Clinical Trial Proposal to Help Find PRP's True Efficacy

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ABSTRACT

INTRODUCTION: Since the early 1970s, Platelet-rich plasma (PRP) has been used in many fields as a potential remedy for having regenerative properties. In dermatology, its use is extended to different hair diseases: alopecia areata, traction alopecia, androgenetic alopecia (AGA), and even cicatricial alopecia. Despite its increasing use in the clinical practice, the protocol used to produce PRP differs greatly between researchers and its efficacy has not been clearly demonstrated. Particularly, AGA is an illness characterized by a progressive hair thinning and miniaturization caused by the effect of dihydrotestosterone (DHT) on the hair follicle. This type of alopecia is the most common in men, increasing its incidence with age. Until the date, there are only two efficient treatments approved by the FDA: Finasteride and Minoxidil. PRP seems to be a potential new candidate according to recent studies, but multiple PRP obtaining techniques have raised ambiguous results about the real efficacy of PRP in patients with AGA. Thus, we have elaborated a methodology to help researchers designing future clinical trials on PRP therapy. We propose a randomized clinical trial with a uniformed PRP obtaining technique in order to properly assess the PRP efficacy as a treatment for AGA.

OBJECTIVE: Analyze the objective data published until the date in order to define the real efficacy of PRP as a treatment for AGA, and offer a design of a clinical phase trial with a standardized methodology to obtain proper scientific evidence of PRP efficacy.

METHODS: Retrospective systematic review of the literature published and a randomized placebo-controlled, double-bind, half-head study clinical trial proposal with a uniformed PRP obtaining methodology to properly assess its efficacy as a future AGA treatment.

Key words: Male-pattern hair loss (MPHL); Androgenetic alopecia (AGA); Platelet-rich plasma (PRP); Efficacy; Growth factors (GFs)

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INTRODUCTION

Androgenetic alopecia (AGA) is an illness characterized by a progressive hair thinning and miniaturization, diminishing both the length and the diameter, transforming it in fuzz so that with time it completely atrophiesand is characterised by the loss of hair from the scalp in a defined pattern. Determining factors appear to be genetic predisposition coupled with the presence of sufficient circulating androgens. The prevalence of this condition is high (up to 50% of white males are affected by 50 years of age [1-3]. AGA affects 80% of men and 50% of women, presenting two different patterns: a central and more diffuse in women (FAGA) and slightly different in men,

in which the main characteristic is the recession of the frontoparietal line (MAGA)[2,3].

MAGA usually starts around the 20 years of age in men, affecting about 50% of the entire male population at 50 years of age and around 70% of men will eventually develop MAGA sometime in their lives[2,4,5]. AGA is an androgenetic dependent illness, modulated by an active testosterone metabolite, called dihydrotestosterone (DHT)[2-4,6-8]. Although the tissue distribution does vary, both enzyme 5α-reductase types are found in the scalp follicles, which specifically concentrate in the dermal papilla[2,9]. To pursue its effect, androgens bind to the human androgen receptor (AR), a member of the steroid-thyroid hormone receptor superfamily[9]. Both testosterone and DHT can bind to the AR domain, which act as a transcription factor, regulating the expression of androgen-sensitive genes[10]. The concentration of DHT, AR and 5α-reductase has been demonstrated to be higher in the balding scalp[11-14]. Moreover, evidence has shown that AGA has a polygenic mode of inheritance, after finding that 81.5% of balding sons had fathers with type 3 or more in the Hamilton-Norwood scale[15], exceeding the autosomal dominant expectation of 50%. However, it has been difficult to encounter a gene related to AGA, since none were found in the Y chromosome neither the aromatase gene nor the 5α-reductase[16,17]. Moreover, it has been demonstrated that patients affected by AGA, especially those in early ages, perceive themselves older than they are, adding stress and considerable preoccupation to their lives. AGA truly becomes a psico-social problem for the patient becoming bald at a young age, who feels unattractive and less socially successful[1,18-22].

At the present time, there are only two approved FDA treatments to deal with MAGA; oral Finasteride, which selectively inhibits the 5α-reducatse enzyme and reduces the concentration of DHT in the scalp follicle around 70%, inhibiting or even reducing the miniaturization of the hair follicle[23-27], and topic Minoxidil, which action mechanism remains unclear[3,27-30]. Even though both treatments are effective, they are not exempt of complications; the first and more importantly, they are both chronic treatments[3,4]. For this reason, Finasteride over time can produce: depression, gynecomastia, hepatic enzymes elevation, cholesterol elevation, sexual dysfunction, impotence, decreased libido and mood disorder[31]. On the other hand, the harmful effects of Minoxidil include: contact dermatitis, facial hypertrichosis and transitory hair loss[3].

An enormous economic and human effort is being done trying to find new strategies and treatments for AGA, and a promising one is the Platelet-rich plasma (PRP)[3,32]. PRP consists of an autologous preparation of concentrated plasma with a threefold to eightfold increase in platelet number, ~1.500.000/µL, compared to normal plasma[33-35], which is extensively used to promote soft tissue healing. PRP started to be used in the 70s for its healing properties for cutaneous ulcers[32]. Moreover, it has been utilized in other specialties like traumatology, plastic surgery and maxillofacial surgery. In dermatology its use is extended to pathologies like: cutaneous ulcers, alopecia areata, tensional alopecia, androgenetic alopecia, skin resurfacing, hair transplant and the scar treatment, to name a few[36-40]. It is considered as a natural source of growth-factors (GFs)[40], able to improve angiogenesis, stem cell recruitment[33], remodel the extracellular matrix, together with effects of cellular proliferation and differentiation[41]. The regenerative potential of PRP seems to depend on the levels of GFs liberated[42,43]. A schematic representation of all platelets granules potentially released upon its activation are shown in Figure 1, for example growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF) or interleukin (IL)-1.

PRP is produced using different methods; open or closed technique (this very last one thanks to the disposable kits), in order to produce a platelet cocktail taken out from plasma[45]. The PRP is initially obtained from 20 to 60mL of the patient's blood, followed by a centrifugation protocol in two stages. The first centrifugation separates the erythrocytes from the less heavy plasma, with a buffy coat in the interphase. The plasma and the buffy coat are aspirated and they are centrifuged at a high spin so that the platelets sediment. A plasma portion is eliminated and the platelet sediment is resuspended again with the remaining plasma[46]. When treating AGA, PRP is injected subcutaneously, intradermal, or in the interfollicular alopecia area[43,47]. A huge commercial offer exists to fabricate PRP[34,47-49], however, a consensus protocol does not exist for the PRP preparation. In the literature we encounter preparation methods that differ from centrifugation, cellular and plasmatic proteins components, platelet activation, and site of injection[50-56,57-63].

Anti-apoptotic effects of activated PRP have been suggested as one of the major contributing factors stimulating hair growth through the Bcl-2 protein and Akt signaling, prolonging dermal papilla cells survival during the hair cycle[60]. TGF-β1 is a known inmune celular system modulator and a cell growth regulator, which together with IGF-1 growth factor, stimulate cellular proliferation and inhibits apoptosis. In addition, the upregulation of FGF-7/b-catenin signaling pathways with the use of PRP suggest the stimulation of hair growth by inducing follicular stem cell differentiation[50,64]. In addition, the release of VEGF and PDGF, known to have angiogenic properties, increase the perifollicular vascular network as previous studies have demonstrated[57,58,60]. All of the platelet's growth factors have been reported to increase notably by the addition of the most used calcium chloride or the PRGF right before the PRP treatment application[35,58].

In this literature review, all the studies published have been systematically analyzed in a unique revision that had the objective to evaluate PRP as a treatment for AGA, and to propose a clinical trial with a reliable PRP obtaining protocol, called the "Ultimate Platelet Rich-Plasma extraction protocol UPRP)".

METHODS

Numerous studies have been conducted lately to assess the efficacy of PRP as a hair restoration treatment option, but the question whether is effective for AGA or not remains unsolved. Thus, a systematic

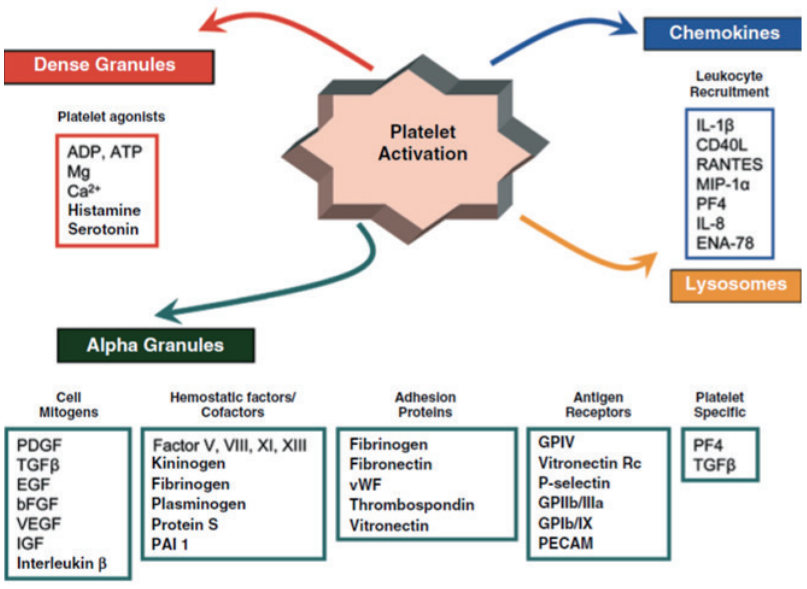

Figure 1 Platelet components[44].

review in June 2017 has been conducted in order to gather all the evidence by searching through PubMed and Cochrane databases, using the keywords "Alopecia AND PRP". This search resulted with a total of 38 articles, which we excluded 11 review articles, 6 studies using PRP to treat other hair diseases, 3 expert opinions, 2 articles were off topic, 1 abstract, and 1 article was conducted only with animals, resulting in a total of 14 studies. We focused on the most recent studies, especially those that included placebo-controlled trial. These 14 studies were carefully evaluated, focusing on the PRP protocol used and the results obtained in hair restoration, all summarized in three tables grouped by: study outcomes, PRP protocol, and PRP application. The first 10 article's data were plotted to graphically show their hair restoration results.

SYSTEMATIC REVIEW

The latest study published by Alves and Grimalt in 2017 brought us an innovative approach on the AGA's treatment, by combining PRP with minoxidil or finasteride. In this study, they recruited 25 patients with AGA, 11 males and 13 females, and treat all of them with PRP on one side of the head and saline solution on the other half-side, in combination of finasteride or minoxidil solution as ongoing treatment. After randomization, thirteen patients were treated with 1mL 5% minoxidil solution twice daily and 12 patients were medicated with 1 mg/day of oral finasteride, for a total of 25 patients.

Results were assessed by TrichoScan analysis at 3 and 6 months after the last treatment dosage, resulting in a statistically significant of mean hair count (hairs/0.65 cm^2), hair density (1/cm^2), and terminal hair density (1/cm^2) in the PRP associated with medication group versus placebo after 6 months ($p < 0.05$; month 6 vs baseline, data not shown). The combination of PRP and 5% minoxidil group showed higher hair restoration than PRP and finasteride, resulting in an increase of the total hair count, total mean hair density, anagen and telogen percentages, and mean anagen/telogen ration ($p < 0.05$: PRP + minoxidil vs PRP + finasteride).

Biologically speaking, it makes sense to obtain a synergic effect by combining two therapies that potentiate both angiogenesis and hair buldge proliferation by two different mechanisms. Minoxidil and PRP together have shown higher restoration hair than the combination with Finasteride, opening new doors to AGA treatment in the future. However, we must take into account that the study was conducted with low grade alopecia in both men and women subjects recruited, and most of them were non-smokers, two aspects that may potentiate the therapeutic effects seen in this trial. As reported later on this review by Gentile *et al*, the effect of PRP therapy seems to decay over time, so future longer follow-up must be accomplished to see the most adequate therapy frequency.

Gentile *et al* also in 2017 made a very interesting approach to assess whether is important to activate the concentrated platelet plasma by comparing CPuntT Preparation System, a non-activated (A-PRP) system, to Arthrex Angel System or Regen Blood Cell Therapy, both being activated PRP (AA-PRP) systems. Proteins released from platelets were quantified before and after its activation with calcium, data shown in the following table.

Table 1 and figure 2 pretends to proof the non-similarity of secreted proteins with the C-PuntT system over the other two that needed previous activation. The amount of VEGF and PDGF-BB in the activated systems is significantly higher over the C-PunT system, and equal to the rest of parameters. IGF-1 was not measured with the C-PunT system that is why it is not represented.

This study shows an improvement in hair density with the C-PunT system of +64 ±3 hairs/cm^2 after 3 months of treatment compared to placebo and about +90 ± 6 hair/cm^2 with the Arthrex system after 6 months of treatment compared to baseline. On the other hand, the Regen system showed a reduction in hair density of -73 ± 24 hairs/cm^2 compared to baseline. The scalp histopathological examination under the microscope was conducted after two weeks of treatment with CPunT system, which showed an increase of epidermal thickness, number of follicles, ki67+ expression, and capillary density assessed by CD31 expression over placebo.

They conducted an interesting study to approach whether platelets needed to be activated, but many questions remain unanswered. The scalp histopathological evaluation was only assessed in the A-PRP treatment group, leaving the AA-PRP group microscopic effects out of the picture. Also, the A-PRP was assessed at 3 months after treatment and the AA-PRP after 6 months, showing an inconsistent analysis between the treatment groups. Finally, the number of patients included in both groups was 3 times higher for the A-PRP group, leaving only 3 patients for each AA-PRP treatment group, and

Table 1 Trichoscan analysis assessed by Alves and Grimalt.

	Placebo		PRP Treatment	
Hair Count (hairs/0.65 cm^2)	Mean	SD	Mean	SD
Finasteride (n = 11)	0.9	16.3	0.6	10.8
5% Minoxidil (n = 13)	3.7	14.5	9.8	26.9
p-value	0.400		0.011	
Hair Density (1/cm^2)	Mean	SD	Mean	SD
Finasteride (n = 11)	1.4	25.1	1.8	16.7
5% Minoxidil (n = 13)	5.1	23.9	12.3	34.2
p-value	0.283		0.010	
Terminal hair density (1/cm^2)	Mean	SD	Mean	SD
Finasteride (n = 11)	-1.9	28.3	3.9	15.2
5% Minoxidil (n = 13)	5.6	21.9	3.2	38.4
p-value	0.138		0.175	
Anagen Hair (%)	Mean	SD	Mean	SD
Finasteride (n = 11)	-2.1	15	-2	12.2
5% Minoxidil (n = 13)	1.5	13.8	5.5	19.7
p-value	0.078		0.002	
Telogen Hair (%)	Mean	SD	Mean	SD
Finasteride (n = 11)	2.1	15	1.8	12.2
5% Minoxidil (n = 13)	-1.7	14	-5.5	19.9
p-value	0.072		0.002	
Anagen/Telogen ratio(%)	Mean	SD	Mean	SD
Finasteride (n = 11)	-40.5	169.7	-6.6	155.7
5% Minoxidil (n = 13)	1.7	192.5	69.6	234
p-value	0.167		0.018	

In bold p-value < 0.05.

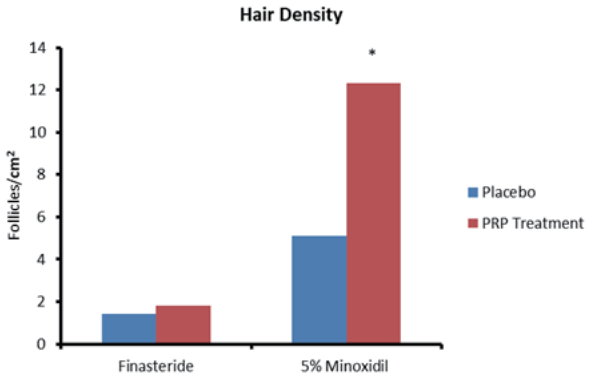

Figure 2 Hair density (hairs/cm^2) (*p-value < 0.05)[65].

placebo data was not shown in the AA-PRP group. The conclusion statement from Gentile *et al* was that PRP does not need to be activated because a greater increase in hair count and total hair density is found in the A-PRP group using the C-PuntT system over the AA-PRP. Although that statement is true when comparing CPunT system to Regen, it is not suitable when looking at the group treated with the Arthrex system, as we can see in the following graph.

Anitua *et al* developed a pilot study in which the use of PRP resulted positive for hair follicle regeneration. To do so, they took 19 patients with AGA, 13 males and 6 females, and treat them with five injections of PRP at months 1, 2, 3, 4 and 7, previously activated by the addition of platelet rich growth factor (PRGF). Compared to baseline, all outcome measures demonstrated a positive result after 5 sessions of PRP injections, assessed at 12 months when compared to baseline. The growth factors released after centrifugation were quantified by ELISA from the supernatant, and the results are described in table 2 and the PDGF, TGFB-1, and VEGF are graphed in figure 4 in order to visually compare to other articles' results.

Some of the results from Anitua *et al* are depicted in figure 5, where hair density, hair diameter and terminal/vellus hair are represented. We can see a statistically significant improvement ($p < 0.05$) in hair density, hair diameter and the ratio of terminal/vellus hair after 5 sessions of activated PRP at 12 months follow-up.

Histopathological examination also showed a statistically significant ($p < 0.05$) increase in epidermal thickness, basal keratinocytes proliferation (ki67+), perifollicular neoangiogenesis, and terminal/vellus hair ratio together with a decrease of the inflammatory perivascular infiltrate and an increase of bulge stem cell population after PRP treatment. On top of these objective positive results, 13 out of 19 patients referred to be satisfied with the results obtained.

Gupta *et al* conducted an open-labeled, not placebo-controlled pilot study with 30 male patients aged 20 to 50 years old. Each participant received 6 PRP treatments, administered 15 days apart, but their PRP treatment administration approach was unique from the other studies. After the scalp was activated by microneedling, PRP solution was massaged on the scalp. Hair density and diameter were measured at the vertex, 10cm from the glabella, by using TrichoScan analysis, photpgraphs analyzed by a blinded observer and self-assessment questionnaires. The results obtained showed an improvement of hair density and hair diameter detailed below, a 30.2 ± 12% average improvement in the photograph analysis, and a mean percentage improvement of 30 ± 13.1 on the basis of self-assessment evaluation. Interestingly enough, treatment response showed a higher efficacy in different clinical scenarios analyzed. Those with a lower grade of alopecia, subjects with shorter duration of disease before undergoing PRP therapy, and those subjects without a family history of alopecia benefit more from PRP therapy than the rest. The following tables summarize the results.

Alves and Grimalt conducted a randomized, placebo-controlled, double-blinded, half-headed clinical trial with 25 subjects. Subjects were grouped according to the half-head treated side; 3 mL of PRP on the right half of the head and 3mL of saline placebo on the left side. Results were assessed after three and six months, resulting in an increase of the mean anagen hair, mean telogen hair, hair density, and terminal hair density in PRP treated areas when compared with baseline. Mean total hair density at 3 and 6 months was the only result found to be statistically significant in the PRP treatment group when compared to placebo ($p < 0.05$).

Table 2 Growth factors and cytokines concentrations.

Protein	Collection System	A-PRP	Ca+2 AA-PRP
PDGF-BB (ng/mL)	Regen (AA-PRP) Athrex (AA-PRP) C-PunT (A-PRP)	1.2 ± 0.3 1.1 ± 0.6 1.8 ± 0.4	4.0 ± 2 3.0 ± 1 -
TGF-β1 (ng/mL)	Regen Athrex	11 ± 2 12 ± 1	15 ± 3 13 ± 0
IGF-1 (ng/mL)	Regen Athrex	130 ± 20 150 ± 40	140 ± 20 150 ± 60
VEGF (pg/mL)	Regen Athrex C-PunT	61 ± 20 61 ± 20 100 ± 20	210 ± 40 260 ± 70 -
FGF (pg/mL)	C-PunT	280 ± 60	-

Table 3 Growth factors and cytokines concentrations.

	Mean	SD
PDGF (ng/mL)	33	±10
TGFB-1 (ng/mL)	21	±12
VEGF (pg/mL)	218	±127
EGF (ng/mL)	826	±221
TSP-1 (µg/mL)	268	±58
Ang-1 (µg/mL)	392	±122

Ang-1: angiopoietin-1; EGF: epidermal growth factor; PDGF: platelet-derived growth factor; PRGF: plasma rich in growth factor; TGFb1: transforming growth factor b1.

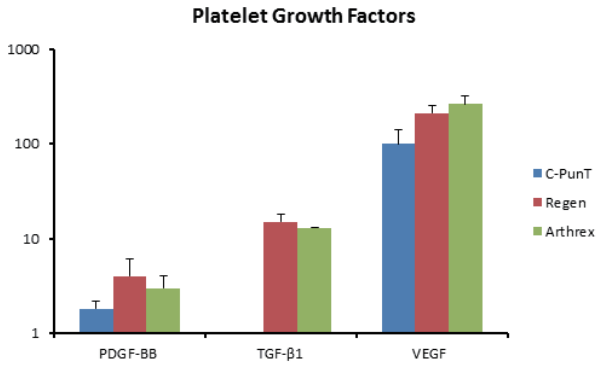

Figure 3 Platelet growth factors (Log scale10). (*p-value>0.05)

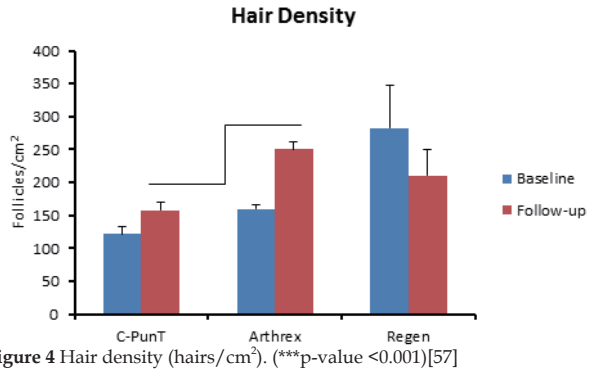

Figure 4 Hair density (hairs/cm^2). (***p-value <0.001)[57]

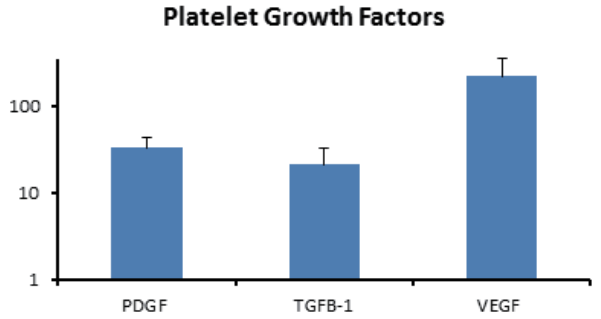

Figure 5 Platelet growth factors (Log Scale[10]).

Looking at the demographic characteristics of patients, treatment with PRP showed a statistically significant correlation between hair density and patients aged ≤ 40 years old, start hair loss ≥ 25 years old, a positive family history, and more than 10 years of AGA's appearance, when compared to placebo. This study also defends a positive correlation in PRP treated areas between anagen hairs (%) and patients aged more than 40 years old and beginning of AGA ≥ 25 years old, at 6 months.

Another similar randomized, placebo-controlled, double-blinded, half-headed clinical trial was done by Gentile *et al* with 23 male patients. PRP was injected in two out of four areas with apparent hair loss, and saline solution was injected into the other two remaining areas. The study found a statistically significant increase in many outcome measures; mean hair count, hair density and terminal hair density, after 3 months of PRP treatment compared to placebo. Specifically, a mean increase of 45.9 hairs/cm^2 in the treated area compared to 3.8 hairs/cm^2 in the placebo treated area after 3 months ($p < 0.0001$). The terminal hair density also improved significantly by 40.1 hairs/cm^2 with PRP over 5.6 hairs/cm^2 in the control area of the scalp ($p < 0.003$). Microscopic evaluation after 2 weeks from the last PRP treatment translated into a significant increase in the number of follicles, epidermal and hair follicular bulge cells, an increase of ki67+ basal keratinocytes, and an increase in small blood vessels around hair follicles in the treated skin area compared to baseline ($p < 0.05$).

The following table and graph summarizes the results obtained from this study.

At the microscopic evaluation after two weeks from the last PRP treatment, a statistically significant increase of epidermis thickness, number of follicles, ki67+ basal keratinocytes expression, and small blood vessels around hair follicles was demonstrated compared to placebo ($p < 0.05$). The clinical visual macroscopically effect of one patient's scalp treated with PRP is shown in the following image, demonstrating the potential therapeutic effect of activated PRP. However, 4 patients reported progressive hair loss around 16 months after the last PRP treatment.

Cervelli *et al* performed a very similar study to Gentile's with 10 men, and found similar positive results after 3 months of PRP treatment. All outcome measures showed statistically significant improvement ($p < 0.05$), compared to placebo. All data is represented in the following table and graph.

Microscopic analysis revealed that epidermal thickness, density of follicles, ki67+ expression, and hair follicle bulge cells were also increased compared to baseline ($p < 0.05$), suggesting an increase of keratinocyte and hair follicle proliferation as well as angiogenesis promotion by the PRP growth factors released during the treatment.

Singhal *et al* conducted a small not placebo-controlled study to check PRP treatment efficacy in 20 subjects; 8 males and 2 females, by using the Hair Pull test and photographs before and after treatment. Hair growth was observed in 6 subjects after just 7 days of treatment, and in 4 subjects after 15 days. By the end of the third month of PRP treatment, the Hair Pull test and the macroscopically evaluation showed superior outcomes, by showing a 65% reduction of hairs pulled and a higher hair density. No statistical analysis was reported on the observed data[61].

On the other hand, there are two studies that have reported no improvement in hair repair after PRP treatment. Mapar *et al* conducted a single-blinded, randomized, placebo-controlled pilot study with 17 male subjects. They underwent a similar protocol to the one used by Alves and Grimalt, in which the same patient had their scalp divided in two square-shaped regions of 2.5 × 2.5 cm in size, at least 3 cm apart from each other and their corners were marked with a tattoo. A volume of 1.5 mL of saline solution and PRP treatment was injected in patients' scalp in 2 consecutive sessions, one month apart. Results were assessed after three and six months, showing no differences in terminal hair increase or vellus hair decrease between the PRP treated

Table 4 TrichoScope ASG analysis assessed by Anitua (In bold *p*-value < 0.05)

	PRP Treatment	
Hair Density(follicles/cm^2)	Mean	SD
Baseline	117	29
12 weeks	156	36
Hair Diameter (μm)	Mean	SD
Baseline	37	10
12 weeks	55	9
Terminal Hair Density (1/cm^2)	Mean	SD
Baseline	48	17
12 weeks	68	18
Vellus Hair Density (1/cm^2)	Mean	SD
Baseline	51	21
12 weeks	32	17

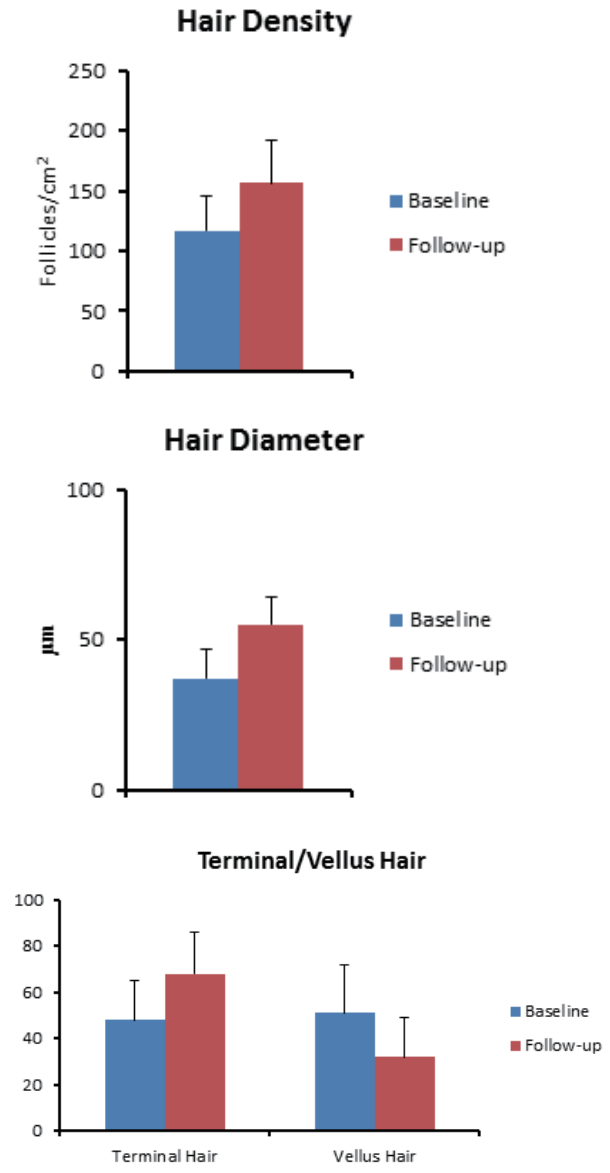

Figure 6 Results at 12 months follow-up. (*p-value < 0.05) [58].

areas compared to placebo neither compared to baseline in either group ($p > 0.05$).

Puig *et al* conducted a randomized, double blind, placebo-controlled trial with 26 women with female pattern hair loss. Women, at least 18 years old with a hair loss at a stage of Ludwig II, were randomized assigned into the PRP treatment or placebo group. Investigators marked a 4-cm^2 hair check data box in the central scalp at an exact distance from the glabella, where Cohen hair check system was used to analyze hair parameters. PRP was obtained from 60 mL of blood sample, using the non-activated Angel centrifuge system. Subjects either received a single 10 mL injection of PRP or normal saline solution at the previously marked area, and the results were analyzed after 26 weeks (4 months). No statistically significant differences were found in patient's hair count or hair mass between the PRP treatments and the control group ($p = 0.503$ and $p = 0.220$, respectively). However, 26.7% of patients treated with PRP reported coarser hair after treatment compared to a 18.2% in the placebo group, and 13.3% of the treated patients reported a substantial improvement in hair loss and hair thickness compared to a 0% in the placebo group[63].

Tables 5, 6, 7 and 8 TrichoScan analysis, and subgroups analysis by alopecia grade, duration of alopecia, and family history assessed by Gupta.

	PRP Treatment	
Hair Diameter (mm)	**Mean**	**SD**
Baseline	0.055	0.015
3 months	0.072	0.017
6 month	0.075	0.019
% Change	39.85	17.21
Hair Density (1/10 mm^2)	Mean	SD
Baseline	6.13	1.72
3 months	7.67	1.88
6 month	8.43	2.06
% Change	39.85	16.54
	6 Months Increase (Mean ± SD)	
Androgenetic Alopecia Grade	**Hair Density (1/10 mm^2)**	**Hair Diameter (mm)**
3	3 ± 0	0.025 ± 0.007
4	3.5 ± 0.71	0.025 ± 0.007
5	2.33 ± 0.58	0.03 ± 0.01
6	1.73 ± 0.47	0.015 ± 0.007
p-value	0.044	0.019
	6 Months Increase (Mean ± SD)	
Duration of Alopecia	**Hair Density (1/10 mm^2)**	**Hair Diameter (mm)**
Up to 5 years	2.68 ± 1	0.025 ± 0.007
6-10 years	1.6 ± 0.52	0.025 ± 0.007
> 10 years	2.0 ± 0	0.03 ± 0.01
p-value	0.048	0.009
	6 Months Increase (Mean ± SD)	
Family History	**Hair Density (1/10 mm^2)**	**Hair Diameter (mm)**
Present	2.15 ± 0.97	0.019 ± 0.007
Absent	3.25 ± 0.5	0.03 ± 0.008
p-value	0.011	0.027

The other older studies conducted by Schiavone *et al*, Takikawa *et al*, Khatu *et al*, and Gkini *et al* are summarized in Tables 1 to 3[45,47,54,55].

PRP treatment has been consistently shown to be effective at the microscopic level, by measuring an increase in the epidermal thickness, basal keratinocytes proliferation (ki67+), and perifollicular neoangiogenesis after 2 to 4 weeks after treatment. The question of how long this effect may last has not been checked by any of the studies analyzed, understanding the obvious inconvenience of the biopsy of the hair scalp for the patient. In order to approach the real effect of the PRP therapy compared to the biological effect from the wound itself made by the needle (recently proven to stimulate epidermal and hair follicle stem cells differentiation[66]), we merged all the published data to date. We are aware of the differences encountered in the studies analyzed, and thus, we have only used hair density data from Trichoscan analysis and placebo-controlled trials, data shown in the following table.

Despite the differences from all the studies analyzed, PRP has shown a statistically significant difference when compared to placebo at 3 months, showing an increase of 30 hair follicles/cm^2. Thus, we can conclude that PRP seems a very promising therapy for AGA, and will bring patients better results once we have it standardized and maybe combined with other approved therapies, such as 5% minoxidil.

Future studies will need to be consistent with the PRP protocol used, use half-head system as placebo, include more patients, analyzed data with Trichoscan, gather the subjective benefit after treatment, and undergo a longer follow-up in order to assess the real efficacy of PRP and approach the best posology for this promising therapy.

DISCUSSION

Many dermatologists worldwide use PRP with the idea of helping patients affected by a hair loss problem, but the assessment of the real efficacy obtained with this treatment, either for the doctor or the patient, is not an easy task. The idea behind this article is to evaluate the different approximations to obtain PRP with scientific evidence, and develop a meticulous study method that will allow us to recognize what is the real effect of the PRP on treating AGA.

According to this review, there is not a consensus protocol in any of the studies reviewed. In many occasions, dermatologists apply what the manufacturer from the PRP isolation machinery suggests as the standard protocol. The exact concentration, dose, deepness of the dosage, frequency of injections or the number of PRP sessions needed to be effective in AGA differ significantly and are not always

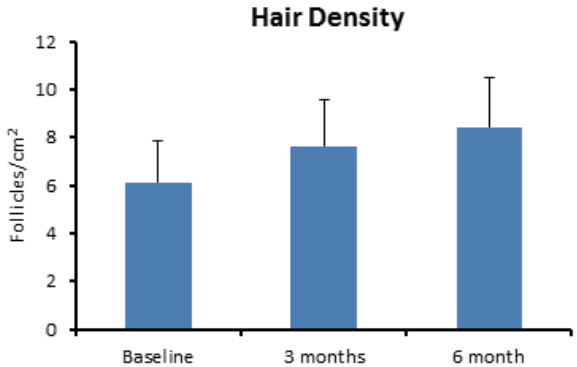

Figure 7 Results at 12 months follow-up. (*p-value <0.05)[59].

specified. However, some common steps exist between the studies analyzed to obtain PRP, which are detailed below.

*Platelet-Rich-Plasma Extraction Common Steps: (1) Peripheral blood is withdrawn from the same patient and transferred it into a tube with 3.8% sodium citrate. (2) The blood obtained is centrifuged at no more than 1.200 rpm. The centrifugation separates the sample into 3 visually recognizable layers; a top yellow layer (plasma), a thin layer in the middle called the buffy coat (white blood cells and platelets), and red blood cells (RBCs) in the bottom layer. (3) The plasma and the top part of the buffy coat (leukocytes rich) is discarded and the remaining buffy coat layer and a fraction of the RBCs are transferred into another tube. (4) Some authors undergo a second spin to achieve a more concentrated solution. (5) The upper ¾ of the supernatant is discarded from the tube. (6) The pellet obtained is homogenized with the remaining fluid and the PRP solution is finished. (6) In many cases, an activator such as calcium is added to the PRP in order to "activate" the platelets. (7) The PRP is injected with a 30-G needle into hair depleted areas.

A standardized method to evaluate the results obtained with the PRP does not exist either, very few patients are included in the studies described or the number between groups are not homogenous, and many others lack proper controls to evaluate results objectively. The main problem was the absence of a standardized, objective, reliable and no invasive method to evaluate AGA's hair loss, as well as the results after using PRP treatment. For these numerous reasons, we would like to propose a methodology for future studies, so that the effectiveness of PRP in AGA can be assessed properly and recommend patients PRP treatment with proper clinical evidence.

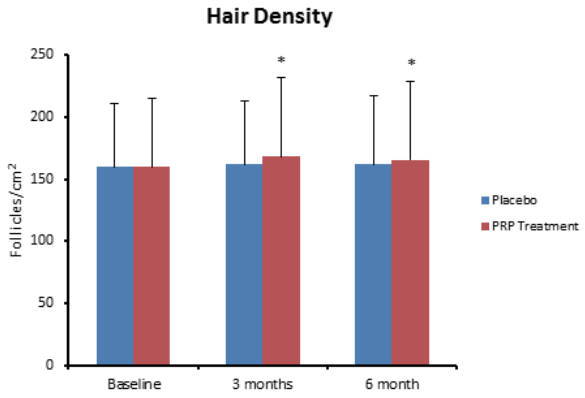

Figure 8 Results at 12 months follow-up.(*p-value < 0.05)[35].

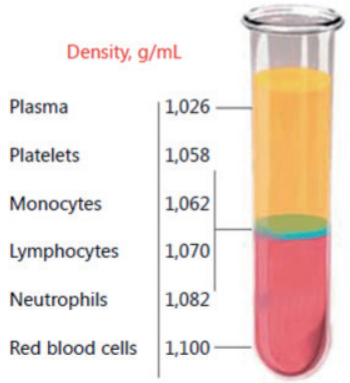

Figure 13 Blood Components[64].

Table 9 Trichoscan analysis assessed by Alves and Grimalt.

	Placebo			PRP Treatment			Placebo *vs* PRP
Anagen Hair (%)	**Mean**	**SD**	***p*-value**	**Mean**	**SD**	***p*-value**	***p*-value**
Baseline	62.1	17.4	> 0.05	62.1	16.1	> 0.05	> 0.05
3 months	63.5	18.9	> 0.05	67.9	13.8	< 0.05	> 0.05
6 month	66.3	15.9	< 0.05	67.6	13.1	< 0.05	> 0.05
Telogen Hair (%)	Mean	SD	*p*-value	Mean	SD	*p*-value	*p*-value
Baseline	37.9	17.4	> 0.05	37.9	16.1	> 0.05	> 0.05
3 months	34.5	16.5	> 0.05	35.8	14.4	< 0.05	> 0.05
6 month	33.7	15.9	< 0.05	32.4	13.1	< 0.05	> 0.05
Anagen/Telogen ratio(%)	Mean	SD	*p*-value	Mean	SD	*p*-value	*p*-value
Baseline	137.5	209.5	> 0.05	128.4	174.8	> 0.05	> 0.05
3 months	159.1	210.1	> 0.05	185.7	199.9	< 0.05	> 0.05
6 month	148.2	173.1	> 0.05	156	164.3	< 0.05	> 0.05
Hair Density ($1/cm^2$)	Mean	SD	*p*-value	Mean	SD	*p*-value	*p*-value
Baseline	167.8	51.2	> 0.05	167.1	55.6	> 0.05	> 0.05
3 months	167.1	51.3	> 0.05	181.9	63.6	< 0.05	< 0.05
6 month	165.7	55.2	> 0.05	179.9	62.7	< 0.05	< 0.05
Terminal hair density ($1/cm^2$)	Mean	SD	*p*-value	Mean	SD	*p*-value	*p*-value
Baseline	160	48.9	> 0.05	159.9	55.1	> 0.05	> 0.05
3 months	161.9	52.2	> 0.05	168.2	60.7	> 0.05	> 0.05
6 month	161.9	52.2	> 0.05	165.8	56.8	< 0.05	> 0.05
Hair Count (hairs/0.65 cm^2)	Mean	SD	*p*-value	Mean	SD	*p*-value	*p*-value
Baseline	111	33.9	> 0.05	110.8	37.6	> 0.05	> 0.05
3 months	112	35.7	> 0.05	115	41.8	> 0.05	> 0.05
6 month	112.7	34.9	> 0.05	113.2	39.4	> 0.05	> 0.05

In bold p-value < 0.05.

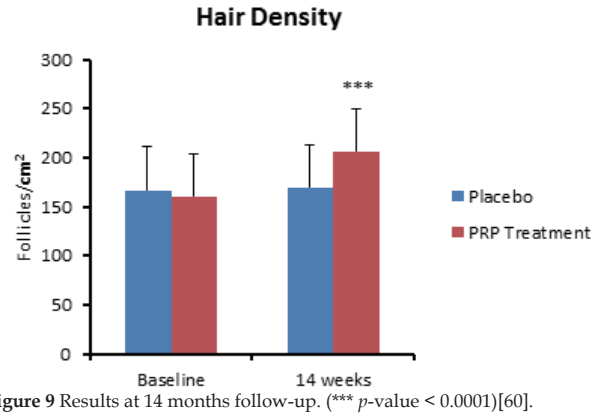

Figure 9 Results at 14 months follow-up. (*** *p*-value < 0.0001)[60].

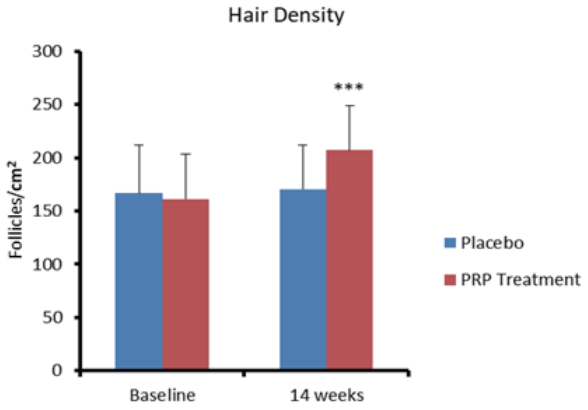

Figure 10 Results at 12 months follow-up. (*** *p*-value < 0.0001)[51].

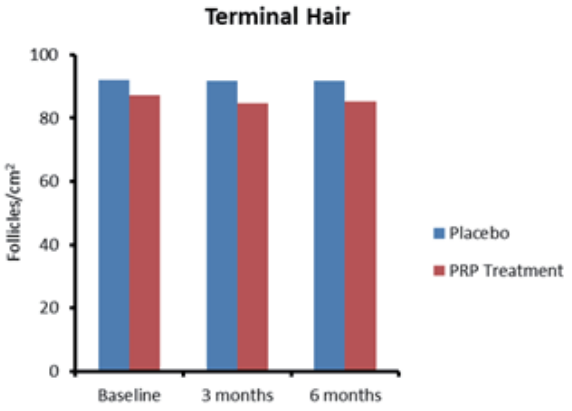

Figure 11 Results at 3 and 6 months follow-up (*p*-value > 0.05)[62].

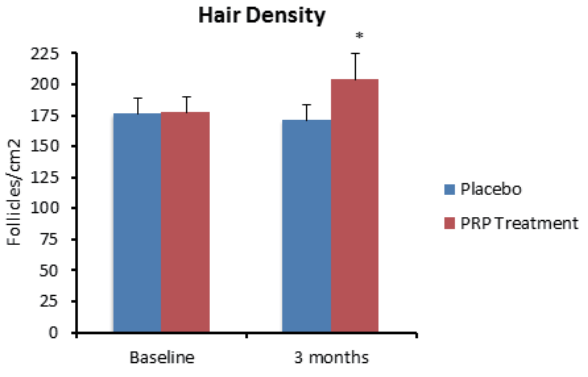

Figure 12 Results merged at 3 months follow-up (**p*-value < 0.05).

Table 10 Trichoscan analysis assessed by Gentile.

	Placebo		PRP Treatment	
Hair Count	**Mean**	**SD**	**Mean**	**SD**
Baseline	91.1	22.4	87.9	20.5
14 weeks	89.6	20.9	123.2	33.7
Hair Density(follicles/cm^2)	Mean	SD	Mean	SD
Baseline	166.5	45.6	161.2	41.9
14 weeks	170.3	42.1	207	56.3
Terminal Hair Density (1/cm^2)	Mean	SD	Mean	SD
Baseline	154.5	40.4	149.1	43.1
14 weeks	148.9	41.1	189.2	46.6
Vellus Hair Density (1/cm^2)	Mean	SD	Mean	SD
Baseline	16.9	11.6	16.6	8.9
14 weeks	17.9	13.8	18.5	8.7

In bold *p*-value < 0.0001.

Table 11 Trichoscan analysis assessed by Cerevelli.

	Placebo		PRP Treatment	
Hair Count (units)	**Mean**	**SD**	**Mean**	**SD**
Baseline	111.3	28.9	103.6	30.9
12 weeks	109.3	28.2	121.6	34.1
Hair Density(follicles/cm^2)	Mean	SD	Mean	SD
Baseline	171.2	44.4	159.4	47.6
12 weeks	168.1	43.3	187.1	52.5
Terminal Hair Density (1/cm^2)	Mean	SD	Mean	SD
Baseline	152.7	39.7	142.7	41.8
12 weeks	150.6	41.7	169.8	47
Vellus Hair Density (1/cm^2)	Mean	SD	Mean	SD
Baseline	16.9	10.4	14.8	9.7
12 weeks	17.4	13.9	15.8	8.5

In bold *p*-value < 0.0001.

Table 12 Magnifying glass analysis assessed by Mapar.

	Placebo	PRP Treatment
Terminal Hair Density (1/cm^2)	Mean	Mean
Baseline	92	87.29
3 months	91.65	84.65
6 months	91.76	85.06
Vellus Hair Density (1/cm^2)	Mean	Mean
Baseline	41.82	43.35
3 months	40.32	42.82
6 months	40.18	41.76

p-value > 0.05.

Table 13 Average hair density from placebo-controlled trials.

	Hair Density	Placebo		PRP Treatment	
	(follicles/cm^2)	Mean	SD	Mean	SD
Alves and Grimalt 2017	Baseline	150.8	46.1	149.5	42
	3 months	153.3	42.2	160.5	47.1
Alves and Grimalt 2016	Baseline	167.8	51.2	167.1	55.6
	3 months	167.1	51.3	181.9	63.6
Gentile 2017 (A-PRP)	Baseline	225	15	218	17
	3 months	227	16	282	20
Gentile 2015	Baseline	166.5	45.6	161.2	41.9
	3 months	170.3	42.1	207	56.3
Cerevelli 2014	Baseline	171.2	44.4	159.4	47.6
	3 months	168.1	43.3	187.1	52.5
Average		Mean	SEM	Mean	SEM
	Baseline	176.26	12.6	171.04	12.1
	3 months	177.16	12.8	203.7	20.9

Results from Trichoscan analysis (In bold *p*-value <0.05).

Supplementary table 1 Study outcomes.

	Study Design					Treatment Outcomes Measured ($p < 0.05$)										
Study	Placebo Controlled	Randomized	Blinded	Half-head	n (M/F) ♂: Norwood Class ♀: Ludwig Class	Hair density (follicles/cm^2)	Hair Diameter (µm)	Hair Count (units)	Anagen Hairs (%) or Terminal (hairs/cm^2)	Telogen Hairs (%) or Vellus (hairs/cm^2)	Epidermal Thickness (µm)	New Blood Vessels (1/mm^2)	Ki67 + (cells/mm^2)	Adverse Efects	Subjective Assessment	Objective Assessment
1. Alves and Grimalt (2017)	Y	Y	Y	Y	24 (11/13) Aged ♂:II-V ♀:I-III	Y	-	Y	Y*	Y*	-	-	-	Not present.	Not described.	Not described.
2. Gentile et al (2017)	Y	Y	Y (3)	N	24 (24/0) Aged 19-63 ♂: II-IV	Y	-	Y	Y	Y	Y	Y	Y	Not described.	Not described.	Not described.
3. Anitua et al. (2017)	N	N	Y (1)	N	19 (13/6) Aged 27-60 ♂: III-VI ♀: II	Y	Y	Y	Y	Y	Y	Y	Y	Erythema and local edema, not present after 24 hours.	Likert scale from patients: 7 very statisfied, 6 satisfied, 5 indifferent, 1 unsatisfied, 0=very unsatisfied.	Clinical evaluation before and after 12 months of treatment were scored in a 3 category scale: 0 (masked evauators found no differences) -1 (if they failed to recognize each image) +1 (if the masked evaluators succeeded in recognizing each image)
4. Alves and Grimalt (2016)	Y	Y	Y(2)	Y	25 (12/13) Aged 18-65 ♂: II-V ♀: I-III	Y*	N	N	N	N	-	-	-	Local injection pain.	Not described.	Not described.
5. Gentile et al. (2015)	Y	Y	Y (1)	Y	23(23/0) Aged 19-63 ♂: IIa-IV	Y	-	Y	Y	Y	Y	Y	Y	Not present.	Physician's and patient's global assessment scale, results not reported.	
6. Cerevelli et al (2014)	Y	Y	Y	Y	10 (10/0) Aged 20 to 52 ♂: IIa-IV	Y	-	Y	Y	-	Y	Y	Y	Not present.	Physician's and patient's global assessment scale, results not reported.	
7. Shingai et al (2016)	N	N	N	N	10 (8/2) Aged 25-35 ♂: I-IV ♀: I-II	Y	-	Y	-	-	-	-	-	Mild headache. No inflammation nor infection.	Not described.	Not described.
8. Puig et al (2016)	Y	Y	Y	N	26 (0/26) ♀:II	-	-	Y	-	-	-	-	-		Not described.	
9. Mapar et al (2016)	Y	Y	Y (1)	Y	17(17/0) Aged 24-45 ♂:IV-VI	-	-	-	Y	Y	-	-	-	Not present.	Not described.	Not described.

10. Puig et al (2016)	Y	Y	Y	N	26 (0/26) >18 years old ♀:II	-	-	Y	-	-	-	-	-	Not present.	13,3% of the treatment subjects claimed to have experienced substantial improvement in hair loss, rate of hair loss, and hair thickness.	Not described.
11. Schiavone et al (2014)	N	-	N	N	64 (42/22) Aged 28-32 ♂:II-V ♀:I-II	-	Y	Y	-	-	-	-	-	Not present.	Not described.	Not described.
12. Gkini et al(2014)	N	N	N	N	20 (18/2) Aged 24-72 ♂:II-Va ♀:I	Y	-	-	-	-	-	-	-	Not present.	Patient Self-assessment Questionnaire	Not described.
13. Khatu et al (2014)	N	N	N	N	11 (11/0) Aged 20-40 ♂:II-IV	-	-	Y	-	-	-	-	-	Not present.	Patient Self-assessment Questionnaire	Not described.
14. Takikawa et al (2011)	Y	-	N	Y	26 (16/10) Aged 28-59 Thin hair	-	-	Y	-	-	Y	Y	-	Not present.	Not described.	No+A12:Q16t described.

No placebo-controlled studies are compared to baseline, if they use placebo, they are compared to placebo.
Numbers 1,2,3 means single-blinded (1), double-blinded (2), triple-blinded (3).
1. *Only in the combination of PRP + Minoxidil
2. AP results from A-PRP only. Hair density and hair count results from Arthrex compared to baselines and C-PunT system comapred to placebo.
5. Differences Alves at 3 and 6 months. Y* <40 years and AGA >25 and N* Age>40 and AGA>25 when compared to placebo.

Supplementary table 2 PRP protocol

Study	Blood Volume (mL)	Activators	Force (g)	Time (min)	Force (g)	Time (min)	Platelet Enrichment (PRP/Plasma)	Other Details
1. Alves and Grimalt (2017)	20	0.15mL of 10% Calcium chloride	460	8	Not performed	-	3	3/4 of the supernatant is discarded (platelet-poor-plasma, PPP) and the resulting solution is used as PRP. Hair count (# of hairs/0,65 cm^2)
2. Gentile et al. (2017)	a. 55 b. 24 c. 120	a. None b and c. 10% calcium gluconate	1200 rpm	10	Not performed	-	5	The top 2mL after centrifugation were discarded. > 40 µm included as terminal hairs. Results not shown.
3. Anitua et al. (2017)	18	PRGF (BTI Biotechnology Institute)	580	8	Not performed	-	2.08	2 cm^3 platelet-rich plasma was gathered, right above the leukocyte buffy coat. 40-60 µm is thin hair >80 µm thick hair
4. Gupta et al (2017)	-	-	-	-	-	-	Not Reported	
5. Alves and Grimalt (2016)	20	0.15mL of 10% Calcium chloride	460	8	Not performed	-	3	3/4 of the supernatant is discarded (platelet-poor-plasma, PPP) and the resulting solution is used as PRP. Hair count (# of hairs/0.65 cm^2)
6. Gentile et al.(2015)	a. 18mL b. 60mL	Ca+2	1100 rpm	10	1200 rpm	10	About 5 (1.48M platelets)	Two-step centrifugation to obtain a platelet pellet that may include leukocytes, a was activated with calcium and b selected by the CPunT system with a light selector. > 40 µm terminal hairs
7. Cerevelli et al (2014)	18 cc	Ca+2	1100	10	Not performed	-	Not Reported	
8. Shingal et al (2016)	20 mL	10 % Calcium chloride	1500 rpm	6	2500 rpm	15	Not Reported	Intermediate PRP rich layer (5% of total volume) was collected with a Finn pipette in another test tube. After the second centrifugation, the PRP poor top layer was discarded.
9. Mapar et al (2016)	9 ml (Plus 1mL of acidic citrate dextrose)	Calcium gluconate (0.1ml per mL of PRP)	3000 rpm	6	3300	3	X 3	
10. Puig et al (2016)	60 ml	No	-	-	-	-	X 2.75 - 3.4	Angel system for PRP obtention
11. Schiavone et al (2014)	a. 60 mL b. 40 mL	No (Scalproller used to favor platelet activation)	-	-	-	-	a. X 6-7 b. X 4 with addition of plasmatic protein concentrate	
12. Gkini et al(2014)	16 ml	Calcium gluconate (0.1 mL per 0.9 ml of PRP; 1:9 ratio)	1500 x g	5	1500	5 min	x 5.8	
13. Khatu et al (2014)	20 ml	Calcium chloride (1:9 ratio)	1500 rpm	6	2500 rpm	15	Not Reported	
14. Takikawa et al (2011)	20 ml	-	1700 rpm	15	3000 rpm	5	Not Reported	

METHODS PROPOSAL

We belief that a randomized, placebo-controlled, double-blinded, half-head study would be the best way to objectively assess the real efficacy of PRP. To better clarify the use of PRP activation, we propose to undergo a two-arm study; in which half of the patients will be randomized into a group receiving either activated PRP with calcium and saline solution in the other half of the head, and the other half will receive PRP without being activated and saline solution in the other half. Only by comparing the micro-trauma done by the injection of saline solution, we will be able to determine whether the therapeutic effects come from the "needle effect" or the PRP itself. All data will need to be processed by Trichoscan analysis, photographs and subjective questionnaires before and after treatment. The other question that remains unanswered is whether the PRP activation is necessary and which technology and protocol steps we need to do to obtain the best quality PRP. A longer follow-up, about 18 months after stopping the treatment, may be of interest in order to discover how long the effect of the PRP therapy lasts[60].

From our own experience, mixed together with recent evidence from this systematic review, we propose the following protocol that might change in the future if new scientific evidence demonstrated other results.

STATEMENT OF ETHICS

We ensure the quality and integrity of our work, in which the entire scientific literature has been analyzed thoroughly and all the papers studying PRP's efficacy were considered in the same manner. No patients were used to write this paper, and thus, no informed consent or money of any sort was needed to conduct this research.

Supplementary table 3 PRP application.

Study	PRP Volume Injection	PRP treatments	Intervals b/w treatments	Follow-up	PRP Application
1. Alves and Grimalt (2017)	4mL			3 and 6 Months	Injected intradermally into 4 selected areas (2 frontal and 2 occipital, marked with a central dot tattoo), at 0.15 to 0.20 mL/cm^2 using a 30 G needle. No anesthesia used. Placebo: 4mL of saline solution at the other half-head
2. Gentile et al. (2017)	a. 9mL b. 9mL c. 8mL	3	1 Month	a. 3 Months b/c. 6 Months	a. Injected intradermally at 5mm depth using a Ultim Gun into 4 selected areas (frontal, pariteal, vertex, and occipital), cleansed with 70% alcohol, at 0.2 mL/cm^2 using 30G needles. No anesthesia used. Placebo: Saline solution at the region not treated (control). Example: Frontal vs parietal or parietal vs vertex (PRP vs placebo). Same number of injections were made in each group. b. Same as above with a Luer-lock syringe with 25G needle.
3. Anitua et al (2017)	3 to 4 mL	5	1 Month (booster at 7 months after the start point)	12 Months	3 to 4 cm3 of freshly activated PRGF was injected intradermally into the hair-depleted areas of the patient's scalp using 30 G needles. For men: 3 transitional areas of hair loss were defined for the phototrichogram analysis: intersection at 12 cm from the left/right eyebrow and 10 cm from the left/right ear, respectively, and 15 cm from the forehead (vertex area). For women, 3 aligned transitional areas involving mild scalp and crown region were defined.
4. Gupta et al (2017)	-	6	15 days	6 months	Scalp was activated by microneedling, then PRP was massaged into the vertex of the scalp (10cm from the glabella).
5. Alves and Grimalt (2016)	3mL	3	1 Month	3 and 6 Months	Injected intradermally into 4 selected areas (2 frontal and 2 occipital, marked with a tattoo), at 0.15 mL/cm^2 using a 30G needle. No anesthesia used. Placebo: 3mL of saline solution at the other half-head
6. Gentile et al. (2015)	a. 9mL b. 20mL-->9mL (CPunT)	3	1 Month	2 Years (Baseline, 2, 6, 12, 16, and 24 months post first treatment)	Injected inerfollicularly using Luer-lock syringe within 2 of the 4 selected areas of the scalp (physiological solution into the other 2 areas) after the scalp was cleansed with 70% alcohol, at 0.1mL/cm^2 using 30G needles. No anesthesia was used.
7. Cerevelli et al (2014)	9mL	3	1 Month	12 Months (Baseline, 14 weeks, 6 months, and 12 months)	Intradermal injections at 0.1mL/cm^2 into two of the 4 selected halves (frontal or parietal), placebo was injected into the other 2 halves after the scalp was cleansed with 70% alcohol. Local anesthesia was not used.
8. Shingal et al (2016)	8-12mL	4	2 weeks	3 Months	Injections using nappage technique (multiple small injections in linear pattern 1cm apart) with an insulin syringe, after area was cleansed with ethanol and povidone-iodine
9. Mapar et al (2016)	1.5 ml	2	1 month	6 months (at 1,3 and 6 months post initial treatment)	Injections (1.5mL of PRP) within one of two 2.5cm x 2.5cm square regions, at least 3cm apart, in the scalp randomly assigned to be a case square (control square received 1.5 mL of normal saline). Randomization of case and control squares was performed using a random number table. Iron oxide and titanium dioxide containing substances were used to tattoo the corners of the squares.
10. Puig et al (2016)	10 ml	1	N/a	26 weeks (at 4 week intervals)	Single subcutaneous injection within the 4 cm^2 area in the central scalp (termed the "hair check data box"), after anesthesia (2% lidocaine and 0.5% bupivacaine) was administered
11. Schiavone et al (2014)	a. 6-8 mL PRP + 3-4 mL of plasmatic protein concentrate= 9-12mL; 0.2-0.3 mL per injection b. same as above	2	3 months	6 months	After local anesthesia (xylocaine 1%, with adrenaline 1:100,000) was administered, cutaneous inflammation was induced via application of gentle pressure using 1.0mm deep Scalproller to favor activation of injected platelets. Then, superficial injections were administered 1cm apart.
12. Gkini et al(2014)	6 ml (0.05-0.1 mL/cm^2)	3 (+1booster)	21 days (booster 6 months after onset)	1 year	Injections (0.05-0.1mL/cm^2) were performed using nappage technique in affected areas to a depth of 1.5-2.5 mm. A specific area was checked at all times by defining a 'V' (Kang's point) as proposed by Lee.
13. Khatu et al (2014)	2 - 3 ml	4	2 weeks	12 weeks	Nappage technique injections (2-3 cc) into a prefixed 1cm squared area over the right parietal area. Anesthetic cream was applied before each treatment after cleaning the skin with cetavlon, spirit and povidone-iodine.
14. Takikawa et al (2011)	3 ml	5	2-3 weeks	12 weeks	Subcutaneous injection (3mL) into selected 1cm x 1cm areas measured from the nasal tip and upper part of the auricular base.

DISCLOSURE STATEMENT

In accordance with Universitat Internacional de Catalunya policies and procedures, and our ethical obligation as researchers, we are reporting that we do not have financial and/or business interests that may be affected by the research reported in the enclosed paper.

Our interest in this publication is to bring a new perspective into the PRP's efficacy, in order to call for a more solid evidence before extending the use of *PRP into our* patients.

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