Platelet-rich plasma for androgenic alopecia treatment: A comprehensive review

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Abstract

Androgenic alopecia (AGA) is a chronic, progressive condition affecting millions of individuals worldwide. Treatment modalities for AGA are limited and our understanding of the pathophysiology underlying the disease is still developing. Platelet-rich plasma (PRP), an autologous collection of concentrated platelets and their respective growth factors, has demonstrated efficacy in limiting AGA. The current understanding of AGA pathogenesis is summarized, including our current understanding regarding androgens, inflammation, and arrector pili muscle loss. Furthermore, the molecular pathways induced by PRP in the context of AGA are discussed to ascertain how PRP can prevent disease progression. Well-designed studies investigating the effect of PRP on AGA patients to provide insight on how PRP should be used to achieve consistent clinical results are also presented. Future investigations should focus on elucidating a unifying theory to connect the currently disparate avenues of AGA pathogenesis. PRP clinical trials should be based on standardized treatment protocols to establish generalizable results.

Keywords: alopecia, androgenetic, androgenic, growth factors, hair loss, mechanism, pathogenesis, plasma, platelet, platelet-rich growth factors, platelet-rich fibrin, platelet-rich plasma, thinning

Introduction

Platelet-rich plasma (PRP) is an autologous source of growth factors derived from platelet sequestration and concentration via gradient density centrifugation. This treatment modality has recently gained popularity in the treatment of androgenic alopecia (AGA), [1].

Platelet-rich plasma was first clinically utilized by Ferrari et al. in 1987 [2]. They employed PRP in cardiac surgery to reduce intraoperative blood loss and subsequent use of blood products [2]. A decade later, Marx et al. described its efficacy in doubling mandibular bone graft maturation rates [3]. More importantly, at that time, their 1998 publication provided the most detailed protocol for PRP preparation [3].

Research regarding PRP has since expanded. It now includes a variety of topics including treatment of venous ulcers, pain reduction in split-thickness skin graft donor sites, burn healing after partial-thickness injury, and skin rejuvenation via dermal fibroblast activation [4-7]. Over the last decade, the use of PRP as a treatment modality for AGA has gained traction given its potential ability to induce and accelerate hair regrowth [8].

Current FDA-approved therapeutic measures for AGA, minoxidil, and finasteride, target vascular and hormonal facets of this condition, respectively. However, owing to the complexity of hair regrowth and maintenance and the continued prevalence of AGA, additional therapies for AGA are being explored. This manuscript will summarize the mechanism of action for platelet-rich plasma, describe its use in the treatment of AGA, and identify

potential opportunities to increase its efficacy in this setting.

Discussion

Method for Acquiring Data

A comprehensive PubMed search was conducted to identify papers published from 1950 to 2017. Search terms included androgenic alopecia, platelet-rich plasma, hair cycle, growth factors, histopathology, pathogenesis, mechanism, finasteride, minoxidil, and platelet-rich fibrin. Articles not related to platelet-rich plasma and androgenic alopecia were excluded.

Platelet-Rich Plasma

A widely accepted definition of PRP is currently pending. Marx et al. proposed that 1 million platelets per milliliter be used [9]. This was based on the results of their soft tissue healing experiments [9].

Whole blood, the source of PRP, consists of red blood cells in a plasma suspension, with platelet counts ranging from 150,000 to 350,000 per microliter. In contrast, PRP consists primarily of platelets, with concentrations ranging from three to five times as high as in whole blood, depending on the protocol used. Platelets house a myriad of growth factors including, but not limited to, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), and vascular endothelial growth factor (VEGF). The growth factors serve to promote angiogenesis, epithelialization, initiation of cell division, and macrophage attraction; hence, the expanded range of applications for which PRP has been used may, in part, be secondary to the accompanying growth factors that are present [10].

Preparation of PRP requires blood collection from the patient. This is followed by anti-coagulation and centrifugation at rates low enough to prevent shearing force induced rupture. Subsequently, plasma aspiration, re-centrifugation, and removal of supernatant is conducted [11].

Platelets are ordinarily inert in the absence of endothelial damage and subendothelial collagen exposure; therefore, activating agents such as calcium chloride ($CaCl_2$) are utilized to induce platelet degranulation. However, unlike platelet activation in the intravascular space, growth factor release from the platelet granules is only localized to the injection site without risk of dispersion secondary to blood flow. Also, in contrast to the setting in which PRP is used, in physiologic conditions, platelet activation secondary to basement membrane collagen-mediated hemostasis completes within one hour, limiting the window of action [12].

Activating factors such as $CaCl_2$ or $CaCl_2$ in conjunction with thrombin have been utilized to alter the release kinetics. In evaluating the time-based release of PDGF, TGF-β, and VEGF, collagen-induced PRP activation results in greater initial release, but decreased availability over the remainder of the platelet lifespan. In contrast, $CaCl_2$ use exhibited gradual release, with initially lower growth factor release, but higher levels at 24 hours [13]. Owing to the short half-life of growth factors (which is on the scale of hours) as compared to the timeframe required for hair growth, the latter method, which utilizes $CaCl_2$, is more applicable to AGA treatment [14]. **Table 1** lists growth factors present in PRP and their suspected mechanism in the treatment of AGA (**Table 1**), [15-30].

Physiologic Hair Cycle

Hair follicles are characterized by numerous parameters, including number, follicular distribution, and size; these traits are referred to as the hair architecture [31]. Three types of hairs can be identified: terminal, vellus, and intermediate hairs. Terminal hairs are characterized by hair bulbs located in the adipose tissue; they appear as long, well-pigmented hairs that are thicker than 0.06 mm [32]. In contrast, vellus hairs have hair bulbs located in the upper dermis and present as shorter, hypopigmented hairs that are thinner than 0.03 mm. Hairs which fail to meet the criteria for either terminal or vellus are classified as intermediate hairs [33]. Hair growth rates vary based on the bodily location; for the scalp, terminal hair in the anagen phase grows at 0.3 mm per day [32].

Hair follicles undergo growth and shedding in a cyclical process involving three key phases, which

Table 1. *Growth factors contained in PRP and their AGA-related functions*

Growth Factor	Function
EGF[a]	Cell growth modulator during follicular differentiation [20] Proliferation and migration of follicular outer root sheath cells [30]
FGF[b]	Anagen phase induction via B-catenin expression [25] Angiogenesis [19] Dermal fibroblast and hair follicle mitogen [20]
HGF[c]	Hair follicle elongation [27] Inhibits catagen phase induction [26]
IGF-1[d]	Hair follicle proliferation during development [28] Increase hair density and inhibit apoptosis [17, 28]
PDGF[e]	Angiogenesis and vascularization [16] Hair follicle dermal stem cell proliferation [22] Mesenchymal stem cell mitogen [15]
TGF-β[f]	Extracellular matrix synthesis [21] Fibroblast and mesenchymal stem cell proliferation [21] Hair folliculogenesis and maturation [23]
VEGF[g]	Elevated expression in dermal papilla cells during anagen phase [24] Endothelial cell-specific mitogen [29] Microvascular permeability and perifollicular vascularization [18]

Abbreviations: [a] EGF, epidermal growth factor; [b] FGF, fibroblast growth factor; [c] HGF, hepatocyte growth factor; [d] IGF-1, insulin-like growth factor-1; [e] PDGF, platelet-derived growth factor; [f] TGF-β, transforming growth factor-beta; [g] VEGF, vascular endothelial growth factor.

vary significantly in length: anagen, catagen, and telogen [31]. The anagen phase consists of hair follicle growth at various speeds, lasting two to six years to produce thick, well-pigmented and deep follicles. Subsequently, the catagen phase involves the initiation of apoptosis and shriveling of the follicular base, lasting one to two weeks [34]. Finally, the telogen phase has varying length, lasting up to 100 days, and allows for shaft shedding. Some investigators include a fourth exogen phase to specifically encompass hair shedding [35].

The distribution of hairs among these three phases is not equal. The majority (up to 90 percent) present in the anagen phase, followed by up to 15 percent in the telogen phase and 1 percent in the transitory catagen phase [31, 36]. More recently, a kenogen phase has been described to account for hairs with delayed entry into anagen phase following shedding [37].

Androgenic Alopecia

Androgenic alopecia is characterized by androgen-sensitive hair follicle miniaturization secondary to elevated levels of dihydrotestosterone (DHT), altered 5α-reductase activity, or over-expression of androgen receptors [38]. Inherited in a polygenic manner with variable penetration, estimates of prevalence have ranged from 30 percent to 80 percent — depending on patient age and race — with more significant hair loss in elderly white men [39, 40]. Various classification scales have been created to grade AGA with the Norwood-Hamilton and Ludwig scale used for males and females, respectively [41, 42]. Notably, the sparing of various hair regions even in higher degrees of AGA implies that hair follicles intrinsically have different sensitivities to androgens. Specifically, in males the vertex and frontotemporal scalp are particularly affected; in contrast, women are affected more diffusely in the mid-frontal region, lacking specific areas of total hair loss unless severe [42, 43].

At a cursory level, the pathophysiology of androgenic alopecia is secondary to androgen receptor activation, resulting in progressively shorter anagen phases [39]. Therefore, the distribution of hairs in the anagen, catagen, and telogen phase is shifted. This results in higher percentages in the telogen phase [31].

Histologically, there is progressive hair follicle miniaturization in androgenic alopecia. This is demonstrated by differing hair bulb depths and shaft diameters on biopsy specimens. One specific histopathologic finding that can be followed for androgenic alopecia includes streamers found deep

to the miniaturized follicle, which represents collapsed connective tissue [31]. Before the influence of androgens, this tissue is vascularized and lined by deep-seated terminal hair; over time, it becomes devascularized.

In addition, the miniaturized hairs in androgenic alopecia are often associated with perifollicular lymphocytic infiltration and subsequent fibrosis. Specifically, this potential inflammatory component of AGA is localized to the follicular bulge, the source of stem cells in the follicular unit [44]. These changes cause a shift in the ratio of terminal to vellus hairs, which is greater than 2:1 in the healthy scalp; in patients with AGA, the ratio is reduced or even reversed [43].

Androgenic Alopecia Pathogenesis

Research has been conducted to elucidate the underlying mechanisms of AGA and to identify plausible solutions. A unifying explanation connecting histologic, biochemical, and clinical findings has not yet been generated. However, our current understanding yields insight into why treatments are efficacious.

The most prominent and well-studied theory is the effect of androgens on hair follicles at the dermal papilla, a mesenchyme-derived population of specialized fibroblasts at the base of the hair follicle [45, 46]. Dihydrotestosterone (DHT), enzymatically produced from testosterone by type II 5-alpha reductase in the hair follicle, has been implicated as the key androgen involved in AGA [47]. For men and women, frontal hair follicles displayed higher levels of 5-alpha reductase and androgen receptors than in the occipital follicles; in contrast, aromatase levels were significantly higher in the occipital region [48].

Concordantly, saturation analysis using non-metabolizable androgens displayed that androgen receptor density in balding scalp was notably higher than in non-balding scalp at the dermal papilla cells [45]. However, androgen receptor content in frontal hair follicles in women has been noted to be 40 percent lower than in men [48]. Similarly, levels of 5-alpha reductase were three-fold higher for men in these regions [48]. These findings are in agreement with the typical clinical distribution of hair loss between genders, with men affected more in the frontotemporal regions.

These observations do not establish elevated serum androgens as the causative factor for AGA. However, studies investigating the effect of different levels of DHT on follicle growth help reach this conclusion. In comparing balding and non-balding scalp regions at baseline, DHT is significantly higher in the former, whereas testosterone levels are similar. In a year-long, randomized, double-blind, placebo-controlled trial with a sample of 1553 men with vertex-predominant AGA, daily oral finasteride treatment (1 milligram) yielded improvement in scalp hair count, verified by photographic and investigator assessment [47]. In this context, it is important to note that finasteride reduces DHT levels in the balding scalp to values similar to non-balding scalps at baseline within 28 days [49].

Other studies have also arrived at similar conclusions; however, they have all investigated only males with AGA [50-52]. Further supporting evidence for the role of DHT in AGA is the lack of hair loss in affected homozygotes with 5-alpha reductase deficiency [53]. Similarly, patients afflicted with androgen insensitivity syndrome lack a functional androgen receptor and fail to exhibit AGA [54].

Androgen receptor crosstalk with the Wnt/β-Catenin pathway regulates hair cycle activity via control of epidermal stem cell renewal and lineage selection [55]. Wnt/β-Catenin pathway activation in adult mouse epidermis induces stem cell expansion and differentiation towards the hair follicle lineage [56, 57]. However, androgen receptor binding results in decreased β-Catenin-induced gene transcription secondary to competitive binding of β-Catenin. As a result, β-catenin cannot bind transcription factors [56, 58-60]. With androgen receptor inhibition, the effects of β-Catenin activation become evident with increased hair follicle proliferation. This reciprocal relationship between these pathways has been similarly documented in human dermal papilla cells as well as hair follicle stem cells in both balding and non-balding scalps [61].

However, this one-dimensional view of AGA pathogenesis may need to be updated based on

recent findings, especially in the context of female pattern hair loss. In stark contrast to studies in men, when evaluating AGA in a year-long, randomized, double-blind, placebo-controlled trial with 137 post-menopausal women, a daily dose of 1 milligram finasteride showed no improvement in either scalp hair count, photographic analysis, or histologic analysis [62]. Owing to the age of the population studied, it must be considered that senescent hair thinning, which may not be androgen driven, could have confounded the results. Notably, baseline DHT levels in post-menopausal women were found to be similar to castrated men, bringing into question the impetus behind follicular miniaturization in this population [62, 63].

Furthermore, a case report — which described an androgen insensitivity syndrome patient who developed female pattern AGA — provides evidence that additional etiologies, besides androgens alone, are responsible for AGA. If the postulate that androgens alone induce and drive AGA, female pattern AGA should not be plausible in this woman since she lacks pubic and axillary hair, which implies a low probability of partial androgen receptor sensitivity [64]. Therefore, it is evident that other mechanisms than androgens alone may be driving the pathogenesis of AGA.

Chronic inflammation is suspected to contribute to AGA; this hypothesis is supported by the presence of perifollicular inflammation in this condition. Histopathologic studies have revealed mononuclear and lymphocytic cells in scalp samples of AGA men. Specifically, recent studies have localized the inflammatory infiltrate to the follicular bulge, a stem cell source for hair follicles [44, 65]. Furthermore, androgens could aid this process through sebum production, in which microbes favoring lipids as nutrients create inflammatory products [66]. Therefore, this is one potential explanation for the effector mechanism through which androgens cause AGA. However, the magnitude of this process and whether it is significant in the pathogenesis of AGA remains to be determined.

A second hypothesis of AGA involves the loss of the arrector pili muscle. Smooth muscle is involved in thermoregulation and sebum secretion; it also connects the hair follicle to the upper dermis and epidermis. Investigators have speculated that arrector pili muscle loss may be associated with the irreversible nature of AGA pathogenesis [67].

This theory was conceived after observing that even though alopecia areata demonstrates similar histologic findings of follicular miniaturization and chronic inflammation, it is a potentially reversible process [68]. One of the observed differences between the two entities is the loss of the arrector pili muscle in AGA, which is replaced by adipose tissue [68, 69]. Since this muscle attaches to the epithelial stem cell niche in the follicular bulge, it is postulated that the muscle may have a role in its maintenance through an undescribed process [70].

Several questions regarding the etiology of AGA remain unanswered. Hence, the exposition of each theory is necessary. However, it is possible that the multiple disparate postulates can be connected in a unifying mechanism of pathogenesis.

Platelet Rich Plasma Mechanism

Platelet-rich plasma, an amalgam of high concentrated growth factors, is utilized in a myriad of processes because these factors are involved in processes such as angiogenesis, cell proliferation, and differentiation [71]. In reference to hindering AGA progression, PRP's therapeutic effect lies in its ability to prolong hair follicle anagen phase and prevent premature catagen phase entry. Given our understanding of hair cycle physiology, PRP as a treatment modality is focused on promoting molecular cell growth mechanisms and preventing the onset of apoptotic processes [54].

The therapeutic goal of PRP is analogous to the two most utilized pharmacotherapies, oral finasteride and topical minoxidil. The mechanism by which finasteride can inhibit AGA has been described in prior sections of this review: it reduces DHT levels in the balding scalp. Minoxidil, however, provides a clue as to how PRP growth factors can mediate hair regrowth.

Minoxidil is an adenosine triphosphate-sensitive potassium channel opener; it mediates six-fold rises in the VEGF mRNA in dermal papilla cells. This process is tightly regulated by adenosine and

sulfonylurea receptors, which enhance and inhibit VEGF production, respectively [72, 73]. VEGF has long been known to promote angiogenesis and is elevated in anagen phase; notably, disappearance of perifollicular capillaries occurs in tandem with the anagen to catagen phase transition [74]. Moreover, VEGF has been shown to act in an autocrine fashion on dermal papilla cells, asserting the importance of these cells in combating AGA [74].

Dermal papilla cells are the source for other growth factors, which serve as the medium by which hair growth progresses. Hepatocyte growth factor (HGF), documented to be present in PRP, has been shown to be generated from dermal papilla cells [71, 75]. In this context, HGF caused hair follicle growth in a mouse system [75]. Indeed, in an organ culture of follicular papilla cells, similar results were observed, with hair follicle elongation occurring secondary to the activity of HGF on bulb-derived keratinocytes [27].

Remarkably, insulin-like growth factor 1 (IGF-1) from the dermal papilla increases secondary to finasteride administration, implying a reciprocal connection between the androgen pathway and growth factor production [76]. Specifically, finasteride application to AGA scalps results in elevated IGF-1 mRNA expression in the dermal papilla [76]. Noting that IGF-1 also stimulates hair growth provides additional evidence that there are not only several pathways for hair growth, but also that the pathways are interconnected [77].

Stem cells in the follicular bulge also demonstrated epidermal growth factor receptors, implying that dermal papilla cell EGF controls their growth [78]. These examples highlight the significant effect dermal papilla-derived growth factors have on hair follicle stimulation. Therefore, it is likely that the clinical effects of AGA occur secondary to androgens reducing growth factor production from this cell population. Platelet-rich plasma may be a vehicle through which the complex process of growth factor production can be bypassed.

Platelet-rich plasma upregulates the downstream effects of growth factor receptor binding by serving as a concentrated source of growth factors. Growth factor binding is known to upregulate protein kinase B (AKT), which engages in stimulatory and inhibitory phosphorylation of a number of downstream targets. Growth factor binding also upregulates mitogen-activated protein kinase/ extracellular signal-regulated kinase (MAPK/ERK) [79-81].

Specifically, Akt phosphorylation of cyclic AMP response element binding protein (CREB) and murine double minute 2 (Mdm2) results in elevated levels of B-cell lymphoma 2 (Bcl-2) and ubiquitin-ligase, respectively [82, 83]. Bcl-2 is an anti-apoptotic protein. Ubiquitin-ligase can induce tumor protein p53 degradation to indirectly drive growth [83].

Furthermore, Akt inhibits two pathways: β-Catenin degradation secondary to glycogen synthase kinase and Bcl-2 associated death promoter (BAD) induced apoptosis [83-85]. These pathways were verified in vitro by Li et al. when application of PRP to dermal papilla cells from human scalp skin resulted in elevated β-catenin and fibroblastic growth factor-7 (FGF-7), which support PRP's role in prolonging anagen phase and preventing apoptosis [86]. This experiment also demonstrated that PRP induced sustained Bcl-2 expression [59].

Finally, when comparing skin cells treated with 5 percent PRP and untreated cells, ERK and AKT were significantly elevated in the former, prolonging cell survival [86]. An in vivo application of PRP on mice yielded faster telogen to anagen phase conversion compared to control groups. This finding supports the hypothesis that anagen phase is associated with higher β-catenin levels [57, 86].

Therefore, it appears that PRP growth factors converge on a set of proliferative and anti-apoptotic pathways; these include not only β-catenin preservation, BAD inhibition, and elevating Bcl-2 levels, but also promotion of angiogenesis. Future investigation is needed to determine if crosstalk occurs among the pathways. Also, research will need to be focused toward creating mechanistic diagrams as this information becomes available. Ultimately, the unique aspect of PRP therapy is not the number of avenues by which it functions compared to prior treatment modalities, but its ability to bypass previously needed structures (such as the dermal

papilla) and thereby allow for hair regeneration at stages of AGA in which previous treatments could not be efficacious.

Current Results and Future Directions

The number of studies evaluating PRP's efficacy in mitigating AGA progression continue to increase during the last decade. However, many of these studies have been conducted in vitro or have additional treatment modalities utilized in conjunction with PRP, limiting the ability to discern the sole effect of PRP on hair loss. Therefore, this section will first highlight well-designed primary literature of PRP use in AGA that contains assessment of objective, qualitative endpoints such as hair density or total hair count. Subjective measurements such as clinical evaluator assessment using rating scales or macrophotography and hair pull testing will not be compared given difficulty with replicability. However, a discussion correlating patient satisfaction scores with objective outcome measures will follow. **Table 2** includes five randomized, double-blind, placebo-controlled studies and compares their outcomes (**Table 2**), [8, 87-90]. **Table 3** demonstrates the respective PRP preparation methods for each study listed in **Table 2** (**Table 3**), [8, 87-90].

Table 2. *Randomized, placebo-controlled, double-blind, half-head primary clinical trials of PRP for AGA within the last 5 years.*

Study	Cervelli, 2014 [87]	Gentile, 2015 [8]	Puig, 2016[a] [90]	Mapar, 2016 [89]	Alves, 2016 [88]
Subjects (Completed) Gender breakdown	10 (10) 10 male	23 (20) 20 male	26 (26) 26 female	19 (17) 17 male 2 female	25 (24) 11 male 13 female
Age Range; AGA Stages	Age 20-52 Stage IIa - IV	Age 19-63 Stage IIa-IV	Stage II	Age 24-45 Stage IV - VI	Age 18-86 Stage I -V
Total follow-up window	1 year	2 years	26 weeks	6 months	6 months
Objective Endpoints	1. Hair Count 2. Total hair density 3. Terminal hair density 4. % Ki67+ and perifollicular vessel density 5. Epidermal thickness & hair follicle density per 3mm punch biopsy	1. Hair count 2. Total hair density 3. Terminal hair density 4. % Ki67+ & perifollicular vessel density 5. Epidermal thickness & hair follicle density per 3mm punch biopsy	1. Hair mass index	1. Terminal hair count 2. Vellus hair count	1. Hair count 2. Total hair density 3. Terminal hair density 4. Anagen hair % 5. Telogen hair % 6. Anagen : telogen ratio
Outcomes statistically significant / Time frame for follow-up	1. Yes ($p < 0.0001$), 3m 2. Yes ($p < 0.0001$), 3m 3. Yes ($p = 0.0003$), 3m 4. Yes ($p < 0.05$), 14wks 5. Yes ($p < 0.05$), 3m 1, 2, 3 compared to placebo 4,5 compared to baseline	1. Yes ($p < 0.0001$), 3m 2. Yes ($p < 0.0001$), 3m 3. Yes ($p = 0.0003$), 3m 4. Yes ($p < 0.05$), 14wks 5. Yes ($p < 0.05$), 14wks 1, 2, 3 compared to placebo 4, 5 compared to baseline	1. No ($p = 0.220$)	1. No ($p = 0.25$) at 6 months 2. No ($p = 0.23$) at 6 months	1. No ($p > 0.05$) 2. Yes ($p < 0.05$), 3m & 6m 3-6. Yes ($p < 0.05$) compared to baseline; No ($p > 0.05$) compared to placebo 1, 2 compared to baseline & placebo

Abbreviations: m, months; wks, weeks

[a] Puig et al. was not a half-head study; however, it was a multi-center trial.

Current results of PRP studies: summary of methods and outcomes

The results of PRP administration are inconsistent regarding objective measures. In addition, non-randomized controlled trials — that also demonstrated a similar positive outcome — were not included Tables 2 and 3 [1, 91-93]. However, the aforementioned studies highlight a positive effect of PRP.

The reason reliable PRP results are not being achieved lies with the state of study design utilized. First and foremost, studies need to consistently report the final prepared platelet concentration. When studying angiogenesis induction in human endothelial cells, it was ascertained that concentrations greater than 1,500,000 platelets per microliter limited angiogenic capacity [94]. Because varying protocols for PRP preparation are utilized, there is significant variations in platelet concentration not only between studies, but also within the studies themselves.

The differences in PRP preparation and administration also include the amount of PRP injected, the choice of activating agents, the time from preparation to administration, and the number of injections over a window of treatment; some studies even go as far as to use inactivated PRP [88, 90, 93, 95]. The most common application protocol consists of three injections of PRP with a one-month interval; however, some opt for only two injections within a three-month window or one injection weekly for four weeks [1, 8, 88, 96]. Considering that PRP does not increase platelet lifespan or growth factor half-life, these protocol differences can impact clinical outcomes [14, 97].

The power of the earlier PRP studies is limited. The sample sizes are small and the measured objective endpoints vary. Furthermore, meta-analyses pooling the information from these small studies are not necessarily an effective approach to evaluate the data, given the variety of endpoints measured and the disparity in PRP administration methods.

Table 3. *Variability of PRP preparation and use in clinical trials.*

Study	Cervelli, 2014 [87]	Gentile, 2015 [8]	Puig, 2016 [90]	Mapar, 2016 [89]	Alves, 2016 [88]
Injection protocol; Anesthesia?	Intradermal injection (0.1 mL/cm^2) in 2 of 4 selected halves (frontal, parietal, etc.) vs. placebo solution into remaining halves No anesthesia	Interfollicular PRP injections (0.1 mL/cm^2) in 2 of 4 selected scalp regions vs. physiologic solution into remaining 2 areas No anesthesia	Subcutaneous 3 mL injection in 4 cm^2 central scalp region 2% lidocaine, 0.5% bupivacaine	1.5 mL injection in two 2.5x2.5 cm^2 regions 3 cm apart (case vs. control) No anesthesia	Intradermal PRP injection into selected hair depleted area No anesthesia
# Sessions	3	3	1	2	5
Interval between sessions	1 month	1 month	N/A	1 month	1 month (1st 4 sessions); 5th session 7 months from initial
Preparation Method	Cascade-Selphyl-Exforax system	1. Cascade-Selphyl-Exforax system 2. Platelet-rich lipotrasform system	Angel RPR system to concentrate platelets 2.75 – 3.4x	Double spin method with Tubex PRR tube	Single spin method
Activators	Ca^{2+}	Ca^{2+}	None	Calcium gluconate (0.1 mL / mL PRP)	Calcium chloride (10%, 0.15 mL)
Centrifugation rate (Time)	1100 g (10 min)	1. 1100 g (10 min) 2. 1200 rpm (10 min)	None	1. 3000 rpm (6 min) 2. 3300 rpm (3 min)	460g (8 min)
Blood Volume vs. PRP Volume	18 mL vs. 9 mL	1. 18 mL vs. 9 mL 2. 60 mL vs. 20 mL	60 mL vs. 10 mL	9 mL vs. 1.5 mL	18 mL vs. 3 mL

Current results of PRP studies: patient perception of outcomes

Six primary studies assessed patient satisfaction in the context of objective endpoints such as hair density, count, and diameter and clinical examination. Of these, only the study by Puig et al. was a randomized controlled double-blinded trial; the remaining investigations were observational studies [1, 90-92, 98, 99]. Questionnaires were used to evaluate patient opinions regarding amount and rate of hair loss, degree of hair regrowth, hair quality, and overall satisfaction with PRP therapy. All of the questionnaires were heterogeneous with respect to questions asked and time of survey administration.

The study conducted by Puig et al. did not report a statistically significant difference in measured objective endpoints; notably, they were the only study to use inactivated PRP [90]. Accordingly, their treatment arm had the lowest patient satisfaction scores across the six studies, with only 13.3 percent of the treatment group reporting improvements in hair loss [90]. However, they did not analyze if perception of improvements in hair loss was significantly different between their control and treatment groups (0 percent versus 13.3 percent), [90]. The results from the other five studies did reach statistical significance [1, 91, 92, 98, 99].

In contrast, Anitua et al. and Gupta et al. stated that 78.9 and 93.3 percent of patients reported decreases in hair loss, respectively [91, 99]. Gkini et al. noted that 65 percent of patients perceived increases in hair density and 85 percent reported greater hair thickness and quality [92]. Furthermore, Khatu et al. noted a mean satisfaction score of seven out of ten with the results of PRP treatment. Patients from Takikawa et al. described diminished hair loss with shampooing and increased maintenance of existing hairs [1, 98]. Although these five studies did not have placebo groups to compare satisfaction scores, it is evident that primary studies with statistically significant differences in objective endpoints had higher rates of patient satisfaction [1, 90-92, 98, 99].

Future directions

Further studies are required to evaluate the magnitude of PRP's effect on AGA. The results of additional research will provide insight as to whether PRP should remain as an adjuvant treatment or become more commonly used. These investigations need to be of larger scale, be multi-centric, and use standardized protocols across studies. Efforts are already being made in this direction, as the importance of a consistent preparation method for obtaining high yields of platelets and growth factors is becoming evident [11, 71].

Recommendations are also needed regarding issues such as the interval of time between PRP treatment sessions and the number of follow-up PRP sessions needed to maintain results. Furthermore, because PRP does not specifically counteract or inhibit androgen activity, new studies need to assess if PRP treatment alone is sufficient to inhibit AGA progression by using longer follow-up periods. If this is not financially feasible, combination trials with PRP and finasteride or minoxidil should be conducted to determine the efficacy of a dual-pronged approach.

Finally, the route and mechanism of PRP delivery needs to be explored. Two approaches — based on the limited time frame of growth factor release — can be taken: augmenting the effect during the same time frame or prolonging the time of action. Regarding the former, Kang et al. explored augmenting PRP with CD34+ cells, since they postulated that the angiogenic potential of the CD34+ cells would aid PRP growth factors [95]. Although they validated clinical efficacy in terms of increase mean hair number and mean hair thickness compared to baseline and placebo, they did not compare this modality with non-CD34+ PRP, limiting the ability to discern if CD34+ cells had any effect compared to PRP alone [95].

Takikawa et al. determined that PRP delivered with dalteparin and protamine microparticles — compared to PPR alone — enhanced growth factor availability, follicle stimulation, and perifollicular vascularization [98]. This approach may be useful in other PRP applications. However, a sustained release may be more effective in AGA treatment due to the slow rate of normal hair growth.

Platelet-rich fibrin (PRF), an autologous platelet gel created without anticoagulation from a single cycle of centrifugation, offers a means for long-term growth factor release [100]. Anticoagulation is not used. High centrifugation rates (up to 3000 revolutions per minute for 10 minutes) must be utilized. After this, the coagulation process immediately begins to generate a fibrin clot via circulating thrombin [101]. The clot has a 3-dimentional structure that traps platelet and cytokines within the fibrin polymer, resulting in time-based release of growth factors as the clot degenerates [101].

Platelet-rich plasma and PRF release of PDGF and TGF-β at 1, 7, 14, 21, and 28 days were compared. Platelet-rich plasma demonstrated highest levels of growth factors released during the first day, with significantly decreased release at all later time points. In contrast, PRF had the highest release of PDGF at day 7 and TGF-β at day 14 [102].

The benefit of PRF was clinically confirmed when evaluating ten male AGA patients with hair follicular unit transplantation in 1×1cm^2 areas in the right and left temporal areas. Three sessions of PRF — at 0, 2, and 3 months — were administered in the right scalp, resulting in increased hair follicles counts at one month (P<0.001), two months (P<0.002), and six months (P<0.005) follow-ups compared to the untreated scalp [100]. However, further experiments comparing PRP and PRF in AGA patients should be conducted prior to making conclusions regarding the relative efficacy of PRF.

Conclusion

Platelet-rich plasma is an efficacious adjuvant treatment modality for AGA. The mechanisms by which PRP can mitigate AGA are being elucidated. Additional research, particularly focused in two areas, is warranted. First and foremost, a multi-dimensional theory connecting androgen, inflammatory, and structural changes should be investigated. In addition, future clinical trials should be performed to confirm the efficacy of using PRP in patients with AGA. As information from new research becomes available, future recommen-dations regarding PRP's role in tandem with other pharmacotherapies, hair transplantation, or both modalities for AGA can be established.

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