# Dissertation

# Platelet-rich plasma for the treatment of androgenetic alopecia

submitted by

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under the Supervision of

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Graz, June 2021 Paul Gressenberger

*Declaration*

*"I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz".*

*Graz, July 2021* *Paul Gressenberger eh*

## Disclosures

The current doctoral thesis was the basis for the preparation of a manuscript, which has been published in the journal “Acta Dermato-Venereologica”. The published manuscript was drafted by the doctoral candidate, Paul Gressenberger. Therefore, significant parts of the doctoral thesis are similar to the published manuscript (with permission of Acta Dermato-Venereologica). It was accepted on July 9, 2020.

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All co-authors have explicitly agreed to the use of their data in the thesis.

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*“It's easier to go down a hill than up it, but the view is much better at the top.”*

-Henry Ward Beecher

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# Abbreviations

| | |
|---|---|
| AGA | androgenetic alopecia |
| AR | androgen receptor |
| BPH | benign prostatic hyperplasia |
| cm | centimeter |
| $cm^2$ | squarecentimeter |
| CYP3A4 | cytochrom P450 3A4 |
| DHEA-S | dehydroepiandrosterone-sulfate |
| DHT | dihydrotestosterone |
| e.g. | *exempli gratia* (for example) |
| EGF | epithelial growth factor |
| EDA2R | ectodysplasin A2 receptor |
| FDA | Food and Drug Administration |
| FGF | fibroblast growth factor |
| FPHL | female pattern hair loss |
| FU | follicular unit |
| GF | growth factors |
| IGF-1 | insulin-like growth factor 1 |
| IGF-2 | insulin-like growth factor 2 |
| i.e. | *id est* (that is) |
| IL-8 | interleukin 8 |
| ml | millilitre |
| mm | millimeter |
| PDGF | platelet-derived growth factor |
| PFS | post finasteride syndrome |
| PRP | platelet-rich plasma |
| RBC | red blood cells |
| RPM | rotations per minute |
| US | United States |
| VEGF | vascular endothelial growth factor |
| WNT | wingless-related integration site |
| µL | microliter |
| µm | micrometer |

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# Zusammenfassung

**Hintergrund**

Injektionen mit plättchenreichem Plasma (PRP) werden als wirksame und nebenwirkungsarme Option zur Behandlung von androgenetischer Alopezie dargestellt. Zuverlässige Studiendaten zu dieser Therapie fehlen jedoch.

**Zielsetzung**

Diese Studie wurde entwickelt, um die Wirksamkeit dieser Behandlung zu untersuchen.

**Materialen und Methoden**

30 gesunde männliche Patienten mit androgenetischer Alopezie wurden in eine randomisierte, placebo-kontrollierte Pilotstudie eingeschlossen. Es wurden fünf PRP-Behandlungen in Abständen von vier bis sechs Wochen durchgeführt. Es wurden zwei Nachuntersuchungen durchgeführt. Zwanzig Probanden wurden mit PRP behandelt und 10 Probanden wurden mit physiologischer Kochsalzlösung (Placebo) behandelt. Die Wirksamkeit der Behandlung wurde durch Messung der Haarzahl und des Haardurchmessers mit dem TrichoScan-System gemessen. Sekundäres Ziel war die klinische Verbesserung der Haardichte, die von einem unabhängigen Gutachter anhand einer Likert-Skala und anhand der Norwood-Hamilton-Skala beurteilt wurde. Zusätzlich wurde die Patientenzufriedenheit mit einem Fragebogen ermittelt.

**Ergebnisse**

In beiden Gruppen gab es keine signifikanten Verbesserungen der Haarzahl und des Haardurchmessers. Die anhand der Likert- und Norwood-Hamilton-Skala beurteilte Veränderung des Haarwachstums im klinischen Erscheinungsbild zeigte keine Verbesserung. Dem Fragebogen zufolge gaben in beiden Gruppen die meisten Patienten eine Verbesserung an, wären bereit gewesen für die Behandlungen zu bezahlen und hätten die Behandlung anderen Betroffenen weiterempfohlen.

**Fazit**

Die Ergebnisse unserer Studie legen nahe, dass plättchenreiches Plasma als Monotherapie das Haarwachstum bei Männern mit AGA nicht verbessert.

# Abstract

### Background

Platelet-rich plasma (PRP) injections are presented as an effective and low-side-effect option for the treatment of androgenetic alopecia, however, reliable study data concerning this therapy are lacking.

### Objective

This trial was designed to explore the efficacy of this treatment option.

### Materials and Methods

Thirty healthy male patients with AGA were enrolled in a randomized, placebo-controlled study. Five PRP-treatments at intervals of four to six weeks were performed. Two follow-up examinations were done. Twenty subjects were treated with PRP and 10 subjects were treated with physiological saline (placebo). Treatment efficacy was measured by calculating the hair number and hair diameter with the TrichoScan system. Secondary objective was the clinical improvement, which was evaluated by an independent reviewer using patient photographs and a 5-point Likert scale as well as the Norwood-Hamilton scale. In addition, patient satisfaction was assessed by a survey.

### Results

No significant improvements in hair number or hair diameter were observed in either group. Hair growth in clinical appearance also did not show any improvement. Surprisingly, despite these negative results, the majority of subjects in both groups declared themselves at least partially satisfied with the result.

### Conclusion

The results of our study suggest that platelet-rich plasma as a monotherapy does not improve hair growth in men with AGA.

# 1 Introduction

## 1.1 Androgenetic Alopecia

Androgenetic alopecia (AGA), also known as male pattern hair loss, is the most common form of hair loss and can affect both men and women [1]. It is caused by a genetically determined sensitivity of hair follicles to androgens, presenting with varying severity and progressing with age [2, 3].

## 1.2 Historical and Cultural Considerations of Hair and Hair loss

There is evidence that already the ancient Egyptians were worried about losing their hair and tried to find methods to prevent and treat hair loss [4, 5]. AGA was first described by the Greek physician Aristoteles (384 – 322 BC) [6], who recognized that hair loss did not occur in eunuchs or before sexual maturity and postulated a correlation between libido and the degree of hair loss [7]. Hair growth and replacement have been studied for many reasons [8]. Animal coats, for example, vary seasonally in terms of weight, density and color, allowing the animal to adapt to changing environmental conditions [8]. These changes can provide protection, have sensory functions or may be used for signaling or communication such as the reproductive cycle [8, 9]. Many experiments which have been carried out on laboratory animals may be transferred to humans and have provided important insights into the complexity of hair growth and hair development [8, 9]. It is theorized that the reduction in human body hair over evolution was a result of the hunter's development, which required mechanisms for the rapid dissipation of body heat. Functional hairlessness permitted the elaboration of eccrine sweat glands, an extraordinarily effective mechanism for heat dissipation and whole-body evaporation [11]. Furthermore, hair reduction in humans evolved along with increasing intellectual capacity, allowing the use of artificial insulation in the form of clothing [10, 11]. Since clothing has met the thermal insulation needs of humans, hair has lost its main thermoregulatory function. Hair growing on the head, however, still has an important role in thermal insulation as well as protection of the scalp from ultra-violet radiation. Eyebrows, additionally, protect the eyes from sweat [12, 13]. In the course of the evolutionary regression of the human hair coat, hair has become

primarily a vestigial structure with a non-verbal signaling function, playing an important role in the development of psychosocial constructs [12, 14]. The high value that is given primarily to the visible hair on the head is closely linked with personal identity and style and plays an important role in psychosocial interactions [12 - 14].

## 1.3 Hair Biology

The scalp contains about 100,000 hair follicles in total [15]. Human hair count and form vary biogeographically, depending on ethnicity (Table I) (Figure 1) [16]. For example, Caucasians have approximately 250 - 310 hairs per square centimeter, Africans have approximately 150 hairs per square centimeter and Asians have approximately 120 hairs per square centimeter (Table I) [16 - 20]. Caucasians hair shafts usually have oval cross sections, with diameters ranging from 50 to 120 micrometers, often depending on the geographical area of origin (Table I) (Figure 1). Light-haired Scandinavians, for example, have the finest diameters—in some cases less than 50 micrometers, whereas dark-haired Caucasians tend to have thicker diameters [21]. The cross sections of Asian hair shafts are round with large average diameters of 100 to 130 micrometers (Table I) (Figure 1) [22]. African hair is tightly curled and varies in diameter along a single shaft (Table I) (Figure 1) [19]. Due to the fusion of ethnicities in modern times, mixed hair types occur frequently, meaning that straight hair and curly hair can occur together on the scalp of one individual [16]. Therefore, in 2007 researchers suggested a global approach to hair type classification based on straightness, curliness and waviness, separating hair types into eight different groups [23]. This provided a more coherent method than a classification based mainly on ethnicity [23].

**Table I. Hair Characteristics by Ethnicity** [15-22]

| ethnicity | diameter (µm) | cross-sections | structure |
|---|---|---|---|
| Asian | 100–130 | round | straight |
| Caucasian | 40–120 | oval | straight or wavy |
| African | 60–100 | elliptical | curly or coiled |

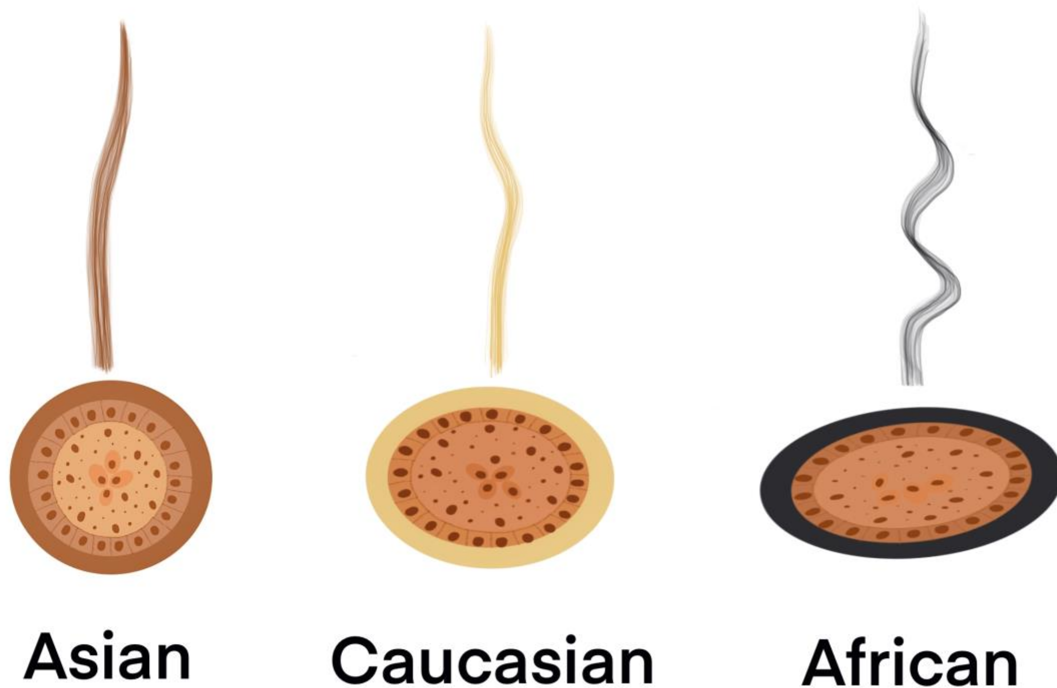


**Figure 1.** Hair Characteristics by Ethnicity. The figure was drawn with procreate on Ipad [15 – 22].

Although human hair characteristics vary widely between individuals, the anatomical structure is always the same [24]. Hair follicle structure (Figure 2) is divided into four parts in relation to the skin, from superficial to deep: The upper segment, called the infundibulum, reaches from the sebaceous gland to the orifice of the follicle (Figure 2) [24, 25]. Two middle parts: the isthmus, which is the area from the sebaceous gland to the arrector pili muscle, and the suprabulbar area, which reaches from the arrector pili muscle to the matrix (Figure 2) [24, 25]. At the base of the hair follicle is the bulb, containing dermal papilla cells, matrix and melanocytes (Figure 2) [25]. The dermal papilla contains blood vessels that nourish the hair follicle (Figure 2) [25, 26]. The hair follicle comprises different layers (Figure 2) [25, 26]. The central part around the dermal papilla is called the hair matrix, which forms the cortex and contains melanocytes that produce melanin which leads to pigmentation of the hair resulting in different hair color (Figure 2) [24 - 26]. This layer is surrounded by the internal layer of the inner root sheath which is further composed of the Huxley's layer and the Henle's layer, from

which the last is already keratinized, an outer root sheath and a fibrous root sheath which consists of collagen bundles that surround the whole hair follicle (Figure 2) [24 – 26].

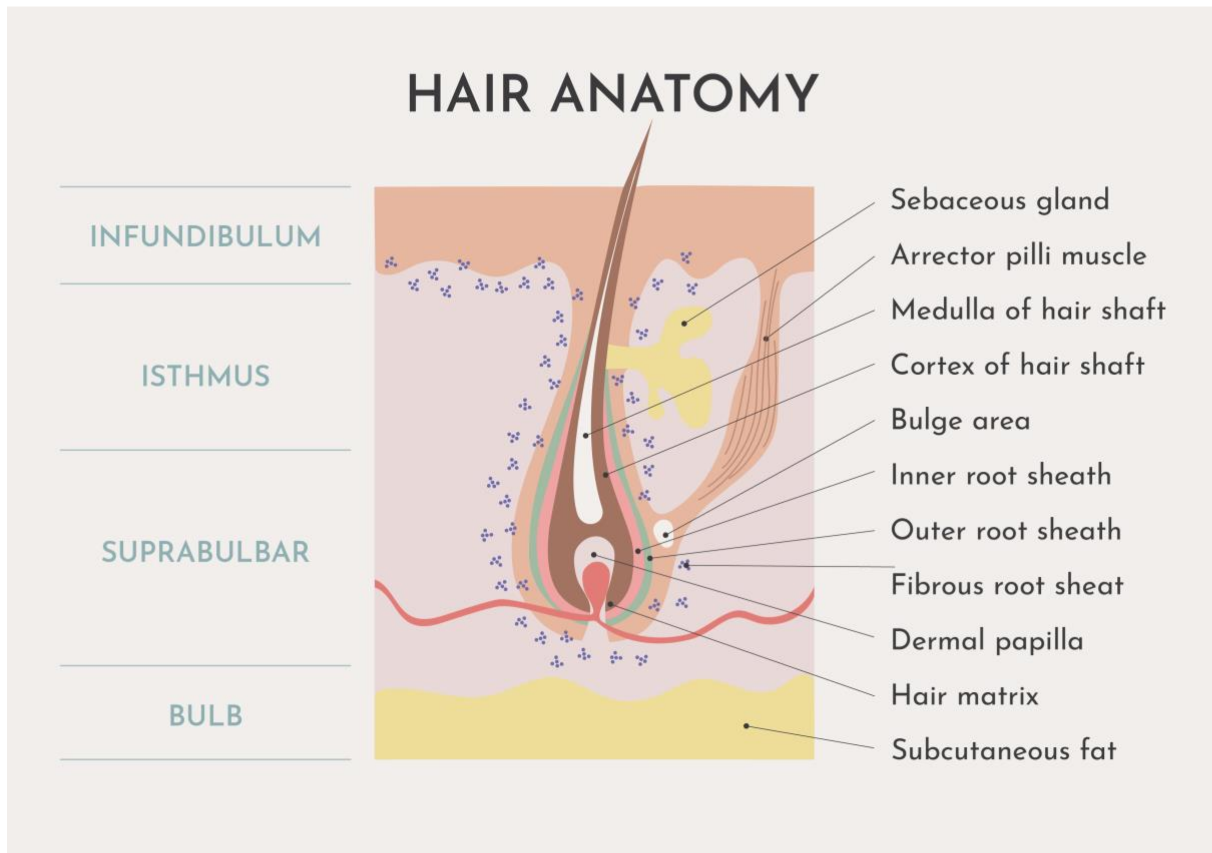


**Figure 2.** Hair Anatomy. The figure was created with Adobe Illustrator [25]

## 1.4 Hair Growth Cycle

Each hair follicle undergoes a recurring cycle with three periods: the anagen phase (growth), the catagen phase (transition), and the telogen phase (resting) (Figure 3) [26]. The anagen phase is the growth period, which lasts from 2 to 8 years (Figure 3) [26, 27]. In this phase mitoses take place and the hair grows about 0.35 mm per day or 1 cm per month [26, 28]. The catagen phase is the second phase of the hair growth cycle, a short transition period lasting from 2 to 3 weeks (Figure 3) [26, 29]. In the catagen phase, hair growth stops due to cessation of mitoses in the hair follicle [26, 28]. In the third phase, the telogen phase, which lasts around 3 months, the hair rests

in the root until a newly formed anagen hair begins to grow beneath it (Figure 3). The new hair pushes out the telogen hair, which then falls out (Figure 3) [26, 28]. Each follicle on the scalp undergoes the growth cycle at different times. At any one time, about 80-90% of hairs are in the anagen phase and 10 to 20% are resting, resulting in an average physiological hair loss of about 100 hairs per day [26, 30]. On the scalp are also found two different types of hair, vellus hair and terminal hair [31]. Vellus hair is very tiny with diameters of 30 micrometer and light hair shafts and covers the whole body except for the palms and the soles of the feet [32]. Terminal hair is thicker, with diameters of over 60 micrometers [33]. In humans, terminal hair is found on the head, the eyebrows, the chest and in the genital region [31 -33].

**Figure 3.** Hair Growth Cycle. The figure was created with Adobe Illustrator [7, 25].

## 1.5 Incidence and Prevalence of AGA

Incidence and prevalence of AGA vary with respect to age, ethnicity and genetic background [34, 35]. Based on the little data available, we know that up to 50% of men will have some degree of AGA by the age of 50 years, and up to 80% by the age of 70 years [34, 35]. Men of Asian and African descent are less affected than Caucasian men [34 - 37]. AGA is gradual in onset and usually manifests at an early age between 15 and 25 years [38]. Onset can occur, however, at any age. The clinical course is episodical with phases of increased hair loss alternating with phases of less hair loss [38].

## 1.6 Pathogenesis

The pathogenesis of AGA is strongly associated with genetic and hormonal factors (Figure 4) [39, 40]. In some men, a genetically-determined response of hair follicles to androgens results in progressive miniaturization of hair follicles on specific areas of the scalp and a resulting change in hair cycle dynamics (Figure 4) [39 - 41]. Male AGA is therefore considered an androgen dependent condition; however, the role of androgens in female pattern hair loss remains unclear [42]. In AGA, the duration of the anagen phase gradually shortens, while the telogen phase duration increases [43]. Because the duration of the anagen phase determines hair length, the maximum length of the new anagen hair is shorter [43, 44]. With each new hair cycle, the miniaturized hair follicle produces a thinner vellus like hair, leading to a higher proportion of telogen follicles in the affected areas and, subsequently, a reduction of hair until balding is visible [45, 46].

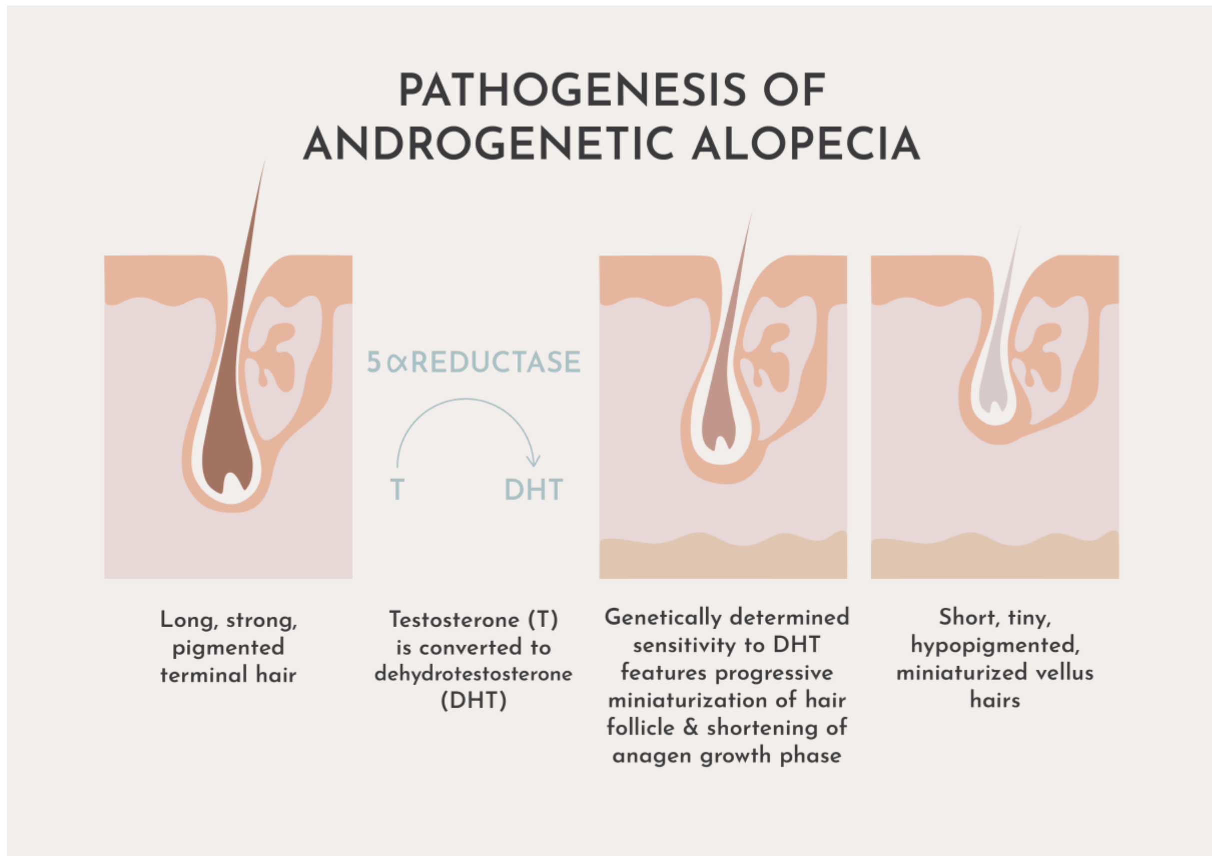


**Figure 4.** Pathogenesis of AGA. The figure was created with Adobe Illustrator [7, 25, 43].

### 1.6.1 Genetics

Androgenetic alopecia, as the name suggests, has a clear genetic involvement and usually there is a family history [47 - 49]. Genetics of androgenetic alopecia are complex. Even though a genetic component to AGA was suggested as early as 1916 by Osborn, the exact mechanism is still not certain [50, 51]. In recent years, several studies have investigated the complex inheritance of androgenetic alopecia [2, 48 – 53]. Some of these studies reported higher expression levels of the androgen receptor (AR) and 5-alpha reductase enzyme in hair follicles of the balding scalp compared to non-balding areas of the scalp, suggesting that these two genes play an important role in the development of androgenetic alopecia [54 - 56]. 5-alpha reductase converts testosterone to dihydrotestosterone and the androgen receptor mediates cellular responses to androgens such as dihydrotestosterone (Figure 4) [2, 56]. Although multiple gene loci are apparently involved in AGA, especially strong associations of

AGA with the androgen receptor (AR) and ectodysplasin A2 receptor (EDA2R) genes [56-59], both located on the X-chromosome at band q12, have been identified [2]. The involvement of the X chromosome is consistent with the known importance of the maternal line in androgenetic alopecia inheritance [2, 57, 59]. In addition to X-chromosome inheritance, a recent study has identified several autosomal gene loci that could also implicate non-androgen-dependent pathways in AGA pathogenesis [60].

### 1.6.2 Hormonal factors

Androgens, male sex hormones, are the main regulators of male body development and play an important role in skin related disorders such as AGA [61]. During puberty, androgens promote the conversion of vellus hair follicles to terminal hair follicles in promoting the growth of hair in pubic and axillary areas, as well as beard growth [3]. Paradoxically, androgens promote both hair growth and the progression of androgenetic alopecia [39, 40]. 5α-reductase, which irreversibly converts testosterone into dihydrotestosterone (DHT) and plays a central role in AGA [2], may be a key factor in the apparently opposing effects of androgens on hair growth. Two different isoforms of 5-alpha reductase, isotype 1 and isotype 2, are expressed in hair follicles, with a slightly higher expression level of isotype 1 [54, 62, 63]. The relative expression level of each isotype in general varies depending on body site [62, 63]. As described above, it has been assumed that the expression of these isotypes is genetically determined [2, 53, 54]. Other hormones that can be metabolized to DHT in dermal papilla cells are the prohormones dehydroepiandrosterone-sulfate (DHEA-S) and androstenedione [64]. Recent studies suggest an association of androgen dependent Wnt-signaling pathways that interact with dermal papilla cells of the hair follicle in AGA [65].

## 1.7 Clinical manifestation

In men, AGA typically affects the top and front of the scalp, while sparing the occipital region. A useful tool for assessing the degree of AGA is the Norwood-Hamilton scale, which uses a scale of I to VII to describe the stage (type) of hair loss (Figure 5) [66 - 68]. Type I is characterized by terminal hair loss in the temporal scalp area and forehead; Type II and III show a typically M-shaped recession of the frontal hairline; types IV, V and VI additionally manifest in hair thinning in the mid-frontal scalp or vertex area of the scalp; type VII shows a complete balding area at the top of the scalp with a fringe of hair at the side and the back of the head. Types IIIa, IVa and Va are characterized by additional hair loss in the middle area of the forehead. The severity and areas of involvement are highly variable and not restrictive [66 – 68]; in some men AGA can even occur in a female pattern (Figure 6) [69].

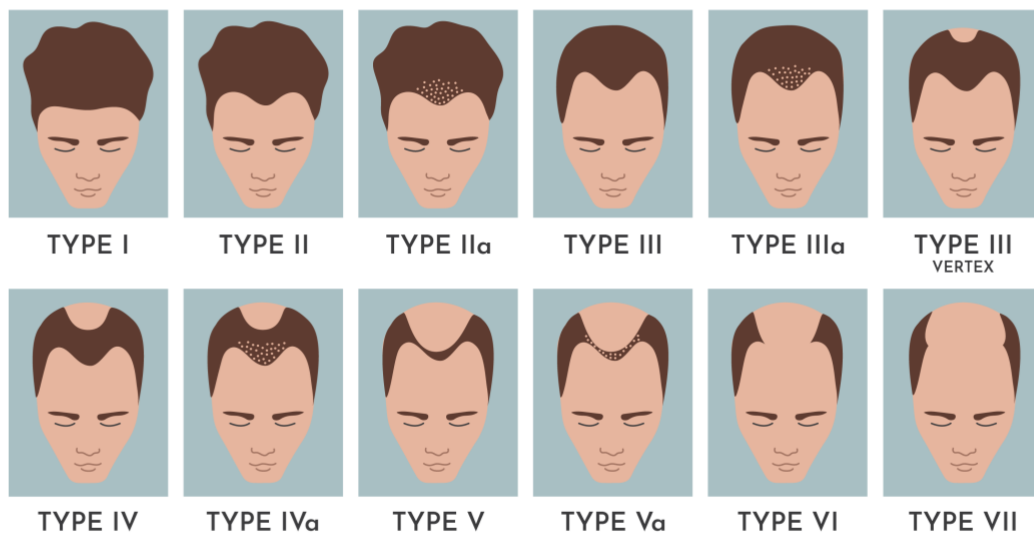


**Figure 5.** Severity assessment of male AGA. The figure was created with Adobe Illustrator [66-69].

In women, AGA presents as a diffuse hair thinning over the central scalp, while the frontal hairline is usually retained [68]. Ludwig described the presentation and severity of female AGA with a scale from grades I to III (Figure 6) [70]. Grade I begins with perceptible thinning on the crown, grade II shows an increased rarefication and noticeable widening of the midline part, and grade III is characterized by full balding of the midline part [68, 70].

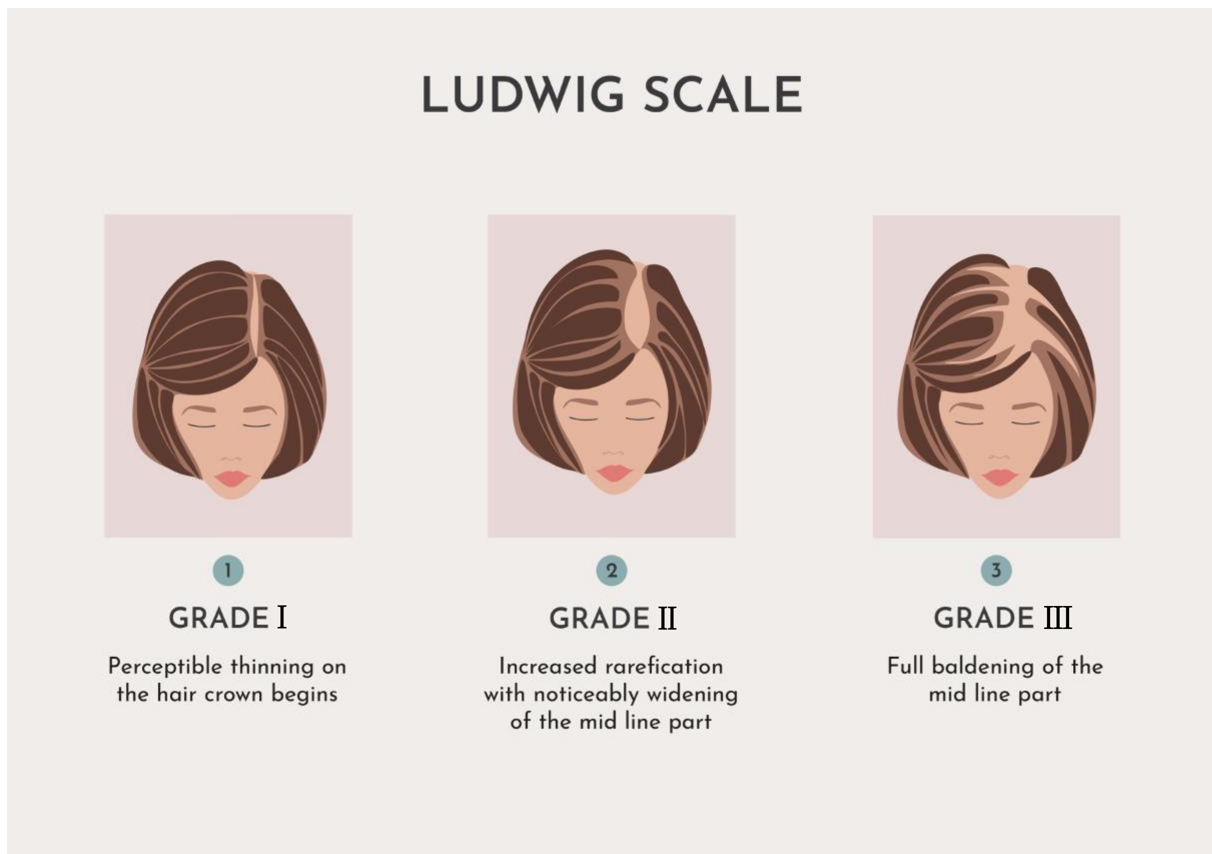


**Figure 6.** Severity assessment of female AGA. The figure was created with Adobe Illustrator [68, 70].

## 1.8 Diagnosis

The diagnosis of AGA is generally based on clinical manifestation and physical examination, therefore a precise examination of the scalp is mandatory [27, 68]. Dermoscopy can be useful for detecting miniaturized hairs [27, 68]. Furthermore, photo documentation can help to track response to therapy or disease progression [27, 68]. To exclude diffuse hair loss, a hair pull test should be done on different areas of the scalp [27, 68]. Other reasons for hair loss should be excluded by medical history or laboratory testing [27, 68].

## 1.9 Treatment options for men

### 1.9.1 Non-surgical treatment options

#### 1.9.1.1 Finasteride

Finasteride, a synthetic 4-azasteroid, was originally developed for the treatment of benign prostatic hyperplasia (BPH) [71]. It was later approved (1997), however, as the first oral pharmacologic therapy for the treatment of men with AGA, with an optimal dose of 1 mg/day [68, 72]. Finasteride is a 5α-reductase inhibitor that specifically and selectively inhibits the isotype II and III of 5α-reductase [68], leading to a reduction in androgen synthesis in general and also significantly decreasing DHT levels in hair follicles [73]. Improvements are noticeable after six months of treatment with 1 mg daily. Cessation of the drug results in loss of all benefits [72]. Finasteride metabolism occurs via CYP3A4 in the liver; therefore, caution is given in patients with liver problems [74]. Although finasteride is generally well tolerated, potential adverse effects include decreased libido, erectile dysfunction, gynecomastia and depression [75]. In a recent study, researchers found an association between suicidality and psychological adverse events in patients younger than 45 years who used finasteride for hair loss [76]. A further topic of discussion is persistent sexual dysfunction in some men despite discontinuing the medication [75]. To collect data about these adverse events and to provide support to victims, the International Society of Hair Restoration Surgery founded the Task Force on Finasteride Adverse Event Controversies in 2012 [75, 77].

#### 1.9.1.2 Dutasteride

Dutasteride, a synthetic 4-azasteroid, is a selective and competitive inhibitor of 5-α-reductase isotype-1 and isotype-2 [78] approved for the treatment of BPH [79]. In several studies, dutasteride has been found to be a more effective treatment for AGA than finasteride [80 - 82]. Despite these promising results, the safety profile of dutasteride remains unclear [83]. Several adverse effects are similar to those of finasteride and include the risk of gynecomastia, erectile dysfunction, loss of libido, or reduced semen volume [81 – 83]. In a subset of patients, erectile dysfunction can persist even after discontinuation of treatment, leading to a lower quality of life or depression [75, 83]. Furthermore, a warning has been added by the US Food and Drug administration (FDA) that dutasteride is associated with a low but increased risk of high-grade prostate cancer; therefore, caution is advised when deciding to start long term treatment with dutasteride [84].

#### 1.9.1.3 Minoxidil

Minoxidil was originally developed as an oral drug for the treatment of arterial hypertension in the United States in the 1970s [85]. Minoxidil works as a potassium channel opener, resulting in widened blood vessels and enhanced blood flow [86]. Increased hair growth was observed as a side effect of the application [87, 88]. The precise mechanism of action of minoxidil in hair loss is not fully understood, but presumably minoxidil ensures a better supply of nutrients to the hair follicles, thus helping to reverse miniaturization of hair follicles and lengthening the growth phases of the hair [88 - 90]. Today, minoxidil is sold as a 5% solution, gel, or foam for the treatment of male AGA [91]. Common side effects are skin irritations, abnormal hair growth on the cheek, burning or headache. At the beginning of treatment, transient shedding may be observed [91].

#### 1.9.1.4 Ketoconazole

Ketoconazole is an antifungal agent used to treat a number of fungal skin infections [92]. It also is an antiandrogen. Some studies have suggested that Ketoconazole improves hair growth when used frequently in a 2% shampoo formulation [92].

#### 1.9.1.5 Low Level Laser Therapy

According to several reports, the use of 600–1,100 nm wavelength laser therapy may be a safe and effective option for promoting hair regrowth [93]. To achieve improvement, treatments must be done over a time period of at least 16 weeks on alternating days for 15 to 30 minutes per day [93]. Despite several studies, the mechanism of action is not clear and methodologically robust trials on that therapy are lacking [93].

#### 1.9.1.6 Wigs and hair pieces

Wigs and hair pieces are a non-invasive treatment option for patients for whom medical treatment or hair restoration surgery has not been successful [94]. They provide excellent cosmetic results and can improve patient quality of life significantly [94].

### 1.9.2 Hair transplantation surgery

Modern hair transplantation techniques provide excellent results and can lead to permanent improvement [95, 96]. The aim of a hair transplant is to transfer the patient's own (autologous) hair follicles from a donor site to the balding areas of the scalp [95]. Hair follicles from occipital areas are preferred as donor follicles, as they are genetically not as sensitive to DHT. Therefore these follicles are not affected by miniaturization and the hairs growing from them do not fall out [3, 96]. In rare cases, axillary hair, hair from the chest, the beard or other sites of the body are also used [97]. Two different methods are utilized for obtaining hair follicles: follicular unit transplantation (FUT) and follicular unit extraction (FUE) [95, 96]. These two techniques are described in the following sections.

#### 1.9.2.1 Follicular Unit Transplantation (FUT) Technique

Follicular unit transplantation (FUT), also known as the strip procedure, has been the gold standard procedure for hair restoration for several years (Figure 7, left panel) [95, 98]. The procedure was developed by Dr. Bobby Limmer, who was the first to use stereoscopic microscopes for aiding visualization during hair transplantation [98].

While performing his procedures, Dr. Limmer observed that hair grows naturally in bundles of 1 to 4 hairs, called follicular units [98]. FUT surgery involves the harvesting of individual strips from the occipital area of the scalp which contains intact follicles [95, 96, 98. 99]. Before strip harvesting, the donor area is carefully evaluated, as wound closure should not be performed under tension. Therefore, the scalp in the donor area must have some degree of flexibility [95, 99]. After shaving, the strip is extracted from the donor area under local anesthesia [99]. While the strip is dissected into follicular units by surgical assistants with the aid of microscopes, the surgeon prepares incisions (slits) in the balding areas of the scalp for insertion of the follicular units [95, 99]. The prepared grafts are stored in chilled saline until they can be inserted, as mechanical damage can result in failure to grow after transplantation or destruction of the graft before insertion [95]. Depending on the number of grafts, the procedure can last from several hours to an entire day [95]. The special trichophytic wound closure is used to close the donor area; i.e., the surgeon trims the overlapping top of the wound edge with a scissor to about 1 mm, allowing the hair to grow over the scar, making it less visible [95]. Precise planning and well-honed surgical skills are mandatory to avoid complications. In some patients, however, a visible scar is unavoidable, especially in those who wear their hair short [95, 96].

In recent years, significant advances have been made in FUE techniques (see below), which are now generally preferred. As a result, the FUT technique is now used mainly in cases where the donor area will not allow sufficient graft numbers with the FUE technique, or if patients don’t intend to cut their hair short, such as women patients [95 - 97, 99 - 101].

#### 1.9.2.2 Follicular Unit Extraction (FUE) Technique

In recent years follicular unit extraction (FUE) has become an increasingly popular surgical technique for hair restoration, as it produces excellent aesthetic results with no risk of scars (Figure 7, right panel) [100 – 104]. The first step of FUE is to fully shave the donor area to 2 mm [95, 100]. Under local anesthesia the surgeon then extracts the follicular units one by one using a special punch device until the required number of grafts have been obtained [100, 101]. Excellent lighting is critical for performing an FUE [100]. Punch sizes range from 0.6–1.2 mm in diameter [100]. After punching, the follicular units are removed from the donor site with a special curved forceps [95, 100]. Follicular unit extraction is time intensive and therefore may require 2 or 3 sessions for completion [103, 104]. The extracted FU are stored in chilled saline until implantation [104]. While the grafts are carefully stored, incisions (slits) are prepared in the balding areas of the scalp for insertion of the follicular units [95]. The main advantage of FUE is that there is no visible scarring after the procedure and, with modern techniques, a high number of grafts can be obtained [95 -104]. The procedure, however, is very time consuming and patience is required on the part of both the physician and the patient.

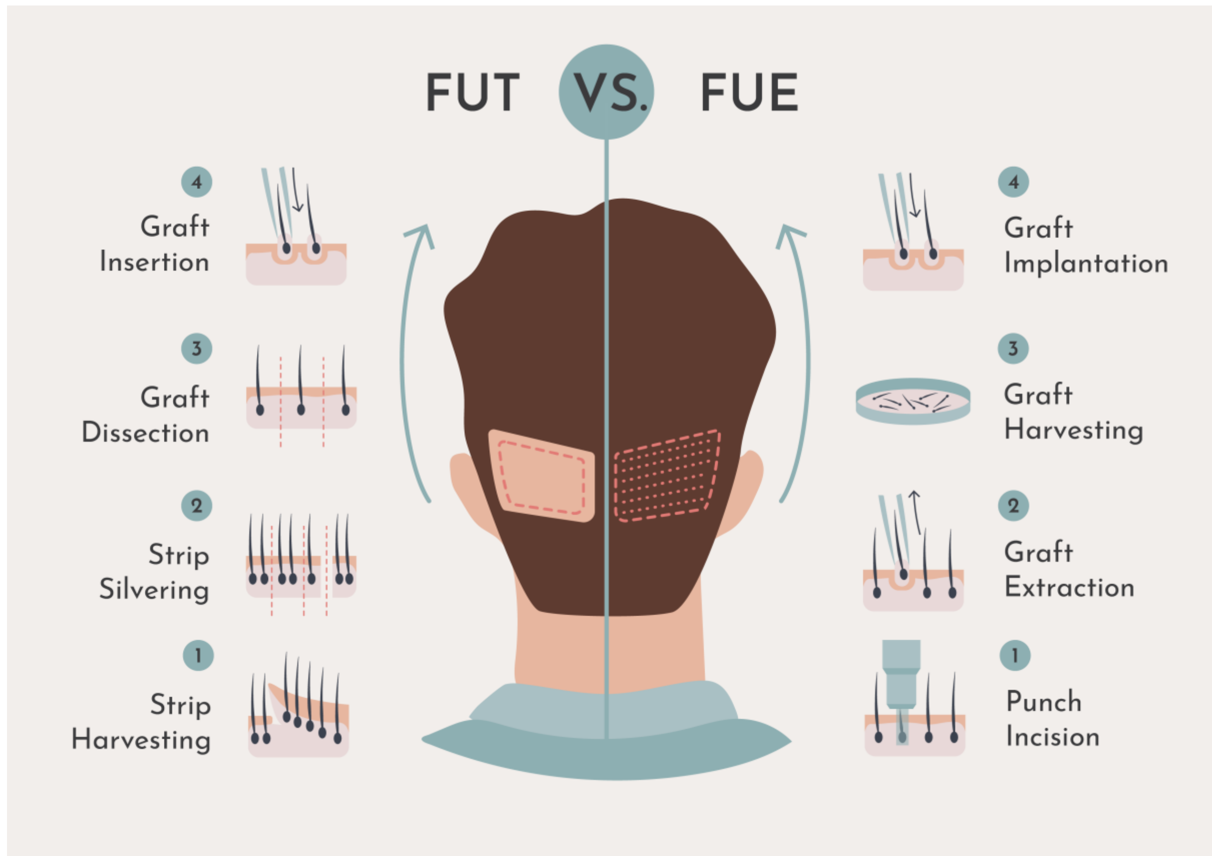


**Figure 7.** Schematic Representation of the Hair Transplantation Techniques. The figure was created with Adobe Illustrator [95 – 104].

### 1.9.2.3 Robotics in hair transplantation

In addition to the two above described procedures, which are performed by a skilled surgeon, there are also attempts to make use of robots in hair transplantation surgery [105, 106]; particularly for performing the punch incision. The hair robot is a machine that automatically extracts hair follicles from the back of the head [100]; however, removal of the hair follicles must still be carried out manually in the same way as in conventional FUE [100]. Furthermore, the slits and the insertion of the grafts also must be carried out manually by the surgeon [100]. In theory, use of a robot should ensure faster and more accurate removal of the hair follicles [105]. However, expenditure in terms of both time and money is very high [100]. Additionally, errors of the robot system include an imprecise planning procedure of the software, which can cause incorrectly determined extraction angles and potential transection of the hair follicles [100]. In order to minimize the transection rate of the hair follicles, very wide-lumen removal

punches are used. These are in turn tissue-damaging, however, and can lead to irreversible complications such as scarring in the removal area [100, 107]. Furthermore, due to the technical nature of the robot, only a small part of the scalp can be used as a donor area [100]. However, advance continue apace in the field of robotics and it will be exciting to see what new innovations will become available and what may be possible in the future of robotics [107 - 109].

### 1.9.3 Hair cloning

Hair cloning is a proposed technique for counting hair loss which is in the early stages of research. At this time, hair cloning is not available for public or commercial use [110 - 114]. Briefly, hair cloning may theoretically generate thousands of new hair follicles by multiplication in the laboratory of dermal papilla cells from only a few extracted hair follicles [110, 111, 113]. The cloned hair follicles may then be injected into the patient's balding areas, where they may stimulate new hair growth [110 -113]. This method could offer new hope, especially to people with increased baldness where medical treatment failed or hair surgery is not a viable option due to sparse donor hair. However, research is still ongoing at this time and the safety profile and efficacy of this method are as yet unknown [110 – 114].

## 1.10 Treatment options for women

As female AGA occurs not only due to the action of dihydrotestosterone but also dihydroepiandrosterone and androstendione the development of balding may be different but for treatment women have at their disposal the same treatment options as men. Still, there are some important differences which are described in the following sections.

### 1.10.1 Minoxidil

Minoxidil is the only FDA approved drug for female pattern hair loss [68]. It is available in 2% or 5% foam, gel, or solution with a twice daily recommended application [68, 115, 116]. Several studies have confirmed a positive effect of minoxidil on hair

regrowth in women [115, 116]. Side effects are the same as in men and include skin irritation, abnormal hair growth on the cheeks, skin burning or headache [68, 115, 116].

### 1.10.2 Finasteride and Dutasteride

Finasteride and Dutasteride are not approved for use in women [68]. Data in the literature regarding treatment of female pattern hair loss with these substances is controversial. Some trials, for example, have reported no improvement after finasteride use compared to placebo, whereas others reported improvement [115, 117,118]. A recent review, however, supported the potential use of finasteride and dutasteride for the treatment of female pattern hair loss, especially in postmenopausal-aged women [118]. Disadvantages are that both drugs are contraindicated during pregnancy, as they can lead to serious fetal malformations [118]. Females of childbearing age must be advised to use effective contraception when using these substances [68, 118]. More research is necessary to evaluate the safety profile and efficacy of these drugs in women.

### 1.10.3 Cyproterone acetate

Cyproterone acetate is a synthetic derivate of 17-hydroxyprogesterone and has antiandrogenic effects [68]. This compound, in combination with ethinylestradiol, is used as a contraceptive. According to some studies, this combination may also be used for the treatment of female pattern hair loss (FPHL) [68, 117]. Possible side effects are irregular periods, weight gain, loss of libido and depression [68, 117].

### 1.10.4 Spironolactone

Spironolactone blocks aldosterone and androgen receptors and works as a mild antiandrogen [68, 117]. Studies have revealed that Spironolactone prevents females from further hair shedding, but has no effect on hair regrowth in FPHL [68, 117]. A recent study has suggested the combination of low-dose oral minoxidil and spironolactone as a promising therapy [119].

### 1.10.5 17-alpha and 17-beta Estradiol

17-alpha- and 17-beta-estradiol are estrogen and 5α-reductase inhibitors [120]. These compounds can prevent hair loss when used topically [68, 120]. Similar to other alopecia drugs, they must be applied frequently to prevent hair loss.

### 1.10.6 Low level laser therapy

Low level laser therapy can be used as a treatment option in women with AGA [121]. Treatments must be done frequently over a longer period of time [121]. However, robust trials on the efficacy of low level laser therapy in women are notably lacking [117, 121].

### 1.10.7 Wigs and hair pieces

Wigs and hair pieces are a non-invasive treatment option that provide excellent cosmetic results and significant quality of life improvement, especially in those patients where medical treatment or hair restoration surgery is not a viable option [122].

### 1.10.8 Hair transplantation surgery

Since in men and women the same hair transplantation surgery techniques are used, see chapter 1.9.2.

## 1.11 Psychosocial impact of hair loss

A change or loss of hair can have an essential impact on an individual’s appearance [123]. While unaffected people often trivialize the negative impact of AGA, many men find it difficult to cope with AGA and sometimes suffer negative psychosocial consequences [123 -125]. Hair is linked with self-image, personal identity and style, not only to the individuals themselves but also in their perception by others [126, 127]. The desire of men to maintain a young and attractive appearance has increased in recent years and may be more important than in years before [128, 129]. Several studies have confirmed that men with hair loss are viewed as less attractive and that

visible hair loss impacts psychosocial interactions and self-image in a negative way [123 – 132]. Studies on physical attractiveness have revealed that hair is a key determinant of attractiveness, to the same degree as the mouth, eyes and nose [130]. Physical attractiveness is described as an interpersonal reciprocation that serves as a sign to gather information about a person [130]. In this regard, the appearance of one's hair impacts not only their optical attractiveness, but may also enhance or detract from perceived character traits [130]. Hair color is another aspect that influences the perception of others [133]. In this regard, researchers have identified sex-specific hair color preferences: men indicated that they preferred blondes and women preferred dark-haired men. Both sexes showed an aversion to persons of the opposite sex with red hair [133, 134]. Hair color may additionally be an indicator of age, since grey hair is associated with aging [130]. Other perceptions related to hair color and style include, for example, the perception that blondes are less intelligent or that excessively hairy men are more sexually potent [130 – 135]. Additionally, scruffy hair is often associated with negative social attitudes [130]. A study from 1980 revealed that women with facial make-up and hair care were not only viewed as more attractive, but were also more likely to be attributed with positive personality traits such as kindness and calmness [136]. Several reports have indicated that, in some cases, hair loss can lead to emotional distress, lower self-confidence, a feeling of physical unattractiveness or even depression [123 – 132]. One study revealed that non balding men could have higher success rates with women than balding men [137]. Younger men who believe their balding is progressive are apparently most distressed by AGA [131]. For the physician, it is important to recognize a patient's emotional response to alopecia and, if necessary, to refer the patient to a professional psychologist [131, 132].

# 2 Platelet-rich Plasma

## 2.1 Definition

Platelet-rich plasma (PRP) is a blood product containing a high concentration of thrombocytes (compared with whole blood) in a small volume of plasma. Autologous PRP is prepared by centrifugation of a patient's own peripheral blood [138].

## 2.2 Brief History of Platelet-rich Plasma

In the 1970s, platelet-rich plasma was reported for the first time in the field of hematology as a treatment for patients with thrombocytopenia [139]. In the 1980s and 1990s, the use of PRP expanded to the surgical disciplines [140]. Particularly in maxillofacial surgery, it was used for tissue regeneration and to promote healing [141]. Subsequently, PRP has been predominantly used to treat sports-related injuries such as muscle and tendon injuries or surgical augmentation of bones [142, 143]. Particularly in sports medicine, PRP has attracted great attention for its use in treating professional athletes who required rapid return to competition [139]. Although there have been many clinical studies regarding the use of PRP in sports-related injuries, the evidence is inconclusive. Several trials have reported no positive wound healing or regeneration promoting effects of PRP [143 – 146]. In the last decade, the use of PRP in cosmetic procedures such as skin rejuvenation has become increasingly popular [147]. Especially in the field of hair loss, PRP is increasing considered an effective and low side effect treatment option [147].

## 2.3 Biology of platelets

Platelets, also called thrombocytes, develop from the bone marrow and are best known for their central role in hemostasis [138, 139, 148]. Platelets are anucleate, discoid cells with a diameter of approximately 2 µm [138, 139]. The physiological circulating platelet count in the blood ranges from 150,000 to 400,000 platelets per microliter (µL) [138, 139]. Research has suggested immune modulatory and anti-inflammatory effects

of platelets [139, 148]. Clinical data indicate that the potential therapeutic effects of PRP is due mainly to a high content of bioactive compounds such as cytokines and growth factors (GF) which can affect inflammation, angiogenesis, stem cell migration, and cell proliferation [139, 147 - 152]. Platelets contain several growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epithelial growth factor (EGF), insulin-like growth factor 1 and 2 (IGF-1, IGF-2) (Table II) [139, 147 – 152].

**Table II. Specific characteristics of growth factors found in platelets**

| **Growth factor** | **Function** |
|---|---|
| VEGF | Increasing angiogenesis, tendon cell proliferation, type 1 collagen synthesis [147 – 152]. |
| FGF | Stimulation of angiogenesis, regulation of cell migration and proliferation, affects angiogenesis and satellite cell numbers [147 – 152]. |
| PDGF | Mitogenic action in mesenchymal stem cells and osteoblasts, endothelial cell replication, stimulation of collagen synthesis and protein synthesis [147 – 152]. |
| EGF | Stimulation of fibroblast and epithelial cell proliferation, Regulation and stimulation of extracellular matrix proteins, Strong stimulation of epithelial and mesenchymal cells [147 – 152]. |
| IGF-1,2 | Stimulation of protein synthesis, proliferation and differentiation of osteoblasts [147 – 152]. |

## 2.4 PRP preparation process

There are a large number of commercially available kits for preparation of PRP that allow application in a reproducible manner. The PRP preparation process, however, is always based on centrifugation [147, 152 - 155].

**1st Step (Blood collection):** At first the physician takes a sample of the patient's peripheral blood by venous puncture in a tube. To prevent the blood from clotting during the preparation process, most tubes contain an anticoagulant agent [138, 139, 152]. Common anticoagulant agents include sodium citrate, acid citrate dextrose, trisodium citrate and trisodium phosphate, and citrate [139, 152]. Citrate ions bind and deactivate calcium resulting in a stop of the coagulation process [138, 139, 152]. With the addition of anticoagulants, PRP is stable for approximately 8 hours after its preparation [138].

**2nd Step (Centrifugation):** Centrifugation of the blood sample separates PRP from platelet poor plasma (PPP) and red blood cell components, resulting in a supernatant of more concentrated PRP, which contains a significantly more platelets than in whole blood (Figure 8) [138, 139, 147, 152]. After centrifugation, there is a small thin layer between the RBC and PRP layers called the buffy coat which contains a high concentration of leukocytes (Figure 8) [138, 152]. A standard laboratory centrifuge or commercial systems can be used for the preparation. The final platelet concentration varies widely depending on the starting number of platelets and the force and time of centrifugation used for PRP preparation [152 – 154].

**3rd Step (Platelet activation):** Some protocols call for activation of platelets with calcium or thrombin after centrifugation [139, 152]. It is known that platelets are activated *in situ* by thrombin, calcium or trauma [152]. Activation of platelets results in the formation of a fibrin network and clot formation, a sequence of events which happens automatically when platelets come into contact with tissue [139, 152]. Due to natural activation of PRP, for example when injected into tissue, it seems doubtful that exogenous activation of PRP is necessary before application.

**4th Step (Clinical application):** Delivery of PRP within 10 minutes of preparation is advised when an activator is added. In an anticoagulated (inactivated) state, PRP is stable for approximately 8 hours after its preparation [138, 139, 152 – 154].

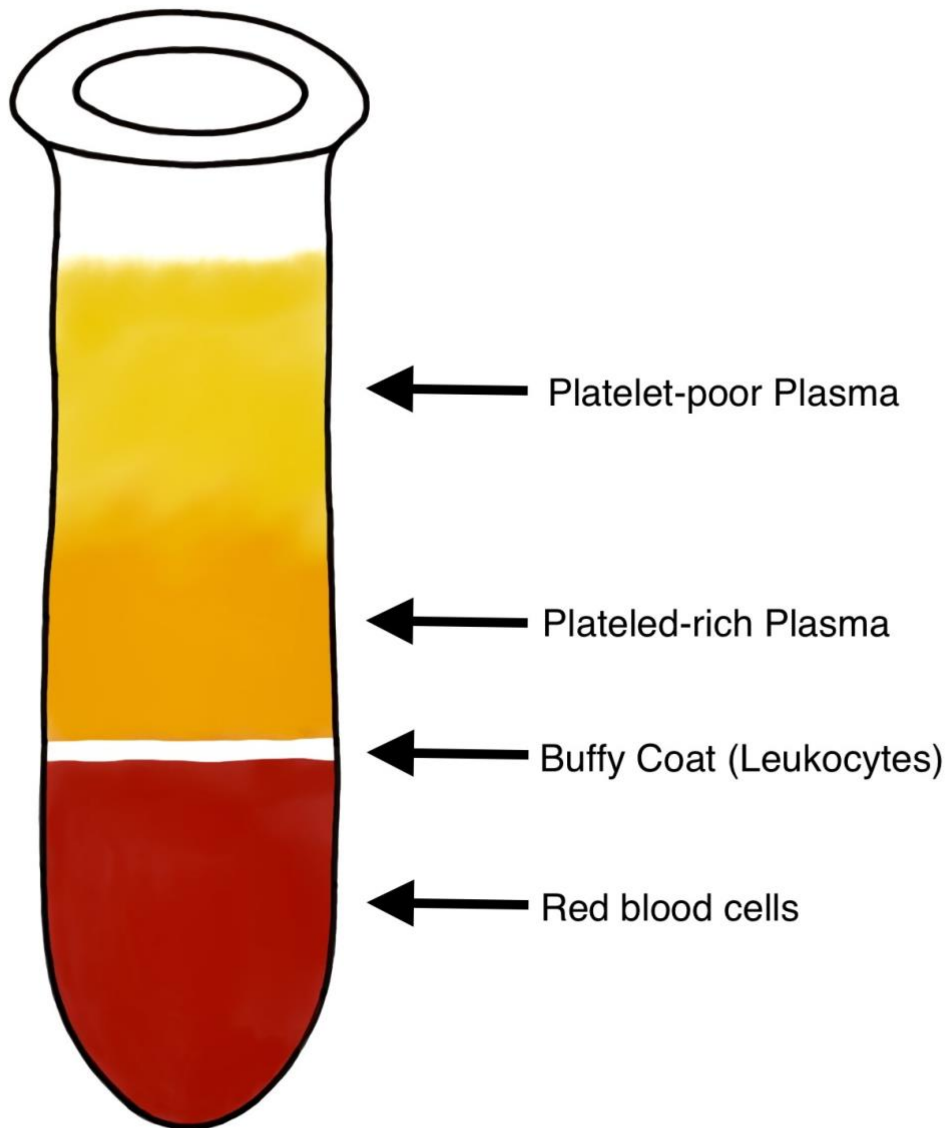


**Figure 8.** Separated blood components after centrifugation of whole blood. The figure was drawn with Procreate on Ipad [152 – 155].

## 2.5 PRP subtypes

According to the literature, depending on cell content and fibrin architecture, four subcategories of PRP can be defined (Table III). These subcategories depend on how the PRP is prepared. The foundation for the preparation of all subcategories, however, is centrifugation resulting in a high concentration of platelets [152 – 155].

There are four broad subcategories of PRP preparations as shown in Table III. Due to substantial differences in PRP preparation protocols and the wide variety of kits available [154, 155], however, two different PRP preparations falling within the same subcategory may not necessarily be identical. Inconsistencies in protocols include centrifugation force, spin process (single spin or double spin), presence of anticoagulant, starting blood volume, and final platelet count [154, 155]. Furthermore, it is unclear which PRP subcategory is best for use in different treatment indications. Further research is necessary to find a consensus regarding PRP preparation protocols.

**Table III. Subcategories of PRP**

| Subcategory | Description |
|---|---|
| Pure-Platelet rich plasma (P-PRP) | Composition of high platelet volume without leukocytes and a low-density fibrin network [153, 154]. |
| Leukocyte-rich PRP (LR-PRP) | In addition to a high volume of platelets, includes leukocytes with a low-density fibrin network [153, 154]. Leukocytes play key roles in the immune defense against pathogens [148, 153, 154]. The rationale of adding leukocytes to the PRP-product is to gain anti -inflammatory effects [152 - 154]. |
| Pure platelet-rich fibrin (P-PRF) | Also called leukocyte-poor platelet-rich fibrin, as the name suggests, is a composition of a high-density fibrin network without leukocytes [153]. These products have a very high, gel-like viscosity and therefore injections are not possible, they are commonly used as fibrin glues [153]. |
| Leukocyte- platelet-rich fibrin (L-PRF) | Composition of a high-density fibrin network together with leucocytes and a gel-like viscosity, usually applied as topical lubricant to promote wound healing [153, 154]. |

## 2.6 Therapeutic Rationale of PRP Therapies

PRP preparations have become an increasingly popular treatment modality for several medical indications. It is hypothesized that the growth factors discussed above are released by the platelets, thus promoting tissue repair and wound healing by stimulation of stem cell differentiation, synthesis of new connective tissue, and revascularization [140, 147, 155]. Although some studies have attempted to investigate the mechanism by which PRP promotes hair growth, it is not yet clear which mechanisms are involved [139, 140, 155]. One hypothesis is that growth factors released by PRP target hair follicles, thus inducing the proliferative anagen phase, as well as activating anti-apoptotic pathways and promoting angiogenesis to increase perifollicular vascularization and regeneration of hair follicles [147, 151].

## 2.7 PRP Applications in Aesthetic Medicine

### 2.7.1 Hair loss

Several studies have reported a hair growth promoting effect of PRP injections [156, 157]. Improvements have been reported in both male and female pattern hair loss, as well as alopecia areata [156 – 159]. The application of PRP to AGA treatment, however, is not without controversy. Some studies have reported no improvement of AGA following PRP treatment, which may possibly be due to the genetically determined progressive nature of the condition [157, 158, 160]. Obstacles to gaining more certainty regarding the benefit of PRP treatment of AGA include a lack of standardized treatment protocols in the field and many unknowns regarding the effect of co-treatments used in studies, such as minoxidil, hormones or microneedling in conjunction with PRP administrations [147, 156 – 160]. Research is ongoing to clarify critical aspects of PRP treatment in hair loss.

### 2.7.2 Skin Rejuvenation and Augmentation

It is proposed that PRP treatment can improve skin texture and elasticity and reduce wrinkles [147, 161 – 163]. It can be applied topically or with injections. Studies have revealed that, compared with laser treatments alone, PRP used in combination with laser resurfacing and microneedling treatments achieves better results [147, 161 –

163]. Microneedling creates small holes in the skin, which enhances PRP uptake. Skin biopsies revealed higher rates of collagen fibers and thicker elastic fibers when comparing PRP-treated versus saline-treated areas [161 – 163]. Successful augmentation of nasolabial folds, cheeks and forehead lines using PRP alone or in combination with hyaluronic fillers, also known as the “vampire facelift”, has also been reported [147].

### 2.7.3 Acne and Traumatic Scarring

Improvement in the appearance of scars has been reported after PRP treatment [147, 164]. In several trials, a decrease in erythema and an improvement in scar appearance, was observed in response to PRP injections [147, 161 – 164]. Improved skin elasticity and an increase in collagen production have also been reported [147, 164]. The combination of PRP with other treatments, such as laser therapy, has also been suggested to improve outcomes in the treatment of scars [147, 161 - 164]. The ideal number of treatment sessions is variable, depending on the treatment goal.

### 2.7.4 Striae Distensae

Striae distensae are atrophic dermal scars (stretch marks) that result from continuous stretching of the skin such as during pregnancy, weight gain or growth spurts [147]. Cosmetic improvements have been reported when combining intradermal radiofrequency and ultrasound devices with PRP administration [147, 162]. Biopsies after treatment revealed an increase in collagen density and elastic fibers. Furthermore, high patient satisfaction has been reported [147, 161 – 164].

## 2.8 Safety and side effects

No serious side effects of PRP treatment have been reported to date. Minor, but fully reversible, side effects may include swelling, erythema, minor bleeding and pain after treatment [160 – 164].

# 3 Study aim

We aimed to investigate the effect of PRP on hair growth in a single-center, blinded, placebo-controlled pilot-setting in male AGA subjects. The primary aim was to determine any changes in hair number or hair diameter of subjects between baseline and two follow up visits, as measured with the TrichoScan system. The secondary objective was to assess any clinical improvement, which was evaluated by an independent reviewer on a Likert scale and the Norwood-Hamilton scale using patient photographs. In addition, patient satisfaction was assessed by a survey. [160]

# 4 Materials and Methods

Study design and population

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval by the Ethics Committee of the Medical University of Graz (EK 28-576 ex 15/16). Informed consent was obtained from each participant. In this single-center, randomized, placebo-controlled, blinded pilot study thirty healthy male subjects with untreated AGA, aged 24-52 years, were enrolled in a 2:1 ratio between 2016 and 2019 (Table IV). Selected subjects had an AGA Norwood-Hamilton score of ≥ III (Table IV). Exclusion criteria were: previous or ongoing treatment for AGA (Finasteride, Minoxidil), previous hair transplantation, malignancy, hematological disorders, thyroid dysfunction, malnutrition, and other dermatological disorders contributing to hair loss. At intervals of 4 to 6 weeks five treatments were performed. Twenty subjects were treated with PRP ("verum" group) and 10 subjects were treated with physiological saline ("placebo" group). The subjects were blinded to treatment and did not know whether they received PRP or saline, therefore they were asked to wear goggles during the treatment sessions. Randomization of the subjects was performed using an online randomizer tool (randomizer.at). With a Nikon D300 12.3-megapixel camera standardized photographs of the affected AGA areas were taken in front of a grey photo wall in a fixed position (Figure 9 – Figure 14). Hair density documentation using a computer-assisted method (TrichoScan technology) for determining hair density and hair root status was performed (Figure 15). For that purpose macroscopic images of the reference areas (Figure 15) were taken with a special microscope camera (Figure 16) at three time-points – at baseline (BL) and at follow-up visits 4 weeks and 6 months after the last treatment (FU1 and FU2, respectively). A point was tattooed in a shaved area of the scalp with an 18-gauge cannula and sterile black ink before the first treatment, to facilitate precise identification of the treated areas at the clinical control examinations (Figure 17). [160]

**Table IV. Baseline characteristics**

| Variables | Verum (n=19) | Placebo (n=9) |
|---|---|---|
| **Age, years** | 29 (25-52) | 29 (24-33) |
| **Sex, male** | 19 (100%) | 9 (100%) |
| **Norwood Hamilton** | | |
| III | 10 (52.6%) | 2 (22.2%) |
| IV | 4 (21.1%) | 4 (44.4%) |
| V | 3 (15.8%) | 3 (33.3%) |
| VI | 1 (5.3%) | |
| VII | 1 (5.3%) | |
| **Hair number, per cm$^2$** | 59.0 (15.0 – 133.0) | 30.0 (11.0 – 92.0) |
| **Hair diameter, μm** | 66.0 (47.5 – 81.9) | 64.6 (55.8 – 72.0) |

Reproduced from Gressenberger P. *et al*. Platelet-rich plasma for androgenetic alopecia treatment - A randomized placebo-controlled pilot study [160]. Results are presented either as median (range) or as n (%). Reproduced with permission of “Acta Dermato-Venereologica”.

**Figure 9.** One subject from the PRP group at baseline.

**Figure 10.** The same subject from the PRP group 4 weeks after the last treatment.

**Figure 11.** The same subject from the PRP group 6 months after the last treatment.

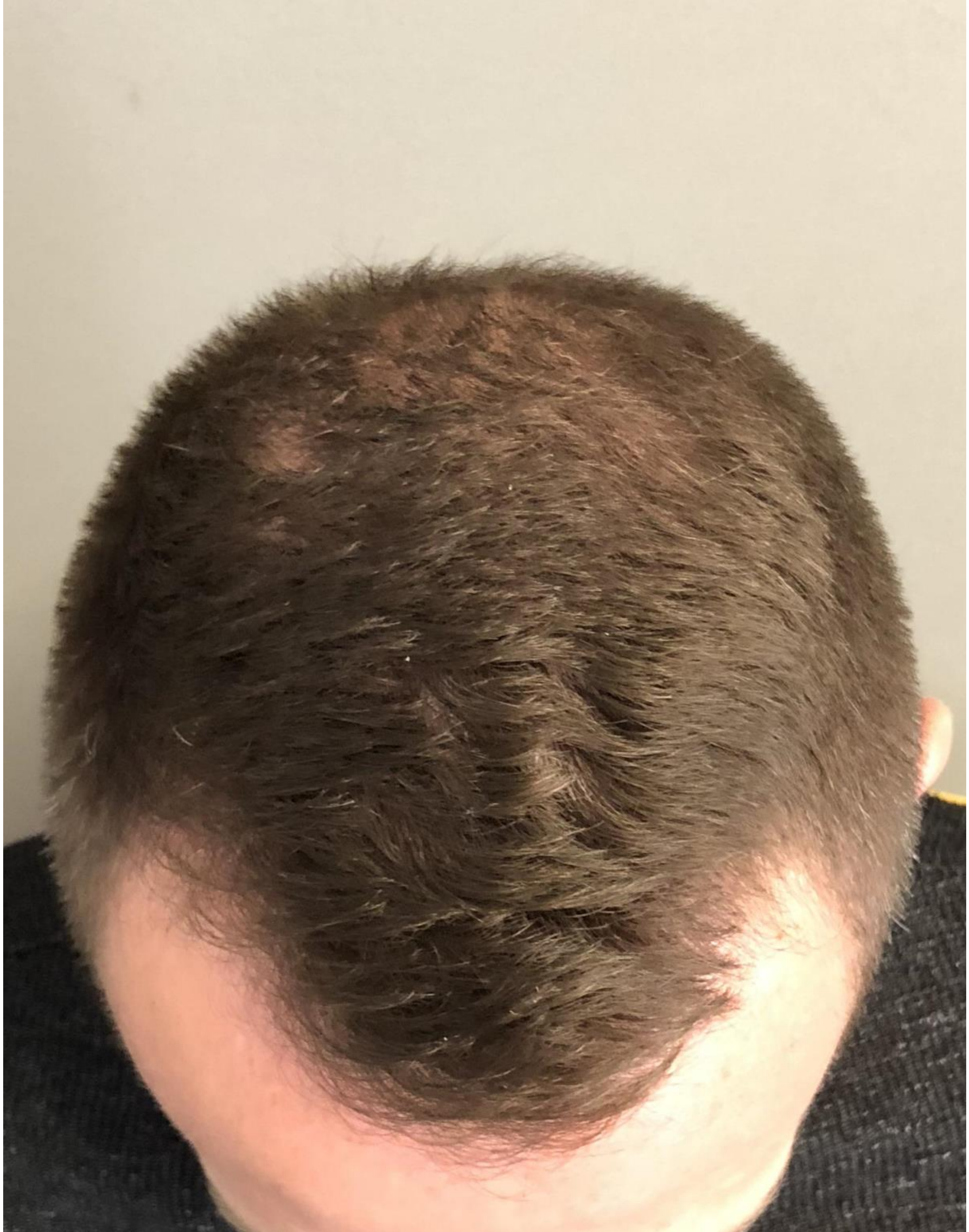

**Figure 12.** One subject from the placebo group at baseline.

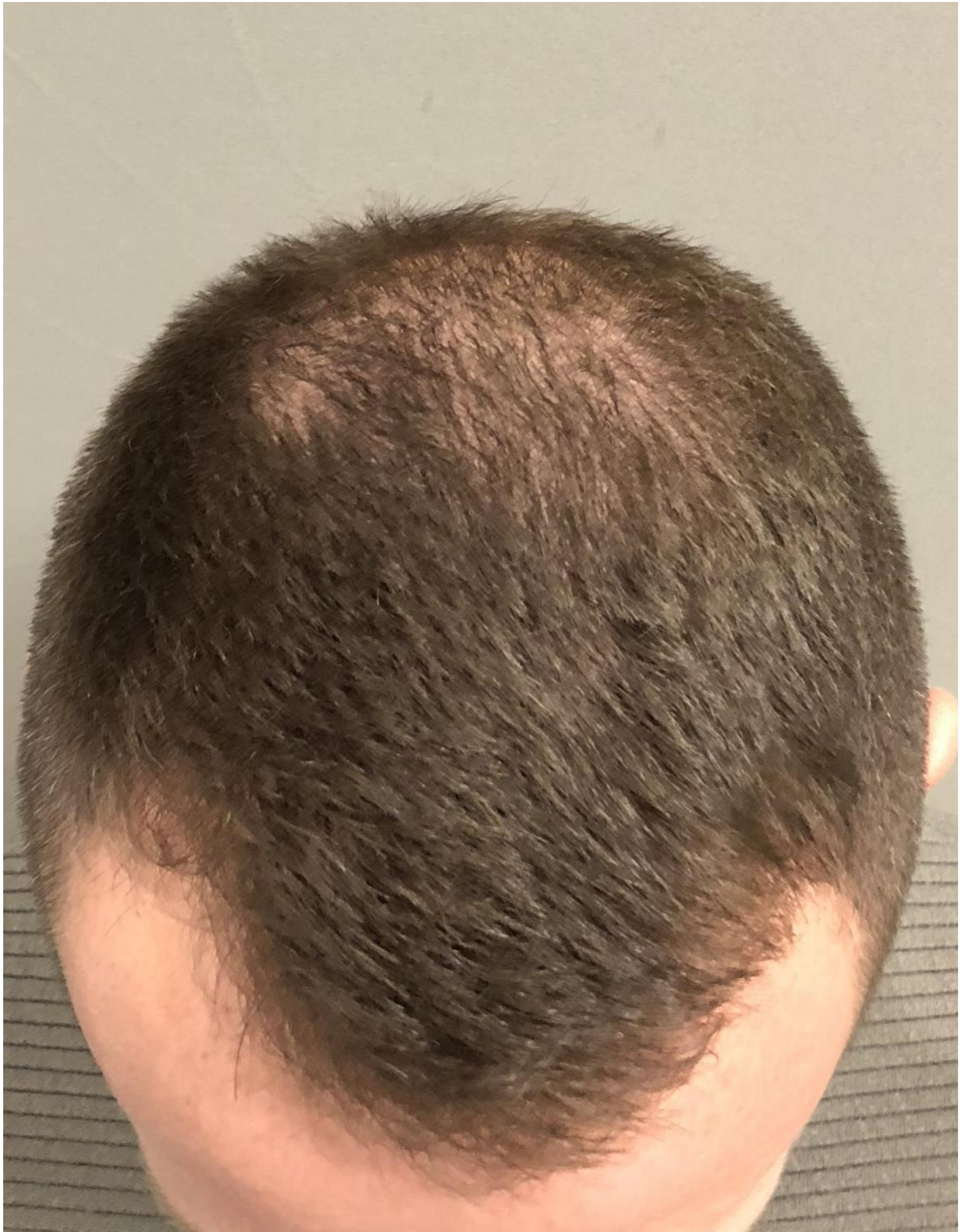

**Figure 13.** The same subject from the placebo group 4 weeks after the last treatment.

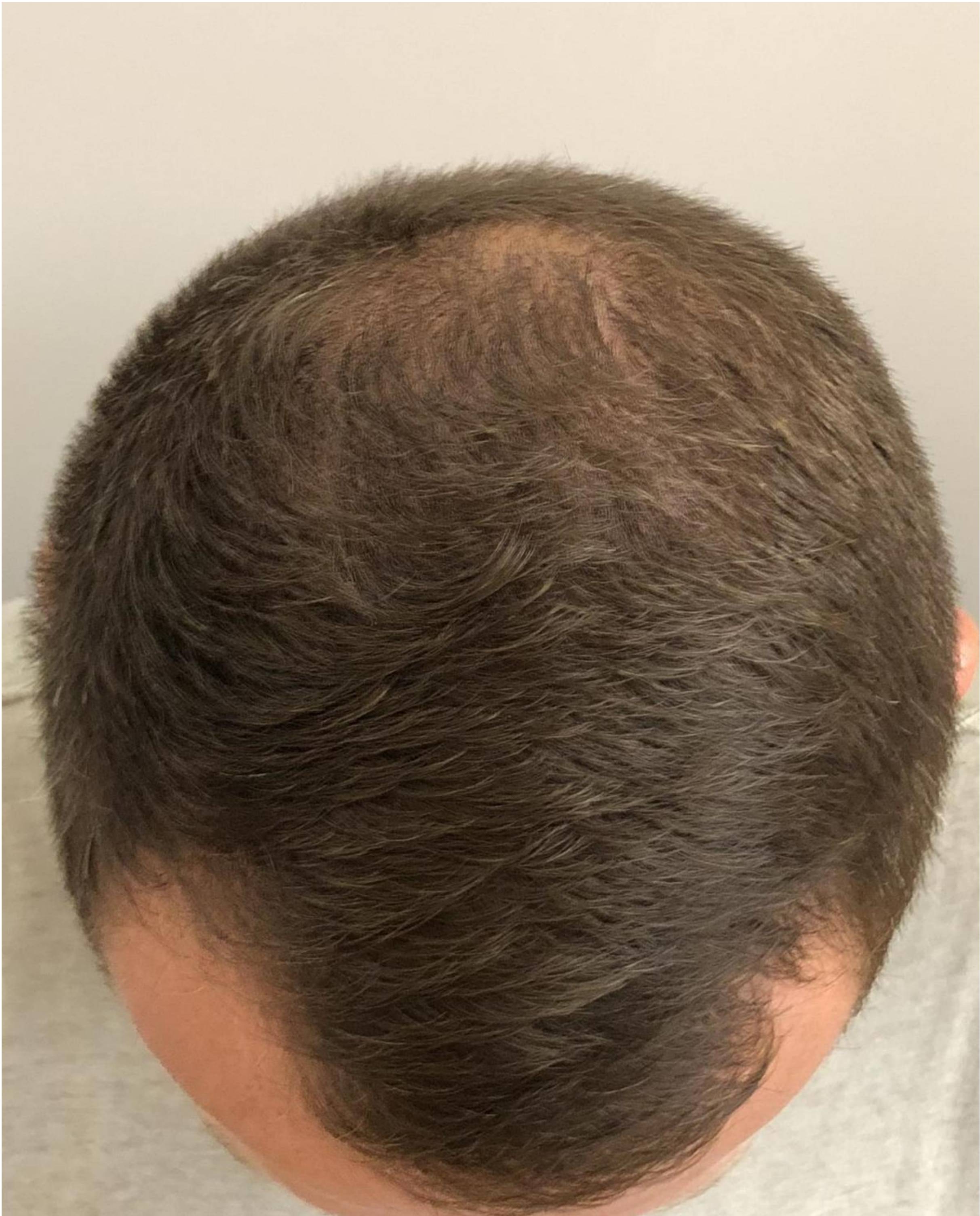

**Figure 14.** The same subject from the placebo group 6 months after the last treatment.

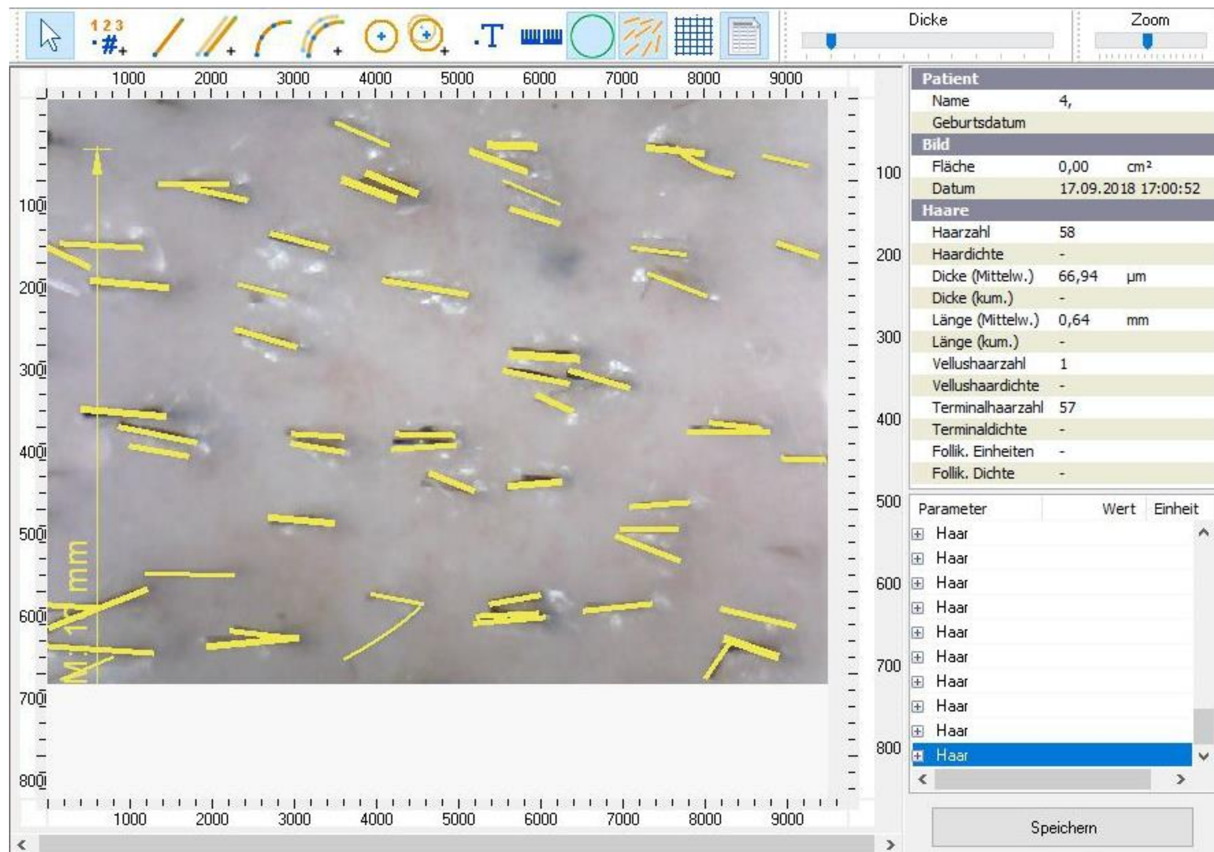


**Figure 15.** Trichoscan measurement at the reference area.

**Figure 16.** Macroscopic images of the reference areas were taken with a special microscope camera.

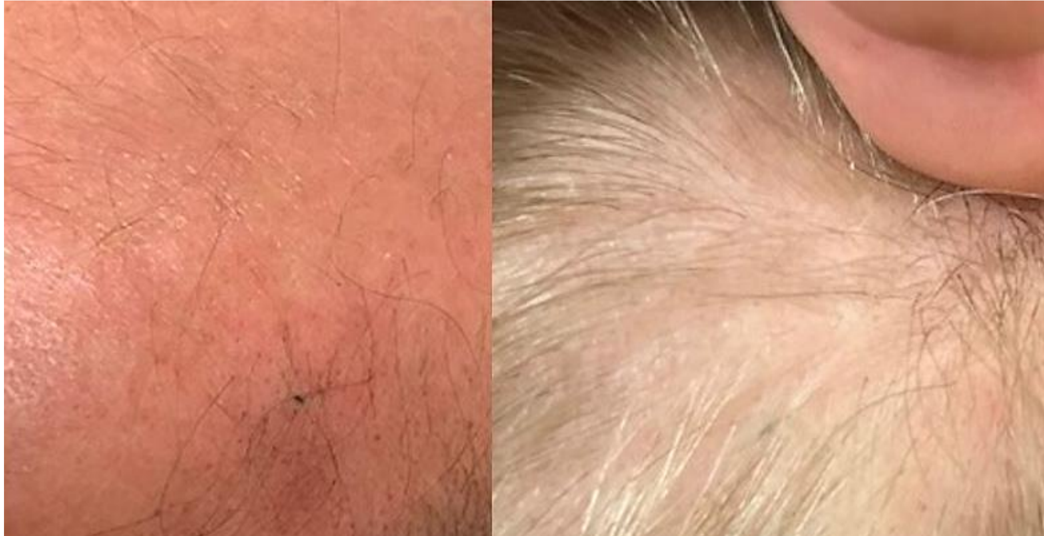

**Figure 17**. A point was tattooed in the scalp with an 18-gauge cannula and sterile black ink before the first treatment. This allowed easy identification of the reference areas for measuring at the follow up visits.

Study procedure

Using Yes” PRP kits (Figure 18) 20 ml of each subject’s blood was collected in a syringe containing sodium citrate to stop clotting (Figure 19), at each treatment. Platelet poor plasma (PPP) and red blood cells were removed from the blood, centrifuging at 2800 rpm for 9 minutes (single spin procedure), and the resulting PRP was extracted into a tube, which was also included in the kit (Figure 18, Figure 19). During the same session, depending on the degree of AGA, about 3 to 4 ml of the concentrated PRP or saline, respectively, was used to deliver 0.1 ml injections intradermal into the affected areas of the scalp. The injections were delivered with a 30 gauge needle at approximately 1 cm intervals in a grid-like pattern. [160]

**Figure 18.** Yes PRP-Kit and the individual components of the kit.

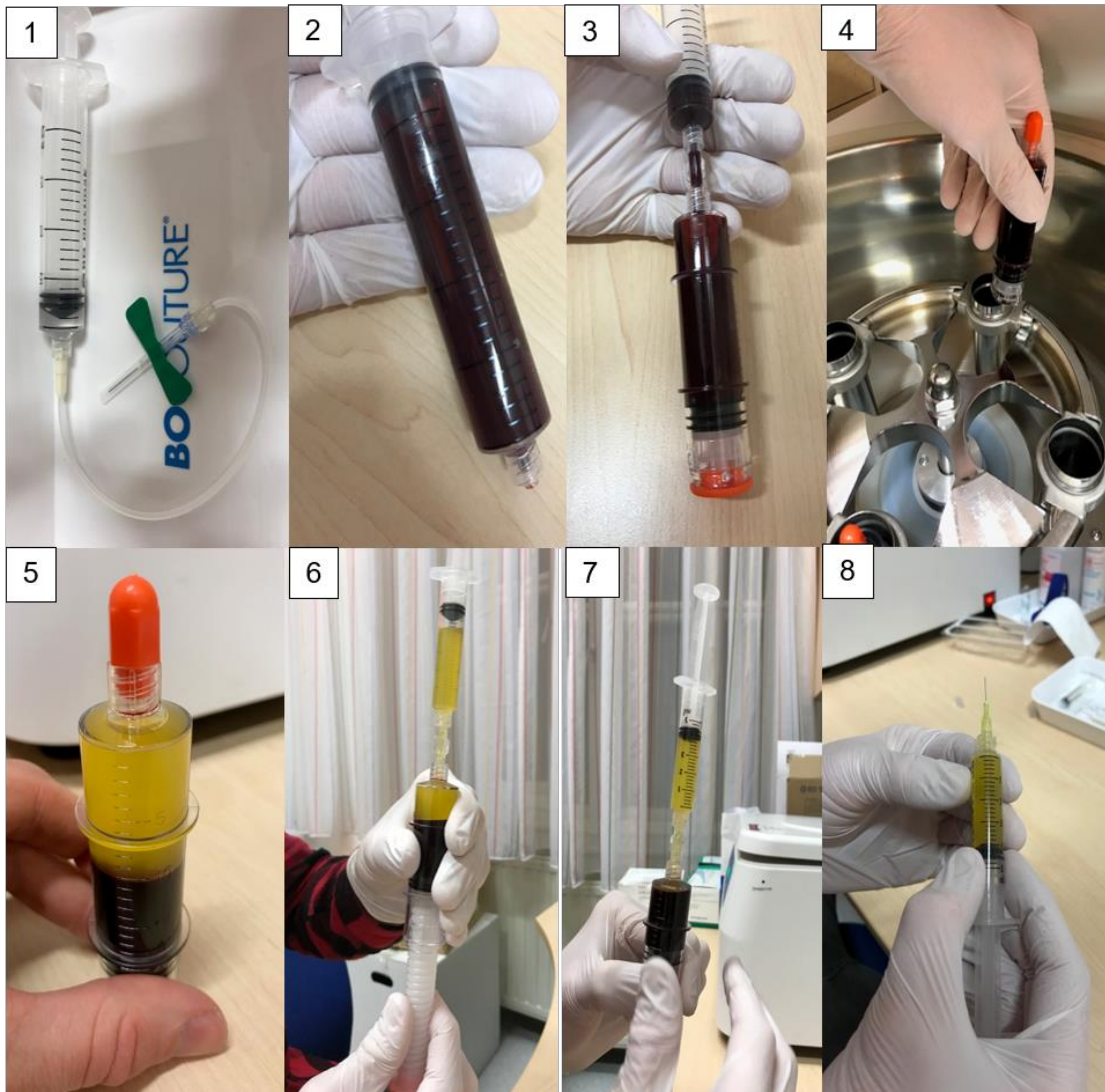


**Figure 19.** Different steps of the PRP manufacturing process.

(1) Syringe containing 2 ml of sodium citrate connected with a butterfly needle. (2) 20 ml of the patient's collected blood (3) were transferred into a piston like tube (4), which was subsequently centrifuged at 2800 rpm for 9 minutes. (5) Centrifugation separated the red blood cells from the plasma. (6) The platelet poor plasma (about 5-6 ml at the top) was discarded resulting in approximately 3-4 ml PRP (middle layer) (7) which was extracted into a syringe. (8) PRP ready for injection.

Outcome measures

The main outcome measures were hair number per square centimeter and hair diameter (µm), both measured using the TrichoScan system. Briefly, images of a shaved area of the scalp (1 cm²) were taken with a digital microscope camera and evaluated with the TrichoScan software for determination of the essential parameters of hair growth. A blinded investigator who was not involved in the enrollment and treatment procedure evaluated the TrichoScan measurements at baseline and at each of the follow-ups. The secondary objective was the clinical improvement, which was also evaluated by an independent reviewer using patient photographs. For this purpose, changes from baseline were evaluated on a five-point Likert-type scale (much worse (1), somewhat worse (2), no change (3), somewhat better (4), much better (5)) at each of the follow-ups. Furthermore, subjects clinical appearance was classified according to the Norwood-Hamilton scale. Therein, male pattern baldness is classified in seven stages, some with several sub-stages, and higher stages corresponding to higher levels of baldness. Additionally, subject satisfaction was assessed by survey after the last treatment. We asked for the perceived level of pain, perceived clinical improvement, willingness to pay for each procedure, and whether they would recommend the treatment to others suffering from AGA. [160]

Statistical Analysis

The study was carried out as a pilot study in order to test platelet-rich plasma as a novel therapy for AGA. Due to the sample size of this study, only large effect sizes (Cohen's d: 1.12) could be detected with a sample size of 30 (power 80%, two-sided significance level 5%). The two primary outcomes, hair number and hair diameter, were summarized in each group at the three time-points using standard statistical measures. Differences between baseline and each of the follow-up measurements were calculated. Further, differences between the groups regarding these calculated changes from baseline were determined. Due to the non-normal nature of the data, which was assessed visually using quantile-quantile plots, the data is presented as medians and ranges (minimum to maximum) and Mann-Whitney U-tests were performed. The results of visual improvement on a five-point Likert-type scale, Norwood-Hamilton-Scale and patient satisfaction are presented as absolute and relative frequencies. Differences between the groups were determined by Mann-Whitney U-Test for visual improvement on the Likert-scale and Fisher's exact test for

the Norwood-Hamilton Scale and items of satisfaction. Missing values are due to the refusal of some subjects to submit to the TrichoScan follow-up examination or technical problems (e.g. picture files or TrichoScan measurements could not be opened). A p-value of $<0.05$ was considered statistically significant. All statistical analyses were performed using R version 3.6.1. [160]

# 5 Results

Twenty-eight of the 30 enrolled subjects (93%) completed all treatments. Two subjects dropped out of the study before completion. One subject dropped out of the verum group after the fourth treatment and did not take part in the follow-up examinations. One subject dropped out of the placebo group after the first treatment and did not appear for further scheduled visits. The following report is based on the 28 participants that completed all treatments. [160]

Hair number

The median (range) hair number per square centimeter at baseline (BL) was 59.0 (15.0 – 133.0) in the treated group and 30.0 (11.0 – 92.0) in the placebo group (Table IV). At the first follow-up visit 4 weeks after the last treatment (FU1), differences to baseline were -6.5 (-38.0 - 4.0) in the treated group and -9.0 (-15.0 – 2.0) in the placebo group; these differences were not statistically significant (p=0.817) (Table V) (Figure 20). At the second follow-up visit 6 months after the last treatment (FU2), differences to baseline were -9.0 (-27.0 – 8.0) in the treated group and -12.0 (-30.0 – 3.0) in the placebo group; these differences were also not statistically significant (p=0.366) (Table V) (Figure 20). [160]

Hair diameter

The median (range) hair diameter at baseline (BL) was 66.0 (47.5 – 81.9) micrometers in the treated group and 64.6 (55.8 – 72.0) in the placebo group (Table IV). At the first follow-up visit 4 weeks after the last treatment (FU1), differences to baseline were 1.7 (-20.5 – 14.2) in the treated group and 1.1 (-7.9 – 9.6) in the placebo group; these differences were not statistically significant (p=0.523) (Table V) (Figure 20). At the second follow-up visit 6 months after the last treatment (FU2), differences to baseline were -0.6 (-18.7 – 12.9) in the treated group and -0.4 (-2.1 – 12.2) in the placebo group; these differences were also not statistically significant (p=0.630) (Table V) (Figure 20). [160]

**Table V. Outcome parameters, follow-up measurements and differences to baseline**

| | **Verum (n=19)** | **Placebo (n=9)** | **p-value** |
|---|---|---|---|
| **Hair number per cm$^2$** | | | |
| **FU1** | 46.5 (8.0 – 127.0) | 20.0 (2.0 – 94.0) | |
| **BL-FU1** | -6.5 (-38.0 – 4.0) | -9.0 (-15.0 – 2.0) | 0.817 |
| **FU2** | 54.0 (12.0 – 133.0) | 18.0 (0.0 -95) | |
| **FU2-BL** | -9.0 (-27.0 - 8.0) | -12.0 (-30.0 – 3.0) | 0.366 |
| **Hair diameter µm** | | | |
| **FU1** | 66.4 (44.5 – 74.8) | 67.0 (47.9 – 74.2) | |
| **BL-FU1** | 1.7 (-20.5 – 14.2) | 1.1 (-7.9 – 9.6) | 0.523 |
| **FU2** | 64.5 (34.2 – 74.8) | 67.9 (60.8 – 73.1) | |
| **FU2-BL** | -0.6 (-18.7 -12.9) | - 0.4 (-2.1 – 12.2) | 0.630 |

Reproduced from Gressenberger P. *et al*. Platelet-rich plasma for androgenetic alopecia treatment - A randomized placebo-controlled pilot study [160]. Results are presented either as median (range). Differences between the groups were assessed with the Mann-Whitney U-test. BL = baseline, FU1 = follow up 1, FU2 = follow up 2, BL-FU1= difference from baseline to follow up 1, BL-FU2= difference from baseline to follow up 2. Used with permission of “Acta Dermato-Venereologica”.

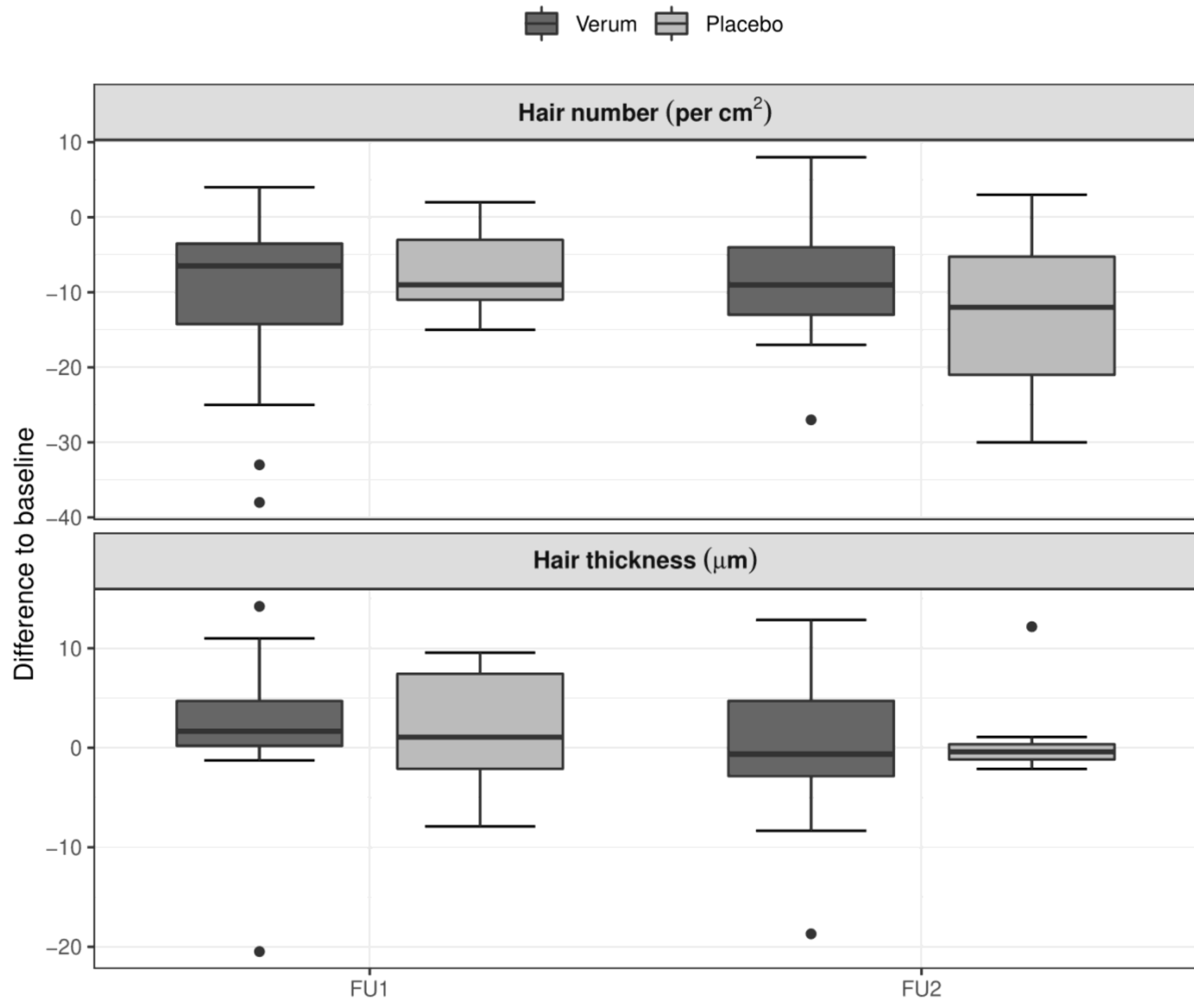


**Figure 20.** Box plots showing the main outcome measures: hair number (per square centimeter) and hair diameter (µm) for both groups. Differences between baseline and each of the follow-up measurements were calculated. No statistically significant differences between the two groups regarding these calculated changes from baseline were found.

Visual improvement on a five-point Likert scale

None of the patients was scored “much better” compared to baseline at either of the two follow-up visits. At the first follow up visit (FU1), in the verum group four subjects (22.2%) were rated “somewhat worse“, eleven subjects (61.1%) were rated “no change“ and three subjects (16.7%) were rated “somewhat better” (Table VI). In the placebo group, four subjects (44.4%) were rated “somewhat worse“, two subjects (22.2%) were rated “no change“, and three subjects (33.3%) were rated “somewhat better“ (Table VI). [160]

At the second follow-up visit (FU2), four subjects (23.5%) were scored “much worse” compared to baseline in the treated group, whereas one subject (14.3%) was scored “much worse” in the placebo group (Table VI). In the treated group, seven subjects (41.2%) were rated “somewhat worse”, three subjects (17.6%) were rated “no change”. and three subjects (17.6%) were rated “somewhat better” (Table VI). In the placebo group, 4 subjects (57.1%) were rated “no change” and two subjects (28.6%) were rated “somewhat better” (Table VI). No statistically significant differences between the groups were observed at either follow-up (p=0.824 and p=0.131, respectively) (Table VI). [160]

**Table VI. Outcome parameters of visual improvement evaluated on a five-point Likert scale**

| | | Verum (n=19) | Placebo (n=9) | p-value |
|---|---|---|---|---|
| **Likert scale** | | | | |
| **FU1** | Somewhat worse | 4 (22.2%) | 4 (44.4%) | 0.824 |
| | No change | 11 (61.1%) | 2 (22.2%) | |
| | Somewhat better | 3 (16.7%) | 3 (33.3%) | |
| **FU2** | Much worse | 4 (23.5%) | 1 (14.3%) | 0.131 |
| | Somewhat worse | 7 (41.2%) | | |
| | No change | 3 (17.6%) | 4 (57.1%) | |
| | Somewhat better | 3 (17.6%) | 2 (28.6%) | |

Reproduced from Gressenberger P. *et al*. Platelet-rich plasma for androgenetic alopecia treatment - A randomized placebo-controlled pilot study [160]. Results are presented as n (%). Differences between the groups were assessed with Mann-Whitney U-Test. FU1 = follow up 1, FU2 = follow up 2. Used with permission of “Acta Dermato-Venereologica”.

Visual improvement according to the Norwood-Hamilton scale

At baseline, in the verum group 10 subjects were rated Norwood-Hamilton stage III (52.6%), 4 subjects stage IV (21.1%), 3 subjects stage V (15.8%), and one subject each stage VI and VII (5.3%) (Table IV). At baseline, in the placebo group, 2 subjects were rated stage III (22.2%), 4 subjects were rated stage IV (44.4%), and 3 subjects were rated stage V (33.3%) (Table IV).

At the first follow-up visit, two subjects (11.1%) had a higher score (i.e. a worse classification) than at baseline in the verum group and one (11.1%) in the placebo group (Table VII) (Figure 21). At the second follow-up visit, six subjects (31.6%) were scored higher than at baseline in the treated group, whereas one (12.5%) was scored higher in the placebo group (Table VII) (Figure 21). Since there were no improvements (i.e. a lower score) in either group at both follow-up visits, the remaining subjects showed no noticeable change**.** There were no statistically significant differences between the groups at either follow-up (p=1.000 and p=0.633, respectively) (Table VII).

**Table VII. Outcome parameters of visual improvement according to the Norwood-Hamilton scale**

| | | **Verum (n=19)** | **Placebo (n=9)** | **p-value** |
|---|---|---|---|---|
| **Norwood-Hamilton** | | | | |
| **FU1** | No change | 16 (88.9%) | 8 (88.9%) | 1.000 |
| | Worsening | 2 (11.1%) | 1 (11.1%) | |
| **FU2** | No change | 13 (68.4%) | 7 (87.5%) | 0.633 |
| | Worsening | 6 (31.6%) | 1 (12.5%) | |

Results are presented as n (%).Differences between the groups for this outcome were determined by Fisher's exact test. BL = baseline, FU1 = follow up 1, FU2 = follow up 2.

**Figure 21.** Bar plots showing that there were no improvements (i.e. a lower score) according to the Norwood-Hamilton scale in both groups at either of the two follow-up visits.

Subject satisfaction survey results

In the verum group, 13 subjects (68.4%) rated the clinical outcome at the end of the study as better, while 6 subjects (31.6%) did not notice any change. In the placebo group, 4 subjects (44.4%) reported an improvement, while 4 subjects (44.4%) did not notice any change, and 1 (11.1%) subject rated a worsening of hair loss. Most subjects reported mild to moderate pain during treatments, however, only one subject in the placebo group reported severe pain. In the verum group, the majority of subjects (14, 73.7%) stated that they would be willing to pay for the treatments, while 5 subjects (26.3%) stated they would not. In the placebo group, 5 subjects (55.6%) stated that they would be willing to pay for the treatments and 4 subjects (44.4%) stated that they would not be willing to pay anything for the treatments. Sixteen subjects in the verum group (84.2%) versus five subjects in the placebo group (55.6%) would recommend the treatment for other individuals suffering from AGA. None of these differences were statistically significant (Table VIII). [160]

**Table VIII. Subject satisfaction at end of study**

| Variable | Verum (n=19) | Placebo (n=9) | p-value |
|---|---|---|---|
| **Pain** | | | |
| **severe** | 0 (0%) | 1 (11.1%) | 0.482 |
| **moderate** | 10 (52.6%) | 4 (44.4%) | |
| **mild** | 9 (47.4%) | 4 (44.4%) | |
| **Clinical outcome** | | | |
| **much better** | 3 (15.8%) | 0 (0%) | 0.272 |
| **somewhat better** | 10 (52.6%) | 4 (44.4%) | |
| **no change** | 6 (31.6%) | 4 (44.4%) | |
| **somewhat worse** | 0 (0%) | 1 (11.1%) | |
| **Willing to pay** | | | |
| **100-200 €** | 1 (5.3%) | 2 (22.2%) | 0.135 |
| **up to 100€** | 13 (68.4%) | 3 (33.3%) | |
| **nothing** | 5 (26.3%) | 4 (44.4%) | |
| **Recommendation** | | | |
| **yes** | 16 (84.2%) | 5 (55.6%) | 0.165 |
| **no** | 3 (15.8%) | 4 (44.4%) | |

Reproduced from Gressenberger P. *et al*. Platelet-rich plasma for androgenetic alopecia treatment - A randomized placebo-controlled pilot study [160]. Results are presented as n (%). Group differences were assessed using Fisher’s exact test. Used with permission of “Acta Dermato-Venereologica”.

No serious adverse events were reported during or after treatment in either group. Common, but fully reversible, side effects included swelling, redness, minor bleeding in treated areas, hematoma and pain (Figure 22, Figure 23). [160]

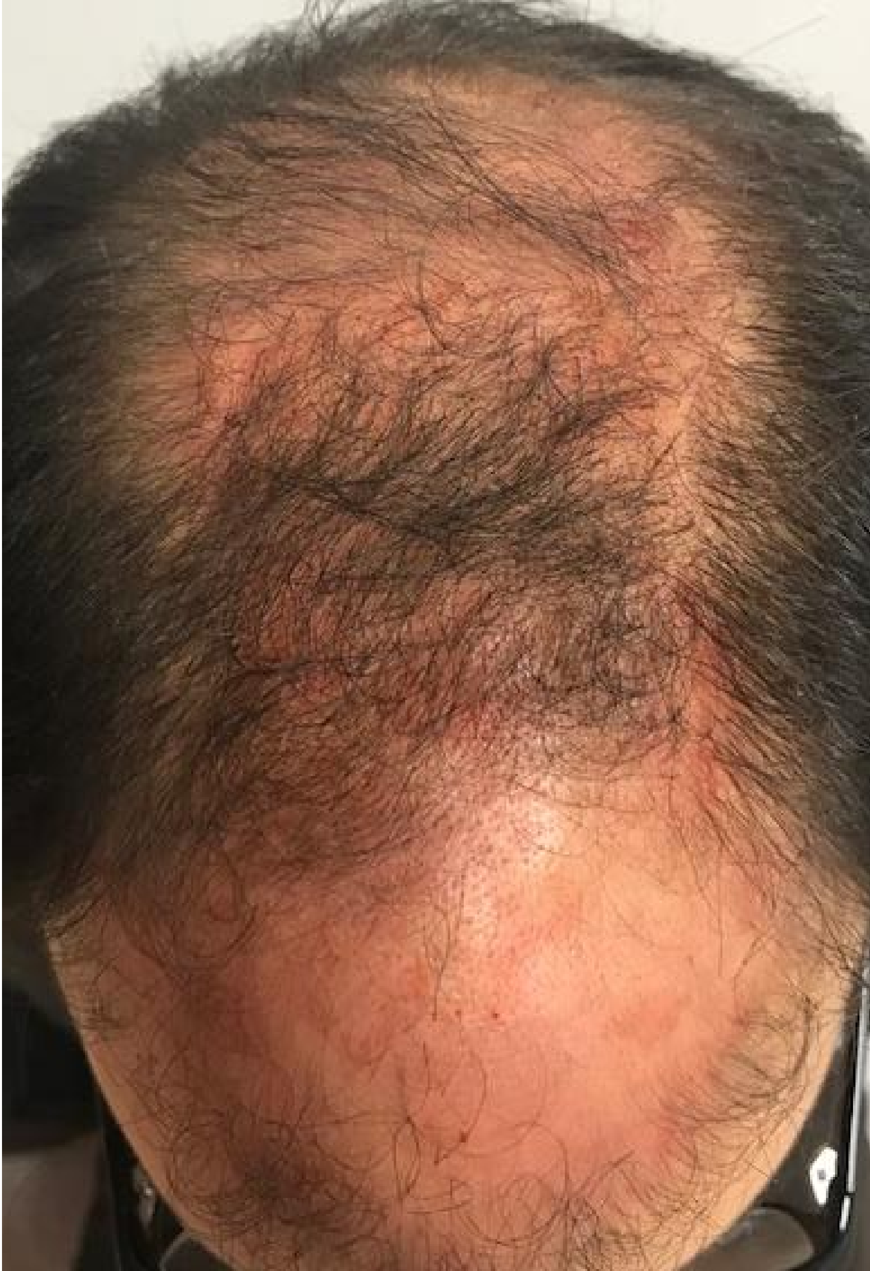

**Figure 22.** Side effects including swelling and redness after treatment.

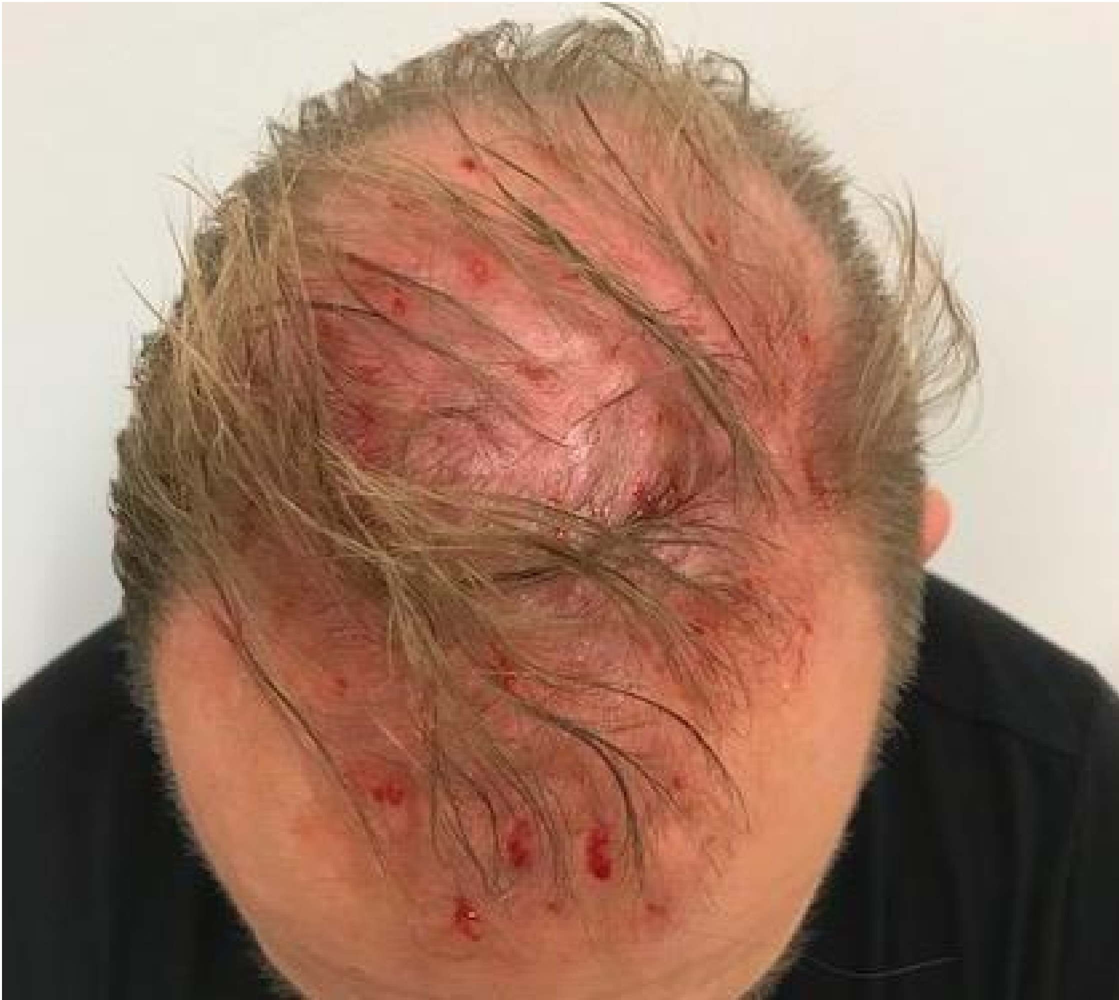

**Figure 23.** Side effects including minor bleeding after treatment.

# 6 Discussion

Hair loss is an issue of immense popular interest [165]. Although the general public may consider AGA to be part of the normal aging process, many men find it difficult to cope with AGA and sometimes even suffer in psychosocial terms [123 – 125, 160]. Voluminous hair is associated with health and youthfulness and is closely linked with personal identity and style [125 – 128, 160]. The desire of men to maintain a vital and attractive appearance has increased in recent years and may have more significance than in decades before [128 – 132]. Although several successful over-the-counter hair loss products are available to the public, only two drugs have been clinically approved for the treatment of AGA [166]. Despite their approval, minoxidil and finasteride do not work for everyone with AGA and can have side effects, some of which can persist after cessation of the drug [76, 77, 166]. Topical minoxidil is generally safe with very rare side effects. Finasteride, however, is associated with a phenomenon called post finasteride syndrome (PFS), a persistent undesirable effect after cessation of finasteride, including erectile dysfunction and depression [76, 77]. Although there are no predictive factors for the development of post finasteride syndrome, Trüeb *et al*. have suggested that patients with a medical history of depression may be at increased risk for PFS [167]. The incidence and prevalence of AGA as well as the demands and expectations of patients for hair loss treatments are very high. In this regard, it is no surprise that millions are spent on hair loss solutions every year letting patients in hope of being able to stop their hair loss [34, 35, 168 -170]. Over-the-counter products seem to be very attractive patient's first treatment choice, since they are easy to obtain and do not require the consultation of a doctor. Supportive evidence regarding efficacy of these products is lacking, however [168 – 170]. Despite this lack of evidence, patients frequently buy and use them, reflecting the high expectations placed on these products, which may be based mainly on aggressive marketing strategies [168 – 175]. A lack of treatment options is a common clinical complaint of AGA patients [160]. Although hair transplantation generally provides excellent and permanent results, because of its invasive nature and high cost, it is not a viable option for many patients [160]. Physicians are aware of these problems and therefore try to find new effective treatment options to offer patients better outcomes. Primarily used in maxillofacial surgery and in the field of sports-medicine such as muscle injuries, tendon repairs or surgical augmentation of bones, in recent years, PRP has become an increasingly popular treatment modality for various dermatological and aesthetic indications. These

indications include facial rejuvenation, treatment of wrinkles and acne scarring, fat grafting and hair restoration [139, 142, 143, 147, 160, 176]. Numerous clinical trials have reported the promotion of hair regrowth by PRP, indicating that PRP may offer hope to affected patients and to the doctors who treat them [160, 176 - 198]. Regardless of the indication, the studies have one thing in common, the best results observed to date have been from methodologically weak trials [185 – 197], giving reason for caution when drawing conclusions [160, 198]. More robust data generated from randomized controlled trials is notably lacking [160, 176 – 201]. In this regard, results from our blinded, placebo-controlled pilot study, wherein no hair growth promoting effect of PRP was observed, are rather sobering [160]. There are many factors which may have influenced the outcome of our trial in comparison to others, however, due to differences in the design of those studies, a direct comparison of their results is difficult [160, 176 – 201]. In fact, no general consensus exists in the field with regard to several potentially critical aspects of PRP treatment for hair restoration [156, 160, 198 -201]. These aspects include the method used for preparing PRP for injection, which characteristics of the PRP itself are critical, optimal number and frequency of treatments, best mode of administration, and optimal volume of PRP injected per unit area [160, 176 – 201]. Other unknowns include the effect of co-treatments, such as concurrent or alternating use of medical treatments together with PRP treatments, or the use of mechanical stimulation methods such as micro-needling in conjunction with PRP administration [160, 202 – 206]. There has also been some indication that certain patients are better candidates for PRP treatment for hair loss than others; i.e., some patients may simply be non-responders, which may confound trial observations [160, 207]. Finally, other variables which may account for differences in results reported from published trials are the methods used and the timing of clinical outcome assessment [160, 168 – 192]. Some of these topics are discussed in more detail below.

## 6.1 PRP preparation mode

Based on the contents of the PRP, such as leukocytes and platelets, as well as the density of the fibrin network, four broad subcategories of PRP preparations have been described [151 – 155]. These subcategories include pure platelet-rich plasma (P-PRP), leukocyte-rich PRP (LR-PRP), Pure platelet-rich fibrin (P-PRF) and Leucocyte-platelet-rich fibrin (L-PRF) [151- 155]. Despite the different PRP subtypes, the PRP preparation

process is always similar and is based on centrifugation of whole blood, which results in the formation of four layers of different blood components [138]. In the bottom layer are red blood cells (RBC), followed by a thin layer called the buffy coat, which contains a high concentration of leukocytes, followed by a platelet-rich middle layer and platelet-poor plasma in the top layer [176]. Although the platelet-poor plasma and the RBCs are always discarded, controversies exist regarding the additional use of the buffy coat layer [153, 176]. Since leukocytes play an important role in immune regulation, prevention of infections, and promotion of angiogenesis, some authors claim that the use of the buffy coat may have beneficial effects on the treated areas, whereas others advocate for the exclusion of leukocytes due to avoid a potential inflammatory response to cytokine release from the leukocytes [176, 208 - 211]. P-PRF and L-PRF consist of a high-density fibrin-platelet network which allows a slower release of growth factors over time. These preparations, however, are highly viscous and therefore not suitable for injection. As such, they are commonly used as fibrin glues or topical gels, making them less suitable for use in hair loss treatment, as it is difficult to deliver them to the hair root [151 – 155, 176]. In light of the large number of different commercially available PRP kits and different PRP subcategories, the best mode for PRP preparation for its use in the treatment of hair loss, as well as its composition, have not been well defined [160]. Furthermore, as illustrated by the large variety of PRP preparation methods, which substantially differ in preparation protocols as described above, a wide range of final platelet concentrations have been reported [201 – 218]. Therefore, no consensus has yet been reached in the field in this regard [154, 160]. Studies comparing the clinical effects of platelet concentrations on tissue have presented contradictory results. For example, some studies have reported that platelet counts over 1 million per microliter was the most therapeutically effective concentration [217], while other studies have recommended lower platelet concentrations [153, 160, 212 - 216]. A potential correlation between platelet count and growth factor levels in PRP and hair growth following subcutaneous PRP injections was analyzed by Rodrigues *et al*. [178], but none was found [160]. Recent studies [161, 218] have suggested that higher platelet concentrations and use of a double-spin procedure may give better results, while studies using a single spin procedure, such as our study, did not impart statistically significant results [160]. Several studies have claimed a dose-dependent response of cells to PRP [169, 176]. *In vitro* studies, however, have indicated that very high platelet counts and thus higher growth factor concentrations

could be counterproductive and may even have destructive effects [161, 219]. The reason for this effect may be that not enough cell membrane receptors are available for such high growth factor levels, which could negatively affect cell function [169, 176, 219, 220]. In our study we utilized the “Yes” PRP kit for pure PRP preparation [160]. According to the supplier (https://yesprpkit.com/yes-prp-kit-2/test-results/) the PRP preparation should comprise six to ten times more platelets per microliter than at baseline in whole blood [160]. The range of platelet counts in healthy Caucasians is from 150,000 to 450,000 per microliter [221]. With such a wide range of starting concentrations, finding a standardized preparation method that produces a consistent product for each patient seems nearly impossible. Using an individual manufacturing process for each patient depending on their baseline platelet count would be a potential solution [160]. In this regards, Trüeb *et al*. have suggested the need for personalized medicine for hair loss patients, i.e., the customization of medical care to the patient's individual characteristics [222]. Such concepts are common in other disciplines such as oncology, where therapies can be specifically tailored to the patient [222]. In sum, the field is currently lacking a standard with regard to preparation methods and platelet concentration in PRP for AGA treatment [160].

## 6.2 PRP treatment frequency

There is no consensus regarding PRP treatment frequency. In published studies, both PRP treatment intervals and the total number of PRP treatment sessions have varied substantially [160]. Treatment intervals, for example, have ranged from weekly to monthly to bimonthly administrations [160, 177 – 186]. Similarly, the total number of treatment sessions have range from only a single session to up to six sessions [160, 177 – 186]. While some authors have reported positive results with 4-week treatment intervals, such as Cervelli *et al*. [182] and Gentile *et al*. [181], it should nonetheless be noted that others have reported negative results with both 4-week and 2-week treatment intervals [183, 184]. In our trial, we chose to administer five treatments at 4- to 6-week intervals, which was roughly in line with the regimen of other studies [160]. Recently Hooper suggested a loading phase of 3 to 4 monthly treatments followed by a booster treatment every 6 months [157].

## 6.3 Subject eligibility

The results of some studies have hypothesized an effect of the degree of alopecia in individual subjects on the outcome of PRP treatment [160]. There is some indication from studies to date that patients with early onset AGA may be better candidates for PRP treatment [157]. For example studies from Dicle *et al*. [179] and Gkini *et al*. [189] supported the hypothesis that patients with low-grade alopecia had a better response to PRP treatment. Data from Quian Qu *et al*. [190] reported a better response to PRP treatments in subjects with low grade AGA. A recent report from Juhasz *et al*. [207] indicates that an apparent lack of efficacy of PRP treatment may be due to the high number of non-responders [160]. Gender may also play a role in PRP efficacy, as concluded in a recent meta-analysis by Gupta *et al*. Sex-based differences in the outcomes of PRP treatment were investigated and it was found that women had an increase in hair diameter but not in hair density as compared with men [157, 223]. A positive effect of PRP has also been shown in AGA patients who were resistant to minoxidil and finasteride therapy [194]. In sum, patient selection appears to be a critical parameter in the efficacy of PRP treatment for hair loss. It would therefore be of great interest to conclusively determine which patients have the best likelihood of benefitting from PRP treatment [160].

## 6.4 PRP delivery and application mode

The best mode of PRP delivery should be intradermal injection, as the proposed therapeutic mechanism of PRP is the activation of stem cells which target the hair bulge and dermal papilla cells located in the dermis [139, 147 160, 224-230]. While in our study multiple intradermal injections in a grid-like pattern were used, other studies achieved better results with subdermal depot bolus injections [160, 230]. The main advantage of bolus injections could be that fewer injections are needed. Since Rodrigues *et al*. [178] also achieved hair growth by subdermal PRP injections, it is possible that the mechanical stimulation alone stimulates hair growth. Similarly, other studies have reported hair growth promoting effects after microneedling or after inserting threads into the scalp, observations which further support this hypothesis [225 – 228].

While we used manual injections [160], other studies have investigated mechanical devices for PRP injections [230]. In these studies, special mechanical devices such as PRP guns were used to administer PRP to the target areas and resulted in better outcome with respect to hair count, hair density, and pain reduction [230]. Moreover, several studies have reported the use of PRP as a coadjuvant in combination with other treatment modalities, such as microneedling, topical minoxidil or oral finasteride. The studies reported additive or synergistic effects which probably significantly influenced the outcome, but gave no information regarding the effect of the individual drugs alone [196 – 198, 229, 230]. A recent study compared the clinical efficacy of PRP, Minoxidil, and their combination with immunohistochemical evaluation of the dynamics of cell proliferation in 69 men with AGA [229]. They reported that a positive additive effect may result by the combination of such treatments, as platelet-rich plasma differs in its mechanism of action from the other treatment modalities [229]. Another study recommended PRP with microneedling in conjunction with medical treatment such minoxidil or finasteride for optimal improvement, since microneedling creates microholes that allow better transdermal delivery and good distribution of treatments to the hair root [230]. Aggarwal *et al.* [231] investigated microneedling as a monotherapy versus microneedling in combination with PRP by dermatoscopic evaluation. In contrast to other trials, this study demonstrated that the effects of microneedling alone were equal to those obtained by microneedling in combination with PRP; i.e., no additional effect of PRP over microneedling monotherapy was observed. The authors of this study reported an improvement in hair diameter of 3 to 6 micrometers and an increase of about 14 hairs per square centimeter in both groups. The proposed therapeutic mechanism of microneedling is the creation of micro-traumas to the skin, which cause superficial bleeding and stimulate growth factor expression and wound healing [231]. The above studies suggest that microneedling and mechanical stimulation alone are effective in promoting hair growth. In this regard, it becomes a matter of debate which therapy is more effective. However, due to the multiple treatment modalities available, their different mechanisms of action and different treatment responses from patient to patient, a combination approach using different procedures and treatments seems reasonable to pursue to achieve the best cosmetic results by synergistic effects.

## 6.5 Timing of follow-up examinations

Another aspect which has varied widely among published studies is the timing of follow-up examinations after treatment [160, 177- 186]. In our trial, 4-week and 6-month follow-up time points were chosen to assess short-term and long-term improvements, respectively. The selection of these time points was loosely based on observations from a previous study by Gkini *et al*. [189], which reported observable hair growth both early in the study (6 weeks after the first PRP injection) as well as 6 months after the last PRP injection. Interestingly, other studies using similar time points have shown apparently conflicting results. For example, Schiavone *et al*. [193], Singhal *et al*. [186], Alves *et al*. [180] and Rodrigues *et al*. [178], they all demonstrated hair growth three months after the last injection, whereas Ayatollahi *et al*. [184] reported negative results at the same time point. Other studies, such as those reported by Cervelli *et al*. [182], reported hair growth using short term follow-up time points directly after the last treatment, whereas Mapar *et al*. [183] did not observe a hair growth promoting effect directly after the last treatment. With the exception of two studies, no long-term follow-ups were performed. In a study from Gentile *et al*. [181], progressive hair loss was observed in 20 percent of subjects 12 months after PRP injections, and in the study from Gkini *et al*. a downward trend of hair growth was observed one year after the last treatment [189]. This seems to be a logical outcome, as AGA is a chronic progressive condition. As such, subsequent treatments may be required to maintain the effect [160]. Two to three periodic treatments per year after the initial treatment have already been recommended by Ferrando *et al*. [195]. Since AGA is an aesthetic condition, treatments are not covered by insurance. In the US, costs for one PRP treatment range from $500 – $2500 per treatment, with patients often receiving multiple treatments [232, 233]. This observation is of some clinical significance, as the high cost of PRP treatment precludes its use over longer time periods for many patients [160, 232, 233]. In light of these observations, it is no surprise that the global PRP market is expected to grow to between 380 million and 4.5 billion US-Dollar over the next 10 years [233]. In any case, the disparity in published results underscores the difficulty of finding appropriate time points for evaluation [160].

## 6.6 General limitations of PRP trials

It should be noted that the inconclusive nature of some PRP studies may be simply related to their general study design. For example, across published studies, there is a lack of more objective assessment methods of hair growth parameters such as digital imaging analysis (e.g., TrichoScan measurements) to measure hair growth [160, 176, 187 – 194]. Furthermore, several trials have assessed hair growth using only a single method, such as the hair-pull test or pre- and post-treatment pictures, to evaluate treatment efficacy, methods which are strongly based on subjective perceptions, rather than collecting multiple and objective measurements [160, 176, 187 – 194]. Also common in the literature are half-head studies [179 – 180]; however, this study design carries the risk of potential carry-over effects, as each patient is included in both the treatment and control groups. Other general limitations of PRP trials have included small sample size [177 – 186] or the absence of control groups [187 – 194]. In general, before the approval of a new drug, the safety and efficacy of the drug must be demonstrated [234 - 236]. The quality of clinical trials for testing efficacy of a new drug may be defined as the confidence that the design, conduct, report, and analysis restrict bias in the intervention comparison [236]. When evaluating healthcare interventions, randomized controlled trials, if appropriately designed, conducted, and reported, represent the gold standard [235]. However, trials can yield biased results if they lack accurate methodological design. Bias is the tendency of a statistic to overestimate or underestimate a parameter which can lead to wrong interpretation and conclusion of study results [236]. To avoid bias and to assess a trial correctly, studies should follow certain standards. Therefore the CONSORT Group, an international and eclectic group comprising doctors, methodologists and medical journal editors developed the CONSORT statement [235]. CONSORT stands for Consolidated Standards of Reporting Trials, which is an evidence-based, minimum set of recommendations for reporting randomized trials and offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation [235]. The statement includes a 25 item checklist that should facilitate clarity, completeness, and transparency of reporting by assiduous adherence to the checklist. The statement does not include recommendations for designing, conducting or analyzing trials. It does, however, indirectly affect design and conduct, as investigators who conduct inadequate trials, but must transparently report, should not be able to pass through the publication process without revelation of their

trial's inadequacies. Therefore, separate quality components should be used such as the presence of a control group to which comparisons are made in an experiment, equal conditions for the experimental and control groups, blinding, as well as an adequate sample size [236]. Determining the right sample size, i.e., the number of participants enrolled in a trial, is an especially crucial aspect when planning a study [237]. The precision of study estimates and the power of a study to draw conclusions are significantly influenced by the sample size; i.e. a larger sample size gives more power. In the PRP studies we analyzed, the total number of study participants has ranged from 10 participants in the smallest study to up to 51 participants in the largest study, with an average number of 28 participants per study [177 – 194]. Normally hundreds to thousands of participants are enrolled in trials to prove the evidence of an indication, with sometimes even numerous trials required before drug approval such as therapeutics approved for cancer indications or for cardiovascular disease [234]. In these trials, new drugs are compared to a control regimen, which is either an already existing drug or a placebo, to ascertain whether the experimental drug is efficacious and safe [234 - 235]. If the experimental group has a significantly better response than the control group, the new drug is considered effective. On the other hand, if the experimental group experiences significantly more side effects than the control group, the new drug may not be considered safe and approval may be denied. In the absence of a control group, drawing concrete conclusions regarding the effects of a novel drug is difficult. In this regard, many PRP trials simply seem to be underpowered with low overall quality. Still, PRP treatment is a time-consuming medical procedure and cannot simply be compared with taking a pill once a day. Conducting procedure-based/surgical trials pose several challenges in comparison to pharmaceutical trials such as blinding, influence of doctor's skills/experience, number of participants and endpoint choice [238]. For example, blinding a treating surgeon is rarely possible. Moreover, some endpoints such as quality of life assessment require patient input and therefore remain subject of patient related bias. Furthermore, because of its invasive nature recruiting a sufficient number of participants in surgical trials could sometimes be challenging. However, based on the current study data available, designing an adequate trial including appropriate sample size and the selection of a meaningful endpoint should be possible to make the trial results more robust.

## 6.7 Treatment efficacy and patient satisfaction

Recently Bigby and Grimalt opined that clinical evidence supporting the efficacy of platelet-rich plasma in AGA is lacking [158]. They criticized clinical trials for reporting statistically significant results which were nevertheless clinically imperceptible. For example, a recent study by Makki *et al*. reported increases in hair density from 261 to 312 ($p < 0.05$) and in hair width from 0.64 to 0.76 ($p < 0.05$) [239]. Another study by Shapiro *et al*. reported a mean increase of approximately 20 hairs/cm$^2$ ($p < 0.05$) compared to baseline. In this study however, the hair count in placebo-treated areas also increased by about 15 hairs/cm$^2$ compared to baseline ($p < .05$) and no significant differences were noted between the two groups ($p > 0.05$) [239]. Gentile *et al*. [181] reported an increase of 45 hairs per cm$^2$ compared to baseline ($p < 0.05$) and Cervelli *et al*. [182] reported a mean increase of 28 hairs per cm$^2$ ($p < 0.05$). Aggarwal *et al.* [231] reported an improvement in hair diameter of 3 to 6 micrometers and an increase of about 14 hairs per square centimeter comparing microneedling as a monotherapy versus microneedling in combination with PRP. These findings are in accordance with results from several other studies noting improvements ranging from 2 to 59 hairs per square centimeter [158, 177 – 182, 186 – 192, 239 - 241]. When evaluated, however, studies have reported high overall patient satisfaction [177 – 197, 231, 239 - 241]. A recent meta-analysis by Gupta *et al*. revealed a standardized mean difference in hair density in patients treated with PRP injections of 0.58 ($p < 0.0004$) and 0.51 ($p <0.0004$) in favor of PRP treatment compared with baseline and placebo treatment, respectively [241]. Caucasians have approximately 250 - 310 hairs per square centimeter resulting in a total number of about 100,000 hair follicles on the scalp, with physiological shedding of about 100 hairs per day [15, 25, 30]. The above studies seem to indicate that, even if hair growth is measurable, it is not necessarily cosmetically visible and patients are affected by AGA both before and after PRP treatments.

Interestingly, out of four published randomized controlled PRP trials reporting no improvement after PRP injections [183 – 185, 242], three reported patient satisfaction despite negative results [184, 185, 242]. A similar observation was made in our study. Although PRP treatment provided no measurable improvement, the majority of subjects in both the placebo and PRP treatment groups were at least partially satisfied with the result and indicated a willingness to pay for the treatment and to recommend the treatment to others. This positive assessment despite no measurable improvement

is likely attributable to the placebo effect, a complex neurobiological phenomenon described in several research articles [243 – 247].

A placebo is a pharmaceutically inert substance or a simulation of a medical intervention that is not directly known to cause an effect [246]. A placebo is commonly used as a comparator group when testing the efficacy of a novel therapy. The placebo effect or response, however, is an apparently therapeutic effect which is paradoxically observed in response to the administration of an inert placebo [246]. Several studies have demonstrated that placebo effects can be robust in both laboratory and clinical settings and are assignable to the overall therapeutic context [243 - 246]. Evidence has also emerged that placebo effects can exist in clinical practice even if no actual placebo is given [246].

The placebo effect can be affected by several factors and the underlying mechanisms can be broadly discussed from psychological and neurobiological viewpoints. Multiple psychological mechanisms may be involved, including expectations, conditioning, learning, memory, motivation, somatic focus, reward and anxiety reduction. Classical conditioning and expectations of the patient seem to play key roles in the placebo effect. The brain placebo response is strongly influenced by verbal conditioning and observational cues which can lead to the release of endogenous opioids and dopamine [243 - 247]. According to the response expectancy theory, a patients can be manipulated using verbal cues as a modulator of expectations [246]. For example, changes in emotions and expectations can be mediated when a trial subject is given a placebo pill for pain in the context of two different cues: the first that the pill is inert and will have no effect and the second, that the pill is a powerful pain killer, which can result in an analgesic placebo effect. Another key mechanism of the placebo effect involves classical conditioning, which is the repeated association between a neutral stimulus and an active drug leading to the ability of the neutral stimulus by itself to induce a response characteristic of the real medical treatment. For example, several studies have demonstrated that administering a placebo in place of a habitually used active drug could result in relief of pain and other symptoms due to a conditioned stimulus [243 - 246]. The psychological stimuli around the treatment could activate a number of receptorial pathways in multiple brain areas associated with pain and emotion such as opioid and non-opioid mechanisms and beta-adrenergic activities, or immune

modulation in different diseases and therapeutic interventions [243 - 247]. Some studies have shown that placebo effects can be reversed by the opioid antagonist naloxone, supporting the involvement of endogenous opioids in analgesic placebo effects. Furthermore, placebo induced brain changes similar to those seen following opioid drug administration have been reported [247]. In fact, opioid mediated placebo responses may extend beyond pain pathways, as some studies have found that placebo induced respiratory depression (a conditioned placebo side effect) and decreased heart rate and β-adrenergic activity can be reversed by naloxone. This finding demonstrates the involvement of opioid mediated mechanisms on other physiological processes, such as respiratory and cardiovascular function [243 - 247]. In sum, these observations indicate that psychological factors are capable of modulating the action of drugs.

A study from 2002 by Moseley *et al.* [248] compared arthroscopic or placebo surgery in patients with osteoarthritis of the knee. In the placebo group patients received skin incisions and underwent a simulated surgery without insertion of the arthroscope. Patients and assessors of outcome were blinded to the treatment-group assignment. No differences between the two groups regarding pain or better joint function could be found [248]. This study demonstrates the power of the placebo effect. Maybe a quite similar phenomenon may appear with PRP treatments. During PRP-treatment sessions, the focus is clearly on the patient. Using PRP a lot of attention is paid to the patients, we talk to them, take a blood sample, centrifuge the blood and finally inject it. The injections are painful and indicate to the patient that something is being done. In short, there are a lot of impressions which might influence the patient's perception of the treatment. A recent study for example revealed that doctors attire alone affect patient-perceived relational overall empathy in terms of confidence and trust in a positive way [249]. More than a century ago, Sir William Osler (1849-1919) already suggested a patient-centered approach: "The good physician treats the disease, the great physician treats the patient who has the disease" [250]. While study results are important, so is the presentation of a treatment, as we know the persuasive power of advertising and marketing [172, 173, 175]. Particularly in sports medicine, PRP has attracted great attention with its use in professional athletes who required rapid return to competition. The general perception seems to be that, if famous athletes are getting the treatment, it must be worthwhile [139, 251]. Interestingly, although a large number

of trials in sports-related injuries and maxillofacial surgery have not reported any benefit of PRP treatment, it remains a popular treatment option [143 -146, 251 - 253]. This positive perception is traceable to the spillover effect, another complex psychological phenomenon that seems to be involved when people decide to get PRP treatment. A spillover effect can occur in a positive or negative way and refers to the process where the behavior of one person affects the behavior of another person [254]. A recent study proposed that group dynamics and the support by a trusted entity are a crucial part of the spillover effect [254]. These observations underscore the importance of the psychological needs of patients and begs the question whether PRP is simply a profit-rich placebo [251].

# 7 Limitations

Several limitations of our study should be mentioned: The study was performed as a pilot study to test this novel recently hyped therapy, using a study protocol developed in 2016. Although the study protocol was already fixed in 2016, it took some time to finally complete the study. In the meantime, data from other studies which might have influenced the design of our study in retrospect, became available. Due to the smaller nature of this study, only large effect sizes could be detected with a sample size of 30. In addition, the area measured for hair density and thickness was quite small and it cannot be excluded that assessment of a larger scalp area or using another treatment protocol may have revealed at least a modest PRP treatment effect.

# 8 Conclusion

In conclusion, our results suggest that treatment with PRP as a monotherapy does not improve hair growth in men with AGA. Although numerous trials have suggested that PRP benefits at least a subset of AGA patients, substantial differences in trial design have made it difficult to draw concrete conclusions about its clinical efficacy. Furthermore, the development of a reliable clinical protocol has not yet been possible.

# 9 Future perspectives

Within the present study further data were collected for PRP treatment in AGA patients. Despite our sobering results, many topics remain to be explored in the field to develop PRP treatment as a viable option for AGA patients. Parameters for further development include identifying the best process for preparing PRP for AGA treatment, selecting subjects who are most likely to benefit from the therapy, the best mode of delivery, timing of the treatments and the potential usefulness of combination therapy. At the present time, patients should be informed about the risk of a potential treatment failure in an unbiased fashion before scheduling a PRP treatment, as it seems that there is no guarantee of efficacy. Thus, PRP and a standardized treatment protocol for its use, must be further evaluated in large, randomized, placebo-controlled multicenter studies in order to determine efficacy and/or best modes of this therapy. Besides, with other recent advances in hair research, including the potential tool of hair cloning, and improved hair transplantation technologies, we have a promising perspective to offer patients better outcomes.

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