A Prospective Double-Blind Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

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DOCTORAL THESIS

A Prospective Double-Blind Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

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CERTIFICA: Que la Tesis Doctoral que se presenta a juicio del Tribunal por el aspirante al grado de Doctor, Rubina Filipa Ascensão Alves bajo el título “A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia” ha sido realizada bajo mi dirección y supervisión, encontrando dicho trabajo adecuado para tal fin.

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(Prof. Dr. Ramon Grimalt)

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Rubina Alves

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LIST OF ABBREVIATIONS

AA	Alopecia areata
ACD	Acid citrate dextrose
AE	Adverse event
AGA	Androgenetic alopecia
Akt	Protein kinase B
BAD	Bcl-2 associated death promoter
BMP-4	Growth factor bone morphogenetic protein-4
CA	Cyproterone acetate
CAD	Coronary artery disease
DHEA-S	Dihydrotestosterone-sulfatase
DHT	Dihydrotestosterone
DP	Dermal papilla
EGF	Epidermal growth factor
ERK	Extracellular signal-regulated kinase
FAGA	Female androgenetic alopecia
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FPHL	Female pattern hair loss
GF	Growth factor
GFR	Growth factor receptor
GSK3-β	Glycogen synthase kinase-3β
HFs	Human follicles
HGF	Hepatocyte growth factor
HS	Hair shaft
IGF-1	Insulin-like growth factor 1
IL-1α	Interleukin-1alfa
IRS	Inner root sheath

IRS	Inner root sheath
KGF	Keratinocyte growth factor
LED	Laser-emitting diodes
LEFT	T-cell factor lymphoid enhancer
LLLT	Low-level laser therapy
MMP	Matrix metalloproteinases
MPHL	Male pattern hair loss
ORS	Outer root sheath
PDGF	Platelet-derived growth factor
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
PSA	Prostatic-specific antigen
RBC	Red blood cells
RCT	Randomized clinical trial
SD	Standart desviation
SHBG	Sex-hormone-binding globulin
Shh	Sonic hedgehog
TE	Telogen effluvium
TGF-β	Transforming growth factor beta
TNF-α	Tumour necrosis factor alfa
TNHL	Transient neonatal hair loss
VEGF	Vascular endothelial growth factor
Wnt	Wingless
µL	Microliter

KEY-WORDS

Alopecia

Androgenetic alopecia

Hair loss

Platelet-rich plasma

Growth factors

Hair growth

Double-blind

Placebo-controlled

Clinical trial

Drug therapy

Humans

1. INTRODUCTION

1.1 JUSTIFICATION

Androgenetic alopecia (AGA) is the most common non-cicatricial alopecia that leads to the progressive miniaturization of the hair follicle.

Topical minoxidil and oral finasteride are the gold-standard therapies for AGA and, currently, the only Food and Drug Administration (FDA)-approved drugs for the treatment of AGA. However, conventional treatments have not achieved the desired results and there's no complete satisfactory treatment for AGA. So finding new therapeutic options for this pathology is of utmost importance.

In recent years, Platelet-rich plasma (PRP) has started to be considered as a potential therapeutic tool for promoting hair growth and has been postulated as a new therapy for alopecia. However, few controlled studies have been published in order to assess the efficacy of PRP in the treatment of AGA.

The objective of this doctoral thesis has been to investigate the efficacy of PRP in the treatment of Androgenetic Alopecia.

We performed a prospective randomized placebo controlled and double-blind study, previously approved by the Ethic Committee and registered in clinical trials.gov.

Our study aims to assess the evolution of AGA, between 3 and 6 months of treatment with PRP and baseline.

The study was divided into two phases: 1) Group A: PRP alone and Group B: PRP with concomitant treatment for AGA. It was designed comparing the treatment of PRP in half-head and the use of placebo (saline solution) in the other half-head of the scalp.

As both treatment and placebo are performed in the same patient, the baseline characteristics are the same for both groups, thus dismounting the incidence of bias.

Although published studies suggest the positive effect of PRP in AGA, most of them are open label and lack a randomized controlled study.

If through our randomized clinical trial is possible to demonstrated the efficacy of PRP as a treatment option for AGA (both in monotherapy or in association with FDA approved medications) it would be novel and important finding in the field of alopecia.

2. STUDY RATIONAL

2.1 ANDROGENETIC ALOPECIA

Androgenetic Alopecia is a nonscarring alopecia that affects both men and women. It is characterized by a progressive miniaturization of hair follicles with a characteristic pattern distribution in genetically predisposed men and women (1). Is the most frequent type of hair loss in both sexes (2-4).

The onset of AGA is usually gradual and the condition slowly develops over the years. The frequency and severity of this type of hair loss increases with age (5). It usually appears in the third and fourth decades (2) and affects 30% to 50% of men by the age of 50 (6-7) and around 80% of caucasian men aged over 70 years (1,8,9).

As in men, the frequency and severity of female pattern hair loss (FPHL) increases with age (9). According to Norwood (10), after examined a total of 1006 caucasian women, he found a prevalence of almost 30% in women over 30 years of age. Gan DC et al. (8) reported that prevalence of mid-frontal hair loss also increases with age and affects 57% of women aged 80 and over.

The prevalence of AGA is different between populations; among asian, native american, and many men of african heritage is lower than that among caucasians with a decreased frequency of frontal hair loss and less extensive hair loss. (1,2,11).

Although AGA is more frequent in adults, it can also appear in adolescents (12), though its prevalence among this younger population is not well established (13). On an average, adolescent AGA onset starts after puberty between 13.5 and 15 years of age (14). Although it is not expected to see AGA in prepubertal patients without androgen levels (15,16). Tosti et al. (15) reported 20 prepubertal children with AGA with a very early onset age between 6 and 10 years. According to the authors, all patients had a strong genetic predisposition to the disease with a strong family history and the dihydrotestosterone-sulfatase (DHEA-S) levels were consistent with postadrenarche, but none of them was affected by premature puberty, as demonstrated by physical and laboratory assessments. In adolescents

with a genetic predisposition, the first signs of AGA usually appear with rising androgens at puberty (17).

Beautiful and well-groomed hair represents youth, vitality and energy and is important in determining one's self-image and self-esteem (18). In today's society, body image is influenced by many factors, such as the media and the fashion industry; and hair plays an important role. People who have thinning hair or any type of hair disease may feel unattractive and have a negative body image (19).

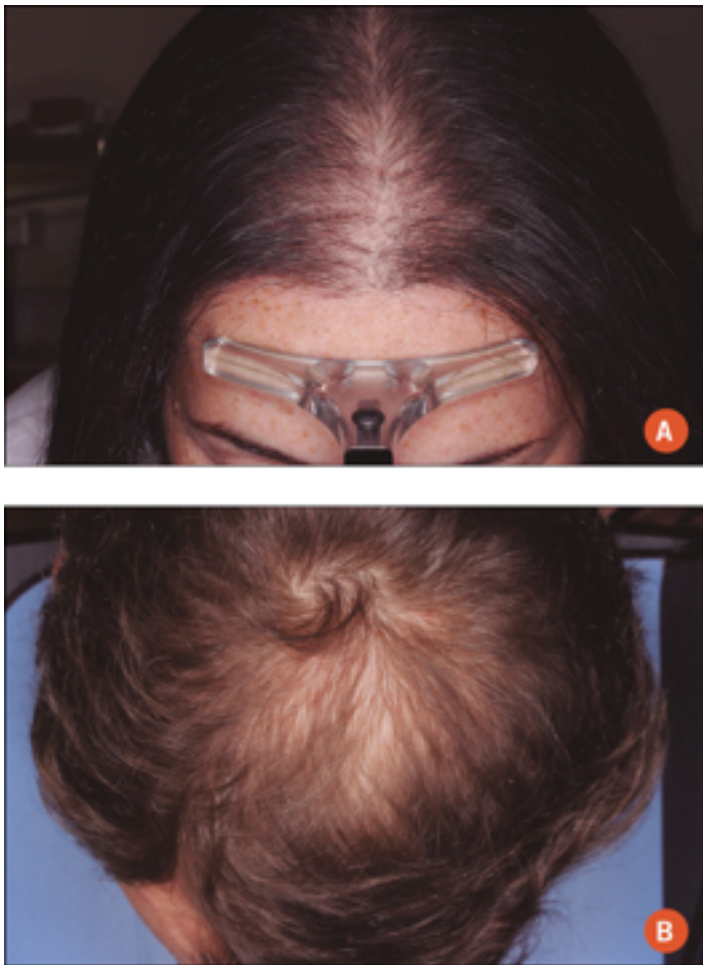

Fig.1. Androgenetic Alopecia in a female (A) and male (B) patient.

2.1.1 Embryology and Normal Hair Development

Hair follicles are derived from an interaction between the embryological ectoderm and mesoderm, which begins between 9 and 12 weeks of gestation. Human hair follicles primary develop in the regions of the eyebrows, upper lip and chin (20).

Then, hair follicles develop over the scalp in a frontal to occipital direction and progress over the body in a cephalocaudal direction (21). On the trunk and limbs, the hair germ development is delayed and appears around the sixteenth week (22).

By 18–20 weeks of gestation, the entire initial population of follicles has formed, including those on the scalp (23), although follicular development is in different stages of evolution.

Human follicles (HFs) develop through complex morphogenetic processes resulting from reciprocal molecular interactions between epithelium and underlying mesenchyme during embryonic development (24).

At birth, within anatomical regions and most of the scalp hair, all hairs are initially synchronous in anagen phase (25,26). However, in the occipital scalp, telogen phase is delayed until after birth and this can give rise to a path of hair loss in this region of the scalp (27).

Initially, this transient neonatal hair loss (TNHL) also known as ‘neonatal occipital alopecia’, was thought to be secondary to friction due to babies sleeping in the supine position (20). A prospective study (23) was performed, and as stated by the authors, TNHL is related to the physiology of hair shaft shedding, it appears in healthy babies from birth until approximately the second month of life without accompanying symptoms and with spontaneous resolution.

Each follicle is capable of producing three different types of hair as follows: lanugo, vellus and terminal hairs. The initial hair produced is the lanugo hair. The type of hair originated by an individual follicle can change with age or under the influence of hormones (28).

Lanugo hair is nonmedullated, fine (typically less than 30 μm in diameter), soft, and usually non pigmented hair which cover the whole body of the newborns (20). Is typically shed between the thirty-second and thirty-six weeks of gestation, although it can remains by 3–4 months after birth in a unsynchronized wave pattern. Then he is replaced by vellus hair (12).

Vellus hair is short, light-colored, barely noticeable, and covers almost the whole body. Around puberty, there is an increase of the circulating adrenal androgens, which leads to a site-specific response from the hair follicles. With the presence of androgens, the hairs of the scalp miniaturize, while the hair of the body enlarges. At this time, the hairs of axillary, pubic, chest, and beard (boys) change from vellus to terminal hair, contributing to the development of the secondary sex characteristics (21, 28).

Terminal hair is larger (approximately 1 to 100 cm of length) and thicker usually with more than 60 μm in diameter. This type of hair is strongly pigmented and is found on the scalp, eyebrows, axillary and pubic areas, chest and face (21). In patients with AGA, terminal hairs follicles in the scalp convert to miniaturised hair follicles (< 30 μm in diameter).

2.1.2 Anatomy and Hair cycle

The hair follicle is anatomically divided into three parts: upper (infundibulum), middle (isthmus) and lower part (inferior segment). The infundibulum and isthmus comprise the permanent portions of the hair follicle and is a relatively constant structure, while the inferior segment is transient and undergoes cyclical regeneration (29). A fourth segment of the pilosebaceous follicular unit would comprise the sebaceous gland (26).

In the permanent part, the infundibulum extends from the skin surface to the insertion of the sebaceous duct at the junction with the isthmus whereas the isthmus begins below the sebaceous duct and extends to the bulge or area of attachment for the arrector pili muscle (22).

The lower segment of the follicle is the site of the majority of activity in the hair follicle. Is comprised of three layers from outermost to innermost: outer root sheath (ORS), inner root sheath (IRS) and hair shaft (HS).

The inner part of hair bulb is invaginated at its base by the dermal papilla (DP). The IRS and HS are derived from epithelial cells surrounding the DP, a region known as the hair bulb matrix or germinative epithelium.

Dermal papilla size is dynamically regulated during the hair cycle (30). Cells emigrate from the DP during catagen and then repopulate it in anagen (31, 32). The DP is a condensation of mesenchymal cells at the proximal end of the hair follicle, which determines hair shaft size and regulates matrix cell proliferation and differentiation. In large follicles the DP often contains a loop of capillary blood vessels. DP cells have the ability to regenerate new hair follicles. These cells tend to aggregate both in vitro and in vivo. This tendency is associated with the ability of papilla cells to induce hair growth (33). The follicle miniaturization seen in AGA is thought to be driven by dysfunctional DP cell.

The human skin supports approximately 5 million hair follicles, of which approximately 100,000 are on the scalp (23).

Human hair grows in a continuous cyclic pattern presents with different phases: the anagen or growth phase, which lasts between 2 and 7 years, the catagen or regression phase, which has a duration of 2-3 weeks, the telogen phase, which lasts 3 months, and the shedding exogen phase (20).

The duration of hair cycle phases can be modulated by different physiological conditions in the same individual, in accordance with stages of postnatal, sexual development or seasonal environment (34). The normal hair cycle results in the replacement of every hair on the scalp every 3–5 years (28).

The anagen phase receives signals from the cells of the germ to regenerate. The interface between the hair follicle epithelium and the mesenchyme increases progressively and the cells of the hair bulb matrix reproduce with a high mitotic rate and stem cells in the bulge give rise to hair germs to create a new HF (27, 35).

When the period of active growth ends, the follicle growth stops and enter in a transitional physiological involution stage, the catagen phase. In catagen, bulbar

portion of the follicle is degraded but HF stem cells are maintained in the bulge (24, 36). At this time, the follicle decreases in size as the DP cells move together (37). After catagen, follicles undergo apoptosis and enter in the telogen phase. The hair filament remains in the telogen phase which later is detached during exogen (38).

The telogen or resting phase is followed by exogen in which the hair filament is detached.

The regenerative hair cycle is regulated by different molecular signals between follicular epithelium and mesenchymal DP cells (24). The growth and development of HFs is influenced by a number of signals, different growth factors (GFs) and their receptors and cytokines. These signals also comprise transcription factors, nuclear receptors, neurotrophins and intracellular signaling pathways (27).

There is a balance of negative and positive influences regarding the activation or down-regulation of hair cycle and that is distinct for each phase of the hair cycle (39).

It has been described that anagen phase is activated by Wnt (wingless) /β-catenin (Beta-catenin) / LEFT (T-cell factor lymphoid enhancer) (40), Sonic hedgehog (Shh) (41) and the proteyoglican versican (33). This molecules have been used as an indicator of hair inductiveness, many of which are also associated with aggregative behaviour.

Other GFs are involved in promoting the hair regrowth including fibroblast growth-factor (FGF), insulin-like growth factor 1 (IGF-1) (42,43), hepatocyte growth factor (HGF) (39), keratinocyte growth factor (KGF) (39) and vascular endothelial growth factor (VEGF) (44). This activation signalling leads to the stimulation of the hair germ in anagen phase.

The hair cycle is regulated through other pathways with a down-regulation effected, which are also essential for the proper hair formation. Others GFs exert their action through an inhibitory effect and down-regulation on hair growth and that may be involved in the beginning of the catagen phase (39). Some of these GFs include: growth factor bone morphogenetic protein-4 (BMP-4) (45), transforming growth factor-β (TGF-β) (46), interleukin-1α (IL-1α) and tumour necrosis factor-α (TNF-α).

The knowledge of these molecular targets could help us to identify the potential therapeutic target of platelet-rich plasma, as described below.

2.1.3 Pathophysiology of Androgenetic Alopecia

The pathogenesis of AGA is influenced by two main factors: heredity and androgen action. AGA is considered a disease with a genetic predisposition, but the mode of inheritance has not been well characterized (47).

Inheritance is almost certainly polygenic, with genetic input from both parents. Studies performed on monozygotic twins showed strong concordance rates between 80 and 90%, reinforcing the implication of a genetic basis (12, 48).

Family history predisposes to early development and rapid progression of the alopecia (3). Its etiology is multifactorial and polygenetic (1, 49).

For the development of AGA, the presence of androgens, in combination with genetically susceptible hair follicles, is necessary (50).

The modulation of hair growth androgens are very important. Androgens such as testosterone are responsible for the conversion of vellus hair into terminal hairs of facial, trunk, extremities, pubic and axillary areas. The growth of these areas are similar in both sexes and begins around puberty. The growth of scalp hair is not dependent of the androgens, although it is well known they are necessary for the development of balding (51).

So, androgens will have a different effect regarding the location of the hair follicle. They are able to stimulate both the growth of hair (pubic, axilar, beard, chest) and also promote the loss of certain hair in the scalp, in genetical susceptible individuals.

The androgen hormones testosterone and dihydrotestosterone (DHT) each have selective roles at puberty. Testosterone is converted to DHT by the enzyme 5-alpha-reductase that has two isoenzymes: 5-alpha-reductase type I (present in the liver and sebaceous glands) and 5-alpha-reductase type II (present in the scalp, beard, chest, liver and prostate gland).

Testosterone is associated with increased muscle mass, growth of the scrotum, voice change, and the presence of terminal pubic and axillary hair fibers. An increased activity of 5-alpha-reductase type II plays an important role in the development of AGA (1); Levels of this isoenzyme are higher in men than in women and in the areas affected by AGA (3).

DHT is associated with temporal scalp hair recession, acne, growth of the prostate gland, and the development of terminal hairs in the beard region, external ears, and limbs.

The hormone DHT activates the genes responsible for the shortening of hair growth cycle as well as the transformation of large hair follicles into smaller follicles that progressively become miniaturized (16).

AGA is characterized by the miniaturization of the hair that only occurs in certain areas: the frontotemporal and vertex area in men and the crown region in women. These scalp regions are susceptible to the effects of androgens. During the gradual transformation of the miniaturized follicles (from terminal to vellus-like follicles), the anagen phase shortens, and for this reason more hairs are in telogen phase (50). There is no loss of hair follicles in AGA, just miniaturized (12). DHT has been shown to inhibit hair growth in mouse models through blocking the actions of growth factors such as insulin-like growth factor 1 (IGF-1) (37, 52).

2.1.4 Diagnosis of Androgenetic Alopecia

Most patients with AGA will be easily diagnosed by a clinical history and clinical examination, according to the condition of the characteristic distribution pattern (53).Male pattern baldness is characterized by a receding hairline at the temples and balding of the vertex, which gradually enlarges to link together. In the female pattern, there is a diffuse decrease in density over the crown of the head and the frontal hairline is preserved.

Some noninvasive techniques such as trichoscopy and hair pull test help to perform the correct diagnosis. In some cases, it might be necessary to do a more extensive examination using other diagnostic tools, such as scalp biopsy. A laboratory examination might be extremely useful in some patients, especially in

women, to evaluate any associated diseases. Global photographies performed at baseline are helpful to compare the efficacy of the treatment, both for patient and physician.

In the presence of a patient with hair loss the first to perform is a detailed clinical history. The history should include all demographic data of the personal history of the patient including age, sex, age at the first manifestation and grade of evolution of the hair loss, through time.

The family history of AGA is important because of the role of genetics in this pathology. Although a positive family history is frequent, a negative family history of AGA does not exclude the diagnosis (1, 54).

An exhaustive history of associated diseases, systemic alterations and drug history are of utmost importance, as there are different factors such as nutritional or endocrine diseases and drug intake that could precipitate or aggravate the severity of the hair loss. Several environmental factors such as exposure to ultraviolet radiation (55, 56) and smoking (57,58) have been associated with the development of AGA, so these aggravating factors should also be considered when performing the clinical history of the patient.

In the physical examination, besides the scalp skin and hair it should also be evaluated the facial and body hair and the nails.

The skin of the scalp should be examined to exclude signs of erythema, scaling, inflammation and atrophy. Usually, in patients with AGA, the scalp skin has no alterations although some patients can present with a discrete erythema and scaling related with seborrheic dermatitis, that can be an aggravating factor. Jang WS et al. (47) in a retrospective review found that seborrheic dermatitis was the most common associated disease in male and female patients with AGA.

In patients with advanced AGA, the skin of the scalp becomes smooth and shiny but in some cases a discrete atrophy can be present (1). Nevertheless, the presence of atrophy should alert to another type of alopecia such as scarring alopecia.

These signs would easily help differentiate AGA from other types of hair loss or infections.

Hair examination includes the hair of the scalp, facial and the whole body.

The scalp hair of patients with AGA is a miniaturized hair with variation of hair calibre, length and regrowth. It follows a characteristic pattern of distribution, that is different between sexes. Male pattern is characterized by recession of hairline, frontotemporal and vertex areas while in the female pattern, there's usually a diffuse distribution of hair loss, more pronounced in frontal and mid scalp areas. Unlike in male pattern, the frontal hairline in women is typically preserved.

Although, these are the most frequent patterns in both genders, there are other types of pattern distribution which have specific classifications, such as Hamilton-Norwood scale for men and Ludwig for women, the most frequently used. The Classifications of AGA are described below.

The examination of the facial and body hair will give further information, regarding differential diagnosis or associated pathologies. The absence or reduced of eyebrows/ eyelashes may suggest a frontal fibrosing alopecia or alopecia areata (AA) (1). Regarding body hair examination, an excessive hair growth could lead the physician to think in hypertrichosis or signs of hyperandrogenism such as hirsutism.

To analyse with more detail the hair scalp, some noninvasive diagnostic tools may be helpful.

A hair pull test, also known as "traction test" or "Sabouraud's sign" helps distinguishes between active and non-active hair loss from follicles. To perform this test, around 50–100 hairs are grasped between the index finger and thumb and then lifted proximally to distally, with gentle traction (20). This procedure is repeated in different areas of the scalp: frontal, occipital and temporal regions. The hair pull test is considered positive if more than 10% of telogen hairs are released, (59),this means that if the pull test has more than 5 or 6 hair indicates ongoing hair activity (54).

False positives can occur if the test is performed on a day in which hair has been washed. A positive hair pull test will indicate an active hair shedding. Hair pull test in positive in telogen effluvium (TE). In patients with AGA, the hair pull test is usually negative, with the exception of active periods when a moderate telogen hair shedding is present in a pattern distribution (1).

Trichoscopy is a noninvasive technique performed using a handheld dermoscopy or a digital videodermatoscopy system. The handle dermoscopy usually has a 10-fold magnification of the skin surface while the videodermoscope, which is equipped with a software, will have the benefit of larger magnification (ranging from 10-70 fold) and the conversion of measurement results into a database (60,61) Trichoscopy adds important information for establishing the correct diagnosis and it is also a useful tool for assessing disease activity and monitoring treatment efficacy (18).

In AGA, trichoscopy will easily identify the abnormalities present in this type of alopecia. Male and female AGA share similar trichoscopic features.

The most important trichoscopic feature is hair shaft thickness heterogeneity, which means the presence of hairs with different thickness such as thin, intermediate and thick hairs (61). Other features include vellus hairs (decreased terminal to vellus hair ratio), predominance of HF units with single hairs, yellow dots (hair follicles filled with hyperkeratotic plugs), perifollicular discolouration, wavy hair and honeycomb pigmentation (60, 62).

The videodermoscope with an automatic digitalised system will add more information of the analysed images regarding hair parameters such as number of hairs (number of hairs/0.65 cm^2), hair density (n per cm^2), terminal hair density (n per cm^2), anagen and telogen hairs (%) and anagen/telogen ratio. In patients with AGA, frontal hair density is decreased when compared with occipital areas and anagen/telogen ration is normal or decreased (1).

The trichogram is a quantitative technique that provides information on the ability of hair growth and its alterations. Although an useful technique, it requires prior training and is time consuming (63). Usually is not a frequent diagnostic technique used for the diagnosis of AGA.

Usually, scalp biopsy is the standard technique for diagnosing different types of scarring alopecia (54). In addition, scalp biopsy could give relevant information in difficult cases of non scarring alopecia, where the diagnosis of the type of alopecia is uncertain. In few cases, the diagnosis of AGA is not clear, such as alterations of the scalp suggestive of scarring alopecia or diffuse AA (1), and a scalp biopsy may be needed to obtain the final diagnosis.

The scalp biopsy should be performed with a cylindrical punch of 4 to 6 mm in diameter, in an area representative of the hair loss (26). Biopsies taken with a smaller punch than 4 mm in diameter are not recommended as it would give less number of follicle units, and as the follicles are not always affected simultaneously by a such disease it would diminishes the probability of a correct diagnosis (26). The biopsy specimen should include the entire follicular unit and reach into the subcutaneous tissue where normally are the bulbs of anagen hair follicles (54, 64).

According to Blume-Peytavi et al. (1), it should be obtained two 4-mm punch biopsies in which 1 biopsy is for vertical sectioning and the other biopsy for horizontal sectioning.

A horizontal sectioning of the scalp biopsy specimen would give more quantitative and morphometric data and allowed a visualization of a larger number of follicles, diameter and grouping whereas the vertical section would provide more information about the length of the hair follicle and full dermal thickness and this was a diagnostic advantage, especially in scarring alopecia (54, 65, 66). It remains uncertain if biopsies should be oriented horizontally or vertically (26).

Recently, DU X et al. (66) published a systematic review meta-analysis to evaluate the value of horizontal sections compared to vertical sections in the diagnosis of alopecia and found “no significant difference exists between horizontal and vertical sectioning techniques in the diagnosis of alopecia”, although more studies are needed.

In areas affected by AGA is possible to identify an increased number of miniaturized follicles, and a ratio telogen-vellus of < 3:1 when compared to a ratio telogen-vellus of < 7:1, in patients without AGA (1, 67).

The main histologic features in AGA are slight perifollicular lymphohistiocytic infiltration with a perivascular degeneration of the lower one third of the connective tissue (68).

The examination of the nails can add important information and is important for the differential diagnosis of other types of alopecia. Nail abnormalities such as pitting can be present in AA and liquen planus. Patients with AGA typically don’t have any alteration in nails.

In women, although the majority have a normal hormonal function, a careful evaluation regarding gynaecological history should be done to exclude hormonal dysregulations (1).

Regarding laboratory examination and according to Camacho and colleagues (63) in their protocol, the most important laboratory tests to be considered are levels of free testosterone, 5-α-DHT, DHEA-S, 17-beta-hydroxyprogesterone, prolactin, delta-4-androstenedione, sex-hormone-binding globulin (SHBG), 3-α-androstanediol glucuronidel and prostatic-specific antigen (PSA) in both, premenopausal (normal levels ≦ 0.02 ng/mL) and postmenopausal women (nL ≦ 0.04 ng/mL);

The global photography of the scalp is a non-invasive and useful method for determining clinical changes in the patient over time in a standardized manner.

2.1.5 Classifications of Androgenetic Alopecia

There are several classifications that help to determine the degree of androgenetic alopecia for male and female patients. This allow staging the degree of alopecia at the time of diagnosis and control the evolution and response to the treatment.

For male pattern hair loss, the classification widely used is:

- Hamilton-Norwood Classification: the most widely classification used for MPHL (10). There are seven stages of evolution, involving the frontotemporal area and evolution of hair loss to the vertex. There are absence of frontal hairline, however, in some cases, men develop diffuse thinning of the crown with retention of frontal hairline that resembles a female pattern observed in Ludwig Classification.

For female pattern hair loss, the most common classifications are:

- Ludwig's Classification: divided into three progressive stages based on hair density: I - minimal alopecia; II - Moderated alopecia and III - severe alopecia (69).

- Ebling's Classification: includes a five stage classification system for FAGA. The first two stages are the same as the Ludwig system. Type III includes the diffuse hair loss and the initial loss of hair from the frontotemporal hair line; Type IV besides the diffuse loss there's evidence of frontotemporal recession; type V, appears like the male pattern of baldness (MPHL) with a there is complete loss of hair on the top of the scalp (70).
- Olsen's Classification: classification proposed by Olsen (71) with 3 degrees of severity. The main characteristic of this classification is the "christmas tree" distribution, with a frontal accentuation in a triangular.
- Savin Scale: a recent classification scale of 8 levels similar to Hamilton–Norwood's classification with frontal and lateral views of the hair loss patterns, that uses a computer analysis based in "the density of hair by unit of area" (63).

2.1.6 Treatment of Androgenetic Alopecia

The treatment of androgenetic alopecia should be initiate as early as possible as untreated androgen-dependent alopecia progressively deteriorates (72).

There are several treatment options available for the treatment of hair loss, although currently, only topical minoxidil and oral finasteride have the approval of Food and Drug Administration (FDA) for the treatment of AGA.

2.1.6.1 Minoxidil

Initially, oral minoxidil was used to treat high blood pressure. As a side effect of the treatment was an increased hair growth. Since then, topical minoxidil has been used to treat hair loss (6).

The 2% topical minoxidil received the approval by US-FDA for the treatment of AGA in male and female patients in 1998 and 2001, respectively.

The 5% topical minoxidil was approved for the treatment of AGA in men, in 2007 and the 5 % foam minoxidil in 2006 also for the treatment of AGA in men (49). The

5% solution and 5% foam are not licensed by the FDA for use in women, but it is commonly used off-label for this purpose (12).

Minoxidil is a pyrimidine derivate that needs to be converted to its active metabolite, minoxidil sulphate, to exert its action. Minoxidil is thought to promote hair regrowth through its ability to open ATP-potassium channels. Although the exact mechanism of action of minoxidil is not fully understood, minoxidil has vasodilatory, proliferative and anti-inflammatory effects (53).

There are different possible mechanisms in which minoxidil could act to induce hair regrowth.

VEGF is known to increase angiogenesis and also promote perifollicular vascularization, hair growth rates, and increased follicle and hair size (44). An VEGF mRNA is strongly expressed in DP cells during anagen phase with a substantial decrease of expression during catagen and telogen phases (73) Topical minoxidil on the hair follicle can increased the expression of VEGF mRNA, presented in the DP cells (49, 74).

Lachgar et al. (73) performed a study to evaluate if minoxidil could affect the expression of VEGF. According to the authors, minoxidil caused an elevation of VEGF and should be consider an indirect inducer of angiogenesis through an up regulation of the expression of VEGF in DP cells, during the anagen phase.

Other effects of minoxidil to induce hair regrowth could be the activation of activation of cytoprotective prostaglandin synthase-1 and increased expression of HGF, both hair growth promoters (49,74,75).

The literature has demonstrated that topical minoxidil is as an effective treatment for AGA by promoting hair growth (76) Minoxidil is available in 2% and 5% solutions and it is used twice a day in doses of 1 mL.

Several studies (5, 49, 77-81) have demonstrated that minoxidil applied twice a day is an effective treatment for AGA, in both men and women.

Olsen et al. (79) performed a randomized clinical trial (RCT) comparing the use of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of AGA in men. The authors refer that the men who used 5 % topical minoxidil had an earlier response to treatment than those who used 2% topical minoxidil. Both 2

and 5% topical minoxidil solutions were well tolerated by the men without evidence of systemic effects.

Concerning the treatment of FPHL, either 2 or 5% (used off-label) topical minoxidil solution appears safe to use in women with AGA. The only additional risk of the 5% topical minoxidil solution over the 2% topical minoxidil solution reported was the higher incidence of facial hypertrichosis (9).

Recently, 5% minoxidil foam has received specially attention because of the good compliance of the patient and as hydro-alcohol-based vehicle with absence of propylene glycol, presented in minoxidil topical solutions.

Blume-Peytavi et al. (82) performed a randomized, single-blind study of 5% minoxidil foam once daily versus 2% minoxidil solution twice daily to compare their efficacy and safety in the treatment of AGA in women. According to the authors, both treatments were equally effective for improving hair loss, although once daily 5% MTF was better tolerated (lower incidence of pruritus and dandruff) with greater aesthetic advantages in comparison with twice-daily 2% minoxidil solution.

Another study (83) reported that 5% minoxidil foam could be more convenient with a better compliance of the treatment and was not associated with greater side effects. The present article provides update and reliable information that 5% minoxidil foam is a very good option for women patients with hair loss.

Some clinicians prescribe minoxidil in combination with other active ingredients, such as tretinoin. Bazzano GS et al. (84) studied the combination of topical tretinoin with minoxidil and found a terminal hair regrowth in 66% of the patients, after 1 year of treatment. Another study (85) compared the efficacy of 5% minoxidil alone (twice-daily) versus the combination of 5% minoxidil solution and 0.01% tretinoin (once daily) for MPHL. In this randomized, double-blind trial, the authors reported that the efficacy and safety of both treatments appear to be equivalent, with no statistical differences between the two treatment groups.

To assess the efficacy the patient should wait at least 6 months before considering if the treatment is effective. To ensure the compliance of the patient to the treatment, the physician have to inform about the transitory hair shedding that occurs after 2 months of the beginning of the treatment. The main side effects reported with the use of minoxidil are hypertrichosis and contact dermatitis.

2.1.6.2 α-Reductase Inhibitors

There are to types of 5α-reductase enzymes, that have to be considered. Type 1 5α-reductase enzyme predominates in the sebaceous gland, liver, skin and scalp while type 2 predominantly is mainly present in the prostate and certain regions of terminal hairs (49) The 5α-reductase inhibitors regulate the transformation of testosterone to DHT in DP cells of the follicular units.

The main 5α-reductase inhibitors are finasteride and dutasteride.

A daily oral dose of 1 mg finasteride reduces scalp DHT by 64% and serum DHT by 68%. Dutasteride can decrease serum DHT by more than 90%, while finasteride decreases serum DHT by 70% (6).

Oral finasteride is a competitive and specific inhibitor of type 2 5α-reductase. In men with AGA, the balding scalp contains miniaturized hair follicles and increased amounts of DHT if compared to the hairy scalp. The administration of finasteride decreases scalp and serum DHT concentrations in bald scalp and increases scalp testosterone, as stated by Dallob and colleagues (86).

Finasteride has been investigated extensively and it is widely accepted as one of the more effective therapies for AGA in men (49, 87).

Mella et al. (88), published a systematic review to assess the efficacy and safety of finasteride therapy for AGA, when compared to placebo. The authors report that moderate-quality evidence of the studied articles showed that daily use of oral finasteride increases hair count and improves patient and investigator assessment of hair appearance.

Treatment with finasteride should be life-long as its interruption is followed by gradual hair loss, with return to the pre-treatment status within 1 year.

The optimal dose of finasteride for male AGA was identified as 1mg/day (63).

Regarding the use and of finasteride in women, its efficacy is not well-established and is used as an off-label treatment of 2.5-5mg/day (89-91). Two RCT (92, 93) have demonstrated that the use of 1mg/day is not effective in women. Price VH et al. (92) published a double-blind, placebo-controlled, randomized, multicenter

study and reported that after 12 months of treatment with finasteride 1mg/day in postmenopausal women there was a decrease in hair count from baseline and that finasteride was not effective in improving hair loss.

Oral dutasteride is an 5α-reductase inhibitors of both type 1 and 2 enzymes and is prescribed as an off-label treatment for AGA.

Although with few studies published until now, the use of dutasteride appears to be safe and efficacious in the treatment of patients with AGA (94, 95).

In a recently published clinical trial, Tsunemi et al. (96) reported that patients who took 0.5 mg of oral dutasteride once daily for 52 weeks improved the hair regrowth, at 26 and 52 weeks. Of notice, between weeks 26 and 52 the proportion of patients who improved from baseline increased, which means that the effects of dutasteride are still progressing and that dutasteride needs a longer follow-up. The present study reports that dutasteride exhibited long-term safety, tolerability and efficacy for male AGA.

Different studies compared dutasteride versus finasteride in order to assess its efficacy and safety.

In 2004, Olsen EA et al. (97) performed study in which men with AGA were randomized to receive daily dutasteride (0.05, 0.1, 0.5, or 2.5 mg), finasteride (5 mg), or placebo. After 24 weeks of treatment, both dutasteride and finasteride were superior to placebo and the group of 2.5mg dutasteride had a statistical significant improvement when compared with 5 mg finasteride.

Tsunemi et al. (96) reported that 0.5 mg of dutasteride exhibited long-term safety, tolerability and efficacy for male AGA. Another study performed by Boersma et al. (98) reported that 0.15 mg of oral dutasteride appears to be significantly more effective than 1.25 mg of oral finasteride in women under 50 years of age.

Both finasteride and dutasteride are frequently prescribed as off-label treatments for women with AGA. These drugs cross the blood-brain barrier and might cause the feminisation of a male fetus, which means that they are contraindicated for use in women with child-bearing potential and during pregnancy (Pregnancy Category X) (6). Thus, special attention is needed in fertile women of childbearing age, for whom contraceptive measures are obligatory (6).

2.1.6.3 Antiandrogens

Oral antiandrogen therapy such as cyproterone acetate, spironolactone or flutamide are used in the treatment of FPHL, although evidence of efficacy for any of these treatments is limited (49). Oral antiandrogens are used in women and are contraindicated in men due to their feminizing action.

Cyproterone acetate (CA) is an androgen receptor blocker and potent progestin It has been in common usage for over 40 years in the treatment of hirsutism but the limited controlled trial evidence has shown little evidence of efficacy in FPHL.

The mechanism of action of CA is to block androgen receptors and decrease testosterone levels by suppressing luteinizing hormone and follicle-stimulating hormone release (63,99). It seams that CA may be more effective in women with hyperandrogenism.

There are no dose-ranging studies, but most practitioners use CA 50 to100 mg/ day from the fifth to the 15th day of the menstrual cycle for a 6-month period. the first 10 days of each menstrual cycle, then followed by 2mg/d from the first day of the cycle to the 21st, with a week of rest, for 18 months (63). In premenopausal women, oral contraceptives such as ethanol estradiol should be added as it stabilizes menstrual irregularities. For postmenopausal women, CA may be used continuously.

The side effects of CA are dose dependent and this medication is absolutely contraindicated in patients with liver disease. In addition, feminization of a male fetus may occur and so patients should be advised to cease the medication before conception. According to Camacho et al opinion' (63), CA is the best treatment for the FPHL.

Spironolactone is a potassium-sparring diuretic, structurally related to aldosterone (antagonist), that acts as an anti-androgenic by reducing the levels of total testosterone and competitively blocking the androgen receptor in target tissues. (99) .Its primary use is as a diuretic and antihypertensive. There are no controlled trials in FPHL.

The dosage range from 50 mg/day to 200 mg/day for at least 6 months. The beginning is made with a starting low dose of 50 mg/day and increasing the monthly dose by 50 mg to a final dose of 200 mg/day (63,100).

Side effects are dose related and include menstrual irregularities (menorrhagia), postmenopausal bleeding, breast tenderness, hyperkalemia and fatigue. In healthy young women, the risk of hyperkalemia is very low, although check of the serum potassium levels should be performed periodically (63).

This drug is category X for pregnancy, as it has as the potential to feminize a male fetus.

Flutamide is a pure, nonsteroidal antiandrogen that acts by inhibiting androgen uptake and by inhibiting nuclear binding of androgen within the target tissue. It is the treatment of choice when hair loss and hirsutism are associated with each other (101). The most severe side effect is hepatotoxicity. Other side effects described include lethargy, mood change, and loss of libido. Is also contraindicated in pregnancy and lactancy as it may cause feminization of the male fetus.

Emerging treatments

The currently available treatments for AGA are sometimes perceived as having limited effectiveness; therefore, the identification of new therapies for this pathology is of utmost importance (102,103). New possible offline treatments for AGA, such as topical finasteride, injectable dutasteride, laser therapy and PRP, have recently emerged and have been claimed to have hair growth-promoting or anti-hair loss effects.

2.1.6.4 Topical finasteride

Topical finasteride is being investigated as a new treatment for AGA with fewer side effects than oral finasteride. According to Caserini et al.(104), a daily application of a 0.25% solution of finasteride (doses of 100 and 200 μL) leads to the inhibition of scalp DHT and may minimize the untoward sexual side effects that are linked to systemic DHT reduction (6).

A RCT (105) compared oral finasteride and topical finasteride in 45 male patients with AGA. Patients were randomized in two groups to receive 1) twice daily finasteride gel 1% preparation and placebo tablets 2) oral finasteride 1% and placebo gel, for 6 months. According to the authors, the results showed similar efficacy for the two therapies, although not statistically significant.

A study conducted by Tanglertsampan C (106) compared the safety and efficacy of 3% minoxidil solution (MNX) versus the combination of 3% minoxidil and 0.1% finasteride solution (MFX) in the treatment of men with AGA. At 24 weeks, there was an increase of hair count from baseline in both groups, but no statistical difference. The assessment by global photography showed significantly greater improvement in the MFX group than the MNX group.

Another study (107) assessed the efficacy of 5% topical minoxidil fortified with 0.1% finasteride in patients with AGA who had been medicated with 5% topical minoxidil and oral finasteride in the previous two years. The aim was to assess the efficacy of maintaining the hair growth. Eighty-four percent maintained a good hair density after 12 months of treatment with minoxidil-finasteride combination.

There are few published articles regarding topical finasteride and usually topical finasteride is used in combination with other medication. At this moment, there is insufficient evidence to support the use of topical finasteride (49). Additional studies using the topical finasteride alone are needed to assess its efficacy.

2.1.6.5 Injectable dutasteride

Injectable dutasteride might be a promising treatment for AGA. In 2013, Moftah et al. (108) published a study that evaluated the efficacy and safety of mesotherapy using a dutasteride-containing preparation (0.5 mg of dutasteride, 20 mg of biotin, 200 mg of pyridoxin and 500 mg of D-panthenol) in female patients with hair loss. The authors concluded that this preparation was effective, tolerable and yielded better response among females with a shorter duration of disease. More studies are needed with the use of injectable dutasteride alone, to assess its efficacy.

2.1.6.6 Low-level laser therapy

The laser therapy is the application of light of low-level laser (LLL) or laser-emitting diodes (LED) with a spectral of 600 - 1000 nm (109) and is used to treat several conditions such as pain reduction, inflammation and to promote wound healing and tissue regeneration (110). Recently, it has been use to promote hair regrowth. For its used in hair loss, most of the available devices use a wavelength of 655 nm.

The first LLLT device, the Hairmax Lasercomb® was approved by the US FDA and received clearance as a safe therapy for the treatment of male AGA in 2007 and female AGA in 2011 (111,112).

Although the exact mechanism of LLLT is not clear, this laser phototherapy might induce proliferation, migration, oxygenation and stimulate the transition from telogen hair follicles to anagen (110,111,113).

A systematic review (114) was carry out and found an that the majority of studies covered in the review found an overall improvement in hair regrowth, thickness, and patient satisfaction following LLLT therapy.

Another evidence-based review (113) stated that LLLT devices are both safe and effective for the treatment of FPHL and MPHL.

Avram MR et al. (115) performed a study in 7 patients to investigate the efficacy of LLLT in hair regrowth and found a not statistical significance decrease in the number of vellus hairs, an increase in the number of terminal hairs, and an increase in shaft diameter. No improvement in hair density was noted.

Although published articles (113,116,117) found that LLLT could improve hair regrowth, the LLLT results are limited with a low number of patients. More studies, including RCT are needed to support its efficacy. In addition, the optimum dose, wavelength, coherence and dosimetric parameters remain to be determined (110).

As stated by Gupta AK et al. (110), clinical studies should be conducted and published in peer-reviewed journals and although manufacturers believe in the quality and efficacy of their device, this might not be “sufficient for physicians to accept LLLT for hair loss and widely promote it, without reservation, to patients”.

2.1.7 Prognosis of Androgenetic Alopecia

There have been a number of studies regarding a relationship between AGA and associated diseases, such as hypertension, hyperlipidemia, diabetes mellitus, and coronary artery disease (CAD).

Ahouansou et al.(118) evaluated the association between AGA and hypertension and found that men, aged between 19 to 50 years and who have early AGA (occurring before 35 years of age), had an increased incidence of hyperinsulinemia and disorders associated with insulin resistance, such as obesity, hypertension (47), and dyslipidemia (119).

Patients with AGA might be at an increased risk of CAD which increases with increasing grade of AGA (120).

Arias-Santiago et al. (121) performed a case control study with 154 participants, to analyze the presence of the cardiovascular risk factors and the prevalence of carotid atheromatosis in male and female patients with AGA in comparison with control subjects. According to authors’s results, the metabolic syndrome was diagnosed in 60% and 48.6% of male and female patients with AGA, respectively; Atheromatous plaques were more frequent in patients with AGA than in controls and that was statistical significant.

Therefore, the determination of metabolic syndrome, ultrasound study of the carotid arteries in patients with AGA may be useful screening methods to detect risk of developing cardiovascular disease in patients with early-onset AGA and signal a potential opportunity for early preventive treatment.

2.2 PLATELET-RICH PLASMA

2.2.1 History of Platelet-Rich Plasma

Platelet-rich plasma is also known as platelet-rich in GFs, platelet-rich fibrin matrix, platelet-rich fibrin and platelet concentrate.

The concept and description of PRP started in the field of Hematology (122). Hematologists created the term PRP in 1970's in order to describe the plasma with a platelet count above that one of the peripheral blood, which was initially applied as a transfusion product to treat patients with thrombocytopenia (123).

Ten years later, PRP started to be used in maxillofacial surgery as a platelet-rich fibrin. Fibrin had the potential for adherence and homeostatic properties and PRP with its anti-inflammatory characteristics stimulated cell proliferation (124).

Subsequently, PRP has been used predominantly in musculoskeletal field in sports injuries. With its use in sports in elite players it draw widespread media attention and is been extensively used in this area (125). Other medical areas that also use PRP are cardiac surgery, paediatric surgery, gynecology, urology, plastic surgery and ophthalmology (126).

More recently, interest has been increasing in the application of PRP in dermatology, for example, in tissue regeneration, wound healing, scar revision, skin rejuvenating effects and alopecia(124,127-132).

Wounds have a pro-inflammatory biochemical environment that impairs healing in chronic ulcer. In addition, it is characterized by a high protease activity, which decreases effective growth factor concentration. PRP is used as an interesting alternative treatment for recalcitrant wounds as it is a source of GFs, and consequently has mitogenic, angiogenic and chemotactic properties (133).

In cosmetic dermatology, a study performed *in vitro* demonstrated that PRP can stimulate human dermal fibroblast proliferation and increase type I collagen synthesis (134). Additionally, based on histological evidence, PRP injected in human deep dermis and immediate subdermis induces soft-tissue augmentation,

activation of fibroblasts and new collagen deposition, as well as new blood vessels and adipose tissue formation (135,136).

Other applications in which PRP is being used is to improve burn scars, postsurgical scars and acne scars (137). According to the few articles available, PRP alone or in combination with other techniques seams to improve the quality of the skin as well as an increase in collagen and elastic fibers.

In 2006, PRP has started to be considered as a potential therapeutic tool for promoting hair growth and has been postulated as a new therapy for alopecia, both AGA and AA. Several studies have been published that refer the positive effect of PRP in the treatment of AGA, although a recent meta-analysis suggested the lack of RCTs. As stated by the authors, controlled clinical trials are considered to be the best way to provide scientific evidence for a treatment and avoid potential bias when assessing efficacy.

The aim of this thesis is through a RCT access the efficacy of PRP as a new possible treatment for AGA.

On May 23rd, 2013, the Spanish Agency for Drugs and Medical Devices of the Ministry of Health, Social Services and Equality published a report concerning the preparation and medical use of PRP and outlined the technical standards to be met and the information to be given to patients. The report established that the use of PRP requires a medical prescription, which is limited to suitably qualified medical doctors, dentists or podologists and excludes other health or non-health professionals (138).

The procedure must be subject to quality guarantees, and if an open technique is used, an administrative and medical authorization is required. The report also recommended the implementation of control, surveillance and traceability measures to prevent the transmission of infectious diseases (136,138).

2.2.2 Platelet Biology

All blood cells derive from a common pluripotent stem cell, which differentiate into different cell lines. Each of these cell series contains precursors that can divide and mature.

Platelets, also called thrombocytes, develop from the bone marrow. Platelets are anucleated, discoid cellular elements with different sizes and a density of approximately 2 μm in diameter, the smallest density of all blood cells. The physiological count of platelets circulating in the blood stream ranges from 150 000 to 400 000 platelets per μL.

Platelets contain several secretory granules that are crucial to platelet function. There are three types of granules: dense granules, α-granules and lysosomes. In each platelet there are approximately 50-80 α-granules, the most abundant of the three types of granules.

Platelets are primarily responsible for the aggregation process. The main function is to contribute to homeostasis trough 3 processes: adhesion, activation and aggregation. During a vascular lesion, platelets are activated and their granules release factors that promote coagulation (139).

Platelets were thought to have only hemostatic activity although in recent years, scientific research and technology has provided a new perspective on platelets and functions. Studies suggest that platelets contain an abundance of GFs and cytokines that can affect inflammation, angiogenesis, stem cell migration and cell proliferation (140).

PRP is a natural source of signalling molecules and upon activation of platelets in PRP, the α-granules are degranulated and release the GFs and cytokines that will modify the peri-cellular microenvironment.

Some of the most important GFs released by platelets in PRP included VEGF, FGF, PDGF, EGF, HGF, insulin-like growth factor 1, 2 (IGF-1, IGF-2), matrix metalloproteinases 2, 9 (MMP-2 and MMP-9) and IL-8 (123,141).

2.2.3 Definition

Platelet-rich plasma is a biologically product defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline (before centrifugation) (102). As such, PRP contains not only a high level of platelets but also the full complement of clotting factors, the latter of which typically remain at

their normal, physiologic levels (142). It is enriched by a range of GFs, chemokines, cytokines and other plasma proteins (125).

The PRP is obtained from the collection of blood of each patient before undergoing centrifugation. After centrifugation and according with their different density gradient's there's the separation of blood components (red blood cells, PRP and platelet-poor plasma).

In PRP, besides the higher concentration of platelets, other parameters need to be taken into account such as the presence or absence of leucocytes and activation. This will defined the type of PRP used in each pathology.

There are several commercial devices available which simplify the preparation of PRP.

According to manufactures, PRP devices usually achieve a concentration of PRP 2 to 5 times the baseline concentration. Although one might think that higher platelet count with higher number of GFs would give better results, this has not been determined yet (140). In addition, one study also suggest that a concentration of PRP 2.5 times above the baseline could have an inhibitory effect (143).

2.2.4 Mechanism of action

The GFs and the bioactive molecules present in PRP promote four main actions in the local environment of the administration such as proliferation, migration, cell differentiation and angiogenesis (37,102,144-146). Various cytokines and GFs are involved in the regulation of hair morphogenesis and cycle hair growth (147).

The DP cells produce GFs such as IGF-1, FGF-7, HGF and VEGF that are responsible for maintaining the hair follicle in the anagen phase of the hair cycle. Therefore, a potential target would be to upregulate these GFs within the DP cells, which lengthen the anagen phase (75).

According to a study performed by Akiyama et al. (148), EGF and TGF are involved in regulating the growth and differentiation of bulge cells, and PDGF may

have related functions in the interactions between the bulge and the associated tissues, starting with follicle morphogenesis (145).

Beside the GFs, the anagen phase is also activated by Wnt / β-catenin / LEFT (40). In the DP cells, the activation of Wnt will lead to an accumulation of β-catenin that in combination with LEFT act as a co-activator of transcription and promote proliferation, survival and angiogenesis. The DP cells then initiate the differentiation and consequently the transition from telogen to anagen phase (37). Beta-catenin signalling is important in HF development and for the hair growth cycle.

Another pathway presented in DP is the activation of extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) signalling that promotes cell survival and prevents apoptosis (127).

The precise mechanism by which PRP promotes hair growth is not fully understood. To explore the possible mechanisms involved, Li et al. (127) performed a well-designed study to investigate the effects of PRP on hair growth using *in vitro* and *in vivo* models. In the *in vitro* model, activated PRP was applied to human DP cells, obtained from normal human scalp skin. The results demonstrated that PRP increased the proliferation of human DP cells by activating ERK and Akt signalling, leading to anti-apoptotic effects. PRP also increased the β-catenin activity and FGF-7 expression in DP cells. Concerning the *in vivo* model, mice injected with activated PRP showed faster telogen-to-anagen transition in comparison to the control group.

Recently, Gupta AK and colleagues (37), also proposed a mechanism for the action of PRP on the HF that includes the “elicitation of the Wnt /β-catenin, ERK and Akt signaling pathways fostering cell survival, proliferation, and differentiation”.

After GF binds with its correspondent GF-receptor (GFR), the signalling necessary for its expression begins. The GF-GFR activates the expression of both Akt and ERK signaling. The activation of Akt will inhibits through phosphorylation two pathways: 1) the glycogen synthase kinase-3β (GSK3-B) that promotes degradation of β-catenin 2) and Bcl-2-associated death promoter (BAD), responsible to induce apoptosis. As stated by the authors, PRP might increase

vascularization, prevent apoptosis and prolong the duration of the anagen phase (37).

The main functions of the GFs presented in the PRP are detailed in table 1.

Growth Factors	Main Functions
PDGF (145)	- Increase hair growth - Vascularization - Angiogenesis stimulators
TGF-β (147)	- Inhibits hair growth *in vitro* - Hair cell proliferation and regeneration
VEGF (44,73)	- Expressed in DP cells the anagen phase - Probably regulates perifollicular angiogenesis - Increase perifollicular vessel size during the anagen growth phase
EGF (147,149)	- Angiogenesis stimulators - Hair cell proliferation and regeneration
HGF (39)	- Angiogenesis stimulators
FGF (147,150-151)	- Increase hair growth by inducing anagen phase of HF - Promote DP cell proliferation - Increase the HF size in mice - Angiogenesis stimulators
IGF-1(127,145,152)	- Increase hair growth - Maintains HF growth *in vitro* - Angiogenesis stimulators

Table 1. Main functions of the growth factors presented in the platelet-rich plasma.

2.2.5 Devices to obtain PRP

Currently, there is a great discussion and no consensus regarding PRP preparation. PRP is prepared through a process known as differential centrifugation, in which acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity (153).

Regarding the obtention of PRP there are two techniques:

- Open technique: one in which the product is exposed to the environment of the working area and comes in contact with different materials that should be used for their production, such as pipettes or product collection tubes. In the blood processing to obtain PRP by open technique, it should guarantee that the product is not contaminated during handling microbiologically.
- Closed technique: involves the use of commercial devices with CE marking (including centrifuge equipment and application) in which the product is not exposed to the environment (recommended).

Several CE medical devices are available for the production of autologous PRP. Most of them are included in one of this three types of devices:

1) The blood collection is obtained to a tube that contains an anticoagulant and this tube is compatible with different can be used in any type centrifuge
2) Medical devices where the blood is collected into a tube that already contains an anticoagulant; the centrifugation can then be made in any type of centrifuge.
3) Medical devices where the blood is collected into a syringe previously filled with an anticoagulant; usually the blood is transferred into a secondary device whose shape imposes the use of the centrifuge supplied by the same manufacturer (154).

The obtention of PRP depends on the type of device chosen and should be followed the manufacturer's instructions (table 2).

Devices	Blood collection/ Anticoagulant	Centrifugation		
		Number of times	Speed/Time	Centrifuge
Selphyl ®	Tube 9 mL/ Sodium Citrate	1	1100 g / 6 min	Classic
PRGF Endoret ®	Tube 9 mL/ Sodium Citrate	1	270 g / 7 min	Classic
Cascade ®	Tube 9 mL Sodium Citrate	2	1100 g / 6 min 1450 g / 15 min	Classic
Plateltex ®	Tube 9 mL ACD	2	180 g / 10 min 1000 g / 10 min	Classic
Regenkit ®	Tube 9 mL Sodium Citrate	1	1500 g / 9 min	Classic
ACP Arthrex ®	Syringe 15 mL ACD or no anticoagulant	1	1500 rpm / 5 min	Adapted
GPS III ®	Syringe 30 or 60 mL ACD	1	3200 rpm / 15 min	Adapted
Genesis ®	Syringe 12 mL ACD	1	2400 rpm / 12 min	Adapted
SmartPrep 2 ®	Syringe 20 or 60 mL ACD	2	2500 rpm / 4 min 2300 rpm / 10 min	Adapted
Proteal ®	Syringe 20 mL Sodium Citrate	1	1800 rpm / 8 min	Adapted
Magellan ®	Syringe 30 to 60 mL ACD	-	-	Adapted device
Angel ®	Syringe 40 to180 mL ACD	-	-	Adapted device

Table 2. Blood collection and centrifugation protocols from different medical devices to obtain PRP. Courtesy of Dr. Jeremy Magalon, adapted from (154); ACD: Acid citrate dextrose.

As described in table 2, there are different PRP systems which facilitate the preparation of PRP in a reproducible manner. All operate on a small volume of drawn blood and on the principle of centrifugation.

Briefly, the procedure requires use of relatively small volumes of blood. The PRP is obtained from the collection of blood of each patient before undergoing centrifugation. The whole blood is obtained by venipuncture in anticoagulated tubes (usually with ACD or sodium citrate solution). The blood is then centrifuge, with a single or a double spin centrifugation, depending on the device. The settings of the centrifuge established to obtain PRP at an adjustable concentration is defined by the manufacture and can't be changed by the physician.

After centrifugation, the tube appears with three basic layers: at the bottom of the tube, red blood cells with leukocytes deposited immediately above; the middle layer corresponds to the PRP and at the top, there's the PPP. (Fig. 2) The PPP is removed and PRP is obtained. Platelets can be activated before application of the PRP, although there is no consensus on whether or not platelets must be previously activated before their application and with which agonist (153).

Thrombin and calcium chloride, aggregation inducers, are used with the aim to activate platelets and stimulate degranulation causing the release of the growth factors (155).

Some authors activate platelets whereas others apply platelets without being previously activated, arguing that better results are obtained. Recently studies have described that the use of such aggregators is not necessary as at the time of administration, the platelets are automatically release and ready to exert their function.

Even though most of devices aim to obtain the best PRP, the systems differ extensively in their ability to collect and concentrate platelets depending on the method and time of its centrifugation. As a result, suspensions of different concentration of platelets and leucocytes are obtained. It is difficult to assess which kit for PRP preparation is better and which is worse (153). Also, each preparation may produce different types of PRP with different applications. There is no consensus about the number of centrifugations required, neither on their speed and duration.

There is intensive ongoing debate regarding the ideal volume of PRP to administer, the frequency of application, the exact site of administration of PRP, and which technique/ preparation system of manufacture to use.

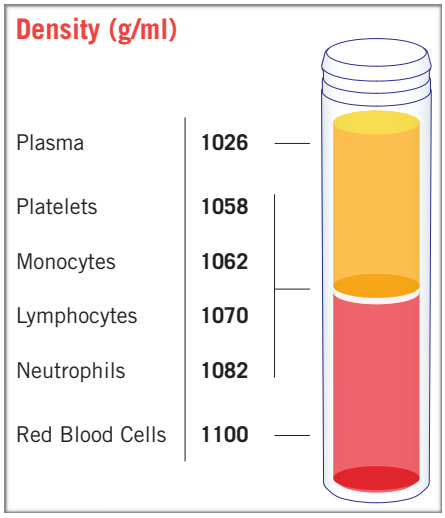

Fig. 2. After centrifugation, the blood components (red blood cells, leukocytes and platelets) are separated from the plasma due to their different densities. The platelets have the lowest density. Adapted from (54).

2.2.6 Classification

Several authors have tried to characterize and classify the numerous techniques available on the market in terms of preparation (centrifugation speed and use of anticoagulant), content (platelets, leucocytes, and GFs), and applications (156).

Although the literature about PRPs developed with all these contradictions, the need for standardized terminology is of maximum importance (157,158). Thus, some classifications have been proposed to achieve a consensus terminology in the field of platelet concentrates (159-163).

Characterizing the type of PRP used (as a pure PRP, in our study) will lead to a better understanding of PRP, and data available will be easier to sort and interpret. Also, this terminology would serve as a basis for further research on the topic.

In 2009, Ehrenfest et al. (160) proposed a classification of four main families of preparations following 2 principle parameters: presence or absence of a cell content (such as leucocytes) and the fibrin architecture.

1. Pure Platelet-rich plasma (P-PRP) or Leucocyte-poor PRP: the preparation obtained is without leucocytes and with a low density fibrin network after activation.
2. Leucocyte and PRP (L-PRP): the preparations contain leucocytes and with a low density fibrin network after activation.
3. Pure platelet rich fibrin (P-PRF) or leucocyte poor platelet rich fibrin: preparations are without leucocytes and with a high density fibrin network. Unlike P-PRP or L-PRP, these products can't be injected and exist in an activated gel form.
4. Leucocyte and platelet rich fibrin (L-PRF): products are preparations with leucocytes and with a high density fibrin network.

Mishra et al. (162) proposed another classification based on the presence or absence of white blood cells, activation status and platelet concentration, based on the coefficients of increase platelets and leukocytes concentration in PRP compared to whole blood baseline, as well as on PRP activation (163).

The classifications were not consensual and there's still the intent to search a classification for PRP that could characterize the injected PRP in order to compare the efficacy of different studies.

An important point of discussion is that in the previous classifications, the authors didn't take into account the final volume of the preparation, the presence or absence of RBC in PRP and the doses of platelets in the final volume obtained of PRP.

In 2016, Magalon et al. (163) proposed the DEPA classification (Dose, Efficiency, Purity, Activation) that focuses on the quantity of platelets obtained by the PRP kits as well as on product purity and on platelet activation prior to injection.

The DEPA classification is based on four different components:

1. **Dose of injected platelets**: calculated by multiplying the platelet concentration in PRP by the obtained volume of PRP. According to the injected dose (measured in billions or millions of platelets) it should be categorised into: A, very high dose of injected platelets of >5 billion; B, high dose of injected platelets, from 3 to 5 billion; C, medium dose of injected platelets, from 1 to 3 billion and; D, low dose of injected platelets, <1 billion.
2. **Efficiency of the production:** corresponds to the percentage of platelets recovered in the PRP from the blood. It is categorised as follows: A, high device efficiency if recovery rate in platelets is > 90%; B, medium device efficiency if recovery rate in platelets is from 70% to 90%; C, low device efficiency if the recovery rate is from 30% to 70% and; D, poor device efficiency for a recovery rate < 30%. corresponds to the relative composition of platelets, leucocytes and RBCs in the obtained PRP.
3. **Purity of the PRP obtained:** correlates to the relative composition of platelets, leucocytes and RBCs in the obtained PRP. It is described as: A: very pure PRP, if percentage of platelets in the PRP, compared with RBC and leucocytes is > 90%; B: pure PRP, between 70-90% of platelets; C: heterogeneous PRP, if percentage of platelets is from 30% to 70%; and D: whole blood PRP, if percentage of platelets in the PRP is <30% compared with RBC and leucocytes.
4. **Activation process**: if an exogenous clotting factor was used to activate platelets, such as autologous thrombin or calcium chloride.

Although this last classification is very complete, this quantification can not be define by the physician and should be registered in each CE medical device available for preparation of PRP. In this manner, the characterization of PRP should provide significant help to clinicians in selecting a system that meets their specific needs for a given indication.

In addition, characterizing the type of PRP used will lead to a better understanding of PRP, and data available will be easier to sort and interpret. Also, this terminology would serve as a basis for further research.

3. HYPOTHESIS

3. HYPOTHESIS

Topical minoxidil and oral finasteride are the gold standard therapies for AGA and the only two drugs currently that have US FDA approved indications for the treatment of AGA. Finding new therapies is extremely important. Recently, PRP have been postulate to improve hair regrowth.

In the current study we tested the hypothesis that treatment with PRP would:

1. Increase hair regrowth in female and male patients with AGA
2. Be an alternative therapy in patients with AGA, if used in monotherapy
3. Be effective in combination with US FDA approved medication for AGA, such as minoxidil or finasteride

4. OBJECTIVES

4. OBJECTIVES

The objectives of the study were:

4.1 MAIN OBJECTIVE

a. To assess the efficacy of PRP on the evolution of AGA, between 6 months of treatment and baseline.

4.2 SECONDARY OBJECTIVES

a. To assess the evolution of AGA, between 3 and 6 months of treatment with PRP alone and baseline.
b. To assess the evolution of AGA between 3 months and 6 months of treatment with PRP in combination with minoxidil or finasteride and baseline.
c. To observe the overall tolerance of the treatment with PRP
d. To evaluate if PRP could be a new therapeutic options for patients with AGA

To achieve the proposed objectives, the study was divided into two phases. The first phase was intended to evaluate the evolution of AGA in patients who performed treatment with PRP alone. The second phase of the study included patients of AGA, with ongoing medication with minoxidil or finasteride.

5. MATERIAL AND METHODS

5. MATERIAL AND METHODS

5.1 STUDY DESIGN

This was a prospective randomized, double-blind, placebo-controlled (half-head), parallel-group study conducted at a private clinic, linked to the University International of Catalunya, Spain.

The study was submitted for consideration and approval to the Clinical Research Ethic Committee of the University International of Catalunya. After approval by the local Ethic Committee *(Annex 13.1)*, the study was registered on ClinicalTrials.gov, with identify number: NCT02393040 *(Annex 13.2)*.

All patients provided written informed consent before participating in the study, which was performed according to the Declaration of Helsinki *(Annex 13.3)*.

Male patients with a clinical diagnosis of AGA (stage II to IV, according to Hamilton-Norwood Classification) and female patients with clinical diagnosis of AGA (stage I to III, according to Ludwig Classification), were recruited.

The selected patients were divided in two groups: Group A and Group B.

Group A included patients without any prescribed medication for AGA, in the previous 12 months.

Group B included patients with an ongoing treatment with 5% topical minoxidil or 1mg oral finasteride for AGA, within the preceding 12 months. The patients were instructed to maintain their usual treatment for AGA during all the protocol, otherwise, they were excluded from the study.

For Group A and Group B, the protocol of treatment and assessment criteria were the same.

Before inclusion in the study, a complete blood cell count was performed in all patients to exclude platelet abnormalities.

All patients were evaluated in 4 visits: V1, baseline and beginning of the study; V2, second treatment; V3, third treatment; and V4, follow-up. In the first 3 visits, a total

of 3 treatments were given with an interval of 1 month from each other. Each patient received injections of PRP in one side of the head and in the other half-head was injected with a placebo solution (saline solution). The follow-up visit was the last visit, at 6 months to assess the efficacy of treatment compared with baseline.

The evaluation criteria were assessed in all patients by global photography and phototrichogram. The evaluator responsible for both global photographs and phototrichogram analyses was blinded with regard to the treatment and placebo areas and was not involved in the administration of treatment.

Safety outcomes were evaluated during the course of all protocol.

5.2 PARTICIPANTS

Eligible participants were healthy adults of both sexes with confirmed diagnosis of AGA. The diagnosis was established based on clinical history, physical examination and trichoscopic features. Further evaluation of the patients was not necessary to complete the diagnosis of AGA.

The patient selection was performed in two different phases, according to presence or absence of medication for AGA. Group A included patients without previous medication for AGA and Group B included patients under medication for AGA.

According to the respective group (A or B), patients were eligible in this study with the following criteria:

5.2.1 Patient selection of Group A: PRP alone

Inclusion criteria:

- Patients aged 18 to 65 years
- Male patients with clinical diagnosis of AGA and with stage II to V, according to Hamilton-Norwood Classification.
- Female patients with clinical diagnosis of AGA and with stage I to III, according to Ludwig Classification.

Exclusion criteria:

- Patients with other hair loss diseases or dermatologic disorders, besides AGA
- Patients who had received topical solutions (minoxidil or any other solution for hair growth), systemic treatment (finasteride, dutasteride, or antiandrogens) or laser therapy for AGA, within the preceding 12 months
- Patients submitted to hair transplant
- Platelet dysfunction syndrome or platelet count < 150 000 platelets per microliter (μL)
- Smokers (> 20 cigarettes/day)
- Pregnancy or lactation
- Patient unable to accomplishing all fases of treatment

5.2.2 Patient selection of Group B: PRP and Medication

Inclusion criteria:

- Patients aged 18 to 65 years
- Male patients with clinical diagnosis of AGA and with stage II to V, according to the Hamilton-Norwood Classification.
- Female patients with clinical diagnosis of AGA and with stage I to III, according to Ludwig Classification.
- Patients with an ongoing treatment with minoxidil or finasteride for AGA, within the preceding 12 months.

Exclusion criteria:

- Patients with other types of alopecia or pathology, besides AGA
- Patients who do not maintain the ongoing treatment (minoxidil or finasteride), during all the protocol.
- Patients submitted to hair transplant
- Platelet dysfunction syndrome or platelet count < 150 000 platelets per microliter (μL)
- Smokers (> 20 cigarettes/day)
- Pregnancy or lactation
- Patient unable to accomplishing all fases of treatment

5.3 PERIOD OF STUDY

The study was initiated on January of 2014, after ethical committee approval and completed on November 2015. It was divided in two phases. Participants included in Group A were admitted between January 2014 and November 2014 while patients of Group B were included in the study between January 2015 and November 2015.

The study took place at the private clinic of Prof. Ramon Grimalt (Terrassa, Spain), linked to University International de Catalunya. Participants with AGA were recruited from the database and consultation of Prof. Ramon Grimalt.

5.4 INTERVENTIONS

For practical proposes, the study was divided in two phases: Group A and Group B. In each group, 2 subgroups were formed (A1 and A2; B1 and B2) to randomly define which half-head (right or left) would be injected with PRP or placebo. Subgroups A1 and B1 received treatment with PRP on the right half-head and the placebo on the left half-head, whereas subgroups A2 and B2 received treatment with PRP on the left half-head and the placebo on the right half-head.

The main difference between the two groups was regarding the criteria of presence or absence of medication.The protocol design, data assessment and interventions were similar in both Group A and B.

All interventions of the study are described bellow to allow replication of the clinical protocol.

For simplicity and continuity the division in the two groups will not be highlighted in the subsequent sections.

5.4.1 Analysis

Platelets are important to a great degree, as they are crucial contributors to the molecular pool present in PRP (123). The mean platelet concentration factor defined as the platelet count in PRP compared to the platelet count in whole blood ranged from 3.01 to 4 (156).Therefore, to achieve the higher concentration of platelet after centrifugation, the number of platelet of each patient should be within the respective reference ranges.

Before inclusion in the study, a complete blood cell count was performed in all patients to exclude platelet abnormalities. All participants included in this study had platelet numbers over 150 000 platelets/μL.

The blood cell count performed in all patients at baseline had a mean platelet counting of 1 523 82 ± 35 000 platelets/mL.

5.4.2 Platelet Rich-plasma

The preparation and medical use of PRP fulfilled the criteria published by the Spanish Agency for Drugs and Medical Devices of the Ministry of Health, Social Services and Equality, on May 23rd, 2013.

PRP is produced using different methods of platelet concentration via centrifugation and cell separation. The PRP system and equipment used for separation, concentration and plasma collection belongs to the manufacture Proteal, *Soluciones Bioregenerativas, SL.*

In this study, for preparation and obtention of PRP we used a closed system with sterile and disposable kits that meet all of the applicable quality criteria (EC certified system).

Samples collected with the Proteal System were handled in accordance with the manufacturer’s instructions.

The material used to obtain PRP:

- Centrifuge *Omnigrafter II - Proteal* (Fig. 3) *(Annex 13.4)*
- Disposable and closed 20 mL Kit (Fig. 4) *(Annex 13.5)*
- Sodium citrate solution 3.8% *(Annex 13.6)*
- Calcium chloride solution 10% *(Annex 13.7)*

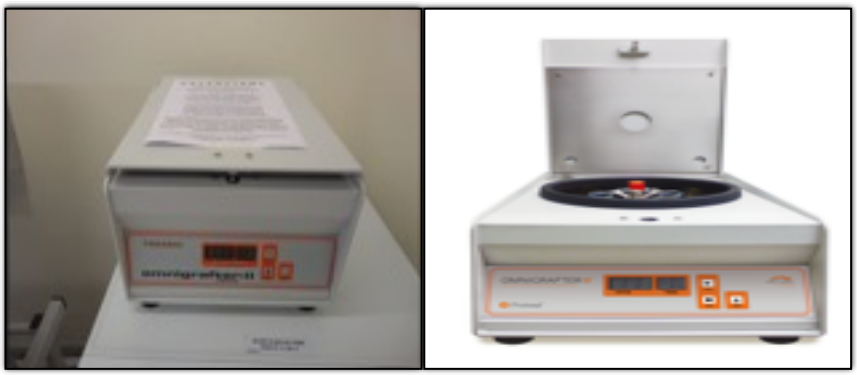

Fig. 3. Centrifuge Omnigrafter II, Proteal,*Soluciones Bioregenerativas*.

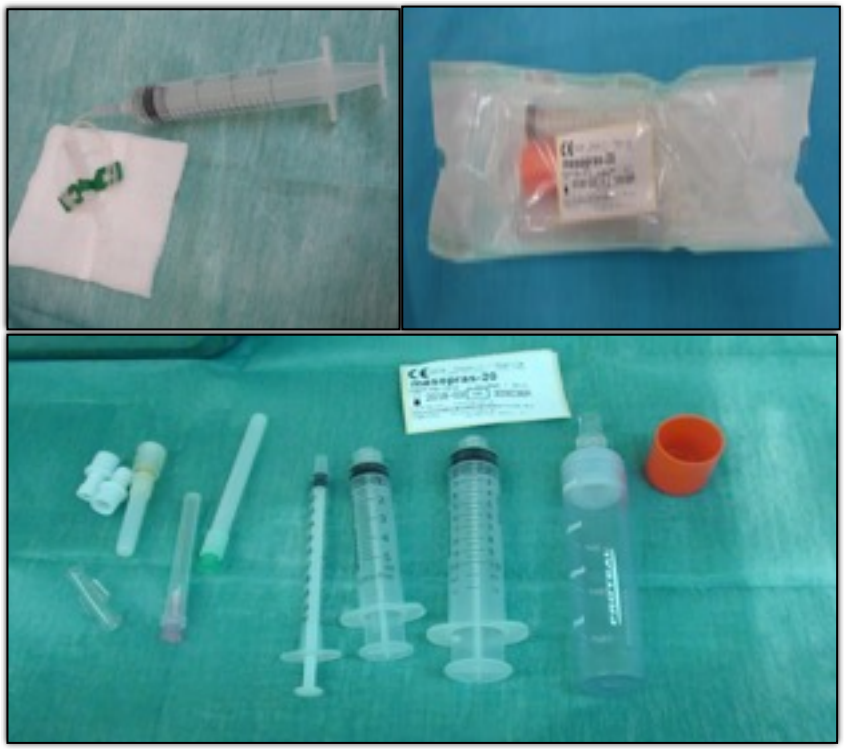

Fig. 4. Illustration of the sterile and disposable kit of 20 mL and its components.

Procedure to obtain PRP

The procedure requires use of relatively small volumes of blood. The PRP is obtained from the collection of blood of each patient before undergoing centrifugation.

First, a vein from the arm is chosen by palpation. Normally, the venipuncture is performed in the right median cubital vein, which lies within the cubital fossa anterior to the elbow. Approximately, 18 mL of blood is drawn into a syringe pre-filled with 2 mL of 3.8% sodium citrate solution (anticoagulant). The anticoagulated blood of the syringe is gently transferred to a cylinder, which is placed inside the metallic adapter of the Omnigrafter® centrifuge.

The settings of the centrifuge are established to obtain PRP at an adjustable concentration defined by the manufacture (approximately 2.2 ± 0.4 x the baseline concentration/mL). A single spin centrifugation is performed at force 460 g, for 8 minutes.

The centrifugation automatically starts the whole process of separation of the whole blood into its different components. Blood components (red blood cells, leukocytes and platelets) are separated from the plasma due to their different densities. The platelets have the lowest density.

After centrifugation, the cylinder appears with three basic layers: 1) the RBC are located at the bottom of the tube with leukocytes deposited immediately above; 2) in the middle layer, there's the greatest platelet concentration that corresponds to the PRP; 3) at the top, there's the PPP layer with a smaller platelet concentration.

PRP fractions in the closed tube are then collected with a specific system (Push-Out®) and transferred into the application syringes. PPP represents approximately ¾ of the supernatant and the other 4 mL obtained correspond to PRP. The PRP obtained is classified as a pure - PRP (P-PRP).

Immediately before injection in the selected areas, the PRP is activated with 0.05 mL of 10% calcium chloride solution, per mL.

The PRP administration is performed in half-head of each patient in the selected areas, previously marked with a central dot tattoo. The infiltration was carry-out with a 30-G needle using the manual mesotherapy technique, in dermal level.

PRP in a total dose of 4 mL, was administer in the amount of 0.15 mL - 0.20 mL, per point of injection. Each injection point is spared of 1 cm from each other (Fig. 5). Local anesthesia was not injected on the treated areas.

Each patient received three treatments of PRP, with an interval of 1 month from each other, in the half-head corresponding to treatment with PRP.

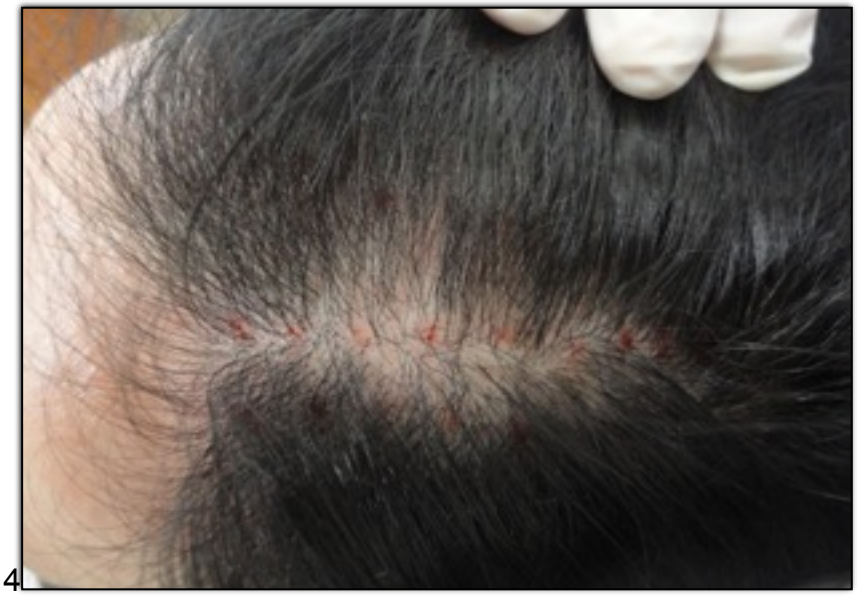

Fig. 5. Each injection point is spared of 1 cm from each other.

The steps to obtain the PRP with the closed system are illustrated in Fig. 6

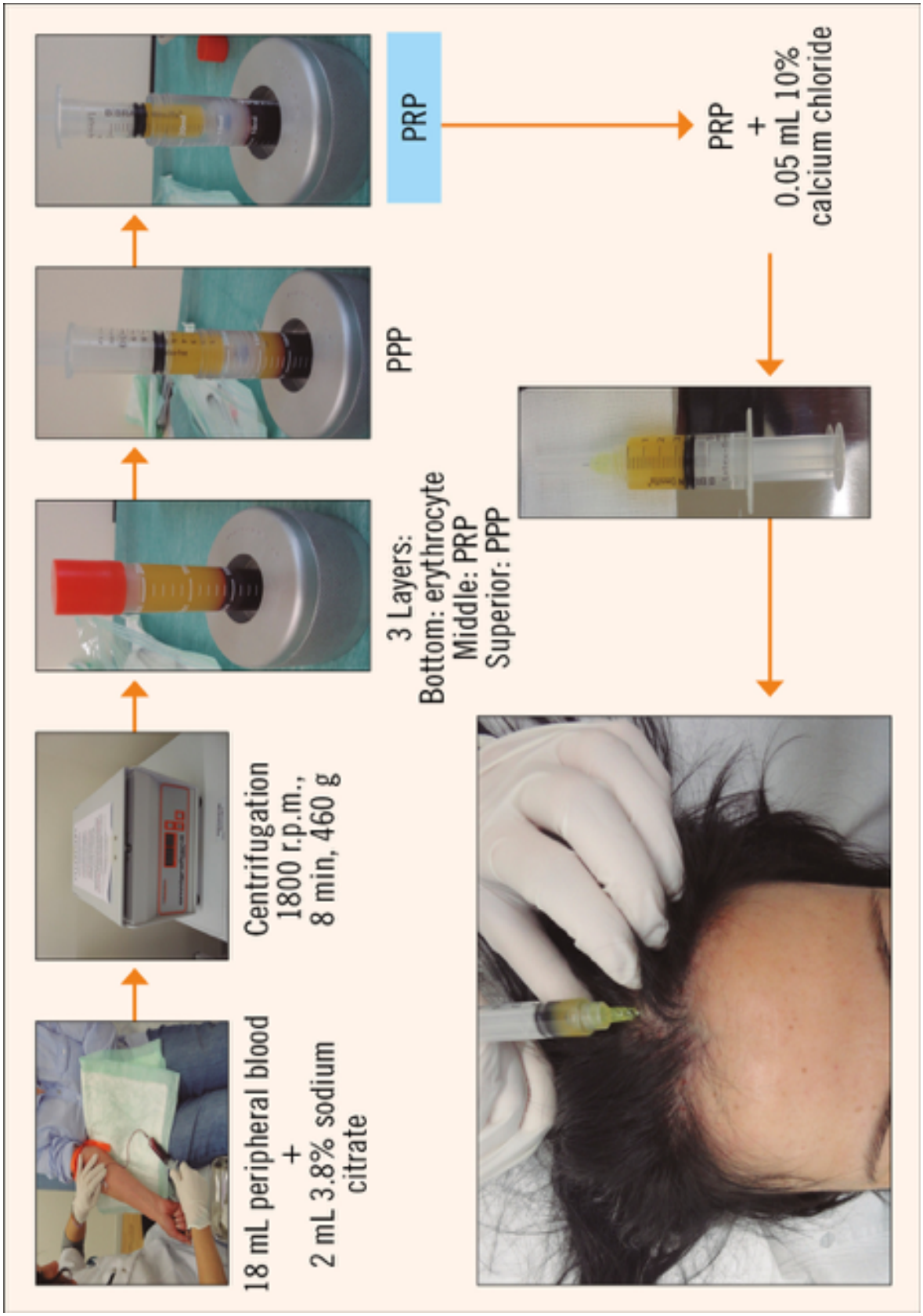

Fig. 6. Diagram of the steps to produce PRP using a closed system.

5.4.2 Placebo

In the opposite half-head of the administration of PRP, the patient received injections with placebo (Fig. 7). The use of placebo solution allows the comparison of the hair regrowth parameters, between treatment target areas versus control areas.

The placebo is a saline solution and infiltration technique was similar to injection of PRP.

The infiltration was carry-out with a 30-G needle using the manual mesotherapy technique, in dermal level. Placebo solution in a total dose of 4 mL, was administer in the amount of 0.15 mL - 0.20 mL, per point of injection. Each injection point was spared of 1 cm from each other.

In total, three injections of placebo were given for each patient, with an interval of 1 month from each other.

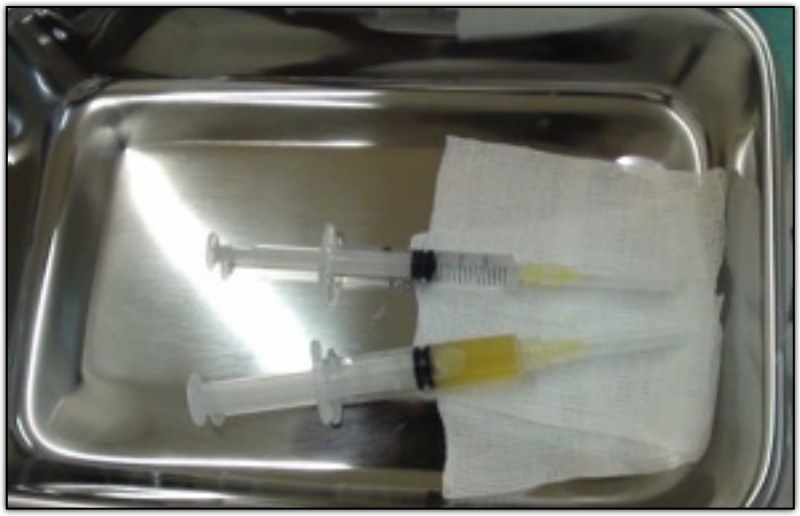

Fig. 7. The saline solution is applied in one half-head while PRP is injected in the other half-head of the same patient. The consistency and colour of PRP (yellow syringe) and placebo solution are different from each other.

5.4.3 Tattoo

Two areas in each half-head of the scalp (one frontal and one occipital) are select to perform the trichoscan analysis and marked with a permanent red tattoo (Fig.8). The dot tattoo guaranties the analysis of the same area and therefore ensures the reproducibility of the study.

The red ink for tattoo was chosen so it wouldn't interfere with color of the hair.

All four clipped areas were landmarked with a central, single red tattoo (*Tattoo ink: Permanent Cosmetic Colors 1/2 oz Bottles, Color: 8016 - Fire Red; Spaulding and Rogers, U.S.*) (Fig. 9) using a 30-G sterile needle, before the beginning of the study (Fig.10).

No local anesthesia was used.

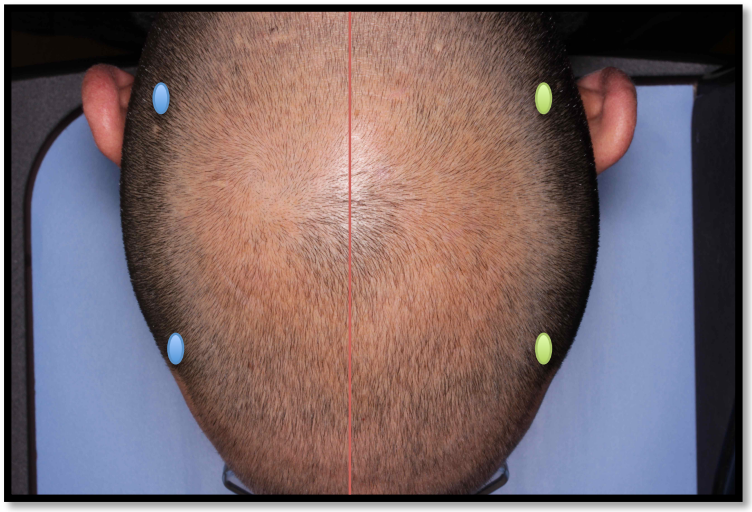

Fig. 8. Two identical circular areas of 1 x 1 cm (1 frontal and 1 occipital) in both the treatment and control half heads were defined for TrichoScan analysis.

Fig. 9. Permanent tattoo ink, color fire red.

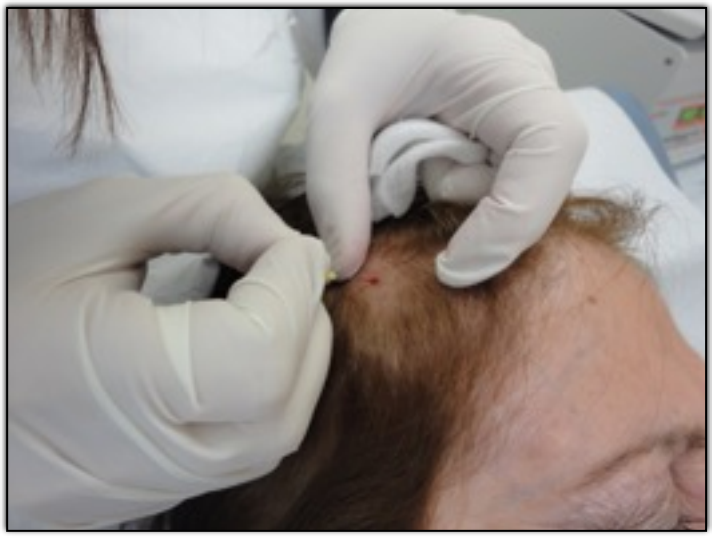

Fig. 10. The tattoo is performed using a using a 30-G sterile needle. The dot tattoo guaranties the analysis of the same area in the Trichoscan analysis.

5.5 EVALUATION CRITERIA

All patients were evaluated in 4 visits: V1, baseline and beginning of the study; V2, second treatment; V3, third treatment; and V4, follow-up. The evaluation criteria was assessed in all patients by global photography and TrichoScan analysis, as described below.

5.5.1 Global photographies

The global photography of the scalp is a non-invasive and useful method for determining clinical changes in the patient over time in a standardized manner.

The images were carried out using the medical photography system and software *Canon Canfield Orthostatic Device®,OMNIA Digital Imaging System*. The system has a special holder where the patient is positioned exactly in the same distance from screen to assessor (Fig. 11). Another holder is fixed to the reflex digital camera and the photographs are performed in three fixed positions: 0º (frontal), 45º (vertex) and 90º (occipital). In addition, the system used provides balanced lighting and color for consistent and uniform patient images.

The global scalp photographs can be directly compared and analyzed for alterations in the different evaluations.

The evolution of AGA was assessed by standardized images of 3 areas of the scalp (vertex, frontal, temporal) on which hair regrowth was evaluated. Patients were instructed to maintain the same hair color and hairstyle through the entire study.

The evaluation of hair growth was assessed by comparison of standardized images between baseline and 6 months (Fig. 12)

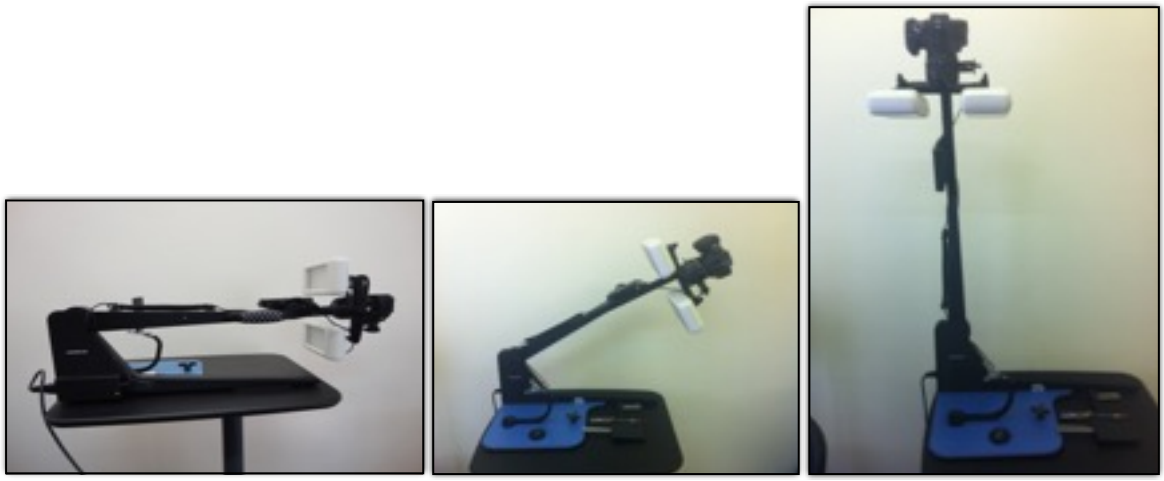

Fig. 11. The global photographies were performed in three fixed positions: 0º - frontal (A), 45º- fronto-parietal (B) and 90º - occipital, using the with the medical photography system: *Canon Canfield Orthostatic Device®,OMNIA Digital Imaging System*.

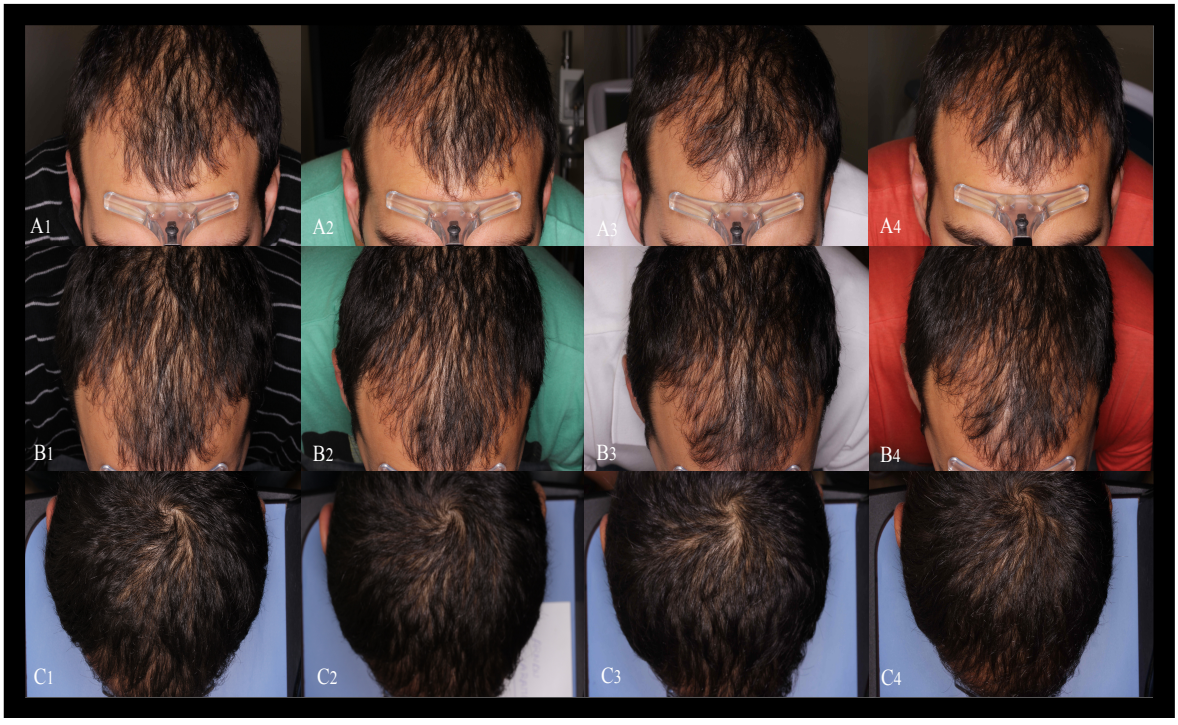

Fig. 12. Clinical photographs of the scalp of a 32 year-old male with AGA who was treated on the left half-head with PRP. The images were performed in three fixed positions: frontal (A), fronto-parietal (B) and vertex, in the four visits of the protocol (1-Baseline; 2 - Month 2; 3-Month 3; 4-Month 6 (follow-up).

5.5.2 TrichoScan analysis

TrichoScan is a quantitative and non-invasive method that combines epiluminescence microscopy (ELM) with automatic digital image analysis for the measurement of hair parameters (67,164) This is an useful and objective toll for monitoring responses to therapy and to compare placebo versus treatment.

Images were obtained using the video system FotoFinder; TrichoScan Professional Version, in the four target areas of the scalp, in all visits (Fig. 13)

The images are analyzed with TrichoScan software and data, regarding hair parameters such as number of hairs (number of hairs/0.65 cm^2), hair density (n per cm^2), terminal hair density (n per cm^2), anagen and telogen hairs (%) and anagen/telogen ratio, is obtained.

The procedure was carried out as follows:

- Before the beginning of the study, four areas of the scalp (2 in the left half-head: 1 frontal and 1 vertex; 2 in the right half-head: 1 frontal and 1 vertex) were selected and marked centrally with a red dot tattoo.The tattoo was visible during all study. The target areas were set symmetrically and according to the hair density.

- A template with a round hole in the centre of approximately 1 cm was placed on the scalp surface and clippings of the hair in the 4 target areas were perform using the *Moser-Trichoscan* hair clipper.

- Forty-eight hours after clipping, images of the selected areas were obtained. The epiluminescence light microscope is pressed on the measurement site and an image of the scalp is taken. The images are standardized, always at the same distance from the scalp surface and with a 20-fold magnification. Also is

important to avoid air bubbles and hair which cross the measurement site from outside as this will give an erroneously count of the hair.

- All recorded protocol-conformed photographs are automatically analyzed using the TrichoScan software. The hair parameters are obtained for each image.

- The data analyzed of the four target areas in the 4 four visits (Baseline, V1, V2, and follow-up), of a total of 16 images analysis per patient is transferred to an excel and then compared statistically (Table 3).

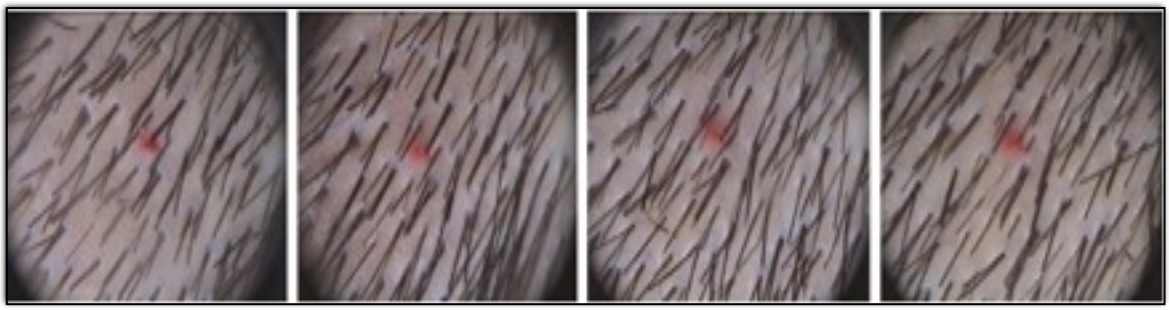

Fig. 13. The figure illustrates a digital image taken at 20-fold magnification. The hair parameters are obtained for each target area. The dot tattoo ensures reproducibility of the study. Images obtained with Trichoscan software.

NAME: M.C.G.A.					NUMBER: 11 Doctorado								SIDE PRP: RIGHT			
	Frontal (Right)				Frontal (Left)				Vertex (Right)				Vertex (Left)			
	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
Area (cm^2)	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651	0,651
Hair Count (n/0.65)	108,5	106	137,5	140,5	90,5	109,5	96,5	96	119	120	137	140	129,5	117,5	156	178
Hair Density (n/cm^2)	166,7	162,8	211,2	215,8	139	168,2	148,2	147,5	182,8	184,3	210,4	215,1	198,9	180,5	239,6	273,4
Anagen Hairs (%)	49	51	56	55	70	54	73	66	72	50	62	76	77	70	67	51
Telogen Hairs (%)	51	49	44	45	30	46	27	34	28	50	38	24	23	30	33	49
Vellus Hair Density ($1/cm^2$)	6,1	11,5	13,1	19,2	6,9	13,8	1,5	5,4	6,9	15,4	7,7	10,8	9,2	8,4	1,2	24,6
Terminal Hair Density ($1/cm^2$)	160,5	151,3	198,2	196,6	132	154,4	146,7	142,1	175,9	169	202,8	204,3	189,7	172	239,6	248
Vellus Hairs (%)	4	7,5	8,5	12,5	4,5	9	1	3,5	4,5	10	5	7	6	5,5	1	16
Terminal Hairs (%)	104,5	98,5	129	128	86	100,5	95,5	92,5	114,5	110	132	133	123,5	112	156	162

Table 3. The table illustrates the TrichoScan results of a 28 years-old female patient with AGA who performed treatment with PRP in the right half-head. Each target area were analyzed in all visits. The data obtained is analyzed statistically. V1: Baseline and first visit; V2: Month 2, V3: Month 3; V4: Month 6 (follow-up).

5.5.3 Safety parameters

Given the autologous nature, PRP is considered a safe product and until now, no systemic effect has been demonstrated after local application of PRP.

Global tolerance was assessed through collection of any adverse events at each visit.

Safety outcomes were evaluated during the course of all protocol. There were no side-effects observed in this study beside the local pain while injecting PRP and placebo.

5.6 DATA COLLECT

The data was collected in the four programmed visits:Visit 1, Baseline visit; Visit 2 and Visit 3, with 1-month interval; andVisit 4, at month 6.

For each patient during the consultation, the physician performed a clinical examination and collection of data, as described below.

5.6.1 Visit 1 (Baseline visit)

- Explanation of treatment protocol in detail and written consent form signed.

- Physician questionnaire with the following information *(Annex 13.8)*:
    e. Confirmation of inclusion and exclusion criteria
    f. Sociodemographic data: gender, age
    g. Medical history (diseases, allergies, surgery, medication, analyses)
    h. AGA description and history: age of onset of AGA, localization and extension
    i. Family history of AGA (yes/no). If yes: mother, father or both.
    j. Past medical care for AGA (yes/no). If yes: topical or systemic, duration, discontinuation date
    k. Current medical care (yes/no). If yes: oral treatment, topical capillary solution, other (specify)

- Standardized global photography areas of 3 areas of the scalp (vertex, frontal, occipital), using the *Canon Canfield Orthostatic Device®, OMNIA Digital Imaging System*

- Standardized Trichoscan® images, using the video system with ELM, *FotoFinder; TrichoScan Professional Version,* in the 4 target areas of the scalp (2 frontal and 2 occipital), in all visits. Clipping was performed in the previous 48h.

- Administration of PRP on one half-head and administration of placebo in the other half-head.

5.6.2 Visit 2 (Month 2) and Visit 3 (Month 3)

- Physician questionnaire with the following information *(Annex 13.9)*:

    a. Confirmation of inclusion and exclusion criteria
    b. Alterations in medical care of AGA since the inclusion
    c. Presence/absence of any adverse effect
    d. Tolerance to the treatment
    e. Patient feeling about global improvement

- Standardized global photography areas of 3 areas of the scalp (vertex, frontal, occipital), using a *Canon Canfield Orthostatic Device®, OMNIA Digital Imaging System.*

- Standardized Trichoscan® images, using the video system with ELM, *FotoFinder; TrichoScan Professional Version,* in the 4 target areas of the scalp (2 frontal and 2 occipital). Clipping was performed in the previous 48h.

- Administration of PRP on one half-head and administration of placebo in the other half-head, at the same locations and side defined at baseline.

5.6.3 Visit 4 (Month 6)

- Physician questionnaire with the following information *(Annex 13.10)*:
    a. Confirmation of inclusion and exclusion criteria
    b. Alterations in medical care of AGA since the inclusion
    c. Presence/absence of any adverse effect
    d. Tolerance to the treatment
    e. Patient feeling about global improvement

- Standardized global photography areas of 3 areas of the scalp (vertex, frontal, occipital), using a *Canon Canfield Orthostatic Device®, OMNIA Digital Imaging System*

- Standardized Trichoscan® images, using the video system with ELM, *FotoFinder; TrichoScan Professional Version,* in the 4 target areas of the scalp (2 frontal and 2 occipital). Clipping was performed in the previous 48h.

5.6.4 Double-blind study

This study is a double-blind study in order to eliminate subjective, unrecognized bias and increase the validation of the protocol.

As the consistency and color of PRP and placebo solution are different from each other, the physician allocated to the intervention group were aware of the treatment side. Participants are blinded regarding the half-head injected with PRP or the half-head injected with placebo.

The evaluator responsible for both global photographs and phototrichogram analyses was blinded with regard to the treatment and placebo areas of the scalp.

5.7 SUMMARY OF PROTOCOL DESIGN

Before the beginning of the study, all patients performed dot tattoos in 4 selected areas of the scalp. This study includes 3 visits (V1, V2, V3) with 1-month interval. During each visit, before the administration of PRP/placebo, standardized global photographies of the scalp are performed and Trichoscan images obtained. In the last consultation (V4), at 6 months, both global photographies and Trichoscan images are obtained. No treatment with PRP/placebo is performed in the last visit (Fig. 14).

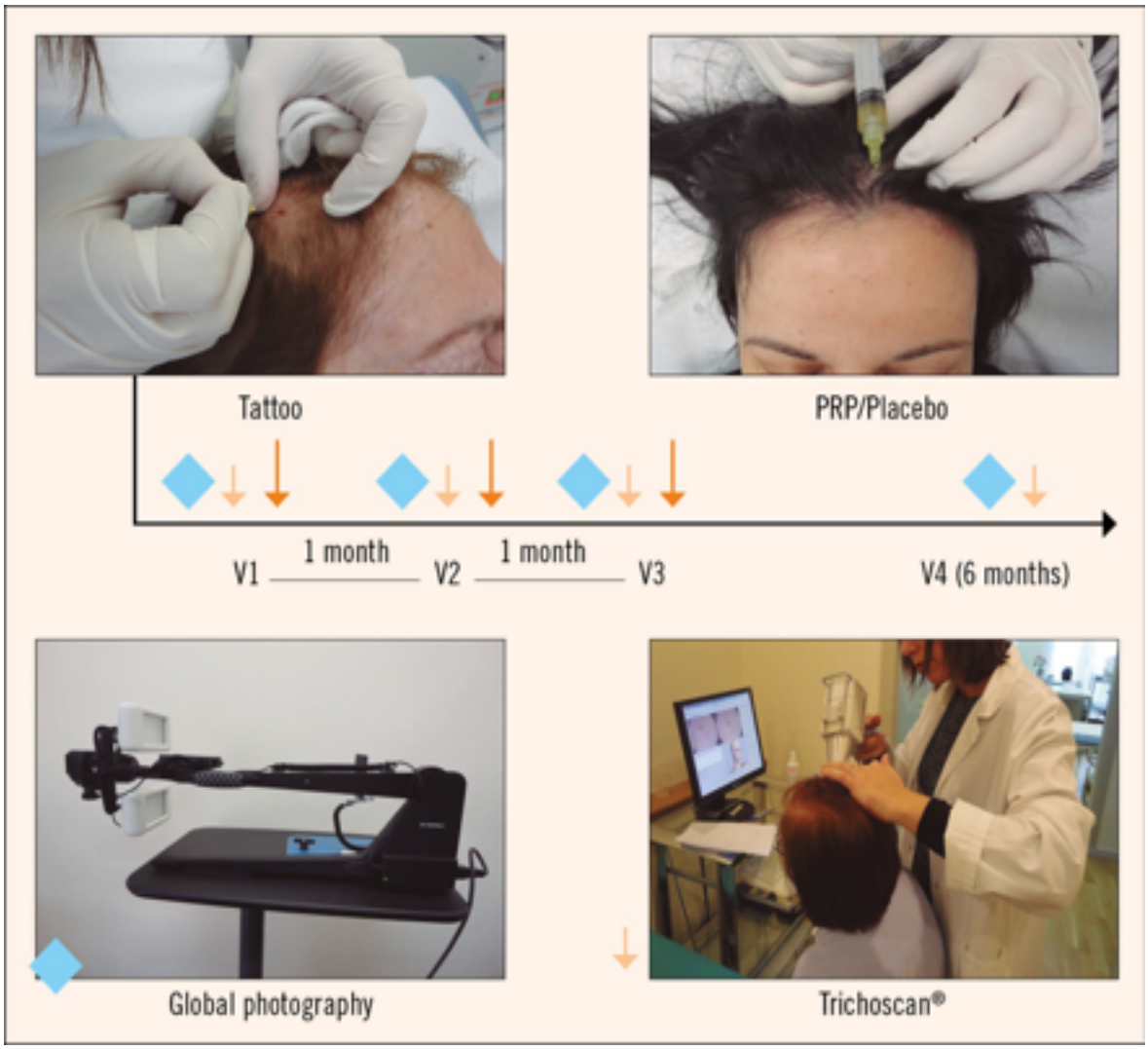

Fig. 14. Illustration of study protocol.

5.8 INFORMED CONSENT

Before participating in the study, each patient was fully informed by the investigator about all aspects related to the research. This included the number of visits, type of interventions and data assessment. In addiction, risks and benefits of the treatment with PRP, in the light of current knowledge, were analyzed and explained. Patients had the opportunity to ask questions about the study and received answers accordingly.

Participation in this research project was voluntary without any source of remuneration. At any time, the patient could revoke its agreement without explanation. The refusal of a patient to participate in the study didn't interfere with the patient–physician relationship.

As this study is a half-head study, patients were also informed that if there was significant evidence of efficacy of the treatment with PRP, the investigators will provide to the patient 3 new treatments of PRP, on the previous non-treated half-head (placebo area).

All select patients provided written informed consent before participating in the study, which was performed according to the Declaration of Helsinki *(Annex 13.3)*.

5.9 CONFIDENTIALITY

All the documents related to the present study are confidential and the investigator can give or communicate them only to the persons namely implicated in the study.

The investigator maintains the confidentiality of the identity of subjects enrolled in the study and the information contained in the study records, according to the Organic Law 15/1999 of 13th December on the Protection of Personal Data.

5.10 CLINICAL RESEARCH ETHICS COMMITTEE

On 17th of July 2013, the study “Prospective double-blind, placebo-controlled study to assess the efficacy of platelet-rich plasma on the treatment of androgenetic alopecia” was submitted for consideration, comment, guidance and approval to the Clinical Research Ethic Committee of the International University of Catalunya (*Comité de Ética en Investigación Clínica, CEIC*).

The documents submitted to CEIC included the design of the study with a research protocol. The protocol for ethical consideration included methodology of the study, information regarding participants with inclusion and exclusion criteria, subjects to interventions, side-effects and sponsors.

The committee was independent of the researchers, the sponsor and any other undue influence.

On 4th December 2013, the clinical study received the Approval by the corresponding Ethic Committee, under the reference of Ref.138/2013 (CEIC, UIC). No change to the protocol approval by the committee was made *(Annex 13.1)*.

5.11 CLINICAL TRIALS REGISTRATION

ClinicalTrials.gov is a registry and results database that provides access to information on clinical studies of human participants, on a wide range of diseases and conditions. The World Health Organization states that the “registration of all interventional trials is considered to be a scientific, ethical and moral responsibility”.

The registration is freely available both for registers and users and provides information of ongoing and previously conducted trails, worldwide.

After approval by the correspondent Ethics Committee, the clinical trial is submitted to the web site ClinicalTrials.gov. The study is registry through “organizational accounts” under an identification number (NCT) and provide information regarding title, description, and design of the study, clinical disease, eligibility criteria, assigned interventions in the protocol, locations where the study is being conducted and contact information for the study locations. Once entry of data elements is finished, the quality assurance staff at ClinicalTrials.gov will review the submission. The quality panel may ask to the investigators some questions or to make corrections. After the analyses is accurate and completed, the record is made public and information is continuous updated (165).

The second phase of registration in clinical trails is results submission. When the study is finished, the investigator must submit information on the results of the study, such as, participant flow, baseline characteristics, outcomes of the study and statistical analyses, and summary of adverse events experienced by study participants.

This clinical trial, after Ethics Committee approval (CEIC/UIC), was registered in ClinicalTrials.gov, under the “organizational account” of Universitat Internacional de Catalunya, with identify Number: NCT02393040. (https://clinicaltrials.gov/ct2/show/NCT02393040). *(Annex 13.2)*

5.12 STATISTICAL ANALYSES

5.12.1 Justification of sample size

The study was designed comparing the treatment of PRP in half-head and the use of placebo in the other half-head of the scalp. As both treatment and placebo are performed in the same patient, the baseline characteristics are the same for both groups, thus dismounting the incidence of bias. For these reason, similar baseline data are important for the variable outcomes measured along the clinical trial. The sample size defined was 50 patients (Group A: 25 patients; Group B: 25 patients). The design of the study (PRP versus placebo in the same patient) justifies the chosen sample size.

5.12.2 Descriptive study

All analysis was performed with SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC, USA)

For each variable, a mixed model regression coefficients with repeated measures was applied. This allowed to calculate the statistical significance of the mean change in month 1, month 2 and month 6 with respect to baseline, and also compare the 2 treatment groups (placebo vs PRP) in global and in each visit, based on the unique mixed model. All results are presented using the model adjusted mean and standard deviation (SD), and p-values less than 0.05 were considered statistically significant (166).

5.13. SPONSOR

This study received a grant by the company *PROTEAL, Soluciones Biogenerativas* in the form of material for the interventions of the study.

The material included:

1) Centrifuge for the separation and concentration of blood: Omnigrafter II - Proteal, Barcelona, Spain,
2) Disposable and closed 20 mL Kit to obtain PRP.

At the end of the study, as there was significant evidence of the efficacy of the treatment with PRP, the sponsor provided material to perform 3 more treatments of PRP in each patient, on the previous non-treated half-head (placebo area).

The sponsors had no involvement in the study design, protocol or analysis of the results obtained. The final decision of the content of the clinical trial was the sole responsibility of the authors.

There was any other type of compensation for patients or researchers in this clinical trial.

6. RESULTS

6. RESULTS

As demographic characteristics and results of the Group A and Group B are different from each other, for simplicity and continuity, the description of each group is analyzed separately in the subsequent section.

6.1 RESULTS OF GROUP A: PRP ALONE

Age-eligible participants were recruited and 27 patients were evaluated for potential enrollment. One patient was excluded from the study and one patient declined to participate.

Twelve men and 13 women with AGA were admitted in the study, of a total of 25 patients. Three patients were lost to follow-up, 2 women and 1 men.

Twenty-two patients (11 male and 11 women) completed the entire protocol and were included in the main analysis (Fig.15).

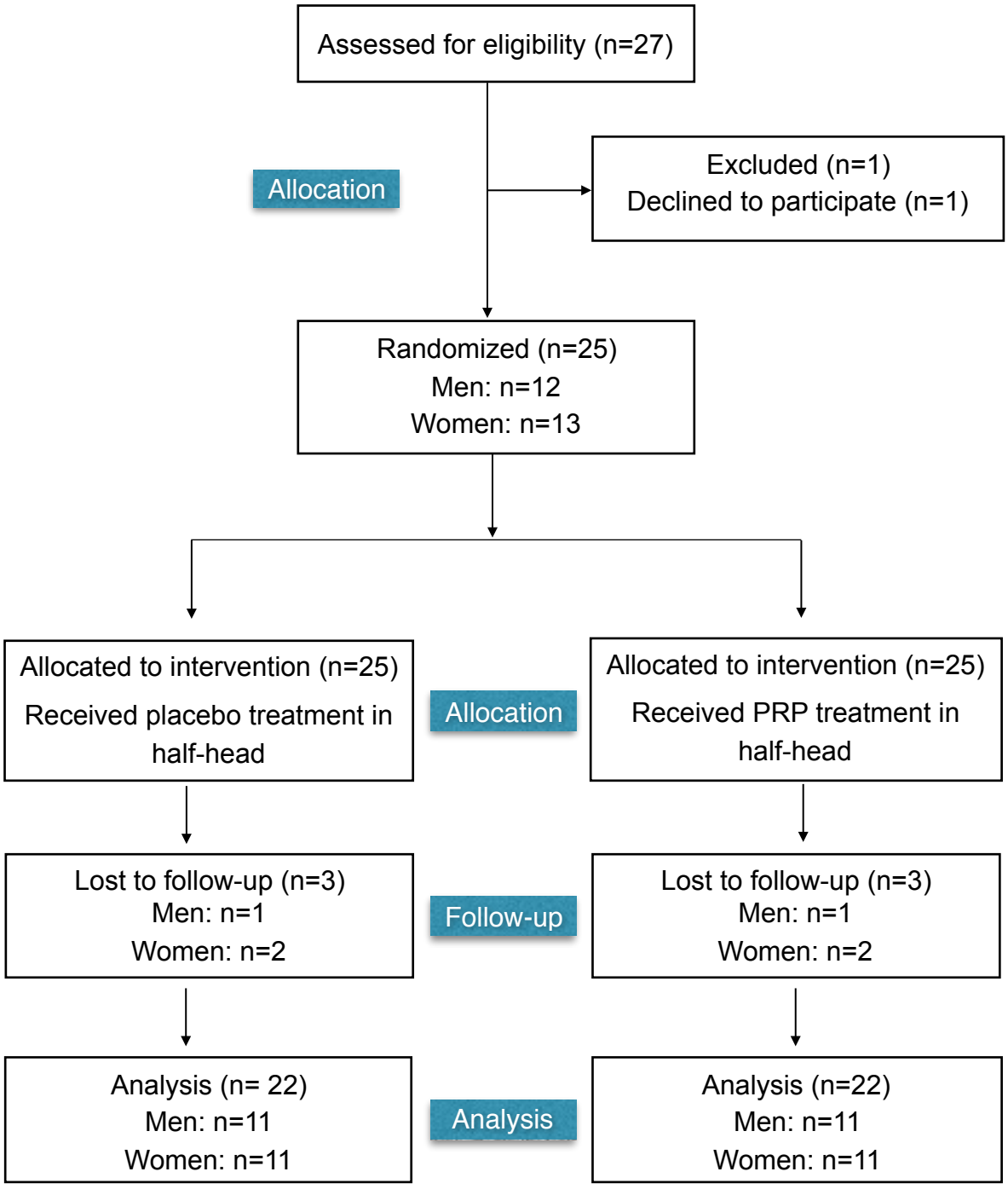

Fig. 15. Flow diagram of the study of Group A. The diagram shows participants enrolled and included in the main analysis.

6.1.1 Demographic Data

Patient demographic data	Group A (n = 22)
Age (mean; years)	39.2 y (21 y.- 62y.)
Sex	
Female	n = 11 (50%)
Male	n = 11 (50%)
Family History	
Positive	n = 17 (77.3%)
Mother	n = 7
Father	n = 13
Both	n = 3
Negative	n = 5 (22.7%)
Beginning of AGA	
< 25 years	n = 14 (63.6%)
≥ 25 years	n = 8 (36.3%)
Smoking status	
Yes	n = 2 (9%)
No	n = 20 (91%)
Ethnic origin	
Caucasian	n = 22 (100%)
Nordwood-Hamilton Classification (n = 11)	
Stage II	n = 1/11 (9%)
Stage III	n = 8/11 (73%)
Stage IV	n = 2/11 (18%)
Stage V	-
Ludwig Classification (n= 11)	
Stage I	-
Stage II	n = 8/11 (73%)
Stage III	n = 3/11 (27%)

Table 4. Baseline patient demographic of patients of Group A

The clinical characteristics of the 22 patients are summarized in table 4.

At presentation, the age of the patients ranged from 21 to 62 years, with a mean age of 39.2 years. There were 11 females (50%) and 11 males (50%), of a total of 22 participants. Ethnicity was documented for all patients; 100% were caucasians.

Concerning family history of AGA, 73.3% and 22.7% had a positive and negative family history, respectively. Most of the patients (63.6%) reported beginning of hair loss under 25 years of age while 36.3% stated the beginning of AGA ≥ 25 years

Clinically, and according to Hamilton–Norwood Classification, in men the most frequent pattern was stage II (73%), followed by stage IV and II, with 18% and 9%, respectively. Regarding Ludwig Classification, 8 of 11 (73%) female patients were in stage II while 27% were in stage III.

6.1.2 TrichoScan Analysis of Group A

The images obtained during the study were evaluated with TrichoScan software and data analyzed between treatment and control areas.

The hair growth parameters were measured after 3 months and 6 months and compared with the baseline (before treatment) and between treatment and control areas (placebo).

The hair parameters were: number of hairs, hair density, terminal hair density, anagen hairs, telogen hairs and anagen/telogen ratio.

At baseline, there were no significant differences in hair count, hair density, terminal density, and anagen or telogen hairs, between the treatment and control areas of the scalp. The detailed hair growth parameters are described below.

6.1.2.1 Hair Count

The mean total hair count for the treatment area with PRP after 3 months showed a mean increase of 4.2 ± 22.5 hairs/0.65 cm^2 versus 1.8 ± 22.2 hairs/0.65 cm^2 in the control area, compared with baseline.

After 6 months, the area treated with PRP had a mean increase of 2.3 ± 17.8 hairs/0.65 cm^2 versus 1.7 ± 17.1 hairs/0.65 cm^2 in the control area. Regarding the mean total hair count there were no significant differences between the two areas, although treatment with PRP exhibited a slight increase in number of hairs compared with the control (Table 5) (Fig.16).

Mean Hair Count (hairs/0.65 cm^2)	Placebo (n=22)	PRP (n=22)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	111 ± 33.9	110.8 ± 37.6	0.9692
Month 1	114.9 ± 35.1	113.5 ± 41.3	0.7670
Month 1 vs Baseline	4 ± 18.9	2.7 ± 19.4	0.7670
P-value (Month 1 vs Baseline)	0.2039	0.0313	
Month 3	112.8 ± 35.7	115 ± 41.8	0.5957
Month 3 vs Baseline	1.8 ± 22.2	4.2 ± 22.5	0.5957
P-value (Month 3 vs Baseline)	0.5517	0.1823	
Month 6	112.7 ± 34.9	113.2 ± 39.4	0.8
Month 6 vs Baseline	1.7 ± 17.1	2.3 ± 17.8	0.8
P-value (Month 6 vs Baseline)	0.3609	0.2091	

Table 5. Mean hair count parameter for the half-head areas treated with PRP and Placebo, at Baseline, Month 1, 3 and 6. Data obtained from TrichoScan analysis.

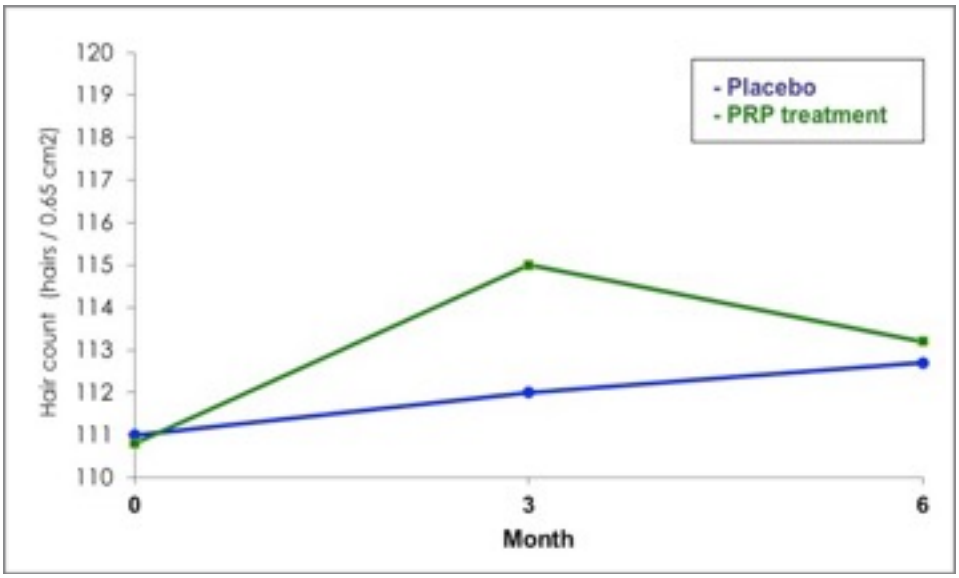

Fig. 16. Evolution of mean hair count, between PRP treatment and placebo, at 3 and 6 months.

6.1.2.2 Hair Density

The mean total hair density for the treatment area after 3 months showed a mean increase of 14.8 ± 32.1 hairs/cm^2 (8.9%) compared with baseline ($p<0.05$; Month 3 vs Baseline), whereas the control area showed a mean decrease of 0.7 ± 32.7 hairs/cm^2 (– 0.42%).

After 6 months, the treatment area with PRP had a mean increase of 12.8 ± 32.6 hairs/cm^2 (7.7%) ($p<0.05$; Month 6 vs Baseline) and the control area a decrease of 2.1 ± 31.3 hairs/cm^2 (– 1.2%).

Regarding the mean total hair density, the increase on the treated side was found to be significant compared with baseline and that on the control side, both at 3 and 6 months ($p<0.05$; control vs treatment) (Table 6) (Fig.17).

Mean Hair Density (1/cm²)	Placebo (n=22)	PRP (n=22)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	167.8 ± 51.2	167.1 ± 55.6	0.9087
Month 1	173 ± 53.9	178 ± 63.4	0.4174
Month 1 vs Baseline	5.2 ± 29.5	10.9 ± 34.7	0.4174
P-value (Month 1 vs Baseline)	0.2912	**0.0313**	
Month 3	167.1 ± 51.3	181.9 ± 63.6	**0.0304**
Month 3 vs Baseline	-0.7 ± 32.7	14.8 ± 32.1	**0.0304**
P-value (Month 3 vs Baseline)	0.8943	**0.0041**	
Month 6	165.7 ± 55.2	179.9 ± 62.7	**0.0367**
Month 6 vs Baseline	-2.1 ± 31.3	12.8 ± 32.6	**0.0367**
P-value (Month 6 vs Baseline)	0.6699	**0.0121**	

Table 6. Mean Hair Density parameter for the half-head areas treated with PRP and placebo at Baseline, Month 1, Month 3 and Month 6; p<0.05 statistical significant.

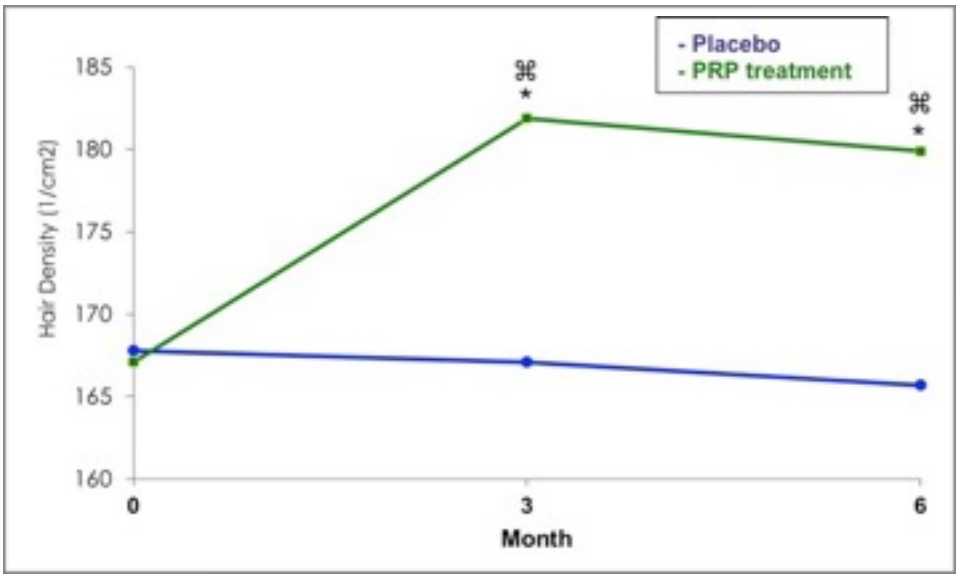

Fig. 17. Evolution of mean hair density, between PRP treatment and placebo target areas; *p<0.05 statistical significant, compared to baseline; ⌘p<0.05 statistical significant, placebo versus PRP.

6.1.2.3 Terminal Hair Density

The parameter terminal hair density showed an mean increase of 8.2 ± 31.3 hairs in the areas treated with PRP versus a slight increase of 1.2 ± 34.1hairs in the placebo area, at 3 months, when compared to baseline.

At 6 months, the data obtained in the PRP target areas showed a statistical significant increase of 5.8 ± 25.7 hairs/cm^2 compared to baseline while the control area showed a slight increase of 1.2 ± 27.3 hairs/cm^2 ($p<0.05$; Month 6 vs Baseline).

No significant differences between the PRP-treated area and the placebo area were found (Table 7) (Fig.18).

Mean Terminal Hair Density (1/cm^2)	Placebo (n=22)	PRP (n=22)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	160.7 ± 48.9	159.9 ± 55.1	0.8948
Month 1	164.1 ± 49.6	165.7 ± 60	0.7135
Month 1 vs Baseline	3.4 ± 29.1	5.8 ± 28.1	0.7135
P-value (Month 1 vs Baseline)	0.4681	0.2165	
Month 3	161.9 ± 52.2	168.2 ± 60.7	0.2817
Month 3 vs Baseline	1.2 ± 34.1	8.2 ± 31.3	0.2817
P-value (Month 3 vs Baseline)	0.8016	0.0798	
Month 6	161.9 ± 50.6	165.8 ± 56.8	0.2372
Month 6 vs Baseline	1.2 ± 27.3	5.8 ± 25.7	0.2372
P-value (Month 6 vs Baseline)	0.6507	**0.0411**	

Table 7. Comparison of mean terminal hair density between the half-head areas treated with PRP versus Placebo; $p<0.05$ statistical significant.

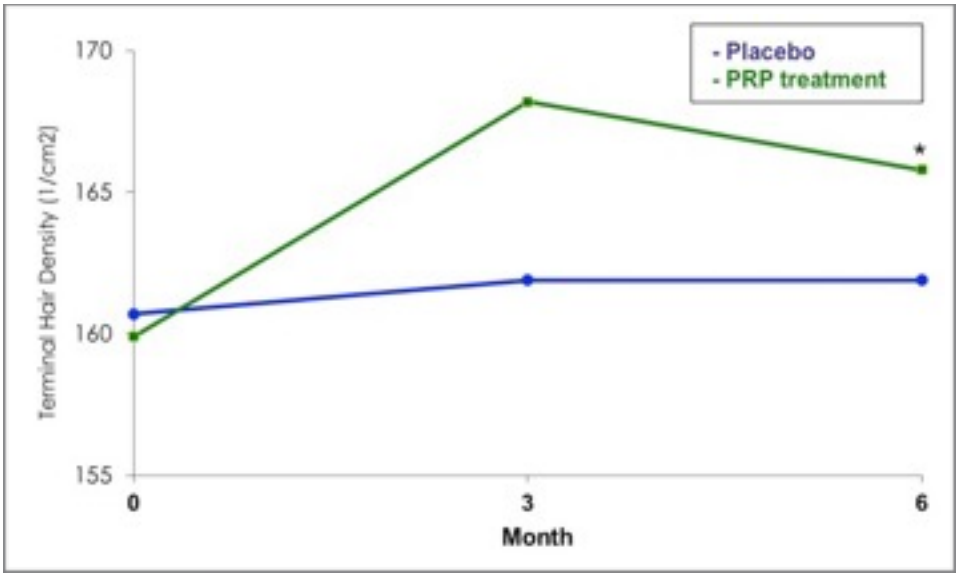

Fig. 18. Chart of mean terminal hair density at baseline, Month 3 and Month 6; *p<0.05 statistical significant, compared to baseline.

6.1.2.4 Anagen Hairs

In the treatment areas, the mean changes from baseline anagen hairs were 5.8 ± 10.9 hairs (9.3%) at 3 months and 5.5 ± 9.7 hairs (8.8%) at 6 months (p<0.05; Month 3, Month 6 vs Baseline).

The placebo group showed at the same time mean changes from baseline between 1.4 ± 13.2 hairs and 4.2 ± 15 hairs, at 3 and 6 months, respectively.

At 6 months, in both PRP and placebo group the changes were statistically significant in comparison with baseline although there were no significant differences between the PRP-treated area and the placebo area (Table 8) (Fig.19).

Mean Anagen hairs (%)	Placebo (n=22)	PRP (n=22)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	62.1 ± 17.4	62.1 ± 16.1	0.9839
Month 1	65.5 ± 16.5	64.2 ± 14.4	0.6001
Month 1 vs Baseline	3.4 ± 14.6	2.1 ± 12.9	0.6001
P-value (Month 1 vs Baseline)	0.0550	0.2268	
Month 3	63.5 ± 18.9	67.9 ± 13.8	0.0763
Month 3 vs Baseline	1.4 ± 13.2	5.8 ± 10.9	0.0763
P-value (Month 3 vs Baseline)	0.4220	**0.0016**	
Month 6	66.3 ± 15.9	67.6 ± 13.1	0.6121
Month 6 vs Baseline	4.2 ± 15	5.5 ± 9.7	0.6121
P-value (Month 6 vs Baseline)	**0.0181**	**0.0027**	

Table 8. Mean anagen hairs of the half-head areas treated with PRP and Placebo at Baseline, Month 1, Month 3 and Month 6; p<0.05 statistical significant.

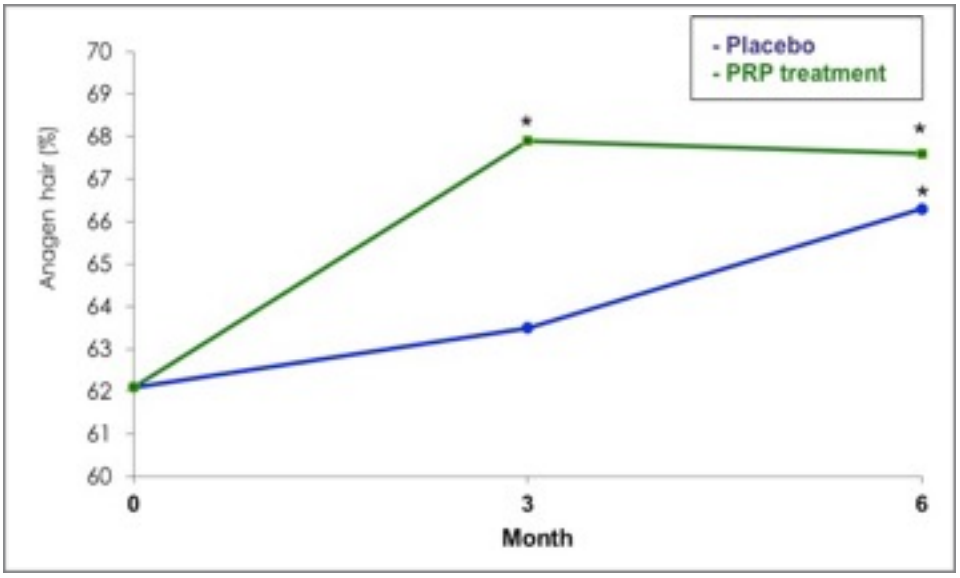

Fig. 19. Evolution of mean anagen hairs of the half-head treated with placebo and PRP, at baseline, Month 3 and Month 6; * p<0.05 statistical significant, compared to baseline

6.1.2.5 Telogen Hairs

The hair parameter telogen hairs showed an mean decrease of 5.5% in the areas treated with PRP versus a decrease of 8 % in the placebo area, at 3 months, when compared to baseline (p>0.05; Month 3 vs Baseline).

The mean telogen hairs (%) for the treatment area after 6 months showed a mean decrease of 5.5 ± 9.7 hairs (–14.5%) compared with baseline while the placebo area displayed a decrease of – 4.2 ± 15 hairs (–2.2%) (p<0.05; Month 6 vs Baseline).

No differences were found between PRP treated area and the placebo area (Table 9) (Fig.20).

Mean Telogen hairs (%)	Placebo (n=22)	PRP (n=22)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	37.9 ± 17.4	37.9 ± 16.1	0.9839
Month 1	36.5 ± 18.9	32.1 ± 13.8	0.0763
Month 1 vs Baseline	-1.4 ± 13.2	-5.8 ± 10.9	0.0763
P-value (Month 1 vs Baseline)	0.4220	**0.0016**	
Month 3	34.5 ± 16.5	35.8 ± 14.4	0.6011
Month 3 vs Baseline	-3.4 ± 14.6	-2.1 ± 12.9	0.6011
P-value (Month 3 vs Baseline)	0.0550	0.2268	
Month 6	33.7 ± 15.9	32.4 ± 13.1	0.6121
Month 6 vs Baseline	-4.2 ± 15	-5.5 ± 9.7	0.6121
P-value (Month 6 vs Baseline)	**0.0181**	**0.0027**	

Table 9. Trichoscan analysis of mean telogen hairs, comparing the half-head treated with placebo versus PRP; p<0.05 statistical significant.

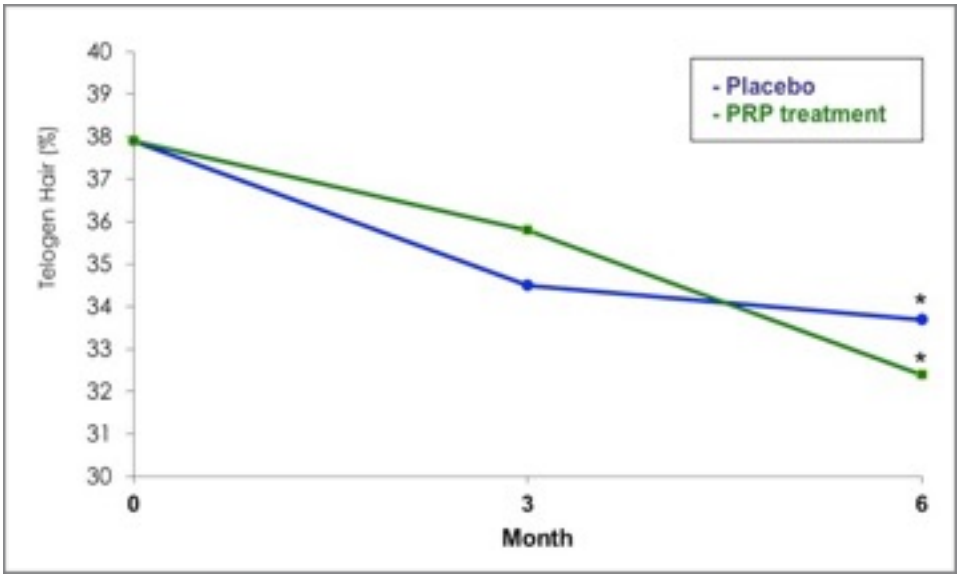

Fig. 20. Evolution of mean telogen hairs, between PRP treatment and placebo, at 3 and 6 months; * $p<0.05$ statistical significant, compared to baseline.

6.1.2.6 Ratio Anagen/Telogen Hairs

After analysis of the mean anagen hairs and telogen hairs, the anagen/telogen ratio was calculated.

The areas treated with PRP exhibit a greater anagen/telogen ratio than the control areas, both at 3 months and 6 months. These increase was statistical significant when compared to baseline ($p<0.05$; Month 3 vs Baseline) ($p<0.05$; Month 6 vs Baseline) (Table 10).

Anagen/Telogen ratio (%)	Placebo (n=22)	PRP (n=22)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	137.5 ± 209.5	128.4 ± 174.8	0.9839
Month 1	141.5 ± 135	118.5 ± 111	0.6001
Month 1 vs Baseline	4 ± 190.8	-9.9 ± 179.1	0.6001
P-value (Month 1 vs Baseline)	0.8500	0.6377	
Month 3	159.1 ± 210.1	185.7 ± 199.9	0.0763
Month 3 vs Baseline	21.6 ± 208	57.3 ± 130.5	0.0763
P-value (Month 3 vs Baseline)	0.3050	**0.0087**	
Month 6	148.2 ± 173.7	156 ± 164.3	0.3165
Month 6 vs Baseline	10.7 ± 169.4	27.6 ± 131.8	0.3165
P-value (Month 6 vs Baseline)	0.3882	**0.0346**	

Table 10. Anagen/telogen ratio between placebo and treatment with PRP; $p<0.05$ statistical significant.

6.1.2.7 Summary of Trichoscan Analysis of Group A

Group A: Summary of TrichoScan analysis	Placebo (n=22)		PRP (n=22)		Placebo versus PRP
	Mean ±SD	P-value	Mean ± SD	P-value	P-value
Hair count (hairs/0.65cm)					
Baseline	111 ± 33.9	P > 0.05	110.8 ± 37.6	P > 0.05	P > 0.05
3 months	112 ± 35.7	P > 0.05	115 ± 41.8	P > 0.05	P > 0.05
6 months	112.7 ± 34.9	P > 0.05	113.2 ± 39.4	P > 0.05	P > 0.05
Hair density (1/cm^2)					
Baseline	167.8 ± 51.2	P > 0.05	167.1 ± 55.6	P > 0.05	P > 0.05
3 months	167.1 ± 51.3	P > 0.05	181.9 ± 63.6	**P< 0.05**	**P< 0.05**
6 months	165.7 ± 55.2	P > 0.05	179.9 ± 62.7	**P< 0.05**	**P< 0.05**
Terminal Hair density					
Baseline	160.7 ± 48.9	P > 0.05	159.9 ± 55.1	P > 0.05	P > 0.05
3 months	161.9 ± 52.2	P > 0.05	168.2 ± 60.7	P > 0.05	P > 0.05
6 months	161.9 ± 50.6	P > 0.05	165.8 ± 56.8	**P< 0.05**	P > 0.05
Anagen Hair (%)					
Baseline	62.1 ± 17.4	P > 0.05	62.1 ± 16.1	P > 0.05	P > 0.05
3 months	63.5 ± 18.9	P > 0.05	67.9 ± 13.8	**P < 0.05**	P > 0.05
6 months	66.3 ± 15.9	**P< 0.05**	67.6 ± 13.1	**P < 0.05**	P > 0.05
Telogen Hair (%)					
Baseline	37.9 ± 17.4	P > 0.05	37.9 ± 16.1	P > 0.05	P > 0.05
3 months	34.5 ± 16.5	P > 0.05	35.8 ± 14.4	**P < 0.05**	P > 0.05
6 months	33.7 ± 15.9	**P< 0.05**	32.4 ± 13.1	**P< 0.05**	P > 0.05
Anagen/telogen ratio (%)					
Baseline	137.5 ± 209.5	P > 0.05	128.4 ± 174.8	P > 0.05	P > 0.05
3 months	159.1 ± 210.1	P > 0.05	185.7 ± 199.9	**P < 0.05**	P > 0.05
6 months	148.2 ± 173.1	P > 0.05	156 ± 164.3	**P< 0.05**	P > 0.05

Table 11. Summary of Trichoscan analysis regarding patients enrolled in Group A, at Baseline, Month 3 and Month 6; p<0.05 statistical significant.

6.1.2.8 Demographic Data and Trichoscan analysis

The demographic data (table 4) and the trichoscan analysis (table 11) were evaluated at the end of the study, at month 6 .

In support of the data obtained, a statistical significant correlation was observed between demographic data and two hair parameters (hair density and anagen hairs). No correlation was found between demographics and the other hair parameters analyzed by TrichoScan.

The treatment with PRP showed a statistically significantly correlation of the mean total hair density between men, patients aged ≦ 40 years, beginning of AGA ≥ 25 years, positive family history, and more than 10 years of disease evolution, when compared with the placebo ($p<0.05$ control versus PRP) (Table 12).

Another correlation was found in the areas treated with PRP alone between anagen hairs and patients aged more than 40 years and beginning of AGA ≥ 25 years ($p<0.05$ control versus PRP) (Table 13).

Hair density (1/cm²)	Placebo (n=22)	PRP (n=22)	Placebo *vs* PRP
	Mean ± SD	Mean ± SD	P - value
Women	2.7 ± 30.7	11.1 ± 29.6	P > 0.05
Men	0.8 ± 31.6	14.3 ± 36.2	**P< 0.05**
Age ≦ 40 years	4.1 ± 31.5	9.4 ± 35.5	**P< 0.05**
Age > 40 years	7.5 ± 30.0	17.6 ± 29.0	P > 0.05
Beginning of AGA < 25 years	5.1 ± 29.3	1.9 ± 27.2	P > 0.05
Beginning of AGA ≥ 25 years	10.6 ± 32.7	30.4 ± 34.3	**P< 0.05**
Family history +	1.9 ± 31.9	14.7 ± 34.1	**P< 0.05**
Family history –	2.9 ± 29.6	5.2 ± 29.4	P > 0.05
Disease duration ≦ 10 years	5.8 ± 38.6	16.4 ±33.2	P > 0.05
Disease duration > 10 years	3.2 ± 23.0	9.7 ± 32.8	**P< 0.05**

Table12. Analysis of mean hair density and subject demographic characteristics; p < 0.05 statistical significance.

Anagen hairs (%)	Placebo (n=22)	PRP (n=22)	Placebo *vs* PRP
	Mean ± SD	Mean ± SD	P - value
Women	1.8 ± 15.5	3.3 ± 11.3	P > 0.05
Men	4.1 ± 12.9	5.5 ± 11.1	P > 0.05
Age ≦ 40 years	5.1 ± 14.8	4.4 ± 11.0	P > 0.05
Age > 40 years	0.8 ± 13.6	4.5 ± 11.3	**P< 0.05**
Beginning of AGA < 25 years	6.0 ± 14.4	4.9 ± 9.9	P > 0.05
Beginning of AGA ≥ 25 years	1.9 ± 13.8	3.9 ± 13.0	**P< 0.05**
Family history +	4.3 ± 12.7	5.2 ± 11.5	P > 0.05
Family history –	2.9 ± 29.0	5.2 ± 29.4	P > 0.05
Disease duration ≦ 10 years	2.6 ± 14.4	4.5 ± 11.8	P > 0.05
Disease duration > 10 years	3.4 ± 14.2	4.4 ± 10.9	P > 0.05

Table 13. Analysis of mean anagen hairs and subject demographic characteristics; p<0.05 statistical significance.

6.2 RESULTS OF GROUP B: PRP AND MEDICATION

Age-eligible participants were recruited and 28 patients were evaluated for potential enrollment. Two patients did not meet the inclusion criteria and one patient declined to participate, because it was unable to accomplishing all fases of treatment.

Thirteen patients medicated with topical minoxidil 5% solution (1ml, twice daily) and 12 patients medicated with oral finasteride (1mg/day) were admitted in the study, of a total of 25 patients. Twenty-four patients completed the entire protocol and were included in the main analysis. One patient was lost to follow-up (Fig.21).

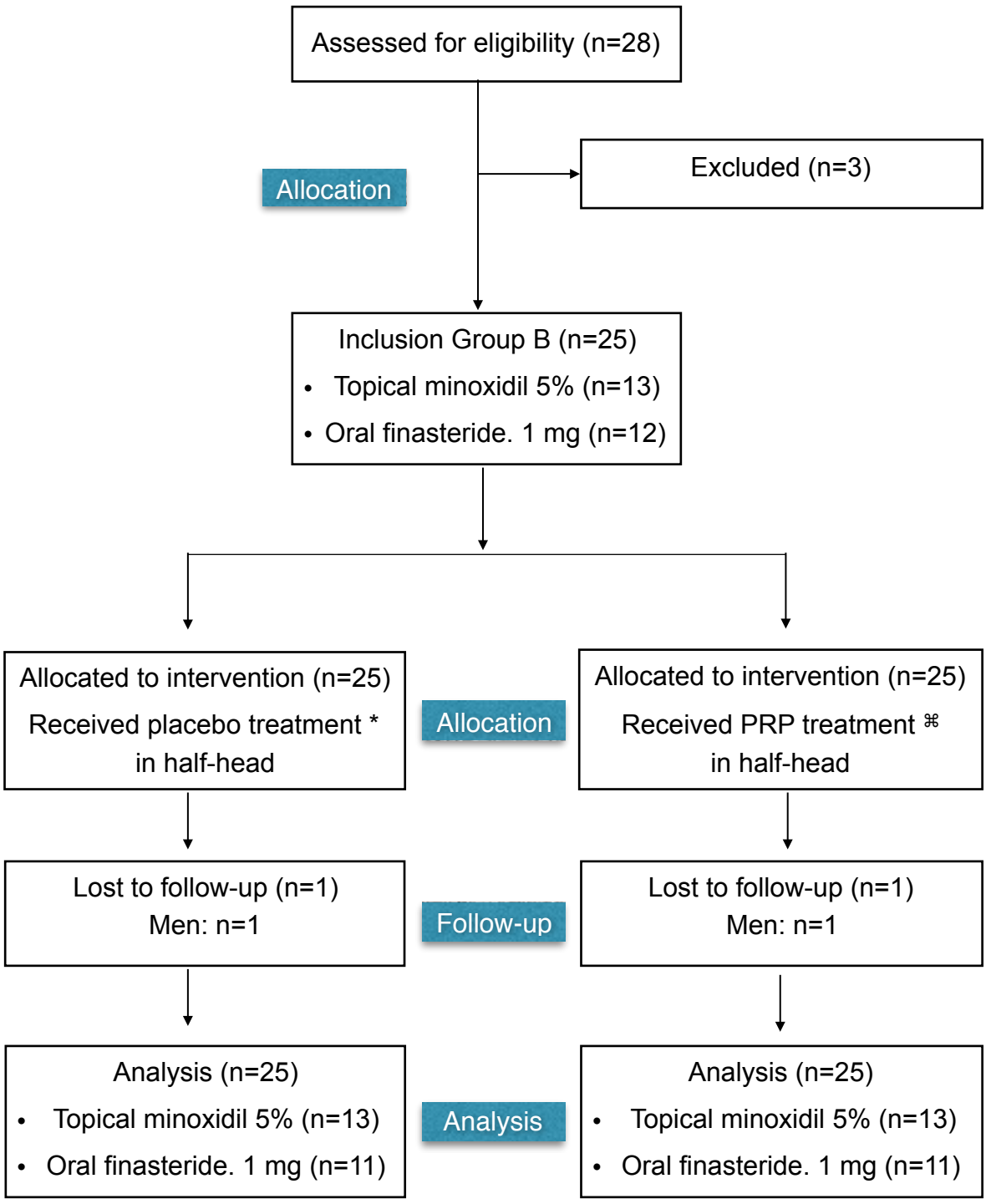

Fig. 21. Flow diagram of Group B. The diagram shows participants enrolled and included in the main analysis. *Placebo treatment (Placebo and minoxidil or Placebo and oral finasteride); ⌘PRP treatment (PRP and minoxidil or PRP and oral finasteride).

6.2.1 Demographic data

Patient demographic data	Group B (n = 24)
Age (mean; years)	39.9 y (18 y.- 65y.)
Sex	
Female	n = 13 (54.2 %)
Male	n = 11 (45.8 %)
Family History	
Positive	n = 21 (87.5 %)
Mother	n = 5 (23.8 %)
Father	n = 16 (76.2 %)
Negative	n = 3 (12.5 %)
Beginning of AGA	
< 25 years	n = 12 (50 %)
≥ 25 years	n = 12 (50 %)
Smoking status	
Yes	n = 4 (16.7 %)
No	n = 20 (83.3 %)
Ethnic origin	
Caucasian	n = 24 (100 %)
Nordwood-Hamilton Classification (Male: n = 11)	
Stage II	n = 2 (18.2 %)
Stage III	n = 7 (63.6 %)
Stage IV	n = 1 (9.1 %)
Stage V	n = 1 (9.1 %)
Ludwig Classification (Female n= 13)	
Stage I	n = 3 (23.1 %)
Stage II	n = 9 (69.2 %)
Stage III	n = 1 (7.7 %)

Table 14. Baseline patient demographic and clinical characteristics of Group B

At presentation, the age of the patients ranged from 18 to 65 years, with a mean age of 39.9 years. There were enrolled 24 participants, with 13 females (54.2%) and 11 males patients (45.8%). Ethnicity was documented for all patients; 100% were caucasians.

Twenty one patients stated a positive family history of AGA and 3 of the 24 patients had a negative family history. Half of the patients reported the beginning of AGA under 25 years of age while the other half after 25 years.

Clinically, and according to Hamilton–Norwood Classification, in men the most frequent pattern was stage III (63.6%). Regarding Ludwig Classification, 9 of 13 (69.2%) female patients were in stage II while 23.1% and 7.7% were in stage II and stage I, respectively.

The clinical characteristics of the 24 patients are summarized in table 14.

6.2.2 TrichoScan Analysis of Group B

At baseline, there were no significant differences in hair count, hair density, terminal density, and anagen or telogen hairs between the treatment and control areas of the scalp.

6.2.2.1 Hair Count

In the treatment areas, the mean change from baseline total hair count ranged between 8.5 hairs/cm^2 and 11.4 hairs/cm^2 (9% – 12%), at 3 to 6 months.

The placebo group showed at the same time mean changes from baseline total hair count between 1.9 hairs/cm^2 (2%) and – 2 hairs/cm^2 (–2%), at 3 and 6 months.

The increase on the treated side was also found to be significant compared to baseline and with that on the control side, at 6 months. ($p<0.05$; control vs treatment) (Table 15) (Fig.22).

Mean Hair Count (hairs/0.65 cm²)	Placebo (n=24)	PRP (n= 24)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	97.9 ± 29.2	96.8 ± 27.5	0.7491
Month 1	104.9 ± 34.1	105.7 ± 29.8	0.8079
Month 1 vs Baseline	7.0 ± 17.0	8.9 ± 21.8	0.9049
P-value (Month 1 vs Baseline)	**0.0362**	**0.0085**	
Month 3	99.8 ± 28.1	105.3 ± 32.3	0.1031
Month 3 vs Baseline	1.9 ± 15.7	8.5 ± 22.1	0.1832
P-value (Month 3 vs Baseline)	0.5573	**0.0116**	
Month 6	95.9 ± 27.0	108.2 ± 33.3	**0.0004**
Month 6 vs Baseline	-2.0 ± 15.1	11.4 ± 20.7	**0.0030**
P-value (Month 6 vs Baseline)	0.5405	**0.0009**	

Table 15. Mean hair count assessed by TrichoScan analysis for the treatment and control half-head areas (p <0.05 statistical significance).

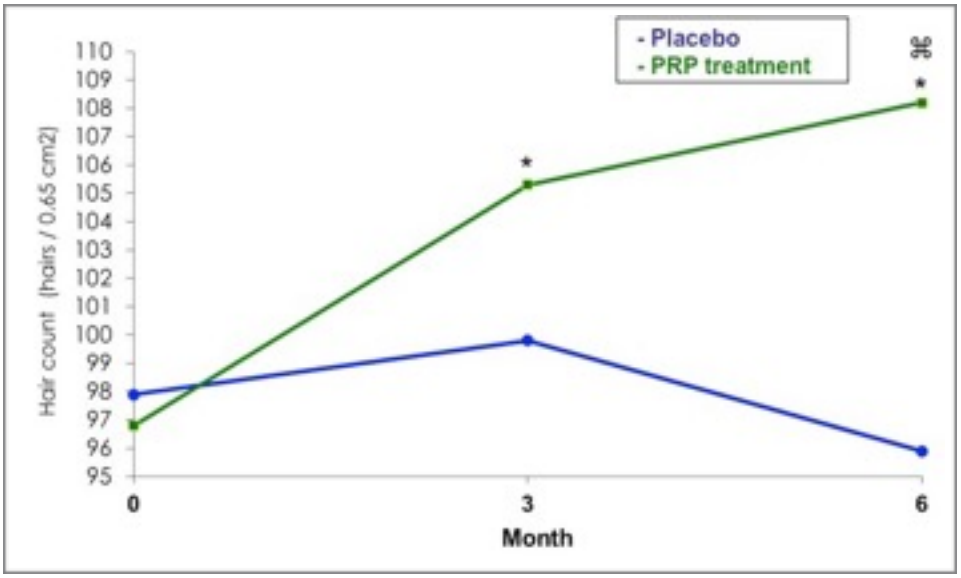

Fig. 22. Evolution of mean hair count, between areas treated with PRP and placebo target areas; *p<0.05 statistical significant, compared to baseline;⌘ p<0.05 statistical significant, placebo versus PRP.

6.2.2.2 Hair Density

Regarding the mean total hair density, the results of Group B showed a significant increase for the treatment area after three months, with a mean increase of 11 number of hairs/cm^2 (7%) in the target area compared to baseline ($p<0.05$; Month 3 vs Baseline), while the control area showed a mean increase of 2.5 hairs (1.6%).

At 6 months of treatment, in the areas treated with PRP, a mean increase in total hair density of 14.1 number of hairs/cm^2 (9.4%) compared to baseline ($p<0.05$; Month 6 vs Baseline) was observed and the control area showed a mean decrease of 3.3 number of hairs/cm^2 (-2%).

In addition, mean total hair density was statistical significant ($p<0.05$; control vs treatment) (Table 16) (Fig.23).

Mean Hair Density (1/cm2)	Placebo (n=24)	PRP (n=24)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	150.8 ± 46.1	149.5 ± 42	0.7896
Month 1	161.1 ± 52.3	162.5 ± 45.8	0.7732
Month 1 vs Baseline	10.3 ± 26.3	13 ± 32.9	0.8619
P-value (Month 1 vs Baseline)	**0.0291**	**0.0067**	
Month 3	153.3 ± 42.2	160.5 ± 47.1	0.1298
Month 3 vs Baseline	2.5 ± 24.2	11 ± 27.1	0.2138
P-value (Month 3 vs Baseline)	0.5935	**0.0208**	
Month 6	147.5 ± 41.6	163.6 ± 47.1	**0.0010**
Month 6 vs Baseline	-3.3 ± 25.1	14.1 ± 24.6	**0.0055**
P-value (Month 6 vs Baseline)	0.4797	**0.0034**	

Table 16. Comparison of mean hair density between the half-head areas treated with PRP versus Placebo; $p<0.05$ statistical significant.

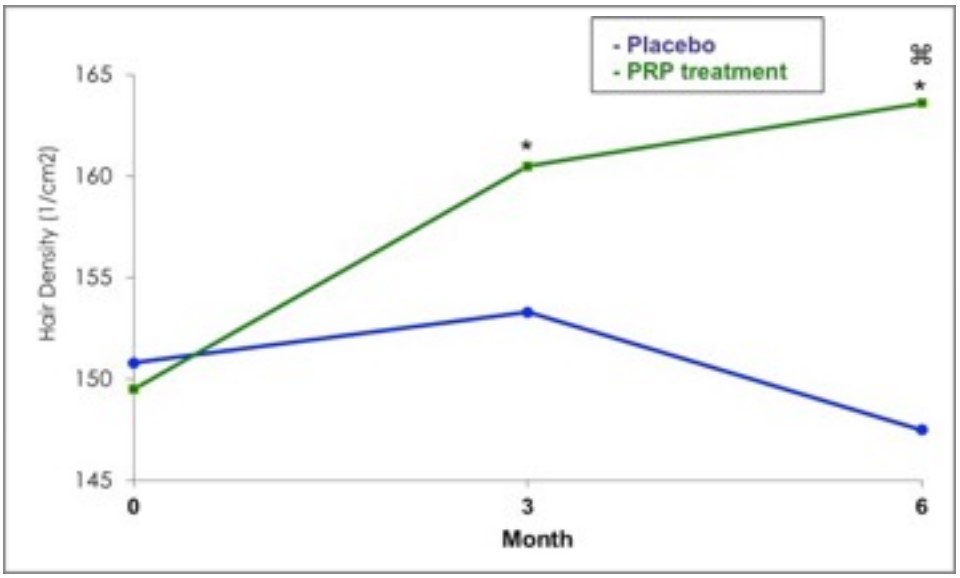

Fig. 23. Evolution of mean hair density, between areas treated with PRP and placebo target areas at baseline, Month 3 and Month 6; *p<0.05 statistical significant, compared to baseline;⌘ p<0.05 statistical significant, placebo versus PRP.

6.2.2.3 Terminal Hair Density

In the areas treated with PRP, the patients presented clinical improvement in the mean terminal hair density, ranged between 4.6 hairs/cm^2 and 11.6 hairs/cm^2 ,at 3 to 6 months.

The placebo group showed at the same time mean changes from baseline total hair count between 1.9 hairs/cm^2 and – 2 hairs/cm^2, at 3 and 6 months.

The increase of the mean terminal hair density on the treated side was found to be significant compared with baseline values and with the placebo area, at 6 months ($p<0.05$: Month 6 vs Baseline) ($p<0.05$; control vs treatment) (Table 17) (Fig.24).

Mean Terminal Hair Density (1/cm²)	Placebo (n=24)	PRP (n=24)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	142.4 ± 43.8	141.3 ± 40.4	0.8296
Month 1	151.4 ± 49.8	151.9 ± 40.8	0.9070
Month 1 vs Baseline	9.0 ± 25.0	10.6 ± 25.7	0.9682
P-value (Month 1 vs Baseline)	0.0671	**0.0312**	
Month 3	145.2 ± 42.2	145.9 ±45.8	0.8933
Month 3 vs Baseline	2.8 ± 23.2	4.6 ± 39.1	0.9562
P-value (Month 3 vs Baseline)	0.5561	0.3466	
Month 6	136.8 ± 44.1	152.9 ± 44.6	**0.0016**
Month 6 vs Baseline	-5.6 ± 28	11.6 ± 23.9	**0.0074**
P-value (Month 6 vs Baseline)	0.2506	**0.0191**	

Table 17. Mean terminal hair density assessed by TrichoScan analysis for the treatment and control half-head areas; p <0.05 statistical significance.

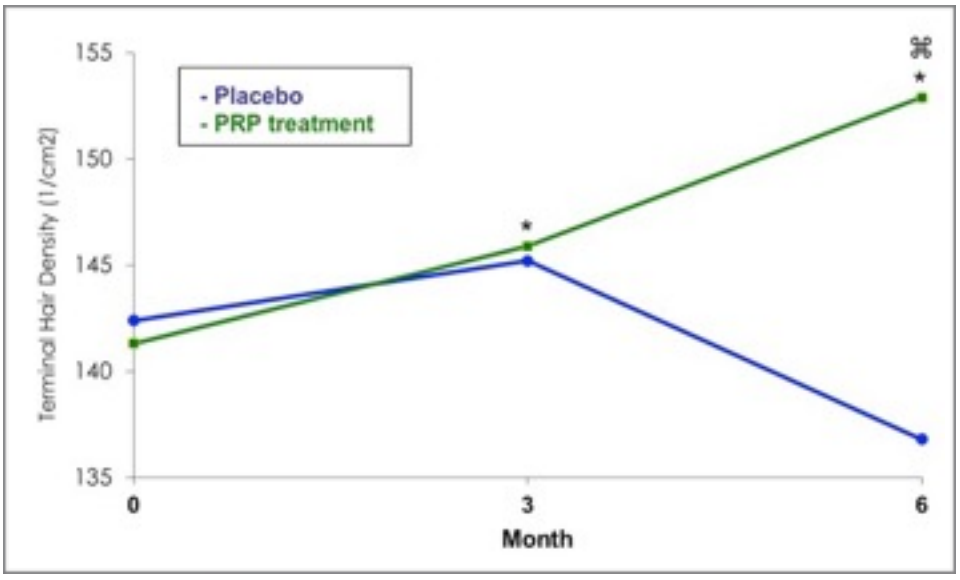

Fig 24. Mean terminal hair density. Comparison between half-head areas treated with PRP versus Placebo; *p<0.05 statistical significant, compared to baseline;⌘ p<0.05 statistical significant, placebo versus PRP.

6.2.2.4 Anagen Hairs

Regarding the mean anagen hairs, area treated with PRP exhibited an increase of 4.4% and 7.7% anagen hairs versus 1.9% and 0.72% in placebo area, at 3 and 6 months respectively.

At 6 months, PRP was statistical significant compared with baseline values although there were no significant differences between the PRP treated area and the placebo area (Table 18) (Fig.25).

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Mean Anagen hairs (%)	Placebo (n=24)	PRP (n=24)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	68.6 ± 13.2	67.5 ± 15.6	0.6319
Month 1	70 ± 10.5	70.3 ± 13	0.8829
Month 1 vs Baseline	1.4 ± 14.1	2.8 ± 15.2	0.9936
P-value (Month 1 vs Baseline)	0.5187	0.2021	
Month 3	69.9 ± 9.9	70.5 ± 14.7	0.7881
Month 3 vs Baseline	1.3 ± 12.5	3.0 ± 19.5	0.9120
P-value (Month 3 vs Baseline)	0.5498	0.1770	
Month 6	69.1 ± 9.9	72.7 ± 10.7	0.1074
Month 6 vs Baseline	0.5 ± 15	5.2 ± 16.1	0.1562
P-value (Month 6 vs Baseline)	0.8049	**0.0194**	

Table 18. Mean anagen hairs and comparison between half-heads treated with PRP versus placebo; $p<0.05$ statistical significance.

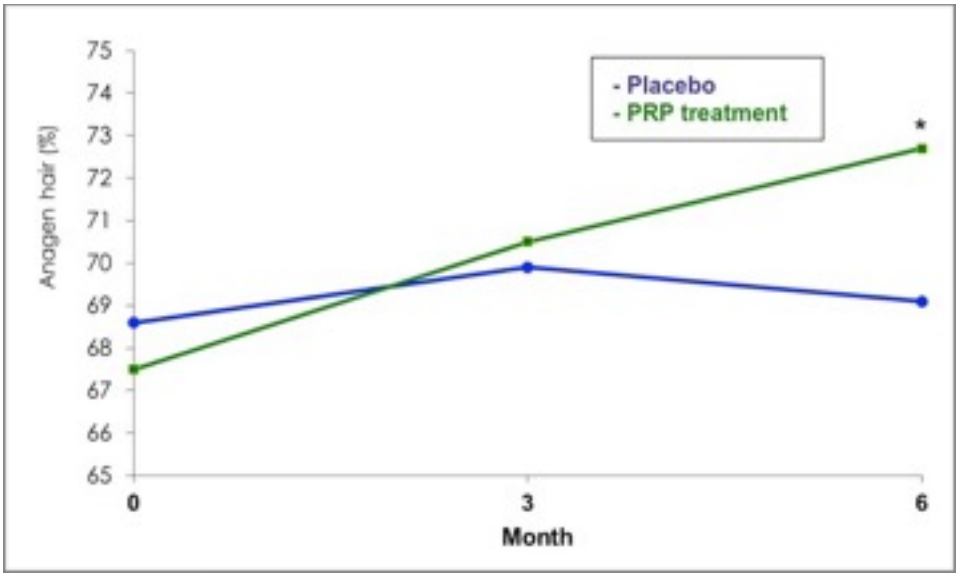

Fig. 25. Evolution of mean anagen hairs at baseline, month 3 and Month 6 in placebo and treatment with PRP. *p<0.05 statistical significant, compared to baseline.

6.2.2.5 Telogen Hairs

The mean telogen hairs (%) for the treatment area after 6 months showed a decrease of 16% of telogen hairs when compared with baseline (p<0.05; Month 6 vs Baseline) while the placebo area displayed a decrease of 2.2%. No differences were found between PRP-treated area and the placebo area.

Mean Telogen hairs (%)	Placebo (n=24)	PRP (n= 24)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	31.6 ± 13.2	32.5 ± 15.6	0.6657
Month 1	30 ± 10.5	29.7 ± 13	0.8910
Month 1 vs Baseline	-1.6 ± 14.2	-2.8 ± 15.1	0.9973
P-value (Month 1 vs Baseline)	0.4776	0.1997	
Month 3	30.1 ± 9.9	29.7 ± 14.6	0.8762
Month 3 vs Baseline	-1.5 ± 12.6	-2.8 ± 19.6	0.9882
P-value (Month 3 vs Baseline)	0.5013	0.2063	
Month 6	30.9 ± 9.9	27.3 ± 10.7	0.1123
Month 6 vs Baseline	-0.7 ± 15.1	-5.2 ± 16.1	0.1580
P-value (Month 6 vs Baseline)	0.7471	**0.0191**	

Table 19. Mean telogen hair parameter for the half-head areas treated with PRP and Placebo, at Baseline, Month 1, 3 and 6. Data obtained from TrichoScan analysis; $p<0.05$ statistical significance.

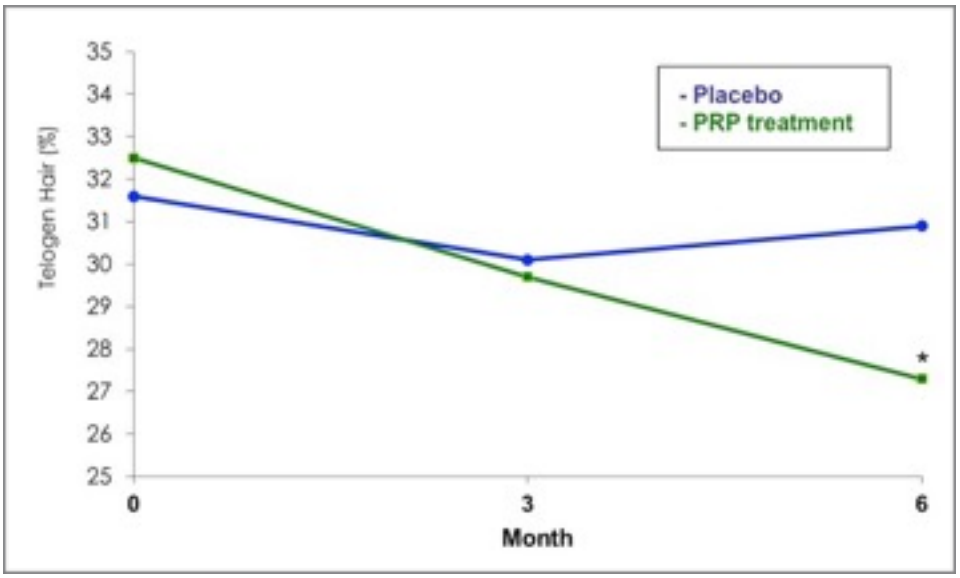

Fig 26. Mean telogen hairs at baseline, month 3 and Month 6 in placebo and treatment with PRP; *$p<0.05$ statistical significant, compared to baseline.

6.2.2.6 Ratio Anagen/Telogen Hairs

After analysis of the mean anagen hairs and telogen hairs, the anagen/telogen ratio was calculated.

The areas treated with PRP exhibit a greater anagen/telogen ratio than the control areas, at 3 months, although not statistical significant.

After 6 months, in the PRP-treated area there was an increase of 21.3% and a decrease of 9% in the placebo area; The increase was statistical significant when compared to baseline ($p<0.05$; Month 6 vs Baseline) (Table 20).

Mean Anagen/telogen ratio (%)	Placebo (n=24)	PRP (n=24)	Placebo versus PRP
	Mean ± SD	Mean ± SD	P - value
Baseline	173.7 ± 159.6	168.9 ± 155.8	0.8749
Month 1	184.1 ± 161.3	204.4 ± 178.5	0.5071
Month 1 vs Baseline	10.4 ± 175.2	35.5 ± 165.3	0.5823
P-value (Month 1 vs Baseline)	0.7285	0.2394	
Month 3	176.6 ± 156.5	228 ± 238.3	0.0948
Month 3 vs Baseline	2.9 ± 175	59.1 ± 257.4	0.1431
P-value (Month 3 vs Baseline)	0.9248	0.0521	
Month 6	169.2 ± 149.3	204.9 ± 184.4	0.0550
Month 6 vs Baseline	-4.5 ± 151	36 ± 176.5	0.0550
P-value (Month 6 vs Baseline)	0.7511	**0.0187**	

Table 20. Mean anagen/telogen ratio and comparison between half-heads treated with PRP versus placebo; $p<0.05$ statistical significance.

6.2.2.7 Summary of Trichoscan analysis of Group B

	Placebo (n=24)		PRP (n= 24)		Placebo versus PRP
	Mean ±SD	**P-value**	**Mean ± SD**	**P-value**	**P-value**
Hair count (hairs 0.65/cm²)					
Baseline	97.9 ± 29.2	P > 0.05	96.8 ± 27.5	P > 0.05	P > 0.05
3 months	99.8 ± 28.1	P > 0.05	105.3 ± 32.3	**P< 0.05**	P > 0.05
6 months	95.9 ± 27	P > 0.05	108.2 ± 33.3	**P< 0.05**	**P< 0.05**
Hair density (1/cm²)					
Baseline	150.8 ± 46.1	P > 0.05	149.5 ± 42	P > 0.05	P > 0.05
3 months	153.3 ± 42.2	P > 0.05	160.5 ± 47.1	**P< 0.05**	P > 0.05
6 months	147.5 ± 41.6	P > 0.05	163.6 ± 47.1	**P< 0.05**	**P< 0.05**
Terminal Hair density					
Baseline	142.4 ± 43.8	P > 0.05	141.3 ± 40.4	P > 0.05	P > 0.05
3 months	145.2 ± 42.2	P > 0.05	145.9 ± 45.8	P > 0.05	P > 0.05
6 months	136.8 ± 44.1	P > 0.05	152.9 ± 44.6	**P< 0.05**	**P< 0.05**
Anagen Hair (%)					
Baseline	68.6 ± 13.2	P > 0.05	67.5 ± 15.6	P > 0.05	P > 0.05
3 months	69.9 ± 9.9	P > 0.05	70.5 ± 14.7	P > 0.05	P > 0.05
6 months	69.1 ± 9.9	P > 0.05	72.7 ± 10.7	**P < 0.05**	P > 0.05
Telogen Hair (%)					
Baseline	31.6 ± 13.2	P > 0.05	32.5 ± 15.6	P > 0.05	P > 0.05
3 months	30.1 ± 9.9	P > 0.05	29.7 ± 14.6	P > 0.05	P > 0.05
6 months	30.9 ± 9.9	P > 0.05	27.3 ±10.7	**P< 0.05**	P > 0.05
Anagen/telogen ratio (%)					
Baseline	173.7 ± 159.6	P > 0.05	168.9 ± 155.8	P > 0.05	P > 0.05
3 months	176.6 ± 156.5	P > 0.05	228 ± 238.3	P > 0.05	P > 0.05
6 months	169.2 ± 149.3	P > 0.05	204.9 ± 184.4	**P< 0.05**	P > 0.05

Table 21. Summary of Trichoscan analysis regarding patients enrolled in Group B, at Baseline, Month 3 and Month 6; p<0.05 statistical significant.

6.2.2.8 Trichoscan Analysis and Medication

The analyses of Group B (n=24) included 11 male patients medicated with finasteride 1 mg daily and 13 female patients medicated with minoxidil 5% solution applied twice daily.

In the table 22, there's a description of hair growth parameters regarding the two different subgroups: PRP and minoxidil 5% topical solution and PRP and oral finasteride, at 6 months.

At baseline, there were no significant differences in hair count, hair density, terminal density, and anagen or telogen hairs between the treatment areas (PRP + minoxidil 5% solution or PRP + oral finasteride) and control areas of the scalp (placebo + minoxidil 5% solution or placebo + oral finasteride).

In the areas treated with placebo, there were no statistical differences between minoxidil and finasteride.

The combination of PRP and minoxidil 5 % solution in comparison to PRP and finasteride 1 mg displayed a total mean hair count change of 9.8 ± 26.9 hairs versus 0.6 ± 10.8 hairs; a total mean hair density of 12.3 ± 34.2 hairs/cm^2 versus 1.8 ± 16.7 hairs/cm^2; a mean anagen hairs (%) of 5.5 ± 19.7 versus -2 ± 12.2, respectively ($p<0.05$: PRP + minoxidil versus PRP + finasteride).

Overall, after 6 months, combination of PRP and minoxidil 5% showed superiority in mean hair count, hair density, anagen and telogen percentages and mean anagen/telogen ratio in comparison to the association of PRP and finasteride 1 mg ($p<0.05$: PRP + minoxidil versus PRP + finasteride).

In this group in which there was an association of PRP with associated medication we found a better improvement with PRP and minoxidil.

MONTH 6	Placebo	PRP	Placebo versus PRP
	Mean ± SD	Mean ± SD	P-value
Hair count (hairs 0.65/cm^2)			
Finasteride (n=11)	0.9 ± 16.3	0.6 ± 10.8	0.9271
Minoxidil 5% (n=13)	3.7 ± 14.5	9.8 ± 26.9	0.0597
P-value	0.4004	**0.0105**	
Hair density (1/cm^2)			
Finasteride (n=11)	1.4 ± 25.1	1.8 ± 16.7	0.9384
Minoxidil 5% (n=13)	5.1 ± 23.9	12.3 ± 34.2	0.1117
P-value	0.2831	**0.0104**	
Terminal Hair density			
Finasteride (n=11)	-1.9 ± 28.3	3.9 ± 15.2	0.6584
Minoxidil 5% (n=13)	5.6 ± 21.9	3.2 ± 38.4	0.7019
P-value	0.1379	0.1749	
Anagen Hair (%)			
Finasteride (n=11)	- 2.1 ± 15	- 2 ± 12.2	0.9384
Minoxidil 5% (n=13)	1.5 ± 13.8	5.5 ± 19.7	0.0504
P-value	0.0777	**0.0015**	
Telogen Hair (%)			
Finasteride (n=11)	2.1 ± 15	1.8 ± 12.2	0.9058
Minoxidil 5% (n=13)	- 1.7 ± 14	- 5.5 ± 19.9	0.0618
P-value	0.0724	**0.0019**	
Anagen/telogen ratio (%)			
Finasteride (n=11)	- 40.5 ± 169.7	- 6.6 ± 155.7	0.3253
Minoxidil 5% (n=13)	1.7 ± 192.5	69.6 ± 234	**0.0261**
P-value	0.1667	**0.0179**	

Table 22. Group B : Minoxidil versus Finasteride

7. DISCUSSION

7. DISCUSSION

The discussion is divided in two parts according to each study, as follows:

7.1 DISCUSSION OF RESULTS OF GROUP A: PRP ALONE

In this double-blind, half-head and placebo-controlled study, the authors have demonstrated that, at three months, the administration of PRP alone attended a statistically significant improvement of mean hair density (14.8 ± 32.1 hairs), mean anagen hairs (5.8 ± 10.9%) and telogen hairs (- 2.1 ± 12.9%) when compared with baseline ($p<0.05$; Month 3 vs Baseline).

At 6 months, we observed that the half-head treated with PRP alone showed a statistically significant improvement of mean hair density (12.8 ± 32.6 hairs), terminal hair density (5.8 ± 25.7 hairs), anagen hairs (5.5 ± 9.7 %), telogen hairs (- 5.5 ± 9.7), when compared with baseline ($p<0.05$; Month 6 vs Baseline).

An increase of the anagen/telogen ratio in the areas treated with PRP alone was observed when compared with control area, both at 3 and 6 months.

When comparing to the control side, the hair on the PRP-treated side showed a statistically significant increase in mean total hair density only, both at 3 and 6 months ($p<0.05$; control vs treatment).

Regarding the mean hair count, we didn't found any statistical differences between treated area and control area, through the duration of the study.

In support of the data obtained, the correlation between demographic data and trichoscan analysis PRP showed a statistically significantly association with two parameters: mean total hair density and mean anagen hairs.

Male patients under 40 years old with a positive family history and beginning of hair loss after 25 years had a better response in mean hair density in the areas treated with PRP when compared with the control areas ($p<0.05$; control vs treatment).

This study also found a correlation in the areas treated with PRP between mean anagen hairs (%), and patients aged more than 40 years and beginning of AGA after 25 years, at 6 months. The other parameters analyzed did not show statistical differences.

The underlying mechanism of how administration of PRP induces a positive effect on AGA is not fully clear.

It is proposed that GFs released from platelets may act on stem cells in the bulge area of the follicles, stimulating the development of new follicles and promoting neovascularization (167).Interactions between the bulge area and matrix as well as with binding GFs (PDGF, TGF-β, and VEGF) activate the proliferative phase of the hair, giving rise to the future follicular unit.

Growth factors appear to play a fundamental role in the life-long cyclic transformation of the hair follicle by controlling the anagen phase and promoting apoptosis to induce the catagen and telogen phases. So, when platelets become activated the GFs are release and act in tissue angiogenesis. Application of these GFs to DP cells can lead to the initiation and prolongation of anagen phase in the hair follicle (37). and together they can stimulate cell survival (127,168), differentiation (127,169), vascularization and angiogenesis (127,144,145).

In 2006, Uebel et al.(144) published a study in which patients subjected to hair transplant surgery had their follicular units embedded in PRP before transplantation. According to the authors, this approach resulted in improved hair growth and an increase in follicular density. Moreover, the authors hypothesized that GFs could act upon dermic papilla, leading to an intense vascular endoneogenesis and the progression of new hairs to the anagen phase.

Three years later, Greco et al. (170) performed a study to determine if PRP had any effect on non-transplanted miniaturized hair. The authors believed that high concentrations of GFs could stimulate the follicular cells in the affected regions without the need for a hair transplant. The results of this small study revealed an average increase of hair shaft diameter after 6 months.

Both studies provided a new perspective concerning the application of PRP for alopecia, in both transplanted and non-transplanted patients.

PRP has started to be considered as a potential therapeutic tool for promoting hair growth, and several studies have been published in recent years.

Li and colleagues (127) studied the effect of PRP on hair growth using *in vitro* and *in vivo* (mice) models. They proposed that the injection of mice with activated PRP induced a faster telogen-to-anagen transition than was seen in the control mice. This study provides further support that pure-PRP may prolong the anagen phase of the hair cycle, as the authors found a superior anagen/telogen ratio (%) in the areas treated with PRP than the areas treated with the placebo, when compared with baseline. Anagen-associated angiogenesis has been suggested to be one of the most important factors in active hair growth (102)

Cervelli et al. (171) conducted a controlled, double-blind study that included a clinical and histomorphometric evaluation of 10 patients with pattern hair loss. The injection of activated PRP into half of the head (the other half was treated with placebo) led to improvements in the mean hair count and mean hair thickness after three cycles of treatment. The authors also reported the clinical efficacy of injection of PRP in addition to an increase in the thickness of epidermis, the number of hair skin follicles and the number of Ki-67^{+} keratinocytes (a marker for cell proliferation).

The findings of the study performed by Cervelli et al. were corroborate with the study conducted by Pietro Gentile and colleagues (167). In this study, the authors investigated the safety and clinical efficacy of autologous PRP injections for pattern hair loss. Three treatments were administered to each patient at 30-day intervals and the other half-head was treated with placebo. At the end of the 3 treatment cycles, the patients presented clinical improvement in the mean number of hairs, with a mean increase of 33.6 hairs in the target area, and a mean increase in total hair density of 45.9 hairs per cm^{2} compared with baseline values.

These authors, also report an increase of Ki67^{+} keratinocytes in the epidermis and small blood vessels around hair follicles in the treated scalp compared with baseline. This finding might support that the GFs presents in PRP may induce angiogenesis and cell proliferation through an increase in vascularization around the follicles.

A prospective study of 11 male patients affected by AGA was performed by Khatu and colleagues (172). The administration of PRP was repeated every two weeks for 4 sessions and outcome assessed after 3 months by clinical examination, macroscopic photos, hair pull test and patient's overall satisfaction. Authors reported a significant reduction in hair loss between first and fourth PRP injection, without major side-effects. This study, despite the absence of controls and statistical analysis, lacks an objective evaluation method (173).

Gkini et al. (174) reported, in a nonrandomized prospective cohort study, the efficacy of PRP injection in 20 patients with AGA (18 males and 2 females). Three treatment sessions were performed with an interval of 21 days and a booster session at 6 months following the onset of therapy. At 6 months, the patients presented a significantly increased in mean hair density compared with baseline (167).

A systematic review was performed by Maria-Angeliki et al. (173). The authors performed an analysis of the literature regarding the PRP mechanism of action, preparation methods and therapeutic potential in patients with noncicatricial alopecias. The analysis included 12 articles about AGA and 2 articles regarding the use of PRP in AA. In most articles, activation and preparation methods were not mentioned, and no standard protocol was employed regarding the frequency of PRP applications. In addition, there’s a lack of controls, small sample size, lack of detailed reports in patients’ characteristics and used statistical methods. As stated by the authors, controlled clinical trials are considered to be the best way to provide scientific evidence for a treatment and avoid potential bias when assessing efficacy.

Recently a meta-analysis was published by Gupta et al. (37) to estimate the value of PRP therapy for treatment of AGA. After an extensive review of PubMed and Google Scholar databases, only 4 studies (145,171,174,175) with adequate data could be included in the meta-analysis.

Two of the studies examined PRP as a treatment for AGA outside of human subjects, using *in vitro* and murine models. In the four studies, hair density was the measure of treatment success.The present meta-analysis reported that there is evidence to support the potential efficacy of PRP, however further investigation is required.

Data analysed from the majority of the studies in the literature demonstrated an increase in number of hairs and/or hair density in patients treated with PRP.

According to our study we also found a mean increase in total hair density in the areas treated with PRP, compared with baseline. In addition, we also found a statistical significant increase when comparing to the control side. This gives further support of the data obtained in our study as eliminate all bias related with the control side. Regarding the mean hair count we also found a slight increase of number of hairs, although not statistical significant.

As mentioned above, Li et al. reported a faster telogen-to-anagen transition and found a superior anagen/telogen ratio (%) in the areas treated with PRP than the areas treated with the placebo, when compared with baseline.These findings are comparable with our study as we also found a statistical significant increase of the anagen/telogen ratio in the areas treated with PRP alone when compared with control area, both at 3 and 6 months.

Besides the Trichoscan analysis, in our clinical trial we showed a statistically significantly association between two parameters of the Trichoscan analysis (mean total hair density and mean anagen hairs) and demographic data of the enrolled patients.

In a study performed by Lopez and colleagues (176), patients treated with PRP showed a significant correlation between male sex and the hair density.

As in the latter article, beside the correlation between male sex and the hair density, in our clinical trial study, we were also able to demonstrate for the first time a statistical significant association between hair density and patients below 40 years with positive family history of AGA and more than 10 years of duration of the disease. In addition, to the best of our knowledge, this study is also the first in which there is a correlation between anagen hairs and patients older than 40 years and beginning of AGA with age superior to 25 years.

Diverse methods are reported as activators can be used to stimulate growth factor release (37). Some studies also included modifications or other types of PRP, such as dalteparin/protamine (145) and leukocyte-PRP to boost results (177).

Two studies (178,179) reported that dalteparin and protamine microparticles (D/P MPs) are a carrier for controlled release of GFs such as FGF-2. And that the

FGF-2 containing D/P MPs displayed a substantial ability to induce vascularization.

Following this effects and as PRP is a source of several GFs, including FGF, Takikawa et al. (145) performed a study to identify the effects of PRP-containing dalteparin and protamine microparticles (D/P MPs) on hair growth. Participants received five local treatments of PRP with D/P MPs at 2 to 3-week intervals and were evaluated for 12 weeks. According to the authors, PRP associated with D/P MPs promoted hair growth as significant differences were seen in hair cross-section, although not in hair numbers. The addition of D/P MPs to PRP resulted in significant stimulation in hair cross-section. The microscopic findings demonstrated a thickened epithelium, proliferation of collagen fibres and fibroblasts, and increased vessels around follicles.

Another study of a different type of PRP was performed by Schiavone et al. (177) that evaluated the effect of L-PRP on hair growth in AGA. The present article enrolled 64 patients with AGA and performed two injections of L-PRP with the addition of concentrated plasmatic proteins administered at baseline (with a single spin centrifugation) and after 3 months (double-spin centrifugation). Follow-up was made at 6 months. The authors didn't performed a trichoscan analysis but a 15-point scale proposed by Jaeschke and colleagues to assess the clinical change between the first treatment and the end of the follow- up. According to authors, L-PRP led to an increase in hair thickness and numbers compared to baseline, resulting in a clinically important difference in over 40% of patients. The article gives rise that L-PRP may also induce hair growth in patients with AGA however, patients were evaluated through subjective investigator assessment making the results of this study difficult to compare with the previous data reported in the literature.

Kang et al. (180) performed a study to investigate the clinical efficacy of injection of $CD34^+$ cell-containing PRP preparation for pattern hair loss. As it has been suggested that $CD34^+$ cells in peripheral blood naturally decrease in the number of circulating cells and lose angiogenic potential with age, the authors hypothesised that $CD34^+$ cell-containing PRP preparation could be effectively used for the treatment of male and female pattern hair loss. In accordance with the data obtained, it was suggest that the injection of autologous CD34+ cell-containing

PRP preparation had a positive therapeutic effect on male and female pattern hair loss with a statistical significant improvement in the mean number of hairs, and mean hair thickness at 3 months, compared with baseline values. At 6 months, the patients presented clinical improvement in mean hair count and mean hair thickness compared with baseline. Thus, the use of $CD34^+$ cell-containing PRP led to a significant increase in hair thickness and numbers compared to baseline

These articles with modifications of the PRP reported a positive effect in hair growth of administration of PRP in the treatment of AGA, with little side-effects. Although the final result of a positive effect of PRP is coincidence with our study, the data is not susceptible of comparison as it's comparing different types of PRP.

7.2 DISCUSSION OF RESULTS OF GROUP B: PRP AND MEDICATION

To our knowledge this is the first randomized double-blind controlled study that reports the use of PRP with associated medication in the treatment of AGA.

The results of the study of PRP associated with medication ((PRP and minoxidil 5% solution or PRP and oral finasteride) revealed that the administration of PRP led to a statistically significant increase in the mean hair count, hair density, terminal hair density, anagen hairs and telogen hairs, when compared with baseline values ($p<0.05$; Month 3 versus Baseline), after 3 months. At this point of the study, the changes were not statistically significant in comparison to placebo ($p>0.05$).

At six months after the first treatment, the parameters analyzed such as mean hair count, hair density, terminal hair density, anagen hairs and telogen hairs, revealed a statistical significant positive effect of PRP regarding baseline values ($p<0.05$; Month 6 versus Baseline). In addition, the changes observed in the mean hair count, mean hair density and mean terminal density, were statistically significant in comparison to placebo ($p<0.05$; control vs treatment).

In our study, a comparison between the two treatments: minoxidil finasteride was also performed.

In the areas treated with placebo, there were no statistical differences between minoxidil and finasteride. When PRP is used in combination with minoxidil or finasteride, the combination of PRP and minoxidil 5% showed superiority in mean hair count, hair density, anagen and telogen percentages and mean anagen/ telogen ratio in comparison to the association of PRP and finasteride 1 mg ($p<0.05$: PRP and minoxidil versus PRP and finasteride).

Minoxidil is known to promote the survival of DP cells by increasing Bcl-2/Bax ratio and by activating ERK and Akt (171). In addition,another possible effect of minoxidil to promote hair regrowth is the increased expression of some GFs such as VEGF in cultured DP cells.

Lachgar et al. (73) described that VEGF mRNA is strongly expressed in DP cells in the anagen phase, while is less expressed during the catagen and telogen phases. This may lead to the formation of new blood vessels to maintain adequate microvascularization, necessary to maintain hair regrowth. In the present study, the authors report that “expression of VEGF is elevated in DP cells after exposure to minoxidil

Other published articles demonstrated that VEGF expressed is induced by interleukin-6 (181), interleukin-1β (182), TGF-β (183) and PDGF (184). Yano K et al. (44) performed a study in order to quantify the cyclic changes of perifollicular vascularization and to characterize the biological role of VEGF for hair growth, angiogenesis, and follicle cycling. Previously, the authors had identified that VEGF enhances microvascular permeability and angiogenesis and that this angiogenesis factor was very important for skin vascularization.

PRP is also known to produce different GFs and that might lead to an intense neovascularization (144) with increased proliferation of human DP cells. These GFs are present in PRP so leading to an expression in VEDF and consequently in hair regrowth, in anagen phase.

As both minoxidil and PRP promote the expression of VEGF, the results of our study may indicate a causal relationship between minoxidil, PRP and the upregulation of the angiogenic VEGF.

Although this has not been previously described, the expression of VEGF to induce proliferation and angiogenesis could be dose-dependent. In addition, the association of both treatments (PRP and 5% minoxidil topical solution) could up regulate the expression thus inducing a greater regrowth in DP of hair follicles from alopecia patients.

Although the association of PRP with minoxidil seams to be better than the association of PRP and finasteride, the data is limited to 6 months. The response to treatment with finasteride should be assessed with the minimal period of 6 months, in some men it may not become evident until 12 months (1).

So, regarding treatment with finasteride one might consider that finasteride might need a longer follow up (for at least 12 months) to have similar or even better

improvement than minoxidil. This efficacy needs to be assessed in another studies with a longer follow up.

7.3 SIDE-EFFECTS

Beside the local and transitory pain at the moment of administration of the treatment with PRP and saline solution, no other side-effects were noted or reported during the 6 months of follow-up

7.4 LIMITATIONS

The two major limitations of this study relate to the sample size and duration of follow-up. This study has the limitation of having a follow-up of 6 months. Furthermore, this follow-up period is short to draw final conclusions about the benefits of treatment with PRP over time.

Most of the published articles included few number of patients with a limited number of randomized controlled studies. In our study, a single patient played the role of both treated and controlled subject. This has advantages because it could correct the possible biases such as gender and grade of hair loss that could affect the results.

The use of Thrichoscan might also be considered as a limitation as some authors question the accuracy of Trichoscan analysis (67,185,186). D. Van Neste et al. (185) reported there's an underestimation of hair density and a lack of detection of thinner hair. Considering AGA is also associated with thin hair this may be a limitation and trichoscan method might require an optimization of the software.

Other methods for monitoring hair cycle are being developed that could give a more accurate evaluation during clinical trials.

8. CONCLUSIONS

8. CONCLUSIONS

In our clinical trial we have demonstrated that the administration of autologous PRP has a positive effect on male and female pattern hair loss without significant side-effects.

Regarding the first phase of the study (Group A: PRP alone) we conclude:

- Administration of PRP alone attended a statistically significant improvement of mean hair density, terminal hair density, mean anagen hairs and telogen hairs, when compared with baseline, at 6 months.
- When comparing to the control side, the hair on the PRP-treated side showed a statistically significant increase in mean total hair density.
- An increase of the anagen/telogen ratio in the areas treated with PRP alone was observed.
- A statistically significantly correlation between demographic data and trichoscan analysis was found with two parameters: mean total hair density and mean anagen hairs. Male sex and beginning of AGA after 25 years seams to respond better to the treatment in both parameters as hair density and anagen hairs.

With respect to the second phase of the study (Group B: PRP and Medication):

- Administration of PRP associated with ongoing medication is effective on the evolution of AGA;
- Application of PRP improved the mean hair parameters, and that was statistically significant versus baseline at month 6;
- Both minoxidil or finasteride with PRP improved hair regrowth;
- PRP in combination with minoxidil showed a greater improvement than PRP with finasteride, at six months.

In conclusion, the use of PRP is effective, safe and worthwhile as a complementary treatment for AGA, but additional placebo-controlled studies with larger samples sizes and longer follow-up periods are needed.

9. FUTURE LINES OF INVESTIGATION

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The research of PRP in the field of alopecia is only in its beginnings. To ensure and validate the efficacy of PRP, future lines of investigation may be considered, as suggested:

- As is of our knowledge, this is the first study that found a correlation with demographic data and two parameters of Trichoscan analysis. More studies are needed to compare it with our study and to confirm these results.

- In the book “Clinical indications and treatment protocols of PRP in Dermatology” (*Annex 13.17*), a protocol regarding PRP and Alopecia was published. More studies are necessary to further investigate and consolidate a treatment protocol.

- Perform other clinical trial that includes a longer follow-up and a larger sample of patients with AGA treated with PRP .

- Evaluate the efficacy of administration of PRP in other types of alopecia.

10. PUBLICATIONS

10. PUBLICATIONS

10.1 ARTICLES

10.1.1 Article 1

Title: Randomized Placebo-Controlled, Double-Blind, Half-Head Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia.

Authors: Alves R, Grimalt R.

Journal: Dermatologic Surgery

JCR Impact Factor (2015): 1.936

Volume: 42

Number: 4

Dermatol Surg. 2016 Apr;42(4):491-7. (*Annex 13.11*)

10.1.2 Commentary on Article 1

Title: Commentary on a Randomized Placebo-Controlled,Double-Blind, Half-Head Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia.

Authors: Nusbaum AG, Tosti A.

Journal: Dermatologic Surgery

Volume: 42

Number: 4

Dermatol Surg. 2016 Apr;42(4):498-9. (*Annex 13.12*)

10.1.3 Article 2

Title: Platelet-rich plasma in combination with 5% minoxidil topical solution and 1 mg oral finasteride for the treatment of androgenetic alopecia: a randomized placebo controlled,double-blind, half-head study

Authors: Alves R, Grimalt R.

Journal: Dermatologic Surgery: in revision (*Annex 13.13*)

10.2 CHAPTERS OF BOOKS

10.2.1 Hair Loss in Children

Chapter: Hair Loss in Children. (*Annex 13.14)*.

Editors: Loannides D, Tosti A .

Book: Current Problems in Dermatology: Alopecias, Practical Evaluation and Management

Year: 2015

Alves R, Grimalt R. Hair loss in children. Curr Probl Dermatol. 2015;47: p. 55-66.

10.2.2 Androgenetic Alopecia in Adolescents

Chapter: Androgenetic Alopecia in Adolescents (Annex 13.15)

Editors: Oranje AP, Al-Mutairi N, Shwayder T

Book: Practical Pediatric Dermatology, Controversies in Diagnosis and Treatment.

Year: 2016

Alves R, Grimalt R. Androgenetic Alopecia in Adolescents. In: Oranje AP, Al-Mutairi N, Shwayder T (Eds.). Practical Pediatric Dermatology, Controversies in Diagnosis and Treatment. Switzerland: Springer; 2016.p.187-196.

10.2.3. PRP in Alopecia

Chapter: PRP in Alopecia (*Annex 13.16*)

Editors: Alves R, Grimalt R.

Book: Clinical indications and treatment protocols with Platelet-rich plasma in Dermatology.

Year: 2016

Alves R. PRP in Alopecia. In: Alves R, Grimalt R. (Eds). Barcelona: Ediciones Mayo; 2016. p. 29-44.

10.3 BOOK

10.3.1 Book of PRP

Editors: Alves R, Grimalt R.

Book: Clinical indications and treatment protocols with Platelet-rich plasma in Dermatology. (*Annex 13.17)*

Year: 2016

Editorial: Ediciones Mayo

11. REFERENCES

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12. ABSTRACT

12. ABSTRACT

Background: Androgenetic Alopecia (AGA) is a nonscarring alopecia that affects both men and women. It is characterized by a progressive miniaturization of hair follicles with a characteristic pattern distribution in genetically predisposed men and women. The currently available treatments for AGA are sometimes perceived as having limited effectiveness, therefore, Platelet-rich plasma (PRP) has been postulated as a new therapy for AGA.

Objective: To assess the efficacy of PRP on the evolution of AGA, between 6 months of treatment and baseline.

Methods: This was a prospective, randomized, placebo controlled, double-blind, half-head study in male and female patients with AGA. The patient selection was performed in two different phases, according to presence or absence of medication for AGA. Group A included patients without previous medication for AGA and Group B included patients under medication for AGA (minoxidil or finasteride). All patients received a total of 3 treatments of PRP on one half-head and saline solution in the other half-head, with an interval of 1 month from each other. Injected areas comprised 4 circular areas marked with a dot tattoo. The follow-up visit was made at 6 months.

Results: In Group A: administration of PRP alone attended a statistically significant improvement of mean hair density, mean terminal hair density, mean anagen hairs and telogen hairs, when compared with baseline, at 6 months. A statistically significantly correlation between demographic data and trichoscan analysis was found with two parameters: mean total hair density and mean anagen hairs. Regarding Group B: administration of PRP associated with ongoing medication is effective on the evolution of AGA. Both minoxidil or finasteride

associated with PRP improved hair regrowth, although PRP in combination with minoxidil showed a greater improvement than PRP with finasteride, at six months.

Limitations: The two major limitations of this study relate to the sample size and duration of follow-up.

Conclusion: Administration of autologous PRP had a positive effect on male and female pattern hair loss without major side-effects.

13. ANNEXES

13.1 ETHIC COMMITTEE APPROVAL

Comitè Ètic d'Investigació Clínica

Clínica Universitària d'Odontologia

Universitat Internacional de Catalunya

CARTA APROVACIÓ ESTUDI PEL CEIC

Número de l'estudi: PRP 1/13
Versió del protocol:1.3
Data de la versió:04/12/2013
Títol:"A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia"

Sant Cugat del Vallès, 19 de desembre de 2013

Dr. Ramon Grimalt

Referència: "A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia"

Benvolgut Doctor,

Els membres del CEIC de la Clínica Universitària d'Odontologia, els hi agraeixen l'aportació científica en el camp de la investigació i la presentació del Protocol en aquest Comité per a la seva avaluació.

Valorades les noves aportacions realitzades a l'estudi, sol.licitades pel nostre CEIC, el passat dia 04 de desembre de 2013, li comuniquem que el dictamen final ha sigut FAVORABLE.

Li recordem que, segons la Normativa del Real Decret 223/2004 art. 27, s'haurà de presentar al Comitè d'Ètica d'investigacions clíniques de la CUO, i a través de la Comissió Científica, un informe preliminar mensual del seguiment de l'estudi i un informe final un cop finalitzat aquest.

1

CEIC Comitè Ètic d'Investigació Clínica

CUO Clínica Universitària d'Odontologia | Universitat Internacional de Catalunya

Quedem a la seva disposició per a qualsevol dubte o aclaració al respecte.

Atentament,

Dr.Magí Brufau
President CEIC

2

Universitat Internacional de Catalunya
COMUNICAT INTERN

Dr. Ramon Grimalt
Investigador Principal

CEIC
Ref.138/2013

Comitè Ètic d'Investigació Clínica

El Comitè Ètic d'Investigació Clínica en sessió del dia 04 de desembre de 2013 va adoptar, entre d'altres, el següent acord:

S'aprova l'estudi número PRP 1/13 "A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia" de Dermatologia (Investigador Principal: Dr. Ramon Grimalt/Investigadora Secundària: Rubina Alves).

Atentament,

Deborah Violant
19 de desembre de 2013

A/c.: Dr. Lluís Giner, Dra. Montse Mercadé, Rubina Alves.

13.2 CLINICAL TRIALS REGISTRATION

ClinicalTrials.gov

A service of the U.S. National Institutes of Health

Now Available: Final Rule for FDAAA 801 and NIH Policy on Clinical Trial Reporting

Trial record **2 of 2** for: Rubina Alves

◂ Previous Study | Return to List | Next Study

Study to Assess Efficacy of Platelet-Rich Plasma in Androgenetic Alopecia (PRP)

This study is ongoing, but not recruiting participants.

Sponsor:
Universitat Internacional de Catalunya

Information provided by (Responsible Party):
Rubina Alves, Universitat Internacional de Catalunya

ClinicalTrials.gov Identifier:
NCT02393040

First received: March 3, 2015
Last updated: March 18, 2015
Last verified: March 2015
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Tracking Information	
First Received Date ICMJE	March 3, 2015
Last Updated Date	March 18, 2015
Start Date ICMJE	January 2014
Estimated Primary Completion Date	July 2015 (final data collection date for primary outcome measure)
Current Primary Outcome Measures ICMJE **(submitted: March 18, 2015)**	Determine the treatment efficacy by measuring hair regrowth score for each side of scalp. [Time Frame: 6 months] [Designated as safety issue: No]
Original Primary Outcome Measures ICMJE	*Same as current*
Change History	No Changes Posted
Current Secondary Outcome Measures ICMJE **(submitted: March 18, 2015)**	• Determine the treatment efficacy by measuring hair density for each side of scalp [Time Frame: 6 months] [Designated as safety issue: Yes] Determine the treatment efficacy by measuring mean hair density for each side of scalp using the trichogram analysis. • Determine the treatment efficacy by measuring anagen/telogen ratio for each side of scalp [Time Frame: 6 months] [Designated as safety issue: Yes] Determine the treatment efficacy by measuring anagen/telogen ratio for each side of scalp using the trichogram analysis. • Global photographs of three areas of the scalp (evaluation of hair growth will be assessed by comparison of standardized images) [Time Frame: 6 months] [Designated as safety issue: Yes] The evaluation of hair growth will be assessed by comparison of standardized images between baseline and 6 months.
Original Secondary Outcome Measures ICMJE	*Same as current*
Current Other Outcome Measures ICMJE	*Not Provided*
Original Other Outcome Measures ICMJE	*Not Provided*

Descriptive Information	
Brief Title ICMJE	Study to Assess Efficacy of Platelet-Rich Plasma in Androgenetic Alopecia
Official Title ICMJE	A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia
Brief Summary	The purpose of this study is to determine the efficacy of treatment with Platelet-rich plasma on Androgenetic alopecia (compared with placebo), between six months and baseline.
Detailed Description	Androgenetic alopecia (AGA) is a non-scarring patterned alopecia, multifactorial and a genetic determined skin condition. This pathology is one of the most common forms of progressive hair loss. It's an increasingly frequent complaint of dermatology clinic patients and has a high impact psychologically. The onset of AGA is gradual and when this pathology progresses, the anagen phase shortens and the telogen phase remains constant. As a result, more hairs are in the telogen phase, and the patient may notice an increase in hair shedding. This area varies from patient to patient and is usually most marked at the vertex in men while women with androgenetic alopecia generally lose hair diffusely over the crown. The incidence and prevalence of AGA increases with age. Topical minoxidil and oral finasteride are the gold standard therapies for AGA and the only two drugs currently that have US Food and Drug Administration (FDA)-approved indications for the treatment of androgenetic alopecia. Minoxidil and finasteride are known to be effective medical treatments in AGA, especially during the initial grades. PRP is a plasma concentrate reaped from the patient's whole blood that comprises predominantly platelets. Numerous growth factors (GFs) are present within platelet α granules. Some of the most important of these include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF). This GFs stimulate cell proliferation and differentiation. PRP was identified as having a beneficial effect on bone grafting with applications in oral and maxillofacial surgery, orthopedic and cardiac surgery. More recently, increasing interest is seen in the application of PRP in dermatology, for example, tissue regeneration, wound healing such and fat grafting. It has also been shown to promote hair survival and growth, both in vitro and in vivo. The production of autologous PRP involves extraction of a specific volume of the patient's whole blood, which is then placed in an automated centrifuge to separate the layers of whole blood by their specific weight into 3 separate layers: (1) platelet-poor plasma, (2) platelet-rich plasma and (3) red blood cells. The patients were divided into two groups (A and B): group A received treatment with PRP on the right half-head and the placebo on the left half-head, whereas group B received treatment with PRP on the left half-head and the placebo on the right half-head.
Study Type ICMJE	Interventional
Study Phase	*Not Provided*
Study Design ICMJE	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Double Blind (Subject, Investigator, Outcomes Assessor) Primary Purpose: Treatment
Condition ICMJE	• Androgenetic Alopecia • Alopecia • Hair Loss
Intervention ICMJE	Other: PRP/Saline PRP/Saline: Two groups were defined: group A and group B. Group A received treatment with PRP on the right half-head and placebo on the left half-head while group B received administration of PRP on left half-head and placebo on the right half-head. Same patient will be injected with PRP and a saline solution. Each patient will be injected on half-head. Other Names: • PRP • androgenetic alopecia • alopecia • hair Loss • placebo • saline
Study Arm (s)	Experimental: PRP/Saline

	PRP/Saline Briefly, for PRP preparation, approximately 18 mL of blood from each patient is drawn into a tube containing 3,8 % sodium citrate. The tubes were centrifuged at 450 g for 8 minutes, resulting in three basic layers: an erythrocyte layer at the bottom of the tube, a PRP layer in the middle, and a platelet-poor plasma (PPP) layer at the top of the tube. After removing the platelet-poor plasma (PPP) layer, the PRP is obtained, activated with 10 % calcium chloride. In the same patient, PRP will be injected to half-head and in the other half-head will be injected with saline solution (placebo). This study includes 4 visits: 3 visits (with 1-month interval) and 1 visit of follow-up (month 6). Intervention: Other: PRP/Saline
Publications *	*Not Provided*

* **Includes publications given by the data provider as well as publications identified by ClinicalTrials.gov Identifier (NCT Number) in Medline.**

Recruitment Information

Recruitment Status ICMJE	Active, not recruiting
Estimated Enrollment ICMJE	50
Estimated Completion Date	November 2015
Estimated Primary Completion Date	July 2015 (final data collection date for primary outcome measure)
Eligibility Criteria ICMJE	Inclusion Criteria: • Patients ≥18 years and < 65 years • Male patients with a clinical diagnosis of AGA (stage II to V according to the Hamilton-Norwood Scale) • Female patients with a clinical diagnosis of AGA (stage I to III according to Ludwig Classification) Exclusion Criteria: • Patients with other types of alopecia, other than AGA • Fasting < 3h prior of each injection • Use of nonsteroidal anti-inflammatory drugs one week before treatment. • Platelet count < 150 000 µL • Alterations of coagulation • Heavy smokers (> 20 cigarettes/day) • Medications: anticoagulants/ acetylsalicylic acid • Patient unable to accomplishing all fases of treatment
Gender	Both
Ages	18 Years to 65 Years (Adult)
Accepts Healthy Volunteers	No
Contacts ICMJE	*Contact information is only displayed when the study is recruiting subjects*
Listed Location Countries ICMJE	Spain
Removed Location Countries	

Administrative Information

NCT Number ICMJE	NCT02393040
Other Study ID Numbers ICMJE	UICatalunya
Has Data Monitoring Committee	No

Plan to Share Data	*Not Provided*
IPD Description	*Not Provided*
Responsible Party	**Rubina Alves**, Universitat Internacional de Catalunya
Study Sponsor ICMJE	Universitat Internacional de Catalunya
Collaborators ICMJE	*Not Provided*
Investigators ICMJE	Principal Investigator: **Rubina Alves**, M.D. — Specialist Dermatology, Universitat Internacional Catalunya Study Director: Ramon Grimalt, M.D.; PhD — Prof. Dermatology, Universitat Internacional Catalunya
Information Provided By	Universitat Internacional de Catalunya
Verification Date	March 2015

ICMJE **Data element required by the International Committee of Medical Journal Editors and the World Health Organization ICTRP**

13.3 INFORMED CONSENT

Información para el paciente

Título del estudio:
Evaluación de la eficacia del tratamiento con Factores de Crecimiento Plaquetarios, en pacientes con Alopecia Androgenetica.

Estimado(a) paciente:

Desearíamos solicitar su participación voluntaria en un estudio científico para la Evaluación de la eficacia del tratamiento con plasma rico en plaquetas con factores de crecimiento (PRP), en pacientes con diagnostico de Alopecia Androgenetica. Antes de decidirse a participar y de firmar el formulario de consentimiento informado, rogamos lea con atención esta información y nos formule las preguntas que le surjan al respecto.

El estudio formará parte de una tesis doctoral. Esta tiene por objeto mostrar la eficacia de un tratamiento complementar a los tratamientos convencionales para, en el futuro, poder ofrecer mejores resultados terapéuticos a los pacientes con diagnostico de Alopecia Androgenetica.

Para que los resultados sean significativos, es preciso contar con la participación del máximo número de pacientes.
Este procedimiento es seguro, porque el PRP y los factores de crecimiento utilizados son del sangre del propio paciente, es decir, autólogos. Los factores de crecimiento son específicos de cada individuo, lo que hace de este sistema un tratamiento personalizado, con muy bajo riesgo de reacciones adversas.

El tratamiento es totalmente compatible con otros tratamientos para la alopecia.

Dentro del estudio se pedirá a los participantes información sobre su calidad de vida, tolerancia e adherencia al tratamiento y se recabarán algunos datos médicos.

Los datos obtenidos se recogerán por escrito mediante una serie de preguntas realizadas pelo medico responsable.

PARTICIPACIÓN VOLUNTARIA:
La participación en este proyecto de investigación es voluntaria. En cualquier momento puede revocar su conformidad sin necesidad de dar explicaciones, momento en que se eliminarán todos sus datos. Dicha revocación no influirá en su asistencia médica.

PERSONA DE CONTACTO:
Para cualquier duda que le pueda surgir, diríjase a las siguientes personas de contacto:
Prof. Dr. Ramon Grimalt / Dra Rubina Alves

SECRETO MÉDICO/PROTECCIÓN DE DATOS:

Todas las personas que le prestan asistencia están sujetas a secreto médico y a la obligación de proteger sus datos.

Los resultados de la investigación realizada en el marco del estudio serán utilizados anónimamente en publicaciones científicas.

Siempre que sea necesario para la correcta recogida de datos, las personas con la oportuna autorización (p. ej. designadas por el cliente o la universidad) podrán consultar las partes de los informes médicos relevantes para el estudio.

En caso de que las personas autorizadas a acceder a dichos datos no estén sujetas a la obligación de secreto médico, los datos personales a los que tengan acceso en el marco del estudio estarán sujetos a secreto profesional y se tratarán como confidenciales.

....

Lugar y fecha (Nombre de la doctora informante)

CONSENTIMIENTO INFORMADO

Nombre del estudio: Evaluación de la eficacia del tratamiento con Factores de Crecimiento Plaquetarios, en pacientes con Alopecia Androgenetica.

Se nos ha informado debidamente sobre el contenido, el procedimiento, los riesgos, la realización de 4 puntos de tatuaje en el cuero cabelludo y el objetivo del proyecto de investigación arriba descrito, así como de la autorización para acceder a los datos recogidos.

He tenido la oportunidad de formular preguntas y he recibido respuesta a las mismas.

He tenido suficiente tiempo para decidir acerca de mi participación en el proyecto.

Accedo a participar en este proyecto de investigación.

.....

(Nombre de la paciente)

.....

(Firma de la paciente)

.....

Lugar y fecha

INFORMACIÓN Y CONSENTIMIENTO RELATIVO A LA PROTECCIÓN DE DATOS

En los estudios científicos se van a recabar datos y resultados médicos sobre mi persona. El almacenamiento, el análisis y la distribución de los datos relativos al estudio se realizarán de conformidad con las disposiciones legales y, antes de la participación en el estudio, requieren mi siguiente autorización voluntaria:

1. Acceso a que los datos personales/médicos recopilados sobre mí queden registrados en forma de cuestionarios o por medios electrónicos y a que se utilicen de forma anónima.

2. Asimismo, accedo a que una persona autorizada y sujeta a secreto profesional (p. ej. designada por el cliente o la universidad) tenga acceso a los datos personales recogidos sobre mí, siempre que esto sea necesario para la validación del proyecto. En lo que se refiere a estos datos, eximo al médico de su deber de secreto médico.

.....

(Nombre de la paciente)

.....

(Firma de la paciente)

.....

Lugar y fecha

13.4 EC CERTIFICATE OF CENTRIFUGE OMNIGRAFTER II

EC CERTIFICATE

J. P. Selecta SA
Ctra. A2 (Km 585, 1) s/n,
08630 Abrera, Spain

Full Quality Assurance System Approval Certificate

Annex II, section 3 of Council Directive 93/42/EEC concerning medical devices

Scope of Certificate:
Design, development and manufacturing of centrifuges for the separation and concentration of blood, bone marrow and adipose tissue

Device Classification:
Class IIa

Device Descriptions:
Centrifuges for the separation and concentration of blood, bone marrow and adipose tissue

Model Type:
Please refer to Attachment 1

File Number	A17917	Cycle Start Date	08 August 2011
Certificate No.	629.110808	Effective Date	08 August 2011
		Expiry Date	08 July 2014

Authorised by

Barry A. Fitch
Certification Manager
For and on Behalf of UL International (UK) Ltd

We hereby declare that an examination of the full quality assurance system has been carried out per report 11CA18207, following the requirements of the national legislation to which the undersigned is subject, transposing Annex II (with the exemption of section 4) of Council Directive 93/42/EEC on Medical Devices. We certify that the full quality assurance system conforms with the relevant provisions of the aforementioned directive and is subject to periodic surveillance as required by 93/42/EEC. Annex II, Section 5. For Class III devices where they are covered by this certificate, an EC Design Examination certificate according to 93/42/EEC, Annex II, Section 4 is required. This certificate is issued with 1 attachment listing model numbers and 0 addendums listing additional locations covered by this certificate.

Notified Body

0843

00-NB-F0413 1.0

UL International (UK) Limited
Wonersh House, The Guildway, Old Portsmouth Road,
Guildford, Surrey, GU3 1LR, United Kingdom

EC CERTIFICATE

J. P. Selecta SA

Ctra. A2 (Km 585, 1) s/n,
08630 Abrera, Spain

Attachment 1 of 1

The products detailed below are covered under the scope of this certificate

Product Family	Product Sub-Group	Model/Type	Classification
Centrifuges		Medigraft II	Class IIa
		Centro-8 8J	Class IIa
		Duografter II	Class IIa
		Omnigrafter II	Class IIa

File Number A17917
Certificate No. 629.110808

Cycle Start Date 08 August 2011
Effective Date 08 August 2011
Expiry Date 08 July 2014

Authorised by

Barry A. Fitch
Certification Manager
For and on Behalf of UL International (UK) Ltd

Notified Body
0843

00-NB-F0413 1.0

UL International (UK) Limited
Wonersh House, The Guildway, Old Portsmouth Road,
Guildford, Surrey, GU3 1LR, United Kingdom

13.5 EC PRODUCTION QUALITY CERTIFICATE

MINISTERIO DE SANIDAD Y POLÍTICA SOCIAL

agencia española de medicamentos y productos sanitarios

ORGANISMO NOTIFICADO Nº 0318

Cert. 93/42/2.2P- Rev.20/02/2003

Pág. 2 de 2

ANEXO Nº/*ANNEX NO:* I

CERTIFICADO CE DE GARANTÍA DE CALIDAD DE LA PRODUCCIÓN
de acuerdo con el Anexo V de la Directiva 93/42/CEE

EC PRODUCTION QUALITY ASSURANCE CERTIFICATE
in accordance with Annex V of Directive 93/42/EEC

PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: 14-06-2000
Fecha de la primera prórroga/*First extension date:* 9-06-2005

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*		ON nº/*NB no*
2000 06 0271 CP	Desde/*From* 24-05-2010	Hasta/*To* 23-05-2015	0318

2. Accesorios quirúrgicos/*Surgical accessories*

2.1. Cintas marcadoras/*Vessel loops* (GMDN 13828)

Tipo de producto/*Devices types*: Equipos para procesar fluidos corporales/*Body fluids processing sets.*

Clasificación/*Classification:* IIa

3. Equipos para procesar fluidos corporales/*Body fluids processing sets.*

3.1 Equipo para obtención de plasma por centrifugación DISPRAS y MESOPRAS (GMDN 15682)/*Plasma centrifugation sets DISPRAS and MESOPRAS (GMDN 16901)*

3.2. Equipo para obtención de concentrados de grasa por centrifugación LIPOPRAS/ *Fat concentration by centrifugation set LIPOPRAS (GMDN 16901)*

Tipo de producto/*Devices types*: Productos para ginecología y obstetricia/*Gynaecological and obstetrical devices*

Clasificación/*Classification:* IIa

4. Productos para ginecología y obstetricia/*Gynaecological and obstetrical devices.*

4.1. Lanceta amniótic/*Amniotic lancet.*

Este certificado ampara todas las marcas de estos productos incluidos por el fabricante en su declaración de conformidad.
This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity.

Madrid, 4 de junio de 2010

La Directora,
Cristina Avendaño Solá

13.6 EC CERTIFICATION OF SODIUM CITRATE

Istituto Superiore di Sanità

Organismo Notificato N° 0373 - Sezione Presso il Dipartimento AMPP
Notified Body N° 0373 – Unit relating to the Department AMPP

ATTESTATO DI CERTIFICAZIONE CE

secondo l'Allegato III della Direttiva Europea 93/42/CEE
(recepita con il Decreto Legislativo n. 46 del 24/2/1997)

CE CERTIFICATION DOCUMENT

according to Annex III of EC Directive 93/42/EEC
(enforced by the Decreto Legislativo n. 46 issued on 24/2/1997)

Allegato al certificato 103 CTP 413 09
(enclosed to certificate 103 CTP 413 09)

Il presente certificato è da considerarsi riferito ai seguenti prodotti:
This certificate refers to following products:

Nome prodotto ***(product name)***	***Codice*** ***(Code)***
Soluzione sterile glicerolizzante per congelamento emazie – Glicerolo 57 %	*XXXXYYYYZZZ*
Soluzione sterile deglicerolizzante per emazia scongelate – Sodio cloruro 1,6 %	
Soluzione sterile deglicerolizzante per emazia scongelate – Sodio cloruro12 %	
Soluzione sterile deglicerolizzante per emazia scongelate – Sodio cloruro0,9 % Glucosio 0,2 %	
Soluzione per la preparazione di plasma ricco di piastrine e di plasma ricco di fattori della crescita (Soluzione anticoagulante sterile – sodio citrato 3,8%; Soluzione ricalcificante sterile – calcio cloruro 3,68%)	
Soluzione eparinizzante sterile per il mantenimento della pervietà dei cateteri ed apparati tubolari (50 U.I./ml – 100 U.I./ml – 5.000 U.I./ml)	

Le lettere del codice di cui sopra hanno il seguente significato, come da criteri di codifica presentati dalla Ditta e conservati presso questo Organismo Notificato: *XXXX:* lettere che indicano la famiglia, il prodotto e il contenitore del dispositivo medico; *YYYY:* numeri che indicano la concentrazione e il volume del dispositivo medico; *ZZZ:* lettere che indicano il tipo di chiusura del dispositivo medico.

13.7 EC CERTIFICATION OF CALCIUM CHLORIDE

Istituto Superiore di Sanità

Organismo Notificato N° 0373 - Sezione Presso il Dipartimento AMPP
Notified Body N° 0373 – Unit relating to the Department AMPP

ATTESTATO DI CERTIFICAZIONE CE

secondo l'Allegato III della Direttiva Europea 93/42/CEE
(recepita con il Decreto Legislativo n. 46 del 24/2/1997)

CE CERTIFICATION DOCUMENT

according to Annex III of EC Directive 93/42/EEC
(enforced by the Decreto Legislativo n. 46 issued on 24/2/1997)

Allegato al certificato 103 CTP 413 09 addendum 01 09
(enclosed to certificate 103 CTP 413 09 addendum 01 09)

Il presente certificato è da considerarsi riferito ai seguenti prodotti:
This certificate refers to following products:

Nome prodotto ***(product name)***	***Codice*** ***(Code)***
Soluzione per la preparazione di plasma ricco di piastrine e di plasma ricco di fattori della crescita (Soluzione attivante sterile – calcio cloruro 10%; Soluzione attivante sterile – calcio cloruro 0,368% e Alcool etilico 66%)	*XXXXYYYYZZZ*
Soluzione eparinizzante sterile per il mantenimento della pervietà dei cateteri ed apparati tubolari (5 U.I./ml – 10000 U.I./ml)	

Le lettere del codice di cui sopra hanno il seguente significato, come da criteri di codifica presentati dalla Ditta e conservati presso questo Organismo Notificato: *XXXX:* lettere che indicano la famiglia, il prodotto e il contenitore del dispositivo medico; *YYYY:* numeri che indicano la concentrazione e il volume del dispositivo medico; *ZZZ:* lettere che indicano il tipo di chiusura del dispositivo medico.

Mod.09.03/ Rev.0 Il presente certificato è costituito di 2 pag. Questa è la pagina 2. / *This certificate consists of* 2 *pag. This is page* 2

13.8 INCLUSION QUESTIONNAIRE

A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of

Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

INCLUSION QUESTIONNAIRE (Part 1)

PATIENT No.: _____

PHONE: _____

DATE OF CONSULTATION: ___ / ___ / ___

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

- Male patients with clinical diagnosis of AGA and with stage II to V, according to the Hamilton-Norwood Classification.
- Female patients with clinical diagnosis of AGA and with stage I to III, according to Ludwig Classification.

Exclusion criteria:

- Patients with other types of alopecia
- Patients that do not maintain the same treatment options, previously prescribed by the dermatologist, during all the protocol.
- Use of nonsteroidal anti-inflammatory drugs one week before treatment.
- Platelet count < 150 000 µL
- Thrombocytopenia
- Alterations of coagulation
- Heavy smokers (> 20 cigarettes/day)
- Medications: anticoagulants/ acetylsalicylic acid
- Patient unable to accomplishing all fases of treatment
- Pregnancy and breastfeeding

1. SOCIODEMOGRAPHIC DATA

Gender: ☐ F ☐ M

Age: |__|__| years

2. MEDICAL HISTORY

Does the patient have been diagnosed as suffering from any disease? ☐ Yes ☐ No

if Yes, specify _____

Does the patient have any history of allergies? ☐ Yes ☐ No

if Yes, specify _____

Does the patient have been submitted to any surgery? ☐ Yes ☐ No

if Yes, specify _____

Does the patient smoke? ☐ Yes ☐ No

if Yes, specify quantity/day _____

Does the patient have any alteration on the analytic? ☐ Yes ☐ No

If Yes, specify_____

Does the patient take any medication? ☐ Yes ☐ No

If Yes, specify _____

_____

_____

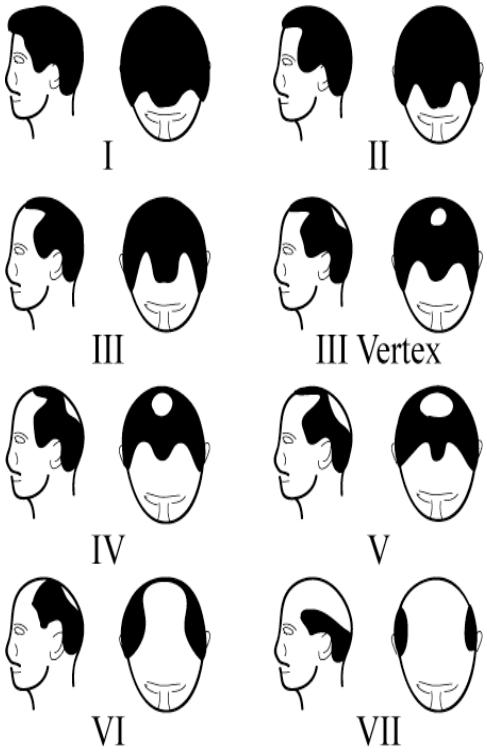
_____

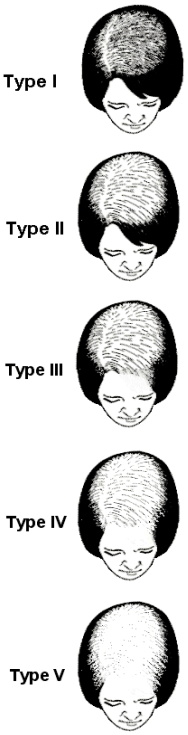
_____

3. ANDROGENIC ALOPECIA (AGA) HISTORY AND DESCRIPTION

How old was your patient when his AGA started? |__|__| years

Localization and Stage: Please, indicate the stage of AGA corresponding to your patient according to the Norwood-Hamilton classification below: |__|__| or to the Ebling's female pattern baldness |__|__|





Does your patient have family history of AGA? ☐ Yes ☐ No

if Yes, specify _____

4. CURRENT MEDICAL CARE FOR AGA

Does your patient take any oral treatment for his alopecia? ☐ Yes ☐ No

If yes, please specify_____

_____

Does your patient use a specific capillary solution for his alopecia? ☐ Yes ☐ No

If yes, please specify_____

_____

Does your patient take dietary supplements for his alopecia? ☐ Yes ☐ No

If yes, please specify_____

_____

4. PAST MEDICAL THERAPY FOR AGA

Did your patient made a treatment for his/her alopecia? ☐ Yes ☐ No

if Yes, please complete:

Name Treatment_____ **Duration (months)** |__|__| **Discontinuation date** __/__/__

Reason for Stop ☐ Inefficacy

☐ Observance

☐ Other

Name Treatment_____ **Duration (months)** |__|__| **Discontinuation date** __/__/__

Reason for Stop ☐ Inefficacy

☐ Observance

☐ Other

Name Treatment_____ **Duration (months)** |__|__| **Discontinuation date** __/__/__

Reason for Stop ☐ Inefficacy

☐ Observance

☐ Other

5. STANDARDIZED IMAGES (PHOTOS)

Please take standardized images of the 3 areas mentioned below:

☐ Vertex ☐ Frontal ☐ Temporal

These images will be compared with those taken at the next visit.

6. STANDARDIZED TRICHOSCAN:

Please take standardized images of the 3 areas mentioned below:

☐ Vertex ☐ Frontal ☐ Temporal

These images will be compared with those taken at the next visit.

7. INFILTRATION PRP:

☐ Vertex ☐ Frontal ☐ Temporal

☐ Left ☐ Right

8. NOTES

_____

_____

A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of

Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

INCLUSION QUESTIONNAIRE

VISIT Nº 1 (Part 2)

BASELINE

VISIT Nº _____ / _____

NAME: _____

PATIENT No.: _____

PHONE: _____

DATE OF CONSULTATION: ___ / ___ / ___

1. STANDARDIZED TRICHOSCAN:

Please take standardized images of the 4 areas mentioned below:

☐ Frontal right ☐ Frontal left

☐ Vertex right ☐ Vertex left

These images will be compared with those taken at the next visit.

2. INFILTRATION PRP:

☐ Frontal right ☐ Frontal left SIDE: _____

☐ Vertex right ☐ Vertex left

_____

3. INFILTRATION SALINE SOLUTION:

☐ Frontal right ☐ Frontal left

☐ Vertex right ☐ Vertex left

4. NOTES:

_____

_____

_____

_____

13.9 QUESTIONNAIRE VISIT 2 AND VISIT 3

A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of

Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

FOLLOW-UP QUESTIONNAIRE

Visit nº 2 (Part 1)

VISIT Nº _____ / _____

NAME: _____

PATIENT No.: _____

DATE OF CONSULTATION: ___ / ___ / ____

DATE OF INCLUSION: ___ / ___ / _____

_____

1. INCLUSION AND EXCLUSION CRITERIA?

Are there any alterations of the inclusion or exclusion criterial? ☐ Yes ☐ No

if Yes, please specify _____

Are there any changes in medical care of AGA since the inclusion? ☐ Yes ☐ No

if Yes, please specify _____

Did your patient experience any adverse effect since the beginning of the treatment? ☐ Yes ☐ No

if Yes, please specify _____

2. PATIENT FEELING ABOUT GLOBAL IMPROVEMENT

How was the overall tolerance of the treatment?

☐ Very good

☐ Good

☐ Average

☐ Poor

Is the patient satisfied with the effectiveness of the treatment?

☐ Very satisfied

☐ Fairly satisfied

☐ Not very satisfied

☐ Not at all satisfied

Does the patient noted any improvement or not with the treatment?

☐ Much improved

☐ Moderally improved

☐ Minimally improved

☐ No change

☐ Minimally worse

☐ Moderally worse

☐ Much worse

3. PHYSICIAN FEELING ABOUT GLOBAL IMPROVEMENT

Are you satisfied with the effectiveness of the treatment?

☐ Very satisfied

☐ Fairly satisfied

☐ No change

☐ Not very satisfied

☐ Not at all satisfied

Did you noted any improvement or not with the treatment?

☐ Much improved

☐ Minimally improved

☐ No change

☐ Minimally worse

☐ Much worse

4. STANDARDIZED IMAGES (SCALP PHOTOS) and PHOTOGRAPHIC EVALUATION

☐ Vertex ☐ Frontal ☐ Temporal

These images will be compared with those taken at the previous visit.

☐ Greatly increased
☐ Moderately increased
☐ Slightly increased
☐ No change
☐ Slightly decreased
☐ Moderately decreased
☐ Greatly decreased

5. PERFORM 4 CLIPING AREAS:

☐ Frontal right ☐ Frontal left

☐ Vertex right ☐ Vertex left

A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of

Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

FOLLOW-UP QUESTIONNAIRE

Visit nº 2 (Part 2)

VISIT Nº _____ / _____

NAME: _____

PATIENT No.: _____

DATE OF CONSULTATION: ___ / ___ / ____

DATE OF INCLUSION: ___ / ___ / _____

_____

1. STANDARDIZED TRICHOSCAN:

Please take standardized images of the 4 areas mentioned below:

☐ Frontal right ☐ Frontal left

☐ Vertex right ☐ Vertex left

These images will be compared with those taken at the previous visit.

☐ Greatly increased
☐ Moderately increased
☐ Slightly increased
☐ No change
☐ Slightly decreased
☐ Moderately decreased
☐ Greatly decreased

2. INFILTRATION PRP:

☐ Frontal right ☐ Frontal left SIDE: _____

☐ Vertex right ☐ Vertex left

3. INFILTRATION SALINE SOLUTION:

☐ Frontal right ☐ Frontal left SIDE: _____

☐ Vertex right ☐ Vertex left

4. NOTES:

_____

_____

_____

_____

13.10 FOLLOW-UP QUESTIONNAIRE

A Prospective Double Blind, Placebo Controlled Study to Assess the Efficacy of

Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

FOLLOW-UP QUESTIONNAIRE

VISIT Nº _____

NAME: _____

PATIENT No.: _____

DATE OF CONSULTATION: ___ / ___ / ____

DATE OF INCLUSION: ___ / ___ / _____

_____

1. INCLUSION AND EXCLUSION CRITERIA?

Are there any alterations of the inclusion or exclusion criterial? ☐ Yes ☐ No

if Yes, please specify _____

Are there any changes in medical care of AGA since the inclusion? ☐ Yes ☐ No

if Yes, please specify _____

Did your patient experience any adverse effect since the beginning of the treatment? ☐ Yes ☐ No

if Yes, please specify _____

2. PATIENT FEELING ABOUT GLOBAL IMPROVEMENT

How was the overall tolerance of the treatment?

☐ Very good

☐ Good

☐ Average

☐ Poor

Is the patient satisfied with the effectiveness of the treatment?

☐ Very satisfied

☐ Fairly satisfied

☐ Not very satisfied

☐ Not at all satisfied

Does the patient noted any improvement or not with the treatment?

☐ Much improved

☐ Moderally improved

☐ Minimally improved

☐ No change

☐ Minimally worse

☐ Moderally worse

☐ Much worse

3. PHYSICIAN FEELING ABOUT GLOBAL IMPROVEMENT

Are you satisfied with the effectiveness of the treatment?

☐ Very satisfied

☐ Fairly satisfied

☐ Not very satisfied

☐ Not at all satisfied

Did you noted any improvement or not with the treatment?

☐ Much improved

☐ Minimally improved

☐ No change

☐ Minimally worse

☐ Much worse

4. PLEASE TAKE STANDARDIZED IMAGES and PHOTOGRAPHIC EVALUATION

In order to evaluate the effectiveness of the treatment on AGA, please take standardized images of the 3 areas mentioned below:

☐ Vertex ☐ Frontal ☐ Temporal

These images will be compared with those taken at the previous visit.

☐ Greatly increased
☐ Moderately increased
☐ Slightly increased
☐ No change
☐ Slightly decreased
☐ Moderately decreased
☐ Greatly decreased

5. PLEASE TAKE STANDARDIZED IMAGES WITH TRICHOSCAN:

In order to evaluate the effectiveness of the treatment on AGA, please take standardized images of the 3 areas mentioned below:

☐ Vertex ☐ Frontal ☐ Temporal

Please check you have answered EVERY question. Thank you.

13.11 ARTICLE 1

Randomized Placebo-Controlled, Double-Blind, Half-Head Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

Rubina Alves, MD and Ramon Grimalt, MD, PhD

BACKGROUND Platelet-rich plasma (PRP) was identified as having a beneficial effect in alopecia and has been postulated as a new therapy for androgenetic alopecia (AGA).

OBJECTIVE To assess the efficacy of PRP for the treatment of AGA.

MATERIALS AND METHODS This was a randomized, placebo-controlled, double-blind study in 25 patients with AGA. Platelet-rich plasma was injected in half-head and the other half-head with placebo. Each patient received a total of 3 treatments of PRP, 1 month apart.

RESULTS Six months after the first treatment with PRP, significant differences were seen in mean anagen hairs (67.6 ± 13.1), telogen hairs (32.4 ± 13.1), hair density (179.9 ± 62.7), and terminal hair density (165.8 ± 56.8) when compared with baseline ($p < .05$). Platelet-rich plasma was also found to increase hair density when comparing with the control side ($p < .05$). For the first time, the authors found a correlation between anagen hairs and patients >40 years and beginning of AGA ≥25 years old ($p < .05$) and hair density and male sex, age ≤40 years, positive family history of AGA and >10 years of duration of the disease ($p < 0.05$).

CONCLUSION Application of PRP showed a positive effect on AGA and could be regarded as an adjuvant therapy for AGA.

The authors have indicated no significant interest with commercial supporters.

Androgenetic alopecia (AGA) is a nonscarring progressive miniaturization of the hair follicle with a typically characteristic pattern distribution in genetically predisposed men and women.[1,2] Until now, topical minoxidil and oral finasteride are the only approved drugs that Food and Drug Administration has for the treatment of AGA. Currently available treatments are at times perceived as having limited effectiveness, and finding new therapies for this pathology is therefore of utmost importance.[3-6]

Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma that contains a platelet concentration above basal concentration (150,000–350,000/L).[7,8] When platelet-alpha granules become activated, they release numerous growth factors (GFs), such as platelet-derived growth factor, transforming growth factor–beta (TGF-β), vascular endothelial growth factor, epidermal growth factor, and insulin-like growth factor.[7-13] These GFs seem to stimulate cell proliferation and differentiation.[10,14-16]

Platelet-rich plasma was identified as having a beneficial effect on bone grafting with applications in oral and maxillofacial surgery, and orthopedic and cardiac surgery.[9,10,17] More recently, interest has been increasing in the application of PRP in dermatology, for example, in tissue regeneration, wound healing, fat grafting, and skin rejuvenating effects.[7,18-22] Recently, PRP has been postulated as a new therapy for AGA.

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In 2006, Uebel and colleagues[14] reported a new application of PRP for male pattern baldness surgery. This study showed that treatment of follicular units with PRP before transplantation resulted in improved hair growth and density. Since then, several studies[3,4,7,10,14,23-26] have been conducted worldwide to investigate the role of PRP for the treatment of hair loss, with a limited number of articles on PRP and AGA.

Despite these previous studies, the precise mechanism by which PRP promotes hair growth has not been properly studied.[7] The level of evidence of various studies in AGA is from low to medium,[27] and defined double-blind trials or split-face trials will definitely increase the confidence of clinicians with this procedure.[28]

Regarding the limited available literature, the authors performed a prospective randomized, double-blind placebo-controlled, half-head study on 25 patients with AGA, including 13 women. The main objective of this study was to evaluate the efficacy of treatment with PRP on AGA, between 3 months, 6 months, and baseline and to determine whether PRP could be considered a new effective treatment option for AGA.

Materials and Methods

Study Design

This study was a randomized, double-blind, placebo-controlled, half-head, parallel-group study on patients with AGA. After having received approval from the Ethical Committee of CEIC-UIC, the clinical protocol for the treatment of AGA was started in a private clinic linked to the Universitat Internacional Catalunya. All patients provided written informed consent before participating in the study, which was performed according to the Declaration of Helsinki.

Participants

Participants were admitted in the study between January 2014 and November 2014. Men aged 18 to 65 years with Hamilton–Norwood Patterns II to V and women aged 18 to 65 years with Stage I to III according to Ludwig classification, who were otherwise healthy, were eligible for inclusion in this study. The exclusion criteria were as follows: use of any topical medication (such as minoxidil or any other solution for hair growth), oral medication (finasteride, dutasteride, or antiandrogens), laser therapy, or chemotherapy, within the preceding 12 months; bleeding disorders, platelet dysfunction syndrome, platelet counts <150,000 platelets per microliter (μL); anticoagulant therapy or nonsteroidal anti-inflammatory drugs in the last 2 weeks; smokers (>20 cigarettes/day); and pregnancy or lactation. Patients were also excluded from the study if they had any chronic active scalp condition other than AGA or a history of hair transplants.

Interventions

A complete blood cell count was performed in all patients at baseline. Only patients whose blood test had all parameters within the respective reference ranges were included in the study.

Eligible subjects were randomized in a ratio of 1:1 to receive a half-head treatment with PRP and the other half-head with placebo (saline solution). The patients were divided into 2 groups (A and B): Group A received treatment with PRP on the right half head and the placebo on the left half head, whereas Group B received treatment with PRP on the left half head and the placebo on the right half head (Fig.1).

Briefly, to prepare PRP, 18 mL of peripheral blood was transferred to a tube with 2 mL of 3.8% sodium citrate.

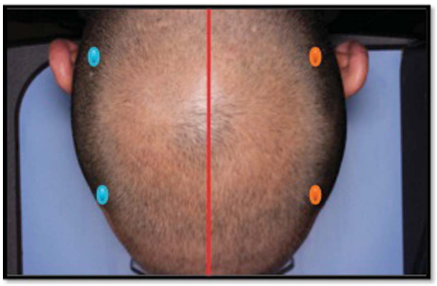

Figure 1. Two identical circular areas of 1 × 1 cm (1 frontal and 1 occipital) in both the treatment and control half heads were defined for TrichoScan analysis. The patient received treatment with PRP on the right half head and the placebo on the left half head (Group A).

The 20 mL citrated blood was centrifuged at 460*g* for 8 minutes (Omnigrafter-Proteal, Barcelona, Spain). The centrifugation process separated blood components owing to their different specific densities: an erythrocyte layer at the bottom of the tube, a PRP layer in the middle, and a platelet-poor plasma (PPP) layer at the top of the tube. Approximately 3/4 of the supernatant is discarded (PPP), and the resulting suspension is used as PRP (3 mL). The PRP obtained is a pure PRP (leukocyte poor), and the platelet count was on average approximately 3 times higher than in whole blood. The PRP fraction was separated and activated with 0.15 mL of 10% calcium chloride immediately before application. The PRP was injected on 4 selected areas of the scalp (marked with a dot tattoo) at the amount of 0.15 mL/cm^2, using a 30-G needle. Local anesthesia was not used. No adverse events were recorded in this study, apart from local injection pain.

As a control, 3 mL of saline was injected into the opposing side of the experimental side. Because the characteristics and color of PRP are different from the placebo, the injecting physician is not blinded to the treatment modalities. The other physician, who is not aware of the side of the treatment, was responsible for the analyses of the data obtained and the evaluation of the treatment efficacy. The subjects remained blinded to the treatment (PRP or placebo) until the end of the study.

At the end of the study, all patients received 3 treatments of PRP on the previously nontreated half head (placebo area).

Assessment Criteria

All patients were evaluated in 4 visits: V1, baseline and beginning of the study; V2, second treatment; V3, third treatment; and V4, follow-up. In the first 3 visits, a total of 3 treatments were given with an interval of 1 month from each other. The follow-up visit was the last visit, at 6 months to assess the efficacy of treatment compared with baseline. At baseline, 2 circular areas (1 frontal and 1 occipital) in both treatment and control half heads (4 circular areas) were defined and marked centrally with a red permanent tattoo. A dot tattoo guaranties the analysis of the same area to ensure the reproducibility of the study. The target areas were set symmetrically and according to the hair density. The results were compared on similar locations: the right frontal area was compared with the left frontal area and the right posterior area was compared with the left posterior area.

The evaluation criteria were assessed in all patients by global photography and phototrichogram. The evaluator responsible for both global photographs and phototrichogram analyses was blinded with regard to the treatment and placebo areas and was not involved in the administration of treatment.

Global photographs of 3 areas of the scalp (vertex, frontal, and occipital) were performed using a medical photography system and software (Canon Canfield Orthostatic Device; OMNIA Digital Imaging System, Fairfield, NJ) to ensure that all patients were photographed consistently.

Phototrichograms were performed in all patients with the help of epiluminescence microscopy with digital image analysis (FotoFinder; TrichoScan Professional Version). Clippings of the 4 target areas were performed 48 hours before the phototrichograms were obtained. Using the TrichoScan software, all protocol-conformed pictures were analyzed to determine the treatment efficacy by measuring hair count (number of hairs/0.65 cm^2), hair density, terminal hair density, anagen (%), telogen (%), and the anagen/telogen ratio.

Statistical Analyses

Statistical analyses were performed using the mixed procedure SAS 9.2 (SAS Institute Inc., Cary, NC). Values of $p < .05$ were regarded to be statistically significant. Data are presented as the mean ± SDs.

Results

Twelve men and 13 women with AGA were admitted in this study, of a total of 25 patients. Twenty-two patients (11 male and 11 women) completed the entire study; 3 patients were lost to follow-up. The mean age of enrolled subjects ($n = 22$) was 39 years (age range: 21–62 years). The blood cell count performed in all patients at baseline had a mean platelet counting of 1.523.82 ± 35,000 platelets/μL.

The hair growth parameters were measured after 3 months and 6 months and compared with the baseline (before treatment) and between treatment and control areas (placebo). At baseline, there were no significant differences in hair count, hair density, terminal density, and anagen or telogen hairs between the treatment and control areas of the scalp. Mean hair growth parameters for the treatment and control areas are shown in Table 1.

The results of this study revealed that the administration of PRP led to a statistically significant increase in the mean anagen hairs, telogen hairs, hair density, and terminal hair density after 3 months and after 6 months when compared with baseline (Fig.2)

Regarding the mean total hair density, the increase on the treated side was also found to be significant compared with that on the control side. The mean total hair density for the treatment area after 3 months showed a mean increase of 14.8 ± 32.1 hairs/cm^2 compared with baseline, whereas the control area showed a mean decrease of 0.7 ± 32.7 hairs/cm^2 (control vs treatment, $p < .05$). After 6 months, the treatment area with PRP had a mean increase of 12.8 ± 32.6 hairs/cm^2 and the control area a decrease of 2.1 ± 31.3 hairs/cm^2 (control vs treatment, $p < .05$).

With respect to the number of total hair count, although there were no significant differences between the PRP-treated area and the placebo area, PRP exhibited a slight increase in number of hairs compared with the control group.

No differences in vellus hair density between the PRP and placebo areas were observed. In this study, an

TABLE 1. Relevant Hair Growth Parameters for the Half-Head Areas Treated With PRP and Placebo at Baseline, 3 Months, and 6 Months

	Placebo		*PRP Treatment*		*Placebo vs PRP*
	Mean ± SD	*p*	*Mean ± SD*	*p*	*p*
Anagen hair (%)					
Baseline	62.1 ± 17.4	>.05	62.1 ± 16.1	>.05	>.05
3 months	63.5 ± 18.9	>.05	67.9 ± 13.8	**<.05**	>.05
6 months	66.3 ± 15.9	**<.05**	67.6 ± 13.1	**<.05**	>.05
Telogen hair (%)					
Baseline	37.9 ± 17.4	>.05	37.9 ± 16.1	>.05	>.05
3 months	34.5 ± 16.5	>.05	35.8 ± 14.4	**<.05**	>.05
6 months	33.7 ± 15.9	**<.05**	32.4 ± 13.1	**<.05**	>.05
Anagen/telogen ratio (%)					
Baseline	137.5 ± 209.5	>.05	128.4 ± 174.8	>.05	>.05
3 months	159.1 ± 210.1	>.05	185.7 ± 199.9	**<.05**	>.05
6 months	148.2 ± 173.1	>.05	156.0 ± 164.3	**<.05**	>.05
Hair density (1/cm^2)					
Baseline	167.8 ± 51.2	>.05	167.1 ± 55.6	>.05	>.05
3 months	167.1 ± 51.3	>.05	181.9 ± 63.6	**<.05**	**<.05**
6 months	165.7 ± 55.2	>.05	179.9 ± 62.7	**<.05**	**<.05**
Terminal hair density (1/cm^2)					
Baseline	160 ± 48.9	>.05	159.9 ± 55.1	>.05	>.05
3 months	161.9 ± 52.2	>.05	168.2 ± 60.7	>.05	>.05
6 months	161.9 ± 52.2	>.05	165.8 ± 56.8	**<.05**	>.05
Hair count (hairs/0.65 cm^2)					
Baseline	111 ± 33.9	>.05	110.8 ± 37.6	>.05	>.05
3 months	112 ± 35.7	>.05	115 ± 41.8	>.05	>.05
6 months	112.7 ± 34.9	>.05	113.2 ± 39.4	>.05	>.05

Data assessed by TrichoScan Analysis.
Bold values indicate statistical significance $p < 0.05$.

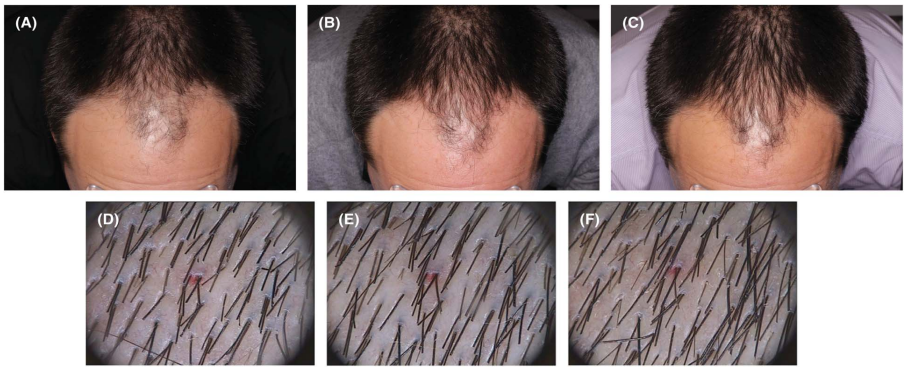

Figure 2. A 34-year-old man with AGA treated with PRP on the right half head and placebo on the left half head. Global photographs of the scalp performed at baseline (A), 3 months (B), and 6 months (C). Phototrichograms (20-fold magnification) performed at baseline (D), 3 months (E), and 6 months (F).

analysis of each hair growth parameter and subject demographic characteristics was performed (Table 2).

In support of the data obtained, treatment with PRP showed a statistically significantly correlation of the mean total hair density between men, patients aged ≤40 years, beginning of hair loss ≥25 years, positive family history, and >10 years of evolution of AGA, when compared with the placebo. This study also found a correlation in the areas treated with PRP between anagen hairs (%), and patients aged more than 40 years and beginning of AGA ≥25 years, at 6 months. The other parameters analyzed did not show statistical differences.

Discussion

In this double-blind, half-head, and placebo-controlled study, the authors have demonstrated that the administration of PRP attended a statistically significant improvement of mean anagen hairs, telogen hairs, hair density, and terminal hair density at 3 months and 6 months, when compared with baseline. However, when comparing to the control side, the hair on the PRP-treated side showed a statistically significant increase in mean total hair density only. The underlying mechanism of how administration of PRP induces a positive effect on AGA is not clear.

Li and colleagues[7] studied the effect of PRP on hair growth using in vitro and in vivo (mice) models. They proposed that the injection of mice with activated PRP induced a faster telogen-to-anagen transition than was seen in the control mice. Anagen-associated angiogenesis has been suggested to be one of the most important factors in active hair growth.[26] This study provides further support that pure PRP may prolong the anagen phase of the hair cycle, as the authors found a superior anagen/telogen ratio (%) in the areas treated with PRP than the areas treated with the placebo, when compared with baseline.

In a study performed by Lopez and colleagues,[29] patients treated with PRP showed a significant correlation between male sex and the hair density. As in the latter article, beside this correlation, in this study, the authors were also able to demonstrate for the first time a statistical significant association between hair density and patients below 40 years with positive family history of AGA and more than 10 years of duration of the disease. In addition, to the best of the authors' knowledge, this study is also the first in which there is a correlation between anagen hairs and patients older than 40 years and beginning of AGA with age superior to 25 years.

This study has the limitation of having a follow-up of 6 months. Furthermore, a follow-up period of 6 months is short to draw final conclusions about the benefits of treatment with PRP over time. However, in this study, a single patient played the role of both treated and

TABLE 2. Analysis of Hair Growth Parameters (Hair Density and Anagen Hairs) and Subject Demographic Characteristics

	Placebo	*PRP*	*Placebo vs PRP*
Mean Total	*Median ± SD*	*Median ± SD*	*p*
Hair density (1/cm²)			
Women	2.7 ± 30.7	11.1 ± 29.6	>.05
Men	0.8 ± 31.6	14.3 ± 36.2	**<.05**
Age ≤40 years	4.1 ± 31.5	9.4 ± 35.5	**<.05**
Age >40 years	7.5 ± 30.0	17.6 ± 29.0	>.05
Beginning of AGA <25 years	5.1 ± 29.3	1.9 ± 27.2	>.05
Beginning of AGA ≥25 years	10.6 ± 32.7	30.4 ± 34.3	**<.05**
Family history, +	1.9 ± 31.9	14.7 ± 34.1	**<.05**
Family history, −	2.9 ± 29.0	5.2 ± 29.4	>.05
Disease duration ≤10 years	5.8 ± 38.6	16.4 ± 33.2	>.05
Disease duration >10 years	3.2 ± 23.0	9.7 ± 32.8	**<.05**
Anagen hair (%)			
Women	1.8 ± 15.5	3.3 ± 11.3	>.05
Men	4.1 ± 12.9	5.5 ± 11.1	>.05
Age ≤40 years	5.1 ± 14.8	4.4 ± 11.0	>.05
Age >40 years	0.8 ± 13.6	4.5 ± 11.3	**<.05**
Beginning of AGA <25 years	6.0 ± 14.4	4.9 ± 9.9	>.05
Beginning of AGA ≥25 years	1.9 ± 13.8	3.9 ± 13.0	**<.05**
Family history, +	4.3 ± 12.7	5.2 ± 11.5	>.05
Family history, −	2.9 ± 29.0	5.2 ± 29.4	>.05
Disease duration ≤10 years	2.6 ± 14.4	4.5 ± 11.8	>.05
Disease duration >10 years	3.4 ± 14.2	4.4 ± 10.9	>.05

Bold values indicate statistical significance $p < 0.05$.

controlled subject. This has advantages because it could correct the possible biases such as gender and grade of hair loss that could affect the results.

Data analyzed from different studies[7,10,14,25,29-33] demonstrated an increase in number of hairs and/or hair density in patients treated with PRP. Most studies are open label and not blinded, making it difficult to reproduce the results and perform comparisons between them.[22] Also, there is a large heterogeneity in PRP preparation with many devices available, which makes more difficult the interpretation of the results. Some studies also included modifications or other types of f PRP, such as dalteparin/protamine[10] and leukocyte PRP.[4]

Although the literature about PRPs developed with all these contradictions, the need for standardized terminology is of maximum importance.[34,35] Thus, some classifications have been proposed to achieve a consensus terminology in the field of platelet concentrates.[36-39] Characterizing the type of PRP used (as a pure PRP, in this study) will lead to a better understanding of PRP, and data available will be easier to sort and interpret. Also, this terminology would serve as a basis for further research on the topic.

Conclusions

This clinical research provides support that the application of PRP may have a therapeutic effect on AGA and can be used as a safe complementary treatment option. However, more controlled and well-designed clinical trials should be conducted to confirm the clinical improvement of AGA with administration of PRP.

Furthermore, other clinical trial that includes a larger sample of patients with AGA, simultaneously treated with PRP and other topical and/or oral medications for hair growth, would also help define the efficacy of PRP as an adjuvant treatment of AGA.

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13.12 COMMENTARY ON ARTICLE 1

COMMENTARY

Commentary on a Randomized Placebo-Controlled, Double-Blind, Half-Head Study to Assess the Efficacy of Platelet-Rich Plasma on the Treatment of Androgenetic Alopecia

Regenerative medicine is gaining increasing popularity in many fields of dermatology, including hair loss, and platelet-rich plasma (PRP) therapy has gained notoriety worldwide partly because it is heavily covered in the media and marketed by doctors. But, is PRP an effective treatment of androgenetic alopecia (AGA) and hair loss in general?

In this study, Alves and Grimalt[1] present the effects of PRP versus placebo in a half-head paired comparison study of 22 patients with AGA. Patients received a total of 3 treatments at monthly intervals to 2 symmetrical areas on each side of the scalp with injections of either saline or activated PRP (3× concentration). Phototrichogram assessments were obtained at baseline, 3, and 6 months. Compared to baseline, PRP resulted in a significant increase in total hair density and terminal hair density at 6 months; however, only terminal hair density was found to be significantly increased (just below 10%) when PRP was compared with placebo. The authors also analyze the data based on demographics and demonstrate a significant therapeutic response in hair density versus placebo in men, patients ≤40 years of age, onset of AGA ≥ the age of 25, positive family history, and AGA duration of greater than 10 years. This study is noteworthy as it included equal numbers of men and women, and it used a half-head design comparing symmetrical left and right target areas, which were tattooed for accurate localization of blinded phototrichogram assessments. The demographic analysis is interesting, however, limited by small sample size and seems to indicate that women in this study group did not exhibit a statistically significant improvement with PRP as compared with placebo.

Platelet-rich plasma therapy for hair loss consists of injecting concentrated autologous platelets into the scalp, which on activation release growth factors and cytokines such as PDGF, TGF, VEGF, IGF, EGF, and IL-1. This treatment application stems from a clinical study showing enhanced growth of transplanted follicular unit hair grafts.[2] Additionally, in vitro evidence supports a role for PRP in promoting hair growth as PRP applied to dermal papilla cells stimulates their proliferation and upregulates components of the Wnt pathway.[3] The hair promoting effects of PRP are likewise supported by in vivo animal studies demonstrating a faster telogen to anagen transition in mice injected with PRP[3] and also an increase in the number of newly formed follicles and earlier hair formation in grafted murine epidermal and dermal papilla cells exposed to human PRP.[4]

The above findings have sparked interest in evaluating the possible benefits of PRP as a treatment option in patients with AGA. In a study of 26 men and women with pattern hair loss, PRP therapy increased target hair counts and also increased hair shaft diameter when combined with carrier molecules for controlled release of growth factors.[5] Target areas were localized by landmarks rather than a tattoo, which should be taken into account when interpreting these results. The largest clinical study of PRP in AGA consisted of a single treatment arm of 64 patients evaluated by global photographic assessment with 2 evaluators observing a "clinically important difference" in 40.6% and 54.7% of the subjects at 6 months.[6] In a recent randomized controlled study of 20 males with AGA, PRP injections resulted in a significant improvement in mean hair counts and hair density at 3 months as compared with baseline and placebo.[7]

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COMMENTARY

Microscopic assessment showed an increase in number of follicles as well Ki67+ basal keratinocytes and hair follicle bulge cells. Platelet-rich plasma and placebo treatment areas varied anatomically based on individual AGA patterns rather than being standardized to the left and right symmetrical target sites.

Furthermore, well-controlled studies are needed to evaluate PRP as a therapy for not only AGA but also other hair loss conditions such as acute or chronic telogen effluvium, alopecia areata, and scarring alopecias. To accurately assess therapeutic efficacy, study protocols should standardize the method of PRP preparation as numerous products are currently available, which differ in the volume of blood processed, centrifugation system, level of platelet concentration, yield of PRP volume, and type of activation used. In addition, studies are needed to determine the ideal platelet concentration, appropriate volume of PRP per injection and proper depth, the distance between injection sites, and also optimal treatment intervals not only to achieve initial results but also to maintain a therapeutic response. Considering the modest degree of efficacy that has been demonstrated in various studies of PRP for hair loss, this treatment modality may be useful not only as monotherapy but also as an adjunct to achieve additive or synergistic effects when combined with other established therapies. Alternatively, the effects of PRP may be optimized by the addition of other biological constituents such as an extracellular matrix or autologous stem cells.

Platelet-rich plasma represents an exciting possibility as an addition to the current hair loss treatment armamentarium.[8] Although current protocols focus on platelets themselves, it is likely that platelet products such as cytokines and growth factors are exclusively needed for achieving efficacy. Following this principle, emerging therapies may consist of preparations containing only purified bioactive molecules.

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13.13 ARTICLE 2

Dermatologic Surgery

Platelet-rich plasma in combination with 5% minoxidil topical solution and 1 mg oral finasteride for the treatment of androgenetic alopecia: a randomized placebo-controlled, double-blind, half-head study

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Manuscript ID	DS-00584-2016.R1
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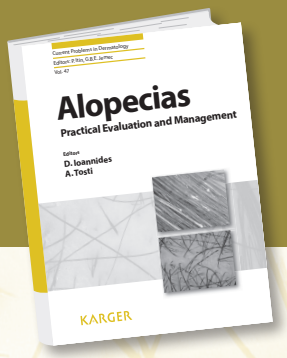
Key Words:	platelet-rich plasma, minoxidil, finasteride, androgenetic alopecia, treatment

13.14 CHAPTER: HAIR LOSS IN CHILDREN

A comprehensive and practical overview

Alopecias – Practical Evaluation and Management



Editors:
Dimitrios Ioannides
Antonella Tosti

Hair is subject to either intrinsic (i.e. physiological) aging or extrinsic (i.e. premature) aging that can be attributed to external factors. The intrinsic factors can be associated with genetic mechanisms and are subject to individual variations, whereas external factors include ultraviolet radiation, air pollution, smoking, and possibly nutrition.

Leading experts have contributed to this book which offers a practical approach to the evaluation and management of various forms of alopecia, including their pathogenesis, the diagnostic procedures involved, medical treatments, and nutritional issues. A completely new surgical technique for hair transplantation that leaves behind sufficient follicle unit tissue to regenerate hairs is also described.

Being comprehensive and easy-to-read, this book should be very useful for dermatologists who wish to diagnose and treat their patients with hair problems more effectively.

Fields of Interest: Dermatology; Immunology; Gerontology; Endocrinology, Surgery

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Hair Loss in Children

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Abstract

Hair diseases represent frequent complaints in dermatology clinics, and they can be caused by a number of conditions reflected by specific diagnoses. Hair loss is not uncommon in the pediatric group, but its patterns in this group are different from those seen in adults. Additionally, in children, these disorders can have psychological effects that can interfere with growth and development. Hair is easily accessible for examination, and dermatologists are in the enviable situation of being able to study many disorders using simple diagnostic techniques. To fully understand hair loss during childhood, a basic comprehension of normal hair growth is necessary. Knowledge of the normal range and variation observed in the hair of children further enhances its assessment. This chapter has been written in an attempt to facilitate the diagnostic process during daily practice by helping to distinguish between acquired and congenital hair diseases. It can sometimes be difficult to differentiate between abnormality and normality in neonatal hair aspects. Management of hair disorders can be quite a daunting task for the attending physician and mandates a holistic approach to the patient. Some hair disturbances have no effective treatment, and for others, no single treatment is 100% successful. If no effective treatment for a hair loss disease exists, a cosmetic approach is important.

Introduction

Hair loss in children occurs in a wide range of conditions that may be congenital or acquired. A congenital abnormality may be an isolated finding in a healthy patient or may occur as a feature of a multisystem syndrome. Recognition of a hair disorder may enable the diagnosis of a particular syndrome. The clinical presentations of pediatric hair disorders range from subtle to disfiguring. Alopecia is not uncommon in the pediatric population but has patterns that are different from those seen in adults. The occurrence of these problems during childhood can cause psychological and emotional stress to both children and their parents. A good knowledge of the normal hair cycle, embryology and clinical features is necessary.

Embryology and Normal Hair Development

Hair follicles are derived from an interaction between the embryological ectoderm and mesoderm, which begins at 9 weeks of gestation. Primary follicles first develop on the eyebrows, upper lip and chin. Then, hair follicles develop over

the scalp in a frontal to occipital direction and progress over the body in a cephalocaudal direction [1]. By 18–20 weeks of gestation, the entire initial population of follicles has formed, including those on the scalp [2]. Each follicle is capable of producing three different types of hair as follows: lanugo, vellus and terminal. Lanugo hair is nonmedullated, fine, soft, and usually nonpigmented, and it can be found on the bodies of newborns. This type of hair is shed by 3–4 months after birth. Vellus hair is short, fine, light-colored, barely noticeable, and covers almost the whole body. During puberty, the androgen hormone causes most of vellus hair to turn into terminal hair. Terminal hair is larger, thicker and strongly pigmented, and it is found on the scalp, eyebrows, axillary and pubic areas, chest and face [1].

Human hair grows in a continuous cyclic pattern known as the hair cycle. The hair cycle presents with different phases as follows: the anagen or growth phase (85–90% of hairs), the catagen or regression phase (<1% of hairs), the telogen or resting phase (4–15% of hairs) and, finally, the shedding exogen phase. At birth, about 5 million hair follicles cover the human body, and approximately 100,000 are scalp hairs [2]. Newborn hairs are all anagen, and during childhood, there is a gradual transition of scalp hairs from vellus to terminal hairs [3].

Diagnosis

A newborn can have the following three presentations of hair (normal variants): a full head of hair, or little or no hair. The beginning of abnormal hair growth sometimes occurs during infancy; thus, it is difficult to predict which newborn will have a hair pathology. Knowledge of a patient's personal and family history, a thorough clinical examination, as well as general and specific diagnostic procedures are important for a correct diagnosis and early treatment [4]. A detailed history is essential for an accurate diagnosis. The key points in a patient's history are the following: age of onset (congenital or acquired); onset of hair loss (sudden or insidious); extent of alopecia (localized or diffuse); physical and mental development (underlying syndrome?); past medical history (surgery, autoimmune disease, or medication); psychiatric disorders; and family history of alopecia [3, 5]. Similar to the history evaluation, an exhaustive physical examination should be performed to assess the following components: type of alopecia (localized or generalized/scarring or nonscarring); hypotrichosis or alopecia; hair shaft anomalies; hair quality; and hair color. A thorough scalp examination is also important to evaluate the existence of erythema, edema, pustules, scaling, atrophy or scarring.

The presence of short stature, abnormal bone development, defective hearing, dysmorphic features, impaired vision or other physical findings could indicate an underlying metabolic or autoimmune disease.

Usually, the diagnosis of the most common forms of hair loss can be made only by clinical and physical examinations. A hair pull test distinguishes between loss from follicles and loss due to hair shaft fragility. To perform this test, around 50–60 hairs are grasped between the index finger and thumb and then lifted with gentle traction. The pull test is considered positive if more than 10% of hairs are released [5]. False positives can occur if the test is performed on a day in which hair has been washed.

To confirm breakage of the hair shaft, a tug test should be performed. The tug test consists of the grasping of a hair between the finger and thumb near its exit from the scalp and the pulling of the distal part [6]. If the hair is fragile, a fracture will occur in the shaft. Trichoscopy, a noninvasive method, has emerged as a valuable tool in the differential diagnosis of most hair and scalp diseases. It is also important to evaluate the therapeutic response of hair loss [7].

Alopecia Areata

Alopecia areata (AA) is a nonscarring, autoimmune and inflammatory pattern of alopecia that occurs in both children and adults. It is a common disorder, affecting different ethnicities with equal incidences between genders. The prevalence of AA is approximately 0.2% in the general population, and its lifetime risk is estimated to be approximately 1.7% [8]. Although AA has been considered rare in young infants, recent studies have suggested that this disorder could occur in 1–2% of patients less than 2 years of age [9]. In children younger than 16 years of age, AA has been reported to occur in 21–24% of patients [9, 10].

The predisposition to AA is genetically determined, with 5–25% of patients possessing a strong family history [10, 11]. There is also an increased frequency of AA in individuals with Down syndrome [12–15]. AA may be seen in association with autoimmune disorders, such as atopic dermatitis, allergic rhinitis, asthma, vitiligo and autoimmune thyroid disease [13].

There are several clinical presentations of AA, which are usually classified according to the hair loss pattern or extent. The classic presentations are as follows: patchy AA (the most common pattern), alopecia totalis (the complete absence of terminal scalp hair), and alopecia universalis (the total loss of terminal scalp and body hair) [13]. Less frequent patterns of AA include the reticulated pattern, ophiasis type, sisaipho type and diffuse thinning over a part of or the total scalp [16, 17]. Another variant, acute diffuse and total alopecia, has been described and is characterized by rapid progression and extensive involvement along with a good prognosis.

The typical appearance of AA is of a well-demarcated localized patch that is asymptomatic, round or oval with a smooth surface. There may be single or multiple lesions with no associated epidermal changes, such as scaling. In some cases, slightly reddened skin can be present. AA can affect the scalp or any hair-bearing area on the body. This disorder is associated with nail involvement, including nail pitting, trachyonychia, brittle nails, onycholysis and koilonychia [16–18].

Trichoscopy examination reveals the presence of short 'exclamation mark' hairs at the periphery of the lesion (pathognomonic of AA) and 'yellow dots' in a follicular distribution. The hair pull test is typically positive in active AA with the presence of telogen club hairs and dystrophic anagen hairs [5, 19]. A scalp biopsy is usually unnecessary to establish the diagnosis of AA, except in the case of diffuse shedding. The hallmark histological finding is a dense lymphocyte infiltrate comprising mainly T cells around the anagen hair bulb matrix and the dermal papillae. The main differential diagnosis of AA in children includes tinea capitis, trichotillomania (TTM), transient neonatal hair loss (TNHL) and congenital triangular alopecia.

Tinea capitis is a common cause of patchy hair loss in infants, although individuals of all ages are occasionally affected. This condition involves the invasion of scalp hairs by dermatophyte fungi, including *Trichophyton* and *Microsporum* species. The clinical picture of this disease varies according to host immunity, the degree of inflammatory response and the type of hair invasion by the pathogen. The key feature is patchy hair loss with various degrees of inflammation and scaling and the easy removal of hairs from the affected area [19]. A trichoscopic hallmark of this disease is the presence of comma hairs. Fungal potassium hydroxide preparations and cultures and Wood's lamp are also helpful in establishing the diagnosis of tinea capitis. Oral antifungal treatment is required to treat this disease. The aim of treatment is a clinical and mycological cure [20].

TTM is classified as an impulse control disorder and is a self-inflicted compulsion or habit-tic to pull or pluck at the hair. It is characterized by single or multiple patches with the presence of

broken hairs of different lengths. TTM can be more difficult to differentiate from AA [21] (see Traumatic Alopecia for more information).

TNHL, which is also known as 'neonatal occipital alopecia', appears as a bald patch on the occipital region. Initially, it was thought to be secondary to friction due to babies sleeping in the supine position. According to a prospective study, 20% of neonates are born with an observable deficiency of the occipital scalp hair. This entity can be present at birth and is related to the physiology of hair shaft shedding. TNHL appears in healthy babies from birth until approximately the second month of life without accompanying symptoms and with spontaneous resolution [2]. It is important to inform parents of the benign course of TNHL and its absence of a relationship with the sleeping positions of babies.

Congenital triangular alopecia is also known as temporal triangular alopecia or Brauer nevus [22, 23]. It is a localized, congenital, nonscarring form of alopecia that might be present at birth or acquired during the first 10 years of life. Typical lesions are triangular or lancet shaped, can be unilateral or bilateral, are several centimeters in width and are confined to the front temporal region [23]. Apparently, the lesions are hairless, although very fine vellus hairs may be seen. Trichoscopy can aid in the differential diagnosis of this disease by the observation of normal follicular openings containing vellus hairs and the absence of specific features associated with AA [7].

There is no consistently reliable treatment for AA. Its natural history is uncertain, and spontaneous remission occurs in some patients.

Depending on the extent of involvement and the patient's age, watchful waiting is often a sensible approach. In the majority of children with patchy AA, hair will regrow entirely within 1 year without treatment. The aim of therapy is cosmetically acceptable hair regrowth.

Therapeutic options available for AA in children are more limited than for adults. Topical corticosteroids are commonly used for the treatment of this disease and are the first-line choice of many dermatologists.

Topical corticosteroids may be applied painlessly and have benign side-effect profiles [1]. Topical potent fluorinated corticosteroids remain an acceptable form of treatment for children with AA but have been reported to have some effects [19, 24, 25]. Children younger than 10 years of age with AA of recent onset tend to be the most responsive [19]. If hair growth occurs, this treatment should be continued with regular monitoring to prevent cutaneous atrophy [26]. Although rare, the development of systemic effects must also be monitored in children. Relapse after discontinuation of treatment could happen [27]. Injectable triamcinolone acetonide can be useful in children with patchy hair loss. Children younger than 10 years of age are not usually treated with intralesional corticosteroids because of the pain caused by its injection [16]. Older children (>10 years) may be prepared to consider this treatment for limited areas of hair loss if they are able to tolerate the injections [19].

Systemic corticosteroids have been considered in the treatment of widespread AA or AA that is refractory to other local treatments, but careful reviews of the protocols (doses, lengths of treatments and side effects) are mandatory.

Topical minoxidil is widely used in children and adults for the treatment of AA. Systemic absorption of the drug is typically minimal with topical therapy. Although side effects are rare, they have to be taken in consideration, especially in young children. According to Herskovitz et al. [28], a 2-year-old male patient developed generalized hypertrichosis after 2 months of treatment with 5% minoxidil foam for AA. This report highlights the risk of serious cutaneous or systemic side effects due to the possibility of the systemic absorption of topical minoxidil.

Other treatments for AA include topical immunotherapy (topical agents that induce hair growth by provoking allergic contact dermatitis,

such as dinitrochlorobenzene, squaric acid dibutylester, and diphencyprone), anthralin (induces inflammatory irritant dermatitis), phototherapy, immunosuppressants and immunomodulators [1]. Widespread forms of AA are generally more difficult to cure, and no treatment appears to prevent relapse.

An earlier onset of AA may be indicative of a more widespread disease, although it is impossible to predict its severity. In patients with few patches of alopecia, prognosis is generally good, and regrowth might occur within 6–12 months. Indicators of a poor prognosis are the presence of immune disease, atopy, a family history of AA, young age at onset, nail involvement, extensive hair loss and an ophiasis pattern [17]. The course of AA is unpredictable, and response to treatment can be changeable.

Androgenetic Alopecia in Children

Androgenetic alopecia (AGA) is a nonscarring, patterned alopecia with a typical pattern of distribution and with genetic involvement [29]. Family history predisposes individuals to the early development of this disease and rapid progression of the alopecia. The onset of AGA is gradual, slowly developing over a period of years. For its development, the presence of androgens is necessary in combination with genetically susceptible hair follicles. The onset of AGA is not expected to occur in children without abnormal androgen levels [30]. If a healthy prepubertal child presents with AGA, an endocrine evaluation is strongly recommended [30]. AGA is not uncommon in adolescents and should be considered as a cause of hair loss. Minoxidil topical solution appears to be effective and well tolerated in adolescent boys and girls with AGA [31]. Finasteride has not been studied in the treatment of males younger than 18 years of age; thus, its safety and efficacy in adolescents with AGA has not been determined.

Disturbances of Hair Cycle

Anagen Hair Loss: Anagen Effluvium and Loose Anagen Syndrome

The hair follicle undergoes periods of cyclic growth in the following phases: the anagen or growth phase, the catagen phase, the telogen phase and the shedding exogen phase. At any given time, 85–90% of follicles are in the anagen phase. During this phase, mitotically active matrix cells in the hair bulb differentiate and divide, resulting in hair growth [32]. The duration of the anagen growth phase of scalp hair varies from 2 to 6 years [1]. About 1% of scalp hairs are in the catagen phase, which lasts about 3 weeks. Approximately 10% of follicles are in the telogen resting phase, which lasts about 3 months, after which these hairs are shed. The final step of the hair cycle, exogen, is when the hair is released from the follicle. Anagen loss results from the shedding of a large amount of hairs during the anagen phase. The daily loss of telogen hairs is considered normal, but if a loss of anagen hair occurs, potential causes should be promptly analyzed. The causes that affect children are the same as those affecting adults, and a similar evaluation should be performed.

Anagen Effluvium

In anagen effluvium, hair loss is profound because up to 90% of scalp hair is normally in anagen. Hair loss characteristically occurs within 1–2 weeks of the insult.

The most frequent and easily recognizable cause of anagen effluvium is radiotherapy and systemic chemotherapy (e.g. doxorubicin, cyclophosphamide, vincristine, or bleomycin) [33]. Other causes that must be considered are exposure to toxic agents, such as mercury and colchicine, boric acid intoxication (e.g. exposure or ingestion of household pesticides), and ingestion of certain plants *(Lecythis ollaria* and *Leucaena glauca)* [34]. Severe protein malnutrition can also lead to anagen effluvium.

Diagnosis is easily made according to the patient's clinical history and the presence of dystrophic anagen hairs. If the insult is removed, normal hair regrowth is expected because the hair cycle was only temporary interrupted.

Loose Anagen Syndrome

In loose anagen syndrome, the anchoring of growing anagen hairs to follicles is impaired, with a lack of adhesion of the hair shaft to the hair follicle [19]. Because the growing anagen hairs are not anchored normally, they can be easily and painlessly plucked from the follicle.

Children between 2 and 7 years of age are the most frequently affected with this syndrome. At birth, the hair is sparse but appears normal, with no fragility or breakage [35]. Around 2–3 years of age, the hair becomes unruly and remains so until it spontaneously becomes normal at 5–7 years.

The hair of affected children does not grow long, and parents commonly state that there is no need for a haircut. Using light microscopy, hairs with distorted anagen roots without inner root sheaths and ruffled cuticles can be observed [35]. Trichogram analysis reveals a preponderance of anagen hairs (>97%) [36].

During childhood, gentle handling decreases hair shedding. No treatment is necessary because the hair spontaneously reverts to normal around puberty, although some physicians have reported the use of a 5% minoxidil solution causing clinical improvement [37, 38].

Telogen Effluvium

Telogen effluvium is an abnormality of hair cycling that can occur at any age. It is a reaction pattern in which a percentage of hairs move prematurely from anagen to telogen, resulting in a diffuse increase in hair shedding. This shedding occurs in response to a pathologic or physiologic alteration in the health condition.

The main causes of telogen effluvium are high fever, surgery, drugs (allopurinol, colchicine, beta-blockers, or antihypertensives), systemic illnesses, endocrine disorders, nutritional disorders (protein-calorie malnutrition, zinc deficiency, or starvation), severe emotional stress, immunization (following bivalent human papillomavirus) [39] and pregnancy. Acute telogen effluvium occurs approximately 3 months after a triggering event. Chronic telogen effluvium is considered when shedding continues longer than 6 months. A detailed history should be sought, including a thorough drug history, and full clinical examination should be performed [40]. A hair pull test is positive when a high percentage of telogen hairs is obtained (>20%) [5]. This test should be performed at the vertex, parietal and occipital areas [33].

Telogen effluvium is less frequent in children than in adults, and in children, it is more likely to be related to sudden disease or trauma. This condition is managed by the treatment of the underlying disorder with appropriate replacement drugs or medical therapy [19]. The prognosis of telogen effluvium is very good if the precipitating event is eliminated.

Traumatic Alopecia

Trichotillomania

TTM is characterized by repetitive and self-induced compulsive hair pulling [41]. The most frequent site is the scalp, but it can also involve the eyebrows, eyelashes and pubic hair. During infancy and early childhood, TTM is more frequent in boys, while during puberty and in adults, there is a strong female predominance [42].

Clinically, patients present with areas of alopecia of different shapes, irregular borders and the presence of hairs of different lengths [21]. This condition is usually a nonpermanent form of alopecia, but if the same area is persistently plucked, scarring may result with persistent hair loss [1]. Typically, there is no scaling on the scalp, although some excoriations can be seen. Hair density is normal, and the pull test is negative [5].

Trichoscopy reveals the presence of flame hairs (specific for TTM) with the v-sign and tulip hairs, which are highly characteristic features of TTM [43].

Three subsets of individuals affected by this condition appear to exist as follows: preschool age children, preadolescents to young adults, and adults. In young children, TTM is usually a habit comparable to thumb sucking, and it normally has a self-limited and benign course of hair pulling with regrowth of the hair if the condition is managed conservatively. Several reviews of childhood TTM have related its onset in young children to stressful situations. This type of traumatic alopecia is more frequent in preadolescents to young adults with an average age of onset of between 9 and 13 years [21]. These patients tend to have more chronic and relapsing courses of hair pulling.

TTM in adults may be secondary to underlying psychiatric disturbances and has a more prolonged course.

Management of this disorder is difficult and is usually based on a person's age. No specific treatment has been established that is completely effective. Preadolescents to young adults without associated psychiatric conditions may benefit from nonpharmacological interventions, such as behavior modification programs. If TTM is associated with psychological/psychiatric disorders, referral to a psychiatrist for evaluation or treatment is recommended. Pharmacological interventions include serotonin-specific re-uptake inhibitors, tricyclic antidepressants and more recently, N-acetylcysteine, a glutamate modulator [44].

Traction Alopecia

Traction alopecia, a pattern of alopecia caused by physical trauma, is induced as a consequence of constant traction while hairstyling (e.g., tight ponytails, cornrows or rollers). It is more frequent in black women and children. Afro-textured hair is particularly easily damaged by such procedures.

Clinical manifestations include hair thinning, focal decreases in hair density and perifollicular erythema [45]. Short, broken hairs, folliculitis and follicular papules may be seen [1]. With prolonged traction, perifollicular scarring and cicatricial alopecia develop, which are more evident along the hairline. During its early stages, alopecia is initially reversible with a change in hairstyling methods. If patients maintain continuous traction, permanent hair loss occurs due to hair follicle loss.

Hair Shaft Disorders

Hair shaft disorders can be inherited or acquired and sometimes can aid in diagnosing an underlying disease. A reliable diagnosis of a hair shaft abnormality can only be made via an evaluation of the wide structural variations found in normal hair.

Hair shaft disorders can be divided in two subcategories as follows: (1) hair shaft abnormalities WITH increased fragility and breakage; and (2) hair shaft abnormalities WITHOUT increased fragility. Table 1 has a description of the principal hair shaft abnormalities.

Special Disorders with Hypotrichosis in Children

There is an extensive list of genetic disorders associated with hypotrichosis. The detailed analysis of these syndromes falls outside of the ambit of this chapter.

Congenital Atrichia and Hypotrichosis

Atrichia congenita is a rare form of irreversible alopecia with an autosomal recessive mode of inheritance that is usually associated with a mutation in the human hairless gene located on chromosome 8 [55, 56].

This condition is characterized by follicular agenesis or programmed follicular destruction.

Table 1. Hair shaft disorders

Disease	Genetic/clinical features	Diagnosis	Associated diseases	Management
Hair shaft abnormalities WITH increased fragility and breakage				
Trichorrhexis nodosa [46–48]	Congenital or Acquired (++) Acquired TN is frequently due to weathering due to external causes, such as shampooing, styling, UV radiation, wetting and natural friction Hairs appear dry and brittle with a tendency to break at different lengths Three variants of acquired TN: Proximal ATN: seen in patients after years of hair straightening Distal ATN: acquired, cumulative cuticular damage Circumscribed ATN: scalp, moustache or beard	Light microscopy: – nodes along the hair shaft that cause hair to break easily; distal nodes generally indicate hair weathering; and the absence of cuticle cells Trichoscopy: – light magnification: nodular thickenings along hair shafts that appear lighter in dark hair shafts – high magnification: small fibers with an appearance suggestive of the ends of two brushes aligned in opposition	Mental retardation Argininosuccinic aciduria Muenke syndrome Defective teeth and nails Its presence in infants or young children should trigger a search for an underlying metabolic problem	Change in hairstyling procedures Sun hair protection
Monilethrix (beaded hair) [1, 49, 50]	Congenital (AD): mutation in the hair cortex-specific keratin genes *KRT86*(++), *KRT81*, *KRT83*, and *DSG4* Clinically, there is normal hair at birth, but within the first months of life, a characteristic moniliform aspect of the hair shaft develops. Perifollicular erythema and follicular hyperkeratosis are common features The hair is short, fragile, brittle and breaks spontaneously or as a result of friction In its mildest forms, only the scalp is involved. In extensive cases, there may be eyebrow, eyelash and nail involvement	Light microscopy: hairs possess a beaded appearance Trichoscopy: – uniform elliptical nodes and intermittent constrictions of the hair shaft, causing variation in hair shaft thickness – hairs are bended regularly at multiple locations and have a tendency to fracture at constriction sites – periodic constriction of the hair shaft	Keratosis pilaris	No specific treatment Retinoids, glycolic acids and minoxidil may be helpful in some cases There is a wide spectrum of severity, and it tends to remain constant throughout life
Pili torti [50, 51]	Congenital (AD (++)/AR) or acquired: isolated or associated with other conditions At birth, the hair is normal. Changes gradually appear between the 3rd month and 3rd year. More common in girls with blond hair Clinically, hairs are fragile, with a twisted and spangled appearance	Light microscopy: twists at irregular intervals along the shaft Trichoscopy: hairs are twisted and rotated at 180°. In a field of view, only part of the hair usually appears abnormal. Twists are better seen with dry trichoscopy using high magnification	Björnstad syndromes (pili torti associated with sensorineural hearing loss) Muenke disease Bazex-Dupré-Christol's syndrome Crandall syndrome The presence of pili torti, requires further evaluation for neurological and ectodermal disorders	No treatment May improve in puberty
Trichorrhexis invaginata (bamboo hair) [50–52]	Congenital (AD): mutation in the *SPINK5* gene Clinically, the hair is short, brittle, sparse and very fragile. May affect the scalp, eyebrows and body hair Defective cornification of the cortex	Light microscopy: intussusceptions of the distal shaft into the proximal shaft, with a bamboo appearance Trichoscopy: golf tee hair (scalp and eyebrow)	Marker for Netherton's syndrome (AR; triad of ichthyosis, atopic diathesis and trichorrhexis invaginata)	No specific treatment Avoidance of trauma

Table 1. Continued

Disease	Genetic/clinical features	Diagnosis	Associated diseases	Management
Trichothio-dystrophy	Congenital (AR), characterized by sulfur-deficient hair Clinically, only the hair may be affected (fragile) or it may be associated with ichthyosis, photosensitivity (50%), decreased growth, and mental handicap	Light microscopy: irregular, undulating contours and transverse fractures throughout the hair shaft ('tiger tail') Trichoscopy: non-specific Diagnosis is performed by sulfur and/or amino acid analysis of the hair	BIDS or Tay's syndromes (brittle hair, intellectual impairment, decreased fertility, and short stature) IBIDS (ichthyosis and BIDS) PIBDS syndrome (photosensitivity and IBIDS)	No treatment currently available Sun protection
Hair shaft abnormalities WITHOUT increased fragility				
Pili annulati (ringed hair) [46, 53]	Rare Congenital (AD) or sporadic: abnormalities appear at birth or during infancy Clinically, the hair looks shiny but otherwise normal, and 20–80% of the hair is affected No fragility, and the hair can grow long	Light microscopy: hair shafts with alternating bright (normal hair) and dark bands (abnormal hair; the medulla is expanded by air cavities within the hair) Trichoscopy: hair shafts with regular light bands corresponding with dark cavities, visible by light microscopy	No hair or systemic abnormalities	No treatment currently available Aesthetic defect
Woolly hair syndrome [46, 50]	Three types of wooly hair: Hereditary wooly hair (AD): mutations in plakoglobin genes and desmoplakin genes. Hair color is variable Familial woolly hair (AR): mutations in the *LPAR6* and *LIPH* genes. Hair is sparse, thin, and short Wooly hair nevus: localized Clinically, hair is fine and tightly curled. Occurs in Caucasians	Light microscopy: ovoid cross sections, 180-degree longitudinal twisting, trichorrhexis nodosa and pili annulati Trichoscopy: Crawling snake with short wave cycles and broken hairs	Hereditary wooly hair: palmoplantar keratoderma and cardiac abnormalities Familial woolly hair: associated with hypotrichosis Wooly hair nevus: associated with linear epidermal or pigmented nevi (>50%)	No specific treatment Can improve with age
Uncombable hair (pili trianguli and canaliculi) [46, 54]	Congenital (AD): hair abnormalities appear around the age of 3 years Clinically, the hair is unruly, dry and impossible to control with a brush or comb	Light microscopy (gold standard): in more than 50% of hairs, there is a triangular or reniform shaft with a longitudinal groove or flattening Trichoscopy: triangular or reniform hair shaft	Absence of systemic abnormalities	Improves spontaneously with aging

TN = Trichorrhexis nodosa; AD = autosomal dominant; AR = autosomal recessive; DSG4 = desmoglein 4; LIPH = lipase H.

Hair at birth can be sparse (or inexistent) and progress to a total and permanent absence of scalp hair over the first 5 years. In another variant of the disease, neonates are born with lanugo hair, which is shed during the first few months of life and is never replaced [19, 50]. Congenital atrichia may occur, either as an isolated phenomenon or in association with a number of rare syndromes. To be distinguished from AA totalis, a scalp biopsy may be required.

Congenital hypotrichosis is a less severe form of atrichia congenita, in which hair is not absent but is diffusely thinned [19]. There is a profound reduction in the number of hair follicles on the

scalp, which usually but not always is present from birth. Hypotrichosis may not be noticed until the age of 2 years because of variation in the quality and quantity of the hair normally present at birth, and it usually occurs as an isolated defect.

Marie-Unna Hereditary Hypotrichosis
Marie-Unna hypotrichosis is an autosomal dominant condition, which was first described in 1925 by Dr. Marie Unna in 27 affected members of a family over seven generations [50]. This rare hypotrichosis is characterized by isolated progressive alopecia without abnormalities of the nails or teeth or intellectual or gross motor development. Typically, this diffuse hair defect occurs as an isolated phenomenon, although some reports have described of its associations with Ehlers-Danlos syndrome and juvenile macular degeneration. At birth, hair may be normal or sparse. Around the third year of life, hair becomes coarse and twisted, resembling an 'ill-fitting wig' [19, 57]. During puberty, the hair is gradually lost from the crown, ultimately resulting in complete baldness due to the destruction of the follicles with scarring [19]. Lashes, eyebrows and body hair are also sparse or absent.

Ectodermal Dysplasia
Ectodermal dysplasia represents a heterogeneous group of inherited disorders affecting more than one ectoderm-derived tissue, leading to abnormalities of the hair, nails, epidermis, teeth and eccrine glands. Scalp hair is usually fine and short but silky in texture [19]. Eyebrows and/or eyelashes are also affected. These patients should be examined thoroughly, with particular attention paid to other ectodermal structures.

Conclusion

Hair problems in children occur relatively frequently in a wide range of conditions that may be congenital or acquired. Not all congenital or hereditary hair disorders are present at birth, and those that are often go unrecognized. Usually, these hair disorders are isolated abnormalities, and the child is otherwise well. A number of disorders occur as part of a multisystem syndrome; thus, recognition of the clinical presentation may enable the diagnosis of a particular syndrome. If a disease is hereditary, genetic counseling should be offered. A detailed clinical history accompanied by examination are essential for the accurate diagnosis of the pathology.

Hair is very important, both cosmetically and functionally. Alterations in the normal appearance of hair can predispose individuals to low self-esteem and a negative body image. Pediatric hair disorders causes psychological and emotional stresses in both children and parents. It is important that parents are provided a clear understanding of the etiology and natural history of the disease.

The management of a hair loss disorder should be adapted according to the age of the patient. An early identification of the pathology leads to better treatment results, including psychological and cosmetic support. Unfortunately, for many hair disorders, there is no effective, reliable therapy.

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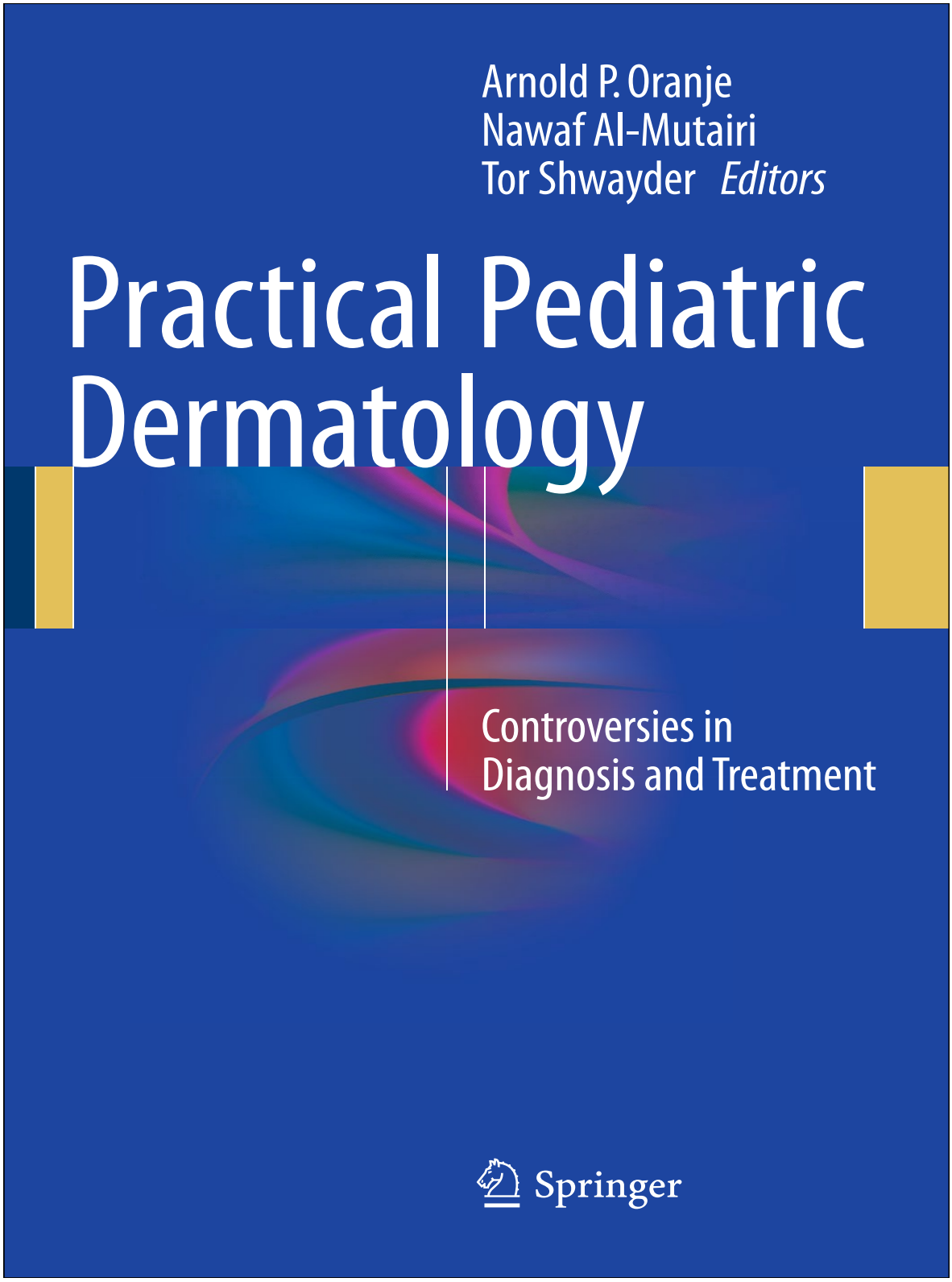
13.15 CHAPTER: AGA IN ADOLESCENTS



Androgenetic Alopecia in Adolescents

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Rubina Alves and Ramon Grimalt

Abstract

Androgenetic alopecia (AGA) is a nonscarring progressive miniaturization of the hair follicle with a usually characteristic pattern distribution in genetically predisposed men and women. Although AGA is the most common form of hair loss in adults, little is known about its prevalence and response to treatments in the pediatric population. As in adults, the diagnosis of this type of alopecia in adolescents is made by recognizing the pattern and progression of hair loss in the context of the family history. A negative family history does not exclude the diagnosis. Early-onset AGA can be the presenting sign of an underlying endocrine disorder.

Adolescents are invariably sensitive about their external features *and*, thus, may easily withdraw psychologically *and* avoid social activities due to AGA development. They can feel anxious and unattractive with a negative body image about themselves. Accurately recognizing AGA in adolescents will help patients and their families understand the diagnosis and its natural progression, allowing timely medical intervention for hair loss and any associated endocrine or psychosocial morbidity. Treatment of adolescent AGA has not been well studied, and currently there are no FDA-approved treatments for this condition. This article provides an overview of the embryology and normal hair development, pathogenesis, diagnosis, and management of adolescent androgenetic alopecia.

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Androgenetic alopecia (AGA) is a common chronic dermatologic disease, affecting both men and women. It is characterized by progressive hair loss usually occurring in a pattern distribution. The onset of AGA is usually gradual and the condition slowly develops over the years.

AGA is undoubtedly the most common form of hair loss (Tosti and Piraccini 1999), affecting around 80% of Caucasian men aged over 70 years (Blume-Peytavi et al. 2011; Rathnayake and Sinclair 2010). As in men, the frequency and severity of AGA increase with age in women (Gan and Sinclair 2005).

Although it's more frequent in adults, it can also appear in adolescents. There are different studies (Gonzalez et al. 2010; Kim et al. 2006; Trancik et al. 2001a, b) that have documented the prevalence and early age at onset of AGA.

In children and adolescents with a genetic predisposition, the first signs of AGA can appear with rising androgens at puberty (Gonzalez et al. 2010; Kim et al. 2006) and have been observed as early as 6 years of age (Tosti et al. 2005). On *an* average, adolescent AGA onset presents between 13.5 and 15 years of age (McDonough and Schwartz 2011).

In our society, strong and dense hair is associated with youth, beauty, health, attractiveness, and success. Independent of age and gender, patients diagnosed with AGA undergo significant impairment in their quality of life.

During the adolescence, self-esteem plays a crucial role when teenagers start to notice changes about their body, own environment, and the outside world. A positive or healthy body image means feeling happy and satisfied about our own body. Perceived or real physical defects may be important for an adolescent trying to establish self-image and adjust socially (Strauch and Greenstein 1997).

Concerning the influence of many factors in body image, such as media and fashion industry, the adolescents who present with hair thinning can feel unattractive with a negative body image (Budd et al. 2000). This source of distress can lead to anxiety, depression, and social isolation (Cash 1992; Cash et al. 1993).

Embryology and Normal Hair Development

Hair follicles are derived from an interaction between embryological ectoderm and mesoderm that begins around 9 weeks of gestation (Moreno-Romero and Grimalt 2014). Then, the development of the hair progresses in a cephalocaudal direction, becoming first visible in the eyebrow, upper lip, and chin regions. Around 16 weeks gestation, hair production begins in the follicles, with lanugo hair formation. At 18–20 weeks gestation, the entire initial population of follicles is completed, including those on the scalp (Cutrone and Grimalt 2005). At birth, about 5 million hair follicles cover the human body, and approximately 100,000 are scalp hairs (Sinclair et al. 1999).

Each follicle can produce three different types of hair: lanugo, vellus, and terminal.

The lanugo hair is a nonmedullated, fine, soft, usually nonpigmented hair that can be found on the body of a newborn. Around the third or fourth month after birth, the initial fine lanugo scalp hairs are shed in a synchronized wave pattern.

The vellus hair is a short, fine, light-colored, *and* barely noticeable *hair* that covers almost the whole body. At puberty, the androgen hormone causes much of the vellus hair to turn into terminal hair and stimulates the growth of new hair in the face, axillary, and the pubic area (Moreno-Romero and Grimalt 2014).

Terminal hairs are larger, thicker, and strongly pigmented hairs found on the scalp, eyebrows, axillary and pubic areas, chest, and face. Both vellus and terminal hairs coexist in the same areas,

and absolute distinction is not always possible (Harrison and Sinclair 2003). The normal ratio of terminal to vellus hairs in a normal scalp is 7–8:1.

The hair follicle is anatomically divided into three parts: upper (infundibulum), middle (isthmus), and lower (inferior segment). The infundibulum and isthmus comprise the permanent portions of the hair follicle, while the inferior segment is transient and undergoes cyclical regeneration (Shapiro et al. 2004).

Human hair grows in a continuous cyclic pattern known as hair cycle. The hair cycle is classically composed of four distinct phases: anagen (growth phase – 85–90 % of hairs), catagen (regression or involution phase – <1 % of hairs), telogen (resting phase – 4–15% of hairs), and finally the shedding exogen phase. The duration of anagen growth phase of scalp hairs typically varies between 2 and 6 years, catagen phase lasts 2 or 3 weeks, and telogen phase approximately 3 months (Moreno-Romero and Grimalt 2014; Harrison and Sinclair 2003). The normal hair cycle results in the replacement of every hair on the scalp every 3–5 years (Harrison and Sinclair 2003).

At birth, within anatomical regions, all hairs are initially synchronous in anagen phase, and during childhood there is a gradual transition of scalp hair from vellus to terminal hairs (Olsen 1994a, b). The type of hair produced by an individual follicle can change with age or under the influence of hormones (Harrison and Sinclair 2003).

Around puberty, there is an increase of the circulating adrenal androgens, which leads to a site-specific response from the hair follicles. With the presence of androgens, the hairs of the scalp miniaturize, while the hair of the body enlarges. At this time, the hairs of axillary, pubic, chest, and beard (boys) change from vellus to terminal hair, contributing to the development of the secondary sex characteristics (Moreno-Romero and Grimalt 2014; Harrison and Sinclair 2003).

Etiology and Pathogenesis

For the development of AGA, the presence of androgens, in combination with genetically susceptible hair follicles, is necessary (Kaufman 1996). Inheritance is almost certainly polygenic, with genetic input from both parents. Studies performed on monozygotic twins showed strong concordance rates between 80 and 90 %, reinforcing the implication of a genetic basis (Chumlea et al. 2004). Family history predisposes to early development and rapid progression of the alopecia (Tosti and Piraccini 1999). Its etiology is multifactorial and polygenetic (Blume-Peytavi et al. 2011; Blumeyer et al. 2011).

The androgen hormones testosterone and dihydrotestosterone (DHT) each have selective roles at puberty. Testosterone is converted to DHT by the enzyme 5-alpha-reductase that has two isoenzymes: 5-alpha-reductase type I (present in the liver and sebaceous glands) and 5-alpha-reductase type II (present in the scalp, beard, chest, liver, and prostate gland).

Testosterone is associated with increased muscle mass, growth of the scrotum, voice change, and the presence of terminal pubic and axillary hair fibers. An increased activity of 5-alpha-reductase type II plays an important role in the development of AGA (Blume-Peytavi et al. 2011). Levels of this isoenzyme are higher in men than in women and in the areas affected by AGA (Tosti and Piraccini 1999).

DHT is associated with temporal scalp hair recession, acne, growth of the prostate gland, and the development of terminal hairs in the beard region, external ears, and limbs.

The hormone DHT activates the genes responsible for the shortening of hair growth cycle as well as the transformation of large hair follicles into smaller follicles that progressively become miniaturized (Price 2003).

AGA is characterized by the miniaturization of the hair that only occurs in certain areas: the frontotemporal and vertex area in men and the crown region in women. These scalp regions are susceptible to the effects of androgens. During the gradual transformation of the miniaturized follicles (from terminal to vellus-like follicles), the anagen phase shortens, and for this reason more hairs are in telogen phase (Kaufman 1996). There is no loss of hair follicles in AGA, just miniaturized.

Clinical Features of AGA in Adolescents

The physician should record age, sex, and age at the first manifestation of hair loss and course of the disorder (chronic or intermittent) in the patient as well as in the family history. In adolescents it's important to differentiate if the hair loss is congenital or acquired. Look for physical and mental development, particularly in case of early onset of puberty (Blume-Peytavi et al. 2011).

If there's any systemic or newly diagnosed disease within 1 year prior to the first signs of hair loss, one should suspect that could be secondary to other causes or aggravating factors. It is known that AGA may be aggravated by chronic disease, metabolic disease, endocrinological disease, stress, emotional change, and medication (Tosti and Piraccini 1999; Kim et al. 2006).

The clinical expression of AGA in adolescents is milder than in adults (Price 2003). A family history for AGA is frequently positive (father, mother, both), but a negative family history does not exclude the diagnosis.

Clinically, a male AGA is relatively easy to diagnose. It is symmetric and progressive, and the pattern of hair loss can vary among individuals. In boys, AGA is recognized by changes in three scalp regions: frontal scalp, vertex region, and bitemporal region. It commonly manifests as bitemporal recession and thinning in the frontal and vertex regions. The frontal and vertex regions may show mild, decreased hair density, and miniaturized, shorter, finer hair (Fig. 19.1) (Price 2003). The occipital density is relatively spared in AGA.

In girls, AGA is more difficult to identify and diagnose than in boys (Fig. 19.2). Early diffuse hair thinning is usually the most evident over the frontal scalp, with an increased spacing between hairs and a widened appearance of the central part. The size of the ponytail is decreased (Price 2003). The most common patterns of hair loss in women are centrifugal loss at the crown and the frontal accentuation or "Christmas tree" pattern (Olsen et al. 2005; Olsen 2003).

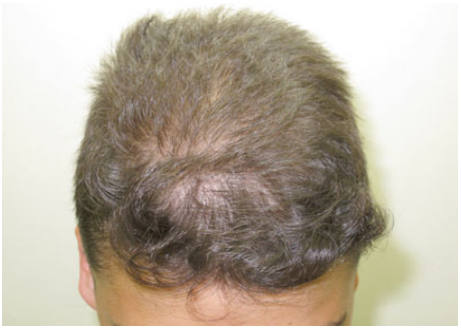

Fig. 19.1 Androgenetic alopecia developed in a 16-year-old boy

The most frequently used scales in practice are Hamilton–Norwood scale (Norwood 1975) for male-pattern distribution and Ludwig scale (Ludwig 1977) or Olsen scale (Olsen 2003) (frontal accentuation/Christmas tree pattern) for a female-pattern *AGA*. Women who develop balding in a pattern similar to men can be classified using the Hamilton–Norwood scale.

In adolescent girls with AGA, a careful evaluation of the gynecological history should include menarche, menstrual cycle, and amenorrhea. The hair loss usually occurs in the absence of any underlying endocrinopathy. Occasionally, it may appear as part of a hyperandrogenemic state such as the one that occurs in polycystic ovary syndrome, hyperprolactinemia, or adrenal or ovarian tumors (Gonzalez et al. 2010).

If indicated, laboratory evaluation may include free androgen index test [FAI = total testosterone × 100/sex hormone-binding globulin (SHBG)], luteinizing hormone/follicle-stimulating hormone, and prolactin as screening parameters (Blume-Peytavi et al. 2011). Depending on the results, further endocrinological investigations may be required.

Because of the clinical features associated with hyperandrogenism, male adolescents typically do not present with signs or symptoms of androgen excess (Witchel 2002).

The diagnosis of AGA is usually clinical. Some specific techniques may be used to improve the diagnostic accuracy.

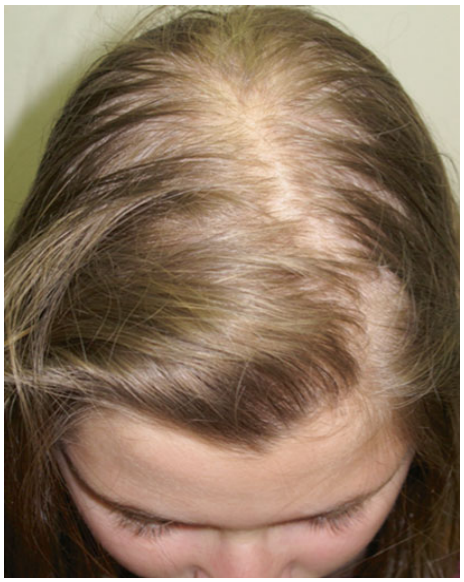

Fig. 19.2 Androgenetic alopecia developed in a 12-year-old girl

The hair-pull test is an examination easy to do in order to analyze active hair shedding. To perform the test, one should grasp around 50–60 hairs between index and thumb and then lift with gentle traction (Blume-Peytavi et al. 2011). The hair-pull test is considered positive if more than 10 % of the hairs are released.

In patients with AGA, the hair-pull test is positive only in the active phase with increased telogen hairs in the affected area. Otherwise, hair-pull test is typically normal in AGA. False positives can occur if the test is performed on the day of hair washing.

Trichoscopy, a noninvasive technique, has emerged as a valuable tool in the differential diagnosis of most hair and scalp diseases. Trichoscopy is useful in the diagnosis of AGA and to evaluate the therapeutic response of hair loss (Rudnicka et al. 2008; Kowalska-Oledzka et al. 2012).

Male and female AGA share similar trichoscopic features, including hair thickness heterogeneity, thin hairs, vellus hairs, single-hair pilosebaceous units, yellow dots, perifollicular discoloration, wavy hair, and honeycomb pigmentation (Rakowska et al. 2009, 2012).

In high-magnification (70×) dermoscopy, three major diagnostic criteria and three minor diagnostic criteria were proposed (Rakowska et al. 2009). The major criteria were the following: more than four yellow dots in four fields in the frontal area, decreased average shaft thickness in the frontal area compared to the occipital scalp, and more than 10 % of thin hairs in the frontal area. Minor criteria included the following: perifollicular discoloration, increased single-hair pilosebaceous units in the frontal area compared to the occipital area, and increased vellus hairs in the frontal area compared to the occiput. Two major criteria and/or one major and one minor criterion have a specificity of 98 % (Werner and Mulinari-Brenner 2012).

Biopsy is usually not necessary for the diagnosis of AGA. It can be performed if there's some difficulties in establishing the diagnosis, for example, in men who present a female pattern of hair loss or in females in order to exclude other causes of diffuse hair loss, such as telogen effluvium (Kaliyadan et al. 2013).

The better site to perform a biopsy is the central scalp in an area representative of hair loss. The punch biopsy should have at least 4 mm in diameter and follow the direction of the hair shafts until subcutaneous fat, where anagen hair bulbs are usually located (Olsen et al. 2005; Kaliyadan et al. 2013; Whiting 1993; Whiting et al. 1999). Biopsies smaller than 4-mm punch reduce the total number of follicles available for examination, and since follicles are not uniformly affected by disease and some are lost in a given process, a smaller biopsy diminishes the probability of making a definitive diagnosis (Olsen 1994a).

Some histologic features of AGA include perifollicular infiltrate (predominantly lymphohistiocytic around the upper or lower follicle), normal total number of follicles, and miniaturization of terminal hairs. The biopsy also allows (when sectioned horizontally) calculating the hair ratio of terminal/vellus (T:V) hairs.

The T:V ratio is reduced in AGA, typically less than 4:1 (Werner and Mulinari-Brenner 2012).

Differential Diagnosis

The diagnosis of AGA is usually made clinically by examination of hair and scalp. A diffuse alopecia may present a diagnostic challenge, and it's important to rule out the more commonly differential diagnosis of AGA such as telogen effluvium (TE) and diffuse alopecia areata (Werner and Mulinari-Brenner 2012).

Telogen effluvium is an abnormality of hair cycling that can occur at any age. It is characterized by a nonscarring, global diffuse pattern of alopecia with an increased shedding of otherwise normal telogen hairs. It may begin with a sudden increase of hair loss and maintenance of the frontal hair density (Werner and Mulinari-Brenner 2012). This shedding occurs in response to a pathologic or physiologic alteration in health condition.

Seasonal changes in hairs are obvious in many mammals. In humans, there are also seasonal changes in hair growth, although it's less clear and not synchronized. The condition tends to run a fluctuating course, and the number of shed hairs reached a peak around August/September, when least follicles were in anagen (Randall and Ebling 1991; Courtois et al. 1996).

The main causes that can precipitate a TE are nutritional disorders (protein–calorie malnutrition, zinc deficiency, starvation), high fever, surgery, drugs (allopurinol, colchicine, B-blockers, antihypertensives), systemic illness, endocrine disorders, and pregnancy (Patel et al. 2013).

Although rare, TE has been reported to be secondary to immunization (Tuccori et al. 2012; Wise et al. 1997). Tuccori et al. (2012) described two cases of TE occurring in two 11-year-old children following bivalent human papillomavirus (HPV) vaccine administration. The two children started to lose their hair following the second HPV vaccine dose, and the alopecia worsened following the third vaccine dose. After a few months, the hair regrew spontaneously. All other causes of hair loss were excluded. According to the authors, it is not recommended to discontinue the immunization as it provides health benefits that overcome the possible adverse effect of transitory TE.

Dietary restrictions, especially protein and iron restrictions, should be excluded, and vegetarians are a risk group for TE. Emotional stress is commonly attributed as a cause of acute TE, but the evidence for this is weak (Harrison and Sinclair 2002). As is of our knowledge, there is no data that suggests the stresses of everyday life are sufficient to induce diffuse hair loss (Cash 2001; Grover and Khurana 2013).

In TE, the hair-pull test is strongly positive, with a high percentage of telogen hairs (>20 %) (Rakowska et al. 2009; Sinclair and Jolliffe 2013). The most widely accepted histological parameter for differentiating the TE from AGA is the number of terminal hairs compared to the number of vellus hairs. In TE, the terminal/vellus ratio is >7:1, while in AGA is <4:1 (Werner and Mulinari-Brenner 2012).

Management of this condition is by treatment of the underlying disorder with appropriate replacement drugs or medical therapy (Harrison and Sinclair 2003). The prognosis for TE is very good if the precipitating event is eliminated.

Alopecia areata (AA) is a nonscarring patterned alopecia that can occur in children, adolescents, and adults, affecting different ethnicities with equal sex incidence. The classic presentations such as patchy AA (most common pattern), alopecia totalis, and alopecia universalis are easy to differentiate from AGA. A diffuse alopecia areata is the principal pattern to rule out.

Diffuse alopecia areata is described as a unique AA that lacks the characteristic patches and, instead, demonstrates widespread scalp hair thinning (Zhao et al. 2012).

Trichoscopy examination is of great value in the diagnosis of diffuse AA and has the same characteristics features of AA. The dermoscopy features that can be found in diffuse AA are short "exclamation-mark" hairs, specially at the margins of the lesion, "yellow dots" in a follicular distribution, broken hairs, and black dots (Rudnicka et al. 2008; Werner and Mulinari-Brenner 2012; Randall and Ebling 1991).

The hair-pull test is positive (Alkhalifah et al. 2010). AA is associated with nail involvement, particularly nail pitting; trachyonychia, brittle nails, onycholysis, and koilonychia

have also been reported (Madani and Shapiro 2000; Tosti et al. 1991).

Treatment

Patients should be informed about the pathogenesis of AGA and the course of the disease. After the diagnosis is confirmed and considering that severity of AGA increases with age, the treatment should begin as soon as possible (Herskovitz and Tosti 2013). Patients must avoid hair care products likely to damage scalp/hair and maintain an adequate diet (Olsen et al. 2005).

In adults, there are two drugs approved by the US Food and Drug Administration (FDA) for the treatment of AGA: topical minoxidil and oral finasteride. There is no approved therapy for AGA in the adolescent population.

Minoxidil, a pyrimidine derivate, is converted to minoxidil sulfate, which opens ATP-sensitive potassium channels in cell membranes, leading to a vasodilatory effect. Topical minoxidil increases the duration of the anagen phase and leads to production of hairs that are gradually thicker and longer (Olsen et al. 2005). This results in partial reversion of miniaturization (Tosti and Piraccini 1999).

The 2% solution was first approved by the US Food and Drug Administration (FDA) in 1988 for the treatment of AGA in men (18–50 years) and in 1991 in women (18 and 45 years). The 5% solution was approved in 1997 for the treatment of AGA in men followed by approval of the 5% foam in 2006 also for the treatment of AGA in men (Blumeyer et al. 2011). The 5% lotion and 5% foam are not licensed by the FDA for use in women, but it is commonly used off-label for this purpose.

Several studies (Alanis et al. 1991; van Zuuren et al. 2012b; Olsen et al. 2002; Krupa Shankar et al. 2009; Lucky et al. 2004; Price et al. 1999) have demonstrated that minoxidil applied twice a day is an effective treatment for AGA in both men and women.

Olsen et al. 2002 performed a randomized clinical trial comparing the use of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of AGA in men. The authors refer that the men who used 5% topical minoxidil had an earlier response to treatment than those who used 2% topical minoxidil. Both 2 and 5% topical minoxidil solutions were well tolerated by the men without evidence of systemic effects.

Concerning the treatment of female-pattern hair loss, either 2 or 5% (used off-label) topical minoxidil solution appears safe to use in women with AGA. The only additional risk of the 5% topical minoxidil solution over the 2% topical minoxidil solution is a higher incidence of facial hypertrichosis (Olsen et al. 2005).

Minoxidil has not been thoroughly studied in adolescents, and data are limited to information from scientific meetings and case series.

Regarding the use of topical minoxidil in adolescents with AGA, a retrospective study Trancik et al. 2001b was performed in 448 boys and girls with a mean age of 15.6 years. After 5% topical minoxidil had been applied for approximately 18 months, 95% of patients responded to treatment; more than 50% had improvement in scalp coverage, and more than 40% had slowing of further hair thinning. Based on these findings, minoxidil appears to be an effective and well-tolerated treatment for adolescents with AGA (Gonzalez et al. 2010; Price 2003; Trancik et al. 2001c).

Topical minoxidil requires proper use of the formulation and it is only effective in the area applied. Approximately 1 ml of solution should be applied directly to dry hair and scalp, twice a day in affected area. Hands should be washed with warm water after application. If hair is to be washed after applying minoxidil, the patient must wait at least 1 h before shampooing (Blumeyer et al. 2011).

Side effects of minoxidil treatment include allergic contact dermatitis, which precludes further use of the drug and reversible hypertrichosis. Minoxidil is contraindicated during pregnancy and lactation (Blumeyer et al. 2011).

Some patients may experience increased hair shedding during the first months of the treatment. This is transitory and only indicates that the drug is stimulating telogen follicles to reenter anagen.

Treatment should be continued for a minimum period of 12 months before deciding about the efficacy, although the first signs of hair regrowth can appear after 4–6 months of therapy. If the treatment prescribed is effective, it should be

maintained, since AGA will relapse after withdrawal of treatment (Tosti and Piraccini 1999).

Olsen et al. (1990) performed a 5-year follow-up of men with AGA treated with topical minoxidil and referred that hair regrowth with topical minoxidil tended to peak at 1 year with a slow decline in regrowth over subsequent years. There are several explanations as to why topical minoxidil could be less effective at 5 years than at 1 year. Perhaps this is secondary to tachyphylaxis or an obligatory cycling in hair growth. Topical minoxidil stimulates the growth of epidermal cells in culture and presumably stimulates hair growth by initiating and promoting the anagen phase of these epidermally derived structures (Olsen et al. 1987; Katz et al. 1987). However, at 4–5 years, maintenance of nonvellus hairs beyond that seen at baseline was still evident.

Finasteride is a synthetic drug approved by FDA in 1993 for therapy of mild to moderate AGA in men with 18 years and older. It is not indicated in women and is contraindicated in pregnant women, because of the risk of feminization of a male fetus (Blumeyer et al. 2011).

Finasteride is a type II 5-alpha-reductase inhibitor which decreases DHT in the serum, prostate, and scalp. A single oral administration of finasteride 1 mg decreases serum DHT as well as scalp DHT up to 70 % compared to baseline.

There is no known interaction with other drugs, such as warfarin, theophylline, digoxin, and propranolol (Blumeyer et al. 2011). Minimal period of use prior to assessing the efficacy is 6 months for reducing hair loss and 12 months for regrowth of hair.

Although it is not approved for AGA in men under 18 years of age, some physicians commonly used finasteride 1 mg off-label for this purpose.

Gonzalez et al. (2010) performed a study in which seven boys, aged 14–17 years, were treated with both 5 % minoxidil and finasteride solutions (1 mg daily), and two were treated with finasteride alone (18 and 19 years old). Follow-up was available for the six of nine patients treated with finasteride; all six boys (four also using minoxidil 5 %) reported increased hair density with no progression in hair loss. One experienced decreased sexual function that resolved despite continued finasteride use. According to authors, finasteride halted hair loss in six adolescent boys. More prospective data evaluating the safety and efficacy of finasteride in adolescent hair loss are needed.

Conclusion

The psychological and cosmetic importance of hair is immense in our society. Disruption in the normal appearance of hair can predispose to low self-esteem and negative body image (Moreno-Romero and Grimalt 2014).

AGA is the most common cause of adolescent hair loss. The teenagers who present with hair thinning can feel unattractive with a negative body image. This source of distress can lead to anxiety, depression, and social isolation.

The typical history of AGA is a chronic hair loss with thinning mainly over the frontal, parietal, or vertex areas. Family history is usually positive for AGA. In female patients, careful attention must be given to assess any associated hormonal dysfunction. If appropriated, further endocrinological investigations may be required.

There is no approved therapy for AGA in the adolescent population.

According to several studies and case series, topical minoxidil appears to be an effective and well-tolerated treatment for adolescents (girls and boys) with AGA (Gonzalez et al. 2010; Price 2003; Trancik et al. 2001a, b, c). Finasteride 1 mg has been used in boy adolescents with increased hair density and no progression in hair loss.

Treatment options for AGA should be further evaluated in the adolescent population to ensure safety and efficacy. Individualized consideration of attitudes, concerns, self-treating efforts, and expectations is crucial for effective management of AGA.

Bulleted List of Controversies

- Currently, there is no FDA-approved treatment for androgenic alopecia in adolescents. Although an off-label indication, physicians

commonly use topical minoxidil and oral finasteride with this purpose.

- Continued use of topical minoxidil seems to prevent further hair loss. Although success rates of minoxidil during long-term follow-up tend to be lower, maintenance of nonvellus hairs beyond that seen at baseline was still evident.
- Emotional stress is widely thought to be a common cause of telogen effluvium. However, how much the role of stress is actually responsible for increased telogen hair loss is not scientifically proven, and, until now, the research literature does not offer a completely consistent answer about this question.

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13.16 CHAPTER: PRP AND ALOPECIA

4. PRP in Alopecia

Rubina Alves, MD

Introduction

Beautiful and well-groomed hair represents youth, vitality and energy and is important in determining one's self-image and self-esteem. In today's society, body image is influenced by many factors, such as the media and the fashion industry; and hair plays an important role. People who have thinning hair or any type of hair disease may feel unattractive and have a negative body image.[1]

For some individuals, especially men, hair loss may be socially accepted as a natural process; for others, hair loss might have a significant impact on quality of life.[2] This source of distress can lead to anxiety, depression and social isolation for both men and women.[3-5] The associated emotional distress and body image concerns often motivate patients to seek professional consultation more often than the medical problem. Patients with hair loss disorders who seek consultation usually have high expectations and aim for rapid results. Medical therapies require continuous use and are limited by patient adherence.[6]

The evaluation of the patient, which includes a clinical history and a patient examination,[7] is a crucial step in making an accurate hair loss diagnosis. After an initial appointment, the clinician is often aware of the type of alopecia, the grade of severity and the disease evolution. However, when the diagnosis is uncertain, some other diagnostic tools (invasive or non-invasive) might be necessary, such as trichoscopy, trichogram and a scalp biopsy.

Hair loss encompasses a range of conditions, and different causes can lead to alopecia. Alopecia is divided primarily into non cicatricial alopecia and cicatricial alopecia. Cicatricial or scarring alopecia is usually a source of frustration for both the physician and the patient. Some conditions destroy the hair follicle and produce a scar. Other conditions produce permanent alopecia without scar evidence. The causes include lupus erythematosous, liquen planopilaris, pseudopelade, folliculitis decalvans and many others.[8] Scarring alopecia usually requires a pathological examination because the clinical appearance is usually undiagnosed.[9]

Noncicatricial alopecias include a number of different types of alopecias with many different causes. These types of alopecias include anagen effluvium, telogen effluvium, androgenetic alopecia and alopecia areata, among many others.

Hair cycle

The human skin supports approximately 5 million hair follicles, of which approximately 100,000 are on the scalp.[10] Human hair grows in a continuous cyclic pattern that consists of different phases, as follows: the anagen phase, which lasts 2 to 7 years, the catagen phase, which has a duration of 2-3 weeks, the telogen phase, which lasts 3 months, and the exogen phase.[11] Periods of active growth (anagen) are followed by a transitional physiological involution stage (catagen), in which the bulbar portion of the follicle is almost degraded, preceding the resting phase of the hair cycle (telogen).[12-15]

The growth and development of hair follicles is influenced by a number of different growth factors (GFs) and cytokines.[16] GFs appear to play a fundamental role in the life-long cyclic transformation of the hair follicle by controlling the anagen phase and promoting apoptosis to induce the catagen and telogen phases.[17,18] The main GFs involved in the establishment of hair follicles are vascular endothelial growth factors (VEGF), transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), insulin-like growth factor, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF).[19] According to a study performed by Akiyama et al.,[20] EGF and TGF are involved in regulating the growth and differentiation of bulge cells, and PDGF may have related functions in the interactions between the bulge and the associated tissues, starting with follicle morphogenesis.[20,21]

Platelet-rich plasma (PRP) is a plasma concentrate isolated from the patient's whole blood and is predominantly comprised of platelets.[22] Numerous GFs are present within platelet α granules. Upon activation, these GFs are released and promote hair growth by stimulating the hair follicle.

Androgenetic alopecia

Androgenetic alopecia (AGA) is the most common noncicatricial alopecia that leads to the progressive miniaturization of the hair follicle. Scalp terminal hairs are converted into vellus hairs, primarily due to two factors:

genetic predisposition and hormonal stimulation.[23] The incidence and prevalence of AGA increases with age.[24] The onset of AGA is gradual, and when this pathology progresses, the anagen phase shortens and the telogen phase remains constant. As a result, more hairs are in the telogen phase, and the patient may notice an increase in hair shedding.[14] The shedding area varies from patient to patient and is usually most marked at the vertex in men, while women with AGA generally lose hair diffusely over the crown (Figure 4.1).

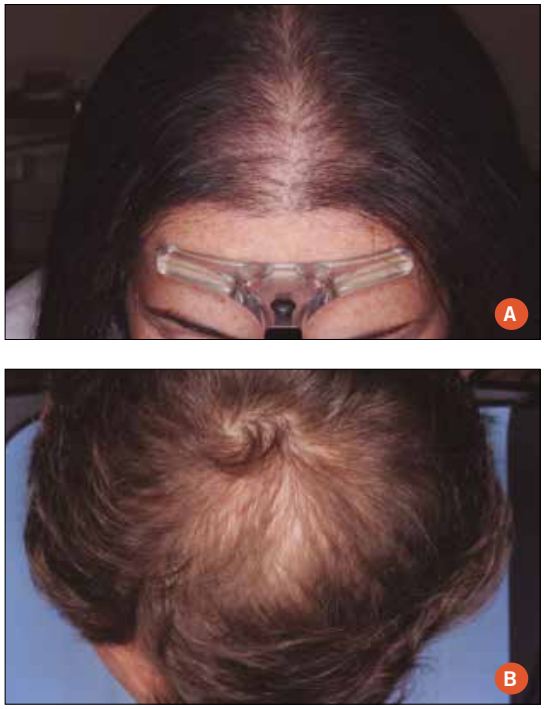

Figure 4.1. A) Decreased hair density over the frontal and vertex areas in a patient with female androgenetic alopecia. B) Hair loss at the vertex area in a 34-year-old man with AGA.

Most patients will be easily diagnosed by a single clinical examination, according to the condition of the characteristic distribution pattern.[2] Trichoscopy adds important information for establishing the correct diagnosis and it is also a useful tool for assessing disease activity and monitoring treatment efficacy. Male and female AGA share similar trichoscopic features, including hair thickness heterogeneity, thin hairs, vellus hairs, sin-

gle-hair pilosebaceous units, yellow dots, perifollicular discoloration, wavy hair and honeycomb pigmentation.[25] In a few cases, other diagnostic tools, such as trichogram or even a scalp biopsy, may be needed.

Management

Topical minoxidil and oral finasteride (5-alpha-reductase type II inhibitor) are the gold-standard therapies for AGA and, currently, the only Food and Drug Administration (FDA)-approved drugs for the treatment of AGA.

Topical minoxidil has been used as a treatment for hair loss since 1984.[26] Although the exact mechanism of action of minoxidil is not fully understood, minoxidil has vasodilatory, proliferative and anti-inflammatory effects.[2] Minoxidil is thought to promote hair regrowth through its ability to open potassium channels. The literature has shown minoxidil to be an effective treatment for AGA by promoting hair growth.[27] Minoxidil is available in 2% and 5% solutions and it is used twice a day in doses of 1 mL. Some clinicians prescribe minoxidil in combination with other active ingredients, such as tretinoin. A new formulation (a 5% foam that is administered once daily) has been shown to be effective, safe and well accepted cosmetically.[28]

Oral finasteride is a competitive and specific inhibitor of Type II 5α-reductase, which is an enzyme that converts the androgen testosterone into dihydrotestosterone (DHT). In men with AGA, the balding scalp contains miniaturized hair follicles and increased amounts of DHT if compared to the hairy scalp. The administration of finasteride decreases scalp and serum DHT concentrations in these men. Finasteride (1 mg/day) has been investigated extensively and it is widely accepted as one of the more effective therapies for AGA in men.

Oral dutasteride (type I and II 5-alpha-reductase inhibitor) has been demonstrated to be safe and efficacious in the treatment of AGA in both men and women, although this drug is prescribed as an off-label treatment. In a recently published clinical trial, Tsunemi et al.[29] reported that 0.5 mg of dutasteride exhibited long-term safety, tolerability and efficacy for male AGA. Another study performed by Boersma et al.[30] reported that 0.15 mg of oral dutasteride appears to be significantly more effective than 1.25 mg of oral finasteride in women under 50 years of age.

Both finasteride and dutasteride are frequently prescribed as off-label treatmentsfor women with AGA. These drugs cross the blood-brain barrier,

which means that they are contraindicated for use in women with childbearing potential and during pregnancy (Pregnancy Category X).[26] Thus, special attention is needed in fertile women of childbearing age, for whom contraceptive measures are obligatory.

Emerging treatments

The currently available treatments for AGA are sometimes perceived as having limited effectiveness; therefore, the identification of new therapies for this pathology is of utmost importance.[19,31] New possible treatments for AGA, such as topical finasteride, injectable dutasteride, laser therapy and PRP, have recently emerged and have been claimed to have hair growth-promoting or anti-hair loss effects.

Topical finasteride is being investigated as a new treatment for AGA and has fewer side effects than oral finasteride. According to Caserini et al.,[32] a daily application of a 0.25% solution of finasteride (doses of 100 and 200 µL) leads to the inhibition of scalp DHT and may minimize the untoward sexual side effects that are linked to systemic DHT reduction.[26] A randomized clinical study[33] compared oral finasteride and topical finasteride and showed similar efficacy for the two therapies. However, additional studies are needed to assess the efficacy of topical finasteride.

Injectable dutasteride might be a promising treatment for AGA. In 2013, Moftah et al.[34] published a study that evaluated the efficacy and safety of mesotherapy using a dutasteride-containing preparation (0.5 mg of dutasteride, 20 mg of biotin, 200 mg of pyridoxin and 500 mg of D-panthenol) in female patients with hair loss. The authors concluded that this preparation was effective, tolerable and yielded better response among females with a shorter duration of disease. A new study of the use of dutasteride alone is being developed to assess its efficacy.

Several *light and laser therapies* are becoming popular for AGA, especially as home wearable devices.[35] An evidence-based review[36] stated that low-level laser therapy (LLLT) devices are both safe and effective for the treatment of female and male pattern hair loss. The optimum wavelength, coherence and dosimetric parameters remain to be determined.

Platelet-rich plasma (PRP)

PRP has also been postulated as a new therapy for AGA.[19] But what is the evidence supporting the use of PRP in patients with AGA?

In 2006, Uebel et al.[37] published a study in which patients subjected to hair transplant surgery had their follicular units embedded in PRP before transplantation. According to the authors, this approach resulted in improved hair growth and an increase in follicular density. Moreover, the authors hypothesized that GFs could act upon dermic papilla (DP), leading to an intense neovascularization and the progression of new hairs to the anagen phase.

Three years later, Greco et al.[38] performed a study to determine if PRP had any effect on non-transplanted miniaturized hair. The authors believed that high concentrations of GFs could stimulate the follicular cells in the affected regions without the need for a hair transplant. The results of this small study revealed an average increase of hair shaft diameter after 6 months.

Both studies provided a new perspective concerning the application of PRP for alopecia, in both transplanted and non-transplanted patients.

The precise mechanism by which PRP promotes hair growth is not fully understood.[19] To explore the possible mechanisms involved, Li et al.[39] performed a well-designed study to investigate the effects of PRP on hair growth using *in vitro* and *in vivo* models. In the *in vitro* model, activated PRP was applied to human DP cells, were obtained from normal human scalp skin. The results demonstrated that PRP increased the proliferation of human DP cells by activating extracellular signal-regulated kinase (ERK) and Akt signalling, leading to anti-apoptotic effects. PRP also increased beta-catenin activity and FGF-7 expression in DP cells. Beta-catenin signalling is important in hair follicle development and for the hair growth cycle, whereas FGF-7, which is located in DP cells, prolongs the anagen phase of the hair cycle and delays progression into the catagen phase.[40] Concerning the *in vivo* model, mice injected with activated PRP showed faster telogen-to-anagen transition in comparison to the control group.

PRP has started to be considered as a potential therapeutic tool for promoting hair growth, and several studies have been published in recent years. Such studies included patients with AGA. After the injection of PRP in affected areas (typically after 3 treatments at 1-month intervals), the application of PRP was found to promote hair growth, with an increase in the total number of hairs and/or the hair density.

Cervelli et al.[41] conducted a controlled, double-blind study that included a clinical and histomorphometric evaluation of 10 patients with pattern hair loss. The injection of activated PRP into half of the head (the other half was treated with placebo) led to improvements in the mean hair count and mean hair thickness after three cycles of treatment. The authors also

found that PRP significantly increases the thickness of the epidermis, the number of hair skin follicles and the number of Ki-67+ keratinocytes (a marker for cell proliferation).

Readers searching for a concise description of the published articles related to the use of PRP in patients with alopecia should read the systematic review by Maria-Angeliki et al.[42] The authors performed an analysis of the literature regarding the PRP mechanism of action, preparation methods and therapeutic potential in patients with noncicatricial alopecias.The analysis included 12 articles about AGA and 2 articles about alopecia areata. In most, activation and preparation methods were not mentioned, and no standard protocol was employed regarding the frequency of PRP applications. As stated by the authors, controlled clinical trials are considered to be the best way to provide scientific evidence for a treatment and avoid potential bias when assessing efficacy.

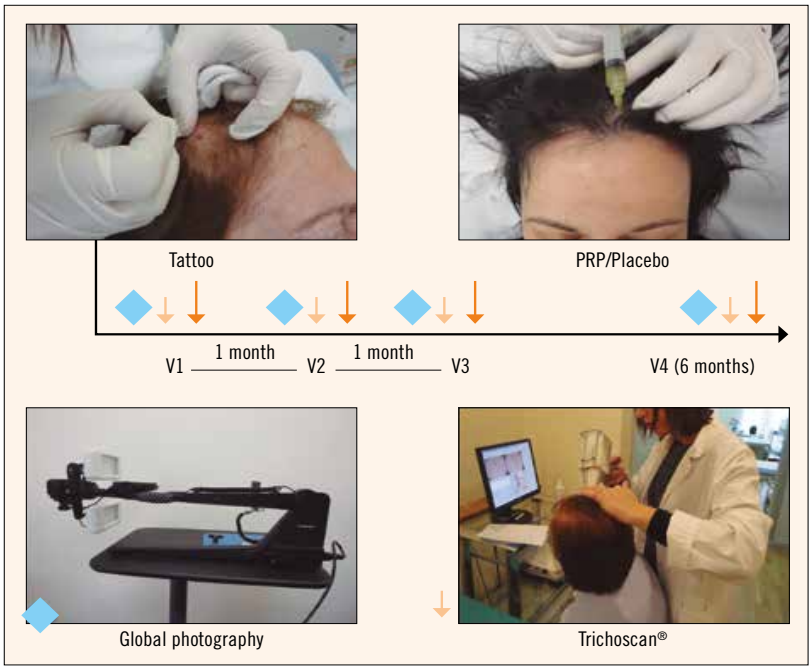

Figure 4.2. Illustration of clinical protocol. Patients received three cycles of treatment (1 month apart), and were observed for 6 months. The evaluation was assessed by global photography and phototrichogram at all visits. A dot tattoo guaranties the analysis of the same area to ensure the reproducibility of the study.

A randomized clinical trial (NCT:02393040) was performed by Prof. Grimalt and myself to assess PRP efficacy in patients with AGA. In our prospective, double-blind, placebo-controlled study,[19] we included 22 patients with AGA, according to the Nordwood-Hamilton (11 men) and Ludwig Classifications (11 women). The patients underwent treatment with pure PRP (P-PRP) on half of the head and a saline solution (placebo) on the other half of the head. A total of three treatments were administered (1month apart), and a follow-up visit was scheduled after 6 months (Figure 4.2).

A complete blood cell count was performed in all patients at baseline. The evaluation criteria were assessed by global photography and phototrichogram. Standardized images of 3 areas of the scalp (vertex, frontal, and occipital) and phototrichograms (epiluminescence microscopy with digital image analysis) were made of all patients at all visits. The data were achived by comparing the images obtained at baseline and the ones obtained after 3 and 6 months. The results of this clinical trial revealed that the administration of PRP led to a significant increase in the mean total hair density, the total terminal hair density and the number of anagen hairs after 3 months and after 6 months in comparison to the baseline. In addition, we observed an increase of the anagen/telogen ratio (%), which led to faster telogen-to-anagen transition, as described by Li et al.[39]

In the clinical trial, we used P-PRP but other authors have focused on other modifications or types of PRP, such as dalteparin/protamine and leucocyte PRP (L-PRP).[19] Takikawa et al.[21] performed a study to establish the efficacy of PRP-containing dalteparin and protamine microparticles (D/P MPs) for hair growth in comparison to PRP alone. Growth factors incorporated onto D/P MPs are gradually diffused and released upon the biodegradation of D/P MPs *in vivo*. According to the authors, hair growth was observed in all patients, but PRP in combination with D/P MPs appeared to generate more substantial changes than PRP alone. Schiavone et al.[43] presented the effects of L-PRP in 64 patients with AGA. The authors reported that patients treated with L-PRP showed "some degree of clinical improvement at 6-month follow-up". The improvement of AGA was assessed only through global photography (using the Jaeschke scale).

PRP and other medications

All of the articles discussed focused on patients who received treatment with PRP alone. Patients who received topical (such as minoxidil, prostaglandin, analogues, retinoids and corticosteroids) and/or systemic treatments for AGA (such as finasteride, dutasteride, and antiandrogens) in the

previous 12 months were typically excluded. Most patients with AGA are undergoing therapy for their alopecia, but at some point of the treatment, the condition appears to stabilize, as the improvement is slow. In these cases, both doctors and patients seek other alternatives.

Can PRP also improve hair regrowth if used simultaneously with the patient's current medication?

To answer this question, the second part of the clinical trial included patients treated with topical medication (5% solution of minoxidil) or oral medication (1 mg/day of finasteride). The present study included 24 patients with AGA, including 11 men and 13 women who were respectively diagnosed according to the Nordwood-Hamilton and Ludwig Classifications. Patients were instructed to maintain their usual AGA treatment throughout the protocol or excluded from the study. They received a cycle of three treatments (1-month interval) with P-PRP on half of the head, and saline solution (placebo) on the other half. A follow-up took place after 6 months. The evaluation criteria were assessed based on global photography and phototrichograms.

Based on the obtained data, treatment with P-PRP in combination with a concomitant medication (topical minoxidil or oral finasteride) significantly increased the number of hairs, hair density, terminal hair density and the number of anagen hairs in comparison to baseline and the control side. In addition, patients who received topical minoxidil along with PRP showed a greater improvement than patients who received PRP treatment in combination with oral finasteride after 6 months.

An intense vascular network characterizes the DP of the hair follicle during the anagen phase.[44] Lachgar et al.[45] demonstrated that minoxidil stimulates the production of GFs, such as vascular endothelial growth factor (VEGF), in cultured DP cells, and that effect might promote hair growth. This up-regulation of VEGF helps maintain DP vasculature and hair growth.[26] PRP also produces different GFs, leading to an intense neovascularization[37] and the increased proliferation of human DP cells.[39] This effect might support the hypothesis that PRP potentiates the effect of minoxidil by promoting the anagen phase and delaying the initiation of the catagen one. Although more studies are needed and the mechanism is not fully understood, the combination of topical minoxidil and PRP appears to lead to a stronger improvement of regrowth.

Prof. Ferrando et al.[46] published a study in which patients with AGA were treated with P-PRP and maintained their current medications (minoxidil

and finasteride). Patients received monthly sessions during the first 3 months, followed by 3 additional sessions (at 2-month intervals) and maintenance with 2 or 3 annual sessions. The authors reported the patients treated with PRP had a "notable improvement of their AGA of at least one grade on the Ebling's scale".

Thus, one might consider PRP to be safe and effective when used concurrently with the patient's current medication.

Other types of alopecia

The available articles concerning PRP use and other types of alopecia are sparse. Only a few articles discuss PRP efficacy in patients with alopecia areata.

Alopecia areata (AA) is a non-scarring and autoimmune pattern of alopecia. AA is a common disorder with several clinical presentations, which are usually classified according to the hair loss pattern or extent.[11] The most typical clinical appearance is a well-demarcated round or oval patch (single or multiple), without inflammation of the skin or scaling in hair-bearing areas. Most common sites are the scalp, eyebrows, beard, eyelashes and eyebrows. Trichoscopy can provide important information for the differential diagnosis of this disease. The presence of short 'exclamation mark' hairs at the periphery of the lesion is pathognomonic of AA. 'Yellow dots' are also frequently found in a follicular distribution.[11] The course of AA and the response to the treatment are unpredictable. Spontaneous regrowth sometimes occurs over several months.

The most frequently used treatments in patients with AA are topical or intralesional corticosteroids (triamcinolone acetonide) and systemic corticosteroids (continuous or as pulse therapy)[47] in more severe cases. Other available treatments include topical minoxidil, topical immunotherapy (squaric acid dibutylester), anthralin, phototherapy, immunosuppressants and immunomodulators.[48]

Regarding treatment with PRP in patients with AA, Trink et al.[31] published the first study to analyse the efficacy and safety of PRP for this type of alopecia. The authors randomized 45 patients to receive intralesional injections of PRP, triamcinolone acetonide (TrA) or placebo in one half of their scalp. The other half of the scalp was not treated. The obtained data showed that the administration of PRP increased hair regrowth in comparison to triamcinolone acetonide or placebo. In addition, the levels

of Ki-67 were significantly higher after PRP administration in humans. Finally, the subjective assessment of a burning or itching sensation decreased after treatment with PRP.

Another article[49] regarding PRP and AA included 20 patients for whom different therapies for AA had proved unsuccessful in the previous 2 years. The patients received a total of 6 PRP sessions at 1-month intervals. According to the authors, after 1 year of follow-up, only one of the 20 patients had a relapse.

Recently, Jeff Donovan described a case report[50] of a patient with corticosteroid-resistant ophiasis AA treated with autologous PRP. According to the author, PRP led to hair regrowth in the first month with "robust regrowth of hairs measuring 2.8 cm by month 3".

These studies demonstrated that PRP is effective for the treatment of patients with AA and that PRP could be a valid treatment option for patients with AA.

To date, no studies have investigated PRP and other types of alopecia. More clinical trials are needed to generate further evidence in the field of alopecias.

Protocol for treating alopecia with PRP

PRP is produced using different methods of platelet concentration via centrifugation and cell separation. First, it is important to choose one of the several commercially available systems. I personally recommend a closed system (majority) that includes disposable kits and everything necessary to prepare PRP. In addition confirm that the PRP systems and equipment used for separation, concentration and plasma collection meet all of the applicable quality criteria (EC certified system).

Patients should be informed about all the important information regarding the treatment and sign an informed consent form.

Standardized global photography of the scalp at baseline and at follow-up is important for monitoring treatment evolution (both for doctors and patients). The room lighting, the distance from the screen to the technician, and the enlargement of the projected photos should be standardized. Medical photography systems and software are available, such as the *Canfield Orthostatic Device®* and the *OMNIA Digital Imaging System*.

A phototrichogram analysis will provide objective data concerning the progression of hair loss. This tool is non-invasive and semi-quantitative, and the analysis can be performed easily.

The mode of PRP preparation can vary among commercial systems. It is important to follow the instructions for each device. A brief description of the protocol for preparing PRP using a certified closed system is presented below (Figure 4.3).

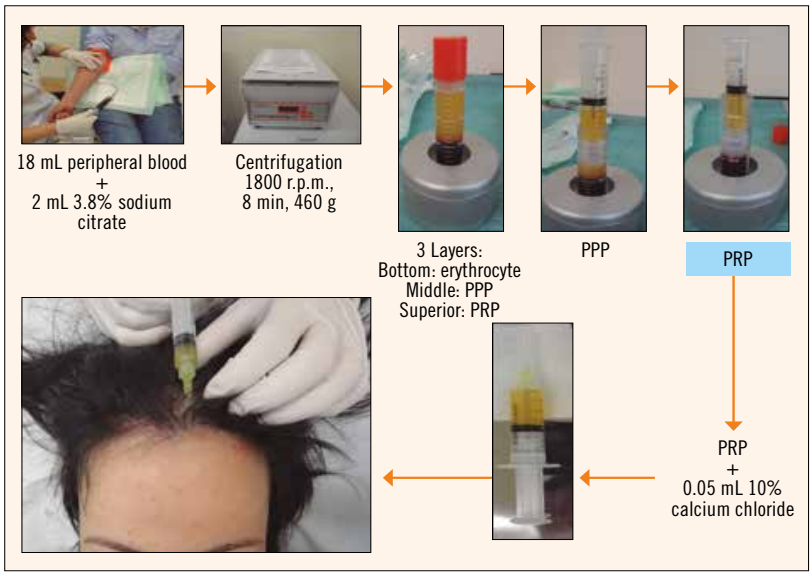

Figure 4.3. Illustration of PRP preparation using a closed system.

This procedure requires the use of relatively small volumes of blood: approximately 18 mL of blood is obtained from the median cubital vein and drawn into a syringe containing 2 mL of 3.8% sodium citrate. The tubes are centrifuged at 460 g for 8 minutes (*Omnigrafter-Proteal, Barcelona, Spain*). After processed, the blood presents in three basic layers: an erythrocyte layer at the bottom of the tube, a PRP layer in the middle of the tube, and a platelet-poor plasma (PPP) layer at the top. After removing the PPP layer (which represents approximately ¾ of the supernatant), the PRP is obtained (3-4 mL). Immediately before injection in the selected areas, the PRP is activated with 0.05 mL of 10% calcium chloride.

The PRP injections may be painful, so the local application of cold before starting the treatment (and after all injections) helps reduce pain. No injected anaesthesia is used.

In affected areas, approximately 0.15-0.20 mL of PRP per point of injection is administered. The space between injections varies from 0.5 cm to 1 cm, depending on the extent of the affected area (Figure 4.4).

Figure 4.4. Women with androgenetic alopecia after PRP treatment. The space between injections varies from 0.5 cm to 1 cm.

After treatment, no special care is required, and the patient can return to work immediately. No sedation or medications that inhibit the ability to drive or use machinery are given during the procedure.

According to the literature and personal experience, a minimum of 3 sessions should be performed 1 month apart. Then, at least 2 or 3 more sessions should be performed after 6 months to maintain the regrowth. As stated by Prof. Ferrando,[46] by performing monthly sessions for the first 3 months followed by 3 additional sessions (2 months apart) and maintenance with 2 or 3 annual sessions, one can achieve excellent results.

All steps of the treatment, including PRP preparation and administration will take approximately 20-30 min.

Conclusions

Based on the published literature and considering that PRP may act in dermal papilla cells to promote new hair growth, contribute to faster telogen-anagen transition and increase hair density, good results are expected with the use of PRP.

PRP treatment can be administered alone or in combination with other therapies for AGA.

As an autologous product, PRP has a good safety profile. To our knowledge, none of the available studies reported any adverse reactions.

To date, no ultimate protocol is available concerning the exact concentration and dosing parameters of PRP, the type of PRP and the number of sessions required for the treatment of androgenetic alopecia and alopecia areata. Efficacy appears to be dependent on the number of sessions, and a minimum of 3 treatments should be performed before achieving some hair growth.

Additional randomized clinical trials are needed, but PRP seemingly has a promising future in the field of alopecias.

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