5. PRP FOR THE TREATMENT OF ALOPECIA

5.1 PATHOPHYSIOLOGY OF ALOPECIA

5.1.1 Pathophysiology of androgenetic alopecia

Androgenetic alopecia (AGA) represents the most common type of progressive hair loss and one of the most common reasons for hair consultation (Kelly et al., 2016; Lolli et al., 2017). In men, hair loss typically involves the temporal and vertex region while the occipital region is spared, forming the characteristic "horseshoe" pattern (Lolli et al., 2017). In women, AGA produces diffuse thinning of the crown region, but the frontal hairline is maintained (Ludwig pattern AGA) (Piraccini and Alessandrini, 2014). AGA affects the quality of life and self-esteem of patients, notably by promoting self-consciousness, worries about aging, helplessness, and feelings of diminished attractiveness, which are often more pronounced in women (Adil and Godwin, 2017; Kelly et al., 2016) Fig. 30.

Epidemiologic data shows that 80 % of Caucasian men and 40–50 % of Caucasian women are affected by AGA over the course of their life, with prevalence increasing with age (Kelly et al., 2016). Prevalence in the Asian and African populations seems lower: AGA was observed in 14.1 % of Korean men at all ages, while its prevalence was found to be 14.6 % in African men and 3.5 % in African women (Kelly et al., 2016). In addition, predisposition to AGA is mainly genetic, as shown by the strong concordance rates (between 80 and 90%) for monozygotous twins (Lolli et al., 2017). Family analysis showed a significantly higher risk of AGA in men with a bald father and with a history of baldness on the mother side. The mode of inheritance however remains uncertain.

Current treatments for AGA such as Finasteride and Minoxidil aim to stop progression and prevent further thinning - improvement and regrowth may not always be achieved. However, these treatments, finasteride in particular, have several adverse effects that affect the patient's life. These treatments need to be taken long-term, even for life to prevent AGA progressing. Non-pharmacological options for AGA include lasers such as low-level light therapy, a fairly new technique used with different types of devices, such as a comb, hood, and helmet, and hair follicle transplantation (Adil and Godwin, 2017). For hair follicle transplantation, hair follicles located in androgen-insensitive areas are transplanted into androgen-sensitive areas of the scalp. Although hair transplantation is a very popular treatment for AGA, the quality of evidence on its efficacy is poor as studies have variable results due to differences in techniques and surgeon abilities as well as in individual characteristics of the patients. It has also been suggested that up to a third of follicles for transplantation display signs of inflammation, which may contribute to treatment failure (Navarro et al., 2018; Nirmal et al., 2013).

5.1.2 Pathophysiology of alopecia areata

Alopecia areata (AA) is an acquired autoimmune skin disorder characterized by patches of hair loss (Fig. 31). It has similarities to vitiligo, another acquired autoimmune disorder, in that it has no or minimal symptoms, hair loss occurs in patches although it can progress to total hair loss, can occur at any age and in any individual but the prevalence is higher in those under 30 years of age, may be associated with other autoimmune diseases and result from an immune attack in genetically susceptible individuals (Barbulescu et al., 2020; Gilhar et al., 2012). An estimated 4.5 million people in the US are affected by this disorder and it has a significant impact on quality of life (Gilhar et al., 2012). Both vitiligo and AA have also been described as TH1-driven diseases, based on the involvement of CD8+ T cells and the clear, consistent production of IFN-γ within lesional skin (Harris, 2013). In AA, the immune attack is directed

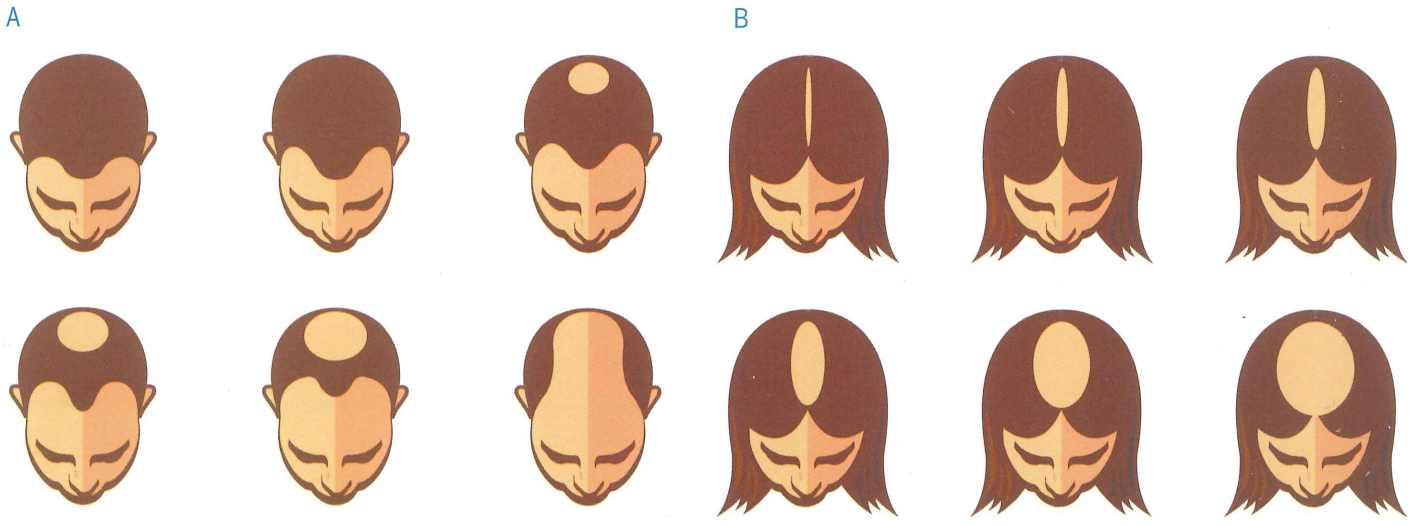


Figure 30: The progression of androgenetic alopecia in males (A) and females (B)

against the dermal hair follicle bulb and in the acute stage an immune infiltrate surrounding the hair bulb of anagen follicles is observed (Marchitto et al., 2019). It has been proposed that since it is primarily the pigmented anagen hair follicle that is targeted in AA, that autoantigens associated with melanogenesis may be involved (Barbulescu et al., 2020; Gilhar et al., 2012).

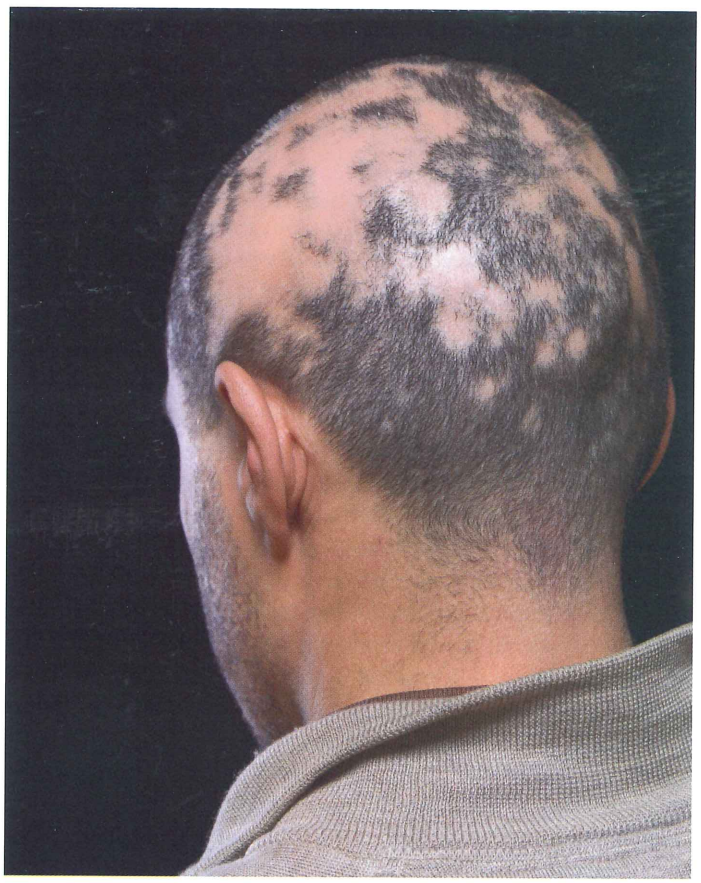


Figure 31: Example of a patient suffering from alopecia areata

There is currently no cure for AA and the available treatments aim to halt the immune attack through the use of immunosuppressive agents and/or to encourage hair growth. This can be successful in 25-67% of patients depending on the product used, but relapse is expected once treatment is discontinued (Gilhar et al., 2012). Minoxidil, commonly used in the treatment of AGA, has also been used for treating AA hair loss. It has been shown to stimulate hair growth in patients with AA but has not given cosmetically acceptable results when used as a monotherapy. New treatments for AA are focused on halting the immune process and activating new bulbs in the anagen phase, and include narrow-band UVB phototherapy, JAK inhibitors, bimatoprost, PRP and minoxidil (Barbulescu et al., 2020). NB-UVB phototherapy activates melanocyte precursors and can modulate the immune response; however, while this treatment approach has demonstrated some benefits in vitiligo patients, it does not appear to be effective for AA patients (Bayramgürler et al., 2011). JAK inhibitors may act to promote the telogen-anagen transition and activate Wnt signaling, which induces proliferation, migration and differentiation of melanocyte precursors needed for epidermal melanocyte regeneration (Barbulescu et al., 2020; Birlea et al., 2017). Hair regrowth has been reported in adults and children with moderate-to-severe AA treated with JAK inhibitors but hair shedding occurs when treatment is discontinued (Barbulescu et al., 2020). Bimatoprost has been reported to increase blood flow to the hair follicle and directly affect the hair follicles themselves by activating the pathways promoting the telogen-anagen transition and anagen duration and has had a demonstrative effect on hair growth (Barbulescu et al., 2020). The rationale for the use of PRP for the treatment of AA is discussed in section 5.3. A recent systematic review of the literature of non-steroidal treatments for AA concluded that PRP demonstrates comparable efficacy to triamcinolone injections and superior efficacy to topical minoxidil 5% (Marchitto et al., 2019). More randomized clinical studies are needed to confirm the efficacy of PRP in AA.

5.1.3 Pathophysiology of endocrine-induced and chemotherapy-induced alopecia

Alopecia is a side-effect of certain therapies for cancer, particularly endocrine therapy, and chemotherapy. Endocrine therapy may be used for the treatment of hormone-sensitive cancers, such as breast cancer. In this case, the aim of the therapy is to reduce the circulating levels of estrogen. Tamoxifen is the most common treatment used for breast cancer. It is a selective estrogen receptor modulator that binds to the estrogen receptor thereby blocking its transcriptional activity (Clifford et al., 2020). Therefore, one way tamoxifen may cause endocrine-induced alopecia (EIA) is by binding to estrogen receptors in the hair follicle, which may result in an arrest of hair follicle growth (Bhatia et al., 2010). Aromatase inhibitors are another endocrine therapy that induce hormonal changes that cause androgenization of hair follicles. It has been observed that the EIA resembles that of female pattern androgenetic alopecia leading to the proposition that EIA may be a result in an artificial shift in the androgen:estrogen ratio resulting in hair loss (Alonso and Rosenfield, 2003). A meta-analysis indicated that the incidence of EIA in cancer patients is 4.4% (Saggar et al., 2013). This can have a devastating impact on patients and a study found that 8% of patients discontinued endocrine therapy secondary to alopecia (Freites-Martinez et al., 2018; Moscetti et al., 2015).

Chemotherapy-induced alopecia (CIA) normally occurs within weeks of initiating the chemotherapeutic drug and approximately 65% of patients may be affected (Freites-Martinez et al., 2019a). Complete hair loss can occur within 2-3 months. Two types of damage response pathways may be initiated depending on whether the hair matrix or hair structure is affected and this may determine whether the CIA is permanent or not (Paus et al., 2013). Chemotherapies that target rapidly proliferating cells, such as tumor cells, also target rapidly dividing cells like keratinocytes and melanocyte stem cells in healthy pigmented mature anagen follicles. Whether hair re-growth occurs may depend on the extent to which the hair follicle stem cells and hair matrix cells of the hair bulb are damaged (Paus et al., 2013). Patients receiving taxane-based chemotherapies appear to have a much greater risk of developing permanent CIA (Kang et al., 2019).

Treatment for EIA follows that of AGA, with topical minoxidil 5% solution resulting in a moderate to significant improvement in 80% of patients with EIA (Freites-Martinez et al., 2018). Systemic treatment with spironolactone 200 mg daily can be used if topical treatment with minoxidil is insufficient – this has resulted in stabilization in 44% of patients and hair regrowth in 44% of patients with female pattern AGA (Sinclair et al., 2005). Minoxidil appears to be more effective in patients with EIA than patients with CIA (Freites-Martinez et al., 2019b). Spironolactone used either alone or in combination with minoxidil 5% can result in a moderate or significant clinical improvement in 60%

of patients with either EIA or CIA, although this is primarily in patients with alopecia grade 1 (Freites-Martinez et al., 2019b). There is also a possible risk of hormonal stimulation of endocrine-sensitive tumor cells with the use of certain systemic therapies for alopecia which should be considered as well (Freites-Martinez et al., 2019b). Once these options are exhausted, there is no other approved treatment available for CIA. The only other approach in use is prophylactic in nature and involves scalp cooling during treatment to induce vasoconstriction and thereby limiting blood flow to scalp and reducing contact of the hair follicles with the chemotherapeutic agents (Freites-Martinez et al., 2019a). Thus, new approaches are urgently needed, and one of these under investigation is the use of PRP.

5.2 RATIONALE FOR THE USE OF PRP FOR ALOPECIA

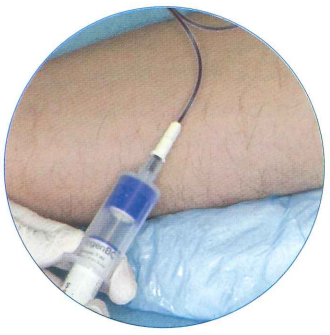
The pathophysiology of alopecia appears to be very complex, with many etiologic factors involved. At the heart seems to be an immune response, that may differ depending on the type of alopecia, and perturbation of the signaling pathways needed for regeneration and normal growth of the hair follicle. The growth factors released from PRP could have several positive benefits on hair follicle regeneration, such as activation of the Wnt signaling pathway needed for melanocyte and keratinocyte proliferation and initiation of hair follicle development (Andl et al., 2002; Barbulescu et al., 2020; Xiao et al., 2019), differentiation and survival and restoring the normal telogen-to-anagen transition, and may increase the duration of the anagen phase. For example, platelet-derived growth factor (PDGF), one of the factors released by activated platelets has been demonstrated to maintain the anagen phase of murine hair follicles (Tomita et al., 2006). Other factors in PRP, such as VEGF, may induce neovascularization and improve blood flow to the hair follicle (see section 3.6 for a more detailed discussion of the effects of PRP on hair growth and other possible etiologic factors of alopecia).

There is great interest in using PRP for the treatment of alopecia due to the low risk of side effects and the need for less frequent application than current treatments. However, despite promising results in clinical trials, a standard protocol regarding the dose, frequency and depth of injection to be used has not yet emerged. This has been impeded due to the myriad of PRP preparations and procedures being used which makes interpretation and comparisons between studies difficult. Moreover, the etiology of alopecia is complex, and treatment may need to be adapted depending on the stage of the disease. Thus, there is an urgent need for the standardization of PRP preparations and the design of appropriate clinical trials with the necessary controls to evaluate the efficacy of PRP for treatment of hair loss. Nonetheless, despite these limitations, a recent systematic review concluded that PRP has demonstrated clinical efficacy and recommends 3 to 4 monthly sessions for the treatment of alopecia (Hesseler and Shyam, 2020). Regen Lab has initiated studies using the standardized RegenPRP in collaboration with Dr Jerry Shapiro, NYU Langone, USA, with the results from the first study published in 2020 (Shapiro et al., 2020). Other studies will follow from the same center of investigation.

The anti-inflammatory activity of PRP may be of particular interest for the treatment of alopecia areata, which is characterized by an extensive inflammatory infiltrate (Barbulescu et al., 2020). However, its effects on proliferation, angiogenesis and modulation of the hair cycle likely also play a role. There are several reports that have already demonstrated that PRP treatment is superior to existing treatments such as minoxidil 5% or corticosteroid therapy (Barbulescu et al., 2020; El Taieb et al., 2017; Shumez et al., 2015; Trink et al., 2013) but further work is needed to elucidate the mechanisms behind these effects.

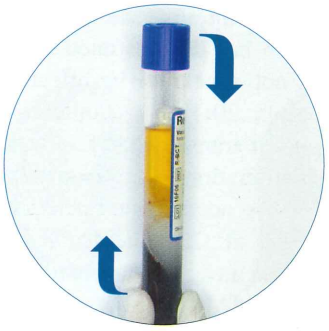
PRP is being investigated as a therapeutic option for endocrine-induced and chemotherapy-induced alopecia. Endocrine therapy that works by blocking estrogen receptor activity may interfere with key signaling pathways involved in estrogen-receptor-mediated regulation of hair growth, including Wnt signaling (Ohnemus et al., 2006). It is known that PRP contains growth factors and other molecules that can activate Wnt signaling as well as other critical pathways for hair morphogenesis. Moreover, PRP has demonstrated efficacy in the treatment of female pattern AGA, whose pathomechanisms resemble that of EIA (Alves and Grimalt, 2016). Whether the molecules and growth factors in PRP can by-pass the endocrine therapy-induced estrogen-receptor blockade and reinitiate hair growth remains to be determined. Treatment of CIA with PRP may prove more challenging. An in vivo study in rats found that PRP was unable to prevent CIA in rats treated with a mild chemotherapeutic agent. Only mononuclear cells were able to provide protection against CIA (Stamatiou et al., 2020). The protective effect of mononuclear cells is believed to be due to their ability to produce large amounts of IL-1 (Stojadinovic et al., 2017). The cellular composition of the PRP used by (Stamatiou et al., 2020) was not reported. However, as both leukocyte-poor and leukocyte-rich preparations of PRP can be made, it may be worth exploring whether a leukocyte-rich PRP has a protective effect.

5.3 REGENPRP FOR THE TREATMENT OF ANDROGENETIC ALOPECIA

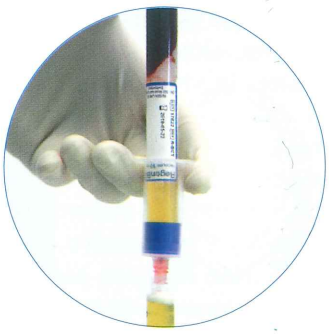


Blood Collection

Centrifugation



Platelet Resuspension



Ready to Use

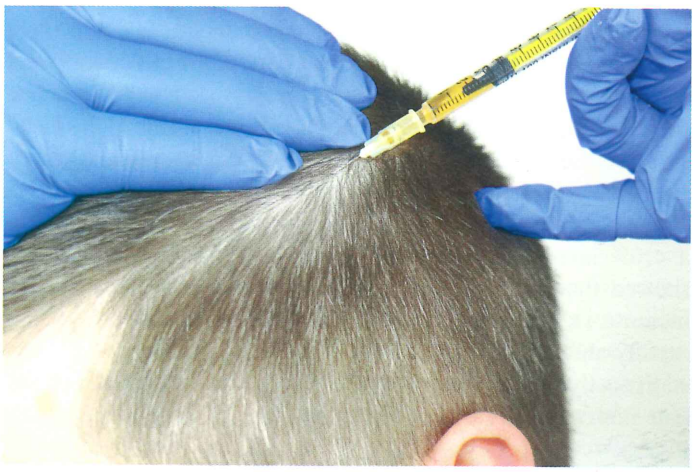


Figure 32: Platelet-rich plasma preparation and intradermal injection for alopecia indications

A recent review and meta-analysis of clinical studies investigating the efficacy of PRP as a treatment for hair loss revealed that of 23 studies meeting the inclusion criteria, among them 4 (17%) used Regen Lab devices for preparation of PRP (Fig. 32). Studies in this review differed in the frequency of injections, depth of injections and whether activated or non-activated PRP was used (Gupta et al., 2019). Nonetheless, there is a growing body of evidence that RegenPRP can improve hair growth in patients with AGA. A summary of the protocols used for treating patients using RegenPRP is given in section 5.7 and the results from the different studies are presented here.

5.3.1 RegenPRP as a monotherapy

Shapiro et al. (2020) conducted a randomized controlled, split-scalp study in which 35 study participants were injected with RegenPRP on one side of the scalp and saline on the other. Subjects underwent three sessions of PRP treatment at monthly intervals with evaluation three months after the final treatment. Hair density in the PRP-treated area was significantly increased compared to baseline at all visits. At the final assessment, hair density in PRP-treated areas increased from 151 ± 39.82 hairs/cm^2 at baseline to 170.96 ± 37.14 hairs/cm^2, a mean increase of approximately 20 hairs/cm^2 ($p < 0.05$). However, hair density in placebo-treated areas also increased, albeit less than on the PRP-treated side, and there was no significant difference in hair density between the PRP and placebo-treated areas. The lack of significant difference in hair growth between the two sides may have been due to PRP diffusion as well as regenerative processes induced by the microinjections. No serious adverse events were reported in this study.

Kadry et al. (2018b) assessed the efficacy of PRP and adipose-derived stem cells (ADSC) for the treatment of AGA. In this study, 60 patients were randomly assigned to receive a scalp injection of either PRP (n=30) or ADSCs and the stromal vascular fraction. PRP was administered intradermally, and patients received a total of three treatment sessions at monthly intervals. At the 3-month follow-up, there was a significant increase in the terminal hair count in both groups. However, although both groups showed an increase in the intermediate hair count and hair diameter, this was only significant in the ADSC-treated group.

Rossano et al. (2017) reported their clinical results from a retrospective study, conducted on 41 patients suffering from AGA that were treated with RegenPRP that was activated prior to use. All patients received three sessions of PRP injections at 3-5 weeks intervals, followed by a booster session at 6 months. Clinical evaluation at all time points was based on the hair pull test, macroscopic photographs, photomicrographs, and a patient satisfaction questionnaire. Overall, PRP treatment led to satisfactory clinical improvements, with a regression of alopecia in 32/41 (78%) patients at 1-year follow-up. Interestingly, the authors identified a correlation between a cytokine IL-1α polymorphism and responsiveness of patients to PRP injection.

Betsi et al. (2013) reported their clinical results of a case series of 42 consecutive patients suffering from AGA and treated with non-activated RegenPRP. All patients received 6 sessions of PRP injections over 2 months. Clinical evaluation was based on subjective outcomes only such as the hair pull test, global pictures, and patient satisfaction. Hair loss evaluated by the pull test significantly decreased to normal levels after the third injection, and global pictures revealed improved hair volume and quality, while 90.5% of patients noticed improvements in hair regrowth and strength. At 3 months, patients had an overall satisfaction score of 7/10.

Gkini et al. (2014) reported their clinical results of a case series of 20 consecutive patients suffering from AGA that were treated with RegenPRP that was activated prior to use. All patients received 3 sessions of PRP injections at 3-week intervals, followed by a booster session at 6-months. Clinical evaluation was based on subjective outcomes only such as the hair pull test, dermoscopic photomicrographs, global pictures, and patient satisfaction. Hair loss had decreased to normal levels at the 6-week and 3-month follow-ups before rising again but remained below baseline levels at 6 months and 1 year. Hair density had increased significantly at the 6-week follow-up and this improvement was still significant at the 1-year follow-up. Overall, 85% of patients reported improvement in hair quality and thickness, 65% an improvement in hair density, and the satisfaction score was 7.1/10.

Borhan et al. (2015) reported their clinical results of a case series of 14 consecutive male patients suffering from AGA treated with non-activated RegenPRP. All patients received 4 sessions of PRP injections at 3-6-week intervals. Clinical evaluation at 4 months involved objective quantitative measures of hair density using the TrichoScan device, subjective assessment of hair quality by investigators and self-assessment of hair quality, volume, shedding, pigmentation, and hair growth. TrichoScan measurements revealed an increased hair density in 11/14 (78.8%) of patients at 4-months follow-up, leading to a self-assessed improvement of hair quality in 100% of patients, a decrease of hair shedding in 71.4% of patients, hair growth in 50% and an improvement in hair volume in 35.7% of patients after PRP treatment.

Ayatollahi et al. (2017) reported their clinical results of a case series of 13 consecutive male patients suffering from MAGA who were treated with activated RegenPRP. All patients received 5 sessions of PRP injections at 2-week intervals. Clinical evaluation was based on objective measurements of hair density and diameter and patient's satisfaction. At the 3-month follow-up, no significant differences were observed in hair density and thickness, while the anagen/telogen ratio significantly decreased. The global physician assessment improved mildly in 46.2% of

patients only. As a result, patient satisfaction was good during the study but decreased at the last follow-up.

5.3.2 RegenPRP in conjunction with other treatment modalities

RegenPRP has also been investigated when used in conjunction with minoxidil. Ho et al. (2020) conducted a retrospective study of 24 patients suffering from AGA. All patients received at least 2 sessions of RegenPRP injections at 1-month intervals. Responders were then selected to receive monthly injections for 4 months followed by maintenance injections every 3-6 months for up to 24 months. All patients received concomitant treatment with minoxidil, and most used oral anti-androgen medications. Clinical evaluation involved objective measurements of hair density and diameter using the Folliscope device (Folliscope, Seoul, South Korea). At the 2-month follow-up, 70.8% of patients exhibited a positive response to PRP, and hair density was significantly increased at follow-up (mean was 6 months; range was 2-24 months).

Juhasz et al. (2020) conducted a retrospective review of patients with AGA that had been treated with RegenPRP using intradermal scalp injections (0.1 mL RegenPRP, 1 cm apart) in the affected areas (n=104). The majority of patients were female with a mean age of 46.0 ± 16.9 years. Almost all patients (n=97) were using other AGA-directed therapies prior to initiating PRP treatment. Of these 97 patients, 17 had had only one PRP treatment session and could not be evaluated and 3 patients with concomitant scarring alopecia did not have trichologic measurements. The patients included in the study (n=77) had received a total of two injections at 4 – 6-week intervals. At this point, the patients were clinically assessed and if a clinical response was observed (i.e., an increase in hair density by 10 hairs/cm^2 from baseline) the patients received four additional PRP injections at monthly intervals, and then received maintenance injections every three to six months. After two treatment sessions, 70% of patients were classified as a PRP-responder. Hair density in these patients had increased by 21.2 ± 12.3%, with 2 patients achieving a 40-49.9% increase and one patient achieving a >70% increase in hair density. Just over half of the non-responders demonstrated a decrease in hair density from baseline, 13% had no change and 34.8% had a <10% increase. Further analysis of the PRP-responder group showed that patients with baseline lower hair density had an increase in hair growth compared to those with normal hair density and subjective thinning although this difference was not statistically significant (p=0.052). No significant improvement of hair shaft diameter was observed in either the PRP-responder or PRP non-responder group.

5.3.3 RegenPRP in hair transplantation

PRP can also be considered for use in hair transplantation, both as a holding solution for the extracted hair follicles and for injection into the area to be treated (Fig. 33).

Cole et al. (2017) examined the survival rates of hair follicles transplanted in the scalp of patients with stage 6 AGA following pre-injection of platelet lysate (PL), activated RegenPRP and saline in the designated areas for transplantation. The transplanted follicular unit growth was highest in regions treated with PL. Although PRP appeared more successful than the control at 14 weeks, there was little disparity between these groups at 18 weeks.

5.4 REGENPRP FOR THE TREATMENT OF ALOPECIA AREATA

Khademi et al. (2019) conducted a pilot study to investigate PRP as a treatment approach for alopecia areata totalis. The study

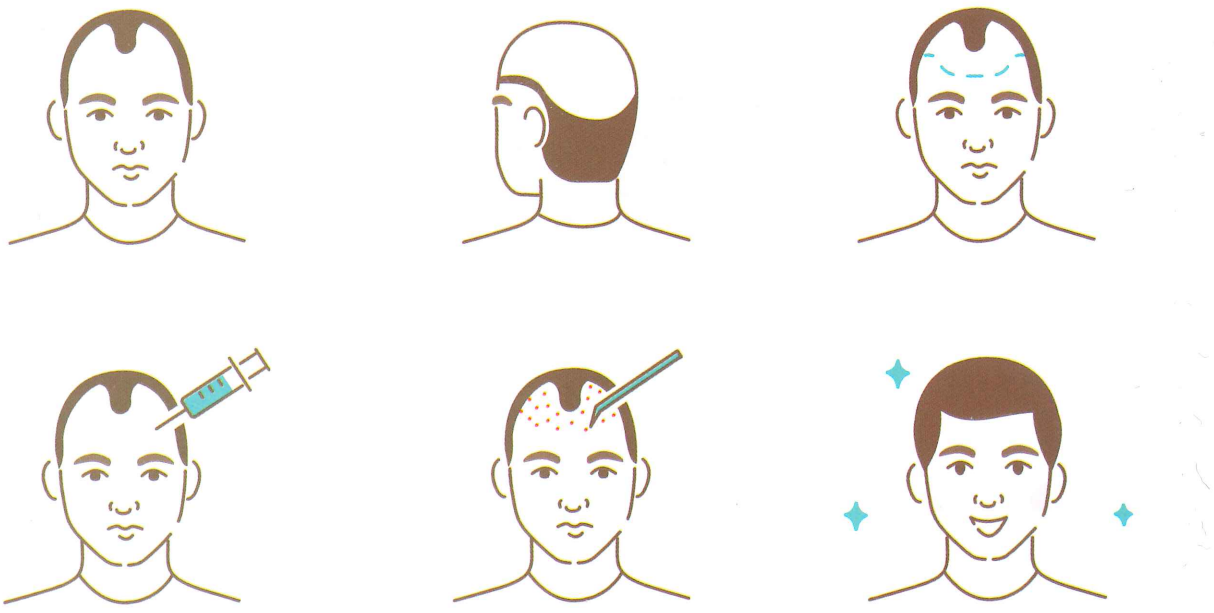


Figure 33: After harvesting, the hair follicles can be stored in a PRP holding solution, which has the advantage of maintaining the viability of the grafts (Garg and Garg, 2019). PRP can also be injected in the areas of the scalp to be treated prior to transplantation of the graft

participants (5 males and 5 females with a mean age of 28.9 ± 6.28 years) had a clinical diagnosis of AA totalis for at least 3 years and had not received any treatment in the three months prior to the start of the study. PRP was prepared according to the manufacturer's instructions and the PRP was collected in 4 mL insulin syringes containing 0.1 mL calcium bicarbonate as a platelet activator substance.

The scalp of each patient was divided sagittally into approximately equal parts and RegenPRP was injected intradermally into either the left or right side of the scalp using a point-by-point technique with a distance of 1.5 – 2 cm between each point. At each point 0.1 mL of RegenPRP was injected for a total volume of 4 mL. Patients were assessed monthly for 4 months. Assessment was by comparison of photographs taken prior to treatment and at each follow-up visit. Each photograph was scored by a dermatologist blinded to the therapy and the score ranged from 100% (complete hair loss) to 0% (complete hair coverage). The severity of AA and the clinical response before and after intervention were also assessed using the severity of alopecia tool (SALT).

In two patients less than 10% hair re-growth was observed, whereas the other patients did not show any hair regrowth and there was no significant change in SALT score 4 months after treatment. No adverse effects were observed during the 4 months of follow-up.

5.5 REGENPRP FOR THE TREATMENT OF CHEMOTHERAPY-INDUCED ALOPECIA

A new pilot study has been initiated by Dr Anthony Rossi at the Memorial Sloan Kettering Cancer Center, USA to investigate the clinical effectiveness of RegenPRP for the treatment of EIA and permanent CIA in breast cancer patients. For this study, it is planned to recruit breast cancer patients, aged 18 years and older that are chemotherapy naïve with EIA (n=14) or have permanent CIA without the use of endocrine therapy (n=14), who have been using minoxidil and/or systemic spironolactone for alopecia for at least 3 months without success. These treatments will be stopped 3 months prior to beginning treatment with PRP. Study participants will receive PRP injections into one half of the scalp and no treatment will be given to the other side. Patients will receive a total of 3 injections at monthly intervals. Assessments will be performed at baseline, and at weeks 12 and 24. To date, 50% of the planned cases have been treated.

5.6 DISCUSSION

Taken together, these results prove the effectiveness of RegenPRP injected intradermally for the treatment of androgenetic alopecia with proven mid-term benefit on objective and subjective outcomes observable up to 12 months after the procedure. RegenPRP can also be used as an adjunct to other treatment modalities such as minoxidil and follicular hair unit transplantation. Interestingly one of the studies demonstrated a polymorphism that affected response to PRP (Rossano et al., 2017). Studies examining the efficacy of PRP for the treatment of AGA have been confounded by patient variables that may affect the outcome, such as duration of the hair loss and stage of the disease (Alves and Grimalt, 2016; Qu et al., 2019). In addition, different protocols, such as the interval between injections and the number of injections may affect clinical outcomes, even if the same protocol for preparing PRP is used.

PRP is also being explored for other alopecia indications for which treatment options are limited. At this point it is premature to speculate on the effectiveness of PRP in these cases due to the lack of data as well as the need to optimize procedures and standardize PRP preparations.

RegenLab kits produce a standardized PRP and this is the first step to the development of standardized protocols for AGA and other forms of alopecia. This is crucial as there are few published long-term, placebo-controlled studies on the use of PRP for the treatment of alopecia, and understanding the components needed for restoring hair growth is essential for optimizing use of PRP for treatment of this disorder.

5.7 Regen Lab Medical Device Clinical Evaluation in Trichology

11 publications were identified that have used RegenKits for the treatment of androgenetic alopecia (AGA) and alopecia areata (AA), including clinical data on 338 patients.

Safety: Safety data included mainly erythema, headache and drowsiness, and mild pain due to scalp sensitivity to the injection procedure; all effects were transient and resolved spontaneously. No serious AE was reported.

Performance: Performance data showed that PRP treatment has a beneficial effect for areas of the scalp affected by androgenetic alopecia, improving quality, density and strengthens of hair, and reducing simultaneously hair loss.

>> These data contribute to the safety and performance profile of PRP prepared with RegenKits.

The key results described in publications are summarized in the following table:

Alopecia

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Evaluation of platelet-rich plasma as a treatment for androgenetic alopecia: a randomized controlled trial Shapiro J, Ho A, Sukhdeo K, Yin L, Lo Sicco K. *J Am Acad Dermatol* 2020; 83: 1298-1303	Randomized controlled clinical study Follow-up: 3 months from the end of treatment	Androgenetic alopecia	35	PRP arm RegenBCT Comparator arm Saline

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared as per instructions for use from 10 ml of whole blood. Prior to injection, the scalp was disinfected and anesthetized with topical lidocaine cream if subject requested. The placebo and PRP were injected at a depth of 3-4 mm (angle 35° to 45°) at a quantity of 0.1-0.2 ml per injection/cm^2 in the pre-designated areas. Excess product was allowed to dry on the scalp. Subjects were requested not to wash their hair for four hours following treatment. Subjects received a total of three treatment sessions at 1-month intervals.	At the final assessment, hair density in PRP-treated areas showed a mean increase of approximately 20 hairs/cm^2 ($p< 0.05$). The mean increase was greater than that seen in placebo-treated areas, but not significantly so. Significant increases in hair diameter were also observed compared to baseline in both treated areas but there was no significant difference between the PRP- and placebo-treated areas	The main reported side effects were pain during the procedure (91.4%) and sensitive scalp or head post-procedure. About half of the patients found the treatment moderately comfortable or tolerable. No serious adverse events were reported.

Alopecia

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Autologous adipose derived stem cell versus platelet rich plasma injection in the treatment of androgentic alopecia: efficacy, side effects and safety Kadry MH, El-Kheir WA, El-Sayed Shalaby M, El-Shahid AR, Metwally HG. *J Clin Exp Dermatol Res* 2018; *9*: 1000447	Randomized comparative clinical study Follow-up: 3 months (from end of treatment)	Androgenetic alopecia	60	PRP group (30 patients) RegenKit Comparator group (30 patients) Autologous adipose derived stem cells (ADSC)
Alopecia and platelet-derived therapies Cole JP, Cole MA, Insalaco C, Cervelli V, Gentile P. *Stem Cell Investig* 2017; 4: 88	Comparative Case series Follow-up: 3.5-7 months	Androgenetic alopecia	3	RegenBCT
The effect of platelet rich plasma on hair regrowth in patients with alopecia areata totalis: a clinical pilot study Khademi F, Tehranchinia Z, Abdollahimajd F, Younespour S, Kazemi-Bajestani SMR, Taheri K. *Dermatol Ther* 2019; 32: e12989.	Prospectve case-series- (Pilot study) Follow-up: 4 months	Alopecia areata totalis	10	RegenTHT

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared as per instructions for use from 8 ml of whole blood. PRP was administered intradermally using a 30G needle. Injections were spaced about 1 cm apart with 0.1 ml PRP delivered per injection. Patients received a total of three treatment sessions at 1-month intervals.	The mean terminal hair count was significantly increased at the final follow-up compared to baseline in both groups. The intermediate hair count and hair diameter were also increased in both groups, but this was only significant in the ADSC -treated group.	All patients treated with PRP reported pain, but this subsided shortly after treatment in the majority of cases and within 48 hours in the others. Headache and itching were reported in a few cases. No serious adverse events were reported. The ADSC treated group also reported pain, headache and erythema after treatment but no serious adverse events reported.
PRP was prepared from 24 ml of whole blood using three collection tubes. The top 2 mL of PRP was discarded from each tube before resuspension of the platelets. PRP was activated with calcium gluconate (10%) just prior to use. PRP was injected using a 25G needle at a depth of 3 mm prior to follicular unit transplantation.	PRP did not significantly improve the outcome of hair follicular unit transplantation compared to the control.	Not formally assessed.
PRP was prepared from 8 ml of whole blood. PRP was activated using calcium bicarbonate. The scalp of each patient was divided sagittally into two approximately equal parts. In each patient, 4 ml PRP was injected intradermally into the left or right side of the scalp at several points 1.5-2 cm apart. At each point, 0.1 ml PRP was injected.	No significant effect of PRP on hair growth was observed.	Not formally assessed

Alopecia

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Platelet-rich plasma by single spin process in male pattern androgenetic alopecia: is it an effective treatment? Ayatollahi A, Hosseini H, Shahdi M, AhmadNasrollahi S, NassiriKashani M, Yadangi S, Firooz FH, Firooz A. *Indian Dermatol Online J* 2017 ; 8 : 460-4	Prospective case series Follow-up: 3 months (from the end of treatment)	Androgenetic alopecia	15	RegenACR
Platelet-rich plasma injection is effective and safe for the treatment of alopecia Betsi E-E, Esnault G, Kalbermatten D, Tremp M, Emmenegger V. *Eur J Plast Surg* 2013; 36: 407-12	Prospective case series Follow-up: 3 months	Androgenetic alopecia	42	RegenACR-C Extra

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared as per instructions for use from 16 ml of whole blood. PRP was injected intradermally in the temporal and frontal areas, 0.05 mL per area at 1-2 cm intervals, with 2-4 mL PRP being injected per session. Patients received a total of 5 sessions, two weeks apart.	The number of hairs increased slightly but not significantly at 3 months after the last treatment session compared to baseline. The thickness of the hairs decreased, but this was not a significant decrease. There was a significant decrease in anagen hairs and increase in telogen hairs, and the anagen/telogen ratio decreased significantly. Patient satisfaction was good during the course of treatment but had decreased at the 3-month follow-up.	All patients had tolerable pain during the procedure. No serious adverse effects reported.
PRP was prepared as per instructions for use from 16 ml of whole blood. 1 ml of upper plasma was removed before platelet resuspension. Anaesthetic cream was applied prior to treatment, and a cold roller was used during the injections. Patients were injected with 8-12 cc of PRP using a 32 G or 30.5 G needle. Patients received a total of 6 injections over a 2-month period.	- The hair pull test switched from positive in 90.5% of patients at baseline to negative in all patients after the third treatment session. - Patients were overall satisfied and their hair had significantly improved quality and volume. Poor results were reported in 1 patient who was previously classified type VI-VII on the Norwood scale. - After 3 months, the hair volume remained stable, and there was a high overall patient satisfaction (7 on a scale of 1–10).	Scalp sensitivity and drowsiness during the first session. Once patients got more comfortable, the anaesthetic cream was no longer needed. No serious adverse events reported.

Alopecia

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Autologous platelet rich plasma as a treatment of male androgenetic alopecia: study of 14 cases Borhan R, Gasnier C, Reygagne P. *J Clin Exp Dermatol Res* 2015; 6: 292	Prospective case series Follow-up: 4 months (from the last treatment session)	Androgenetic alopecia	14	RegenBCT
Study of platelet-rich plasma injections in the treatment of androgenetic alopecia through an one-year period Gkini MA, Kouskoukis AE, Tripsianis G, Rigopoulos D, Kouskoukis K. *J Cutan Aesthet Surg* 2014; 7: 213-9	Prospective case series Follow-up: 6 and 12 months (from the start of treatment)	Androgenetic alopecia	20	RegenBCT

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared as per instructions for use from 8 ml of whole blood. Each patient received a total of 4 PRP injections, at W0, W3, W6 and W12. Follow-up at W16. PRP was injected in the superficial dermis over the vertex using a 32G needle. Each injection consisted of 0.05 to 0.1 ml of PRP (4-5 ml in total).	- TrichoScan assessment showed an increase in hair density in 11 patients. A decrease in hair density was observed in 3 patients. - Self-assessment questionnaires showed improvement in texture (100% patients) and volume (35.7%), decreased shedding (71.4%), and hair re-growth (50%). DLQI questionnaire showed improvement in the patients' quality of life (57%). These results were not consistently observed during the cosmetic assessment. Two patients with seborrheic dermatitis reported improvement of their symptoms after PRP injection (probably due to PRP's anti-inflammatory effect).	A few side effects such as erythema or headaches were reported. Darkening of hair was observed in two patients with blond hair. No serious adverse events reported.
PRP was prepared as per instructions for use from 16 ml of whole blood using two tubes. After removal of 2 ml of the upper supernatant plasma from each tube, the platelets were resuspended by gentle inversion. The total yield of PRP was about 6 ml. PRP was activated with calcium gluconate in a 1:9 ratio. After local anaesthesia and cleaning the scalp, PRP (0.05-0.1 ml/cm^2, 27 G needle) was injected into the androgen-related areas of the scalp (men) and into the problematic areas (women) at a depth of 1.5-2.5 mm using the nappage technique. Patients had 3 treatment sessions at 3-week intervals and a booster session at 6 months.	- There was a remarkable decrease in hair loss, which reached normal values 3 months after treatment. - Hair density reached a peak at 3 months ($p < 0.001$) and remained stable during the 1 year of follow-up. - Patient satisfaction was high, with a score of 7.1 on a scale of 1-10. - 85% of patients reported an improvement in hair quality and thickness, while 65% reported an increase in hair density.	All treated patients felt mild pain, despite application of local anaesthesia. After PRP treatment, 25% of them felt mild pain, lasting 4 hours, while 60% had scalp sensitivity during the first hair wash after treatment injections. None reported any worsened hair shedding, infection or ecchymosis.

Alopecia

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Trichologic response of platelet-rich plasma in androgenetic alopecia is maintained during combination therapy Ho A, Sukhdeo K, Lo Sicco K, Shapiro J. *J Am Acad Dermatol*. 2020; 82: 478-479	Retrospective study Follow-up: 2 months (mean 6 months, from the start of treatment)	Androgenetic alopecia	24	RegenBCT
Stratifying clinical response to adjuvant platelet-rich plasma in patients with androgenetic alopecia Juhasz MLW, Sukhdeo, K, Lo Sicco K, Shapiro J. *Br J Dermatol* 2020; 183: 580-582	Retrospective case series Follow-up: 2 months (from the start of treatment)	Androgenetic alopecia	104	RegenBCT

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared as per instructions for use from 8 ml of whole blood. Each patient received at least 2 injections of PRP at 1-month intervals, that consisted of multiple injections of 0.1 ml of PRP that were injected 1 cm apart at a depth of 1 cm in areas of alopecia on the scalp. Patients were then reassessed after 2 months; if hair density had increased by more than 10 hairs/cm^2 over baseline, monthly treatments were continued for an additional 4 months followed by maintenance injections every 3-6 months.	- A positive response to PRP was seen in 70.8% of patients two months after the initial injections. - Hair density after PRP treatment showed a statistically significant increase from baseline on the anterior crown (+24.5 hairs/cm^2, p=0.022). PRP treatment increased hair density by more than 10% over baseline in 62.5% of patients and by more than 20% over baseline in 33.3% of patients. One patient had an increase in hair density of more than 50%. - Changes in hair shaft diameter did not reach statistical significance.	Not formally assessed.
PRP was prepared according to the manufacturer's instructions from 8 or 16 ml of whole blood. Patients received intradermal scalp injections (0.1 ml, 1 cm apart) in clinically affected areas. Patients underwent two PRP sessions at four- to six-week intervals, after which they were assessed for a clinical response, defined as an increase in hair density by 10 hairs/cm^2 from baseline. If patients were "PRP-responders" they received a further four monthly PRP sessions and maintenance injections every three to six months.	A positive response to adjuvant PRP was reported in 70.1% of patients after two sessions, with patients showing an increase in hair density to varying extents. No significant improvement in hair shaft diameter observed.	Not formally assessed.

Alopecia

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Correlation between individual inflammation genetic profile and platelet rich plasma efficacy in hair follicle regeneration: a pilot study reveals prognostic value of IL-1a polymorphism Rossano F, Di Martino S, Iodice L, Di Paolo M, Misso S, *et al.* *Eur Rev Med Pharmacol Sci.* 2017; 21: 5247-5257	Retrospective case-series Follow-up: 12 months (from the start of treatment)	Androgenetic alopecia	41	RegenBCT

Treatment protocol	Key results on performance	Safety / adverse events (AE)
6 ml of PRP was prepared as per instructions for use from 16 ml of whole blood. PRP was activated with CaGlu in a 1:10 ratio. Note: PRP was frozen at -20 °C until use. Using a 27-G needle 0.1 ml/cm^2 of PRP was injected into the affected areas (frontal, occipital, parietal) of the scalp in men and into the problematic areas in women, using 1 ml syringes at a depth of 1.5-2.5 mm using the nappage technique. The protocol consisted of 3 treatment sessions with an interval of 3-5 weeks. A booster session was performed 6 months after the start of treatment.	- A significant increase in hair density was noticed after the third month of treatment in 32/41 (78%) of the subjects. - This study demonstrated a correlation between the individual genetic inflammatory profile and the efficacy of the PRP treatment in males. On the contrary, in females, it showed a negative correlation. IL-1α could be used as a prognostic value for PRP efficacy.	Not formally assessed.