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Plasma rich in growth factors as an adjuvant treatment for the management of frontal fibrosing alopecia: a retrospective observational clinical study

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Abstract

Background: Frontal fibrosing alopecia (FFA) is a scarring alopecia in which the exact etiopathogenesis has not been completely elucidated and the available treatments are not very effective. Plasma rich in growth factors (PRGF) has shown to induce folliculogenesis in hair loss related disorders. However, the scientific evidence when facing FFA is scarce.

Objectives: The aim of this study was to retrospectively analyse the adjuvant use of PRGF compared to the conventional treatment in the management of FFA.

Methods: Participants with clinically diagnosed FFA who had been treated with either conventional therapy (Control Group) or conventional therapy combined with PRGF (PRGF Group) were identified from the centre's medical records. The clinical assessment was based on the "Frontal Fibrosing Alopecia Severity Score" (FFASS), which was fulfilled during a period of two and four years.

Results: This study included 118 patients with clinically diagnosed FFA (Control Group: 57 and PRGF Group: 61). No adverse effects related to the treatments were observed. Both treatments showed to halt the steady progression of hair loss compared to baseline. PRGF treatment also induced significant hair regrowth compared to the Control Group. The scalp inflammation was reduced in response to treatments. The FFASS score indicated that PRGF Group improved the symptoms and severity of FFA in a significant manner.

Conclusions: The adjuvant use of PRGF may exert long-term beneficial effects on hair loss reduction and might reduce the symptoms and severity of FFA.

1. Introduction

Frontal fibrosing alopecia (FFA) is a primary lymphocytic scarring alopecia with a distinctive clinical pattern of progressive frontotemporal hairline recession and eyebrow loss [1]. Although this type of alopecia has been considered as a variant of lichen planopilaris due histological similarities, the differing pathological characteristics have led to classify FFA as a new disease within the scarring alopecia group [2]. FFA is usually associated with cicatricial changes on the scalp surface, smooth skin, loss of follicular orifices and uniform pallor [3]. In 50% of patients, perifollicular erythema occurs, while in 30-60% of patients follicular hyperkeratosis appears [4]. The clinical presentation is characterized by a receding frontotemporal hairline leaving an area of pale alopecia with subcutaneous atrophy and depression of the frontal veins. Total or partial alopecia of the eyebrows is very frequent and body hair may also be lost in approximately 25% of patients [5]. Most cases occur among postmenopausal women but recently, the number of premenopausal cases and men with FAA has increased significantly. In fact, nowadays FFA is the most frequent cicatricial alopecia [6].

The exact etiopathogenesis of FFA has not been completely elucidated. It is thought that there is a genetic predisposition, a hormonal component and various environmental factors involved. Additionally, the association of FFA with autoimmune diseases, such as hypothyroidism, vitiligo or Sjögren's syndrome suggest an autoimmune aetiology [5]. With respect to the treatment of the disease the main objective has been focused on halting the steady progression of hair loss. In this sense 5alpha-reductase inhibitors, vasodilators, intra-lesional corticosteroids, antibiotics, hydroxychloroquine, topical and oral immunomodulators and tacrolimus have reported acceptable stabilization of FFA [7]. However, the available treatments in general are not very effective and the desired

efficacy is not usually reached [8]. Since no definitive treatment is available, early diagnosis is important because it allows physicians to initiate treatment at the first signs and symptoms, minimizing disease progression and sequelae [9].

General uncertainties about this entity start with the unknown pathogenesis and continue with the difficulty of finding an effective treatment. Hence, given the chronic and recurrent nature of FFA, it is necessary to explore innovative strategies including the treatment with platelet rich plasma (PRP) [10]. PRP is based on the removal of a small volume of the patients´ blood to obtain a platelet enriched plasma fraction that has proven to promote tissue regeneration [11]. When used as an autologous therapy for hair loss related disorders such as androgenetic alopecia (AGA) or alopecia areata (AA), it has shown to induce folliculogenesis and hair growth in a clinically relevant manner [12]. However, the scientific evidence of PRP when facing other types of hair loss such as scarring alopecia or FFA is scarce.

In this retrospective study, plasma rich in growth factors (PRGF) has been proposed as an adjunctive treatment for FFA treatment. PRGF is a specific type of PRP that is depleted of leucocytes and erythrocytes, presents a moderate platelet concentration and is activated in a controlled way with calcium chloride [13]. PRGF is a 100% autologous technology that provides a continuous supply of growth factors (GF) which are directly involved in tissue regeneration [14]. PRGF has previously demonstrated its efficacy and safety in oral and maxillofacial surgery [10,15] and other medical fields such as sports medicine, traumatology, ophthalmology and dermatology [16]. In this line, PRGF monotherapy in trichology has proven to stimulate new hair follicle growth and reduce other AGA symptoms such as hair miniaturization or anagen/telogen phase ratio involution [17-19]. PRGF has been also used as an adjunctive therapy during micrograft transplantation techniques such as

follicular unit extraction (FUE) with excellent results [20]. Proteins within PRGF are also involved in anti-inflammatory and anti-fibrotic responses that may exert an adjunctive effect when applied in combination with conventional drugs against FFA [21-23]. Hence, the aim of this study was to retrospectively analyse the adjuvant use of PRGF compared to the conventional treatment in the management of FFA.

2. Materials and methods

2.1. Study design and data source

This retrospective study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines (Supplementary Table S1) [24]. The study protocol (CDE-01-ER/22/ALOPECIA) was approved on 13 June 2022 by the Institutional Review Board of the General University Hospital of Alicante (Spain) in accordance with the international ethical standards from the revised World Medical Association Declaration of Helsinki amended in 2013 [25]. In the present study an anonymized database of patients with clinically diagnosed frontal fibrosing alopecia and treated between January 2018 and December 2021 at the "Dermatologic and Aesthetic Centre" (Alicante, Spain) was retrospectively reviewed.

The inclusion criteria were the following: adult patients of both sexes, clinical diagnosis of FFA, treatment with either conventional therapy or conventional therapy combined with PRGF, have completed at least one evaluation scale during their follow up. The exclusion criteria were the following: lack of records of the variables to be studied, concomitant diagnosis of an alopecia phenotype other than FFA.

2.2. Treatments

This study included medical treatments and clinical procedures that are applied in the regular practice under healthcare professional prescription [26].

The conventional treatment for FFA consist in a personalized treatment based on the following: use of topical medication (steroid clobetasol 0.05%, steroid dexamethasone 2.5%, minoxidil 3%, alpha tocopherol, -alpha reductase inhibitor dutasteride 0.05%), oral prescription (daily nutricosmetics and biotin 20mg/ginkgo biloba 40mg) and monthly local injections (steroid triamcinolone acetonide 4 mg/mL, 5-alpha reductase inhibitor dutasteride 0.01%, non-reticulated hyaluronic acid, complex B vitamins and amino acids). Topical treatments are revised every four months.

The combined treatment for FFA consists in periodical intradermal injections of PRGF every two to four months in combination with the conventional treatment. Following the regular clinical practice, the combined treatment is applied under healthcare specialist criteria when the patient presents an inflammatory process and frontal line extension over 25% in the FFASS scale.

Manufacture´s instructions were followed for PRGF preparation (KMU-Aesthetics, BTI Biotechnology Institute, Vitoria, Spain). Tubes with 3.8% (wt/vol) sodium citrate were used to obtain patient´s peripheral blood (9mL/tube). After blood centrifugation, (System V centrifuge, BTI) the plasma column was aspirated. For each tube, the 2mL of platelet rich plasma just above the buffy coat were collected. Before the injection, platelets were activated with PRGF-activator (BTI) and an evenly distributed mesotherapy was applied using 30G/32G microneedles, following micropuncture technique over the affected area. PRGF contains neither leukocytes nor erythrocytes and can therefore be classified as pure-PRP, specifically P2-x-Bβ category [27], and 24-00-11 [28] according to two classifications that have been proposed for PRPs.

When analysing the database, patients that followed the conventional treatment for FFA were included in the Control Group and patients that followed the combined therapy were included in the PRGF Group.

2.3. Clinical assessment

Patients were clinically assessed at baseline and two and four years after the beginning of treatments. Any undesired side effects were recorded. The clinical assessment was performed by trained technicians. During the treatment period, the Frontal Fibrosing Alopecia Severity Score" (FFASS) was completed for each patient with the aim of objectively measuring the severity of FFA and the response to treatments. The FFASS is a statistically validated tool for the reliable measurement of FFA severity that is used in regular clinical practice [29]. Apart from the total score, clinical manifestations such as alopecia extent or inflammation level were determined using FFASS scale. Results are expressed as the percentage of change compared to baseline status. Standardized macrophotographs were also taken during the follow up period to visualize the progression of FFA over time.

2.4. Statistical analysis

The statistical analysis was performed using a specialized software (SPSS statistics version 15, IBM, Armonk, NY, USA). Continuous variables were expressed as mean, standard deviation and range. Prior to statistical analysis the normal distribution of continuous variables was assessed by the Saphiro-Wilk normality test. Statistical differences for each group compared to baseline were evaluated by the Wilcoxon signed-rank test. Statistical differences between groups at each time point were assessed using the Mann-Withney test. The applied statistical significance level was 5% ($p<0.05$).

3. Results

Patient demographics at baseline are shown in Table 1. This study included 118 patients with clinically diagnosed frontal fibrosing alopecia whose age ranged from 29 to 87 years old. Fifty-seven patients had undergone conventional treatment for FFA (Control Group) while sixty-one had followed the combined therapy with PRGF (PRGF Group). Table 2 summarizes the treatments followed by both groups. At baseline, the most common concomitant diseases included arterial hypertension, hypercholesterolemia and hypothyroidism. The most common concomitant medications included thyroid drugs and statin drugs. No statistical differences were found between age and baseline FFA severity score when comparing treatment groups. During the study, no adverse effects related to the treatments were observed, apart from transient erythema due to local injections that resolved within 48 hours. Patients did not refer procedural discomfort. Table 3 summarizes the number of PRGF sessions that were performed for each patient. The most common procedure was 11 sessions of PRGF which was performed on 9 patients (14.8%). All patients underwent clinical assessment at baseline and at two years follow up. After four years, 31 patients and 30 patients from the Control Group and PRGF Group respectively, underwent clinical assessment.

Both treatments showed to halt the steady progression of hair loss that is usually associated with FFA (Figure 1A). In the Control Group, the alopecia extent increased only 14% and 14.2% after two and four years respectively (*p<0.001 and *p=0.001 respectively). Combined PRGF treatment not only achieved disease stabilization, but also showed to induce a slight hair regrowth of 4.8% and 5.7% after two and four years respectively (*p=0.003 and*p=0.007 respectively). Statistically significant differences

were found between treatment groups at two and four years, being the PRGF treated patients who experienced a better clinical outcome (#p<0.001 at each time point).

The inflammation level of patient's scalps, which considers local erythema, hyperkeratosis, itching and pain severity, also showed a significant reduction in response to the conventional and the combined therapy (*p<0.001 at each time point for both groups). No statistical differences were found between groups for the inflammation level (Figure 1B). Similarly, the total FFASS score indicated that both treatments were able to slow down the disease progress and decrease the symptoms and signs of FFA. FFASS score at baseline was 9.4±4.5 and 9.3±3.5 for Control and PRGF groups respectively. After two years, it was 9.9±4.3 and 8.3±3.3 for Control and PRGF groups respectively. After four years, it was 10.2±4.8 and 8.6±3.6 for Control and PRGF groups respectively. PRGF Group achieved better clinical results compared to Control Group as 10.1% and 11.4% FFASS score improvement was observed after two and four years respectively (#p<0.001 at each time point) (Figure 2). Representative pre- and post-treatment macrophotographs of FFA affected patients are shown in Figure 3. The images illustrate that FFA was successfully stabilized in response to the conventional treatment (Figure 3A), while PRGF treated group also showed a slight hair regrowth over the affected areas (Figure 3B).

4. Discussion

Although hair loss is a non-life threatening and benign medical condition, people who suffer from different types of alopecia develop clinically relevant psycho-emotional stress and secondary morbidity. As a scarring alopecia, in FFA the steady hair loss progression is usually irreversible and has a significant impact on the confidence and quality of life of the affected patients [30]. Hence, there is a growing interest in developing new strategies

for the management of the disease with the aim of overcoming the current therapeutic limitations.

It seems that it is the ensemble of multiple processes that triggers and leads to disease progression. There is growing evidence that neurogenic inflammation can play a role within FFA. Stress-associated neuropeptides such as substance P can induce hair follicle immune-privilege (HFIP) collapse through the upregulation of mayor histocompatibility complex (MHC) class I/II, beta-2-microglobulin and INF-γ [31]. This could lead to damage of epithelial stem cells within the follicle bulge triggering FFA development. In fact, is precisely around these cells that a higher density of inflammatory infiltrate is observed [32]. In this line, neurogenic inflammation would expose bulge stem cells to cytotoxic T inflammatory infiltrate and promote their destruction. This aberrant immune response could be related to neo-autoantigen formation during the hair follicular miniaturization process [33]. Other studies point out the role of transforming growth factor-β1 (TGF-β1) and fibrotic pathways such as the epithelial-to-mesenchymal transition (EMT) in the occurrence of FFA [5]. Downregulation of the peroxisome proliferator-activated receptor gamma pathway (PPAR-γ) in the bulge region has also shown to be involved in sebaceous gland atrophy and FFA initiation. In this sense, PPAR-γ dysfunction contributes to oxidative stress and inflammation of the follicular microenvironment and facilitates the illness progression [34]. Thus, inhibiting the production of reactive oxygen species and proinflammatory cytokines such as IL1-β, IL-6, IFN-γ and TNF-α would help in the reduction of FFA derived symptoms [5].

This retrospective observational clinical study suggests that the adjuvant use of PRGF promotes hair growth and reduces the severity of FFA. Results reported herein point out that both the conventional and the combined therapy were able to stabilize the disease,

which is the main goal of current treatment modalities against FFA. In fact, the hair loss due to FFA progression would have been significantly higher in the absence of treatment. In addition, PRGF treated patients showed a significative FFASS score improvement when compared to the Control Group, suggesting a synergistic effect between conventional medicaments and PRGF. These results are consistent with previous reports in which platelet-based products have been preliminarily tested as an alternative modality for different types of scarring alopecia such as central centrifugal cicatricial alopecia (CCCA), lichen planopilaris (LPP), frontal fibrosing alopecia (FFA) or discoid lupus erythematosus (DLE) [35-38].

Recent studies reported several patients that underwent PRP treatment for non-responding CCCA. Results showed normal follicular regrowth of the temporal line, 50% improvement in hair density along the scalp vertex and residual perifollicular erythema/scaling [35,39]. Other reports have also provided preliminary data regarding the clinical outcome after PRP alone or in combination with minoxidil when facing LPP. Results showed that significant hair thickening was achieved, with regression of itching and no perifollicular erythema or scaling [36,40]. The satisfactory outcome with intralesional PRP injections in unresponsive FFA has been also reported in terms of disease progression, erythema, scaling and lichenoid papule improvement within the frontotemporal hairline [37]. Additionally, clinical symptoms of DLE have shown to improve after treatment with concentrated growth factors and corticosteroids as lighter erythema, alleviation of hair loss and 90% hair regrowth was achieved [38].

The biological mechanisms behind the therapeutic potential of PRGF could be related to the high load of GF and bioactive proteins within the alpha granules that are released from platelets upon activation [41]. These include transforming growth factor-β (TGF-β1),

platelet derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and insulin like growth factor-I (IGF-I), among others [11]. These morphogens are attached to the autologous fibrin mesh that is formed beneath the epidermal layer upon intralesional PRGF injection, and are gradually released ultimately promoting tissue regeneration [23].

GF have been found to upregulate the transcriptional activity of beta catenin, which leads to differentiation of stem cells into hair follicle cells [42]. In addition, they play an essential role in hair restoration since inflammation and apoptosis are mechanisms involving follicular destruction and scarring formation at later stages of FFA. In this sense, PRGF have previously shown to modulate inflammation by the attenuation of nuclear factor-κB pathway and the reduction of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α [22]. GF within PRGF may also terminate inflammation by restoring local cells to a non-inflammatory phenotype. This effect could be mediated by VEGF and HGF, which additionally protect the function of the endothelial barrier and promote angiogenesis increasing nutrient supply to the hair bulb [43]. In addition, several studies highlight the potential of PRGF to reduce reactive oxygen species and oxidative stress from these hostile microenvironments, which could stimulate diverse regenerative responses in the nearby milieu of the follicular unit [44]. Additionally, PRGF can play a pivotal role in the remodelling of scarred issue as it prevents from perifollicular fibrosis via modulation of myofibroblast transition and collagen deposition in response to TFG-β1 pathway [19,23].

PRGF has also proven to improve the anagen/telogen hair ratio and revert follicular miniaturization [17,19]. This could be associated to the increase of local bFGF levels that induce cell growth through ERK signaling activation and suppression of apoptotic cues

by discharging Bcl-2 while actuating at Akt pathways [45]. Other anagen-maintaining factors such as IGF-1, bFGF and VEGF, have shown to improve the ischaemic tissue and considerably increase the blood flow around the hair follicles [46]. Bulge cells express nephronectin which is known to mediate the interactions with dermal papilla cells (DPC) and human hair germinal matrix cells (HGMC) among others [47]. The biological activity of these cell types has proven to be upregulated in response to PRGF [20]. Additionally, the deposition of nephronectin in basement membrane creates a unique niche leading to a series of signals for hair follicle survival and integrity that have also been proved to be modulated by GF released by platelets [48]. Therefore, the interactions between DPC, HGMC and bulge cells as well as the signals triggered by binding growth factors (PDGF, TGF-β, and VEGF) activate the proliferative phase of the hair, giving rise to the future follicular unit [49].

The adjuvant use of platelet-based products and other hair loss-related products might enhance the final performance of the treatment. These synergistic approaches include medicaments such as finasteride or minoxidil, bioactive macromolecules, corticosteroids and cell-based therapies [50]. Although this study presents several limitations such as the retrospective design and the lack of histological analysis, here we suggest that the combination of PRGF and conventional drugs may exert long-term beneficial effects on hair loss reduction and might reduce the symptoms and severity of FFA. Nevertheless, additional prospective randomized and controlled trials are encouraged to clinically assess the efficacy and safety of PRGF in the management of FFA.

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Conflict of interest: EA is the scientific director and AP is researcher at BTI Biotechnology Institute, the company that has developed the Endoret®PRGF® technology. The rest of the authors declare no conflict of interest.

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Tables

Table 1: Demographic characteristics of patients diagnosed from frontal fibrosing alopecia.

Demographic information		Total	Control Group	PRGF Group
Number of patients (n)		118	57	61
Age (years, mean ± SD)		64 ± 11	65 ± 12	64 ± 10
Women (number, %)		115 (97.5%)	54 (94.7%)	61 (100%)
Men (number, %)		3 (2.5%)	3 (5.3%)	0 (0%)
Common concomitant diseases (number, %)	Arterial hypertension	31 (26.3%)	14 (24.7%)	17 (27.9%)
	Hypercholesterolemia	24 (20.3%)	7 (12.3%)	17 (27.9%)
	Hypothyroidism	21 (17.8%)	7 (12.3%)	14 (23%)
	Arthrosis	11 (9.3%)	4 (7%)	7 (11.5%)
	Anxiety	10 (8.5%)	4 (7%)	6 (9.8%)
	Type II diabetes	8 (6.8%)	5 (8.8%)	3 (4.9%)
	Osteoporosis	8 (6.8%)	5 (8.8%)	3 (4.9%)
	None	27 (22.9%)	14 (24.6%)	13 (21.3%)
Common concomitant medication (number, %)	Eutirox	23 (19.5%)	7 (12.3%)	16 (26.2%)
	Atorvastatin	9 (7.6%)	3 (5.3%)	6 (9.8%)
	Simvastatin	7 (5.9%)	3 (5.3%)	4 (6.6%)
	Hidroferol	7 (5.9%)	5 (8.8%)	2 (3.3%)
	None	43 (36.4%)	20 (35.1%)	13 (21.3%)
Alcohol consumption		48 (40.7%)	19 (33.3%)	29 (47.5%)
Tobacco consumption		31 (26.3%)	16 (28.1%)	15 (24.6%)

Table 2: Treatments followed for frontal fibrosing alopecia.

Treatment	Control Group (number, %)	PRGF Group (number, %)
Topical minoxidil 3%	57 (100%)	61 (100%)
Topical corticosteroids (clobetasol 0.05%, dexamethasone 2.5%)	57 (100%)	59 (96.7%)
Intradermal corticosteroids (triamcinolone acetonide 4 mg/mL)	57 (100%)	59 (96.7%)
Oral complex B vitamins	57 (100%)	61 (100%)
Intradermal complex B vitamins	57 (100%)	61 (100%)
Topical 5-alpha reductase inhibitor (dutasteride 0.05%)	53 (93%)	57 (93.4%)
Intradermal 5-alpha reductase inhibitor (dutasteride 0.01%)	52 (91.2%)	56 (91.8%)
Intradermal PRGF	0 (0%)	61 (100%)

Table 3: Total PRGF sessions performed.

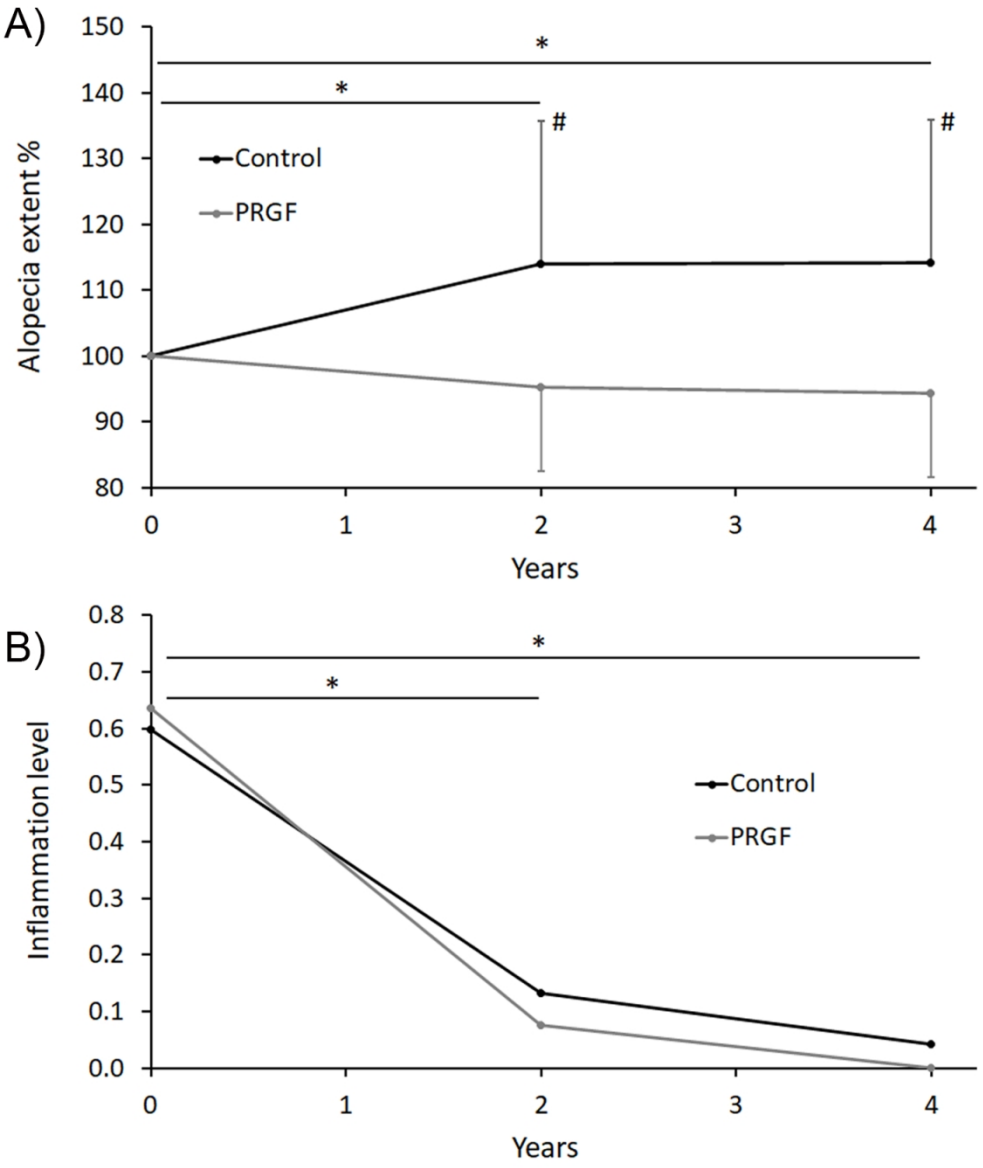
Total PRGF sessions	Number of patients	Percentage (%)
6 sessions	1 patient	1.6%
8 sessions	1 patient	1.6%
9 sessions	8 patients	13.1%
10 sessions	7 patients	11.5%
11 sessions	9 patients	14.8%
12 sessions	5 patients	8.2%
13 sessions	5 patients	8.2%
14 sessions	1 patient	1.6%
15 sessions	2 patients	3.3%
16 sessions	3 patients	4.9%
17 sessions	7 patients	11.5%
18 sessions	4 patients	6.6%
20 sessions	2 patients	3.3%
21 sessions	2 patients	3.3%
22 sessions	1 patient	1.6%
24 sessions	2 patients	3.3%
26 sessions	1 patient	1.6%
Total	61	100%

Figure legends

Figure 1: A) Alopecia extent percentage during the treatment period. Control group: baseline vs two years (*p<0.001), baseline vs four years (*p=0.001). PRGF group: baseline vs two years (*p=0.003), baseline vs four years (*p=0.007). Statistical differences between groups: at baseline (p=1), after two years (#p<0.001), after four years (#p<0.001). B) Inflammation level during the treatment period. Control group: baseline vs two years (*p<0.001), baseline vs four years (*p<0.001). PRGF group: baseline vs two years (*p<0.001), baseline vs four years (*p<0.001). No statistical differences between groups: at baseline (p=0.715), after two years (p=0.631), after four years (p=0.325).

Figure 2: Total Frontal Fibrosing Alopecia Severity Score (FFASS) during the treatment period. *p<0.05 (statistically significant differences compared to baseline). #p<0.05 (statistically significant differences between treatment groups). Control group: baseline vs two years (*p=0.019), baseline vs four years (*p=0.094). PRGF group: baseline vs two years (*p=0.003), baseline vs four years (*p=0.007). Statistical differences between groups: at baseline (p=1), after two years (#p<0.001), after four years (#p<0.001).

Figure 3: Representative macrophotographs of patients diagnosed from frontal fibrosing alopecia who had underwent conventional treatment (A) or combined therapy with PRGF (B).



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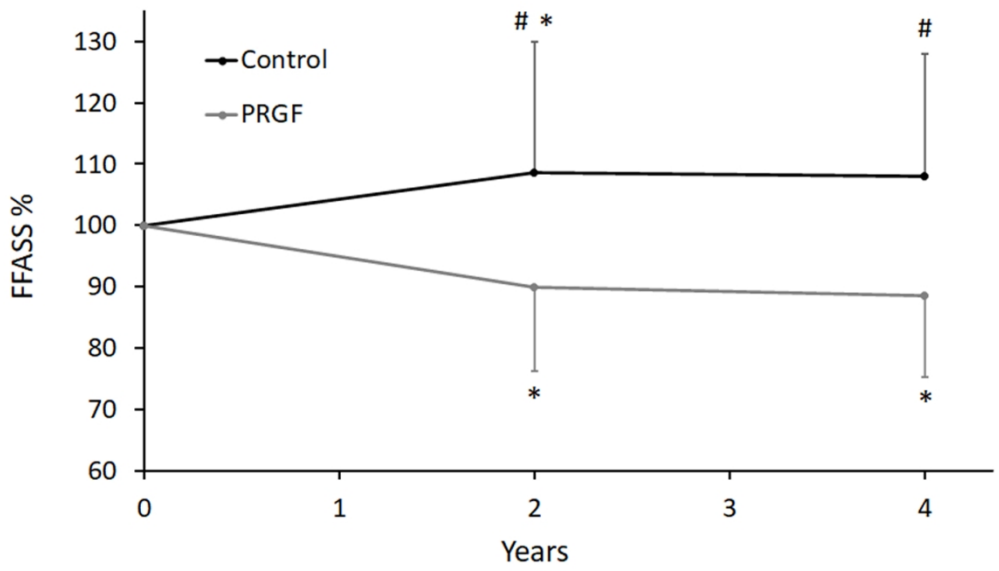

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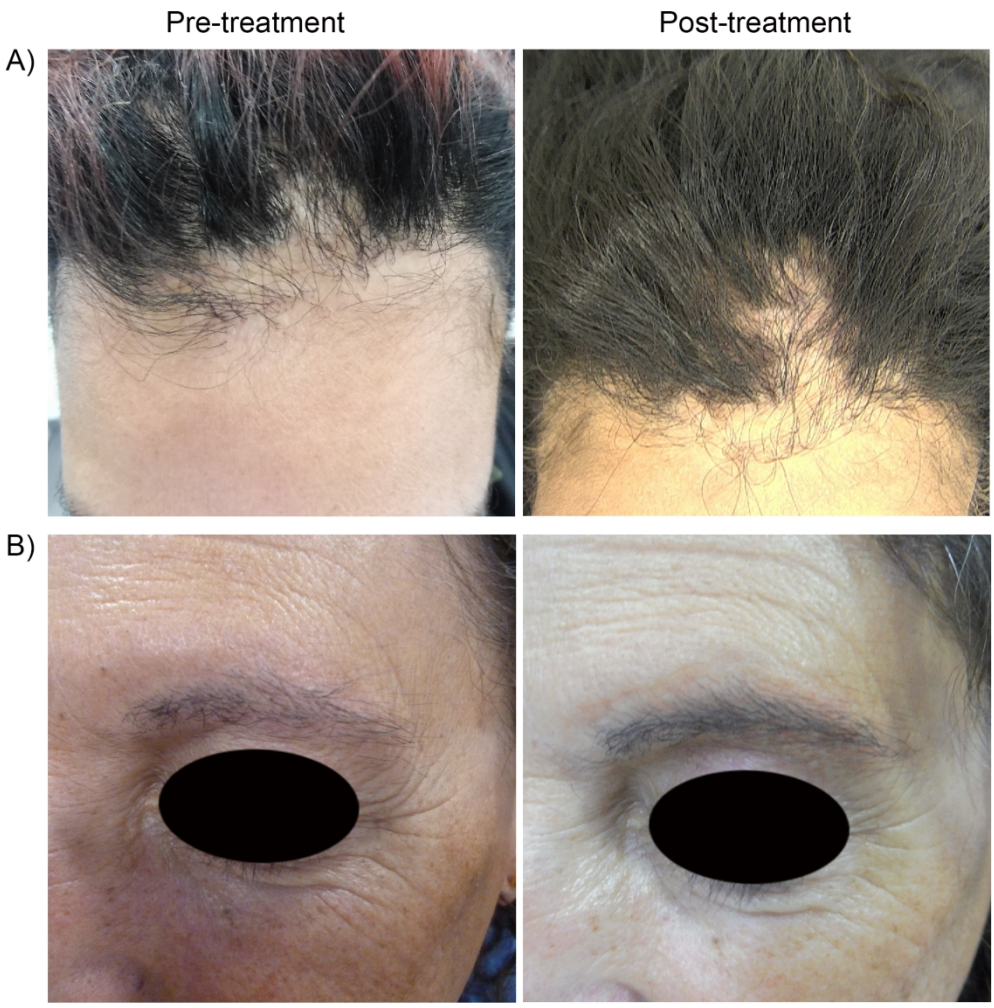

721x729mm (72 x 72 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	A retrospective observational study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	Background, Objective, Materials and methods, Results, Conclusion
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4	FFA is a primary lymphocytic alopecia…, The exact etiopathogenesis of FFA has not been completely elucidated…, It is necessary to explore innovative strategies including the treatment with PRP…
Objectives	3	State specific objectives, including any prespecified hypotheses	4	The aim of this study was to retrospectively analyse the adjuvant use of PRGF…
Methods				
Study design	4	Present key elements of study design early in the paper	4-6	This retrospective study was reported following the STROBE statement guidelines…., The study protocol was approved by the Institutional Review Board…,
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-6	In the present study an anonymized database of patients with clinically diagnosed FFA and treated between January

				2018 and December 2021 at the Dermatologic and Aesthetic Centre (Alicante, Spain) was retrospectively reviewed…
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-6	The inclusion criteria were the following: adult patients of both sexes…, The exclusion criteria were the following: lack of records…,
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-6	When analysing the database, patients that followed the conventional treatment for FFA were included in the Control Group and patients that followed the combined therapy were included in the PRGF Group…
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6	Patients were clinically assessed at baseline and two and four years after the…, The FFASS scale was completed for each patient…
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-6	The FFASS is a statistically validated tool for the reliable measurement of FFA…
Bias	9	Describe any efforts to address potential sources of bias	1, 4	Conflict of Interest: ES is the scientific director…, The clinical assessment was performed by trained technicians…

Study size	10	Explain how the study size was arrived at	4-6	118 patients were identified from the centre's medical records…

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-6	Apart from the total score, clinical manifestations such as alopecia extent or inflammation level were determined using FFASS scale. Results are expressed as the percentage of change compared to baseline status…,
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7	The statistical analysis was performed using a specialized software (SPSS statistics version 15) …,
		(*b*) Describe any methods used to examine subgroups and interactions	6-7	The statistical analysis was performed using a specialized software (SPSS statistics version 15) …,
		(*c*) Explain how missing data were addressed	6-7	The statistical analysis was performed using a specialized software (SPSS statistics version 15) …,
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	6-7	The statistical analysis was performed using a specialized software (SPSS statistics version 15) …,
		(*e*) Describe any sensitivity analyses	6-7	The statistical analysis was performed using a specialized software (SPSS statistics version 15) …,
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-8	This study included 118 patients with clinically diagnosed frontal fibrosing alopecia whose age

				ranged from 29 to 87 years old. Fifty-seven patients had undergone conventional treatment for FFA (Control Group) while sixty-one had followed the combined therapy with PRGF (PRGF Group)
		(b) Give reasons for non-participation at each stage	7-8	Exclusion criteria: concomitant diagnosis of an alopecia phenotype other than FFA
		(c) Consider use of a flow diagram	7-8	None
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8	Patient demographics at baseline are shown in Table 1
		(b) Indicate number of participants with missing data for each variable of interest	7-8	None
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7-8	Patients were clinically assessed at baseline and two and four years after the beginning of treatments.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7-8	Figure 1: A) Alopecia extent percentage during the treatment period. B) Inflammation level during the treatment period. Figure 2: Total Frontal Fibrosing Alopecia Severity Score (FFASS) during the treatment period.
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	7-8	Figure 1: A) Alopecia extent percentage during the treatment period. B) Inflammation level during the treatment period. Figure 2: Total Frontal Fibrosing Alopecia Severity Score (FFASS) during the treatment period.
		Cross-sectional study—Report numbers of outcome events or summary measures	7-8	Figure 1: A) Alopecia extent

				percentage during the treatment period. B) Inflammation level during the treatment period. Figure 2: Total Frontal Fibrosing Alopecia Severity Score (FFASS) during the treatment period.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8	In the Control Group, the alopecia extent increased only 14% and 14.2% after two and four years respectively (*p<0.05). Combined PRGF treatment not only achieved disease stabilization, but also showed to induce a slight hair regrowth of 4.8% and 5.7% after two and four years respectively (*p<0.05), …
		(*b*) Report category boundaries when continuous variables were categorized	7-8	None
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7-8	None

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-8	None
Discussion				
Key results	18	Summarise key results with reference to study objectives	8-11	This retrospective observational clinical study suggests that the adjuvant use of PRGF promotes hair growth and reduces the severity of FFA, …
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-11	Although this study presents several limitations such as the retrospective design and the lack of histological analysis,…
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11	Here we suggest that the combination of PRGF and conventional drugs may exert long-term beneficial effects on hair loss reduction and might reduce the symptoms and severity of FFA. Nevertheless, additional prospective randomized and controlled trials are encouraged to clinically assess the efficacy and safety of PRGF in the management of FFA, …
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-11	These results are consistent with previous reports in which platelet-based products have been preliminarily tested as an alternative modality for different types of scarring alopecia
Other information				

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1	Funding source: this study did not receive any specific grants

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.