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Correlation between individual inflammation genetic profile and platelet rich plasma efficacy in hair follicle regeneration: a pilot study reveals prognostic value of IL-1α polymorphism

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Abstract. – OBJECTIVE: **Hair loss generates severe psychosocial implications. To date, exploring the prognostic factors of possible clinical benefit of autologous blood concentrate platelet rich plasma (PRP) was failed.**

The aim of our pilot study was to explore the correlation between the individual inflammation genetic profile and PRP efficacy in the treatment of hair follicle regeneration.

PATIENTS AND METHODS: 41 volunteers (25 men, 16 women) took part in this retrospective study. All the patients were scheduled for 4 sessions of PRP application with intervals of 40-60 days. All the patients were checked up at 6 weekly intervals for 6 months and, then, at the end of the first year. A panel of 5 polymorphisms on 4 genes (IL-1α, IL-1β, IL-6, and IL-10) implicated in the individual genetic inflammation profile were performed.

RESULTS: A significant increase rate in hair density was noticed after the third month of treatment in 32/41 (78%) of the subjects.

We found an interesting association between the pro-inflammatory cytokine IL-1α polymorphism C>A (rs17561) and responders to PRP treatment. The cases carrying C/C genotype (coding for Ser114) were 21 (66%) in responders and only 2 (22%) in non-responders ($p<0.05$).

In addition, about IL-1α, the frequency of G/G genotype in responder patients was over two times lower in responder (31%) than in non-responder patients (78%).

CONCLUSIONS: Our pilot 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. Also, these results provide preliminary evidence that may encourage the design of controlled clinical trials to properly test this modus operandi on a large number of subjects.

Key Words:
Alopecia, Platelet rich plasma, Cytokines, IL-1a, Inflammation genetic profile, Polymorphisms.

Introduction

Platelets play a fundamental role in hemostasis and thrombosis[1]. Following the lesion of endothelial cells, the underlying collagen fibers are exposed. Platelets adhere to the damaged area and, releasing other substances attracting other platelets, led to the formation of a fibrin clot and bleeding cessation[2-4]. However, the function of platelet rich plasma (PRP) in the wound healing process has not yet been clearly elucidated. Moreover, the attention for PRP therapy is growing in different fields of regenerative medicine like as orthopedics, aesthetic, cancer biology and dermatology. The rationale of PRP therapy is that an injection of concentrated PRP at sites of injury may affect tissue healing via growth factors released after platelets degranulation. Platelets contain several types of granules carrying growth factors involved in coagulation, angiogenesis, atherosclerosis,

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antimicrobial host defense, and inflammation[5,6]. Most abundant growth factors are platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), basic fibroblast growth factor (BFGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF). They play an important role in cell proliferation, migration, and differentiation and are released from α-granules. Differently, adenosine diphosphate (ADP), serotonin and calcium are released from dense granules and are important in the recruitment of new platelets and coagulation cascade[7,8]. The regenerative process is triggered by inflammation cascade mediated by cytokines and immunologic system cells. Cytokines are soluble proteins secreted by immune system cells to modulate the functions of other cells, by inducing proliferation or stimulating a specific function. Wide ranges of pro-inflammatory cytokines produced by epithelial cells are interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ) while interleukin-4 (IL-4) and interleukin-10 (IL-10) are the anti-inflammatory cytokines[9-11]. So far, two different types of IL-1 have been identified, IL-1α and IL-1β. They have similar biological activities but diverse structures: in fact, they are codified by two different genes and have a limited homology at the nucleotide and peptide levels[12]. The gene encoding IL-1 is allocated to chromosome 2q13–21[13]. Greater occurrence and severity of cardiovascular disease are related to the carriage of defined alleles of IL-1alfa and IL-1beta because these carriers produce more IL-1 in response to atherosclerosis plaque than genotype negative individuals[14,15]. The level of IL-1α protein is affected by the presence of a single base-pair C-T tranpositional polymorphism upstream of the IL-1 alpha gene at position -889. An almost fourfold increase in IL-1alpha levels in gingival crevicular fluid was associated with IL-1α -889 T allele[16]. A weak positive association was found concerning IL-1beta T(-511)C and chronic periodontal disease[17]. No association was found for IL-1alfa C(-889)T and IL-1beta T(-511)C with aggressive periodontitis in other studies[17,18].

TNF-α gene maps to chromosome 6p21.3 within the major histocompatibility complex[19]. In the promoter region of this gene have been examined eight single nucleotide polymorphisms (SNPs) at positions -308G/A, -857C/T, -1031T/C, -863C/A, -575G/A, -376G/A, -244G/A, and -238G/A[20-23]. The G to A polymorphism at the -308 position of the TNF-α promoter region was suggested to affect TNF-α production and monocytes of patients with periodontitis; this leds many researchers to investigate the possible link between periodontitis and the -308 polymorphism in the TNF-α gene[24,25]. Higher allele 2 frequency was observed in patients with chronic periodontitis than in healthy individuals[26]. Aggressive periodontitis, instead, was not linked to this locus[27-29]. Apart from one investigation that showed a link between the -1031, -863, -857 SNPs and chronic periodontitis severity in the Japanese population, the remaining promoter polymorphisms were not associated with periodontitis[27,30,31].

The IL-6 gene maps to chromosome 7p21. Different SNPs in the promoter region of this gene have been studied from researchers[32,33] at positions -190C/T, -597G/A, -174G/C, -1363G/T, -572C/G, -6106A/T and 1480C/G. In periodontitis, the G/C SNP at the -174 position correlated with chronic periodontitis susceptibility in Brazilians and Caucasians, but not the Japanese[34,35]. Significantly higher serum C-reactive protein (PCR) and IL-6 concentrations were demonstrated in periodontitis patients carrying the rare C/C allele in the IL-6 -174 polymorphism[36]. A less reduction in probing depths among chronic periodontitis patients after delivery of standard non-surgical periodontal therapy was associated with carriers of the rare allele at this position[37]. Instead, another work[38] showed reduced plasma levels of IL-6 and lowered IL-6 gene transcriptional activity in -174 allele carrier individuals, suggesting that individual's defense against periodontal pathogens may be prevented by low IL-6 response pathogens[38]. IL-6 -174 polymorphism did not display any relation with chronic periodontitis (CP) in a meta-analysis of cytokine gene polymorphisms of 53 investigations[17].

Gingivitis, probing depths, and alveolar bone loss were affected by the mRNA expression and/or concentration of IFN-γ in gingival crevicular fluid, serum and gingival tissues[39].

To date, only some studies have examined the link of polymorphism of IFN-gamma with periodontitis. Polymorphism in gene IFN-γ was found to be functionally relevant and produces differences in the immunoregulatory activity of its cytokine molecules. The T allele of the IFN-γ 874 T/A is found in high producers of IFN-γ[40]. No association was found in the only existing study dealing with the IFN-γ polymorphism 874T/A and CP[41].

Table I. Overall patients age.

Parameters	Males	Females	IQR (Interquartile range)
Median age (years)	33	38.5	10
Age range (years)	18-49	15-65	31.5

The gene for IL-4 maps to chromosome 5q31.1[42]. The presence of IL-4-producing cells and the percentage of IL-4-expressing cells were notably higher in determined and serious periodontitis lesions than in gingivitis tissues. IL-4 levels did not match with the degree of bone loss, even if these levels in the serum of patients were higher in chronic periodontitis[43]. Mout et al[44] identified a 70-bp variable numbers of tandem repeat (VNTR) polymorphism at intron 2 and the promoter SNP at position -590.

The gene encoding IL-10 has been mapped to chromosome 1q31–32[45]. The -1082 G/A locus was linked to CP severity but not related to chronic periodontitis susceptibility in most Caucasian populations, except in one Swedish study[46]. The -1082 single nucleotide polymorphism was combined with high in vitro IL-10 level[47]. Among the Japanese, there was a complete absence of the N-allele carriage at position -1082 in contrast to Caucasians where the -1082 N-allele is the most showed variant[47,48]. The aim of this work is to compare genotypes of pro and anti-inflammatory cytokines genes in subjects who had PRP-based treatment in hair follicle regeneration.

Patients and Methods

Samples and Inclusion Criteria

From October 2014 to November 2016, 41 volunteers (25 males, 16 females) were enrolled in our retrospective study. There was no particular randomization done since all of them were healthy adults without any co-morbidities. Age distribution between the recruited subjects are presented in Table I. All participants had not received any topical or systematic treatment for their hair loss during the last 12 months from the first PRP application. Subjects with present or a history of pharmacological immunosuppression (chemotherapy, steroid therapy), dermatological diseases affecting the scalp, autoimmune disorders, hematologic disorders, platelet dysfunction were excluded from our investigation. Patients under therapy with either anti-coagulants and/or non-steroidal anti-inflammatory drugs (NSAIDs) discontinued their use 10 days before PRP treatment. Topical minoxidil and finasteride for at least 6 months without much improvement were considered for PRP therapy. All the patients were explained about the procedure in detail approved by local etichs committee and written informed consent was obtained.

All the patients were followed up at 6 weekly intervals for 6 months and, then, at the end of 1 year. All the patients were scheduled for 4 sessions of PRP application each 40-60 days. Diagnosis of androgenic alopecia was made in all patients based on a detailed medical history (any drugs causing hair loss), clinical examination, and laboratory tests. The stage of alopecia was evaluated according to the Hamilton-Norwood scale for men and Ludwig scale for women. All

Table II. Assessment of genetic predisposition of the inflammatory profile.

Genetic predisposition to inflammatory profile	Overall assigned score	Allotype scoring criteria*				
		IL-1α A114S	IL-1β (-511)	IL-6 (-174)	IL-10 (-1080)	IL-10 (-592)
Low	1	CC	GG	GG	GG	AA
Medium-low	2	CA	GG	GG	GG/TG	AA/AG
Medium	3	CA	GA	GC	GG/TG	AA/AG
Medium-high	4	AA	GA/AA	GC	GG/TG	AA/AG
High	5	AA	AA	CC	TT	GG

*Score is based on the allelic combination resulting from the 5 polymorphisms. Each allelic profile was assigned a score depending from the either direct and/or indirect influence on the inflammation phenotype. These criteria were calculated as results from current literature data (Di Francia R, unpublished report).

included patients were tested for several analyzes and together with platelet count in PRP, respect to the basal value, before administration.

Patients were enrolled and treated in multicentric slots (CETAC regenerative medicine, Marini polidiagnostic center, Health Park) with the same protocols. The PRP production and laboratory tests were centralized to centro trasfusionale "A. Lubrano" P.O. S.G. Moscati Aversa, Italy, and CETAC Lab, Caserta, Italy, respectively.

Clinical Laboratory Tests

Laboratory tests were assessed to exclude other hair loss causes, such as anaemia, poor nutrition, thyroid dysfunction, or polycystic ovary syndrome: (a) Blood cytometric count assay (Red/ white Blood Cells and Platelets); (b) Inflammation Genotyping panel assay (Table I); (c) HIV, HBS Ag, HCV by ELISA; (d) AB0 typing blood group; (e) T3, T4, TSH, fT3, fT4, anti-TPO; (f) Electroliter (Na, K, Mg, Ca, Cl); (g) For women, female hormone profile (DHEAS, testosterone and prolactin, follicle stimulating and luteinizing hormone).

PRP Production

Complete procedures were performed by "centro trasfusionale "A. Lubrano" P.O. S.G. Moscati, Aversa, Italy, in according to Italian policy in the field of good manufacturer procedures of hemoderivates. For the preparation of PRP, we used RegenKit BCT-3 (Regenlab®). Initially, whole pheripheral blood was collected from the vein of the patient (16 ml). Then, the blood was injected into two tubes (RegenBCT) and centrifugated for 5 min at 1500 g using the laboratory centrifuge RegenA-PRP Centri. Each tube contained a thixotropic gel made up of a mix of polymers for plasma separation and sodium citrate solution, as an anticoagulant, was placed above the separator gel. After centrifugation, the blood was fractionated with cellular elements settling on the surface of the gel and red blood cells being trapped under the gel. After removal of 2 ml of upper supernatant plasma (PPP, platelet-poor plasma) from each tube, 3 ml of PRP was produced, which was resuspended by gentle inversions of the tube. Total amount of yielded PRP was about 6 ml and it was loaded in 1 ml syringes, ready for injections or alternatively was frozen at -20°C until application session. The activation process included the addition of calcium gluconate in a 1:10 ratio (0.1 ml calcium gluconate per 0.9 ml of PRP). After platelets count assessment using a microscope, mean platelet counts were 1.9 × 105 in whole blood and 5.5 × 105 platelets/µl in PRP, respectively. The concentration of platelets was approximately 5 times greater in PRP than in whole blood.

PRP Application

Before the PRP applications, all patients were notified about the process and its eventually adverse effects and they signed a consent form. Moreover, they were informed on will not have their hair washing one day after to the treatment. Local anesthesia was referred in the treatment area (who only on demand) and scalp was cleaned using 0.1% citidine chloride spray. None of the subjects received any other treatment for hair loss during PRP treatment.

Using a 27-G needle 0.1 ml/cm2 of PRP was injected into the hair loss-related areas (frontal, occipital, parietal) of the scalp in men and into the problematic areas in women, using BD-Luer LokTM 1 ml syringes. Nappage technique was executed in a depth of 1.5-2.5 mm. Our protocol proposed three treatment sessions with an interval of 3-5 weeks. After 6 months from the beginning of the treatment, a booster session was also performed.

Evaluation and Validation of an Inflammation Genotyping Panel Assay

Several criteria are used to select polymorphisms for inflammation panel tests (all URL accessed until may 23th, 2017): (A) Searching the most qualified genetic variants known to affect the inflammation in regenerative medicine (www.medscape); (B) Revising the most recent studies upgrading in clinical research, in particular, trials considering polymorphisms related to inflammation (www.clinicaltrials.gov); a review of all the literature was consulted and all the pertinent articles were manually investigated and analyzed matching concordant key words: "individual genetic profile"[MeSH] AND "inflammation"[-MeSH], limited to, regenerative Medicine AND scalp AND hair loss, limited to human subjects and the English language (www.ncbi.nlm.nih.gov/sites/entrez); (C) Issues evaluating the satisfactory impact of the hair grew, likely providing answers for policy making in the incorporation of these procedures into clinical practice.

The most significant Single Nucleotide Polymorphisms (SNPs) were:

IL-1α rs17561 C>A (Ala114Ser)

IL-1β rs16944 G>A (nt-511)

IL-6 rs1800795 C>G (nt-174)
IL-10 rs1800872 T>G (nt-592)
IL-10 rs1800896 G>A (nt-1082)

However, the fine molecular function of these SNPs remains unclear and controversial. Furthermore, there are many genes whose effects on regeneration of hair follicle have yet to be extensively studied.

Genotyping Assay

High molecular weight genomic DNA was isolated from pheripheral blood samples by conventional phenol-extraction method. After spectrophotometrically determining concentration at 260/280 nm (SmartSpec 3000, BioRad, Hercules, CA, USA), DNA samples were stored at 4°C. Real time PCR ABI/Prism 7700 sequence detector (Applied Biosystem, Foster City, CA, USA) platform was used throughout this study for Allelic Discrimination Assay method following by manufacturer's protocol of Ampli-Inflammation profile Kit (Dia-Chem srl, Naples, Italy). Briefly, 50 ng of DNA sample and standards were amplified in duplicate in a total volume of 25 uL. Sample was heated for 2 min at 50°C (for UNG digest), 2 min at 95°C and 45 cycles of 30 s at 95°C, 30 s at 61°C, 70 s at 72°C. In parallel Albumin reference reaction, (50 ng of DNA sample) was performed in duplicate in the same previously described conditions.

Statistical Analysis

Statistical analysis of the data was executed using Stata Package, version 13.1 (StataCorp LP, College Station, TX, USA). The normality of quantitative variables was tested by Shapiro-Wilk test. Normally distributed variables were expressed as mean ± standard deviation (SD), otherwise as median, min-max, and interquartile range. Genetic determined inflammatory profile was categorized in 1-5 classes (Table II). Hair density differences between the different time points were classified as delta (0-5) at the end of the study, according to the Hamilton-Norwood scale for men and Ludwig scale for women. These differences were tested by running a one-way analysis of variance (ANOVA), separately for males and females. Pairwise correlation was performed for the variables delta, inflammatory profile, and age. A stepwise multiple regression model among these variables was also performed, in order to identify possible confounding factors. The x2 test was applied to assess the difference in genotype distribution between responders and non-responders. All tests were two tailed and statistical significance was evaluated for p-values less than 0.05.

Results

Recording PRP Treatment

In our research, we assessed hair loss, hair density (hair/cm2), and patients' satisfaction. We also observed any referred adverse effects. The evaluation of results was executed by two independent evaluators, who were not implicated in the administration of PRP treatment. Evaluation methods included hair pull test, macroscopic photographs dermoscopic, photomicrographs, and a satisfaction questionnaire. All patients were assessed at six time points: T0, beginning of study; T1, after the second administration (about 12 weeks); T2, after the third administration (about 18 weeks); T3 after the fourth administration (about 24 weeks); T4, follow-up (about 6 months) and T6, follow-up of 1 year.

To check the same area at all time points, we used the same distance and the same light exposure for the photo documentation. As proposed by Lee et al[49], we measured the point of intersection between the coronal line connecting the tips of the tragus (called V, Kang's point) and the midsagittal line. This point is situated approximately 3 cm in front of the anterior margin of the headband and note the distance from the headband to the midpoint of the line connecting the lower margins of the eyebrows.

Overall, PRP treatment was clinically satisfactory. 32/41 (78%) total patients (male and female) obtained a regression of alopecia with different grade of regression.

Genotyping Results

To evaluate a possible association between the pro-inflammatory cytokines IL-1α, IL-1β and IL-6 and the anti-inflammatory cytokines IL-10 with PRP treatment, we divided our patient's group into responder and non-responder patients.

About IL-1α, the frequency of C/C genotype was significantly higher in responder (66%) than in non-responder patients (22%). As a consequence, the frequency of A/A genotype was lower in responder (3%) than in non-responder patients (11%). Finally, the frequency of C/A genotype in responder patients was over two times lower in responder (31%) than in non-responder patients (67%).

About IL-1α, the frequency of G/G genotype was over two times lower in responder (31%) than in non-responder patients (78%). The frequency of A/A genotype was much more higher in responder (16%) than in non-responder patients (0%). The frequency of A/G genotype in responder patients, instead, was 53% compared to 22% in non-responder patients.

About IL-6, the frequency of G/G genotype in responder was 44%, compared to 33% in non-responder patients. The frequency of G/C genotype was 40%, compared to 67% in non-responder patients. Finally, the frequency of C/C genotype in responder patients was 16%, compared to 0% in non-responder group.

About IL-10-(nt-592), the frequency of G/G genotype was 13%, compared to 0% in non-responder patients. The frequency of T/T genotype in responder was 26%, compared to 44% in non-responder patients. Finally, the frequency of T/G genotype was quite the same in responder (61%) and non-responder patients (56%).

About IL-10-(nt-1082), the frequency of G/A genotype was 48%, compared to 34% in non-responder patients. The frequency of G/G genotype in responder patients was 15%, compared to 22% in non-responder patients. Finally, the frequency of A/A genotype in responder was 37%, compared to 44% in non-responder patients (Table III).

Overall, statistically significant differences were found in the distribution of IL-1α, IL-1β– between responder and non-responder patients, whilst for IL-6, IL-10(-592) and IL-10(-1082) any statistically significant differences were found.

In addition, we tried to identify differences in the distribution of improvements in the five complete inflammatory profiles, separately for males and females, by running a one-way analysis of variance (ANOVA). Unfortunately, we didn't find any statistically significant difference. We tested the correlation between the efficacy of the treatment after 4 application of PRP (delta) and the complete inflammatory profile and a mild association for both males and females was found (for males: R= 0.29; $p<0.05$; for females: R= -0.05; $p<0.05$). Moreover, we tested the correlation between the age and the efficacy of the treatment after 4 appli-

Table III. Genotyping results.

Genotypes	Responder N=32	Non-responder N=9	Responder vs. non-responder OR (95%CI), *p*-value
IL-1α rs17561 C>A (Ala114Ser)			
C/C	21 (66%)	2 (22%)	6.68 (0.99-72.95) $p<0.05$
A/A	1 (3%)	1 (11%)	
C/A	10 (31%)	6 (67%)	
IL-1β rs16944 G>A (nt-511)			
G/G	10 (31%)	7 (78%)	0.13 (0.12-0.89) $p<0.05$
A/A	5 (16%)	0	
A/G	17 (53%)	2 (22%)	
IL-6 rs1800795 C>G (nt-174)			
G/G	14 (44%)	3 (33%)	1.55 (0.27-11.21) $p=0.57$
C/C	5 (16%)	0	
G/C	13 (40%)	6 (67%)	Not significative
IL-10 rs 1800872 T>G (nt-592)			
G/G	4 (13%)	0	Not determinable
T/T	8 (26%)	4 (44%)	
T/G	20 (61%)	5 (56%)	
IL-10 rs 1800896 G>A (nt-1082)			
G/G	6 (15%)	2 (22%)	0.81 (0.11-9.94) $p=0.82$
A/A	11 (37%)	4 (44%)	
G/A	15 (48%)	3 (34%)	Non significative

IL-1α: A/A + C/A vs. C/C; IL-1β: A/A + A/G vs. G/G; IL-6: C/C + G/C vs. G/G; IL-10 (-592): T/T+T/G vs. G/G; IL-10 (1082): G/A + A/A vs. A/A. OR, odds ratio; CI, Confidential interval; Significant difference from the control, $p < 0.05$

cation of PRP, separately for males and females (for males: R= -0.36; $p<0.05$; for females: R= -0.42; $p<0.05$). We then performed a multiple linear regression models with efficacy, inflammatory profile, and age in order to check if age was acting as a confounder but we didn't find any statistical significance.

Discussion

The results presented here demonstrate a correlation between the complete inflammatory profile and the efficacy of the treatment after 4 application of PRP. In particular, males showed a positive correlation between the complete inflammatory profile and the efficacy of the treatment after 4 application of PRP (R= 0.29; $p<0.05$). Females, instead, showed a negative correlation between the complete inflammatory profile and the efficacy of the treatment after 4 application of PRP (R= -0.05; $p<0.05$). Both males and females, instead, showed a negative correlation between their age and the efficacy of the treatment after 4 application of PRP. This correlation was performed considering the two different scales of evaluation of alopecia between males and females.

Nevertheless, main limitations of our study were: (A) It is not a randomized, double blind, controlled trial; (B) Sample size was small, despite the fact that we had statistically significant results; (C) To date, we didn't find a universal standardized protocol for production and application of PRP; (D) A method to report precise hair improvements in the treated PRP area.

These results indicate the necessity for further investigations in randomized controlled trials, even in series with a wider number of patients and provided controls. Moreover, like our work, in recent reviews is common opinion that the larger part of reported clinical studies[50,51] do not have enough statistical power to give conclusive results. Comparative analyses of different clinical scenarios would be useful in view of multiple potential PRP applications in orthopedics, sports medicine, respiratory medicine and reparative surgery. These comparisons are not feasible to our data, mainly because PRP is a biological product, prepared using diverse protocols, sometimes without even controlling whether platelets were effectively purified and concentrated or whether an early activation presented, discarding all of the secreted growth factors within the poor platelet plasma (PPP). Another question not also mentioned in clinical reports is about the correlation between the PRP volume or platelet concentration applied per injured volume or area. Although still not deeply characterized, the leukocyte content was also shown to be an important factor, increasing inflammation, and reducing tissue regeneration[52]. Many investigations demostrate the relevance of preparation procedures on the osteo-inductive and chondro-inductive potential of PRP, which is reported to be lost by thrombin activation and retained after freeze-thaw activation[53]. Certainly, standardized protocols to produce PRP, together with a full characterization of the final product would highly improve the comparability of studies[54,55]. To give better results, we employed a manual double spin as described in literature with important additional issue allowing high quality of platelet integrity. One last consideration is that our hair pull test evaluation method was performed in a standardized way (according to the Hamilton-Norwood scale for men and Ludwig scale for women) by the two independent external evaluators (see acknowledgments), but it remains a subjective evaluation method (Figure 1). However, an adequate standard of measuring hair growth over time in a reproducible, cheap and non-invasive manner is not available and all the above methods give a quite precise evaluation of the results (outcomes) after treatment[49].

Conclusions

Our pilot study demonstrated a positive correlation between the individual genetic inflammatory profile and the efficacy of the PRP treatment in males. In particular, for IL-1α, the frequency of C/C genotype was higher in responder (66%) than in non-responder patients (22%). On the contrary, in females, this research showed a negative correlation. Despite IL-1α could be used as prognostic value for PRP efficacy, in all these cases the strength of these correlations is weak and this could be due to the small width of our sample. For this reasons, further investigations with a more wide sample could confirm or deny our results. This could be the first example for a personalized therapy for the medicines of 3rd millennium[56-58].

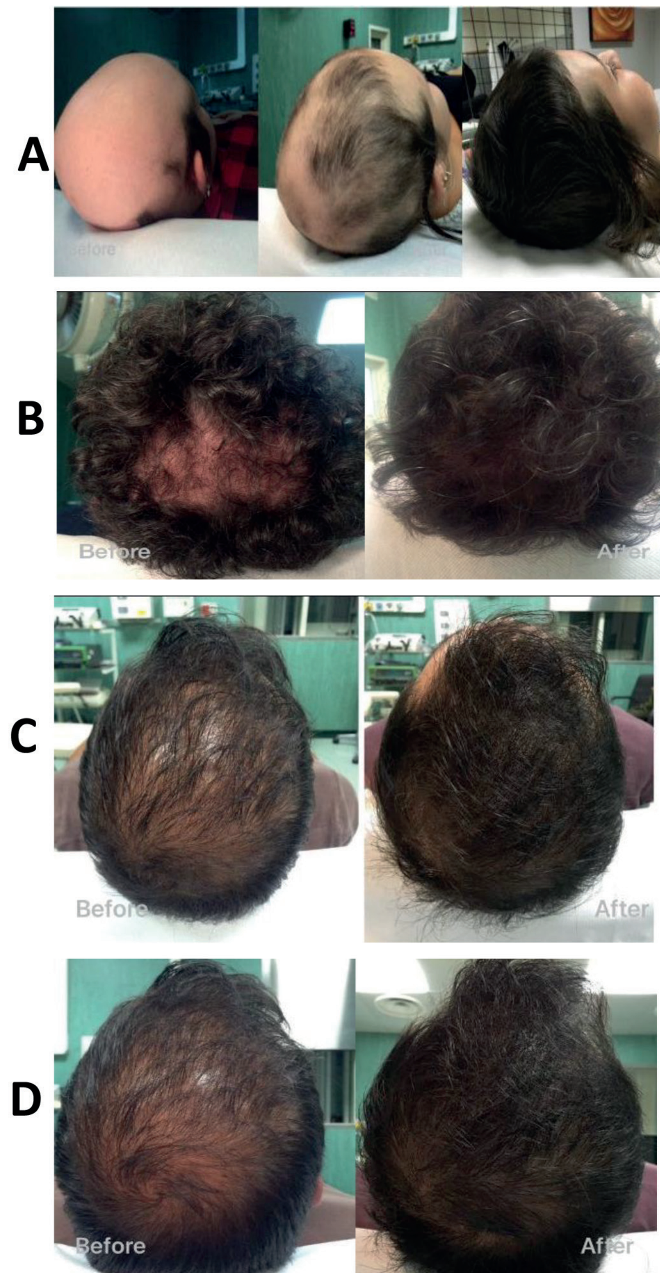

Figure 1. Same examples of PRP treatment and efficacy. ***A,*** Young female (AO1) with alopecia areata, before and after 4 PRP applications with inflammation profile "medium-low" IL-1α CC; IL1β AG; IL-6 GG, IL-10(-592)TT; IL-10(-1080) GA. ***B-D,*** Young males (median age 27 years old) with androgenetic alopecia, before and after 4 PRP applications with "low predisposition" inflammation profile IL-1α CC; IL-1β GG; IL-6 GG, IL-10(-592)GG; IL-10(-1080) GA.

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Conflict of interest

The authors declare no conflicts of interest.

References

1) Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation 2005; 111: 3481-3488.
2) Brass LF. Thrombin and platelet activation. Chest 2003; 124(3 Suppl): 18S-25S.
3) Offermanns S. Activation of platelet function through G protein-coupled receptors. Circ Res 2006; 99: 1293-1304.
4) Angiolillo DJ, Ueno M, Goto S. Basic principles of platelet biology and clinical implications. Circ J 2010; 74: 597-607.
5) Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood Rev 2009; 23: 177-189.
6) Badimon L, Vilahur G, Padró T. Lipoproteins, platelets and atherothrombosis. Rev Esp Cardiol 2009; 62: 1161-1178.
7) Cattaneo M, Gachet C. ADP receptors and clinical bleeding disorders. Arterioscler Thromb Vasc Biol 1999; 19: 2281-2285.
8) Jin J, Quinton TM, Zhang J, Rittenhouse SE, Kunapuli SP. Adenosine diphosphate (ADP)-induced thromboxane A(2) generation in human platelets requires coordinated signaling through integrin alpha(IIb)beta(3) and ADP receptors. Blood 2002; 99: 193-198.
9) Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. Cytokine dysregulation, inflammation and wellbeing. Neuroimmunomodulat 2005; 12: 255-269.
10) Malkowska A, Kasprzak A, Stopa J. Proinflammatory cytokines in pathogenesis of periodontal disease. Pol Merkur Lekarski 2006; 20: 93-98.
11) Prasanna SJ, Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. PLoS One 2010; 5: e9016.
12) Yano S, Sone S, Nishioka Y, Mukaida N, Matsushima K, Ogura T. Differential effects of anti-inflammatory cytokines (IL-4, IL-10 and IL-13) on tumoricidal and chemotactic properties of human monocytes induced by monocyte chemotactic and activating factor. J Leukoc Biol 1995; 57: 303-309.
13) Gabay C, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. Nat Rev Rheumatol 2010; 6: 232-241.
14) McDevitt MJ, Wang HY, Knobelman C, Newman MG, di Giovine FS, Timms J, Duff GW, Kornman KS. Interleukin-1 genetic association with periodontitis in clinical practice. J Periodontol 2000; 71: 156-163.
15) Cullinan MP, Westerman B, Hamlet SM, Palmer JE, Faddy MJ, Lang NP, Seymour GJ. A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population. J Clin Periodontol 2001; 28: 1137-1144.
16) Shirodaria S, Smith J, McKay IJ, Kennet CN, Hughes FJ. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1a protein in gingival crevicular fluid of teeth with severe periodontal disease. J Dent Res 2000; 79: 1864-1869.
17) Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG. Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls. J Clin Periodontol 2008; 35: 754-767.
18) Shete AR, Joseph R, Vijayan NN, Srinivas L, Banerjee M. Association of single nucleotide gene polymorphism at interleukin-1beta +3954, -511, and -31 in chronic periodontitis and aggressive periodontitis in Dravidian ethnicity. J Periodontol 2010; 81: 62-69.
19) Liz-Graña M, Gómez-Reino Carnota J. Tumour necrosis factor. Genetics, cell action mechanism and involvement in inflammation. Allergol Immunol Clin 2001; 16: 140-149.
20) Brinkman BM, Kaijzel EL, Huizinga TW, Giphart MJ, Breedveld FC, Verweij CL. Detection of a C-insertion polymorphism within the human tumor necrosis factor alpha (TNFA) gene. Hum Genet 1995; 96: 493.
21) D'Alfonso S, Richiardi PM. A polymorphic variation in a putative regulation box of the TNFA promoter region. Immunogenetics 1994; 39: 150-154.
22) Uglialoro AM, Turbay D, Pesavento PA, Delgado JC, McKenzie FE, Gribben JG, Hartl D, Yunis EJ, Goldfeld AE. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-alpha gene promoter. Tissue Antigens 1998; 52: 359-367.
23) Di Francia R, Siesto RS, Valente D, Spartà D, Berretta M. Pharmacogenomics panel test for prevention toxicity in patients who recieve Fluoropirimidine/oxaliplatin-based therapy. Eur Rev Med Pharmacol Sci 2012; 16: 215-298
24) Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R, Belaiche J. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998; 113: 401-406.
25) Fassmann A, Holla LI, Buckova D, Vasku A, Znojil V, Vanek J. Polymorphisms in the +252(A/G) lymphotoxin-alpha and the -308(A/G) tumor necrosis factor-alpha genes and susceptibility to chronic periodontitis in a Czech population. J Periodontal Res 2003; 38: 394-399.
26) Kornman KS, di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. Ann Periodontol 1998; 3: 327-338.
27) Endo M, Tai H, Tabeta K, Kobayashi T, Yamazaki K, Yoshie H. Analysis of single nucleotide polymorphisms in the 5'-flanking region of tumor necro-

sis factor-alpha gene in Japanese patients with early-onset periodontitis. J Periodontol 2001; 72: 1554-1559.

28) Perez C, Gonzalez FE, Pavez V, Araya AV, Aguirre A, Cruzat A, Contreras-Levicoy J, Dotte A, Aravena O, Salazar L, Catalan D, Cuenca J, Ferreira A, Schiattino I, Aguillon JC. The -308 polymorphism in the promoter region of the tumor necrosis factor-alpha (TNF-alpha) gene and ex vivo lipopolysaccharide-induced TNF-alpha expression in patients with aggressive periodontitis and/or type 1 diabetes mellitus. Eur Cytokine Netw 2004; 15: 364-370.

29) Trevilatto PC, Tramontina VA, Machado MA, Goncalves RB, Sallum AW, Line SR. Clinical, genetic and microbiological findings in a Brazilian family with aggressive periodontitis. J Clin Periodontol 2002; 29: 233-239.

30) Craandijk J, van Krugten MV, Verweij CL, van der Velden U, Loos BG. Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. J Clin Periodontol 2002; 29: 28-34.

31) Soga Y, Nishimura F, Ohyama H, Maeda H, Takashiba S, Murayama Y. Tumor necrosis factor-alpha gene (TNF-alpha) -1031/-863, -857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. J Clin Periodontol 2003; 30: 524-531.

32) Holla LI, Fassmann A, Stejskalova A, Znojil V, Vanek J, Vacha J. Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. J Periodontol 2004; 75: 30-36.

33) Nibali L, D'Aiuto F, Donos N, Griffiths GS, Parkar M, Tonetti MS, Humphries SE, Brett PM. Association between periodontitis and common variants in the promoter of the interleukin-6 gene. Cytokine 2009, 45: 50-54.

34) Komatsu Y, Tai H, Galicia JC, Shimada Y, Endo M, Akazawa K, Yamazaki K, Yoshie H. Interleukin-6 (IL-6)-373 A9T11 allele is associated with reduced susceptibility to chronic periodontitis in Japanese subjects and decreased serum IL-6 level. Tissue Antigens 2005; 65: 110-114.

35) Trevilatto PC, Scarel-Caminaga RM, de Brito RB, de Souza AP, Line SR. Polymorphism at position -174 of IL-6 gene is associated with susceptibility to hronic periodontitis in a Caucasian Brazilian population. J Clin Periodontol 2003; 30: 438-442.

36) D'Aiuto F, Parkar M, Brett PM, Ready D, Tonetti MS. Gene polymorphisms in pro-inflammatory cytokines are associated with systemic inflammation in patients with severe periodontal infections. Cytokine 2004; 28: 29-34.

37) D'Aiuto F, Ready D, Parkar M, Tonetti MS. Relative contribution of patient-, tooth-, and site-associated variability on the clinical outcomes of subgingival debridement. I. Probing depths. J Periodontol 2005; 76: 398-405.

38) Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998, 102: 1369-1376.

39) Cesar-Neto JB, Duarte PM, de Oliveira MC, Casati MZ, Tambeli CH, Parada CA, Sallum EA, Nociti FH. Smoking modulates interferon-gamma expression in the gingival tissue of patients with chronic periodontitis. Eur J Oral Sci 2006; 114: 403-408.

40) Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFNgamma production. Hum Immunol 2000; 61: 863-866.

41) Babel N, Cherepnev G, Babel D, Tropmann A, Hammer M, Volk HD. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, nterleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with hronic periodontitis. J Periodontol 2006, 77: 1978-1983.

42) Sutherland GR, Baker E, Callen DF, Hyland VJ, Wong G, Clark S. Interleukin 4 is at 5q31 and interleukin 6 is at 7p15. Hum Genet 1988; 79: 335-337.

43) McFarlane CG, Meikle MC. Interleukin-2, interleukin-2 receptor and interleukin-4 levels are elevated in the sera of patients with periodontal disease. J Periodontal Res 1991; 26: 402-408.

44) Mout R, Willemze R, Landegent JE. Repeat polymorphisms in the interleukin-4 gene (IL4). Nucleic Acids Res 1991; 19: 3763.

45) Kim JM, Brannan CI, Copeland NG, Jenkins NA, Khan TA, Moore KW. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. J Immunol 1992; 148: 3618-3623.

46) Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, Camargo LE, Line SR. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. J Clin Periodontol 2004; 31: 443-448.

47) Eskdale J, Kube D, Tesch H, Gallagher G. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. Immunogenetics 1997; 46: 120-128.

48) Berglundh T, Donati M, Hahn-Zoric M, Hanson LA, Padyukov L. Association of the -1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. J Clin Periodontol 2003; 30: 249-254.

49) Lee EH, Kang JS, Kang DS, Han CS, Oh SH, Cho SB. Facilitated scalp is measuring using phototrichogram with a headband and tapeline. Dermatol Surg 2011; 37: 1150–1152.

50) Mazzocca AD, McCarthy MBR, Chowaniec DM, Cote MP, Romeo AA, Bradley JP, Arciero RA, Beitzel K. Platelet-rich plasma differs according to preparation method and human variability. J Bone Joint Surg Am 2012; 94: 308-316.

51) Tang TY, Zhou XX, Huang H, Huang QD. Relationship between IL-1β polymorphisms and obstructive sleep apnea syndrome. Eur Rev Med Pharmacol Sci 2017; 21: 3120-3128.
52) McCarrel TM, Minas T, Fortier LA. Optimization of leukocyte concentration in platelet-rich plasma for the treatment of tendinopathy. J Bone Joint Surg Am 2012; 94: e143.
53) Zaky SH, Ottonello A, Strada P, Cancedda R, Mastrogiacomo M. Platelet lysate favours in vitro expansion of human bone marrow stromal cells for bone and cartilage engineering. J Tissue Eng Regen Med 2008; 2: 472-481.
54) Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med 2009; 37: 2259-2272.
55) Redler LH, Thompson SA, Hsu SH, Ahmad CS, Levine WN. Platelet-rich plasma therapy: a systematic literature review and evidence for clinical use. Phys Sportsmed 2011; 39: 42-51.
56) Berretta M, Di Francia R, Tirelli U. Editorial – The new oncologic challenges in the 3RD millennium. WCRJ 2014; 1: e133.
57) Rinaldi S, Bussa M, Mascaro A. Update on the treatment of androgenetic alopecia. Eur Rev Med Pharmacol Sci 2016; 20: 54-58.
58) Cillo M, Di Paolo M, Pugliese S, Troisi A, Crescente G, Marotta G. Costs and quality of genomics tests in the oncology field. WCRJ 2016; 3: e801.