Treatment of male pattern alopecia with platelet-rich plasma: A double-blind controlled study with analysis of platelet number and growth factor levels

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Background: Promising results with platelet-rich plasma (PRP) in androgenetic alopecia that could be associated with platelet number and growth factor levels were described.

Objective: Analyze the platelet countand growth factor levels in PRP and their correlation with hair growth parameters evaluated by using the TrichoScan (Tricholog GmbH, Freiburg, Germany).

Methods: A total of 26 patients were randomized to receive 4 subcutaneous injections of PRP or saline. Hair growth, hair density, and percentage of anagen hairs were evaluated by using the TrichoScan method before injection, 15 days after the last injection, and again 3 months after the last injection. Growth factors (platelet-derived growth factor, epidermal growth factor, and vascular endothelial growth factor) were measured by the Luminex method (Millipore, Bedford, MA).

Results: We demonstrated a significant increase in hair count (P = .0016), hair density (P = .012) and percentage of anagen hairs (P = .007) in the PRP group versus in the control group, without correlation with platelet counts or quantification of the growth factors in PRP.

Limitations: Other growth factors that could be related to response to PRP were not evaluated.

Conclusion: Our data favor the use of PRP as a therapeutic alternative in the treatment of androgenetic alopecia. The lack of association between platelet count, platelet-derived growth factor, epidermal growth factor, and vascular endothelial growth factor levels and clinical improvement suggest that other mechanisms could be involved in this response. (J Am Acad Dermatol 2019;80:694-700.)

Key words: alopecia; hair loss; platelet-rich plasma; PRP.

Androgenetic alopecia (AGA) is characterized by pattern hair loss and is considered the most common type of alopecia in both men and women.[1] A progressive process of hair follicle miniaturization develops until follicles become ineffective at producing hair.[2] The development and progression of AGA are the result of action of

Abbreviations used:

AGA:	androgenetic androgenetic alopecia
EGF:	epidermal growth factor
PDGF:	platelet-derived growth factor
PRP:	platelet platelet-rich plasma
VEGF:	vascular endothelial growth factor

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androgen hormones on the scalp: testosterone is converted into dihydrotestosterone by the 5-α-reductase type II enzyme that is highly expressed in the AGA affected areas.[3]

The use of platelet-rich plasma (PRP) for hair loss has been described by several authors.[4-10] The platelets contain growth factors involved in many phases of hair growth. During the adult phase, some growth factors act on follicle growth and maintenance: platelet-derived growth factor (PDGF) stimulates stem cell mitosis, transforming growth factor-β activates the dermal papilla and inhibits apoptosis during the cell cycle, and vascular endothelial growth factor (VEGF) helps promote microcirculation.[11,12]

CAPSULE SUMMARY

- Platelet-rich plasma has shown promise for treatment of androgenetic alopecia.
- We found that injections of platelet-rich plasma significantly increased hair growth compared with placebo but the response was not correlated with platelet or the measured growth factors in platelet-rich plasma.

The majority of studies found beneficial effects of PRP on AGA.[4-10] However, there are some controversies regarding the results, mainly because of different end points selected to evaluate the benefits of treatment. In general, more rigorous study design features, including large samples, objective effect measurements, and longer periods of follow-up, are needed to consolidate the utility of PRP for treating patients with AGA. A recent study showed significant differences in mean percentages of anagen, telogen, and terminal hairs and hair density when 3 PRP applications and placebo were compared in 22 patients with AGA who were assessed with use of the TrichoScan (Tricholog GmbH, Freiburg, Germany).[13] Another study did not present PRP as an effective product for increasing terminal hair number; however, the TrichoScan was not the evaluation method used.[13,14]

As an original study, we investigated the correlation between platelet number and PDGF, VEGF, and epidermal growth factor (EGF) levels in PRP with growth hair parameters determined by using the TrichoScan method in patients with AGA treated with 4 local injections.

MATERIALS AND METHODS

Patients

The diagnosis of AGA was based on a personal history of progressive hair loss for more than 2 years and on TrichoScan findings such as the inversion of percentages of anagen and telogen hairs and a decrease in hair density. As a pilot study, a minimum of 30 patients were selected and 26 were included. These patients were randomized into 2 groups: a treatment group treated with application of PRP and a control group treated with application of saline solution.

The inclusion criteria comprised diagnosis of AGA, patient age between 18 and 50 years, and presentation of AGA-III-vertex profile according to the Norwood-Hamilton scale.[15] The exclusion criteria comprised female sex; previous hair transplantation; history of any disease related to hair loss, such as thyroid disease and/or iron deficiency; and present or past neoplasia, kidney, liver, infectious, hematologic, or rheumatoid diseases. Patients who were using antiplatelet and/or anti-inflammatory drugs were also excluded.

All patients provided written informed consent approved by the ethics committee from the Faculty of Medical Sciences of the University of Campinas.

Treatment protocol

This was a double-blinded investigative pilot prospective study. The protocol comprised 20 subcutaneous injections of 100 μL of PRP or saline solution into the scalp, totaling 2 mL. A 32-gauge needle with a 1-mL syringe was used. In total, 4 applications were carried out every 15 days, and the treated area was anesthetized with a 10% lidocaine ointment 15 minutes before each application.

Obtaining PRP

For each patient, peripheral blood was collected in six 8.5-mL acid citrate dextrose tubes, one 4-mL ethylenediaminetetraacetic acid tube, and 1 tube without anticoagulant (BD Vacutaner, BD Biosciences, San Jose, CA). A preparation of PRP with leukocytes was prepared according to the methodology of Amable et al.[16] The platelets were counted in the baseline and in the PRP samples by using Siemens Advia2120i hematology analyzers (Siemens, Knoxville, TN). Autologous serum was prepared from the tube without anticoagulant: after centrifugation at 1258 g for 15 minutes, 1 mL was separated and then added to the PRP syringe right before the application. The entire procedure was carried out in a laminar flow cabinet, and all materials were sterile. PRP samples were normalized to a concentration of 1200×10^6 platelets/μL and activated with autologous serum.

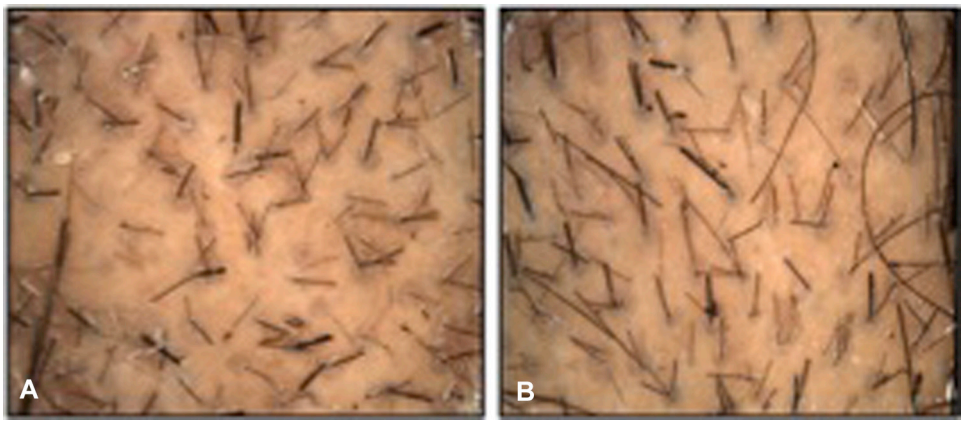

Fig 1. Photographic documentation from the scalp of 1 patient measured by the TrichoScan before (**A**) and after (**B**) the injections. *PRP*, Platelet-rich plasma.

Peripheral blood of patients from the control group was also collected; however, PRP was not prepared, as a saline solution was injected instead.

Growth factor concentration

The growth factors PDGF-AA, EGF, and VEGF were determined by the Luminex technique (Millipore, Bedford, MA), which is a multiplex methodology. The method involves the use of magnetic beads coated with monoclonal antibodies specific to the human protein to be determined. The Luminex technique has a higher evaluation spectrum than the enzyme-linked immunosorbent assay method, and it is more sensitive to lower limits of protein detection even in plasma samples.

To evaluate the intraindividual pattern of variability, only the growth factor levels from the first and the second PRP preparations were analyzed.

TrichoScan

The analysis of the effects of PRP on hair growth parameters was carried out by using the TrichoScan method,[17] which is a noninvasive and objective method to diagnose AGA and carry out the treatment follow-up. TrichoScan consists of standard epiluminescence microscopy, enabling an efficient evaluation of hair density, hair diameter, hair growth, and vellus and terminal hair. After a 1.5-cm^2-diameter section of the area affected by hair loss was shaved, a 1.016-cm^2 area was enlarged with ×40 magnification by using dermoscopy. The parameters were analyzed with specific software (TrichoScanHD 2.0.4.204). This method was executed 3 times: before the injections, 15 days after the last injection, and 3 months after the last injection (Fig 1). The parameters analyzed at the determined times were number of hairs, hair density, percentage of anagen and telogen hairs, and percentage of vellus and terminal hairs.

Statistics

Statistical analyses were carried out by using the Wilcoxon and Spearman tests. To assess statistic normality, the Shapiro test was conducted, and once normality was established, a *t* test between the times was performed in both groups. A *P* value less than 0.05 was considered significant.

RESULTS

During the period from August 2014 to October 2016, a group of 30 male patients were selected for this study. Of these 30, 26 patients were included according to the exclusion criteria and 4 were not: 1 presented with liver disease, 2 were undergoing another treatment for hair loss, and 1 did not present the AGA-III-vertex profile.

The median age of the patients included was 32 years (±7.2 years).

These patients were randomized into 2 groups: the PRP group (n = 15) and the control group (n = 11).

PRP characterization

PRP was characterized according to platelet counts and growth factor concentrations. The median number of platelets was increased 5-fold in each of the 4 PRP preparations, with a minimum of 728.9 and a maximum of 1901.90 (Fig 2). The median platelet count was 1.082×10^6 cells/uL (range, 608-2023). The platelet count in the peripheral blood samples and in the PRP preparations showed a significant correlation ($r = 0.839$, $P < .0001$). The variability in platelet counts for each individual during the applications was 19.7%, with a minimum of 0.50% and a maximum of 56.3% (range, 0.50%-56.3%).

Despite the increase in platelet count in PRP, there was no correlation with any of the growth

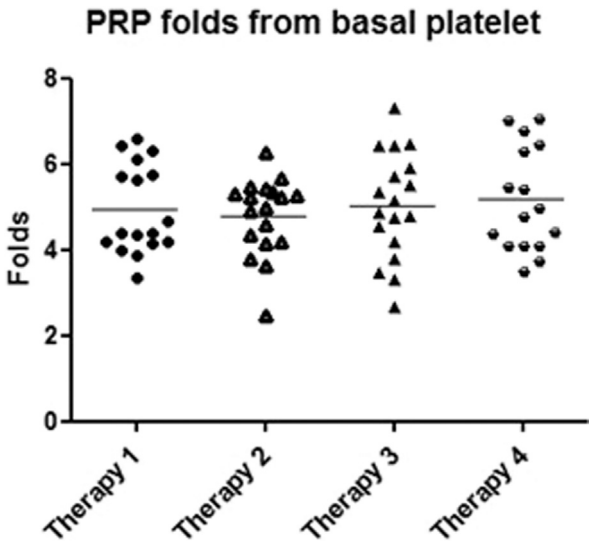

Fig 2. Degree of platelet enrichment from baseline platelet numbers in platelet-rich plasma (PRP) from 4 preparations.

parameters evaluated in this study (P = .123 for hair count, P = .086 for hair density, P = .171 for percentage of anagen and telogen hairs, and P = .174 for percentage of vellus and terminal hairs).

The growth factors from 2 different PRP preparations were measured. There was a similar intra-individual variation between these 2 samples in terms of all growth factor levels, with a mean coefficient of variability of 18.4% for VEGF, 20.9% for PDGF, and 21.6% for EGF.

The EGF and PDGF concentrations showed a significant correlation with platelet number in PRP (r = 0.8287 and $P < .0001$ and r = 0.6925 and P = .0014, respectively).

Regarding EGF, there was no correlation with any of the hair parameters analyzed (P = .389 for hair count, P = .340 for hair density, P = .903 for anagen and telogen percentages, and P = .423 for vellus and terminal hair percentages). The same result was observed regarding VEGF (P = .336 for hair count, P = .364 for hair density, P = .096 for anagen and telogen percentages, and P = .494 for vellus and terminal hair percentages) and PDGF (P = .558 for hair count, P = .193 for hair density, P = .064 for anagen and telogen percentages, and P = .773 for vellus and terminal hair percentages).

Clinical evaluation

The parameters of hair growth evaluated by using the TrichoScan were analyzed in both groups, and the pretreatment and post-treatment results were then compared.

Before treatment, the patients in control group presented more hair and higher hair density than the patients in the PRP group did, and this difference was statistically significant (P = .041).

Regarding hair count, the PRP group showed a significant increase when the counts before application and 3 months after the last application were compared (P = .016). The control group also showed a slight, albeit not significant, increase in hair count (P = .320).

Regarding hair density, the PRP group also showed a significant increase between the baseline and follow-up values (P = .012), whereas no difference was observed in the control group during analysis (P = .206).

Anagen percentage was significantly increased in the PRP group when t0 and t1 were compared (P = .007); however, this result was not maintained during the third evaluation (P = .703). The control group showed no significant increase at any of the evaluation times. As telogen percentage is proportionally inverse to anagen percentage, there was a significant decrease when t0 and t1 were compared (P = .007), and as observed with anagen, this result was not maintained during the follow-up.

The terminal-to-vellus ratio showed no significant difference in the PRP group or in the control group when the ratios before and after treatment were compared (P = .955 for the PRP group and P = .206 for control group).

All these results are shown in Fig 3. The individual mean values for all patients according to time of evaluation are shown in Fig 4.

DISCUSSION

In this study we analyzed the platelet number and growth factor levels in PRP samples and their correlation with the clinical response to AGA treatment. This analysis could contribute to the knowledge in this field, as diverse protocols for PRP preparation could promote different therapeutic responses.

Our results show that PRP promoted increase in hair count, hair density, and the percentage of anagen hairs in patients with AGA. Even though the patients in the PRP group showed worse baseline characteristics, with a lesser number of hairs and lower hair density than the patients in control group showed (which could have resulted in a lower likelihood of response), our results favor the use of PRP. The inclusion of only patients with the AGA-III-vertex profile and the use of TrichoScan for outcomes may be considered strengths of our study. We limited the study to a specific group of patients to promote a more homogeneous group, thereby limiting confounding factors. TrichoScan is

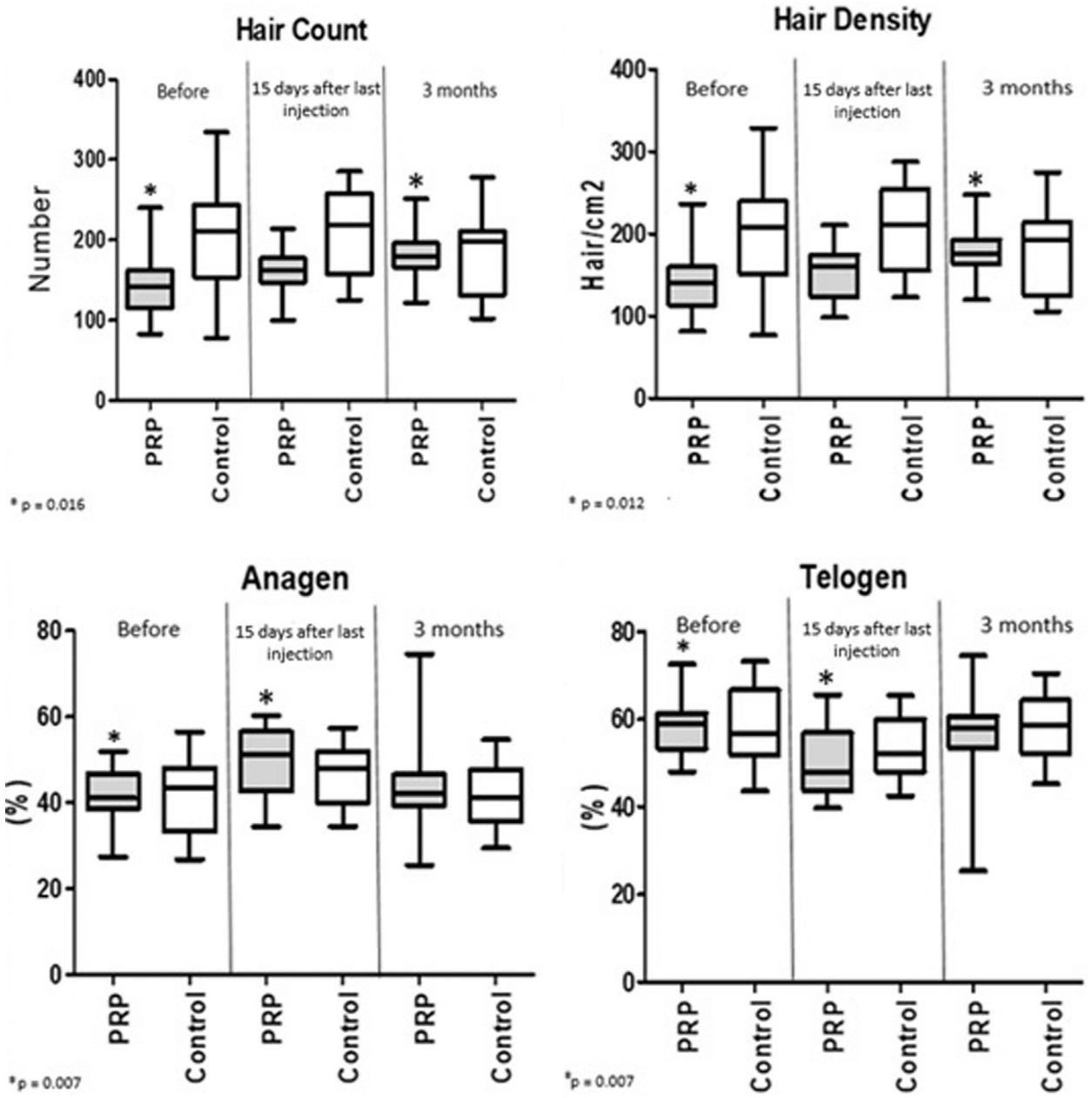

Fig 3. Graphs of the clinical results. *PRP*, Platelet-rich plasma.

an objective, noninvasive, and non—observer-dependent method that allows reliable results. Most previous studies that analyzed the effect of PRP solely on the basis of before-and-after global photographs present some limitations, as doing so makes it more difficult to obtain objective results.[18-20]

Our results demonstrate that the increase in hair count and hair density was maintained for up to 3 months after PRP injections with no other treatment for hair loss. Another interesting point is that PRP did not require the use of daily medication, which is a common cause of nonadherence to conventional treatments.

We chose to inject a PRP with 1 million platelets/mL; however, we could not obtain this concentration in 3 PRP samples. Nevertheless, it is important to point out that in every preparation there was a 5-fold increase in the baseline platelet counts.

Despite the relationship between hair growth and growth factors, no previous study had yet correlated concentrations with the responses obtained.[4-10,13,14] We evaluated PDGF, EGF, and VEGF levels in PRP; however, we were not able to demonstrate any correlation with hair parameters. We observed an intraindividual and a high interindividual variation in platelet numbers and growth

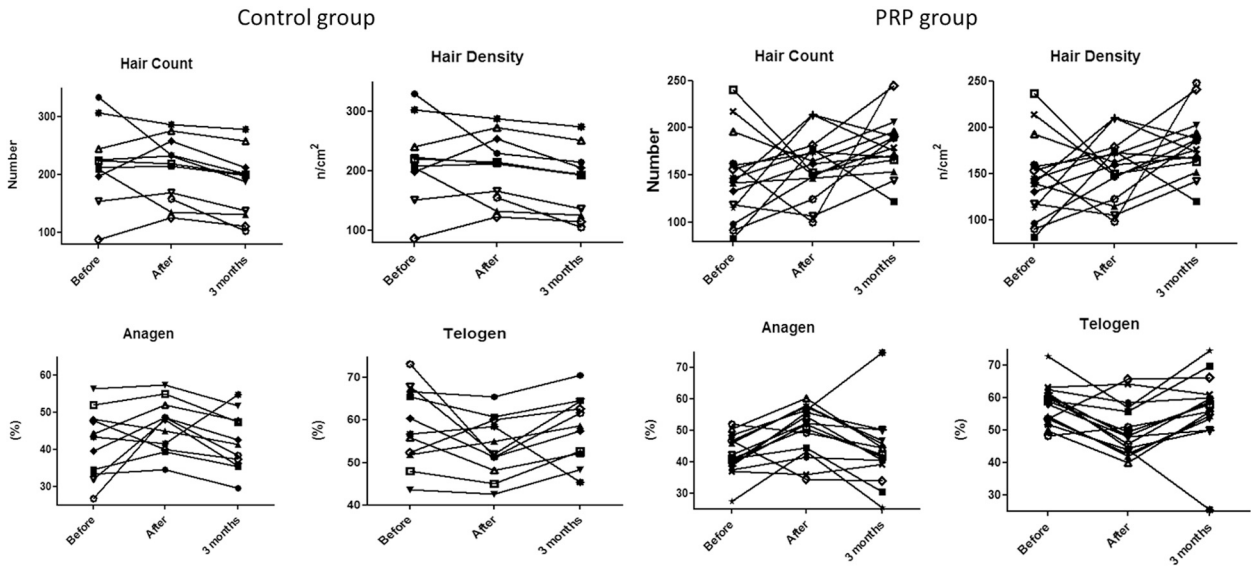

Fig 4. Individual mean values of all patient parameters evaluated according to time of evaluation. *PRP*, Platelet-rich plasma.

factor levels in PRP, but with no association with clinical outcome.

One possibility is that as the effects of PRP depend on orchestration of many mechanisms involved in the increase in hair count and growth, other factors, including local receptors, may play a role in this response. Another hypothesis regarding the lack of correlation between PDGF, EGF, and VEGF levels and the clinical findings is that the receptor saturation, according to which once the concentration threshold has been exceeded, growth factors are left with no additive effect.

Despite the new understanding regarding growth factors involved in the treatment of AGA with PRP that is offered by our results, a limitation of our study is that we did not measure all growth factors or signaling pathways that could contribute to hair growth.

One of the controversies when using PRP is the effect that needling may exert, including inducing the release of growth factors (PDGF and EGF) and activating stem cells in the hair bulge area under conditions of wound healing.[21] Dhurat et al[22] reported the microneedling technique as a promising tool for the treatment of AGA in men and women. As PRP is applied by injections, it was important to demonstrate that there was no difference in the evolution of AGA in our group treated solely with injections of saline solution into the scalp. Therefore, our results suggest that the needling had no effect on hair parameters in these patients with AGA.

Despite the fact that all patients included in this study had been clinically classified as presenting the AGA-III vertex profile, the TrichoScan data showed that the control group had a milder course of the disease, with higher density and hair count at baseline, than did those in the PRP group at the same time. Even with this advantage, there was no clinically significant improvement with needling in the control group. This enhances the results obtained with PRP.

In conclusion, our data favor the use of PRP as a therapeutic alternative in the treatment of AGA. The lack of association between platelet counts, growth factor (PDGF, EGF, and VEGF) levels, and clinical improvement pointed to other mechanisms or other growth factors that could be involved in this response.

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