Skin Appendage Disorders

Methods

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DOI: 10.1159/000533796

Received: June 21, 2023
Accepted: August 17, 2023
Published online: September 21, 2023

Hair-to-Hair Trichoscopy: An Objective Method to Assess Effectiveness of Botulinum Toxin in a Clinical Trial for Androgenetic Alopecia

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Keywords
Androgenetic alopecia · Botulinum toxin · Hair count · Hair-to-hair matching · Alopecia

Abstract
Introduction: Androgenetic alopecia (AGA) is the most common alopecia affecting both genders leading to a potential decrease in quality of life and self-esteem. A current concern in trichology is how to accurately measure clinical response in both daily medical practice and academic research. Hair-to-hair (H2H)-matching technology™ has recently emerged as a technique to evaluate variations in follicular units, hair shaft number, and thickness. This study aimed to describe the methodology employed in a clinical trial using this technology to test the efficacy of botulinum toxin (BT) for male AGA. ***Methods:*** This pilot study is a triple-blind, randomized, split scalp, placebo-controlled clinical trial. Patients enrolled were submitted to injections half of the scalp with 50 IU of BT and the other half with 1 mL of normal saline as a control. The trial involved three visits (weeks 0, 12, and 24) and 8 global clinical photographs followed by H2H-matching trichoscopy were captured before the injections at each visit. Paired *t* test analysis was employed for matched pairs of the following parameters: total hair count, the total number of terminal hair strands, average shaft thickness, and the number of hairs lost or gained during each visit. Then, the software compared the differences between the two sides (BT vs. placebo) per scalp zone and a long time. ***Conclusion:*** The combination of manually corrected image processing, follicular map, and H2H-matching technology™ appears to be the most precise way to evaluate changes in hair count and thickness over time. The design is reproducible and can help other researchers and dermatologists in their clinical practice to obtain reliable results in similar scientific research.

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Introduction

Androgenetic alopecia (AGA) is the most common alopecia, affecting both genders leading to a potential decrease in quality of life (QoL) and self-esteem [1]. The search for more effective treatments for AGA continues to motivate numerous clinical trials [2]. A current concern in trichology is how to accurately measure clinical response in both daily medical practice and academic research. Before and after global pictures and regular trichoscopic images, though useful, are limited tools, sometimes failing to provide adequate comparative measurements for appropriate follow-up [3]. Manual hair counts are susceptible to bias, and standardized QoL questionnaires are subjective [4]. Hair-to-hair (H2H)-matching technology™ has recently emerged as a technique to evaluate variations in follicular units, hair shaft number, and thickness. These evaluations are achieved through a follicular map (FMap) generated from digital trichoscopy images [5]. This article aimed to describe the methodology employed in a triple-blind, randomized, split scalp, placebo-controlled clinical trial using this technology to test the efficacy of botulinum toxin (BT) for male AGA. (Registry: ensaiosclinicos.gov.br/rg/RBR-5z2rr58).

Methods

We enrolled 13 male patients between the ages of 18 and 45 in triple blind a clinical trial to evaluate the efficacy of BT for AGA. Baseline epidemiologic and clinical data of the patients are presented in Table 1. The patients should be either virgin of any treatment or treatment-free for a minimum of 6 months. We excluded women and individuals with other inflammatory, infectious, or neoplastic scalp conditions. Written informed consent was obtained from patients to participate in the study. The trial involved three visits scheduled at week 0, week 12, and week 24).

At the first visit, we marked eight spots of the scalp with a temporary tattoo ink that could last up to 2 years – two tattoos were placed on each of the following four scalp zones: left frontotemporal recess, right frontotemporal recess, right side of the vertex, and left side of the vertex area. In each tattoo pair, the marks were distant 1 cm apart. This pigmentation served as landmarks to ensure precision of the H2H-matching trichoscopic analysis performed on the subsequent visits. The procedure was performed with aseptic standards and only after informed consent.

In all three visits, a total of 8 global clinical photographs followed by H2H-matching trichoscopy were captured before the injections. The photographic incidences were front, top, right, and left sides at 45° and 90°, and two posterior views (in orthostatic position and with posterior neck flexion). These pictures were thoroughly compared to assess treatment response.

The trichoscopy was obtained shortly after the global photos with a Fotofinder leviacam™ camera and the software Tricholab® H2H-matching technology™. The spots tattooed on the four scalp zones were customized for this research. The software predetermined the exact camera positioning to obtain three images per scalp zone. Notably, the hair did not need to be trimmed or shaved but was re-combed between each image acquisition. The camera had two virtual circles that should perfectly fit the pair of tattoos. The area subjected to trichoscopy analysis measured 0.98 cm^2.

All captured images were uploaded to the Tricholab® system for differential before-and-after statistical analysis of the following parameters: total hair count, the total number of terminal hair strands, average shaft thickness, and the number of hairs lost or gained during each visit.

For this matched-pair analysis, half of the scalp with 50 IU of BT and the other half with 1 mL of normal saline as a control were injected. The treatment side received ten shots of 5 IU BT equally distributed around the tattoos of each zone. As for the control side, the corresponding areas received an equal volume of 0.9% sodium chloride.

Table 1. Summary of baseline epidemiological and clinical characteristics of 13 patients

Variables and statistics/categories	Values
Age of onset, years	
Mean (standard deviation)	37.2 (5.8)
Median (interquartile range)	38 (7)
Minimum; maximum	25; 44
Duration of the disease, years	
Mean (standard deviation)	4.1 (1.7)
Median (interquartile range)	4 (2)
Minimum; maximum	2; 8
Fitzpatrick skin phototype, *n* (%)	
1	1 (7.7)
2	1 (7.7)
3	8 (61.5)
4	2 (15.4)
5	1 (7.7)
Hair type, *n* (%)	
Caucasian	10 (76.9)
African	2 (15.4)
Asian	1 (7.7)
Norwood-Hamilton classification, *n* (%)	
2	1 (7.7)
3	6 (46.2)
4	3 (23.1)
5	3 (23.1)
Comorbidities, *n* (%)	
No comorbidity	10 (76.9)
Asthma	1 (7.7)
Hypertension	1 (7.7)
Pre-diabetes	1 (7.7)
Family history, *n* (%)	9 (69.2)
Body mass index, *n* (%)	
Normal (BMI: 20–24.9)	6 (46.1)
Overweight (BMI: 25–29.9)	5 (38.5)
Obese (BMI: 30 and above)	2 (15.4)

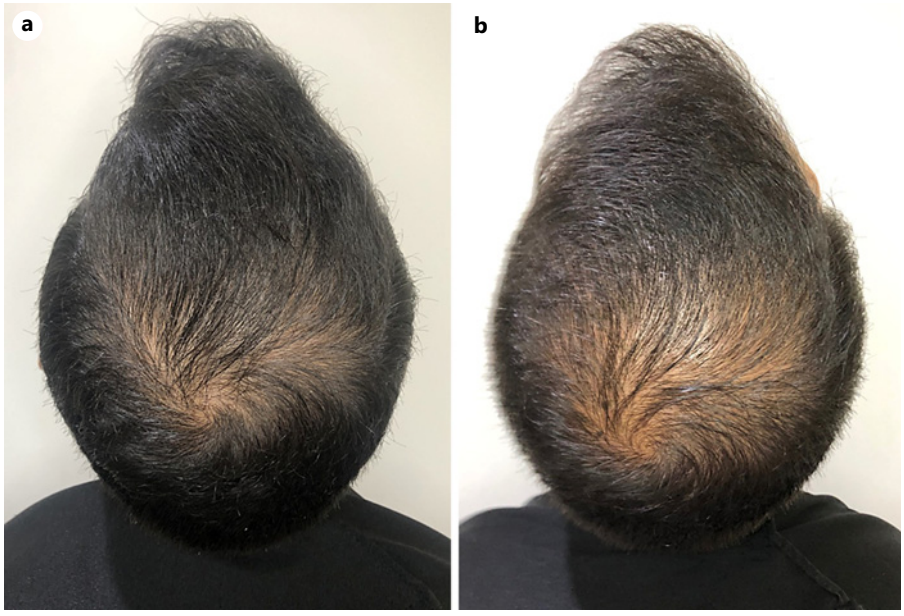

Fig. 1. Comparison of global clinical photographs of the vertex showing apparent worsening of hair density in 1 of the patients after 24 weeks of treatment. Before (**a**) and after (**b**).

In this triple-blind study, neither the patient, the physician, nor the data analyst was aware of what was the intervention each side of the scalp received. The injections were administered on the first two visits.

To analyze data provided by the H2H-matching technology™, paired *t* test analysis for matched pairs of the parameters described above was used. Initially, the intervention and control sides of the scalp were individually evaluated during each follow-up visit (images from the first visit were compared to those from the second, those from the second visit with the third, and first with the third). Then, the software compared the differences between the two sides (BT vs. placebo) per scalp zone and a long time. The statistical significance level considered was 5% ($p < 0.05$). Finally, the percent changes in average hair shaft thickness above 0.06 were calculated using matched odds ratio.

Discussion

Since the 1980s, comparative hair counting before and after intervention in a predetermined area has been considered the gold standard of treatment efficacy outcome [6]. Manual hair counting is time-consuming and subject to human error, and some automated systems require hair shaving [7]. Comparative global clinical photography is subjective, and the perception of the patient may be influenced by haircut, hairstyle, coloring, washing, and psychosocial factors. The distance between the camera and the patient, variation in the environment light, hair color changes, camera angle, and zoom during each picture are all technical variables leading to bias in one's perception of improvement (shown in Fig. 1). Moreover, regular trichoscopy is inefficient for objective analysis if not performed precisely on the very same spot [8].

Tricholab® H2H-matching technology™ uses the relative position of the hair follicles from trichoscopy images to create a map of the scalp – FMap [5]. Similar to a fingerprint, the hair follicle distribution is unique to individuals and scalp areas, and it is not significantly affected by non-scarring alopecia [9]. So, through this technology, we were able to accurately compare BT with placebo for AGA per anatomic zone of the scalp over time without shaving the hair (shown in Fig. 2).

The combination of manually corrected image processing, FMap, and H2H-matching technology™ appears to be the most precise way to evaluate changes in hair count and thickness over time [5]. An increase in hair density is the most objective measure of effectiveness in AGA treatment. However, it does not necessarily correlate with patients' perception of improvement or QoL [10]. Clinical trials should use multiple tools and adopt different endpoints to assess efficacy (e.g., hair counts, global photographs, and QoL questionnaires). The stronger the positive correlation between the tools, the more robust the clinical trials results will be.

Despite its valuable insights, our study is subject to limitations such as a small sample size, the high cost of equipment, the necessity of scalp tattooing, and the considerable time investment required for the examinations. In conclusion, the high prevalence of AGA and its detrimental impact on patients' QoL make the plea for

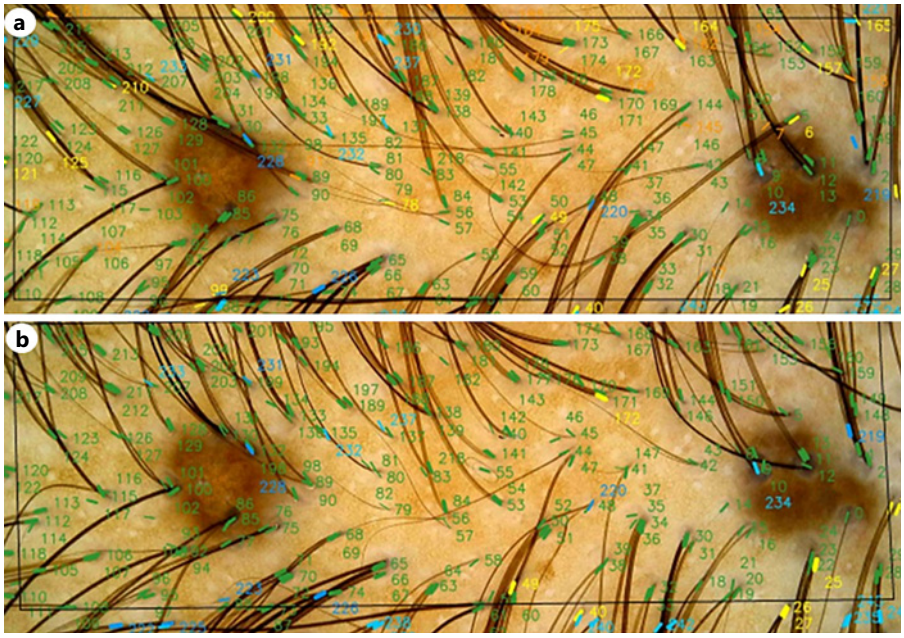

Fig. 2. According to H2H-matched before-after statistics, the average hair thickness was stable: 0.056 mm before (**a**) vs. 0.057 mm after (**b**). There was a reduction in the number of vellus (152 vs. 148) and an increase in terminal hairs (70 vs. 82). These data are discrepant from the apparent clinical worsening seen in Fig. 1.

more effective treatments. Accurate assessment of changes in hair count, thickness, and density is crucial to monitor hair loss, thinning, and the therapeutic effect. This article described in detail the methodology of our triple-blind, randomized, split scalp, placebo-controlled clinical trial evaluating the efficacy of BT for male AGA. The design is reproducible and may help other researchers and dermatologists in their clinical practice to obtain reliable results in similar scientific research.

Acknowledgments

The authors would like to thank Tricholab for providing Leviacam™ throughout the clinical trial period and customizing the H2H-matching technology™ software, especially for our research model. We would also like to thank Allergan for providing Botox® vials for the study, regardless of the final research outcomes.

Statement of Ethics

Written informed consent was obtained from patients to participate in the study. Written informed consent was obtained from the patients for publication of the details of their medical case and any accompanying images. Ethical approval was obtained for this study. https://ensaiosclinicos.gov.br/rg/RBR-5z2rr58. This study protocol was reviewed and approved by Faculdade de Ciências Médicas – Hospital Universitario Pedro Ernesto – State University of Rio de Janeiro, approval number (4.495.668).

Conflict of Interest Statement

Dr. Antonella Tosti is Editor-in-Chief of Skin Appendage Disorders Journal and acts as a consultant for DS Laboratories, MONAT Global, Almirall, Thirty Madison, Eli Lilly, Bristol Myers Squibb, P&G, Pfizer, Myovant, Ortho Dermatologics, and Curallux, LLC. The other authors have no conflict of interest to declare.

Funding Sources

The authors declared that no grants were involved in supporting this work.

Author Contributions

Daniel Fernandes Melo conceived the idea, wrote the manuscript, and approved the final version to be published. André Luiz Vairo Donda and Rita Fernanda Cortez de Almeida prepared the draft, wrote the article, and approved the final version to be published. Daniela Alves Pereira Antelo, Paulo Muller Ramos, and Carla Jorge Machado designed the study, wrote the draft, and approved the final version to be published. Sidney Frattini wrote the draft, reviewed the text, and approved the final version to be published. Antonella Tosti and Carlos Baptista Barcaui critically reviewed the article and approved the final version to be published.

Data Availability Statement

All data are presented within the article. Further inquiries can be directed to the corresponding author.

References

1 Meyer-Gonzalez T, Bacqueville D, Grimalt R, Mengeaud V, Piraccini BM, Rudnicka L, et al. Current controversies in trichology: a European expert consensus statement. J Eur Acad Dermatol Venereol. 2021 Nov;35(Suppl 2):3–11.

2 Melo DF, Ramos PM, Antelo DAP, Machado CJ, Barcaui CB. Is there a rationale for the use of botulinum toxin in the treatment of Androgenetic Alopecia? J Cosmet Dermatol. 2021 Jul;20(7):2093–5.

3 Blume-Peytavi U, Blumeyer A, Tosti A, Finner A, Marmol V, Trakatelli M, et al. S1 guideline for diagnostic evaluation in androgenetic alopecia in men, women and adolescents. Br J Dermatol. 2011 Jan; 164(1):5–15.

4 Shimizu GKM, Wedy GF, Schaefer LV, Ramos PM, Miot HA. Translation into Portuguese language (Brazil), transcultural adaptation and validation of the quality-of-life questionnaire in female pattern hair loss (WAA-QoL-BP). Bras Dermatol. 2018;93(5): 701–6.

5 Bokhari L, Cottle P, Grimalt R, Kasprzak M, Sicińska J, Sinclair R, et al. Efficiency of hair detection in hair-to-hair matched trichoscopy. Skin Appendage Disord. 2022 Sep; 8(5):382–8.

6 Adil A, Godwin M. The effectiveness of treatments for androgenetic alopecia: a systematic review and meta-analysis. J Am Acad Dermatol. 2017;77(1):136–41.e5.

7 Blume-Peytavi U, Orfanos CE. Microscopy of the hair – the trichogram. 2nd ed. In: Serup J, Jemec GBE, Grove GL, editors. Handbook of non-invasive methods and the Skin. Boca Raton, FL: CRC Press; 2006. p. 875–81.

8 Shih H. A precise automatic system for the hair assessment in hair-care diagnosis applications. Skin Res Technol. 2015 Nov;21(4): 500–7.

9 Kasprzak M, Sicińska J, Tosti A. Follicular map: a novel approach to quantitative trichoscopy. Skin Appendage Disord. 2019 Jun; 5(4):216–20.

10 Gupta S, Goyal I, Mahendra A. Quality of life assessment in patients with androgenetic alopecia. Int J Trichology. 2019 Jul–Aug;11(4):147–52.