Aesthetic Medicine 2024; Vol. 10, N. 2: e2024011 DOI: 10.57662/am.v10i2.15836

Original article

Regenerative medicine and carboxytherapy: A promising combination for androgenetic alopecia

Antonio Luca Amore[1], *Emanuele Bartoletti*[2], *Alessandro Gennai*[3]

[1]Private Practice in Aesthetic Medicine, Bologna; [2]Outpatient Service of Aesthetic Medicine and psychophysical wellbeing in pathological patients, Isola Tiberina - Gemelli Isola Hospital, Rome; [3]Private Practice, Bologna

Abstract. *Background:* Androgenetic alopecia, a disease that affects 70% of men and 40% of women, is characterized by the gradual miniaturization of the hair follicle, resulting from the alteration of the dynamics of the hair cycle. *Aim:* One of the most recent and promising advanced medical therapies is regenerative medicine. The important action of Adipose Derived Stem Cells (ADSCs) on the hair follicle is that of inducing anagen by stimulating the stem cells of the hair follicle. The Fibroblastic growth factor expressed by ADSCs also plays an important role in regulating the bulge and in the basal layer of the epidermis, inducing follicular neogenesis. In association with regenerative therapy, carboxytherapy was used with its neoangiogenic potential, exerts its synergistic action. *Methods:* This study aims to evaluate the effectiveness of the technique used for the therapy of androgenetic alopecia. The protocol involved the association of carboxytherapy and regenerative therapy through the grafting of vascular stromal fraction and mesenchymal stem cells of adipose origin. Through trichoscopic images and the use of innovative software, the data relating to the clinical variations of the pathology examined were extrapolated and subsequently statistically analysed. *Results:* 10 patients were enrolled (6 men and 4 women). To evaluate the clinical evolution of the pathology in question, both the clinical scales (Norwood in men and Ludwig in women) and the data extrapolated from the trichoscopic images relating to the ratio between the number of terminal hairs present for each vellus hair (PT/PV) were considered. The variation proved to be statistically significant both by analyzing the average of all areas (P = 0.0018) and by analyzing the areas with the best and worst ratios (P = 0.0032 and P = 0.0106 respectively). *Conclusions:* In consideration of the significance of the data obtained, this study demonstrates once again how the association of regenerative therapy and carboxytherapy is an optimal combination in the treatment of androgenetic alopecia.

Key words: regenerative medicine, androgenetic alopecia, stem cells, dermatology, carboxy therapy, aesthetic medicine

Introduction

Androgenetic Alopecia (AGA) is characterized by the gradual miniaturization of the hair follicle, resulting from the alteration of the dynamics of the hair cycle, which leads to the transformation of the terminal hair follicle into fleece[1]. Understanding hair follicle biology over the past 20 years has established the pivotal role of mesenchyme-derived dermal papillae in maintaining hair growth, with multipotent epithelial stem cells in the bulge area resulting in proliferation and differentiation. Other autocrine and paracrine factors and signaling pathways are involved in this crosstalk between the dermal papillae and hair follicle stem cells[2-4]. Many of the studies have accepted the role of androgens and the interaction between the dermal papillae and the hair follicle as critical process involved in the miniaturization of the hair follicle. The higher the

concentration of androgens and androgen receptors, the greater the effect on the expression of genes that control the follicular cycle. The transmission of the signal between the dermal papillae and hair follicle in the bald person causes the premature cessation of the anagen (hair growth) associated with the premature entry into the catagen (follicular regression phase). Catagen occurs as a consequence of the reduced expression of the anagen maintenance factor (such as growth factor IGF-1, bFGF and VEGF). Furthermore, an increased expression of cytokines (TGF-beta1, IL-1 alpha and TNF alpha) promotes apoptosis[5]. Recently it has been discovered that DKK-1 is a gene upregulated by DHT with consequent inhibition of the cells of the outer root sheath and triggering of apoptosis[6]. The different causes that therefore make Androgenetic Alopecia a multifactorial pathology include: genetic predisposition, food problems, endocrinopathies, use of certain drugs, psychological causes, inflammation of the hair follicle, environmental factors and some microbial toxins[7,8].

Our research group has already highlighted the effectiveness of the association of autologous regenerative therapy and caboxytherapy in a work based on the qualitative analysis of trichoscopic images, overall images and on the analysis of a satisfaction questionnaire. The aim of this new study is to consolidate the efficacy data by analyzing quantitative measures by extrapolating data via new generation trichoscopic images[9].

Therapy

Over the years, various therapeutic strategies have been developed for androgenetic alopecia; from pharmacological treatments (Minoxidil, 5 alpha reductase inhibitors, hormone therapy), to surgery, botulinum toxin, carboxytherapy. In recent years it has become necessary to resort to experimentation with some alternative therapeutic strategies. The use of genes, cells and tissues as a new therapeutic resource is one of the characteristics of contemporary medicine. Advances in regenerative medicine have increased interest in the application of stem cells for the reconstruction of damaged tissues and to develop regenerative therapies for the skin[10,11]. Therapies based on human mesenchymal stem cells (HMSC) have been used in regenerative medicine in various medical areas such as orthopedics, neurology, cardiology and dermatology. HMSCs come from the mesoderm and share their origins with the skin. When these cells are implanted in a damaged tissue, they are able to repair and regenerate the anomalies[12-14]. These cells showed important immunomodulatory activity and are capable of secreting various cytokines and growth factors such as IL-6, IL.8, Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF) which promotes tissue regeneration[15-18]. Although the first tissue used to obtain HMSC was the bone marrow, studies today have shown that subcutaneous adipose tissue is an excellent source of HMSC both from a qualitative and quantitative point of view[19-24]. Some preclinical studies have shown promising results using HMSCs in the treatment of AGA. Byun et al. He developed a pilot study to demonstrate the immunomodulatory effect of mesenchymal stem cells in androgenetic alopecia. In recent years, attention has been paid to the importance of the dermal macroenvironment surrounding the hair follicle. Some studies show how follicles function within a large exchange network and not in a closed system; there are many cellular signals of follicle-follicle and macroenvironment-follicle exchange. The macroenvironment includes cells such as dermal fibroblasts, skin adipocytes, preadipocytes and other extracellular components such as blood vessels and intradermal nerve plexus and immune cells. Therefore, in the light of the previous considerations relating to the pathophysiology of AGA, the ADSCs therapy protocol is also capable of inducing a transformation of the dermal macroenvironment by amplifying those communication signals between the hair follicle and cells of the macro-environment. When we take adipose tissue, in addition to adipocytes we take Stromal Vascular Fraction (SVF), that part rich in stem cells (ADSCs) which includes fibroblasts, pericytes, preadipocytes, hematopoietic cells, cells of the immune system (granulocytes, endothelial cells, monocytes, lymphocytes) and growth factors. As with all therapies it is very important to know the dose that we administer to patients, so it is important to know that in 1ml of adipose tissue we take, we have, on average, from 100,000 to 1,000,000 mononuclear cells of the SVF

and of these from 1 10% are real stem cells. However, for the purpose of our therapy, not only is the action of stem cells important, but also that of all the cells of the SVF and the growth factors present. The rationale used in the development of the SEFFI system (Superficial Enhanced Fluid Fat Injection)[25,26], used to conduct this study, was to take the cellular micro clusters already appropriately sized, through the use of specific cannula, to have a fluid tissue ready to be injected with minimal manipulation that does not interfere with cell viability. The fluidity of the tissue is essential to allow grafting into the superficial dermal and subdermal layers, to improve cell engraftment and expand the possibility of using the method.

As for carboxytherapy, it induces a state of relative hypercapnia and decreases the local pH, which causes a strong vasodilator response, ultimately increasing blood flow to the injection site. Since the pathogenesis of hair loss in Androgenetic Alopecia is caused by a combination of factors including a reduced vascularity of the scalp, it seemed interesting to evaluate its efficacy and safety in the treatment of this pathology combined with regenerative medicine[27].

Materials and methods

This observational study aimed at evaluating the efficacy of the technique used for the treatment of androgenetic alopecia.

The inclusion criteria involved patients with androgenic alopecia who had not received any other therapy for that condition during the study period and in the 4 months before the treatment with regenerative medicine and carboxytherapy.

Before the treatment, trichoscopic images were acquired using the HairMetrix by Canfield® Scientific. Thanks to this type of trichoscopic image (Figure 1) and the integrated software, it was possible to obtain data relating to the characteristics of the hair within the examined area. In particular, the most important data refers to the ratio between the number of terminal hairs present for each vellus hair (PT/PV), a value taken into consideration in this study to evaluate the clinical evolution of the pathology in question.

In order to standardize the image acquisition technique and to make the evaluations reproducible in the different phases of the study, the "Configuration 10-20" protocol established by the International Federation in Electroencephalography and Clinical Neurophysiology, normally used in the positioning of the electrodes for performing EEG (Figure 2). The choice of the image acquisition site was customized according to the distribution of the patient's alopecic areas. Patients enrolled in the study were classified clinically, to assess the degree of alopecia, according to the Hamilton-Northwood scale and the Ludwig scale, for men and women respectively[28].

Photos were also taken of the overall view of the scalp before carrying out the therapy and 2 months

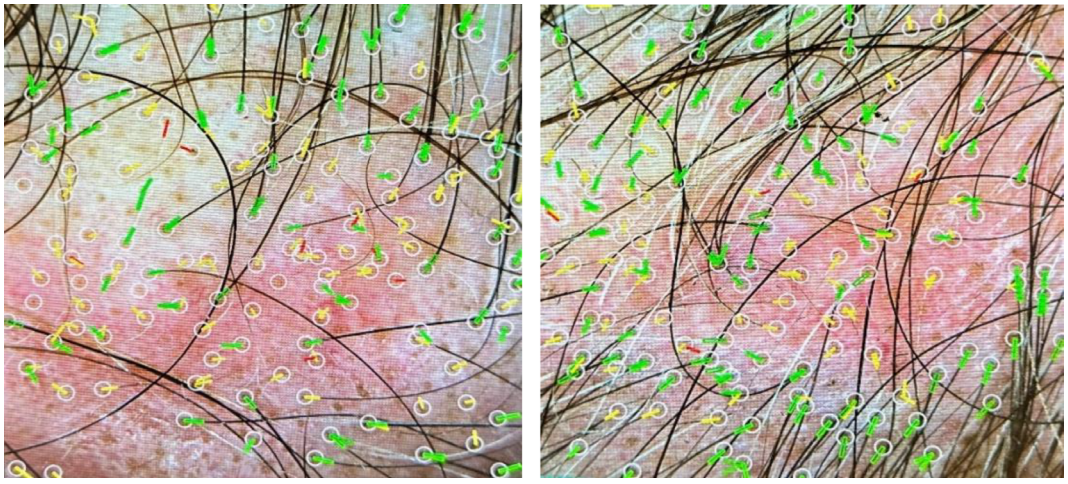


Figure 1. Example of pre/post trichoscopic images used for analysis.

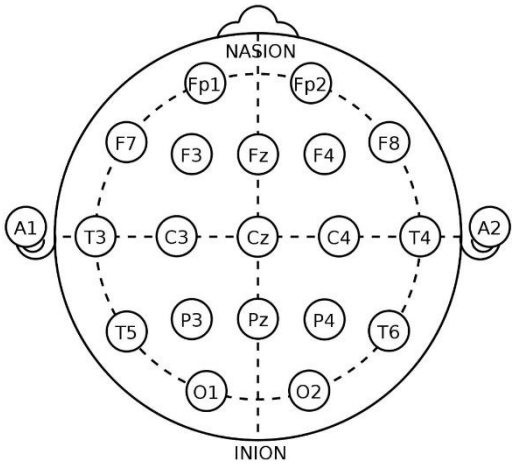


Figure 2. Protocol "Configuration 10-20" https://it.m.wikipedia.org/wiki/File:21_electrodes_of_International_1020_system_for_EEG.svg

after the treatment. The protocol used in this study involved the association of Carboxytherapy and Regenerative Therapy through the grafting of Vascular Stromal Fraction (SVF) and Adipose derived Mesenchymal Stem Cells (ADSCs). For the regenerative therapy, a guided medical device was used in order to standardize the level of tissue sampling, preparation and grafting of the tissue (SEFFIHAIR produced by SEFFILINE srl Bologna Italy). The device has the CE and ISO 13485 mark and is currently undergoing FDA authorization. In order to prepare the scalp for the grafting of SVF and ADSCs, a treatment with carboxytherapy was carried out using the CARBO2HPT® International Patent with Controlled High Flows device using the following protocol: Needle 30G / 4mm, Flow 15-20 cc / 15sec, Temperature 40°, total volume 10-20 cc for single area circumferentially to the skull and inside with treatment in the occipital area of the origin of the vascular axes[27]. After the carboxytherapy treatment, the removal of adipose tissue was performed. Numerous studies have shown that the subcutaneous adipose tissue is composed of adipose cells and stromal tissue (SVF) containing mesenchymal cells and in particular stem cells of adipose origin (ADSCs). This procedure involves grafting the tissue taken and prepared with SEFFIHAIRR in the same patient (autologous graft) in order to obtain the stimulation for hair growth, an improvement in microcirculation (angiogenic action) and an anti-inflammatory and antifibrotic action. The system can be used in different anatomical areas in the same patient and in the same procedure. Absolute contraindications to this treatment included: ongoing infections in the area of sampling or grafting of the tissue; presence of malignant neoplasms in the area of sampling and grafting of the tissue; pregnancy or breastfeeding; anticoagulant therapy or severe bleeding disorder; allergy to local anesthetics; dysmorphophobia; immunosuppressive therapies in place; debilitated subjects. No side effects or complications were noted during the study. We believe that the results that can be obtained with autologous regenerative therapy may vary depending on the patient's age, state of health, lifestyle, surgical site, and the amount of experience of the doctor who performs it. The amount of tissue removed must be limited to what is actually needed. The operating field must be totally sterile. The device is disposable and sterile, not resterilizable. The choice of the tissue sampling area must be made on the basis of the following criteria: adequate presence of subcutaneous adipose tissue (pinch test equal to or greater than 4 cm); size of the area which must have a minimum diameter of 20 cm; the most common sampling areas are abdomen, flank, trochanteric region.

Regerative therapy

The adipose tissue harvesting procedure is performed under local anaesthesia. The adipose tissue was harvested with a 2 mm diameter microperforated cannula with 1 mm side port holes, mounted inside the special patented guide. Both cannula and guide are included in the SEFFIHAIRTM medical device (produced by SEFFILINE S.r.l. Bologna-Italy) (Figure 3)[29-33]. The guide is addressed to standardize the procedure, to guarantee tunneling is performed in the subcutaneous tissue adjacent to the dermis; this layer has been proven to hold more mesenchymal and vascular stem cells[34,35]. Once the adipose tissue is harvested, it is gently washed. The tissue harvested and washed was emulsified with 20 passages from one syringe to another. Subsequently the tissue was subjected to centrifugation (3500 rpm for 10 minutes) in order to separate the liquid (infranatant) tissue from the adipose stromal part. Once the separation had taken place, the infranatant (portion containing the cells of

Figure 3. Seffihair™ medical device produced by SEFFILINE S.r.l. Bologna-Italy.

the stromal vascular fraction and adipose derived stem cells) was aspirated and injected with the mesotherapy technique into the scalp (Figures 4 and 5a).

For statistical analysis the data for continuous variables was reported as means, and initially, the D'Agostino-Pearson test for normal distribution of differences was used to confirm that the data had a normal distribution. Once it was ensured that the data had a normal distribution, a student's t test for paired data was used to verify the significance of the data collected. $P < 0.05$ was considered to indicate significance. MedCalc was used for statistical analyses.

Figure 4. SVF and ADSCs pickup procedure.

Figure 5a. Extraction and grafting of stromal vascular fraction cells.

Results

10 patients were enrolled, 6 men and 4 women who have undergone regenerative therapy with the use of mesenchymal stem cells derived from adipose tissue and carboxytherapy for the treatment of androgenetic alopecia. Clinical improvement was analyzed taking into consideration the starting degree of the scales relating to the classification of androgenetic alopecia (Norwood in men and Ludwig in women) (Figures 5b and 6, Tables 1 and 2, Graphs 1a and b) and it was seen how in 5 out of 6 men and in 3 out of 4 women there was an improvement of one degree in the respective scales.

The VECTRA equipment also allowed the data relating to the PT/PV ratio to be extrapolated from the trichoscopic images.

Table 3 shows all the data (PT/PV ratios in the analyzed areas) of all enrolled patients. The pre/post treatment variation of this type of relationship was analyzed differently based on the areas of the scalp:

- Average of the PT/PV ratios of all the analyzed areas;
- Area with the best starting PT/PV ratio;
- Area with the worst starting PT/PV ratio

The variation proved to be statistically significant both by analyzing the average of all areas (P = 0.0018) and by analyzing the areas with the best and worst ratios (P = 0.0032 and P = 0.0106 respectively).

Discussion

Regenerative medicine certainly represents the most recent approach for the treatment of androgenetic alopecia. As can be seen from the results of this

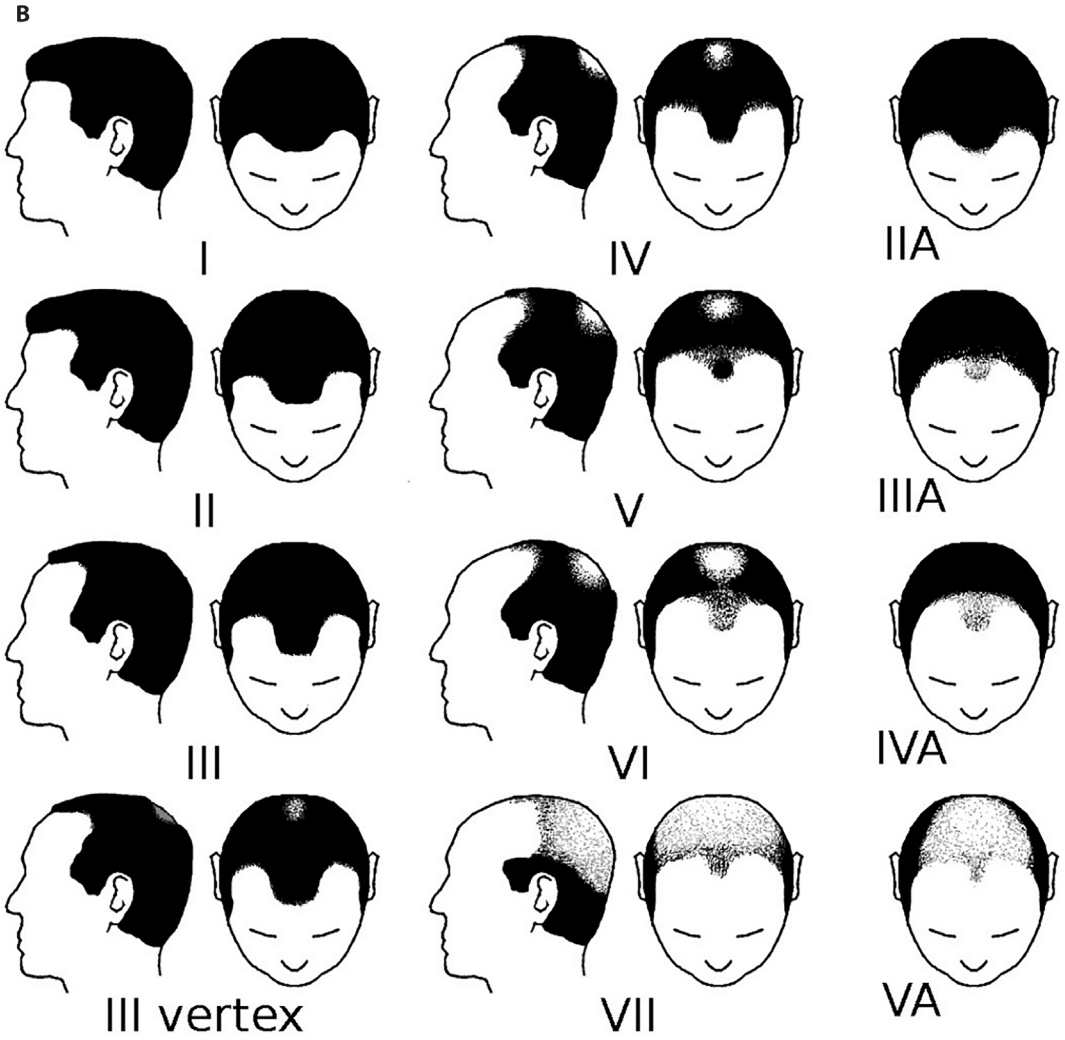


Figure 5b. Male androgenetic alopecia classification table.

study regenerative therapy, through the use of stem cells derived from adipose tissue, has proved effective both considering the trichoscopic parameters and the clinical parameters.

From the analysis of the results obtained in the previous study, it is clear that the combination of regenerative therapy and carboxytherapy is a valid, promising technique and is part of a multidisciplinary approach more suited for the treatment of a pathology such as androgenetic alopecia.

This study strengthens previous findings by confirming clinical improvements upon evaluations with the Norwood and Ludwig scales: in 83% of the men and 75% of the women examined there was a clear clinical improvement.

The strength of the new analysis lies in the introduction of a new quantitative parameter: the terminal hairs / vellus hair ratio (PT/PV) which shows the total number of terminal hairs per single vellus hair, giving an idea of the increase in hair density in the analyzed area.

The change in the PT/PV ratio shows a statistically significant improvement (as shown in Tables 4, 5 and 6) if both the average of all the areas analyzed and the best and worst starting values (respectively P = 0, 0018, P = 0.0032, P = 0.0106) are taken into consideration. This data demonstrates how this therapeutic

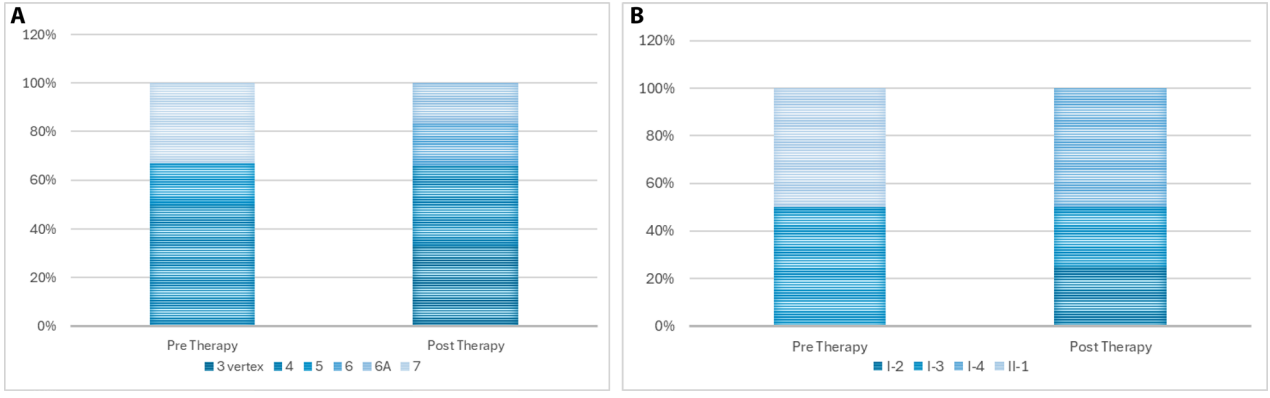
Figure 6. Female androgenetic alopecia classification table.

Table 1. Clinical modification (Norwood scale) pre and post therapy in male patients.

Male Patients	Pre Therapy	Post Therapy
1	5	4
2	4	3 VERTEX
3	7	6
4	4	3 VERTEX
5	4	4
6	7	6 A

Table 2. Clinical modification (Ludwig scale) pre and post therapy in female patients.

Female Patients	Pre Therapy	Post Therapy
1	II-1	I-4
2	I-3	I-2
3	I-3	I-3
4	II-1	I-4



Graph 1. a. Clinical modification (Norwood scale) pre and post therapy in male patients. b. Clinical modification (Ludwig scale) pre and post therapy in female patients.

Table 3. Collection of data relating to the PT/PV ratios in the different areas analyzed, the respective averages, the best and worst values before and after treatment.

Patient	PT/PV Pre	PT/PV Post	PT/PV Average Pre	PT/PV Average Post	PT/PV Best Pre	PT/PV Best Post	PT/PV Worst Pre	PT/PV Worst Post
1	60,7	94,7	61	195	97	225	21,5	27,5
	25,1	265						
	97	225						
2	15,1	73,2	19,4	117,1	29,8	145,7	13,2	132,5
	13,2	132,5						
	29,8	145,7						
3	72	183	75,5	137,7	102,5	152	52	78
	52	78						
	102,5	152						
4	18	46	11,4	35,2	18	46	4	46,3
	4	46,3						
	12,3	13,4						
5	63	102,5	43,4	180,5	63	102,5	33	198
	34,1	241						
	33	198						
6	140	315	104,1	178,2	140	315	81,7	92,7
	90,5	127						
	81,7	92,7						
7	6,7	18,2	7	22	8,3	39	5,8	8,6
	5,8	8,6						
	8,3	39						
8	3,2	120	15	191	19,5	242	3,2	120
	21,3	210						
	19,5	242						
9	58,2	331	40,7	282	58,2	331	26,1	285
	26,1	285						
	37,8	230						
10	50,5	96,5	32,6	70,8	50,5	96,5	21,5	27,5
	23,9	81						
	23,5	34,8						

protocol is effective even in the areas most compromised by androgenetic alopecia, providing uniform improvement.

The literature already contains data relating to the use of microcannulas, in combination with a mechanical digestion by means of an emulsification and centrifuge procedure capable of facilitating the isolation in the infranatant component of SVF cells for regenerative treatment[36]. Furthermore, in the literature, there is already data on how tissue regeneration through Autologous Fat Transfer can significantly improve hair growth in the area of scarring alopecia, emphasizing, once again, the enormous potential of this approach.

After our previous study published in 2022[9], this study confirms the validity of regenerative therapy through the use of stem cells derived from adipose

Table 4. Statistical study of the values relating to the averages of the total PT/PV ratios pre and post treatment.

	PT/PV media PRE	**PT/PV media POST**
Sample size	10	10
Arithmetic mean	41,0100	140,9500
95% CI for the mean	18,6120 to 63,4080	82,9782 to 198,9218
Variance	980,3321	6567,3161
Standard deviation	31,3103	81,0390
Standard error of the mean	9,9012	25,6268
Paired Samples t-test		
Mean difference		99,9400
Standard deviation of differences		72,4413
Standard error of mean difference		22,9080
95% CI of difference		48,1186 to 151,7614
Test statistic t		4,363
Degrees of Freedom (DF)		9
Two-tailed probability		P = 0,0018
Differences		
D'Agostino-Pearson test for Normal Distribution of differences		Accept Normality (P= 0,5495)

Table 5. Statistical study of the values relating to the best pre- and post-treatment PT/PV ratios.

	PT/PV media PRE	**PT/PV media POST**
Sample size	10	10
Arithmetic mean	58,6800	169,4700
95% CI for the mean	27,9118 to 89,4482	94,6088 to 244,3312
Variance	1849,9440	10951,3534
Standard deviation	43,0110	104,6487
Standard error of the mean	13,6013	33,0928
Paired Samples t-test		
Mean difference		110,7900
Standard deviation of differences		87,8614
Standard error of mean difference		27,7842
95% CI of difference		47,9377 to 173,6423
Test statistic t		3,988
Degrees of Freedom (DF)		9
Two-tailed probability		P=0,0032
Differences		
D'Agostino-Pearson test for Normal Distribution of differences		Accept Normality (P= 0,4499)

Table 6. Statistical study of the values relating to the worst pre- and post-treatment PT/PV ratios.

	PT/PV media PRE	PT/PV media POST
Sample size	10	10
Arithmetic mean	26,1800	124,8800
95% CI for the mean	8,3487 to 44,0113	55,6665 to 194,0935
Variance	621,3240	9361,3084
Standard deviation	24,9264	96,7539
Standard error of the mean	7,8224	30,5963
Paired Samples t-test		
Mean difference		98,7000
Standard deviation of differences		97,1194
Standard error of mean difference		30,7118
95% CI of difference		29,2250 to 168,1750
Test statistic t		3,214
Degrees of Freedom (DF)		9
Two-tailed probability		P = 0,0106
Differences		
D'Agostino-Pearson test for Normal Distribution of differences		Accept Normality (P= 0,4442)

tissue associated with carboxytherapy in androgenetic alopecia. For several years now carboxytherapy has represented one of the most significant approaches in this type of pathology: the combination with regenerative medicine, described in this study, aims to underline how the association of several methods is the winning weapon in fighting a pathology with a multifactorial etiology such as Androgenetic Alopecia.

Conclusions

This new quantitative analysis supports the data by demonstrating that combining regenerative therapy and carboxytherapy is a valid, promising technique and is part of a multidisciplinary approach more suitable for the treatment of pathologies such as androgenetic alopecia.

In the future, these acquisitions will have to be consolidated by increasing the number of the population examined and inserting new trichoscopic parameters.

Conflict of Interest: Antonio Luca Amore and Emanuele Bartoletti have no conflict of interest. Alessandro Gennai is the scientific director of Seffiline Academy; Seffiline™ srl produces the device used to perform the study.

References

1. Girman CJ, Hartmaier S, Roberts J, Bergfeld W, Waldstreicher J. Patient perceived importance of negative effects of androgenetic alopecia in women. J Womens Health Gend Based Med. 1999; 8(8):1091–5.
2. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell. 2004; 118(5):635–48.
3. Botchkarev VA, Kishimoto J. Molecular control of epithelial–mesenchymal interactions during hair follicle cycling. J Investig Dermatol Symp Proc. 2003; 8(1): 46–55.
4. Kishimoto J, Burgeson RE, Morgan BA. Wnt signaling maintains the hair inducing activity of the dermal papilla. Genes Dev. 2000; 14(10):1181–5.
5. Paus R. Control of the hair cycle and hair diseases as cycling disorders. Curr Opin Dermatol 1996; 3:248–58.
6. Kwack MH, Kim MK, Kim JC, Sung YK. Dickkopf 1 promotes regression of hair follicles. J Invest Dermatol 2012:132:1554–60.
7. Mahé YF, Michelet JF, Billoni N, et al. Androgenetic alopecia and microinflammation. Int J Dermatol. 2000; 39(8):576–84.
8. Philpott MP, Sanders DA, Bowen J, Kealey T. Effects of interleukins, colony-stimulating factor and tumour necrosis factor on human hair follicle growth in vitro: A possible role for interleukin-1 and tumour necrosis factor alpha in alopecia areata. Br J Dermatol. 1996; 135(6):942–8.

9. Amore AL, Bartoletti E, Gennai A. The role of regenerative medicine in androgenetic alopecia treatment: an uncontrolled clinical trial. Aesthetic Medicine. 2022; 8(1).
10. Strong AL, Neumeister MW, Levi B. Stem Cells and Tissue Engineering: Regeneration of the Skin and Its Contents. Clin Plast Surg. 2017; 44:635–650.
11. Campos-Muñoz A. Advanced therapies in current Medicine. Actual Med. 2017; 102(802):133.
12. Wang L, Ji H, Zhou J, et al. Therapeutic potential of umbilical cord mesenchymal stromal cells transplantation for cerebral palsy: a case report. Case Rep Transplant. 2013; 2013:146347.
13. Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA. Concise review: role of mesenchymal stem cells in wound repair. Stem Cells Transl Med. 2012; 1(2):142–149.
14. Kim S, Lee SK, Kim H, Kim TM. Exosomes Secreted from Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Accelerate Skin Cell Proliferation. Int J Mol Sci. 2018; 19(10):3119.
15. Yates CC, Rodrigues M, Nuschke A, et al. Multipotent stromal cells/mesenchymal stem cells and fibroblasts combine to minimize skin hypertrophic scarring. Stem Cell Res Ther 2017; 8(1):193.
16. Almalki SG, Agrawal DK. Key transcription factors in the differentiation of mesenchymal stem cells. Differentiation. 2016; 92(1-2):41–51.
17. Park JS, Yang HN, Woo DG, Jeon SY, Park KH. The promotion of chondrogenesis, osteogenesis, and adipogenesis of human mesenchymal stem cells by multiple growth factors incorporated into nanosphere-coated microspheres. Biomaterials. 2011; 32(1):28–38.
18. Miyagawa I, Nakayamada S, Kondo M, Tanaka Y. Regulatory Mechanism of The Induction of Regulatory T Cells through Growth Factors Released by Human Mesenchymal Stem Cells. Crit Rev Immunol. 2018; 38(6):471–478.
19. Puissant B, Barreau C, Bourin P, et al. Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. B J Haematol. 2005; 129(1):118–129.
20. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue- derived stromal vascular fraction and culture expanded adipose tissue- derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013; 15(6):641–8.
21. Gimble JM, Bunnell BA, Frazier T, et al. Adipose-derived stromal/stem cells. Organogenesis. 2013; 9(1):3–10.
22. Nguyen A, Guo J, Banyard DA, et al. Stromal vascular fraction: a regenerative reality? Part 1: current concepts and review of the literature. J Plast Reconstr Aesthetic Surg. 2016; 69(2):170–9.
23. Debnath T, Chelluri LK. Standardization and quality assessment for clinical grade mesenchymal stem cells from human adipose tissue. Hematol Transfus Cell Ther. 2019; 41(1):7–16.
24. Silva JD, Lopes-Pacheco M, Paz AHR, et al. Mesenchymal Stem Cells from Bone Marrow, Adipose Tissue, and Lung Tissue Differentially Mitigate Lung and Distal Organ Damage in Experimental Acute Respiratory Distress Syndrome. Crit Care Med. 2018; 46(2):e132–e140.
25. Zare H, Jamshidi S, Dehghan MM, Saheli M, Piryaei A. Bone marrow or adipose tissue mesenchymal stem cells: Comparison of the therapeutic potentials in mice model of acute liver failure. J Cell Biochem. 2018; 119(7):5834–5842.
26. Tesauro P, Trivisonno A, Gennai A, Marliani A, Clauser L. Hair Transplantation in Cicatricial Alopecia: The Role of Autologous Fat Transfer. Int J Regen Med. 2020; 3(2):1–11.
27. Doghaim NN, El-Tatawi RA, Neinaa YME, Abd El-Samd MM. Study of the efficacy of carboxytherapy in alopecia. J Cosmet Dermatol. 2018; 17(6):1275–1285.
28. Guarrera M, Cardo P, Arrigo P, Rebora A. Reliability of hamilton-norwood classification. Int J Trichology. 2009; 1(2):120–2.
29. Gennai A, Zia S, Roda B, et al. SEFFI (Superficial Enhanced Fluid Fat Injection) for Aesthetic and Clinical Regenerative Treatments. Global J Dermatol Venereol. 2020; 8:32–40.
30. Gennai A, Bernardini FP. Superficial enhanced fluid fat injection (SEFFI and MicroSEFFI) in facial rejuvenation. CellR4. 2017; 5(1):e223.
31. Gennai A, Zambelli A, Repaci E, et al. (2016) Skin Rejuvenation and Volume Enhancement with the Micro Superficial Enhanced Fluid Fat Injection (M-SEFFI) for Skin Aging of Periocular and Perioral Regions. Aesthet Surg J. 2017; 37(1):14–23.
32. Bernardini FP, Gennai A. Fluid Fat Injection for Volume Restoration and Skin Regeneration of the Periocular Aesthetic Unit. JAMA Plastic Facial Surg. 2015; 18(1):68–70.
33. Bernardini FP, Gennai A, Izzo L, et al. Superficial Enhanced Fluid Fat Injection (SEFFI) to Correct Volume Defects and Skin Aging of the Face and Periocular Region. Aesthet Surg J. 2015; 35(5):504–515.
34. Clauser L, Lucchi A, Trussardi TI, Gardin C, Zavan B. Autologous fat transfer for facial augmentation and regeneration: Role of mesenchymal stem cells. Atlas Oral Maxillofac Surg Clin North Am.2018; 26(1):25–32.
35. Trivisonno A, Di Rocco G, Cannistra C, et al. Harvest of superficial layers of fat with a microcannula and isolation of adipose tissue-derived stromal and vascular cells. Aesthet Surg J. 2014; 34(4):601–613.
36. Gennai A, Tesauro P, Colli M, Zia S, Roda B, Zattoni A. Infranatant Portion of Microfragmented Adipose Tissue: A Promising Source of SVF for the Management of Androgenetic Alopecia. Int J Regenr Med. 2021.

Correspondence:
Received: 19 march 2024
Accepted: 29 may 2024
Antonio Luca Amore, MD
Via Mazzini 152/1, Bologna
E-mail: Dr.antonioamore@gmail.com