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CASE REPORT

Infiltrative Therapy of Adipose Vital Micrografts in a Metabolic and Protective Solution in Peyronie's Disease: A Case Report in a Pilot Study

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Abstract

Peyronie's disease or *induratio penis plastica*, is characterised by fibrotic plaque formation within the *tunica albuginea*, leading to penile curvature and pain. Current minimally invasive treatments provide limited outcomes. Aim of the research was to explore the preliminary feasibility and tolerability of intralesional injections of viable adipose-derived micrografts composed of mesenchymal stem cells (MSCs) and their exosomes, emulsified with a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acid chains and sodium bicarbonate. A 52-year-old male presented with a 12-month history of penile curvature and painful erections. He underwent a single injection of adipose-derived micrografts (20-40 microns) composed of mesenchymal stem cells (MSCs) and their exosomes and emulsified with a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acids branches and sodium bicarbonate for pH stabilisation directly into the plaque. Outcomes were assessed at baseline and 6 months post-treatment using goniometry, Visual Analogue Scale (VAS) for pain and International Index of Erectile Function-5 (IIEF-5). At 6 months, penile curvature decreased from 28° to 22°, a 21 % reduction. Pain resolved completely (VAS from 5 to 0) and erectile function improved (IIEF-5 from 17 to 21). No adverse events were reported. This single case report provides preliminary, hypothesis-generating observations suggesting that intralesional injection of adipose-derived micrografts combined with a metabolic and protective hyaluronic acid solution is feasible and apparently well tolerated in one patient with Peyronie's disease. The observed numerical improvements in curvature, pain and erectile function cannot be attributed to the treatment with confidence in the absence of a control group and may reflect the natural history of the disease. This approach was investigational and not currently endorsed by European Association of Urology (EAU) or American Urological Association (AUA) guidelines. Larger randomised controlled studies are required before any conclusion on efficacy can be drawn.

Key words: Mesenchymal stem cells, adipose-derived; Stem cells; Adipose tissue; Penile induration; Exosomes.

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Introduction

Peyronie's disease (*induratio penis plastica*) is a pathophysiological condition characterised by an abnormality in wound healing events. The events leading to the disease can be traced back to an abnormal healing response within the tunica albuginea.[1, 2] The characteristic pathological aspect of Peyronie's disease derives from an excess production of extracellular matrix deposited by fibroblasts with the formation of fibrotic tissue which leads to a retracting scar outcome.[3, 4] Clinically it

is characterised by the presence of a pathological curvature on the shaft of the penis and consequent sexual dysfunction due to the sensation of pain. Among patients suffering from this condition, only a low percentage report having had a traumatic event during sexual intercourse.[5, 6] The clinical course is characterised by an acute, very painful phase which induces a progressive curvature of the penis and a subsequent chronic phase with the stabilisation of the symptoms. In this second phase, a slight improvement in symptoms may occur.[7] The signs, symptoms and therapy were described for the first time by Francois Gigot de la Peyronie in 1743.[8, 9]

Although the disease can arise between the fifth and sixth decade of life, it can present itself at any age[10] with a notable impact on the couple's sexuality and the consequent quality of relationship life with the partner.[11] The treatment is different if the disease is in the acute or chronic phase or if deformation or "hinge" phenomena coexist, in which the penis bends on itself in the area of the deformity caused by axial pressure. The retracting plate must be identified by palpating the penis along its entire length, identifying the precise point or points of the positioning of the scarring. A possible ultrasound study can complete the clinical evaluation by indicating the haemodynamics and dimensions of the plaque or plaques, allowing the appropriate therapeutic path to be activated.[12, 13]

In patients with minimal complaints, a follow-up period and possible early pharmacological intervention are implemented which can also prevent the need for subsequent invasive treatment, such as surgical straightening of the penis. In this way, a less invasive approach is facilitated by the reduction of the plaque through an improvement in the quality of the fibroblasts implicated in the pathophysiological events with a consequent decrease in the deposition of fibrotic collagen.[14, 15]

One of the usual least invasive treatments during both the acute and chronic phases is local infiltration therapy[16] and refers to the process of injecting a drug directly into the plaque. This therapeutic approach represents a treatment modality with a very low invasiveness but with proven benefits[16] probably for the recovery of the circumference and length of the penile shaft.[17, 18] A variety of agents have been studied in recent decades, including botulinum toxin, thiocolchicine and hyaluronic acid. Hyaluronic acid has long been used to reduce symptoms and inflammation in Peyronie's disease through intralesional injection and has been shown to reduce plaque volume and curvature due to its own intrinsic protective characteristics against reactive species of the oxygen and nitrogen generated during inflammation and limits their penetration especially to the cell membrane.[19]

This case report aimed at exploring a possible role also of adipose-derived tissue progenitors with the characteristics of adult mesenchymal stem cells as micrografts of vital tissue[20, 21] using a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acids branches and sodium bicarbonate directly into the plaque as a scaffold[20, 21] in the therapy of *induratio penis plastica* and to evaluate the efficacy and the safety of using this possible therapeutic approach also in the treatment of Peyronie's disease. Also, was to verify the possibility of using the possible therapeutic role of tissue progenitors with the characteristics of adult mesenchymal stem cells derived from viable micrografts from a sample of adipose tissue deprived of the inflammatory component through a simultaneous disaggregation and filtration[22, 23] in the treatment of Peyronie's disease.

Emerging therapies show that mesenchymal stem cells (MSCs) have immunomodulatory and antifibrotic effects by secreting regulatory miRNAs, proteins and exosomes with autocrine and paracrine activity with a consequent improvement in scarring due to the influence that some factors have in counteracting pro-fibrotic pathways.[23, 24] The therapeutic approach described in this case report – intralesional injection of viable adipose-derived micrografts combined with a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acid chains and sodium bicarbonate as a scaffold is not currently approved by international guidelines for the management of Peyronie's disease (European Association of Urology (EAU) Guidelines 2023; American Urological Association (AUA) Guidelines 2015, revised 2022). Guideline-approved minimally invasive treatments for Peyronie's disease include intralesional *Clostridium histolyticum* collagenase, verapamil, interferon alpha-2b and penile traction therapy; surgical correction remains the standard for stable chronic-phase disease with functional impairment. This case report is presented strictly as a hypothesis-generating, preliminary observation; all mechanistic claims regarding MSC markers, exosomal activity and microRNA upregulation represent working hypotheses derived from the published biological

literature and are not conclusions supported by data generated in this case.

Case history

A 52-year-old man presented with a 12-month history of progressive penile curvature (~28°) and pain during erection. The patient reported no significant trauma, but had a history of smoking and mild diabetes mellitus. Erectile function was moderately impaired (International Index of Erectile Function-5 (IIEF-5) score of 17). Inclusion criteria were present: history of symptoms characterised by penile curvature and painful erections. Exclusion criteria: history of mental disorders or emotional instability; history of allergic reaction to hyaluronic acid products; current or past treatment with an investigational drug and/or medical device, or participation in another clinical trial were absent.

The patient underwent an injection of a suspension containing 2.0 mL of viable adipose-derived micrografts,[22] composed of tissue progenitors with characteristics of mesenchymal stem cells (MSCs) and containing exosomes (as characterised in published biological literature on analogous preparations; no laboratory validation of MSC markers or exosomal content was performed on the product used in this case), emulsified in 2.0 mL with a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acid ramifications and sodium bicarbonate to stabilise the pH in the corpora cavernosa at the plaque level. The adipose tissue was extracted following a standardised protocol. This standardised protocol ensures the extraction of numerous viable adipose tissue progenitors (ADSCa)[23] by applying filtration between 20 and 40 microns. Indeed, using some disposable devices, it is possible to extract and process optimal quantities of adipose tissue, followed by the necessary microfiltration to the maximum adipose tissue progenitor size of 30.4 microns.[25]

This microfiltration allows us to obtain a suspension containing only viable tissue progenitors, excluding all interfering material such as connective tissue and cell envelope fragments. The procedure to obtain fat using this protocol involves four steps, as illustrated below: (1): A regional local anaesthesia was performed. (2): The adipose tissue was then removed. Both procedures were performed using a multi-hole cannula connected to a 10 mL Luerlock® syringe to simplify the procedure. (3): The extracted tissue was immediately defragmented through a three-way valve to obtain a suspension containing the tissue progenitors. (4) The adipose tissue, during defragmentation, was microfiltered to a size of 20-40 microns to isolate the lateral population of tissue progenitors, excluding interfering material. Microfiltration between 20 and 40 microns did not alter the structure or biological function of the defragmented tissue because it occurs without the use of enzymes, but only mechanically. The sterility of the entire procedure was guaranteed by the closed circuit in which it takes place. The resulting suspension was mixed with 1.5 mL of non-cross-linked hyaluronic amino acid branches and sodium bicarbonate as a protective scaffold, by connecting two syringes. The use of non-cross-linked hyaluronic acid served only as a passive carrier and does not alter the biological function of the defragmented adipose tissue. All these procedures were performed in a medical office.

Detailed procedure

After administering local anaesthesia to the abdomen or supratrocanteric donor area With Klein's solution through a 10 mL syringe and a multi-hole cannula, the extraction of a total of 10 mL of lipoaspirate was undertaken (Figure 1). After adipose tissue extraction, 5 mL of saline was added to dilute the suspension and anaesthesia fluids and the suspension was left to settle for 15 min, in order to eliminate all anaesthesia fluids (Figure 2). Seven mL of adipose tissue was processed as described by Tonnard 2013[26] and filtered at 20/40 microns to preserve the side population of tissue adipocyte progenitors (Figure 3).[23] The elimination of connective tissue and cell shells an debris by microfiltration allows to obtain a better therapeutic quality by excluding the interfering material with the ability to activate the toll-like system,[27] obtaining a final suspension of 3.5 mL. The microfiltrate is mixed with a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acids branches and sodium bicarbonate for pH stabilisation through a three-way tap with a very gentle back and forth movement (made for 4-5 times) in order to emulsify the two parts and we identified the plaques (Figure 4). The suspension thus obtained was injected directly into the plaques that are present along the corpora cavernosa with needle 30 G (Figure 5). The patient

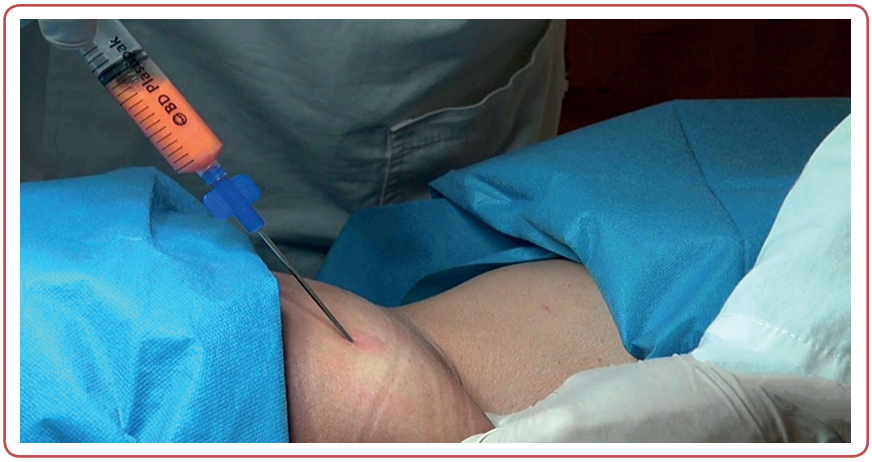

Figure 1: Adipose tissue extraction with multi-hole cannula after local anaesthesia with Klein's solution. Note adrenaline-induced skin whitening

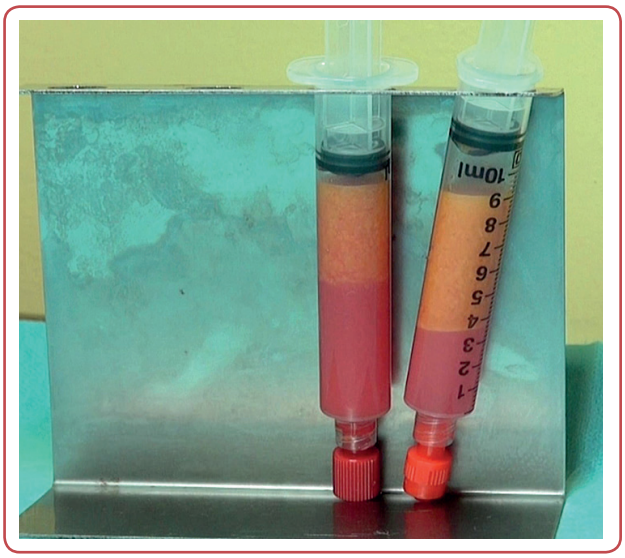

Figure 2: Decantation for the removal of anaesthesia liquids

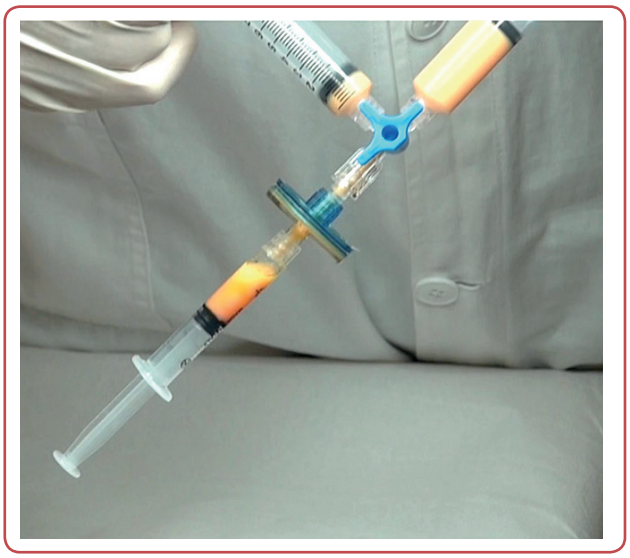

Figure 3: Disaggregation of adipose tissue in the 10 mL syringe and simultaneous 20-40 microns filtration with harvesting in the 2.5 mL syringe

Figure 4: Physical examination to identify the plaque

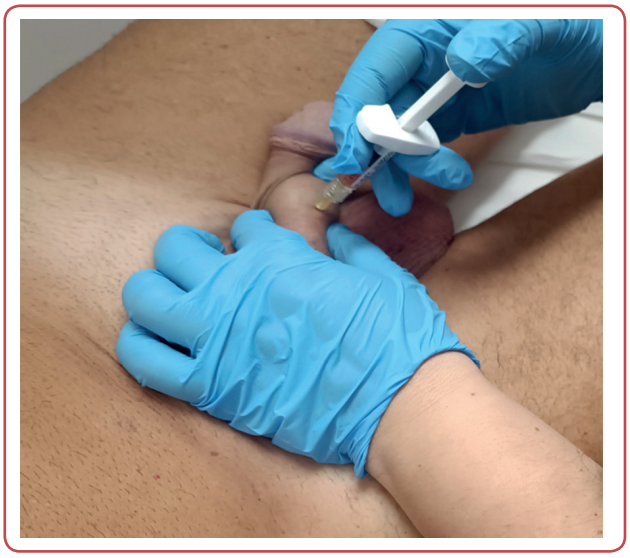

Figure 5: Injection of the microfiltrate in suspension with metabolic and protective solution directly into the plaques of the penile shaft

did not complain of pain in the area of injection of the suspension. Mild to moderate pain for less than 10 days in the area of fat harvesting was reported. Patient was discharged after 30 minutes of observation and evaluated one month, three months and 6 months after treatment.

Follow-ups

The patient was monitored at 1, 3 and 6 months post-injection. Outcomes assessed included penile curvature by goniometry, pain via Visual Analogue Scale (VAS), erectile function by IIEF-5 and ultrasound evaluation of plaque size and vascularisation.

The patient underwent a baseline and dynamic penile ultrasound in the pre-treatment phase and at the 6-month follow-up. The study had as its primary outcome the degree of curvature measured with a goniometer during an erection pharmacologically induced with prostaglandins. As a secondary outcome, pain assessment was taken into consideration, using a VAS scale for the latter symptom (Table 1). The patient reached 6 months of follow-up.

Table 1: Clinical characteristics of patients at baseline

Parameter	Baseline value	6-Month value	Absolute change	Percentage change
Pain (VAS)	5	0	-5	-100.0 %
Penile curvature (°)	28°	22°	-6°	-21.0 %
Erectile function (IIEF-5)	17	21	+4	+23.5 %

VAS: Visual Analogue Scale; IIEF-5: International Index of Erectile Function-5;

At 6 months, the patient reported complete resolution of pain (VAS 0). Penile curvature decreased from 28° to 22°, representing a 21 % improvement. Erectile function improved with IIEF-5 increasing from 17 to 21. Ultrasound showed a slight reduction in plaque size and a qualitative improvement in perilesional vascular flow on colour Doppler ultrasound (in this case we did not consider it useful to record standardised quantitative Doppler parameters, such as peak systolic velocity or resistance index, but we do not believe that for the purposes of this clinical case study this represents a limitation of this observational report). No adverse events occurred at the injection or donor sites. Mild to moderate discomfort at the fat harvesting site resolved within 10 days without the need for medication.

At 6 months post-treatment, the patient reported complete resolution of pain, with a VAS score decreasing from 5 to 0. It should be noted, however, that spontaneous resolution of pain is a well-documented feature of the natural history of Peyronie's disease, occurring in many patients during the transition from the acute inflammatory phase to the stable chronic phase, typically within 12–18 months of symptom onset; therefore, this result cannot be attributed to treatment in the absence of a control group but is also indicative of the decrease in curvature. Indeed, penile curvature decreased from 28° to 22°, a reduction of 6° (21 %), which represents the observed change and whose clinical relevance and causal attribution cannot be established from a single, albeit present, uncontrolled case. Erectile function scores increased from 17 to 21 on the IIEF-5, a change that exceeds the threshold for a minimal clinically relevant difference but, similarly, cannot be causally attributed to the intervention without a comparison group. These results are reported as observational data to inform future hypothesis-driven research. No causal attribution to the treatment is intended or implied.

Discussion

Human mesenchymal stem cells (hMSCs) have been identified in the published literature as a promising cell source for regenerative medicine in a variety of tissue regeneration settings. The present case report was designed as a preliminary, hypothesis-generating observation to explore whether tissue progenitors and their exosomes, obtained from viable adipose micrografts and conveyed by a metabolic and protective solution of non-cross-linked hyaluronic acid, amino acid chains and sodium bicarbonate, might constitute a rationale for a future controlled study in Peyronie's disease. The following considerations on mechanisms of action represent working hypotheses derived from the published literature on biological mechanisms for similar preparations and have not been directly investigated in this case. They are presented solely to provide biological plausibility for future experimental studies.

It was hypothesised, on the basis of published biological evidence from analogous preparations, that this scaffold may support progenitor sur-

vival through activation of CD44 and may exert protective effects on mesenchymal/endothelial lineage markers (CD73, CD90, CD105) through the formation of protective niches.[28] The clinical hypothesis is that hyaluronic acid used as a scaffold in the treatment of Peyronie's disease allows an improvement in physiological neo-collagenogenesis through the ability to react with the clusters of differentiation CD 44 present on the progenitors and allows their survival in an environment hostile and inflammatory like that of plaques, as they are protected by an environment that allows the formation of niches by means of a viscoelastic scaffold. Additional markers present on the progenitors such as CD 73, 90 and 105[22] would influence neo-vasculogenesis due to the hypoxia induced by the procedure in the syringe whit metabolic solution of amino acids branches and sodium bicarbonate for pH stabilisation with consequent improvement in the intra- and perilesional vascularisation of the plaque.[28]

In order to improve a possible prevention strategy of an exclusively surgical chronic phase of this pathology, it has been postulated that this infiltrative method is mainly concentrated on the reduction of inflammation and local hypoxia and both these mechanisms can be activated by the tissue Progenitors of adipose derivation and its exosomes.[23] In fact, although the aetiology of Peyronie's disease is still uncertain today and consists of a chronic inflammation of the *tunica albuginea* due to the deficit in the physiological healing of wounds and deposition of fibrous components of the extracellular matrix in the soft tissue of the penis, in this context the capacity of the tissue. Progenitors present in the adipose microfiltrate thus obtained[22] which allows to favour the induction of regeneration factors through the up regulation of miR-144, miR-30, miR-150, miR-342, miR-29, mi-R223, miR-183 and miR-139[24] in its exosomes[29] represents a potentially relevant mechanism for influencing disease progression, as hypothesised on the basis of published evidence from similar models. This mechanism has not been directly investigated in the present case and deserves to be further explored in future specific mechanistic studies.

The specific objectives of future research should include analysis of the mechanisms of action of adipose-derived stem cells and their exosomes in the clinical context *of induratio penis plastica* and identification of prospects for clinical application of this therapy. The regenerative effects of mesenchymal stromal cells in conditions requiring tissue repair have already been demonstrated.[22]

Adipose tissue is an easily accessible source of tissue progenitors that express typical adult stem cell markers, even after mechanical disaggregation according to the Tonnard 2013 method.[26, 30] Disaggregation by the Tonnard protocol yields a progenitor-rich product that also contains suspended fibrous strands and potentially inflammatory cellular debris; therefore, filtration of the disaggregated adipose at 20–40 microns is recommended.[24] This protocol preserves a numerically high viable side population[23] while removing most of the degraded tissue fraction[23] that can activate Tolllike receptors,[4, 26] and the remaining cells can secrete regenerative exosomes during the brief hypoxia of the procedure.[29]

Like other connective tissues, adipose tissue derives from embryonic mesenchyme and is rich in tissue progenitors that can be isolated by microfiltration.[25] Tissue progenitors or adult stem cells in adipose tissue are abundant—approximately one progenitor per 50 adipocytes—and comprise two size populations: small cells (mean diameter 17.9 µm) and large cells (mean diameter 30.4 µm). For this reason, filtration size has been chosen to retain the mean diameter of the large tissue progenitors.[24] No significant differences have been observed in morphology or immunophenotype among adult stem cells derived from bone marrow, umbilical cord and adipose tissue.[25]

Limitations

This study has limitations that must be acknowledged. First, it is a single, uncontrolled case. Second, the administered product was not analytically characterised for this case, but the characterisation of the tissue progenitors as similar to MSCs due to their possession of the same surface markers (cluster of differentiation) and the presence of exosomes was inferred from the widely demonstrated literature on similar preparations. Third, quantitative Doppler parameters (peak systolic velocity, end-diastolic velocity, resistance index) were not systematically recorded; the reported vascular improvement is based exclusively on a qualitative assessment with colour Doppler. Fourth, standardised photographic documentation of penile curvature was not obtained before and after treatment. Fifth, the Peyronie's Disease Questionnaire (PDQ), the recommended patient-reported outcome measure, was not used. These limitations are intrinsic to the single-case

observational design and must be fully addressed in any future controlled studies.

Conclusion

This case report describes an uncontrolled, single-patient observation of intralesional injection of a suspension of adipose-derived tissue progenitors with characteristics of adult mesenchymal stem cells, along with their exosomes, delivered within a scaffold of non-cross-linked hyaluronic acid, amino acid chains and sodium bicarbonate as a scaffold and for pH stabilisation in a patient with *induratio penis plastica*. The procedure was well tolerated, with no adverse events at the injection site and only mild, self-resolving discomfort at the donor site. At 6 months, numerical improvements in curvature (from 28° to 22°), pain (VAS from 5 to 0) and erectile function (IIEF-5 from 17 to 21) were observed. These observations are strictly preliminary and do not constitute evidence of clinical efficacy in the absence of a control group; the observed changes may reflect the natural history of Peyronie's disease rather than a treatment effect. This approach remains investigational and outside current EAU/AUA guidelines. A definitive randomised controlled trial should be designed with an adequately sized cohort, a control arm, validated patient-reported outcome measures (including the PDQ and penile Doppler data) and a follow-up period of at least 12 months.

Ethics

Our institution does not require ethics approval for reporting individual cases or case series. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki (revised 2013). As this is a single-case observational report of a procedure performed in a standard clinical setting and not a prospective experimental interventional study, formal prospective ethics committee registration was not required under the applicable regulatory framework. Written informed consent was obtained from the patient for the procedure, for the recording and use of clinical measurements and for the anonymised publication of data and photographs for scientific purposes. The patient was explicitly informed of the investigational and off-guideline nature of the treatment. Future prospective or controlled studies employing this approach will require full ethics committee approval and prospective registration (eg, *ClinicalTrials.gov* or equivalent).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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Validation: LS
Formal analysis: LS
Investigation: FS, LS, AM
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