Fractional Microablative CO_2 Laser-Related Histological Changes on Vulvar Tissue in Patients With Genitourinary Syndrome of Menopause

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Background and Objectives: Fractional CO_2 laser has been proposed as an effective treatment for the genitourinary syndrome of menopause (GSM). However, the effects of laser treatment on vulvar tissue have never been assessed. We aimed to assess histological changes related to fractional CO_2 laser in vulvar tissue from GSM patients.

Study Design/Materials and Methods: A single-center observational prospective cohort study was performed enrolling all GSM patients from July 2017 to October 2018. Patients underwent three outpatient vulvovaginal applications of fractional CO_2 laser and vulvar biopsy before and after treatment. Rates of histological changes in vulvar tissue, the difference in means of Vulva Health Index (VuHI), Vaginal Health Index (VHI), Visual Analogue Scale scores for GSM symptoms, and procedure-related pain, and rate of patient's overall satisfaction with treatment were assessed. Univariate comparisons between continuous variables were performed by using the paired *t-test* (α error = 0.05).

Results: Of 20 enrolled patients, 18 underwent all laser applications, and 15 underwent both vulvar biopsies. 93.3% of patients showed remodeling of vulvar connective tissue; 80% showed improvement in vulvar epithelium trophism and 86.7% showed neovascularization. Differences in means between before and after treatment were significant for VuHI, VHI, and all GSM symptoms. Means ± standard deviation of the degree of pain at each laser application were 4.4 ± 0.9, 3.7 ± 1.6, and 2.9 ± 1.9. The rate of overall satisfaction with the treatment was 72.2%.

Conclusions: Fractional CO_2 laser leads to a restoration of the normal architecture of vulvar tissue, with significant improvement in GSM-related signs and symptoms, and overall satisfaction with the treatment in most GSM patients. Lasers Surg. Med. © 2020 Wiley Periodicals LLC

Key words: menopause; HRT; hormone replacement therapy; histology; pathology; morphology

INTRODUCTION

Genitourinary syndrome of menopause (GSM) affects about 50% of menopausal women and includes histological, morphological, and clinical changes due to the drop in circulating estrogen during menopause [1–4]. In particular, collagen fibrils lose their trabecular disposition becoming flattened, elastic fibers and vascularization appear reduced, and the vaginal epithelium becomes thin and flattened, possibly developing hyperkeratosis [4]. Clinically, GSM results in several symptoms, such as dryness, itching, burning, irritation, dysuria, and dyspareunia, reducing the patient's quality of life and sexual function and tending to worsen over time [1,3,5–10].

Local estrogens have been considered as the first-line treatment, especially for persistent cases [11–14]. However, women's compliance is poor, and data about their long-term safety and use for high-risk patients are lacking [11–16]. Moreover, recurrence of symptoms is high after cessation of treatment, probably due to the treatment effect limited to the superficial layer of the vaginal walls [14,17]. In the last few years, by applying the principles of regenerative and anti-aging medicine to the vaginal

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mucosa, the fractional CO_2 laser has been successfully proposed to be used in GSM patients [14,18–20]. In fact, it has shown to be feasible, safe, and effective in reducing the severity of GSM symptoms and improving the patient quality of life and sexual function [14,19–22]. Fractional CO_2 laser would induce a remodeling of vaginal connective tissue without damage to surrounding tissue [16]. In particular, it would stimulate the production of collagen, hyaluronic acid, fibroblasts, and proteoglycans through heat shock proteins (HSPs) 43, 47, and 70 [14,16,22–30]. However, to date, only two studies investigated the histological changes related to fractional CO_2 laser in vaginal tissue, both including only five GSM patients [16,31], and no study assessed these changes in vulvar tissue.

The objective of this study was to assess histological changes related to fractional microablative CO_2 laser in vulvar tissue from GSM patients.

METHODS

Study Protocol

The study protocol was *a priori* defined, and the whole study was reported according to the STROBE guidelines and checklist [32]. The study was designed as a single-center observational prospective cohort study. We enrolled all consecutive postmenopausal women with GSM meeting the study selection criteria at the Department of Neurosciences, Reproductive Sciences, and Dentistry of the University of Naples Federico II from July 2017 to October 2018. Enrolled patients underwent GSM treatment with three outpatient vulvovaginal applications of fractional microablative CO_2 laser (every 4 weeks), and vulvar biopsy before and after treatment. Histological changes in vulvar tissue, vulvar and vaginal appearance, and GSM symptoms after laser treatment were compared with baseline. Procedure-related pain at each laser application and patient overall satisfaction after the last laser application were also assessed.

Patient Selection Criteria

Inclusion criteria were:

(1) symptoms of GSM, such as reduced sensitivity during sexual intercourse, vulvodynia, vaginal dryness, vulvovaginal burning/itching, vaginal discharge, dyspareunia and/or dysuria [33];
(2) age >50 years;
(3) absence of menstruation for ≥12 months;
(4) not responding/being unsatisfied with previous local estrogen therapies.

Exclusion criteria were:

(1) use of any hormone replacement therapy (either systemic or local) within the 6 months prior to inclusion in the study;
(2) use of moisturizers, lubricants or any local preparation within the 30 days prior to inclusion in the study;
(3) acute or recurrent urinary tract infections;
(4) active genital infections (e.g., herpes genitalis, candida);
(5) prolapse staged ≥II according to the pelvic organ prolapse quantification system [34];
(6) previous reconstructive pelvic surgery;
(7) any serious disease or chronic condition that could interfere with study compliance;
(8) psychiatric disorders precluding informed consent.

Primary and Secondary Outcomes

The primary outcome was the rate of remodeling of vulvar connective tissue after laser treatment compared with baseline.

The secondary outcomes were:

(1) the rate of improvement in vulvar epithelium trophism after laser treatment compared with baseline;
(2) the rate of neovascularization in vulvar connective tissue after laser treatment compared with baseline;
(3) the difference in the means of Vulva Health Index (VuHI) after laser treatment compared with baseline;
(4) the difference in the means of Vaginal Health Index (VHU) after laser treatment compared with baseline;
(5) the difference in the means of intensity of each GSM symptom after laser treatment compared with baseline;
(6) the mean of the degree of pain caused by each single laser application;
(7) the rate of patients with overall satisfaction with the treatment.

The remodeling of vulvar connective tissue was defined as the increase of the reticular pattern of vulvar connective tissue assessed on hematoxylin-eosin stained sections [16].

The improvement in vulvar epithelium trophism was defined as the at least partial restoration of the normal architecture and thickness of vulvar epithelium from the GSM-related alterations (thinning, flattening, and/or hyperkeratosis) [4].

The neovascularization in vulvar connective tissue was defined as an increased number of vessels in the vulvar connective tissue compared with the pretreatment status, counted in five high-power fields (×400) of the connective immediately deep to the epithelium. An increase of at least 25% was considered significant.

The VuHI was composed of eight items labia majora, labia minora, clitoris, urethra (caruncle, trauma, inflammation), introitus & elasticity, color, discomfort and pain, other findings (petechiae, excoriation, ulceration, etc.). The severity of each item was assessed by using a 4-point scale and added for a total VuHI score. VuHI could range from 0 to 24, with severe vulvar atrophy defined by a score of 3 in any one category or a total of >8 (Supplemental File S1).

The VHI was composed of five vaginal items, which were overall elasticity, fluid secretion, pH, condition of epithelial mucosa, and moisture. The severity of each item was assessed by using a 5-point scale and added for a total

VHI score. VHI could range from 5 to 25, with higher VHI scores indicating better vaginal health [35].

The intensity of GSM symptoms was measured by using a 10 cm Visual Analogue Scale (VAS), where the left extreme of the scale indicated "absence of symptom" and the right indicated "symptom as bad as it could be," as previously reported [23].

The degree of pain caused by laser was assessed by using a 10 cm VAS, where the left extreme of the scale indicated "absence of pain" and the right indicated "pain as bad as it could be."

As previously reported [23], the overall degree of satisfaction was assessed by answering the following question: "Taking into consideration the variations in GSM symptoms, in overall well-being and quality of life, as well as the adverse effects experienced, if any, how would you define the level of satisfaction with the laser treatment?" Patient answers were scored on a 5-point Likert scale (very satisfied, satisfied, uncertain, dissatisfied, and very dissatisfied). Satisfaction with the treatment was defined when the answers were "very satisfied" or "satisfied."

Fractional CO_2 Laser Treatment

GSM treatment was performed with the fractional microablative CO_2 laser system (SmartXide2 V2LR, Monnalisa Touch; DEKA, Florence, Italy). Vaginal tissue was treated by using the following setting: dot power 30 W, dwell time 1000 microseconds, dot spacing 1000 μm, and the smart stack parameter from 1 to 3. Laser treatment was delivered through a vaginal probe which was slowly inserted and rotated along the vaginal canal. External genitalia was treated by using the following setting: dot power 24 W, dwell time 1000 microseconds, dot spacing 700 μm, and the smart stack 1. Where requested a local anesthetic was placed before the procedure and wiped off before starting. Laser treatment included three laser applications (every 4 weeks) in outpatient. Vulvar biopsy was conventionally performed in the area between the introitus and left labia minora, to avoid operator bias and reduce intra- and inter-variability in selecting areas with different atrophic status.

Histological Methods

All specimens were obtained by vulvar biopsies and fixed in buffered formalin for 6–24 hours. Fixed specimens were processed in toto through an automatized procedure, which involved dehydration with alcohol at increasing concentrations and treatment with xylol. Specimens were then embedded in liquid paraffin at 50°C. Four micrometer-tick sections were obtained from paraffin-embedded tissue blocks and stained with hematoxylin-eosin by using an automatized VENTANA BenchMark® (Ventana Medical Systems, Inc., Oro Valley, Arizona, USA) stainer. Histological examinations were independently performed by three blinded pathologists (LI, AT, EG); finally, disagreements were solved by discussion at a multiheaded microscope.

Ethical Statement

The study received approval by the local Institutional Review Board of the University of Naples Federico II, Italy, and was performed in accordance with the Declaration of Helsinki. All enrolled patients signed informed written consent, and their data were anonymized.

Statistical Analyses

Rate of histological changes (i.e., remodeling of vulvar connective tissue, improvement in vulvar epithelium trophism, and neovascularization) was calculated as the ratio between the number of the events and the total number of patients.

Range intervals and means ± standard deviation (SD) were calculated for VuHI, VHI, and VAS scores at baseline (T_0), and after the first (T_1), second (T_2), and third (T_3) laser cycle.

Univariate comparisons between continuous variables were performed by using the paired *t-test* with α error set to 0.05.

Data analysis was performed as per-protocol; a *post hoc* data analysis of dichotomous variables was added as an intention to treat to avoid the effects of dropout.

Given the primary outcome and the lack of previous data in the literature, a sample size calculation was not possible to be performed. We *a priori* defined to enroll 20 patients in the study.

Statistical analyses were performed by using Statistical Package for Social Science (SPSS) 18.0 package (SPSS Inc., Chicago, IL).

RESULTS

Study Population

As planned, 20 patients were enrolled in the study. Eighteen patients underwent all laser applications, and 15 underwent both pre- and post-treatment vulvar biopsies; the remaining patients were lost to follow-up.

Mean age was 58.7 ± 6.6 years, and the mean body mass index was 24.5 ± 3 kg/m^2.

Range intervals and means ± SD of VHI, VuHI, and VAS scores for GSM symptoms and procedure-related pain at T_0–T_3 were respectively reported in Tables 1 and 2.

Primary and Secondary Outcomes

By assessing the 15 patients who had undergone both vulvar biopsies, the rate of remodeling of vulvar connective tissue after laser treatment was 93.3% (14 of 15 patients). The rates of improvement in vulvar epithelium trophism and of neovascularization in vulvar connective tissue after laser treatment were 80% (12 of 15 patients) and 86.7% (13 of 15 patients), respectively (Figs. 1 and 2).

Between T_3 and T_0, the differences in the means of VuHI and VHI were -8.3 ± 1.6 and 5.3 ± 1.5 points ($P < 0.001$), respectively (Table 2).

Between T_3 and T_0, the difference in the means of VAS scores were -3.8 ± 2.1 ($P < 0.0001$) for reduced sensitivity during sexual intercourse, -3.1 ± 4.2 ($P = 0.006$) for vulvodynia, -4.7 ± 2.5 ($P < 0.0001$) for vaginal dryness, -3.7 ± 3.4 ($P < 0.0001$) for vulvovaginal burning/itching, -1.4 ± 2.1 ($P = 0.011$) for vaginal discharge, -4.6 ± 1.6 ($P < 0.0001$) for dyspareunia, -2.6 ± 2.6 points ($P < 0.0001$) for dysuria (Table 2).

TABLE 1. Range of VHI, VuHI, VAS Scores for GSM Symptoms and Procedure-Related Pain in the Enrolled Patients

ITEM (range)	T_0	T_1	T_2	T_3
VuHI	9–17	5–11	1–7	1–5
VHI	10–17	15–20	17–21	19–22
Bulging feeling	0–8	0–7	0–6	0–3
Reduced sensitivity during sexual intercourse	4–10	0–10	0–10	0–9
Vulvodynia	0–10	0–7.5	0–7	0–4
Vaginal dryness	5–10	5–10	0–10	0–9
Vulvovaginal burning/ itching	0–10	0–8	0–7	0–6
Vaginal discharge	0–10	0–10	0–7	0–4
Dyspareunia	7–10	0–10	0–10	0–9
Dysuria	0–10	0–10	0–6	0–3
Procedure-related pain	–	3–6	0–8	0–6

GSM, genitourinary syndrome of menopause; SD, standard deviation; VAS, Visual Analogue Scale; VHI, Vaginal Health Index; VuHI, Vulva Health Index.

The means ± SD of the degree of procedure-related pain were 4.4 ± 0.9 at T_1, 3.7 ± 1.6 at T_2, and 2.9 ± 1.9 at T_3 (Table 2).

The rate of patients with overall satisfaction with the treatment was 72.2%.

Post Hoc Intention-to-Treat Analysis

The rate of remodeling of vulvar connective tissue after laser treatment was 70% (14 of 20 patients). The rates of improvement in vulvar epithelium trophism and of neovascularization in vulvar connective tissue after laser treatment were 60% (12 of 20 patients) and 65% (13 of 20 patients), respectively.

DISCUSSION

This study shows that microablative fractional CO_2 laser treatment leads to remodeling of connective tissue with restoration of the normal pattern of collagen fibrils, neovascularization, and improvement in epithelial trophism of vulvar tissue in the vast majority of patients with GSM. These histological changes would explain the significant improvement in VuHI, VHI, and each GSM symptom that patients reported. Moreover, most patients show overall satisfaction with treatment and a procedure-related pain decreasing from application to application.

To the best of our knowledge, this is the first study that assesses the histological changes related to microablative fractional CO_2 laser on vulvar tissue from GSM patients. Moreover, this study may be the first histological study to have a prospective design in the field. In addition, the number of assessed patients appeared relatively high compared with the other histological studies in GSM patients [16,31].

The observed histological changes in vulvar tissue support a tissue reactivation and remodeling similar to those reported for vaginal tissue and skin in previous studies [16,18,31]. In the skin, it has been demonstrated that these histological changes were accompanied by local increase of some cytokines, such as transforming growth factor-β, epidermal growth factor, vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived growth factor [36]. Such a local increase of these cytokines would be related to a controlled heat shock response that stimulates the activation of HSPs (especially HSP 43, 47, and 70), which have a crucial role in collagen biosynthesis, acting as chaperones of collagen [37–39].

The observed histological changes in vulvar tissue subsequent to laser treatment seem to act in a rejuvenating sense, making the vulvar tissue more similar to that of premenopausal women. In particular, they support an increase in fibroblast activity with production of new molecular components of the connective tissue matrix, neocollagenesis, and restoration of the trabecular architecture of the collagen. Moreover, the number of vessels of the vulvar connective tissue also appears increased. In addition, these changes in the connective are also accompanied by changes in the vulvar epithelium, with a restoration of its normal architecture and thickness from the flattening, thinning and/or hyperkeratosis related to

TABLE 2. Means ± SD of VHI, VuHI, GSM Symptoms and Procedure-Related Pain in the Enrolled Patients

ITEM (mean ± SD)	T_0	T_1	T_2	T_3	$\Delta_{T3\text{-}T0}$ (P value)
VuHI	11.5 ± 1.9	8.2 ± 1.5	3.9 ± 1.6	3.2 ± 1.1	-8.3 ± 1.6 (<0.0001)
VHI	14.6 ± 1.5	16.8 ± 1.3	19.1 ± 1	19.9 ± 1	5.3 ± 1.5 (<0.0001)
Bulging feeling	2.1 ± 2.9	1.9 ± 2.4	1.6 ± 2.1	0.6 ± 1.2	-1.5 ± 2.1 (0.009)
Reduced sensitivity during sexual intercourse	7.4 ± 2.2	5.7 ± 2.9	4.5 ± 2.8	3.6 ± 2.3	-3.8 ± 2.1 (<0.0001)
Vulvodynia	4.2 ± 4.4	2.4 ± 2.9	2.5 ± 2.5	1.1 ± 1.5	-3.1 ± 4.2 (0.006)
Vaginal dryness	8.9 ± 1.5	7.3 ± 1.7	5.3 ± 2.4	4.2 ± 1.9	-4.7 ± 2.5 (<0.0001)
Vulvovaginal burning/itching	5.9 ± 4	4.1 ± 3.7	2.8 ± 2.7	2.3 ± 2.1	-3.7 ± 3.4 (<0.0001)
Vaginal discharge	2.4 ± 3.2	1.8 ± 3.3	1.3 ± 2.1	0.9 ± 1.4	-1.4 ± 2.1 (0.011)
Dyspareunia	8.6 ± 1.3	6.8 ± 2.9	5.4 ± 2.7	4.1 ± 2.2	-4.6 ± 1.6 (<0.0001)
Dysuria	3.4 ± 3.3	2.5 ± 3.4	1.1 ± 1.7	0.8 ± 1.1	-2.6 ± 2.6 (<0.0001)
Procedure-related pain	–	44.3 ± 8.8	36.8 ± 16.1	28.9 ± 18.8	-

GSM, genitourinary syndrome of menopause; SD, standard deviation; VAS, Visual Analogue Scale; VHI, Vaginal Health Index; VuHI, Vulva Health Index.

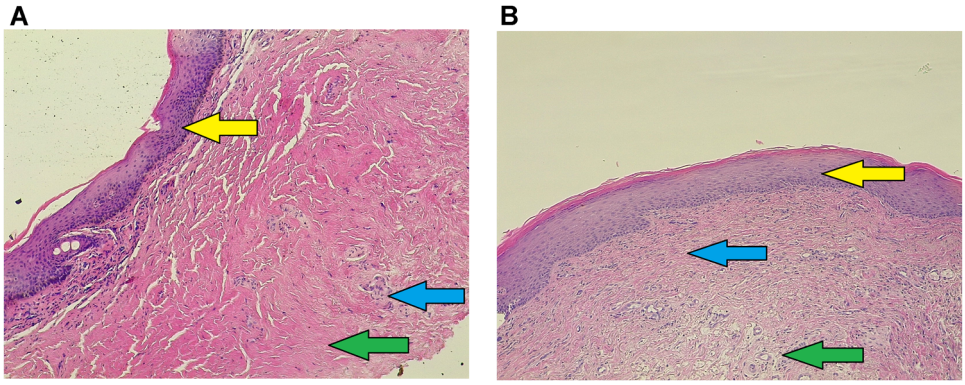

Fig. 1. (**A**) and (**B**) Pretreatment biopsies (magnification ×100): vulvar epithelium was thin and flat (yellow arrows); connective tissue was fibrotic (i.e., dense and homogenous, green arrows); only rare blood vessels were observed in the deep connective (blue arrows).

GSM [4]. In this way, the restored vulvar tissue might also be more sensitive to the residual ovarian hormone activity of the menopause for a better hormone diffusion in tissue [31]. This might lead to a long-term effect of laser treatment on tissue, avoiding hormone therapy, and subsequent side effects to GSM patients, especially in high-risk patients [31]; further studies with long-term follow-up are encouraged in this regard.

Clinically, the histological changes observed were accompanied by a significant improvement in VuHI, VHI, and each GSM symptom from the first laser application. This improvement was growing at each laser application and was accompanied by a decrease in the procedure-related pain. Despite the improvement being significant in all clinical outcomes, the main improvement was found in VuHI, which appears as a further novel finding. This finding makes fractional microablative CO_2 laser as an effective tool for vulvar symptoms and signs of GSM, as well as for vaginal ones (as reported in previous studies and also confirmed by our results [14,16,19–23,40–44]). Other major improvements were observed for dyspareunia, vaginal dryness, reduced sensitivity during sexual intercourse, and vulvodynia. This great improvement appears to be at the basis of a better sexual function previously reported [14,19–22]. The improvement in VuHI, VHI, and GSM symptoms accompanied by a decreasing procedure-related pain explains the overall satisfaction with treatment in most patients. This decrease in pain is even more relevant as pain has been reported as the most common adverse event related to the procedure [45]. Therefore, the effectiveness of laser treatment in GSM patients appears consistent and supported by histological changes in vulvovaginal tissue.

Finally, given that no patient showed worsening of histological status, GSM symptoms and/or signs, this study also defined the adopted microablative fractional CO_2 laser parameters (dot power 24 W, dwell time 1000 microseconds, dot spacing 700 μm and the smart stack 1) as effective and safe for the treatment of vulvar tissue.

Although the effectiveness and safety of microablative fractional CO_2 laser in GSM appear encouraging and supported by clinical evidence, it is currently difficult to define whether it could be preferable to local estrogens, which represents the current first-line treatment [11–14].

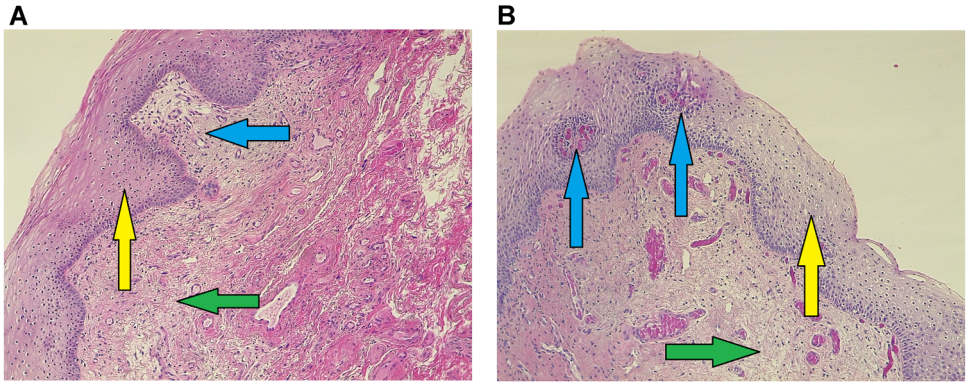

Fig. 2. (**A**) and (**B**) In post-treatment biopsies (magnification ×100), the thickness and architecture of vulvar epithelium were restored (yellow arrows); connective tissue showed restored architecture (i.e., reticular disposition of collagen fibrils) and hydration (i.e., looser pattern) (green arrows); a proliferation of blood vessels was observed in the subepithelial connective (blue arrows).

In fact, the use of local estrogens is supported by the evidence of superiority over placebo in several trials, for both clinical and cytological outcomes [46,47]. About improvement in symptoms, few studies compared fractional CO_2 laser to local estrogens [48–51]. These studies showed similar results between the two treatments [48,51], or even better results for the fractional CO_2 laser (alone or in combination with local estrogens) [49,50]. In addition, another study showed that the fractional CO_2 laser was effective in women dissatisfied with previous local estrogens [52]. In contrast, to the best of our knowledge, no study compared histological effects of fractional CO_2 laser and local estrogens on vulvovaginal tissue. In fact, most studies assessed the effects of local estrogens on vaginal rather than vulvar epithelium and were based on cytological rather than histological findings [46,47]. On this account, it appears difficult to compare our results to those of local estrogens. Further studies are encouraged in this field [44,53].

CONCLUSION

Microablative fractional CO_2 laser appears as an effective treatment for GSM, leading to a restoration of the normal architecture of vulvar tissue in the vast majority of patients. The histological changes are accompanied by a significant improvement in GSM-related signs and symptoms, and by an overall satisfaction with treatment in most patients.

Further studies are needed to evaluate the long-term effects of laser treatment on GSM.

AUTHORS CONTRIBUTIONS

Tiziana Pagano: study conception, protocol development, methods design, patient enrollment, data collection, manuscript writing, and supervision. Antonio Travaglino: methods design, data collection, histologic examination, and manuscript writing. Antonio Raffone: protocol development, methods design, data collection, and manuscript writing. Roberta Vallone: methods design, patient enrollment, data collection, and manuscript writing. Cira Buonfantino: patient enrollment, data collection, and manuscript writing. Pasquale De Rosa: protocol development, data collection, and patient enrollment. Mariavittoria Locci: methods design, patient enrollment, and manuscript writing. Elia Guadagno: methods design, histologic examination, and data collection. Luigi Insabato: study conception, methods design, histologic examination, and supervision. Stefano Salvatore: study conception, protocol development, methods design, and supervision. Giuseppe De Placido: study conception, protocol development, methods design, patient enrollment, and supervision.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.