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CO_2-laser for the genitourinary syndrome of menopause. How many laser sessions?

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ABSTRACT

Objectives: The aim of this prospective study was to assess the efficacy of 3, 4 or 5 CO_2-laser sessions for the management of the genitourinary syndrome of menopause (GSM).

Methods: Postmenopausal women with moderate to severe symptoms of dyspareunia, wanting to resume/retain sexual activity, were treated with 3–5 laser sessions depending on symptom severity/presence, sexual function, clinical findings and women's preference following the third laser application.

Main outcomes: Severity of dyspareunia, dryness, sexual function, sexual satisfaction and frequency of sexual intercourse defined the primary outcomes. Vaginal Maturation Value (VMV) and Vaginal Health Index Score (VHIS) defined the secondary ones.

Results: Fifty-five women received three sessions, 53 an extra fourth and 22 an extra fifth. Following the third, fourth and fifth laser sessions, respectively: dyspareunia completely regressed in 15/55 (27%), 32/55 (58%) and 38/47 (81%) of participants; dryness completely regressed in 20/55 (36%), 36/55 (66%) and 44/51 (86%); normal sexual function resumed in 23/55 (41%), 37/54 (69%) and 41/49 (84%); VMV regained non-atrophic values in 29/55 (53%), 38/55 (69%) and 42/50 (84%); and VHIS regained non-atrophic values in 44/55 (80%), 53/55 (96%) and 55/55 (100%) of participants.

Conclusion: Results of this study indicate that CO_2-laser therapy may contribute to complete regression of dyspareunia and dryness and reestablishment of normal sexual function in postmenopausal women, in a dose-response manner. An extra fourth or fifth session may further increase the GSM symptom-free rate.

1. Introduction

Genitourinary syndrome of menopause (GSM) is a chronic progressive condition presenting in menopause, involving clinical signs and symptoms from the lower genital and urinary tract system [1,2]. Hence, women with GSM may present with one or more of the following symptoms: genital dryness, decreased lubrication with sexual activity, discomfort or pain related to sexual activity, post-coital bleeding, decreased arousal/orgasm/desire, irritation/burning/itching of vulva or vagina, dysuria and urinary frequency/urgency [1]. However, the most common and bothersome symptoms are vaginal dryness and dyspareunia [1,4–8] with a negative impact in sexual intimacy, sexual desire and quality of life [4,7,8]. Furthermore, due to its chronicity nature, a long-term therapy is required [2].

Intravaginal laser therapy is one of the non-pharmacologic treatments for the management of the GSM symptoms [1]. Microablative fractional CO_2-laser (CO_2-laser) (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy) is one of the available laser-technologies used in the current literature [9,10]. Pilot studies evaluating the efficacy of intravaginal CO_2-laser therapy in symptoms of GSM of postmenopausal women, used a therapeutic protocol of 3 CO_2-laser applications at monthly intervals based on an ex vivo study [11–13]. Likewise, all subsequent studies used 3 CO_2-laser therapies for the management of GSM symptoms [14–21].

Indications of GSM symptoms alleviation and improvement of sexual function-satisfaction following the 3 CO_2-laser therapies have

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been reported consistently in all published articles [11,13–20]. The improvement rates were impressive. However, mean values of symptoms intensity after the 3 therapies, implied that absence of GSM symptoms did not occur in all participants. Data from our recently published study indicated that 34% of participants with moderate-severe symptoms before the initiation of the therapeutic protocol, had absence of GSM symptoms 1-month after the 3rd CO_2-laser application [19]. Furthermore, the threshold of 26.55 of the Female Sexual Function Index (FSFI) [22,23], that differentiates women with and without sexual dysfunction, may not be surpassed by all participants following 3 laser-sessions [11,14,20].

In the treatment guide of MonaLisa Touch, by the official website of DEKA, a full cycle of 4–5 laser-sessions is normally recommended [24]; the number of laser-sessions can be changed according to vulvovaginal atrophy (VVA) level [24]. However, to our knowledge, this recommendation has not been evaluated and it is unspecified whether a 4th or 5th laser-session may contribute to symptoms complete regression.

The objective of this study was to investigate whether an extra 4th or 5th CO_2-laser session in postmenopausal women with moderate to severe GSM symptoms could add to treatment's efficacy and results in favour of women's sexual function. Specifically, we intended to detect differences of objective and subjective measurements of GSM comparing their values before the therapeutic protocol and following 3, 4 or 5 CO_2-laser sessions.

2. Methods

The current prospective observational pilot study was carried out at the outpatient clinic of the Urogynecologic Unit of a tertiary referral Hospital, following the Helsinki's declaration. Approval from the local Ethics Committee was obtained and participants signed an informed consent form.

Postmenopausal women with dyspareunia, of moderate to severe intensity, willing to maintain or resume sexual activity, were qualified for inclusion in this study.

Women that had used lubricants or moisturizers the previous month, any form of hormone therapy the previous 6 months, active genital infections, prolapse stage ≥II according to the pelvic organ prolapse quantification (POP-Q) system [25] and medical conditions that could impair compliance to the study's protocol, were disqualified from this study.

At the screening visit eligible women were assigned to a unique random number that was different at each visit. Participants received 3 sessions with CO_2-laser system (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy) at monthly intervals. One month after the 3rd therapy, a thorough discussion with participants was performed by an independent physician, with no other involvement to the protocol. Depending on symptom's presence-severity, normal sexual function, physical examinations' findings and participants' preference, a 4th-session was offered. One-month after the 4th-session a 5th one was offered following the above described pattern. The settings and procedures of the CO_2-laser system were performed as previously described [13,19,20].

The methodology for the assessment of the CO_2-laser efficacy and the efforts to reduce potential sources of bias (i.e independent evaluators, blinding to all clinical information and participants' treatment status) was performed as previously reported [20]. CO_2-laser efficacy was evaluated using subjective measurements for symptoms of GSM, as well as objective ones for clinical signs of GSM.

Subjective measurements involved the following questionnaires:

1) a 10-cm Visual Analogue Scale (VAS 0–10): 0 applied to absence of symptoms, > 0 and < 4 mild symptom's intensity, ≥4 and < 8 moderate intensity and ≥8 a severe one.
2) Female Sexual Function Index (FSFI) [22,23]: It measures sexual function evaluating 6 domains (desire, arousal, lubrication, orgasm, satisfaction and pain) resulting in a total score. Higher scores define better sexual function. The threshold of 26.55 of the total FSFI score distinguishes women with and without sexual dysfunction [23].
3) A 10-cm VAS for the overall sexual satisfaction.

Objective measurements included:

1) Vaginal Health Index Score (VHIS): calculation was performed summing its 5 components (elasticity, fluid volume, pH and epithelial integrity). Each one of the components could receive values from 1 (poorest) to 5 (best). The sum of the 5 components could receive an upper bound score of 25 and a lower of 5. A Score of ≤15 defined the presence of vaginal atrophy [26].
2) A cytological evaluation: calculation of the Vaginal Maturation Value (VMV) using the formula (1 × %superficial) + (0.5 × %intermediate) + (0 × %parabasal) [26]. Vaginal Maturation Value ≤40% defined atrophy on the vaginal smears [26].

Subjective measurements of GSM symptoms were regarded as primary outcomes. All other measurements were regarded as secondary ones. All outcomes were evaluated before the initiation of the protocol (baseline), 1-month after the 3rd laser-session, 1-month after the 4th laser-session and 1-month after the 5th laser session. Women were not allowed to use other GSM therapies (i.e lubricants, moisturizers etc) during the study protocol.

2.1. Statistical analysis

A priori calculation of the sample size was not performed, on the basis that this was an exploratory study. Shapiro-Wilk test and normality plots were used for the assessment of the data distribution. Abnormally distributed continuous variables were assessed using Wilcoxon signed-rank test for related paired samples, while normally ones with *t*-test for paired samples. Categorical variables (presence or absence of dryness/dyspareunia and presence or absence of sexual dysfunction) were evaluated by Mc-Nemar test for related samples. Three comparisons were performed for each outcome, absence or presence of symptoms and sexual dysfunction: Baseline with 3 laser-sessions, 3-laser sessions with 4th and 4-sessions with 5th. Continuous outcomes of independent samples (baseline characteristics and dyspareunia, dryness and total FSFI score of women receiving 4-sessions and of women receiving 5-sessions) were analyzed using Mann-Whitney *U* or student's *t*-test for abnormally or normally distributed data, respectively. Dyspareunia free-rate was calculated using a specific fraction. The nominator was participants without dyspareunia after the particular session and the denominator was baseline participants minus participants with dyspareunia that did not have the particular session. Vaginal dryness free-rate was calculated in a similar manner. The nominator was participants without vaginal-dryness after the particular session and the denominator was baseline participants minus participants with vaginal dryness that did not have the particular session. Similar fractions were used to calculate normal sexual function rate and non-atrophic values rate of clinical findings. All tests were based on a significance level of 5% (p-value < 0.05). Family Wise Error Rates (FWER) were controlled using the Holm-Bonferroni Method (Holm-Bonferroni Sequential Correction, Justin Gaetano, 2013) [27]. Unadjusted and adjusted p-values are reported. Data are presented as median, interquartile range (IQR), and as percentages (%). SPSS statistical software was used for the analyses.

3. Results

Seventy-one postmenopausal women with GSM symptoms were screened for inclusion in the current study. Sixteen were excluded because they were not sexually active for reasons other than severity of

Table 1
Baseline characteristics of the participants in the study.

Number (N)	55
Age	57/14[a]
BMI	25.3/4.4[a]
Years since last menstrual period	7/8[a]
Level of Education	
Secondary	10/55 (18)
University	45/55 (82)
Smokers	
Yes	20/55 (36)
No	35/55 (64)
Number of cigarettes/day	11/11.5[a]
Married	
Yes	49/55 (89)
No	6/55 (11)
No sexual intercourse due to severity of symptoms	23/55 (42)

[a] Data presented as median/interquartile range.

GSM. Hence, 55 postmenopausal women were eligible to be included in the present protocol. Specifically, baseline data and data after 3rd session on 40 of these patients were previously reported [20]. The baseline characteristics of the participants in this study are presented in Table 1. All 55 participants completed the standard protocol of the 3 laser-sessions; 53 of them received an extra 4th- and 22 an extra 5th-session. After the 3rd-session 1 participant did not have normal sexual function but did not receive a 4th-session. After the 4th-session, 8, 4, 5 and 5 participants had dyspareunia, vaginal dryness, sexual dysfunction and atrophic values of VMV but did not receive the extra 5th-session, respectively. The reasons for not having the extra 5th were: a) participants' perception of not bothersome enough symptoms to have a 5th-session (7/8 participants) and b) personal reasons (1/8 participants).

Primary and secondary outcomes are presented in Table 2. After 3 laser-sessions all outcomes presented a significant improvement. After 4 laser-sessions a further significant improvement was noted in all but

Table 3
Presence of symptoms severity and normal sexual function at baseline and following the 3rd, 4th and 5th laser-session.

Intensity of symptoms	Baseline*	After 3 laser-sessions*	After 4 laser-sessions*	After 5 laser-sessions*
Dyspareunia				
Zero	0/55 (0)	15/55 (27)**	32/55 (58)**	38/47 (81) **
Mild	0/55 (0)	21/55 (38)	21/55 (38)	8/47 (17)
Moderate	21/55 (38)	7/55 (33)	3/55 (5)	1/47 (2)
Severe	34/55 (62)	1/55 (2)	0/55 (0)	0/47 (0)
Dryness				
Zero	6/55 (11)	20/55 (36)#	36/55 (66)#	44/51 (86) #
Mild	2/55 (4)	17/55 (31)	16/55 (29)	7/51 (14)
Moderate	17/55(31)	17/55 (31)	3/55 (5)	0/51 (0)
Severe	30/55 (55)	1/55 (2)	0/55 (0)	0/51 (0)
FSFI*				
> 26.55	2/55 (4)	23/55 (41)##	37/54 (69)##	41/49 (84) ##
≤26.55	53/55 (96)	32/55 (58)	17/54 (31)	8/49 (16)

* Statistical analysis using Mc-Nemar test for related samples was performed only based on presence or absence of dryness/dyspareunia and presence or absence of sexual dysfunction. The comparisons were performed between baseline and after 3 sessions, after 3-sessions and after 4, after 4-sessions and after 5. Statistical significance was set at 5% (p-value < 0.05); FSFI: Female Sexual Function Index. Values > 26.55 defined normal sexual function, while ≤26.55 sexual dysfunction [23].

** Dyspareunia presence decreased significantly following the 3rd (unadjusted p-value < 0.001, adjusted p-value < 0.001), the 4th (unadjusted p- value < 0.001, adjusted p-value < 0.001) and 5th laser-session (unadjusted p-value 0.02 and adjusted p-value 0.5).

Dryness presence decreased significantly following the 3rd (unadjusted p-value < 0.001, adjusted p-value < 0.001), the 4th (unadjusted p value < 0.001, adjusted p-value < 0.001) and the 5th laser-session (unadjusted p-value 0.008 and adjusted p-value 0.2).

The presence of normal sexual function increased significantly following the 3rd (unadjusted p-value < 0.001, adjusted p-value < 0.001) and the 4th (unadjusted p-value 0.001, adjusted p-value 0.04) and the 5th laser-session (unadjusted p-value 0.1 and adjusted p-value 1.0).

Table 2
Changes of the primary and secondary outcomes at baseline, after 3, 4 and 5 laser-sessions.

	Baseline (n = 55)	After 3 laser-sessions (n = 55)			After 4 laser-sessions (n = 53)			After 5 laser-sessions (n = 22)		
	Median/ IQR*	Median/ IQR*	Unadjusted p-values	Holm's Adjusted p-values*	Median/ IQR	Unadjusted p-values	Holm's Adjusted p-values*	Median/ IQR	Unadjusted p-values	Holm's Adjusted p-values*
Dyspareunia***	8/4	3/4	< 0.001	< 0.001	0/2	< 0.001	< 0.001	0/2	0.001	0.04
Dryness***	8/4	2/4	< 0.001	< 0.001	0/2	< 0.001	< 0.001	0/1	0.001	0.04
FSFI**										
Desire	2.4/1.8	3.6/1.2	< 0.001	< 0.001	4.2/1.2	0.001	0.04	4.2/1.2	0.1	1.0
Arousal	2.1/2.7	3.6/1.2	< 0.001	< 0.001	3.9/1.2	< 0.001	< 0.001	3.6/1.2	0.1	1.0
Lubrication	2/3.6	4.5/0.9	< 0.001	< 0.001	5.1/1.1	< 0.001	< 0.001	5.3/0.9	< 0.001**	< 0.001
Orgasm	2/3.2	4.4/1.2	< 0.001	< 0.001	4.4/1.2	0.001	0.04	4.4/1.2	0.07	1.0
Satisfaction	2.4/2.4	4.8/1.2	< 0.001	< 0.001	4.8/1.2	0.003	0.09	4.8/1.3	0.03	0.7
Pain	1.2/3.6	4.8/1.6	< 0.001	< 0.001	5.6/1.2	< 0.001	< 0.001	5.4/0.9	< 0.001	< 0.001
Total	13.4/15.7	25.3/5.9	< 0.001	< 0.001	28.4/4.7	< 0.001	< 0.001	28/5.9	< 0.001**	< 0.001
Overall sexual satisfaction***	2/4	7/3	< 0.001	< 0.001	8/3	< 0.001	< 0.001	8/2.6	0.001	0.04
Frequency of sexual intercourse per month	1/2	4/1	< 0.001	< 0.001	4/2	0.07	1.0	4/1	0.006	0.2
VHIS***	8/3	20/6	< 0.001	< 0.001	23/4	< 0.001	< 0.001	22.5/3.3	< 0.001	< 0.001
VMV***	0/30	50/17.5	< 0.001	< 0.001	50/12.5	0.004	0.1	50/8.1	0.003**	0.09

* Data are presented as median and Interquartile range (IQR). Holm-Bonferroni Method was performed to deal with family wise error rates (Holm-Bonferroni sequential correction: An Excel Calculator. Gaetano 2013) [28]. Three comparisons were performed: baseline with after 3 sessions, after 3-sessions with after 4 and after 4-sessions with after 5. Statistical significance was set at 5% ($p < 0.05$).

** Data were analyzed using *t*-test for paired samples. All other analyses were performed using Wilcoxon signed-rank test for paired samples.

*** Dyspareunia and Dryness were assessed using 10-cm Visual Analogue Scale: "0" defined absence of symptoms and "10" symptoms as bad as could get; Desire, Arousal, Lubrication, Orgasm, Satisfaction, Pain and Total were assessed by the Female Sexual Function Score (FSFI) [22,23]: The highest the score the better the sexual function; VHIS: Vaginal Health Index Score. It includes 5 components: VHIS is calculated by adding the scores of the 5 components: Elasticity, fluid volume, pH, epithelial integrity and moisture [26]. Each component could receive a score from 1 (poorest) to 5 (best). The sum of the 5 components could receive an upper bound score of 25 and lower bound of 5. A Score of ≤15 defined the presence of vaginal atrophy [26]; VMV: Vaginal Maturation value was calculated using the formula%parabasal epithelial vaginal cells ×0 +%intermediate epithelial vaginal cells × 0.5 +% superficial × 1[26]. Values ≤40% defined the atrophic smears [26].

one outcome. Frequency of sexual intercourse did not differ between the 3rd- and 4th-session. After 5-sessions all but 4 outcomes had a significant further improvement. Desire, arousal, orgasm and satisfaction did not differ between the 4th and 5th laser-session. Baseline characteristics did not differ between women receiving 4-sessions (n = 31) and those receiving 5 (n = 22) (all adjusted p > 0.05). After 4 laser-sessions dyspareunia, dryness and total FSFI score were statistically significant better in women receiving 4-sessions (n = 31) compared to those receiving 5 (n = 22) (adjusted p-values < 0.001, 0.02 and 0.02, respectively). Outcomes were not statistically significant different following the last laser-session between the women receiving 4-sessions (n = 31) and 5-sessions (n = 22) (all adjusted p > 0.05).

Number of participants with zero, mild, moderate or severe intensity of dyspareunia or dryness and number of participants with or without sexual dysfunction are presented in Table 3. All participants responded to treatment. At baseline 23/55 (42%) did not have sexual intercourse due to severity of symptoms. Following the 3rd laser-therapy sexual activity was resumed by all of them.

The percentage of participants with VHIS > 15 increased from 0/55 (0%) at baseline to 44/55 (80%), 53/55 (96%) and 55/55 (100%), following the 3rd-, 4th-, and 5th-session, respectively. Furthermore, percentage of participants with non-atrophic vaginal smears increased from 0/55 (0%) initially to 29/55 (53%), 38/55 (69%) and 42/50 (84%) following the 3rd-, 4th- and 5th-session, respectively.

None of the participants had any serious adverse events. Some of the participants reported a mild irritation at the introitus during the procedure and/or immediately after, that resolved spontaneously. Vaginitis of any kind was not present at any time point of the study.

4. Discussion

This prospective pilot study suggests that dyspareunia and dryness of postmenopausal women decreased in intensity and presence progressively following 3, 4 and 5 CO_2-laser sessions. Likewise, the sexual function of these participants appeared to improve significantly with normal sexual function restart rate varying from 40 to 84%, depending on the number of treatments. Specifically, desire, arousal, lubrication, orgasm and pain improved significantly after 3 and 4 laser-sessions. An extra 5th-session seemed to add in a significant further improvement of lubrication and pain during sexual intercourse. Furthermore, the regression of GSM symptoms seemed to be in accordance to the changes of objectives measures of vaginal atrophy such as VHIS and VMV.

Various Surveys have been conducted to estimate symptoms related to vaginal atrophy during menopause [3,4,7,8]. Despite country of origin and ethnicity, vaginal dryness and dyspareunia were rated as the most common and bothersome symptoms [3–8]. The results of our study indicated that the management of patients with moderate-severe intensity of vaginal dryness and dyspareunia using CO_2-laser therapy, may result not only in significant improvement of symptoms but also in symptom-free rates up to 86%, depending on number of therapies. Perhaps, this rate would be even higher if the participants with persistent symptoms following 4-sessions had completed a therapeutic protocol with 5-sessions. Nevertheless, for these participants' intensity of symptoms was not considered bothersome enough to receive an additional 5th-therapy.

Moreover, the markedly improvement of dyspareunia and dryness following 3 laser-sessions that was detected in this study, was in accordance with previously published studies [11,13–18,20]. However, application of an extra 4th- or 5th-session resulted in a further reduction. Initially, all participants had moderate to severe intensity of symptoms. Severe intensity disappeared following the extra 4th and finally only 2% of the participants had moderate symptoms after 5 laser-sessions. Although after 4 laser-sessions women receiving 4-sessions compared to those receiving 5-sessions felt better in terms of dyspareunia and dryness, this seemed eliminating when the 5th-session was applied. The latter finding implies that in certain women 5-sessions are necessary for an optimum laser-effect to be achieved, while in others 3- or 4-sessions are adequate. From our data, it does not appear possible to distinguish which women will require 4- or 5-sessions, relying solely on baseline characteristics.

Furthermore, our data suggested an increase of sexual intercourse frequency per month and resumption of normal sexual function in up to 84% of the participants. Vaginal discomfort it is more likely to have a negative impact in self-esteem of postmenopausal women, sexual life-intimacy, loving relationship with their partner and overall quality of life [4,7]. More than 20% of women cease sexual activity due to GSM symptoms, while 50–62% have less sex, less satisfying sex, avoid intimacy or are upset that their body does not work as it used to [7]. Initially, in our study 42% of participants had ceased sexual activity due to severity of GSM symptoms and loss of libido. After the 3 laser-sessions all participants resumed sexual activity and reported an increasing frequency of sexual intercourse and overall sexual satisfaction following subsequent therapies.

Female sexual dysfunction includes disorders of desire, arousal, lubrication, orgasm, satisfaction, and pain related to sexual intercourse [22,23]. A markedly improvement of all the above factors was indicated following the 3-laser sessions in a similar manner with the previously published studies [11,14]. Lubrication and pain were the 2 factors that continued to increase significantly to the 5th laser-session, as assessed by the Holm-Bonferonni correction. Desire, arousal and orgasm improved significantly following the 4th laser-session but after the 5th laser-session only a trend was noticed. However, sexual function is influenced by psychological and social factors [28] and it is encouraging that following 3 or 4 laser-sessions a significant improvement was found. Hence, a possible positive impact in women's' psychology following the CO_2-laser therapy is implied.

The improvement of vaginal health as reflected by the subjective measurements of 10-cm VAS and FSFI, was also confirmed by the significant improvement of the objective ones. VHIS and VMV increased following each subsequent therapy, resulting in 80%-100% of participants surpassing the thresholds of non-atrophic clinical findings. After 3 laser-sessions the values of the VHIS did not vary from the previously reported [11,13–18,20]. However, the further improvement following the 4th- and 5th-session seemed to have an additional positive effect on symptoms' complete regression.

This study has several limitations. It is not a controlled study comparing CO_2-laser efficacy to other treatment modalities and a possible placebo effect of the therapy cannot be estimated. However, a valid self-report measurement such as FSFI, designed to detect differences in all sexual factors in a specific period of 4-weeks, was used. Another possible limitation could be the lack of a priori sample size calculation. Nevertheless, this is an exploratory novel study aiming to assess whether postmenopausal women with moderate to severe symptoms, wishing to maintain/regain sexual activity may or may not benefit by a 4th- or a 5th-session. Additionally, a potential breech of the blindness of the evaluators cannot be overruled, although every possible effort for blinding the assessors to all data, parameters, samples and details of the participants (i.e baseline characteristics, treatment status, etc) was performed. Furthermore, an estimation of the efficacy of a 5th-session could not be assessed in 8 participants that were not symptom-free following the 4th-session, as they did not receive a 5th-one. Additionally, this study has a relevant short follow-up period and the long-term efficacy of the extra 4th- or 5th-session cannot be predicted.

5. Conclusions

The results of this study suggest that vaginal CO_2-laser therapy may improve dryness and dyspareunia, resulting to a better sexual function of the postmenopausal women. This effect is possibly produced in a dose-response manner. An extra 4th or 5th laser-session may add further to treatments efficacy, increasing the symptom-free percentage of

participants. Hence, indications that even severe cases with highly impaired sexual function may benefit by a 4th or 5th CO_2-laser therapy are provided. However, randomized controlled trials, comparing 3rd to 4th and 5th laser-session with long-term follow-up and testing for uniformity of laser impulses applied through sessions, are needed for safe conclusions to be deducted.

Contributors

All five authors contributed equally to the preparation of this paper.

Conflict of interest

Stefano Salvatore has had financial relations (expert testimonies and lectures) with DEKA Laser. The other authors report no potential conflicts of interest.

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Ethical approval

The study protocol was approved by the Ethics Committee of the "Alexandra" Hospital, Athens, Greece.

All participants signed an informed consent form.

Provenance and peer review

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