Fractional CO2 Laser Therapy (MonaLisa Touch™) for Vaginal and Vulvar Skin Conditions

This roundtable discussion, sponsored by the International Academy of Pelvic Surgery (*www.academyofpelvicsurgery.com*), is intended to discuss the use of a unique fractional CO2 laser therapy called MonaLisa Touch for treating vaginal and vulvar skin disorders. The original research done on this treatment was performed at the San Raffaele Hospital in Milan, Italy. Dr. Eric Sokol and I were fortunate enough to be exposed to this treatment in January of 2014 and initiated the first US trial. The therapy has now been commercially available in the United States since January of 2015 and the initial response has been extremely positive. This roundtable is a discussion by six of the early adopters as well as Dr. Sokol regarding their initial experience with the therapy. Also discussed is the mechanism of action, how best to market the therapy to your patients, as well as tips and tricks on performing the procedure.

—Mickey Karram, MD

The technology, trial and treatment experience

Dr. Karram: Dr. Sokol, you and I performed the first study in the United States using MonaLisa Touch laser therapy. Can you tell us your initial thoughts about it prior to doing the study?

Dr. Sokol: I thought it was intriguing. We see primarily postmenopausal women and they come to us from all over the country and all over the world for complex pelvic floor problems. We also see cancer patients referred with pelvic floor complaints. Particularly for breast cancer patients, we often run into the problem that they're not really candidates for traditional therapies such as vaginal estrogen. So this was intriguing for that reason; it was very different. The most intriguing part, and the way it was posited to me before I had any experience with it, was that this was a very fast, relatively painless way to induce changes in vaginal skin that didn't require hormones.

Dr. Karram: What were your initial impressions after seeing it in action?

Dr. Sokol: After seeing it done in Italy and talking to patients six months to a year after having had it done, I remember thinking, this is almost too good to be true. It seemed super easy to do, and as someone who develops technologies, I know that you want something that is easy to do, reproducible and good for patients—kind of the holy grail of treatment. That's when I wanted to research it and get involved.

Dr. Karram: Could you review with us the results of the study?

Dr. Sokol: We just presented the official three-month outcome data at the North American Menopause Society meeting in Las Vegas (Sept. 30–Oct. 3). It was a prospective study of 30 postmenopausal women designed to evaluate the safety and efficacy of the laser to treat what at the time was called vulvovaginal atrophy but is now called genitourinary syndrome of menopause (GSM). We had some secondary objectives as well, designed very similarly to the Italian trials. We looked at the vaginal health index (VHI) score and we assessed the effect of treatment on the pliability of the vaginal wall, by looking at the maximum dilator size patients could use before and after the treatments. We looked at the change in vaginal pH. We also looked at sexual quality of life using the FSFI (Female Sexual Function Index), and overall quality of life with the short-form SF-12 questionnaire.

Generally speaking, we were looking at global vaginal atrophy symptoms, including pain, burning, itching, vaginal dryness and dyspareunia as well as dysuria. We followed up with each patient a week after treatment to make sure there were no adverse events, and treatments were spaced six weeks apart.

We also looked at what I consider very important: overall patient satisfaction using the PGI-I (Patient Global Impression of Improvement) scale, but also physician satisfaction. A goal for new technologies should be to simplify both the patient and physician experience.

In general, all symptoms of GSM significantly improved, as did maximum tolerable dilator size and FSFI scores. We also had several women who were able to become sexually active after many years of not being able to do so. What was most impressive, to me, was the rapid resolution of vaginal dryness. We're coming up on one-year follow-up for all 30 patients and will be sharing that data in the future, but I can say that at one year out, my overall impression is very positive.

Dr. Karram: Can you describe the mechanism of action of the MonaLisa Touch treatment?

Dr. Sokol: Stefano Salvatore[1] has published on that topic and we are starting a small study looking at that very question. But basically, under electron microscopy, we see fine collagen that is laid down after the laser treatment, probably by inducing a very small thermal injury, and then getting a new ingrowth of cells and then a laydown of extracellular matrix. It's like a heat-shock response; the heat-shock proteins get activated and other growth factors come into play to induce type 1 versus type 3 collagen.

Dr. Karram: What has been the feedback from patients?

Dr. Sokol: My patients have loved it, and I've enjoyed being able to treat someone on the spot and even just days later, having them call to say they feel much better. And I've asked patients when I've seen them if things improved soon after treatment, and many said yes, within days, in terms of vaginal lubrication. I think this holds tremendous promise.

Tips and Tricks for Performing MonaLisa Touch Treatment

Early adopters of the technology should be aware of the following pearls regarding successful administration of the treatment.—*Mickey Karram, MD*

1. Feel free to use some anesthetic gel at the introitus in patients with severe atrophy to avoid significant pain and discomfort with initial insertion of the probe. We prefer EMLA cream applied a few minutes before the treatment and then it needs to be wiped off prior to inserting the probe.
2. Make sure that the probe reaches the top of the vagina. This is an area of extreme atrophy especially in post-hysterectomy patients, so you want to make sure that the probe comes in contact with the vaginal vault or the cervix.
3. The actual withdrawal of the probe is intended to be at 1 cm intervals. This should be as precise as possible; however, there is room for error in that regard.
4. Many patients with Genitourinary Syndrome of Menopause have more severe atrophy in the introital area due to the combination of vulvar atrophy as well as vaginal atrophy. As the circular probe reaches the introitus, at times it is difficult to efficiently treat the area of the vestibule and posterior fourchette with this probe. We have now added in these patients a dual probe technique in which we will switch to the vulvar or flat probe to more effectively treat this area. This is done using the mild settings and treatment of this area is under direct visualization, with overlapping of the treatment areas being well expected. I usually treat the vestibule from three o'clock to nine o'clock, the entire posterior fourchette, and at times the upper part of the perineum. The regime I would recommend would be traditional vaginal treatment for the first treatment. For those who report that they had significant improvement in the vaginal canal but continue with insertional dyspareunia, I would initiate the dual probe technique on the second and third treatments.
5. For patients undergoing vulvar treatment, we have patients apply a topical anesthetic similar to EMLA, 20–25 minutes prior to treating the area. In patients with excessive pubic hair in the area to be treated, we recommend trimming this hair prior to placement of the EMLA cream.

Dr. Karram: Dr. Dell, approximately how many patients to date have you treated with the MonaLisa Touch laser treatment?

Dr. Dell: We've treated approximately 130 patients and have delivered more than 450 treatments. While the standard protocol is three treatments per patient, my center is very much involved in developing the protocols for the vulvar treatment for external skin atrophy and lichen sclerosus, so some of those patients are getting vulvar procedures as well.

Dr. Karram: How does this type of laser treatment in the vagina compare to fractional CO2 lasers that historically have been used on the face?

Dr. Dell: Great question, and I think we have to address it in two parts as it relates to the dual-probe approach. The fractional CO2 laser of course has become the gold standard for treatment on the face in the hands of dermatologists and plastic surgeons. There are tremendous benefits of the fractional approach with its matrix penetration of dots, if you will. It's this same technology we now can deliver both vaginally and to the vulvar tissue. There are two separate aspects: the vaginal probe utilizes a different laser pulse or emission mode, specifically developed for vaginal mucosa to maximize the benefit of how that laser pulse is delivered. We then change the emission for the vulvar probe to use the exact same approach that would be used on the face because this is outer skin and a different tissue type than vaginal mucosa. So it is fractional CO2 but using different emission modes to treat both the vaginal and the vulvar tissue.

Next: Patient selection and technology potential

1. Stefano Salvatore, MD, head of urogynaecology, San Raffaele Hospital, Milan, Italy. Dr. Salvatore conducted the original studies of MonaLisa Touch treatment.

Patient selection and technology potential

Dr. Karram: Dr. Dell spoke of his experience treating both vaginal and vulvar tissue. Are others on the panel looking at other ways to utilize this technology?

Dr. Sophocles: Yes—if you think about how this works, it should work on the vulvar tissue as well. It's a matter of how the settings need to be tweaked and how many treatments and how far apart. I think that bladder symptom improvement in postmenopausal women with urinary urgency and frequency is also an underappreciated advantage of the treatment.

Dr. Kubricht: Chronic cystitis in postmenopausal women is probably the number one secondary indication that I treat patients for. Since 2002, when the association between estrogen and breast cancer became familiar to the public, the incidence of recurrent vaginitis, vaginal atrophy, vulvovaginal atrophy and recurrent UTIs has gone up dramatically. Until now we've fought the battle of, "I don't want to be on a hormone and therefore the end result is I'll have more urinary tract infections." So when I see someone with recurrent UTIs and they're postmenopausal, this comes to the top of my list of things to offer the patient, because I know the probability of their wanting to be on a hormone is low if they're not already on one. So I strongly advocate that.

Recurrent UTIs is the most common reported symptom I treat for, followed by vaginal atrophy and third, vaginitis. We counsel patients that vaginal atrophy is what we're treating and that anything beyond that is what we in Louisiana call "lagniappe"—it's all extra.

Dr. Dell: One of the obvious targets is inflammatory disease process conditions, of which lichen sclerosus is the most common. We're seeing very encouraging results by using external probe therapy on these patients. These are patients who in many cases for years have suffered tremendously despite all of the various creams and steroids and topical treatments we've had to offer. We've just completed a 15-patient pilot trial for treating lichen sclerosus and are seeing very encouraging results clinically overall for that condition.

Aside from that, for a tremendous number of patients who don't have a clear disease process of the vulvar skin but have significant atrophy, loss of elasticity, tenderness, discomfort and the like of the outer skin tissues, we're finding that the standard vaginal approach of the MonaLisa Touch does not adequately treat those anatomic areas. So we are finding that we are using the vulvar probe more and more in conjunction with the standard vaginal approach to address the posterior fourchette, the perineal body and the surrounding vulvar tissue.

Dr. Karram: How do you think this treatment compares with other more conventional treatments for atrophy or GSM, such as vaginal estrogen cream or Osphena®?

Dr. Castilla: I think you have some self-selected patients who do well on conventional treatments—"I'm used to this, it works for me." But overall, their results are about average. We have not seen the dramatic improvements we are seeing with the laser when we treat women with vaginal estrogen or Osphena.

Dr. Karram: Dr. Dell, your practice is a urogynecology subspecialty practice. Does this technology have a place in a community-based gynecology practice?

Dr. Dell: Absolutely, I think it does. The ideal fit of this technology is the common patient who is the middle of the bell curve and who is in the hands of the busy community gynecologist. Where I think caution needs to be exerted is that as we get into expanded vulvar protocols where we're pushing the envelope, or for certain patient types such as someone experiencing mesh erosion, which need to be handled with extreme caution, I think protocol development for difficult patient types may need to be handled in the training and teaching centers, but that doesn't prevent the heavy use and wide availability of the standard approach in the hands of community physicians.

Dr. Karram: Dr. Sophocles, could you tell us who in your mind is the most ideal patient to benefit from this treatment?

Dr. Sophocles: I think the ideal patient is the postmenopausal female with dyspareunia or vaginal dryness as her most bothersome symptom, plus or minus LUTS symptoms. The restoration of healthier tissue with enhanced capability to generate moisture, and improved elasticity makes for improved quality of life. Many of my patients with chronic UTIs, or urinary symptoms not

helped with medication or biofeedback are finding improvement after CO2 micro ablative laser.

Breast cancer patients who have taken antineoplastics such as aromatase inhibitors suffer severe debilitating atrophy of genital tissues. While centers such as MD Anderson and Memorial Sloan Kettering have found positive safety profiles in administration of low-dose vaginal estrogen to breast cancer patients, the practicing gynecologist still shies away from prescribing vaginal estrogen for treatment of GSM in breast cancer patients. Most breast cancer patients then suffer doubly from GSM due to lack of estrogen and an exacerbation from the medications they take to prevent recurrence of their breast cancer. We have never had tools in our toolbox to help these women. Until now. The CO2 micro ablative laser is the only safe option for these women, and it is exciting and gratifying to have something to offer them.

My experience with vulvar applications of the laser has been promising but I think we need robust trials comparing various treatment regimens in order to elucidate the proper number of treatments and to create practice algorithms for women with vulvar dystrophies and inflammatory vulvar processes.

Dr. Karram: Could you take us through the typical regime of treatments that are recommended in patients that you feel are good candidates for this treatment?

Dr. Sophocles: The treatment options are threefold: option one is topical vaginal estrogen, either cream, pill or a ring. Option two, a non-estrogen, non-hormonal daily tablet—ospemifene or Osphena. And three, the MonaLisa Touch. (Lubricants are not really a treatment option; they're a symptom reliever. Lubricants and vaginal moisturizers do of course have an important place in our armamentarium to combat dyspareunia.) I present all three unless the patient has contraindications for any. I present them in that order—in the order of what I believe the patient might have heard of first to what they may know the least.

Dr. Karram: How do they react when you tell them about the laser?

Dr. Sophocles: Some are worried that it will be painful. I explain that lasers have been used in gynecology for decades, and that the gentle, microscopic "pinpricks" that the MonaLisa makes are just tiny "injuries" which stimulate a healing response, which creates new collagen and blood vessel formation. Once patients understand that it is a natural body response and lacks risks and side effects of medical therapy, they are usually very interested. Most patients fear pain and side effects. Once you help mitigate those fears, they are very accepting of the procedure. I explain also that it is new in this country, but that somewhere between 15,000 and 20,000 procedures have been done worldwide with an excellent safety profile.

Dr. Karram: Dr. Croak, as a urogynecologist, how have you utilized MonaLisa Touch treatments in your practice?

Dr. Croak: It's been a very nice addition to my practice. Treatments started in early June, and they have ramped up in volume ever since. I will say that the therapeutic results have lived up to the patients' expectations. Nine out of 10 women coming back for their second treatment reported some form of benefit from the first treatment alone. There seems to be a myriad of symptom improvement, the most common being less dryness and painful intercourse, and better lubrication. I have also seen reductions in urgency, frequency and UTIs. I have had four patients with vulvar lichen sclerosus who are still in treatment, but were having no problems after their first session based on a one-week follow-up phone call.

Dr. Karram: Dr. Kubricht, as the only urologist on this panel, can you tell us how you got involved with this treatment and how you have been offering it to your female patients?

Dr. Kubricht: My practice focuses on female pelvic medicine, so I have a primary female practice. I got involved with lasering about four years ago doing laser hair removal, and once I was in that space I became aware of what was happening with the MonaLisa overseas. I then became involved with the technology once it came to the United States and was cleared by the FDA.

With a primary female practice, I use the laser quite heavily. I use the laser for treatment of atrophic vaginitis, vulvovaginal atrophy. I currently use it as a primary treatment modality by giving my patients an opportunity to choose between hormonal therapy, topical hormonal therapy or the MonaLisa.

In the event of patients who have contraindications to hormones, I explain what the contraindications are and why they are of concern and uniformly offer this as an option to all patients with breast cancer, clotting disorders, history of strokes, TIAs and similar coagulation abnormalities.

Dr. Karram: How many patients have you treated?

Dr. Kubricht: We've treated well over 100 patients and have actually had such a good experience that we appear to be on par with all the outcomes and findings reported in the FDA trial as well as in the European literature.

We have well over 60 patients who are past the three-month mark since their final treatment. I arbitrarily established three to four months as a follow-up time after the last MonaLisa treatment and have advised patients that after one year they may begin to have recurrence of symptoms that would require retreatment. At these follow-ups, patients are reporting good results and they're happy.

Dr. Karram: Do you think this is a treatment that could be adopted by most urologists who are comfortable doing pelvic exams?

Dr. Kubricht: Without a doubt. Urologists have been using lasers in surgery for many years—stone surgery, bladder tumor surgery, prostate surgery—so even though the average urologist may not have a heavy female practice, they do all see the same problems that come from vulvovaginal atrophy. The recurrent UTIs, the dyspareunia, the recurrent vaginal infections—even though they're urologists, they still see a lot of that as well. So it's an easy extension to begin to offer that just in the generalist practice.

Dr. Karram: Are you seeing many breast cancer patients, and if so, how do they come to you?

Dr. Kubricht: I would say the majority of the patients we treated initially were all breast cancer survivors. We have a steady referral pattern already in place now from medical oncologists, radiation oncologists and breast surgeons, who are all referring patients now for this treatment because it's had such an impact on their care. All of our specialties deal with the negative aspects of breast cancer treatment and the absence of hormones, and thus the cancer surgeons and oncologists have become some of our biggest physician advocates.

This referral pattern developed once the technology became available and it honestly did not take much to educate these physicians and say: we have a population of patients who are suffering and here is something that could potentially change their lives with very little effort.

Dr. King: Initially I thought that breast cancer patients would be the majority of who I'd see for MonaLisa, but interestingly, in about 90 percent of breast cancer patients I've seen, their oncologist has allowed them to be on vaginal estrogen. I'd say they account for about 25 percent of my MonaLisa patients, and for them it has worked incredibly well. And they are so relieved to find out that there's another option. I'm also seeing uterine cancer survivors, none of whom were on estrogen.

Next: Practice building with MonaLisa Touch marketing

Practice building with MonaLisa Touch marketing

Dr. Karram: Dr. Croak, I understand you have been very successful doing some direct-to-consumer marketing around this treatment. Could you describe how you approached patients in the community regarding educating them on the potential benefits of MonaLisa Touch treatment?

Dr. Croak: I have a rather large practice, so my staff sent a letter to our peri- and post-menopausal patients. In the first three months we sent over 3,000 letters in a graduated allotment so as to not overwhelm the schedule with potential appointments. The letter talked about the technology, its indications, and the fact that there was good scientific evidence behind the therapy. The letter also explained why the MonaLisa Touch could help women who couldn't take estrogen, and it even employed some of the marketing testimonial language such as "life-changing" and "game-changing."

In addition, the letter invited these patients to a seminar. I started offering monthly educational seminars at my preferred hospital, whose staff was kind enough to lend me the meeting space. I also distributed brochures promoting the seminar in the hospital and had flyers available in my waiting room. Patients who attended the seminar and signed up for treatment that evening received a 20 percent discount.

I think the one-two punch of the letters and the seminars really was effective. The first lecture given in June had about 30 women attend; 17 signed up for treatment and 12 actually followed through with therapy. In July I had over 40, and in August I had 58 attend, with over 20 people signing up each time for the discounted treatment package. Consequently, I've been busy. I think what you lose in the higher fee you gain back with more volume.

My practice also ran nice color ads, quarter-page ads in *Toledo Healthy Living News* and *The Maumee Mirror*, a suburban paper, which brought in about a dozen patients. These local papers graciously ran articles as part of the advertisement package. So, the advertising paid for itself. I am now starting to see patients who don't want to wait for a seminar and want to be treated right away; that's a paradigm shift. I am seeing increased demand and subsequently, have raised the fee slightly. Overall, my price point is still a good deal, especially with the discount.

Practice Pearls from the Panel:
Promoting MonaLisa Touch Laser Treatment

- Don't underestimate the effectiveness of a simple letter (or if you have EMR, an email) to patients announcing this technology.
- Offer monthly seminars to the public with a discounted fee to those who sign up for treatment while at the seminar. Ask your hospital to lend you the meeting space.
- Track seminar attendees to know which MonaLisa patients have had many of their questions already answered, to minimize unnecessary counseling time.
- Consider hiring or assigning a dedicated employee to book, counsel and consent MonaLisa Touch patients to save physician time.
- Inform oncology colleagues about the benefits of MonaLisa Touch for their patients for whom estrogen is contraindicated, e.g., an open house at your offices.
- Reach out to the female cancer survivor community.
- Ask area hospitals if they will allow distribution of seminar flyers or patient brochures.

Dr. Karram: Can others talk a little bit about some of the things that you have done within your practice to let women be aware of this therapy?

Dr. King: First, we promote it on our website. I do go on the radio regularly, local talk shows. I speak at women's luncheons. I haven't done any ads yet. The vast majority have come from talking with my patients, and if they have any issues whatsoever, I let them know that this is available. We have the brochures, but it is mostly me speaking with patients when they come in for their exams.

Dr. Castilla: Our first step was to hire an employee specifically for the program, because there were going to be a lot of questions and we needed someone to be an advocate for the patient and navigate her through the process. It's expensive to do that but well worth it. We have a dedicated phone line for this employee and any time a patient has questions, they can call. We've also done a lot of advertising.

About half of the women we've treated have been our own patients, but half have come from outside the practice. Advertising is pricey, but it's been worth it; I know it's driving patients to the website, where they're finding out more about the procedure.

We've also held monthly seminars in our offices. Patients can come in and meet the physicians and learn a little bit more about the procedure. We give patients a discount if they come to the seminar. We promote the seminar in our advertising and we also direct market to our patients through our EHR using their email addresses. We stratified the list; we only emailed those over the age of 40.

It's been really successful for us. I would say that the other two mature practitioners and I are doing two to three new cases a week, which is huge when you extrapolate that monthly.

Dr. Kubricht: We've held open houses for the general public for several months now. Turnout has been very good, very consistent. We also offer a discount to patients who attend the seminar and sign up for treatment. We keep track of who attends and we put that in our records so if they sign up we know that they were educated. You then don't have to spend as much time explaining the procedure because they already know what there is to know.

Dr. Karram: How did you educate your oncology community? What was your process?

Dr. Kubricht: When we first got the laser, we had an open house, a 45-minute seminar to show the technology. We invited every oncology practice in our city to attend, their nurses, specifically, as well as the physicians. For those who did not attend, all it took was a phone call.

Dr. King: I took the local oncologist out to lunch to tell him about MonaLisa. He hadn't heard of it, and breast cancer patients are the majority of his practice.

Dr. Karram: Are patients calling up out of the blue to schedule the procedure?

Dr. Castilla: We actually direct them to the seminar first, and I'll tell you why: if I have a patient I've never seen before, I have to meet her, take a history and eventually I'll do a physical exam on her. But it takes a lot of time for me to walk someone I've never met before through the entire process. So I want our employee, Jennifer, to do that. And if they come to the seminar, it saves a lot of time explaining, counseling them and so on. In other words, if the patient comes to the seminar, then I can see them, consent them, take a quick medical history and do a physical exam, and my total time has been the seminar and maybe 15 minutes. So we want them to go to the seminar first.

Dr. Karram: So it's like having that counseling once with 60 people versus having to do it 60 times, one by one.

Dr. Castilla: Exactly. And for the patient, there's less pressure to decide about the procedure. Patients want that anonymity. If they have further questions, they can always call Jennifer afterward and follow through.

Dr. Karram: Do any of you get patients via word of mouth?

Dr. Sokol: We have people calling every day asking for this. They're calling from all over; we've had calls from Singapore and Moscow. I don't know how they're hearing about it. We have a long waiting list.

Dr. Castilla: Our first seminar was in June; we had about 60 people. We also had 60 people in our August seminar, but the interesting thing in August was that many people had come from the community who had heard about the treatment through word of mouth—already within a couple of months. And that seminar yielded a higher percentage of people signing up for the procedure the same night.

Dr. Sophocles: We've had people come from pretty far away after hearing word of mouth. I've had walk-ins. I had a walk-in from six hours away: she had heard about the MonaLisa Touch and she arrived in tears. She said, "I haven't had sex in eight years, my marriage is almost destroyed because of it, and I thought there was something wrong with me. My friend came here and had this done and she called me, and I just got in the car and drove." So we treated her that day and she's over the moon.

Dr. Karram: Have you had difficulty getting patients to pay out of pocket for this procedure?

Dr. Castilla: To be honest, we live where the demographic doesn't really balk at the cost. Some have said they'll wait for their healthcare or flexible spending account to get enough money in it. But we haven't had anyone say it's way too expensive. A lot of my patients do Botox and fillers and have had plastic surgery, so it hasn't been an issue for us.

Dr. Sophocles: Initially, cost of the procedure was an issue for my patients. We have a mixed population, and for many of these patients, if cost were not an issue, they'd do it, because they would not have to worry about compliance and messy creams and inserting something; they don't want to deal with side effects or risks of any of the meds. One of the reasons I've done so many cases—more than 350—is that I've done many gratis so I could get a few hundred cases under my belt. So initially, most patients opted for the procedure. Now that I charge it's a different percentage, but with 6,000 patients in my practice, I'm having this conversation 10 times a day, and I'd say I'm now treating five patients a day.

Dr. King: In my practice they pay per treatment, but it's important to understand that many of these patients are already on estrogen. For the woman in her 50s who is not on estrogen, I tell her she's probably going to need three treatments to bring her vagina back, and I offer a slight discount for paying up front for all three treatments. That does encourage them to come back. But I've had several people who were on vaginal estrogen and just weren't getting to where they needed to be, and they have chosen to pay per session even though they're not getting that discount. In those cases, all have come back for a second treatment, while some have not come back for the third because after the second they're completely fine.

Dr. Karram: So you're using MonaLisa Touch as an adjunct treatment to vaginal estrogen?

Dr. King: Exactly. I've found that if they've been primed with estrogen, two treatments gets them perfect. Let me offer one example where I was most astounded: My patient was 60 years old, 10 years postmenopausal, no estrogen, severe pelvic prolapse. Absolutely, positively needed surgery. But her tissue was nowhere near ready. I put her on vaginal estrogen. She was one of the first people I treated with the MonaLisa and we talked about it as a possibility. She asked if we could do both and I said of course we can. She received her first MonaLisa treatment that very first day. Six weeks later she came back to see if her tissue was healthy enough for surgery and I said, you know, it's almost there but let's do one more MonaLisa. So we did a second treatment that day. Three weeks later I did her surgery. Her tissue dissected like somebody who was 30 years old.

Dr. Karram: When patients do pay, do they feel the results were worth the investment?

Dr. Castilla: We're still in the infancy with this; most of my patients are on their first or second treatment. But those who have commented have said, "This is a godsend. It's saving our marriage, our relationship. It's saving *me*." They cannot believe what a difference it's made.

Dr. Sophocles: Out of more than 350 patients, including more than 150 who paid out of pocket, I haven't had anyone say it wasn't worth it.

Dr. King: I've had zero complaints. People think of Aspen as only wealthy people, but there are only 6,000 people here year-round, and it is mostly blue-collar. There is a large Hispanic population and I've seen that most are used to paying cash for everything, so it hasn't really been an issue. There's a mentality that insurance should pay for this, but if you point out the cost of vaginal creams, which have shot up in price and are very expensive, say, $100 or $200 a month, plus the hassle, the MonaLisa Touch actually compares very well from a cost standpoint as well as a hassle-free standpoint.

Dr. Dell: After hundreds of treatments in over a hundred patients, I would say the clear quality of life improvement is in the range of 95 percent. For properly selected patients with appropriate expectations, it truly is an unbelievably rare case that the patient does not notice significant improvement from a quality of life standpoint, and I would say that is mirrored by what we see clinically and upon physical exam as the patients move through the treatment.

Dr. Kubricht: The interesting point that I've made to others is that this is the first treatment modality I can think of in my career where patients will pick up the phone to take the time to tell you how good they are doing as opposed to what is traditional in medicine, which is that we don't hear from you unless you're having a problem. That's one of the biggest differences I've seen with this treatment over anything else.

End of series

Moderator

Mickey Karram, MD

Dr. Karram is an internationally renowned urogynecologist and pelvic surgeon. He is currently the Director of Urogynecology and Reconstructive Pelvic Surgery at The Christ Hospital and Professor of Obstetrics & Gynecology and Urology at the University of Cincinnati. He has published more than 190 scientific articles and book chapters and has co-authored textbooks entitled "Urogynecology & Reconstructive Pelvic Surgery" and "Atlas of Pelvic Anatomy and Gynecologic Surgery." He is past president of the American Urogynecology Society and previous editor in chief of the International Urogynecology Journal.

Panel

Julie Anne Castilla, MD, FACOG

Dr. Castilla is a partner in Arizona Women's Care, a five-physician obstetrics/gynecology practice in Scottsdale, AZ, with a history of bringing new technologies to the region. Dr. Castilla's areas of interest include infertility and high-risk obstetrics.

Andrew J. Croak, DO, MS, FACOOG

Dr. Croak is medical director of the Northwest Ohio Center for Urogynecology and Women's Health, where he leads a solo practice. Dr. Croak also teaches at Ohio University College of Osteopathic Medicine, Mercy St. Vincent Medical Center, Toledo.

Jeffrey R. Dell, MD, FACOG, FACS

Dr. Dell is medical director of the Institute for Female Pelvic Medicine in Knoxville, TN. One of the nation's first board-certified urogynecologists, Dr. Dell participates in clinical research trials and regularly serves as organizer, invited lecturer and visiting professor at local, national and international events. He is currently conducting research on MonaLisa Touch.

Gail King, MD, FACOG

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The use of pulsed CO_2 lasers for the treatment of vulvovaginal atrophy

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Purpose of review
This article reviews the literature regarding the safety and efficacy of the use of a pulsed CO_2 laser for the treatment of vulvovaginal atrophy (VVA).

Recent findings
Prospective observational studies have demonstrated histological changes after the use of pulsed CO_2 laser vaginally in atrophic conditions. Increased collagen and extracellular matrix production has been reported together with an increase in the thickness of the vaginal epithelium with the formation of new papilla. Three different observational studies reported a significant improvement of VVA assessed subjectively (with a 10-point visual analogue scale) and objectively (using the Vaginal Health Index) after a cycle of three treatments of pulsed CO_2 laser. Also sexual function (assessed with the Female Sexual Function Index) and quality of life (evaluated with the SF12 questionnaire) significantly improved. No complications or side-effects were reported during or after the laser procedure that was performed in an outpatient setting.

Summary
Increasing evidence with histological and clinical data supports the use of pulsed CO_2 lasers in the treatment of VVA; however, no randomized control trial (sham versus treatment) has yet been produced and no data on the duration of therapy are currently available.

Keywords
dyspareunia, pulsed CO_2 laser, quality of life, sexual function, vaginal dryness, vulvovaginal atrophy

INTRODUCTION

Vaginal atrophy is a common and bothersome condition that occurs following estrogen decline during menopause. Available treatments have specific contraindications or poor patient compliance despite good efficacy. This review covers the use of a novel treatment with pulsed CO_2 laser for the treatment of vulvovaginal atrophy (VVA), describing its rationale, mechanism of action, and the emerging histological and clinical evidence of this therapy for such a bothersome condition. Future applications and research are also explored.

TEXT OF REVIEW

Vulvovaginal atrophy

VVA is a common condition secondary to estrogen depletion; this could happen as a consequence of natural menopause or as the result of iatrogenic events (such as surgery, chemotherapy, or radiotherapy for oncological disorders). VVA can affect 40% of postmenopausal women [1] with a great impact on quality of life and self-image of the sufferers [2].

The definition of VVA is not standardized and many authors have proposed different classification. A recent review [3] evaluated the available literature for VVA terminology and assessment; the authors finally proposed their own definition of VVA as a common manifestation of estrogen deficiency associated with specific symptoms of which the most common are: vaginal dryness, itching/irritation, and dyspareunia. However, even this new proposal does not cover all the aspects consequent to estrogen deficiency. In May 2013, a consensus conference of the Board of Directors of the International Society for the Study of Women's Sexual Health and the Board of

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KEY POINTS

- Vulvovaginal atrophy (VVA) is a common condition with great impact on quality of life.
- Pulsed CO_2 laser is a novel treatment for VVA performed in an outpatient setting.
- Histological samples after pulsed CO_2 laser treatment show changes in the vaginal lamina propria (increased collagen production and extracellular matrix), as well as in the vaginal epithelium with restoration of new papilla and thickness increase.
- Vaginal dryness, dyspareunia, and all the VVA symptoms significantly improve after a cycle of three laser treatments.
- Improvement of VVA symptoms after pulsed CO_2 laser determines a better sexuality and quality of life.

Trustees of The North American Menopause Society [4**] agreed that the term genitourinary syndrome of menopause was medically more appropriate, emphasizing the common involvement of the genital and lower urinary tract in this condition. So called 'irritative bladder symptoms' such as frequency of micturition, urgency, and dysuria are, in fact, common complaints in women with VVA.

The lack of estrogens determines tissue modifications in the female urogenital tract including thinning of the epithelium layer, reduced vascularization, reduced elastic fibers, changes in the content of collagen, reduced lubrication, and increased vaginal pH [5]. All of these changes contribute to the development of increasing tissue friability and of all the VVA symptoms, including sexual dysfunction and dyspareunia.

Available treatments for vaginal atrophy

International guidelines recommend local estrogens, lubricants, moisturizers, and ospemiphene, a new selective estrogen receptor modulators (SERM) molecule, for the treatment of VVA [6,7]. However, all of these options have some limitations:

Local estrogens, despite showing good efficacy in treating VVA symptoms, should be prescribed with caution and after appropriately counselling women with a previous history of malignant conditions sensitive to estrogens and with a previous history of thrombo venous embolism. Moreover, no safety data are available on a long-term basis and patient adherence to treatment is not good.

Lubricants and moisturizers do not really treat the condition but can help in alleviating the symptoms and they are used when needed.

Ospemiphene is a new drug (SERM) approved by European Medical Agency and Food and Drug Administration for VVA symptoms [8,9]. However, its effect on breast tissue and lower urinary tract still needs to be clarified.

CO_2 pulsed laser

'Laser' is an acronym that stands for 'Light Amplification by Stimulated Emission of Radiation'. Lasers can differ for many different aspects and different light sources are commonly used in medicine with specific wavelength. CO_2 laser falls in the infrared spectrum with a wavelength of 10.600 nm and it is highly absorbed by water. These two characteristics determine the superficial action of this laser.

The mode of delivery of the laser beam can also vary: it can be continuous or pulsed (fractional). The latter avoids possible tissue damage secondary to overheating. It is, in fact, possible to set the fractional mode not just for the power delivered but also for the dot spacing and the dwell time, preserving the treated tissue from any unwanted effect. Tissue modifications produced in this way can, therefore, vary depending on different machine settings and can include vaporization, ablation, coagulation, collage shrinkage, or collagen neosynthesis and remodeling.

CO_2 pulsed lasers in medicine

Pulsed CO_2 lasers are widely used in dermatology for many conditions including scars and atrophic changes caused by different factors (acne, ageing and so on) [10–13]. In 2003, Capon and Mordon [14] reported the mechanism of action of pulsed CO_2 laser in regenerating atrophic tissue through a micro ablative and thermal effect. The latter induces some changes in cells' metabolism. The heating shock induces the production of some proteins called heat shock proteins. Heat shock protein 70 stimulates the action of Transforming Growth Factorβ in activating fibrolites to become fibroblasts that are responsible for the synthesis of new extracellular matrix, of new collagen and new elastic fibers. Thirty days are needed for this cascade of events to occur.

Pulsed CO_2 lasers have also been used in dentistry for severe conditions such as oral leukoplakia [15].

In all of these conditions, the regenerative effects of the pulsed CO_2 laser were able to produce remodeling of the affected tissue into healthy tissue.

Ex-vivo and feasibility study of pulsed CO_2 laser for vulvovaginal atrophy

The feasibility of using a pulsed CO_2 laser to treat VVA symptoms was first explored in an ex-vivo

study [16••] wherein the safety and the effects on the vaginal tissue were evaluated. In this study, the most appropriate setting of the laser machine was also tested.

Five different treatment protocols were evaluated. The study population consisted of postmenopausal women with VVA symptoms who were undergoing surgery for anterior vaginal wall prolapse. None of them was on hormonal replacement therapy and all consented to participate in the study. The five different protocols were used treating one side of the excessive vaginal wall that had to be trimmed after fascial plication, whereas the contralateral part was always used as control. The excised samples of the vaginal wall were sent for histological evaluation. In all cases, the control side of the trimmed vagina confirmed an atrophic state showing a flattened epithelium, loss of papilla, and absence of activated fibroblasts in the lamina propria. The five different protocols were associated with different degrees of changes in the epithelium and in the lamina propria in relation to mild ablative effects, fibroblast activation, modifications of collagen, elastic fibers, and mucopolysaccharides in the lamina propria. On qualitative analysis, the protocol with the best profile of safety and effectiveness was protocol 3 (30 W of DOT power, 1000 μs of DOT dwell, 1000 μm of DOT spacing, and smart stack 3), wherein the more pronounced ablative effects and the presence of activated fibroblasts in the lamina propria are evident. These results were confirmed in all five participants. Under an electron microscope, fine collagen microfibrils could be clearly observable, with fine molecular filaments (arrows) in close relationship with collagen microfibrils in the course of fibrillogenesis.

The microablative pulsed CO_2 laser produces a controlled heat shock response that stimulates the production of a small family of proteins: the heat shock proteins [17]. Heat shock proteins 43, 47, and 70 (a protein subtype which is a chaperone of collagen, which is overexpressed after laser irradiation) could play a role in inducing the production of many growth factors.

Transforming growth factor-A is known to be a key element in the inflammatory response and in the fibrogenic process, wherein fibroblasts produce collagen and, most generally, the extracellular matrix. In this study, we observed interesting histological and intracellular changes in the vaginal wall of postmenopausal women irradiated with the microablative pulsed CO_2 laser. These modifications can be interpreted as tissue remodeling in a rejuvenating sense. The most evident effects produced by the microablative fractional CO_2 laser are the neocollagenesis with a return of the collagen fibers to a trabecular disposition, which is how it appears in premenopausal women.

Clinical study on the use of pulsed CO_2 laser for vulvovaginal atrophy symptoms and sexual function

The clinical efficacy of the pulsed CO_2 laser has been reported in three different prospective observational studies.

In 2014, for the first time Salvatore *et al.* [18••] reported a 12-week evaluation of the use of pulsed CO_2 laser for the treatment of VVA symptoms in 50 postmenopausal women. The treatment protocol included three outpatient sessions with an interval of one month in between. All women were assessed on entry and one month after each single laser procedure as follows:

Subjectively, with a 10-point visual analogue scale for each single VVA symptom (vaginal dryness, burning, itching, dyspareunia, and dysuria);

Objectively, using the Vaginal Health Index [19], a composite score including five different variables (overall elasticity, fluid secretion type, pH, epithelial mucosa, and moisture);

Table 1. Assessment at baseline and at 30 days after each single pulsed CO_2 laser treatment for VHI and VVA symptoms (evaluated with a 10-point VAS) expressed as mean value ± SD

	Baseline	4-week follow-up	8-week follow-up	12-week follow-up
VHI (mean ± SD)	13.1 ± 2.5	17.1 ± 1.9[a]	22.1 ± 1.9[a,b]	23.1 ± 1.9[a,b,c]
Vaginal dryness (*n*, mean ± SD)	8.3 ± 2.1	5.5 ± 2.9[a]	3.4 ± 2.5[a,b]	2.7 ± 1.9[a,b]
Vaginal hitching (*n*, mean ± SD)	6.1 ± 3.0	3.3 ± 3.1[a]	2.1 ± 2.8[a,b]	1.5 ± 1.7[a,b]
Dyspareunia (*n*, mean ± SD)	8.1 ± 2.8	5.7 ± 3.2[a]	4.5 ± 3.0[a,b]	3.3 ± 2.3[a,b]
Dysuria (*n*, mean ± SD)	5.0 ± 2.4	2.8 ± 1.3[a]	2.0 ± 0.9[a,b]	1.1 ± 1.1[a,b,c]

VHI, Vaginal Health Index. Adapted from [18••].
[a]Statistical significant difference with baseline.
[b]Statistical significant difference with 1-month follow-up.
[c]Statistical significant difference with 2-month follow-up.

Table 2. Self-assessment of pain experienced during each single laser treatment expressed as mean value ± SD

	First laser application	Second laser application	Third laser application
Pain experienced during insertion of the probe (mean ± SD)	4.7 ± 1.6	2.6 ± 1.5[a]	0.4 ± 0.5[a,b]
Pain experienced due to movements of the probe (mean ± SD)	2.6 ± 1.5	1.0 ± 0.8[a]	0.2 ± 0.4[a,b]
Pain experienced during laser application (mean ± SD)	0.6 ± 0.8	0.3 ± 0.5[a]	0.1 ± 0.4[a]

Adapted from [18▪▪].
[a]Statistical significant difference with first laser application.
[b]Statistical significant difference with second laser application.

With a 10-point visual analogue scale to grade pain (with the left extreme of the scale indicating the absence of pain, and the right indicating severe) experienced by women and caused by the insertion of the probe, the movements of the probe during the treatment and the application of laser.

At the end of the treatment cycle statistically significant improvement compared with baseline was observed with each single evaluation tool used in this study (Table 1). The procedure was well tolerated and only mild pain was reported during the insertion of the probe during the first treatment (Table 2). No complications or side-effects were reported.

In this study vaginal samples were taken for histological evaluation on entry and after the first CO_2 laser treatment in five women. In all of these samples important vaginal wall changes were observed with a remodeling of the lamina propria and the epithelial layer. In the latter new and/or larger papilla were produced; a size increase in the epithelial cells rich in glycogen exfoliating superficially was also evident. A more detailed description of histological changes of the vaginal wall secondary to pulsed CO_2 laser treatment in atrophic vagina was reported by Zerbinati *et al.* [20▪].

In 2015, Perino *et al.* [21▪] replicated the Salvatore study, recruiting 48 patients and using the same evaluation instruments. VVA symptoms significantly improved ($P < 0.0001$) after three sessions of vaginal fractional CO_2 laser treatment compared with baseline. Objectively, the Vaginal Health Index score showed also a significant improvement ($P < 0.0001$). Overall, 91.7% of patients reported that they were satisfied or very satisfied with the procedure and experienced considerable improvement in quality of life. No adverse events due to fractional CO_2 laser treatment were reported.

VVA can have a very important impact on sexual function and deeply influence intimacy. The Closer Survey by Nappi *et al.* [22] showed, in fact, that because of VVA 58% of women and 61% of men reduced their sexual activity, whereas 35% of women and 14% of men decided to put off having sex. The reason for sexual abandonment was attributed to painful sex by 55% of women and 61% of men, respectively.

In another prospective observational study, Salvatore *et al.* [23▪] investigated the effects of the fractional microablative CO_2 laser on sexual function and overall satisfaction with sexual life in postmenopausal women with VVA. Seventy-seven postmenopausal women with VVA symptoms were included and treated with three sessions of fractional microablative CO_2 laser system (SmartXide2 V(2)LR, Monalisa Touch, DEKA, Florence, Italy), at 30 days interval. Sexual function and quality of life were evaluated with the Female Sexual Function Index (FSFI) [24] and the Short Form 12 [25], respectively, both at baseline and at 12-week follow-up. A significant improvement in the total score and in each single specific domain of the FSFI was observed at 12-week follow-up compared with baseline ($P < 0.001$) as illustrated in Table 3.

Table 3. Sexual function evaluation using the Female Sexual Function Index at baseline and at 12 weeks after pulsed CO_2 laser treatment expressed as mean value ± SD

	FSFI DOMAINS					
	Desire	Arousal	Lubrication	Orgasm	Satisfaction	Pain
Baseline (T_1)	2.4 ± 1.0	2.8 ± 1.7	2.2 ± 1.5	2.8 ± 1.8	2.9 ± 1.9	1.8 ± 1.5
12-week follow-up (T_4)	3.6 ± 0.7	4.8 ± 1.0	4.5 ± 1.4	5.0 ± 1.0	5.1 ± 1.0	4.2 ± 2.0
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

FSFI, Female Sexual Function Index. Adapted from [23▪].

Seventeen (85%) out of 20 women not sexually active because of VVA severity at baseline regained a normal sexual life at the 12-week follow-up.

CONCLUSION

A pulsed CO_2 laser can be successfully and safely used for the treatment of VVA symptoms, with improvement of sexuality and quality of life. However, further evidence should be produced focusing on the duration of efficacy of the CO_2 laser treatment, as well as the amount of placebo effect, with a randomized controlled trial comparing treatment versus sham.

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Conflicts of interest

S.S. received fees from the laser manufacturer (DEKA MELA Srl) to act as a speaker in national and international meetings. For the remaining authors none were declared.

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ORIGINAL ARTICLE

The effect of microablative fractional CO_2 laser on vaginal flora of postmenopausal women

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ABSTRACT

Objectives: To assess the effect of microablative fractional CO_2 laser ($MFCO_2$-Laser) therapy on the vaginal microenvironment of postmenopausal women.

Methods: Three laser therapies at monthly intervals were applied in postmenopausal women with moderate to severe symptoms of genitourinary syndrome of menopause, pH of vaginal fluid >4.5 and superficial epithelial cells on vaginal smear <5%. Vaginal fluid pH values, fresh wet mount microscopy, Gram stain and aerobic and anaerobic cultures were evaluated at baseline and 1 month after each subsequent therapy. Nugent score and Hay-Ison criteria were used to evaluate vaginal flora.

Results: Fifty-three women (mean age 57.2 ± 5.4 years) participated and completed this study. $MFCO_2$-Laser therapy increased *Lactobacillus* ($p < 0.001$) and normal flora ($p < 0.001$) after the completion of the therapeutic protocol, which decreased vaginal pH from a mean of 5.5 ± 0.8 (initial value) to 4.7 ± 0.5 ($p < 0.001$). The prevalence of *Lactobacillus* changed from 30% initially to 79% after the last treatment. Clinical signs and symptoms of bacterial vaginosis, aerobic vaginitis or candidiasis did not appear in any participant.

Conclusion: $MFCO_2$-Laser therapy is a promising treatment for improving the vaginal health of postmenopausal women by helping repopulate the vagina with normally existing *Lactobacillus species* and reconstituting the normal flora to premenopausal status.

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Introduction

The vaginal microenvironment of postmenopausal women differs from that of women in premenopausal status mainly due to a lack of estrogens[1,2]. In premenopausal women, estrogens are considered essential not only for the proliferation of the vaginal epithelial cells but also for the production of glycogen, an essential factor for the growth of vaginal lactobacilli. Vaginal lactobacilli play a key role for a healthy vaginal equilibrium due to their competitive activity to other pathogens but also because they are responsible for maintaining an acidic pH by producing lactic acid and H_2O_2[3–6]. In postmenopausal women, a decrease in *Lactobacillus* species and a rise of vaginal pH values over 4.5 are observed, resulting in the loss of the local vaginal defense mechanisms against bacterial pathogens, which may predispose to local inflammation and/or infections[7,8].

The disturbance of Lactobacillary flora has been correlated with the presence of *Gardnerella vaginalis, Trichomonas vaginalis, enterococci,* group B *streptococci,* and *Escherichia coli*[9]. Available data indicate that pre-existing vaginal colonization with pathogenic *enterobacteriae* is essential for the appearance and recurrence of urinary tract infections (UTIs)[10]. Indeed, postmenopausal women are prone to UTIs with an incidence of about 8% per year and a 4% likelihood of recurrence, while asymptomatic bacteriuria has been estimated in up to 15% of women[11,12].

Various therapeutic strategies, hormonal or not, oral or local, have been proposed for the improvement of the vaginal microecosystem of postmenopausal women (e.g. estrogens, probiotics, combination of vaginal estrogens with live *Lactobacillus*)[13–23]. Hormonal therapy (oral or local) has been associated with a healthier vaginal microecosystem by repopulating the *Lactobacillus* species to a premenopausal status and by reducing the pH of vaginal fluid[12,14–24]. The use of vaginal estrogens compared to placebo decreased the incidence of UTIs in postmenopausal women[25]. However, the ideal management for the achievement of the optimal benefit–risk balance is still under investigation. The choice of estrogen therapy should be guided by clinical experience and patient preference[12]. Moreover, possible adverse events of estrogens and controversial guidance from physicians make the women reluctant to use them, particularly those with a history of estrogen-sensitive cancers, such as endometrial and breast cancers[26].

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Supplemental data for this article can be accessed here

Recently, an intravaginal microablative fractional CO_2 laser ($MFCO_2$-Laser) procedure has been introduced for the treatment of postmenopausal women with vulvovaginal atrophy (VVA)[27–29]. Initial reports indicated that the $MFCO_2$-Laser treatment improves significantly the VVA symptoms, sexual function and quality of life of postmenopausal women, as well as the vaginal health index[27–29]. Other studies found that $MFCO_2$-Laser therapy restores the thickness of the squamous stratified epithelium of the vaginal mucosa with a significant storage of glycogen in the epithelial cells and remodels the vaginal connective tissue with the production of neocollagen and ground substance molecules[30,31].

Genitourinary syndrome of menopause (GSM) is a new terminology more accurate than the terms of VVA/atrophic vaginitis, because it describes the clinical signs and symptoms of the vulva, vagina and lower urinary system. In contrast, VVA/atrophic vaginitis describes the appearance of the vulvovaginal structures only[32]. The GSM has a prevalence of more or less 50%, depending on country of origin, with a negative impact on sexuality, quality of life and well-being[33–37]. There are many different strains of lactobacilli (e.g. *L. crispatus, L. Jenseni, L. iners*) and to study such a population is relevant for GSM. However, as the status of vaginal glycogen is indicative of the estrogenic effect, the general population of lactobacilli, as assessed in the routine practice, provides indirect information regarding the estrogenic status. To our knowledge there is currently no evidence regarding the potential impact that the intravaginal $MFCO_2$-Laser therapy may have on the vaginal microenvironment in women with symptoms of GSM.

The aim of the current study was to assess the effect of $MFCO_2$-Laser laser therapy on the vaginal microenvironment of postmenopausal women.

Material and methods

Participants and study design

This prospective study has been conducted at the Urogynecologic Unit of a tertiary referral hospital. The local Ethics Committee approved the study protocol. All women eligible for inclusion in the study provided written informed consent before initiating the therapeutic protocol.

Postmenopausal women with at least one moderate to severe symptom of GSM, as defined by the International Society for the Study of Women's Health and The North American Menopause Society[38], were eligible to participate in this study. Women were also required to have an objective evidence of menopause; the percentage of superficial vaginal epithelial cells, in the maturation index of vaginal smear (MI) and the vaginal fluid pH had to be $\leq$5% and >4.5, respectively. The typical proportion of superficial epithelial cells in postmenopausal women with VVA is less than 5%[7], whereas vaginal fluid with a pH >4.5 has a sensitivity of 84.9% for menopausal diagnosis, better than follicle stimulating hormone (sensitivity of 77.4%)[8].

We excluded from the study women who had used any form of hormone therapy (systemic or local) within the previous 6 months, lubricants or vaginal moisturizers within the last month, suffering from active genital infections (e.g. bacterial vaginosis, genital herpes), with prolapse stage $\geq$ II according to the pelvic organ prolapse quantification (POP-Q) system[39], and any disease that would interfere with compliance to the protocol.

The selection procedure for participation in the study involved: a questionnaire in which women were asked to report the intensity of each individual GSM symptom (dyspareunia, vaginal dryness, vaginal itching, vaginal burning, dysuria, frequency and urgency) measured by a 10-cm visual analogue scale, in which zero applied to 'absence of symptom' and ten to 'symptom as bad as could be'. A score of 4–7 was considered moderate and a score of 8–10 severe. Women with moderate to severe symptom/s were then clinically examined in order to assess signs of VVA and pelvic organ prolapse. A week after the vaginal examination, vaginal samples for microbiological and cytological processing were obtained from all potential participants in the study protocol. Eligible postmenopausal women for participating in the therapeutic protocol were assigned a randomly generated number and entered in a database. In each subsequent therapy, a new number, different from those previously used, was randomly assigned to each participant. In this way the microbiologist was blind to all information regarding participants' clinical findings and treatment status. In addition, an experienced nurse, who was blind to all information regarding participants' clinical findings and treatment status, performed the evaluation of pH indicator strips.

Participants in the study protocol received intravaginal therapy monthly for 3 months, with $MFCO_2$-Laser system (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy). The following settings of the $MFCO_2$-Laser were used: D-Pulse mode, dot power, 40 W; dwell time, 1000 μs; and dot spacing, 1000 μm. The smart stack parameter from 1 to 3 was used for the treatment of the vaginal canal and the dot power was reduced to 24 W; dwell time, 400 μs; and dot spacing, 1000 μm for the treatment of the vaginal introitus. The procedure of $MFCO_2$-Laser was performed as previously described[29]. The participants were not allowed to use any lubricants and/or moisturizers during the study protocol.

Before each laser application, a sterile swab was inserted into the vaginal canal and a sample was obtained from the posterior fornix of the vagina. Vaginal samples were placed in a transport gel (Stuart). pH indicator strips (MColorpHast™, Merck, Germany) were applied against the lateral vaginal wall using sterile forceps, followed by a vaginal lavage for wet mount. Smears from vaginal samples were placed on a glass slide, observed under microscope for *Trichomonas vaginalis* and *Candida* species and stained afterwards according to standard Gram stain procedure. Cultures for aerobic and anaerobic bacteria species were also performed, using MacConkey agar, blood agar, Sabouraud dextrose agar, chocolate agar as culture media, focusing on bacteria with potential clinical impact (e.g. aerobic vaginitis[39], UTIs, vaginal candidiasis). Mycoplasma, ureoplasma and/or corynebacterium species were not assessed. Lactobacilli, *Mobiluncus, Gardnerella* and *Bacteroides* identified by Gram stain were not further evaluated by cultures. All samples were collected at baseline and at each subsequent therapy.

The vaginal flora was evaluated according to the European guidelines on the management of vaginal discharge, using the Nugent score and Hay-Ison criteria[39–42]. Predominant bacteria were defined as a single bacterium prevailing from all visible bacteria under Gram staining at baseline and subsequent laser therapies. The bacteria that could be identified by microscopy of Gram-stained smears were categorized as gram-positive or gram-negative.

Normal vaginal epithelial cells and leukocytes were evaluated using Gram stain in a similar manner to Nugent scoring for morphotypes, receiving scores of 0, 1, 2, 3 and 4. When vaginitis was assessed based only on laboratory findings without any clinical signs and symptoms, pharmaceutical therapy was not recommended according to the Centers for Disease Control and Prevention guidelines[43].

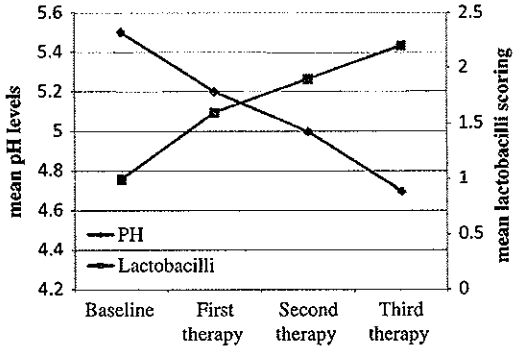

Figure 1. Decrease in the mean vaginal pH levels and increase in the growth of lactobacilli at baseline, and after subsequent therapies. The mean values of pH at baseline and after the first, second and third laser therapy were 5.5 ± 0.8, 5.2 ± 0.5 ($p = 0.002$), 5.0 ± 0.6 ($p < 0.001$) and 4.7 ± 0.5, respectively ($p < 0.001$). Increase in the growth of Lactobacilli was observed after the first ($p = 0.001$), the second ($p < 0.001$) and the final laser therapy ($p < 0.001$).

Statistical analysis

Statistical analysis was performed comparing baseline and subsequent therapies. The distribution of data was assessed using the Shapiro–Wilk test. For abnormally distributed variables, Wilcoxon signed rank test for related paired samples and Spearman correlation coefficient were used. For categorical variables, χ^2 test was used. Logistic regression analysis was performed using as dependent variable the threshold of 4.5 for pH values of vaginal fluid at the final visit. All tests were based on a significance level of 5% ($p < 0.05$). Data were presented as mean ± standard deviation and as percentages (%). SPSS statistical software was used for the analyses.

Results

Eighty-four postmenopausal women with symptoms of GSM sought treatment in our department. Thirty-one women were excluded as they did not meet the inclusion criteria; 18 had pH <4.5, two had a POP-Q stage ≥ II, and 11 reported mild symptoms of GSM. Thus, 53 postmenopausal women (mean age 57.2 ± 5.4 years, mean body mass index 26.0 ± 4.8 kg/m^2, and mean years since the last menstrual period 8.9 ± 6.6) were eligible for inclusion in the study protocol. The majority of the participants had natural menopause (77%), were non-smokers (70%), and married (85%), with dyspareunia and dryness being the most common symptoms (77% and 87%, respectively). Seven participants had a history of hysterectomy; in six participants hysterectomy was the cause of menopause and in one woman the uterus and adnexals were removed after the onset of menopause due to endometrial cancer stage Ia. All participants completed the study protocol. The baseline demographic characteristics of the study participants are shown in Table S1 (see Supplementary Table 1 at http://dx.doi.org/10.1080/13697137.2016.1212006).

A significant progressive reduction of vaginal pH between baseline and 1 month after the third laser therapy was observed (Figure 1). At the end of the therapeutic protocol, the pH of the vaginal fluid decreased in 47 (89%) participants. After the completion of the therapeutic protocol, 43 (81%) participants had pH <5 and 17 (32%) pH <4.5. The pH decreased <4.5 independently of the participant's baseline demographic characteristics (e.g. years since the last menstrual period) and the characteristics of vaginal flora at baseline (pH, vaginal flora according to Nugent scoring and to Hay-Ison criteria). A significant progressive increase of *Lactobacillus* morphotypes was observed (Figure 1).

At baseline, the predominant bacteria identified were *Lactobacillus* (30%), *Bacteroides* (2%), *Mobiluncus* (2%), *Gardnerella* (6%), gram-positive (6%) and gram-negative (11%). The bacteria were evenly grown in 28% of participants while 15% did not have morphotypes of any bacteria species (i.e. complete absence of vaginal flora). In contrast, 1 month following the last therapy, all treated women had some growth of morphotypes. Specifically, 79% of women had *Lactobacillus* as the predominant bacteria species, 2% had *Gardnerella*, 8% gram-positive, 4% gram-negative, 2% had *Candida* and in 6% no predominant bacteria were present.

The changes observed in the vaginal flora according to the Nugent scoring system and Hay-Ison criteria are presented in Table 1. The presence of microorganisms in Gram stain and cultures are presented in Table 2. The detailed changes of *Lactobacillus*, *Gardnerella*, *Mobiluncus* and *Bacteroides* based on the Nugent scoring system are presented in Table S2 (see Supplementary Table 2 at http://dx.doi.org/10.1080/13697137.2016.1212006).

Normal vaginal epithelial cells significantly increased after the second and third therapies compared to baseline ($p = 0.007$ and $p < 0.001$, respectively). At baseline, 33% of participants' epithelial cells had a score of 3 or 4, 21% a score of 2, 40% a score of 1, and 6% a score of 0. After three laser therapies, 58% of participants had a score of 3 or 4, while 26% a score of 2 and 16% a score of 1. Leukocyte proportion remained unchanged through the therapeutic protocol.

All women had no clinical signs and symptoms of urogenital infections and did not receive pharmaceutical therapy at any stage of the protocol. At baseline, 16 participants were not sexually active, due to the intensity of the GSM symptoms. After treatment, 15 of these women resumed sexual activity. No serious adverse events occurred during the

Table 1. The vaginal flora assessed according to the Nugent scoring and the Hay-Ison criteria during the study period with the fractional microablative CO_2 laser system. Data are given as *n* (%)

	Baseline (n = 53)	*After 1 laser therapy (n = 52)*	*After 2 laser therapies (n = 53)*	*After 3 laser therapies (n = 53)*	*p Value*[c]
Nugent flora[a]					
Normal	25 (47.2)	39 (75)	45 (84.9)	46 (86.7)	<0.001
Intermediate	23 (43.4)	13 (25)	7 (13.2)	6 (11.3)	
Bacterial vaginosis	5 (9.4)	0 (0)	1 (1.9)	1 (1.9)	
Hay-Ison flora[b]					
Grade 0	17 (32.1)	5 (9.6)	7 (13.2)	0 (0)	
Grade 1	24 (45.3)	37 (71.2)	40 (75.5)	48 (90.6)	<0.001
Grade 2	3 (5.7)	6 (11.5)	2 (3.8)	4 (7.6)	
Grade 3	7 (13.2)	1 (1.9)	1 (1.9)	1 (1.9)	
Grade 4	2 (3.8)	3 (5.8)	3 (5.7)	0 (0)	

[a], Nugent flora refers to vaginal flora according to the Nugent scoring system. It was obtained by using the sum of scores resulting from the synthesis of *Lactobacillus, Gardnerella, Bacteroides* and *Mobiluncus* morphotypes. A score of 0–3 indicated normal flora, a score of 4–6 was considered intermediate and a score of ≥7 was defined as bacterial vaginosis[40,41]; [b], Hay-Ison Flora refers to vaginal flora according to Hay-Ison criteria. Grade 0: absence of bacterial species (lactobacilli, *Gardnerella*-like and gram-positive species) and presence of only epithelial cells; Grade 1 (normal flora): predominantly *Lactobacillus* morphotypes; Grade 2 (intermediate): mixed flora with some lactobacilli present, but *Gardnerella* or *Mobiluncus* morphotypes also present; Grade 3 (bacterial vaginosis, BV): predominantly *Gardnerella* and/or *Mobiluncus* morphotypes, clue cells, with few or absent *Lactobacillus morphotypes;* Grade 4 (aerobic vaginitis flora): growth of gram-positive cocci only without *Lactobacillus* morphotypes, not related to BV[41,42]; [c], *p* value at level <0.05 is significant. The *p* value was calculated comparing the number of women with normal flora (according to Nugent and Hay-Ison) before and after the completion of the therapeutic protocol

Table 2. Presence of microorganisms in the vaginal fluid of the women included in this study. Data are given as *n* (%)

Microorganisms[a]	*Baseline (n = 53)*	*After 1 laser therapy (n = 52)*	*After 2 laser therapies (n = 53)*	*After 3 laser therapies (n = 53)*	*p Value*[b]
Lactobacillus	36 (67.9)	45 (86.5)	46 (86.8)	53 (100)	<0.001
Gardnerella vaginalis	5 (9.4)	2 (3.8)	4 (7.5)	4 (7.5)	0.7
Bacteroides	5 (9.4)	5 (9.6)	4 (7.5)	2 (3.8)	0.2
Mobiluncus	4 (7.5)	2 (3.8)	2 (3.8)	0 (0)	0.04
Streptococcus agalactiae	5 (9.4)	2 (3.8)	1 (1.9)	2 (3.8)	0.2
Enterococcus faecalis	12 (22.6)	8 (15.1)	7 (13.2)	6 (11.3)	0.1
E. coli	20 (37.7)	10 (19.2)	6 (11.3)	9 (16.9)	0.02
Klebsiella	3 (5.7)	2 (3.8)	1 (1.9)	0 (0)	0.08
Proteus	1 (1.9)	2 (3.8)	3 (5.7)	1 (1.9)	1
Candida spp.	1 (1.9)	1 (1.9)	1 (1.9)	1 (1.9)	1

[a], *Lactobacillus, Gardnerella vaginalis, Bacteroides, Mobiluncus* and *Candida* were identified by Gram stain. *Streptococcus agalactiae, Enterococcus faecalis, E. coli, Klebsiella, Proteus* and *Candida* were identified by cultures using MacConkey agar, blood agar, Sabouraud dextrose agar, chocolate agar as cultures media. Microscopy of wet mount was also used for *Candida. Candida albicans* was identified at baseline and after three laser therapies, while *Candida glabrata* was identified after one and two laser therapies; [b], *p* values presented were calculated at a level of significance of 0.05 comparing baseline with 1 month after the three laser therapies

period of the laser treatment, but only a temporary mild irritation of the introitus was noted. This irritation started immediately after the laser treatment, lasted up to 2 h and resolved spontaneously.

Discussion

The intravaginal $MFCO_2$-Laser has recently been introduced to ameliorate symptoms of GSM without the adverse events of other modes of therapy, especially the hormonal ones[26–28]. Other laser technologies are available (e.g. erbium laser)[44,45]. Erbium has a thermal effect while the $MFCO_2$-Laser has both ablative and thermal effects, stimulating heat shock proteins and other factors (e.g. TGF-β), prompting neocollagenesis and neoangiogenesis, resulting in tissue rejuvenation[45,46]. The results of the present study show that $MFCO_2$-Laser therapy improved the vaginal flora with a significant reduction of the pH of the vaginal fluid, and a significant increase of *Lactobacillus* flora. To our knowledge, this is the first study to assess the effect of $MFCO_2$-Laser on the vaginal microenvironment of menopausal women in a clinical aspect.

In the present study, the vaginal fluid pH values gradually declined after each laser therapy, as would be expected if the women had used estrogens. Although these results were not compared to a control group and should thus be considered with caution, this effect was observed as early as the first treatment session with a further reduction of pH after each session. After completion of the treatment protocol, the mean pH value was 4.7 with the vast majority of participants (81%) having pH <5. In the literature, the reported mean pH values after 12 weeks of vaginal estrogen ranges from 4.6 to 4.9, depending on the type of estrogen, modes of delivery and dosage[16–20,22,23]. In a randomized, controlled trial comparing hyaluronic acid to estradiol vaginal tablets, 14.3% of participants receiving estradiol had pH <5 after the end of the therapeutic protocol and 86% of participants that received estradiol had pH 5.0–5.49[21].

The progressive reduction of the pH during the study protocol was concomitant with a statistically significant increase in normal vaginal epithelial cells after each monthly treatment. Moreover, the observed increase of the normal vaginal epithelial cells in the current study confirms the results of the histological study of Zerbinati and colleagues[30]. In this study, it was demonstrated that one of the effects of the $MFCO_2$-Laser therapy on the vaginal mucosa was a high degree of epithelial exfoliation, with superficial cells filled

with glycogen shedding at the epithelial surface[30]. In the present study, although only 32% of the participants reduced pH levels to <4.5, the statistically significant increase of normal vaginal epithelial cells between baseline and the third laser therapy possibly indicates that, at the end of the study protocol, the re-establishment of vaginal health was still in progress and that the pH may have not reached its lowest value.

The decline of pH described above was in accordance with the increase of *Lactobacillus* morphotypes, to almost a premenopausal status. In a randomized, controlled trial, the prevalences of *Lactobacillus* after the use of vaginal estriol cream and after the application of hyaluronic acid were 55% and 43%, respectively (baseline prevalences were 34% and 31%, respectively)[20]. The prevalence of lactobacilli in the present study was 30% before treatment and reached 79% after the three laser therapies. The lactobacilli predominance is of great importance and is considered as one of two criteria that distinguishes bacterial community groups; the other criterion is considered to be the particular Lactobacillus species present[47]. Even though the assessment of the particular *Lactobacillus* species was not conducted in this study, the significant increase of *Lactobacillus* predominance implies that the laser therapy restores the vaginal mucosa in a beneficial manner for the vaginal microecosystem.

After the completion of the therapeutic protocol, the prevalences of gram-negative, gram-positive, *Gardnerella*-like species and *Candida* were 4%, 7.5%, 2%, and 2%, respectively. Although significant decreases were observed only for *E. coli and Mobiluncus*, all *Lactobacillus* antagonists had a trend of lowering their growth. Asymptomatic bacterial vaginosis with vagina colonized with pathogens occurs more often than previously thought in postmenopausal women, but is usually not recognized[48]. The prevalences of *Gardnerella*-like species and *Candida* in postmenopausal women without receiving hormone therapy (HT) have been reported as 10–40% and 1%, respectively, while, when HT was administered, the prevalences were 8.3–33.3% and 23.3%, respectively[24]. However, there are no data regarding the prevalence of *Lactobacillus* antagonists in Greek healthy postmenopausal women with or without receiving HT. In the studied population, at the baseline, the colonization of *Gardnerella*-like species was present in 6% of participants, while after the end of the therapeutic laser protocol it was 2%. The low percentage of *Gardnerella*-like species at baseline could be explained by the hygiene habits and the sexual intercourse behavior of the women. Most importantly, the decrease from 6% to 2% of *Gardnerella*-like species shows that $MFCO_2$-Laser improves the vaginal equilibrium status and does not predispose to vaginal infections.

Although our study was not designed to include postmenopausal women with a history of recurrent UTIs and/or vaginitis, these women could potentially benefit from the improvement of vaginal flora resulting from treatment with the $MFCO_2$-Laser technique. Vaginal colonization with uropathogens is believed to predispose to UTIs and/or recurrences. Hormonal therapy with or without probiotics, according to the current data, is the only option for maintaining vaginal health in menopausal women and reducing the incidence of UTIs[12,14–25]. Future studies could therefore evaluate whether the $MFCO_2$-Laser represents a valid alternative to vaginal estrogens alone or in combination with lactobacilli for relapse prevention, particularly for women with contraindications to hormonal therapies.

A weakness of this study is the lack of a control group. The findings could thus be due to a placebo effect, or other factors (e.g. time). Furthermore, the follow-up period was relatively short and it remains to be seen whether the above-described effect will be persistent in a longer time interval. Another potential weakness is that the study was not designed to evaluate comprehensively the whole vaginal microbiota. However, the microbiological evaluation was based on diagnostic procedures that are performed in the daily clinical practice and was focused on common pathogens related with urogenital infections. On the other hand, the strengths of the study include its prospective design and the use of clearly defined objective and subjective criteria. Moreover, the assessors of all samples, parameters and data were blinded to all the details related to the study participants.

Conclusion

The novel non-pharmaceutical therapeutic approach of $MFCO_2$-Laser in women with symptoms of GSM was found to have a beneficial effect on the vaginal microenvironment. The laser therapy restored the vaginal equilibrium to a healthier status, as would normally be expected if estrogen levels were sufficient. The predominance of *Lactobacillus* species and the more acidic pH of vaginal fluid achieved after the $MFCO_2$-Laser therapy could protect postmenopausal women from vaginal infections and inflammation and possibly from UTIs. However, studies with larger sample sizes and longer follow-up period, focusing on the comparison of $MFCO_2$-Laser to other therapeutic modalities or placebo, regarding the impact on the prevention of vaginal infections and UTIs, are needed.

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Is vaginal fractional CO_2 Laser treatment effective in improving overactive bladder symptoms in post-menopausal patients? Preliminary results

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Abstract. – **OBJECTIVE: To evaluate the role of vaginal fractional CO_2 laser treatment in the relief of Overactive Bladder (OAB) symptoms in post-menopausal women.**

PATIENTS AND METHODS: Post-menopausal women who complained of one or more symptoms related to vulvo-vaginal atrophy (VVA), who experienced symptoms of OAB and who underwent vaginal treatment with fractional CO_2 laser were enrolled in the study. At baseline (T0) and 30 days post-treatment T1), vaginal status (using Vaginal Health Index – VHI), subjective intensity of VVA symptoms (using a visual analog scale – VAS) and micturition diary were evaluated. OAB symptoms were also assessed using a validated questionnaire.

RESULTS: Thirty patients were enrolled. A statistically significant improvement in VVA symptoms was observed and in VHI at T1 ($p<0.0001$). A significant improvement was also identified in the micturition diary, in number of urge episodes and OAB-q ($p<0.0001$). Nine of the 30 patients suffered from incontinence episodes, and had improved at T1.

CONCLUSIONS: In our experience, fractionated CO2 laser vaginal treatment has proved to be effective in improving OAB symptoms in post-menopausal women. Moreover, it is a safe and efficacious measure for the relief of VVA related conditions. Further long-term studies are needed to confirm these preliminary results.

Key Words:
CO_2 laser, Overactive bladder, Menopause, Vulvo-vaginal atrophy.

Introduction

Vulvo-vaginal atrophy (VVA) is defined as the progressive involution of urogynecological mucous membranes and tissues due to the menopausal drop in oestrogen levels[1,2]. It may lead to the occurrence of bothersome symptoms (vaginal dryness, itching, burning, irritation and dyspareunia), with a significant impact on quality of life (QoL) and sexual function[3,4].

Resolving "vulvo-vaginal aging symptoms" is a very timely issue, firstly because, considering the progressive aging of the general population, women may complain of these symptoms for more than one-third of their lives[5]. Many hormonal and non-hormonal strategies have been proposed to alleviate VVA symptoms[5-8], with the aim of identifying an effective, safe and long-term therapeutic option. Recently, by applying the principles of regenerative and anti-aging medicine, the use of fractional CO_2 laser has been extended to treating patients with VVA symptoms[9,10].

Through the topical remodeling of connective tissue and the production of new collagen, elastic fibers and other components of the extracellular matrix, laser CO_2 effects appear to overcome the negative vaginal changes related to climacteric estrogenic fall, with significant relief of related symptoms[9,10].

Data from our recent study indicate a significant improvement in VVA symptoms ($p<0.0001$) in women who underwent 3 sessions of fractional CO_2 laser vaginal treatment, with a relevant increase in vaginal health index (VHI) scores ($p<0.0001$) and a good level of satisfaction for the procedure[11]. During data collection for this study, an unexpected event occurred. A sample of menopausal women who had complained of urinary

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problems, such as urgency or frequency, (in addition to VVA symptoms), reported a concomitant relevant improvement in urinary symptoms, after CO_2 laser treatment.

Starting from these clinical observations, we decide to evaluate the possible role of fractional CO_2 laser vaginal treatment in the relief of over active bladder (OAB) symptoms in post-menopausal women. Moreover, VVA symptom and VHI score improvements were also analyzed in the same study population.

Patients and Methods

Study Patients

The enrolled patients for this prospective observational pilot study were menopausal women who had complained of one or more symptoms related to VVA, who experienced symptoms of OAB and who underwent vaginal treatment with fractional CO_2 laser from January 2014 to January 2015. The inclusion criteria consisted of menopausal status (including early forms), one or more vulvovaginal symptoms (e.g., itching, burning, reduced lubrication, superficial and/or severe dyspareunia), non-response to previous oestrogen or local therapies, and diagnosis of OAB syndrome.

We defined OAB syndrome as all the cases which in woman complained for ≥3 months frequency of micturition on average ≥8 times per 24 h and at least three episodes of urgency (grade 3 or 4), with or without incontinence, during a 3-day micturition diary period at baseline[12].

Exclusion criteria at study entry were considered: clinically significant bladder outflow obstruction, significant post-void residual (PVR) volume (>200 ml), associated stress urinary incontinence (SUI), diabetic neuropathy, use of concomitant urinary incontinence medications, symptomatic urinary tract infection, active genital infections, previous pelvic radiation therapy, or previous or current malignant disease of the pelvic organs, pelvic organ prolapse (POP) stage >II (according to the *Half Way System* for the quantification of POP)[13] and/or the use of HRT (systemic or local) up to 6 months before the study recruitment period.

Patients who used vaginal lubricants or any other local preparations were asked to suspend the application of these treatments and were included in the study after 30 days. Women who were using psychotropic drugs were excluded.

The study was approved by the Hospital Research Committee. All patients who were recruited for the study signed an informed consent form.

Laser Device

A fractional CO_2 laser system (SmartXide^2 V^{2}LR, Deka m.e.l.a., Florence, Italy) was equipped with a Vulvovaginal Laser Reshaping (V2LR) scanning system and appropriate probes for the vaginal area. This treatment modality is based on the interaction between a specific CO_2 pulsed laser and the vaginal mucosa.

A laser beam is emitted fractionally, and the CO_2 laser is focused in small spots (called DOTs) that are separated by healthy tissue. The laser beam penetrates the tissue and releases heat only when the set depth is reached. With software control and a radiofrequency system that feeds the laser source, it is possible to select the D-Pulse mode, the depth (SmartStak parameter, from 1-3) and the quantity (power, dwell time and spacing) of heat to be transferred to the tissue. The SmartStak function allows for the careful control of the vaporization depth and thermal action. Successive pulses are emitted in the same area for a Stack variable of 1-3 (in the vaginal application).

Every pulse is composed of a constant high-energy peak power to produce rapid ablation of the epithelial component of the atrophic mucosa, followed by longer emission times (dwell time) that allow the CO_2 laser to penetrate further into the mucosa. The pulses are distributed over the vaginal wall and are spaced (DOT spacing) to cover the entire treatment area. A specific probe is used to deliver the pulses, which allows for energy emission at 360°.

In this study, a calibrated probe was specifically utilized for vaginal application, easily inserted into the vaginal canal. The laser is projected towards a 45°-oriented mirror that is placed at the tip of the probe to be reflected on the vaginal walls but not the uterine cervix. To completely treat the vaginal area, it is necessary to emit many laser spots while progressively extracting the probe from the vaginal fundus up to introitus.

Each treatment spot consists of two passages: after the first energy release, the probe is rotated approximately 2 cm (using the regulatory tool) clockwise while remaining at the same vaginal distance.

Laser Treatment

Each patient was treated with the fractional CO_2 laser system using the vaginal probe. All patients underwent a complete cycle of three treat-

ment sessions that were spaced over a period of at least 30 days.

For each patient, a Pap test and vaginal swabbing were performed to rule out local lesions or infections. The procedure was performed in the outpatient clinic by two operators (A.P. and G.C.), and the patients did not receive analgesia or anaesthesia. The settings for intra-vaginal treatment were a *DOT power* of 40 watts, a *dwell time* of 1,000 μs, D*OT spacing* of 1,000 μm, *SmartStak* 2 and the *D-Pulse* mode; when necessary, the *DOT power* was reduced to 30 or 20 watts for the treatment of the vaginal introitus, which is a highly sensitive area (Figure 1).

As previously reported[11], before all treatment sessions, we proceeded to positioning of the speculum and observation of the vagina using colposcopic vision (Vaginal Health Index – VHI scoring was performed during this phase).

No local therapy was recommended after the laser sessions. To avoid vaginal irritation during the healing process, patients were advised to avoid coital activity at least for a week following each laser application. Any secondary or collateral effects of the treatment were recorded. For study analysis, two relevant time points were considered for the evaluation of treatment results: baseline (T0) and 30 days after the last laser application (T1).

Study Data

Relevant demographic characteristics, pre-treatment clinical data and inclusion/exclusion criteria were recorded at T0. At T0 and T1, the vaginal status of the patients was evaluated using VHI score (obtained using colposcopic vision), which consisted of the following 5 parameters: elasticity, fluid volume, pH, epithelial integrity and moisture. Each parameter was graded from 1 (worst condition) to 5 (best condition).

Intensity of VVA symptoms was also evaluated at T0 and T1 (vaginal itching, vaginal burning, vaginal dryness and dyspareunia) using a visual analog scale (VAS), which is based on a score from 1 to 10, where 1 indicates the absence of symptoms and 10 indicates severe symptoms ("as bad as it could be").

At T0 and T1, eligible patients adduced a micturition diary, which was to be completed during the 3 days preceding the visit. In the diary, patients were asked to specify the number of micturitions, number of urgency episodes and number of incontinence episodes, for every considered day.

Moreover, patients rated the degree of associated urgency on the five-point Patient's Perception of Intensity of Urgency Scale (0, no urgency; 1, mild urgency; 2, moderate urgency; 3, severe urgency; and 4, urge incontinence)[12], and only cases

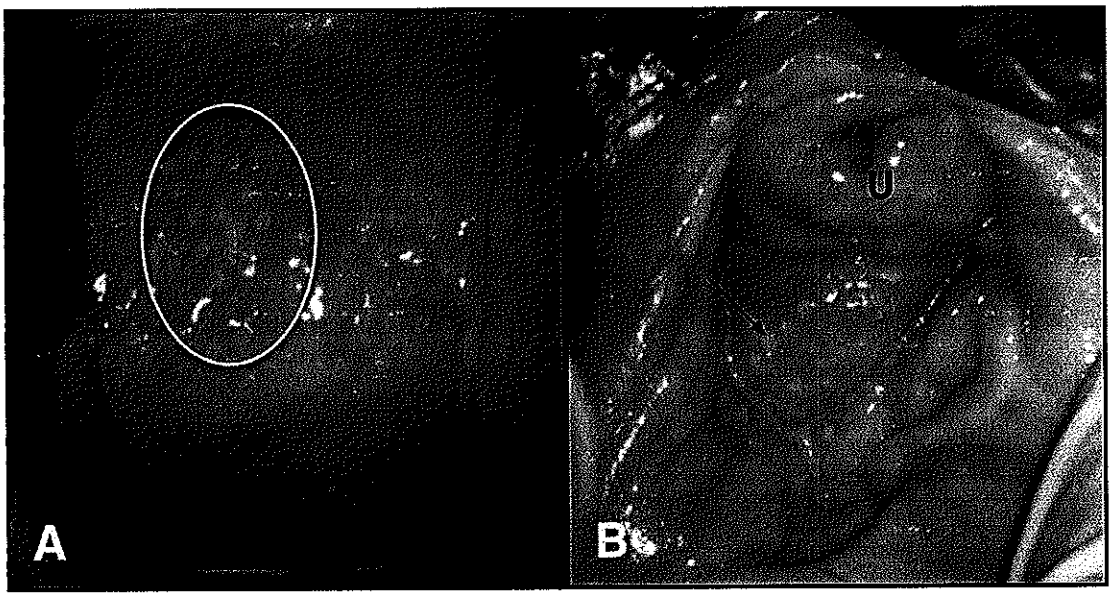

Figure 1. *(A-B)* Colposcopic view of vaginal walls immediately after a session of fractional CO_2 laser therapy. In ***A*** e ***B***, macropores of thermal ablation zones are highlighted in anterior vaginal wall (withe ring) and sub-urethral area *(arrow)*; U: urethral meatus.

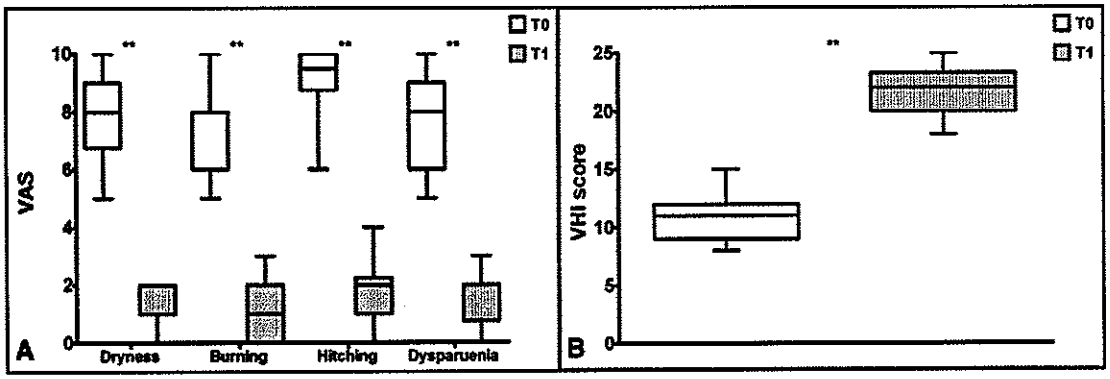

Figure 2. *(A-B).* VAS – dryness, – burning, – itching, – dyspareunia *(A)* and VHI score *(B)* are represented by Box-whiskers (medians, 1st and 3rd quartiles, minimum and maximum values). Statistical analysis was performed using Wilcoxon test, "means $p<0.0001$.

of 3 and 4 degrees of intensity were considered for analysis.

Overactive bladder symptoms were assessed using the validated Overactive Bladder Questionnaire Short Form (OAB-Q SF)[14].

Eventual adverse events which occurred or were referred by patients (during, immediately after treatment sessions, and until the end of study) were recorded. We considered any disorder, discomfort or injury, both local and general, arising in relation to the application of the vaginal fractional CO_2 laser as an adverse event.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows (version 17.0, SPSS Inc., Chicago, IL, USA). Data were presented as median/IQR. Differences between VAS at T0 and T1 were analyzed with Wilcoxon test. Statistical significance was set at $p<0.05$.

Results

In the study period, 30 patients were enrolled. Demographic characteristics are reported in Table I. Patients had used systemic HRT and/or vaginal estriol with no benefit, before vaginal CO_2 laser treatment, in 20% and 36.7% of cases respectively. All patients included into the study completed the study-protocol and carried out the final evaluation at T1. We observed a statistically significant improvement in VAS parameters concerning dryness (8/3 *vs.* 2/1.25; $p<0.0001$), burning (8/2.25 *vs.* 1/1; $p<0.0001$), itching (8/2 *vs.* 1/2; $p<0.0001$) and dyspareunia (9.5/1.25 *vs.* 2/1.25; $p<0.0001$) (Figure 2A).

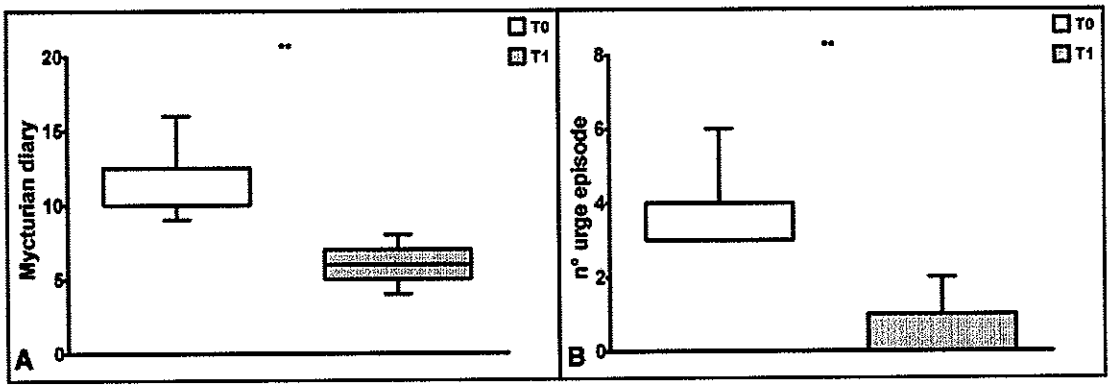

Figure 3. *(A-B)* Micturition diary *(A)* and n° urge episodes *(B)* are represented by Box-whiskers (medians, 1st and 3rd quartiles, minimum and maximum values). Statistical analysis was performed using Wilcoxon test, "means $p<0.0001$.

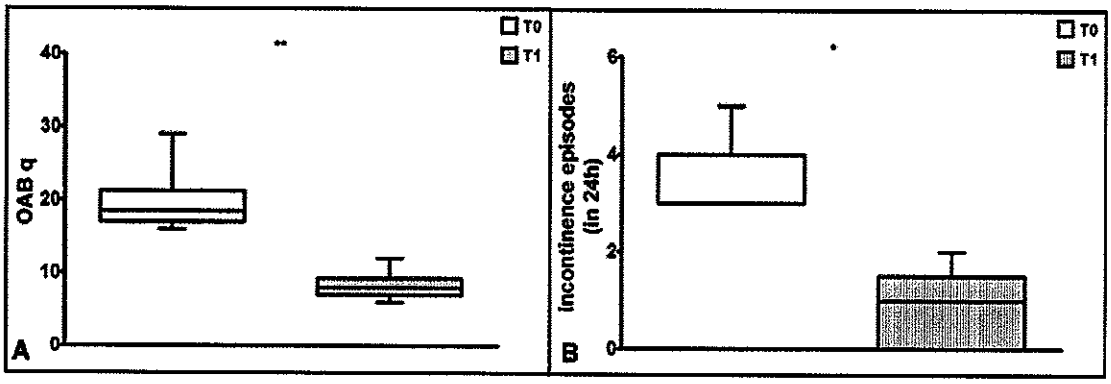

Figure 4. *(A-B)* OAB q *(A)* is represented by Box-whiskers (medians, 1st and 3rd quartiles, minimum and maximum values). Statistical analysis was performed using Wilcoxon test, **means $p<0.0001$. In *B*, incontinence episodes (in 24h) are represented by Box-whiskers (medians, 1st and 3rd quartiles, minimum and maximum values); statistical analysis was performed using Wilcoxon test, *means $p<0.05$.

We also reported a significant improvement in VHI at T1 (11/3 *vs.* 22/3.25; $p<0.0001$) (Figure 2B), in micturition diary (10/2.5 *vs.* 6/2; $p<0.0001$) (Figure 3A), in number urge episodes (3/1 *vs.* 0/1; $p<0.0001$) (Figure 3B) and in OAB-q (18.5/4.25 *vs.* 8/2.25; $p<0.0001$) (Figure 4A). Nine of the 30 patients suffered from incontinence episodes, and had improved at T1 (3/1 *vs.* 1/1.5 episodes in 24 h; $p=0.006$) (Figure 4B).

No significant differences were observed between patients or between the sessions for the same patient. No adverse events due to fractional CO_2 laser treatment occurred. In no case was it necessary to stop the procedure because of patient pain or intolerance. No local therapies were prescribed to any patient after the sessions of laser treatment.

Discussion

Overactive bladder is a wide-spread problem characterized by symptoms of urinary urgency, frequency and nocturia, with/without urgency incontinence (UI) and in the absence of lower urinary tract infection[15,16]; it may cause significant disability, reduced QoL, along with social relationship and sexual function deterioration[17,18]. It is known that this chronic condition requires life-long therapy to control symptoms, with the aim of restoring QoL while balancing efficacy and side effects[19].

Generally, an early approach to the problem involves conservative measures such as dietary controls, fluid modification and pelvic floor muscle rehabilitation, but the results are frequently poor. Antimuscarinic agents are used as first-line pharmacotherapy in the management of OAB, with well-documented effectiveness in the clinical literature[18,20]. However, patients often discontinue this therapy for many reasons including intolerable side effects (dry mouth, constipation and blurred vision) or/and lack of sufficient symptom relief[21,22].

A recent pharmacological alternative is mirabegron, a β3-adrenoceptor agonist that elicits bladder relaxation during the storage phase of the micturition cycle, without inhibiting bladder voiding[23]. It provides treatment benefits in OAB patients and its tolerability profile suggests that it may represent a valuable therapeutic option[24,25]. However, randomized prospective trials are still lacking[23]. Likewise, with regard to intravesical botulinum toxin for management of OAB, a few controlled trial data exist on its benefits and safety[26]. Reported adverse events following botulinum toxin administration may be related to the drug (constipation, transitory asthenia, dry mouth) or the associated procedure (pain, haematuria)[26]; moreover, robust data are required on long-term outcomes and optimal dose of botulinum toxin.

Table I. Demographic characteristics of the study population.

Age (median/IQR)	56/8.5
Body Mass Index (median/IQR)	23.9/3.49
Parity (median/IQR)	1/1.25
Smokers (n, %)	5 (16.7)
Urge incontinence (n, %)	7 (23.3)
Previous HRT (n, %)	6 (20)
Previous vaginal estriol (n, %)	11 (36,7)
Other previous therapy (n, %)	21 (70)

Finally, neuromodulation therapy could be another possible approach[19]; in particular, sacral nerve stimulation with an implantable device has demonstrated the efficacy in managing OAB symptoms[27]. However, it has been limited in clinical practice due to several factors including invasiveness, associated costs, limitations in older adult patients and those who are frail or who have several medical comorbidities[28].

In this pilot study, we assessed the clinical effects of fractional CO_2 laser vaginal treatment on the main symptoms associated to OAB syndrome, such as frequency, urgency and eventual incontinence episodes, in a sample of post-menopausal women. Our results indicated a significant reduction of number of micturitions and number of urge episodes ($p<0.0001$) in women who underwent 3 sessions of vaginal CO_2 laser treatment. Concerning the subgroup of patients suffering from urge incontinence, a significant reduction in the number of daily episodes was shown ($p<0.0001$). Moreover, the changes of OAB-q score in T1 indicated a subjective significant improvement ($p<0.0001$), with particular reference to the incidence of OAB problems in QoL of the patients.

Finally, results of this study confirmed that CO_2 laser treatment was effective in improving VVA symptoms and VHI scores ($p<0.001$) at a T1 follow-up.

The observed phenomenon on OAB symptoms is explained starting from the anatomical characteristics of the urogenital tissue. It has been well demonstrated that urogenital organs are highly sensitive to the influence of oestrogen; oestrogen receptors have been found in the urethra and bladder trigone, as well as in the round ligaments and levator ani muscles[29]. As occurs in vaginal tissue, the progressive decline of estrogens during the climacteric produces atrophy of the urethral and bladder mucosa, causing urinary urgency, frequency and nocturia-OAB symptoms; accordingly, atrophy of muscles and reduction of collagen content may be important factors in the increased prevalence of urinary incontinence[30].

As previously reported[9], fractional CO_2 laser system can irradiate deeper layers of the vaginal wall and ultimately reactivate the extracellular matrix and collagen synthesis, with beneficial effects in the 3 layers of the vaginal wall (in contrast to estrogens or other local therapies that only treat the epithelium). In this way, it is possible to presume that the "regeneration" effect of the vaginal laser CO_2 treatment also involves the lower urinary tract (urethra and bladder), with a significant improvement of urogenital aging symptoms.

To the best of our knowledge, this is the first experience on the topic in international literature; however, this study has some limitations. First of all, the absence of randomization or a control group of patients; secondary, the small sample size considered and lack of long-term follow-up. Further studied are needed to confirm these results and to define the potentialities of this approach.

Based on these preliminary results, it would be advisable to perform a new study that includes a control arm (e.g. intravaginal estriol administration) to compare laser CO_2 with other proposed therapeutic options, in the contemporaneous relief of VVA and OAB symptoms, evaluating the long-term outcomes.

Conclusions

Our preliminary results suggest that fractionated CO_2 laser has a role in improving OAB symptoms in post-menopausal women. Moreover, achieved data confirmed that it is a safe and effective measure for the relief of VVA related problems. Considering the lack of a shared guideline for the management of OAB syndrome, we think that this study could lead the way to an alternative approach, tailored for menopausal women complaining of VVA and OAB symptoms.

Conflicts of interest

The authors declare no conflicts of interest.

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Microablative fractional CO_2-laser therapy and the genitourinary syndrome of menopause: An observational study

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ABSTRACT

Objectives: This study aimed to assess the effect of the Microablative Fractional CO_2 Laser (CO_2-laser) therapy on vaginal pathophysiology and the symptoms of the Genitourinary Syndrome of Menopause (GSM).

Methods: Postmenopausal women with moderate to severe symptoms of GSM underwent three sessions of CO_2-laser therapy at monthly intervals. Participants were evaluated at baseline and 4 weeks after the last treatment.

Main outcome measures: The primary outcomes were Vaginal Maturation Value (VMV) and Vaginal Health Index Score (VHIS). Secondary outcomes included symptoms of GSM, Female Sexual Function Index (FSFI), International Consultation on Incontinence Questionnaire of Female Urinary Tract Symptoms (ICIQ-FLUTS) and Urinary Incontinence Short Form (ICIQ-UI SF), Urogenital Distress Inventory (UDI-6) and King's Health Questionnaire (KHQ).

Results: Fifty-three postmenopausal women completed this study. VMV, VHIS and FSFI increased significantly. Dyspareunia, dryness, burning, itching, dysuria, frequency, urgency, urgency incontinence, stress incontinence and scores on the ICIQ-FLUTS, ICIQ-UI SF, UDI-6 and KHQ decreased significantly. Factors predicting for which women the CO_2-laser therapy was more effective were not identified.

Conclusion: This study suggests that intravaginal CO_2-laser therapy for postmenopausal women with clinical signs and symptoms of GSM may be effective in improving both vaginal pathophysiology and reported symptoms.

1. Introduction

The Genitourinary Syndrome of Menopause (GSM) affects menopausal women due to the decline mainly of estrogens involving clinical signs and symptoms from both the genital and the lower urinary tract (LUTS) [1]. Women may present with some or all of the clinical signs and symptoms [1]. The GSM definition was introduced to describe more accurately the urogenital changes and the local symptoms appearing at menopause in comparison to the terms of vulvovaginal atrophy (VVA)/atrophic vaginitis [1].

The principles of treatment of VVA are the restoration of urogenital physiology and the alleviation of symptoms [2]. The therapeutic options for VVA can include non-hormonal local therapies (i.e. lubricants, moisturizers) or hormonal ones (i.e. low-dose vaginal estrogens). However, the 2 principles of treatment usually are not achieved. Lubricants offer a temporary relief of vaginal symptoms, without restoration of urogenital physiology. Moisturizers improve lubrication but have no effect on the overall vaginal maturation index/value (VMI/VMV) [2,3]. Lubricants and moisturizers are less effective than the local hormonal therapy [2]. Low-dose vaginal estrogens fulfill both treatment principles for symptoms of VVA [2,4] and LUTS [5] and may provide substantial alleviation of symptoms in patients with more severe GSM[5].

Intravaginal Microablative Fractional CO_2 Laser (CO_2-Laser) is a new non-hormonal proposal for the management of the VVA [6–9].

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Current data are promising regarding the effectiveness of CO_2-Laser in postmenopausal women [6–9]. However, there are limitations in the available data; there are pilot studies focusing mainly in the symptoms of VVA and not the GSM as a whole (VVA-symptoms, sexual function and LUTS). Furthermore, sample size and effect size of the analyzed finding have not yet been calculated and there is lack of data regarding the impact of the CO_2-Laser therapy in the LUTS and the changes of the VMI/VMV.

The aim of the current study was to assess the effectiveness of the CO_2-Laser in postmenopausal women with clinical signs and symptoms of GSM focusing in both the genital and the lower urinary tract and both principles of the treatment. Additionally, we aimed to address possible factors of predicting the improvement of symptoms of CO_2-laser therapy.

2. Methods

This prospective observational study was performed at the outpatient clinic of the Urogynecologic Unit of a tertiary Hospital. The study protocol was approved by the Ethics Committee. All participants signed an informed consent form.

Eligibility criteria for participating in the current study were: menopause, ≥1 symptom of GSM with moderate-severe intensity and clinical signs of GSM [1].

Exclusion criteria were: use of any form of hormone therapy (systemic or local) within the previous 6 months, use of lubricants or vaginal moisturizers within the last month, active genital infections, prolapse stage ≥II according to the pelvic organ prolapse quantification (POP-Q) system [10], and any disease that would interfere with compliance to the protocol.

Postmenopausal women with symptom/s of GSM, were asked to complete a questionnaire reporting their symptom/s (dyspareunia, genital dryness, burning, itching, dysuria, urinary frequency, urgency), followed by a 10-cm Visual Analogue Scale (VAS) of the severity of each symptom, in which zero applied to "absence of symptom" and ten to "symptom as bad as could be". A score of 4–7 was considered moderate and a score of 8–10 severe. Women with moderate/severe symptom/s were further evaluated by clinical examination assessing clinical signs of GSM and POP-Q. The clinical signs assessed were pallor, erythema, loss of vaginal rugae, epithelial integrity, petechiae and tissue fragility [1].

Eligible participants were allocated at a randomly generated number that was different in each visit and entered in a database. The cytologists and the pH-evaluator were blind to all clinical information. The cytological samples were evaluated by 2 independent cytologists and any discrepancies were resolved by reporting the mean value of findings. A vaginal smear was obtained from the lateral vaginal wall using a spatula and stained according to the Papanicolaou technique. The VMI was evaluated by defining the percentage of superficial, intermediate and parabasal epithelial cells on the smear, followed by the calculation of the VMV [(1×%superficial)+(0.5×%intermediate)+(0×%parabasal)] [11]. VMV is considered to be an indicator of the estrogenic stimulation, whereas values of 0–49%, 50–64% and 65–100% indicate absent/low, moderate and high estrogenic effect on the vaginal epithelium, respectively [11].

The evaluation of the clinical signs was performed by 2 independent gynecologists blind to all clinical information. Any discrepancies were resolved by reporting the mean value of findings. During the clinical examinations the evaluators could not guess the identity of the participant and thus the treatment status, using a curtain at the median abdominal site of the participant. The clinical signs evaluated according to Vaginal Health Index Score (VHIS), which includes elasticity, fluid volume, pH and epithelial integrity as components [8,9,11].

Participants of the study protocol received intravaginal therapy once a month for 3 months, with CO_2-Laser system (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy). The settings and procedures were performed as previously described [9,12].

The efficacy of the CO_2-Laser on VVA-symptoms was measured using the following questionnaires: frequency of sexual intercourse/month, 10cm-VAS questionnaire for the intensity of dyspareunia, genital dryness, burning, itching and dysuria, 10cm-VAS questionnaire for the overall sexual satisfaction, Female Sexual Function Index (FSFI) [13] and 10cm-VAS questionnaire for the pain during the insertion of the vaginal speculum. The efficacy of the CO_2-Laser on the LUTS was measured using the International Consultation on Incontinence Questionnaires (ICIQ) modules [14–16]: ICIQ-Female Urinary Tract Symptoms (ICIQ-FLUTS Filling Domain) and ICIQ- Urinary Incontinence Short Form (ICIQ-UI SF). Patients also completed the Urogenital Distress Inventory (UDI-6), and the King's Health Questionnaire (KHQ). The participants completed all the questionnaires at the baseline and 4-weeks after the last laser therapy (12-weeks follow-up). Satisfaction of the treatment was assessed with the Patients Global Impression of Improvement (PGI-I) at 12-weeks' follow-up. The participants completed the questionnaires without any interference from the researchers involved in the current study. For the KHQ the minimal important clinical difference (MICD) (the smallest change in score that patients perceive as beneficial) was set at −5 points for each KHQ domain [17].

As primary outcomes were regarded the changes of the objective assessment tools of VMV and VHIS. As secondary outcomes were regarded the changes of the subjective assessment tools as evaluated by the above mentioned questionnaires and the evaluation of possible predicting factors of the CO_2-Laser efficacy.

2.1. Statistical analysis

Statistical analysis was performed comparing baseline data and 12-weeks' follow-up data. A priori calculation of the sample size required for the primary efficacy outcomes to achieve a power of 90%, level of significance 5% and effect size derived from the previously published pilot study [12]. Due to the large size effect of the VHIS in the pilot study and the small sample size required for the current study, we calculated a sample size based on the VMV. The changes of the VMV after the CO_2-Laser have not been reported in previous studies, thus we used a level of significance 5%, power of study 90% and a hypothetical effect size of 0.5 (medium effect). The required sample would be 47 participants. Considering a 10% drop-out rate, 52 participants would be necessary to be included. Post-hoc computing of the power of study and effect size of all the outcomes was performed using the G-power statistical software. The distribution of data was assessed using the Shapiro-Wilk test. For abnormally distributed variables Wilcoxon signed-rank test for related paired samples and Spearman correlation coefficient was used. For categorical variables chi-square test was used. Logistic regression analyses were performed using as dependent variables the presence or absence of symptom/s and thresholds of 15 and 49 for VHIS and VMV, respectively at the 12-weeks' follow-up. All tests were based on a significance level of 5% (p-value < 0.05). Data were presented as mean ± SD and as percentages (%). SPSS statistical software was used for the analyses.

3. Results

Fifty-eight women were eligible to be included in the study. Three women declined the protocol, as it was difficult for them to follow the protocol schedule and 2 hesitated to participate due to the novelty of the therapy. The protocol was possible in all eli-

Table 1
Baseline characteristics of the participants in the study.

Number (N)	53
Age	57.2 ± 5.4[a]
BMI	26.0 ± 4.8[a]
Years since last menstrual period	8.9 ± 6.6[a]
Level of Education	
University	42/53 (79)
Secondary	11/53 (21)
Smokers	
Yes	16/53 (31)
No	37/53 (70)
Number of cigarettes/day	13.9 ± 6.8[a]
Married	
Yes	45/53 (85)
No	8/53 (15)
Sexual activity	
Yes	25/53 (47)
No	28/53 (53)
Causes for no sexual activity	
Vulvovaginal symptoms	16/28 (57)
No sexual partner	7/28 (25)
Other	5/28 (18)

[a] Data are displayed as mean ± SD. All other data are displayed as percentages.

gible women despite the severity of the vaginal atrophy. Thus, fifty-three participants (mean age 57.2 ± 5.4 years) were enrolled in this study, their baseline characteristics are presented in Table 1. All participants completed the study protocol without any serious side effects. Only a temporary mild irritation of the introitus was noted that started immediately after the laser treatment, lasted up to 2 h and resolved spontaneously.

The VMV and the VHIS increased significantly after the completion of the study protocol (Table 2). At the baseline none of the participants had VMV > 49% and VHIS > 15. At the 12-weeks' follow-up 57% (30/53) and 89% (47/53) of the participants had VMV > 49% and VHIS > 15, respectively. The participants that could not overpass the thresholds of VMV > 49% and VHIS > 15, had an improvement of 10–40% and 57–180%, respectively. At the baseline VMV was correlated with the years since last period (r-0.4, p = 0.003), but at the 12-weeks' follow-up this correlation was not present.

The severity and the presence of VVA-symptoms decreased significantly, while the sexual function (as assessed by the FSFI) improved significantly (Table 3). At the baseline the presence of severe and moderate intensity of the VVA-symptom/s was 49% (26/53) and 51% (27/53), respectively. At the 12-weeks' follow-up the presence of severe, moderate, mild and zero intensity of the VVA-symptoms was 2% (1/53), 13% (7/53), 51% (27/53) and 34% (18/53), respectively. The 8 participants presenting with moderate to severe symptoms at the 12-weeks' follow-up had severe symptoms at the baseline. The improvement of symptoms in these participants varied from 20 to 60%.

All but one of the participants (15/16) who did not have sexual activity at baseline, due to GSM symptoms, resumed their sexual activity. The participant who did not resume sexual activity was due to personal reasons. The frequency of sexual intercourse/month increased significantly from a mean 1.6 ± 2.1 to 4.1 ± 2.1 ($p < 0.001$, effect size 1.5).

LUTS were improved significantly as reflected by the significant reduction of the scores of ICIQ-FLUTS, UDI-6, ICIQ-UI SF and KHQ (Table 4). At the baseline 40% (21/53), 34% (18/53), 23% (12/53) and 4% (2/53) of the participants passed urine during the day 1–6, 7–8, 9–10 and ≥13 times, respectively. At the 12-weeks' follow-up 47% (25/53), 47% (25/53), 4% (2/53) and 2% (1/53) passed urine during the day 1–6, 7–8, 9–10, and 11–12 times, respectively. The change of KHQ questionnaire was >5 points in all participants. A history of recurrent urinary tract infections was present in 4% (2/53) of the participants. However, relevant symptoms were not reported at the baseline or during the study protocol.

At the PGI-I 30% (16/53), 60% (32/53) and 9% (5/53) of the participants reported very much better, much better and a little better, respectively.

At the logistic regression analyses the results were as follows: 1) VMV and VHIS increased independently of the participants' baseline characteristics (i.e years since last period), 2) The presence or absence of VVA symptoms at the 12-weeks' follow-up was independent of the participants' baseline characteristics and 3) No predictor of the intensity of the VVA symptoms at the 12-weeks' follow-up was identified.

4. Discussion

This study assesses the efficacy of CO_2-Laser therapy in postmenopausal women with clinical signs and symptoms of GSM as a whole, focusing not only in the genital tract but also in the lower urinary tract. This efficacy was assessed in both principles of treatment (improvement of local pathophysiology and alleviation of symptoms) and it was independent to the participants' baseline characteristics (i.e years since last period). In particular, improvement following the CO_2-Laser was observed on VMV, VHIS, VVA symptoms, LUTS, sexual female function and general health perception of the women. This improvement was not only statistical significant but also clinically meaningful. This finding was ratified by the PGI-I. The majority of the participants expressed they felt "much better" or "very much better" after the end of the therapeutic protocol.

The VMI and the VMV are considered an inexpensive measurement of evaluating the hormonal status of women [11]. A negative correlation between the years since last period and VMV exists [18]. Hence, VMV is recommended in the evaluation of the VVA [11,18] and it is used often as a measurement of the local vaginal therapy [3,11,18–21]. The vaginal estrogens have been proved to induce the vaginal mucosal maturation with a significant rise of the VMV [18–21]. In our study the significant increase of the VMV by the use of CO_2-Laser indicates the estrogen-like effect of the therapy. At the baseline VMV was correlated with the years since last period indicating the hormonal status. However, at the 12-weeks' follow-up such correlation was absent, confirming further the estrogen-like effect of the CO_2-Laser on the vaginal mucosal.

Moreover, the VHIS increased significantly indicating the positive effect of the CO_2-Laser on vaginal health. This effect was in accordance to the findings of previous studies [7–10]. The improvement of the VHIS was observed as soon as 1 month after the first therapy [10]. However, the percentage of the participants that reached non-atrophic values of VHIS after the completion of the CO_2-Laser therapy, have not been previously reported. In our study, at the 12 weeks' follow-up, non-atrophic values of the VHIS were observed in 89% of the participants, independently of their baseline characteristics.

The VVA-symptoms at the end of our therapeutic protocol decreased in both prevalence and intensity. All participants experienced improvement in VVA-symptoms. Indeed, even those that reported moderate to severe intensity of VVA symptoms at the end of the protocol, showed a symptomatic improvement up to 60%. Additionally, the sexual function of the participants improved significantly as reflected by the FSFI. It is noteworthy, that all the components of the FSFI (desire, arousal, orgasm, lubrication, satisfaction and pain) changed to clinically meaningful better status as

Table 2
Changes of the primary outcomes (Vaginal Health Index (VHIS), Vaginal Maturation Value (VMV) and their components) of the study.

	Baseline (n = 53)[b]	12 weeks follow-up (n = 53)[b]	Mean of difference ± SD of difference (n = 53)[b]	p-value[b]	Effect size[b]	Power of the study[b]
VMV[a]	11.7 ± 15.6	44.2 ± 13.7	32.4 ± 15.2	<0.001	2.1	100%
Parabasal epithelial cells[a]	74.6 ± 31.6	16.2 ± 23.9	−57.6 ± 29.9	<0.001	1.9	100%
Intermediate epithelial cells[a]	24.6 ± 31.7	80.0 ± 23.4	55.6 ± 30.6	<0.001	1.8	100%
Superficial epithelial cells[a]	0 ± 0	3.3 ± 4.9	3.3 ± 4.9	<0.001	0.7	99%
VHIS[a]	8.4 ± 2.5	20.1 ± 3.0	11.6 ± 2.9	<0.001	4	100%
Elasticity	1.6 ± 0.8	4.2 ± 0.9	2.6 ± 0.9	<0.001	2.9	100%
Fluid Volume	1.0 ± 0	3.1 ± 0.9	2.1 ± 0.9	<0.001	2.3	100%
pH	2.7 ± 1.1	4.2 ± 1.7	1.5 ± 1.0	<0.001	1.5	100%
Epithelial Integrity	1.3 ± 0.9	4.1 ± 1.7	2.8 ± 1.8	<0.001	1.6	100%
Moisture	1.8 ± 1.2	3.1 ± 0.9	2.7 ± 1.2	<0.001	2.3	100%

[a] Parabasal epithelial cells, Intermediate epithelial cells and superficial epithelial cells were quantified as percentages in the vaginal smear. VMV was calculated using the formula ((1×%superficial) +(0.5×%intermediate) +(0×%parabasal)) [11]. VHIS is calculated by adding the scores of the 5 components: Elasticity, fluid volume, pH, epithelial integrity and moisture [12–14]. 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 [8,9,11].

[b] Mean ± SD values of the outcomes are presented. Wilcoxon signed rank-test for paired samples was used for the statistical analysis. Effect size was calculated based on Cohen's d, derived from the mean of difference ±SD of difference using the G-power statistical program. The thresholds for the interpretation of the effect size were 0.2, 0.5 and 0.8 for small, medium and large respectively. The power of study was calculated post-hoc for the effect size of each outcome at a level of significance 5% and the given number of participants.

Table 3
Changes of the symptoms from the Genital Tract System and the Female Sexual Function Index (FSFI) of participants in the current study.

	Baseline[a]	12 weeks follow-up[a]	Mean of difference ± SD of difference	p-value[b]	Effect size[b]	Post –hoc power of the study[b]
Dyspareunia	7.7 ± 2.5 (98)	2.3 ± 2.2 (71)	5.4 ± 2.6	<0.001(<0.001)	2.1	100%
Genital dryness	6.1 ± 3.1 (87)	1.7 ± 1.9 (53)	4.3 ± 2.9	<0.001(<0.001)	1.5	100%
Burning	1.3 ± 2.9 (22)	0.3 ± 0.9 (12)	1.1 ± 2.4	0.003 (0.04)	0.5	94%
Itching	1.7 ± 3.2 (22)	0.3 ± 1.2 (8)	1.6 ± 3.1	0.002 (0.03)	0.5	94%
Dysuria	0.9 ± 1.7 (34)	0.3 ± 0.7 (13)	0.7 ± 1.1	0.01(<0.001)	0.6	98%
Total	7.4 ± 2.1	1.8 ± 1.8	5.6 ± 1.9	<0.001	3	100%
Pain during the insertion of the speculum	4.9 ± 2.6	1.5 ± 1.9	3.4 ± 2.7	<0.001	1.3	100%
FSFI						
Desire	2.4 ± 0.9	3.8 ± 1.0	1.5 ± 1.0	<0.001	1.5	100%
Arousal	2.1 ± 1.5	3.9 ± 0.9	1.7 ± 1.4	<0.001	1.2	100%
Lubrication	2.5 ± 1.9	4.5 ± 0.9	2.0 ± 1.6	<0.001	1.3	100%
Orgasm	2.2 ± 1.8	4.4 ± 1.0	2.2 ± 1.7	<0.001	1.3	100%
Satisfaction	2.6 ± 1.3	4.6 ± 0.9	1.9 ± 1.4	<0.001	1.3	100%
Pain	1.9 ± 1.6	4.7 ± 1.0	2.8 ± 1.8	<0.001	1.6	100%
Total	13.7 ± 8.1	25.9 ± 4.6	12.1 ± 8.1	<0.001	1.5	100%
Overall satisfaction with sexual life	2.3 ± 2.8	6.5 ± 2.3	3.5 ± 3.6	<0.001	1	100%

[a] The presence of each symptom and the calculated p-value of the presence decrease is presented in the parenthesis. The mean values and the presence of all but one symptoms were calculated using the overall number of participants (n = 53). FSFI (mean values) and dyspareunia (mean values and presence) was calculated using the number of participants having sexual intercourse or willing to have sexual intercourse but vulvovaginal symptoms forbade them (n = 40). Mean ± SD values of the outcomes are presented.

[b] Wilcoxon signed rank-test for paired samples was used for the statistical analysis. Effect size was calculated based on Cohen's d, derived from the mean of difference ± SD of differences using the G-Power statistical program. The thresholds for the interpretation of the effect size were 0.2, 0.5 and 0.8 for small, medium and large respectively. The power of study was calculated post-hoc for the effect size of each outcome at a level of significance 5% and the given number of participants.

it was implied by the large effect sizes (all ≥ 1.3) of the statistically significant results. Furthermore, the significant increase in the frequency of the sexual intercourse, ratified the findings of the FSFI. The above described findings are in accordance to those of previous studies [6–9].

In addition, in our study LUTS improved significantly (in severity and prevalence), as it was shown by the ICIQ-FLUTS, ICIQ-UI SF and UDI-6 after the CO_2-Laser. Even though in UDI-6 the difficulty of emptying the bladder or the lower abdominal pain, did not reduce significantly after the CO_2-Laser, these are not considered GSM symptoms. Nevertheless, the improvement of the LUTS after the CO_2-Laser was also observed in the KHQ. The MID threshold of 5-point improvement [16], was exceeded in our study in all of its domains, indicating its important effect at the patient level.

In previous studies the efficacy on the Quality of life of the women treated with the CO_2-Laser was assessed by the SF-12, which is a generic questionnaire, not orientated in women with urinary incontinence [7,9,21]. ICIQ-FLUTS, UDI-6, ICIQ-SF and KHQ are considered to be reliable instruments for assessing the LUTS and their impact in the Quality of Life [16,23–26]. They are usually used for the evaluation of the treatment success of urinary incontinence [22,25]. The findings of the current study indicate that the CO_2-Laser has a positive effect to the lower urinary tract resulting in treatment success of LUTS.

A limitation of the current study is the lack of a control group (i.e pelvic floor physical therapy), as the objective was to evaluate changes before and after the CO_2-Laser therapy in women with GSM as a whole and not to compare its efficacy with other treatment modalities. Thus, a hypothesis of placebo effect of the CO_2-Laser therapy cannot be overruled. However, pathophysiological findings had similar improvement to the decrease of GSM symptoms, implying that probably placebo is not the mechanism of the CO_2-Laser effect. Another potential limitation is the relatively small sample size. Moreover, this study was designed to include women with ≥1 symptom of GSM with moderate/severe intensity regardless of symptoms' origin (genital or lower urinary tract). VVA-symptoms in contrast to LUTS were regarded as the most bothering symptoms by all participants. Moreover, voiding times were calculated

Table 4
Changes of the International Consultation on Incontinence Questionnaire Female Lower Urinary tract systems (ICIQ-FLUTS Filling Domain) and Urinary Incontinence Short-Form (ICIQ-UI SF), Urogenital Distress Inventory (UDI-6) and King's Health Questionnaire (KHQ).

	Baseline[a]	12 weeks follow-up[a]	Mean of difference ± SD of difference	p-value[b]	Effect size[b]	Post-hoc power of the study[b]
ICIQ-FLUTS (n = 53) (Filling Domain)	3.6 ± 2.4	1.6 ± 1.5	2.0 ± 1.6	<0.001	1.3	100%
Day frequency	0.9 ± 0.9	0.6 ± 0.7	0.4 ± 0.5	<0.001	0.8	99%
Nocturia	1.3 ± 1.0	0.6 ± 0.7	0.7 ± 0.6	<0.001	1.2	100%
Urgency	1.2 ± 1.0 (74)	0.3 ± 0.5 (28)	0.9 ± 0.8	<0.001(<0.001)	1.1	100%
Bladder pain	0.2 ± 0.6	0.08 ± 0.3	0.2 ± 0.5	0.02	0.4	80%
ICIQ-UI SF (n = 35)	8.1 ± 5.6	3.4 ± 4.3	4.7 ± 4.1	<0.001	1.1	100%
UDI-6 (n = 35)	30.2 ± 21.4 (66)	11.9 ± 16.6 (34)	18.3 ± 17.9	<0.001(<0.001)	1	100%
Frequency	1.8 ± 1.8 (38)	0.7 ± 1.3 (15)	1.1 ± 1.6	0.001 (0.008)	0.7	98%
Urgency incontinence	1.6 ± 1.7 (34)	0.6± 1.2 (17)	0.9 ± 1.4	0.001 (0.04)	0.6	92%
Stress incontinence	1.6 ± 1.5 (38)	0.5 ± 0.9 (19)	1.2 ± 1.5	0.001 (0.03)	0.8	99%
Drops	1.8 ± 1.7 (38)	0.5± 1.1 (15)	1.3 ± 1.4	<0.001 (0.008)	0.9	99%
Difficulty emptying bladder	0.2 ± 0.8 (6)	0.2 ± 0.9 (6)	0.0 ± 0.6	1 (1)		
Pain discomfort from the lower abdomen or genital region	0.5 ± 0.9 (13)	0.2 ± 0.6 (4)	0.3 ± 0.9	p = 0.1 (p = 0.08)		
KHQ (n = 35)	235.9 ± 226.1	114.1 ± 165.8	121.8 ± 114.9	<0.001	1.1	100%
General Health Perception	35.3 ± 20.6	20.7 ± 14.2	14.6 ± 16.6	<0.001	0.9	99%
Incontinence impact	38.1 ± 37.2	17.6 ± 26.5	20.8 ± 28.0	0.001	0.7	98%
Role limitations	29.5 ± 35.5	12.4 ± 24.4	17.1 ± 23.4	0.001	0.7	98%
Physical limitations	28.1 ± 33.8	11.6 ± 21.7	16.5 ± 22.3	0.001	0.7	98%
Social limitations	17.5 ± 29.4	10.8 ± 23.6	6.7 ± 12.3	0.007	0.5	80%
Personal relationships	18.9 ± 33.3	6.1 ± 20.7	12.8 ± 24.2	0.01	0.5	80%
Emotions	28.9 ± 38.9	10.8 ± 24.9	18.5 ± 28.2	0.001	0.7	98%
Sleep/Energy	20.5 ± 28.9	12.9 ± 23.9	7.9 ± 14.5	0.002	0.5	80%
Severity measures	28.8 ± 29.8	12.8 ± 22.6	15.9 ± 18.7	<0.001	0.9	99%

[a] Mean ± SD values of the outcomes are presented. The presence and the calculated p-values of the presence decrease is presented in the parenthesis. The presence of each LUTS was calculated using the number of the included participants (n = 53).

[b] Wilcoxon signed rank-test for paired samples was used for the statistical analysis. Effect size was calculated based on Cohen's d, derived from the mean of difference ± SD of difference, using the G-Power statistical program. The thresholds for the interpretation of the effect size were 0.2, 0.5 and 0.8 for small, medium and large respectively. The power of study was calculated post-hoc for the effect size of each outcome at a level of significance 5% and the given number of participants.

using the ICIQ-FLUTS and not voiding dairies. In addition, this study was not designed to include women with a history of recurrent UTIs and/or vaginitis, and we cannot derive conclusions of the CO_2-Laser effect in this group of women. Furthermore, there is a relevant short follow-up period and it remains to be seen for how long these results would last.

Despite the above potential limitations, this study has several strengths. This is a prospective study, with a well-defined group of participants. The assessors of all samples, parameters and data were blinded to all details related to the participants, although a possible breech in the blindness of the evaluators cannot be excluded. The assessment of the CO_2-Laser effect was performed with the intention to investigate both principles of the treatment. Furthermore, confirmation of the results from the previously published studies on the CO_2-Laser and its effect on the VVA-symptoms, FSFI and VHIS, is provided. Additional strengths of this study are: the performance of a power calculation (a priori and post-hoc), the estimation of the effect size of the results and the assessment of baseline characteristics as potential predictors of the women who could benefit the best from the CO_2-Laser therapy.

5. Conclusions

The CO_2-Laser therapy could be a valid non-hormonal therapeutic option for the clinical signs and symptoms of the GSM. This finding is of great importance particularly for women that are not allowed to use vaginal estrogens due to a history of estrogen-sensitive cancer or not willing to use them due to personal perceptions. The results of our study indicates that CO_2-Laser may be effective in decreasing both the VVA symptoms and LUTS and improving the VMV, VHIS, sexual function and general health perception of the women with clinical signs and symptoms of GSM. The indication of restoration of the local pathophysiology and the alleviation of GSM symptoms by the use of CO_2-Laser, decreases the possibility of a placebo effect of the therapy. However, larger studies with a control arm are needed, for our findings to be safely generalized, as well as studies for the cost-effectiveness of the treatment.

Contributors

E.P. was responsible for data extraction and analysis, and drafted the manuscript.

T.G. evaluated the participants' clinical signs and drafted the manuscript.

A.T. was responsible for cytological evaluation.

D.Z. was responsible for the random allocation of participants and data collection and entry.

S.A. conceived the idea of the study, acted as project manager, evaluated the participants' clinical signs, and drafted the manuscript.

All authors contributed to the interpretation of the data or the work, were involved in drafting the work or revising it critically for important intellectual content, and read and approved the final version of the manuscript.

Conflict of interest

S.S. has had financial relations (expert testimonies and lectures) with DEKA Laser.

All other authors declare they have no conflicts of interest.

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Genitourinary syndrome of menopause: Current and emerging therapies

With more than 1 billion menopausal women likely to be affected by vulvovaginal atrophy worldwide by 2025, the need for effective remedies is acute. Here, 3 experts survey the options.

Mickey Karram, MD; Eric R. Sokol, MD; and Stefano Salvatore, MD

Genitourinary syndrome of menopause (GSM) is the new terminology to describe symptoms occurring secondary to vulvovaginal atrophy.[1] The recent change in terminology originated with a consensus panel comprising the board of directors of the International Society for the Study of Women's Sexual Health (ISSWSH) and the board of trustees of the North American Menopause Society (NAMS). At a terminology consensus conference in May 2013, these groups determined that the term GSM is medically more accurate and all encompassing than vulvovaginal atrophy. It is also more publicly acceptable.

The symptoms of GSM derive from the hypoestrogenic state most commonly associated with menopause and its effects on the genitourinary tract.[2] Vaginal symptoms associated with GSM include vaginal or vulvar dryness, discharge, itching, and dyspareunia.[3] Histologically, a loss of superficial epithelial cells in the genitourinary tract leads to thinning of the tissue. There is then a loss of vaginal rugae and elasticity, leading to narrowing and shortening of the vagina.

In addition, the vaginal epithelium becomes much more fragile, which can lead to tears, bleeding, and fissures. There is also a loss of the subcutaneous fat of the labia majora, a change that can result in narrowing of the introitus, fusion of the labia majora, and shrinkage of the clitoral prepuce and urethra. The vaginal pH level becomes more alkaline, which may alter vaginal flora and increase the risk of urogenital infections—specifically, urinary tract infection (UTI). Vaginal secretions, largely transudate, from the vaginal vasculature also decrease over time. These changes lead to significant dyspareunia and impairment of sexual function.

In this article, we survey the therapies available for GSM, focusing first on proven treatments such as local estrogen administration and use of ospemifene (Osphena), and then describing an emerging treatment involving the use of fractional CO_2 laser.

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Dr. Karram reports being a speaker and consultant for Cynosure. Dr. Salvatore reports being a speaker and consultant for DEKA Medical. Dr. Sokol reports no financial relationships relevant to this article.

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In a recent international survey, 77% of respondents believed that women were uncomfortable discussing vaginal atrophy

How prevalent is GSM?

Approximately half of all postmenopausal women in the United States report atrophy-related symptoms and a significant negative effect on quality of life.[4-6] Few women with these symptoms seek medical attention.

The Vaginal Health: Insights, Views, and Attitudes (VIVA) survey found that 80% of women with genital atrophy considered its impact on their lives to be negative, 75% reported negative consequences in their sexual life, 68% reported that it made them feel less sexual, 33% reported negative effects on their marriage or relationship, and 26% reported a negative impact on their self-esteem.[7]

Another review of the impact of this condition by Nappi and Palacios estimated that, by the year 2025, there will be 1.1 billion women worldwide older than age 50 with specific needs related to GSM.[8] Nappi and Palacios cite 4 recent surveys that suggest that health care providers need to be more proactive in helping patients disclose their symptoms. The same can be said of other symptoms of the urinary tract, such as urinary frequency, urgency, and incontinence, as well as pelvic floor relaxation.

A recently published international survey on vaginal atrophy not only depicts the extremely high prevalence of the condition but also describes fairly significant differences in attitudes toward symptoms between countries in Europe and North America.[9] Overall, 77% of respondents, who included more than 4,000 menopausal women, believed that women were uncomfortable discussing symptoms of vaginal atrophy.[9]

Pastore and colleagues, using data from the Women's Health Initiative (WHI), found the most prevalent urogenital symptoms to be vaginal dryness (27%), vaginal irritation or itching (18.6%), vaginal discharge (11.1%), and dysuria (5.2%).[4] Unlike vasomotor symptoms of menopause, which tend to decrease over time, GSM does not spontaneously remit and commonly recurs when hormone therapy—the dominant treatment—is withdrawn.

What can we offer our patients?

Vaginal estrogen

The most common therapy used to manage GSM is estrogen. Most recommendations state that if the primary menopausal symptoms are related to vaginal atrophy, then local estrogen administration should be the primary mode of therapy. The Society of Gynecologic Surgeons Systematic Review Group recently concluded that all commercially available vaginal estrogens effectively can relieve common vulvovaginal atrophy–related symptoms and have additional utility in women with urinary urgency, frequency, stress incontinence, urge incontinence, and recurrent UTIs.[10] Although their meta-analysis clearly demonstrated that estrogen therapy improves the symptoms of GSM, investigators acknowledged that a clearer understanding is needed of the exact risk to the endometrium with sustained use of vaginal estrogen, as well as a more precise assessment of changes in serum estradiol levels.[10]

A recent Cochrane review concluded that all forms of local estrogen appear to be equally effective for symptoms of vaginal atrophy.[11] One trial cited in the review found significant adverse effects following administration of cream, compared with tablets, causing uterine bleeding, breast pain, and perineal pain.[11]

Another trial cited in the Cochrane review found significant endometrial overstimulation following use of cream, compared with the vaginal ring. As a treatment of choice, women appeared to favor the estradiol-releasing vaginal ring for ease of use, comfort of product, and overall satisfaction.[11]

After the release of the WHI data, the US Food and Drug Administration (FDA) released a "black box" warning on postmenopausal hormone use in women, which has significantly reduced the use of both local and systemic estrogen in eligible women. NAMS has recommended that the FDA revisit this warning, calling specifically for an independent commission to scrutinize every major WHI paper to determine whether the data justify the conclusions drawn.[12]

Ospemifene

This estrogen agonist and antagonist selectively stimulates or inhibits estrogen receptors of different target tissues, making it a selective estrogen receptor modulator (SERM). In a study involving 826 postmenopausal women randomly allocated to 30 mg or 60 mg of ospemifene, the 60-mg dose proved to be more effective for improving vulvovaginal atrophy.[13] Long-term safety studies revealed that ospemifene 60 mg given daily for 52 weeks was well tolerated and not associated with any endometrial- or breast-related safety issues.[13,14] Common adverse effects of ospemifene reported during clinical trials included hot flashes, vaginal discharge, muscle spasms, general discharge, and excessive sweating.[12]

Vaginal lubricants and moisturizers

Nonestrogen water- or silicone-based vaginal lubricants and moisturizers may alleviate vaginal symptoms related to menopause. These products may be particularly helpful for women who do not wish to use hormone therapies.

Vaginal lubricants are intended to relieve friction and dyspareunia related to vaginal dryness during intercourse, with the ultimate goal of trapping moisture and providing long-term relief of vaginal dryness.

Although data are limited on the efficacy of these products, prospective studies have demonstrated that vaginal moisturizers improve vaginal dryness, pH balance, and elasticity and reduce vaginal itching, irritation, and dyspareunia.

Data are insufficient to support the use of herbal remedies or soy products for the treatment of vaginal symptoms.

An emerging therapy: fractional CO_2 laser

In September 2014, the FDA cleared for use the SmartXide^2 CO_2 laser system (DEKA Medical) for "incision, excision, vaporization and coagulation of body soft tissues" in medical specialties that include gynecology and genitourinary surgery.[15] The system, also marketed by Cynosure as the MonaLisa Touch treatment, was not approved specifically for treatment of GSM—and it is important to note that the path to device clearance by the FDA is much less cumbersome than the route to drug approval. As NAMS notes in an article about the fractional CO_2 laser, "Device clearance does not require the large, double-blind, randomized, placebo-controlled trials with established efficacy and safety endpoints required for the approval of new drugs."[16] Nevertheless, this laser system appears to be poised to become a new treatment for the symptoms of GSM.

This laser supplies energy with a specific pulse to the vaginal wall to rapidly and superficially ablate the epithelial component of atrophic mucosa, which is characterized by low water content. Ablation is followed by tissue coagulation, stimulated by laser energy penetrating into deeper tissues, triggering the synthesis of new collagen and other components of the ground substance of the matrix.

The supraphysiologic level of heat

Most data back local estrogen as treatment for GSM

In 2013, the North American Menopause Society (NAMS) issued a position statement noting that the choice of therapy for genitourinary syndrome of menopause (GSM) depends on the severity of symptoms, the efficacy and safety of therapy for the individual patient, and patient preference.[1]

To date, estrogen therapy is the most effective treatment for moderate to severe GSM, although a direct comparison of estrogen and ospemifene is lacking. Nonhormonal therapies available without a prescription provide sufficient relief for most women with mild symptoms. When low-dose estrogen is administered locally, a progestin is not indicated for women without a uterus—and generally is not indicated for women with an intact uterus. However, endometrial safety has not been studied in clinical trials beyond 1 year. Data are insufficient to confirm the safety of local estrogen in women with breast cancer.

Future research on the use of the fractional CO_2 laser, which seems to be a promising emerging therapy, may provide clinicians with another option to treat the common and distressing problem of GSM.

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Fractional CO_2 laser: A study in progress

Two authors (Mickey Karram, MD, and Eric Sokol, MD) are performing a study of the fractional CO_2 laser for treatment of genitourinary syndrome of menopause (GSM) in the United States. To date, 30 women with GSM have been treated with 3 cycles and followed for 3 months. Preliminary data show significant improvement in all symptoms, with all patients treated in an office setting with no pretreatment or posttreatment analgesia required.

The laser settings for treatment included a power of 30 W, a dwell time of 1,000 µs, spacing between 2 adjacent treated spots of 1,000 µs, and a stack parameter for pulses from 1 to 3.

Laser energy is delivered through a specially designed scanner and a vaginal probe. The probe is slowly inserted to the top of the vaginal canal and then gradually withdrawn, treating the vaginal epithelium at increments of almost 1 cm (**FIGURE 3**, page e6). The laser beam projects onto a 45° mirror placed at the tip of the probe, which reflects it at 90°, thereby ensuring that only the vaginal wall is treated, and not the uterine cervix.

A treatment cycle included 3 laser treatments at 6-week intervals. Each treatment lasted 3 to 5 minutes. Initial improvement was noted in most patients, including increased lubrication within 1 week after the first treatment, with further improvement after each session. To date, the positive results have persisted, and all women in the trial now have been followed for 3 months—all have noted improvement in symptoms. They will continue periodic assessment, with a final subjective and objective evaluation 1 year after their first treatment.

generated by the CO_2 laser induces a rapid and transient heat-shock response that temporarily alters cellular metabolism and activates a small family of proteins referred to as the "heat shock proteins" (HSPs). HSP 70, which is overexpressed following laser treatment, stimulates transforming growth factor-beta, triggering an inflammatory response that stimulates fibroblasts, which produce new collagen and extracellular matrix.

The laser has emissions characteristics aligned for the transfer of the energy load to the mucosa while avoiding excessive localized damage. This aspect of its design allows for restoration of the permeability of the connective tissue, enabling the physiologic transfer of various nutrients from capillaries to tissues. When there is a loss of estrogen, as during menopause, vaginal atrophy develops, with the epithelium deteriorating and thinning. The fractional CO_2 laser therapy improves the state of the epithelium by restoring epithelial cell trophism.

The vaginal dryness that occurs with atrophy is due to poor blood flow, as well as reduced activity of the fibroblasts in the deeper tissue. The increased lubrication that occurs after treatment is usually a vaginal transudate from blood outflow through the capillaries that supply blood to the vaginal epithelium. The high presence of water molecules increases permeability, allowing easier transport of metabolites and nutrients from capillaries to tissue, as well as the drainage of waste products from tissues to blood and lymph vessels.

With atrophy, the glycogen in the epithelial cells decreases. Because lactobacilli need glycogen to thrive and are responsible for maintaining the acidity of the vagina, the pH level increases. With the restoration of trophism, glycogen levels increase, furthering colonization of vaginal lactobacilli as well as vaginal acidity, reducing the pH level. This effect also may protect against the development of recurrent UTIs.

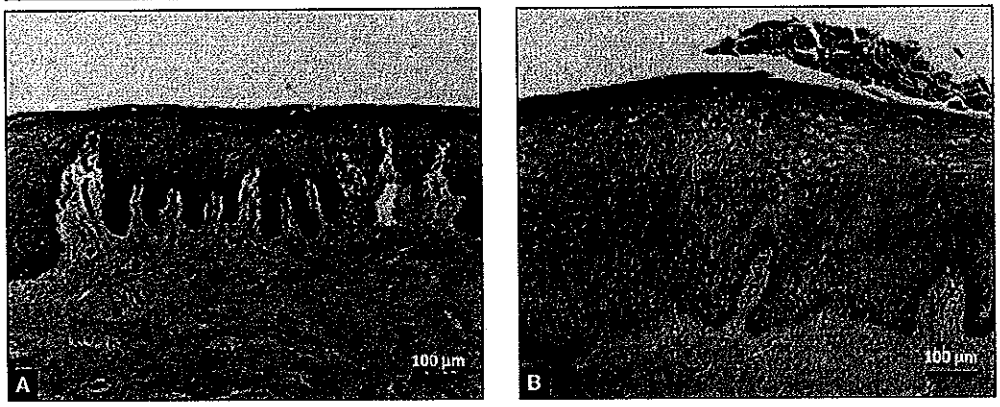
A look at the data

To date, more than 2,000 women in Italy and more than 10,000 women worldwide with GSM have been treated with fractional CO_2 laser therapy, and several peer-reviewed publications have documented its efficacy and safety.[17-21]

In published studies, however, the populations have been small and the investigations have been mostly short term (12 weeks).[17-21]

A pilot study reported that a treatment cycle of 3 laser applications significantly improved the most bothersome symptoms of vulvovaginal atrophy and improved scores of vaginal health at 12 weeks' follow-up in 50 women who had not responded to or were unsatisfied with local estrogen therapy.[17] This investigation was followed by 2 additional studies involving another 92 women that specifically addressed the impact of fractional CO_2 laser therapy on dyspareunia and female sexual function.[19,20] Both studies showed statistically significant improvement in dyspareunia as well as Female Sexual Function Index (FSFI) scores. All women in these studies were treated in an office setting

FIGURE 1 Early-stage vaginal atrophy



This histologic preparation of vaginal mucosa sections reveals untreated early-stage vaginal atrophy (A), with thinning epithelium and the presence of papillae, and the same mucosa 1 month after treatment with fractional CO_2 laser therapy (B). *Reprinted with permission from DEKA M.E.L.A. Srl (Calenzano, Italy) and Professor A. Calligaro, University of Pavia, Italy.*

with no pretreatment anesthesia. No adverse events were reported.

Recently published histology data highlight significant changes 1 month after fractional CO_2 laser treatment that included a much thicker epithelium with wide columns of large epithelial cells rich in glycogen.[21] Also noted was a significant reorganization of connective tissue, both in the lamina propria and the core of the papillae (**FIGURES 1 AND 2**).

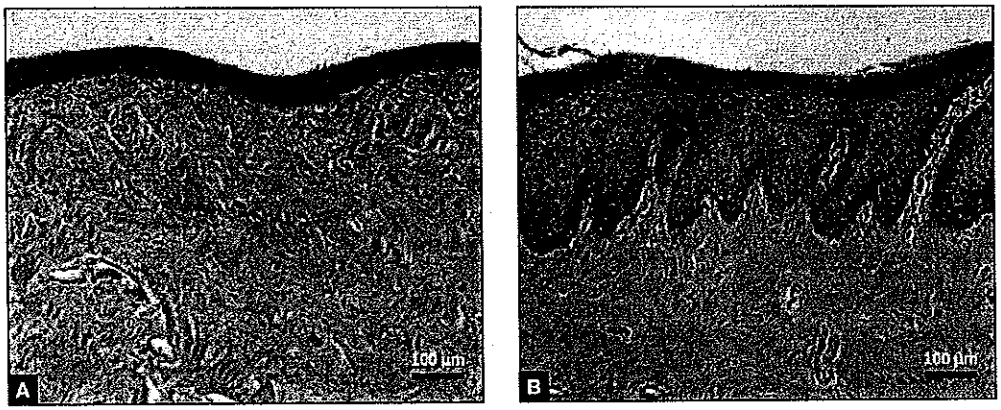
Caveats

No International Classification of Diseases (ICD) 9 or 10 code has been assigned to the procedure to date, and the cost to the patient ranges from $600 to $1,000 per procedure.[16]

NAMS position. A review of the technology by NAMS noted the need for large, long-term, randomized, sham-controlled studies "to further evaluate the safety and efficacy of this procedure."[16]

NAMS also notes that "lasers have become a very costly option for the treatment of symptomatic [GSM], without a single trial comparing active laser treatment to sham laser treatment and no information

FIGURE 2 Atrophic vaginitis



This histologic preparation of vaginal mucosa sections shows untreated atrophic vaginitis (A) and the same mucosa 1 month after treatment with fractional CO_2 laser therapy (B). *Reprinted with permission from DEKA M.E.L.A. Srl (Calenzano, Italy) and Professor A. Calligaro, University of Pavia, Italy.*

FAST TRACK

No ICD 9 or 10 code has yet been assigned to fractional CO_2 laser therapy

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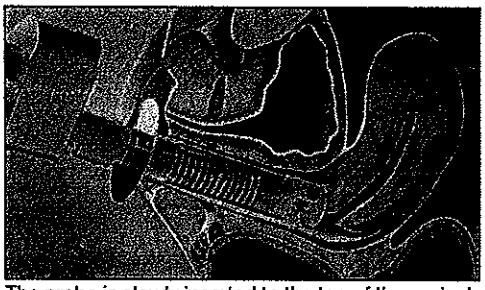
FIGURE 3 Fractional CO_2 laser treatment



The probe is slowly inserted to the top of the vaginal canal and then gradually withdrawn, treating the vaginal epithelium at increments of almost 1 cm.

on long-term safety. In all published trials to date, only several hundred women have been studied and most studies are only 12 weeks in duration."[16]

Not a new concept. The concept of treating skin with a microablative CO_2 laser is not new. This laser has been safely used on the skin of the face, neck, and chest to produce new collagen and elastin fibers with remodeling of tissue.[22,23]

Preliminary data on the use of a fractionated CO_2 microablative laser to treat symptoms associated with GSM suggest that the therapy is feasible, effective, and safe in the short term. If these findings are confirmed by larger, longer-term, well-controlled studies, this laser will be an additional safe and effective treatment for this very common and distressing disorder.

FAST TRACK

The use of local estrogen for genitourinary syndrome of menopause is backed by a large body of reliable data

Bottom line

Although preliminary studies of the fractional CO_2 laser as a treatment for GSM are promising, local estrogen is backed by a large body of reliable data. Ospemifene also has FDA approval for treatment of this disorder.

For women who cannot or will not use a hormone-based therapy, vaginal lubricants and moisturizer may offer at least some relief.

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Sexual function after fractional microablative CO_2 laser in women with vulvovaginal atrophy

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ABSTRACT

Objective To investigate the effects of fractional microablative CO_2 laser on sexual function and overall satisfaction with sexual life in postmenopausal women with vulvovaginal atrophy (VVA).

Method This prospective study included 77 postmenopausal women (mean age 60.6 ± 6.2 years) treated for VVA symptoms with the fractional microablative CO_2 laser system (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy). Sexual function and quality of life were evaluated with the Female Sexual Function Index (FSFI) and the Short Form 12 (SF-12), respectively, both at baseline and at 12-week follow-up. A 10-mm visual analog scale was used to measure the overall satisfaction with sexual life and the intensity of VVA symptoms (vaginal burning, vaginal itching, vaginal dryness, dyspareunia and dysuria) before and after the study period.

Results We observed a significant improvement in the total score and the scores in each specific domain of the FSFI at 12-week follow-up compared to baseline ($p < 0.001$). After concluding the laser treatment, the overall satisfaction with sexual life significantly improved ($p < 0.001$). Seventeen (85%) out of 20 (26%) women, not sexually active because of VVA severity at baseline, regained a normal sexual life at the 12-week follow-up. Finally, we also found a significant improvement in each VVA symptom ($p < 0.001$) and in quality-of-life evaluation, both for the scores in the physical ($p = 0.013$) and mental ($p = 0.002$) domains.

Conclusions Fractional microablative CO_2 laser treatment is associated with a significant improvement of sexual function and satisfaction with sexual life in postmenopausal women with VVA symptoms.

INTRODUCTION

Vulvovaginal atrophy (VVA) defines a progressive age- and estrogen-dependent condition that may lead to the occurrence of symptoms, such as dryness, burning, itching, irritation, discharge and dysuria[1,2]. VVA symptoms can affect up to 50% of postmenopausal women[3–8] with a significant impact on quality of life and sexual function[9,10]. The drop of estrogen after menopause determines histological involution both in the vulva and in the vagina[11,12], such as thinning, reduced vascularization and elasticity, decreased engorgement and lubrication. All these changes are likely to produce an altered response to sexual stimuli and to dyspareunia[13]. VVA is therefore generally associated with female sexual dysfunction (FSD)[14]; pain during sexual intercourse, in fact, often co-exists with a decline in women's desire, arousal, orgasm and frequency of sexual activity throughout the menopausal transition and beyond[15–17].

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The management of urogynecological and sexual health should be individualized according to the VVA-associated conditions[18]; many hormonal and non-hormonal strategies have been proposed to alleviate VVA symptoms and to restore urogenital physiology[12,19–21]. A variety of treatment strategies for FSD are available[22]; non-pharmacological approaches would be welcome especially in those postmenopausal women with contraindications to hormones[23].

Fractional microablative CO_2 laser is safe in many body regions, such as the skin of the face, neck and chest, and has the ability to produce new collagen and elastic fibers with remodelling tissue properties[24–26]. Recently, a pilot study demonstrated that the use of fractional microablative CO_2 laser in the treatment of VVA was feasible, efficacious and safe at 12-week follow-up[27]. Its positive effects on VVA symptoms and quality of life[27] could improve not only the aspect related to sexual pain (dyspareunia, secondary vaginismus, and non-coital pain) but also other dimensions of women's sexual response, such as desire, initiative and receptivity to their sexual partner.

On this basis, we have carried out the present study aiming to investigate sexual function and overall satisfaction with sexual life in postmenopausal women treated for VVA symptoms by using the fractional microablative CO_2 laser.

METHODS

Study design

This prospective study was conducted between January 2013 and March 2014 at the Department of Obstetrics and Gynecology of the IRCCS San Raffaele Hospital and Vita-Salute San Raffaele University of Milan (a University teaching hospital and tertiary referral center in Northern Italy). It included postmenopausal women referred to our menopause clinic because of symptoms related to VVA. The study protocol was approved by the Hospital Research Review Committee and it represented an extension of an already published pilot study demonstrating the efficacy and feasibility of fractional CO_2 laser in the treatment of VVA[27]. Women entered the study after an informed written consent was obtained.

Study population

Out of 84 patients selected to participate in the present study, 77 women (91.6%) were recruited. Each woman completed the 12-week follow-up and was included in the final analyses. For an intention-to-treat analysis, women unable to tolerate the insertion of the probe due to the severity of VVA and/or vaginal stenosis were considered to have the worst possible results. Inclusion criteria for referral were: symptoms of VVA (vaginal dryness and/or dyspareunia rated as moderate/severe most bothersome symptoms[28]); age >50 years; absence of menstruation for ≥ 12 months; not responding/being unsatisfied with previous local estrogen therapies; wishing to maintain an active sexual life. Exclusion criteria were: use of any hormone replacement therapies (either systemic or local) within the 6 months prior to inclusion in the study; use of vaginal moisturizers, lubricants or any other local preparation within the 30 days prior to inclusion in the study; acute or recurrent urinary tract infections; active genital infections (e.g. herpes genitalis, candida); prolapse staged $\geq$ II according to the pelvic organ prolapse quantification system[29]; previous reconstructive pelvic surgery; any serious disease or chronic condition that could interfere with study compliance; psychiatric disorders precluding informed consent.

Study protocol

Postmenopausal women were treated intravaginally with the fractional microablative CO_2 laser system (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy), using the following setting: dot power 30 watt, dwell time 1000 μs, dot spacing 1000 μm and the smart stack parameter from 1 to 3. The laser beam was provided using a vaginal probe gently inserted up to the top of the vaginal canal and subsequently withdrawn and rotated in order to provide a complete treatment of the vaginal wall. At the level of the vaginal introitus, we decreased the dot power to 20 watt[27]. A treatment cycle included three laser applications (every 4 weeks). The procedure was performed in the outpatient clinic and did not require any specific preparation (e.g. analgesia/anesthesia). Patients were recommended to avoid coital sexual activity for at least 3 days after each laser application because a mild inflammatory reaction may last up to 48 h.

The primary outcome of the study was to investigate the changes in sexual function of women treated for symptoms related to VVA with fractional CO_2 laser. Secondary outcomes were: (1) changes in overall satisfaction with sexual life; (2) effects of the laser treatment on VVA symptoms and quality of life.

Data collection

Sociodemographic characteristics of the study sample were collected at baseline and inclusion/exclusion criteria were verified before starting the first laser application. The intensity of VVA symptoms (vaginal dryness, vaginal burning, vaginal itching, dyspareunia and dysuria) was measured using a 10-cm visual analog 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 in VVA[30,31]. The intensity of VVA symptoms was evaluated at baseline, before starting the first laser application, and at 12-week follow-up, after 4 weeks from the last laser application. At the same time points, postmenopausal women filled in:

(1) The Italian version of the Female Sexual Function Index (FSFI), a 19-item questionnaire developed as a brief, multidimensional, self-reported instrument for assessing the key dimensions of sexual function in women[32]. The questionnaire has been already used in the Italian gynecological

population[33,34] and in randomized clinical trials to investigate sexual function[35]. Indeed, it allows to obtain individual domain scores on a five-point scale (desire, arousal, lubrication, orgasm, satisfaction and pain) and total scale score (ranging from 2 to 36);

(2) A 10-cm VAS to measure the overall satisfaction with sexual life[36], where the left extreme of the scale indicated 'the worst level of satisfaction' and the right indicated 'the best level of satisfaction';

(3) The Italian version of the Short Form 12 (SF-12) to assess physical (PCS12) and mental (MCS12) component summary scores of quality of life[37], as previously reported[38].

Questionnaires were anonymous and a dedicated specialist nurse, who was not involved in the clinical evaluation and was unaware of the clinical data, collected each questionnaire after completion and labelled it by using a random computer-generated enrolment number. An Excel database was created containing the name of the patient, the enrolment number attributed to that particular case and the results of the questionnaires. The principal investigator created another Excel database (clinical database) containing the name of the patient and the clinical information but not the enrolment number. At the end of the study, the clinical database was given to the nurse and she created a third Excel database (final database) containing the enrolment number, the clinical information and the results of the questionnaires for each patient but not their names. All the analyses were performed by the principal investigator using the final database.

Statistical analysis

The sample size was defined in an arbitrary fashion on the basis of the novelty of the study and the lack of available literature on the role of the fractional microablative CO_2 laser in the treatment of VVA on sexual function.

The normal distribution of continuous variable data was evaluated with the Kolmogorov–Smirnov test. Categorical variables were analyzed using the McNemar test. Continuous variables, before and after treatment, were analyzed by using the paired *t*-test and the Wilcoxon Rank Sum Test accordingly to data distribution. The Spearman's rank correlation coefficient was used to assess whether PCS12 and MCS12 scores were correlated with FSFI total score and overall satisfaction with sexual life, either at baseline or at 12-week follow-up. Data were presented as mean ± standard deviation. Data were analyzed using the SPSS software version 21.0 (SPSS Science, Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

The main characteristics of our study population are described in Table 1. Seventy-five patients (97.4%) enrolled in the study and completed the treatment with fractional CO_2 laser and returned at the 12-week follow-up. Two patients (2.6%; 95% confidence interval (CI) 0–6.2%) could not be treated because their vagina was too narrow and not compliant with the vaginal probe.

Table 1 Baseline characteristics of the study population ($n = 77$). Data are given as mean ± standard deviation, *n* (%) or median (range)

Age (years)	60.6 ± 6.2
Body mass index (kg/m^2)	23.5 ± 2.0
Smokers	24 (31.2%)
Level of education	
primary	5 (6.5%)
secondary	45 (58.4%)
university	27 (35.1%)
Patients with previous live births	69 (89.6%)
Parity	2 (1–5)
Sexually active women	57 (74.0%)
Previous systemic HRT	29 (37.6%)
Duration of previous HRT (months)	28 (2–60)

HRT, hormone replacement therapy

Changes in sexual function and overall satisfaction with sexual life

At baseline, 57 women (74.0%; 95% CI 64.2–83.8%) were sexually active, while 20 (26.0%; 95% CI 16.2–35.8%) reported not having coital sexual activity because of the severity of symptoms related to VVA. Already after one cycle of laser treatment, 17 of these patients (85.0%; 95% CI 69.4–100%) resumed coital sexual activity, whereas three women (15.0%; 95% CI 0–30.7%) remained inactive at the 12-week follow-up due to the persistence of VVA symptoms. Therefore, at the end of the study period, a total of 74 women (96.1%; 95% CI 91.8–100.0%; $p < 0.001$) were sexually active. Figure 1 reports the FSFI total and domain scores at baseline and after the study period. A significant improvement ($p < 0.001$) of FSFI total score was observed among sexually active women at the 12-week follow-up (27.2 ± 5.6; 95% CI 25.8–28.7) in respect to baseline (14.8 ± 7.7; 95% CI 12.8–16.8). Similarly, each individual FSFI domain score was ameliorated after fractional CO_2 laser application ($p < 0.001$, all) (Figure 1). When compared with baseline (4.3 ± 1.3), the overall satisfaction with sexual life was significantly higher at the 12-week follow-up (7.7 ± 1.6; $p < 0.001$) in sexually active women (Figure 2).

Changes in VVA symptoms and SF-12

Table 2 reports the presence and severity of VVA symptoms at baseline and after the study period. Before starting the laser treatment, vaginal dryness was reported by 69 women (89.6%; 95% CI 82.8–96.4%), vaginal burning by 66 (85.7%; 95%

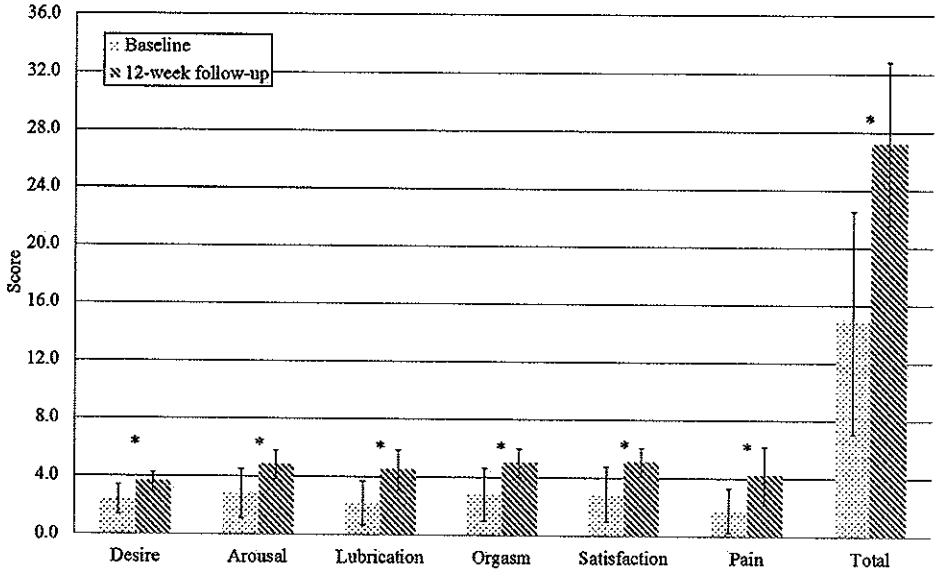

Figure 1 Female Sexual Function Index scores at baseline and at 12-week follow-up

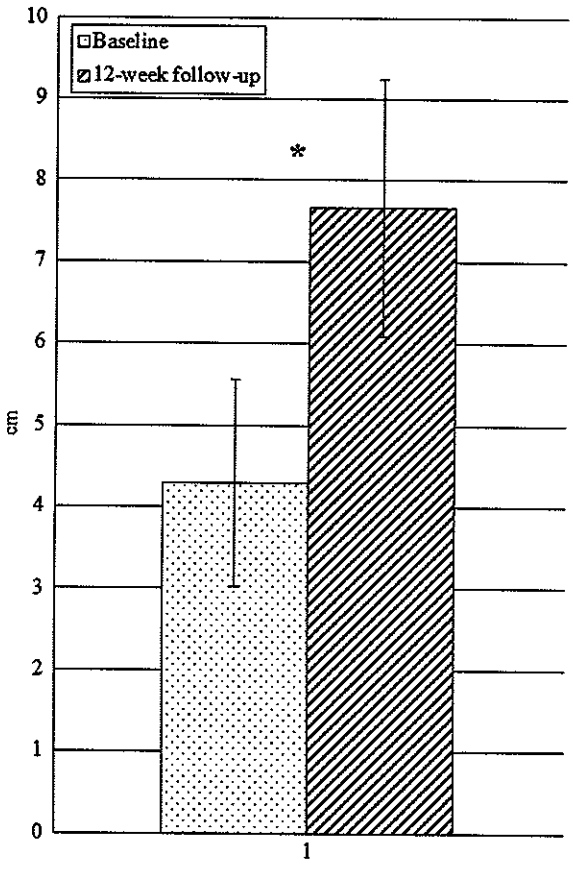

Figure 2 Overall satisfaction with sexual life (expressed as cm) at baseline and at 12-week follow-up

CI 77.9–93.5%), vaginal itching by 64 (83.1%; 95% CI 74.7–91.5%) and dysuria by 60 (77.9%; 95% CI 68.6–87.2%). Dyspareunia was experienced by 52 (91.2%; 95% CI 84.9–97.5%) women who were sexually active. At the 12-week follow-up, fractional CO_2 laser treatment was confirmed to be associated with a significant improvement in each VVA symptom ($p < 0.001$, all) (Table 2).

As far as quality of life measured by SF-12 was concerned, the PCS12 significantly improved ($p = 0.013$) at the 12-week follow-up (50.7 ± 6.5; 95% CI 49.3–52.2) in comparison with baseline (48.8 ± 6.4; 95% CI 47.4–50.2). The same significant improvement was observed for the MCS12 (43.2 ± 8.3, 95% CI 41.4–45.1 at baseline vs. 46.1 ± 7.6, 95% CI 44.4–47.8 at 12-week follow-up; $p = 0.002$). However, no significant correlation was evident between physical and mental components of quality of life and sexual function and overall satisfaction with sexual life, either at baseline or at the 12-week follow-up ($p > 0.05$, all).

DISCUSSION

This prospective study demonstrated that fractional microablative CO_2 laser is associated with a significant improvement in sexual function and overall satisfaction with sexual life in postmenopausal women complaining of VVA symptoms. After one cycle of laser treatment, 85% of sexually inactive women, presumably as a consequence of VVA, resumed an active sexual life. Such a positive effect was likely related to both restoration of genital tissues and alleviation of urogenital symptoms. Indeed, our current data confirmed the efficacy of fractional microablative CO_2 laser treatment in improving

Table 2 Presence and severity of vulvovaginal atrophy symptoms in our study sample. Data are given as mean ± standard deviation (95% confidence interval)

	Number of women (%)	*Baseline*	*12-week follow-up*
Vaginal dryness† (cm)	69 (89.6%)	8.4 ± 2.0 (7.9–8.9)	2.8 ± 1.8* (2.4–3.2)
Vaginal burning† (cm)	66 (85.7%)	6.2 ± 2.7 (5.6–6.9)	2.2 ± 2.8* (1.5–2.9)
Vaginal itching† (cm)	64 (83.1%)	6.4 ± 2.7 (5.7–7.1)	2.1 ± 2.0* (1.6–2.6)
Dyspareunia† (cm)	52 (91.2%)‡	8.4 ± 2.4 (7.8–9.1)	2.8 ± 2.1* (2.2–3.4)
Dysuria† (cm)	60 (77.9%)	5.7 ± 2.8 (5.0–6.4)	2.6 ± 1.9* (2.1–3.1)

*, Statistically significant difference in comparison with baseline ($p < 0.001$ for each); †, measured on a 10-cm VAS scale (range: 0–10); ‡, calculated out of the 57 women who were sexually active at baseline

VVA symptoms and quality of life at the 12-week follow-up; these results are consistent with the ones of our previous pilot study showing also a significant improvement in the vaginal health index[27].

A strength of our study was the evaluation of women's sexual function by using a standardized specific questionnaire[32–34] to measure, in a statistically significant manner, multiple dimensions of their sexual response; we also added a subjective evaluation based on patients' perception of satisfaction with sexual experience and of VVA symptoms[27,36]. Moreover, the high rate of women becoming sexually active and the significant reduction in dyspareunia were further elements supporting fractional microablative CO_2 laser as an effective treatment for postmenopausal women complaining of VVA symptoms and concomitant FSD. The most important limitation of our study is the absence of a control arm with a sham procedure (given the high placebo response reported in interventional trials on FSD[39]) or with hormonal treatment. Furthermore, the lack of randomization did not allow an effective control of confounding factors (i.e. higher motivation for sexual activity because of participation in the study, partners' higher interest for sexual intercourse as they know their partners underwent the treatment, selection bias toward higher inclusion rate among women more motivated for the change in their sexual life) which may affect the findings of the current study. In addition, at the present time, the short-term follow-up limits our ability to document the duration of such a treatment in term of sexual well-being; however, in dermatology the persistence of skin collagen remodeling has been reported after 3 months since the last laser session[40]. Another potential limitation of our study is that we cannot be completely sure that women did not use any vaginal products throughout the study period, despite our clear instruction to avoid any kind of product. Indeed, it is well known that postmenopausal women with symptoms related to VVA widely use non-prescription lubricants when needed, as a personal strategy to counteract sexual pain at intercourse, because they do not easily recognize the chronic nature of the VVA condition[7,41].

The strong association between fractional microablative CO_2 laser treatment and significant improvement in sexual function, perception of sexual health and VVA symptoms encourages us to continue with further research in different clinical populations and with a long-term follow-up. Moreover, whether such a technique may exert a synergic effect with systemic and/or local hormonal treatments and other non-hormonal substances/devices to treat VVA remains to be fully investigated.

It is well established that the clinical expression of sexual symptoms in menopause is influenced by several factors, ranging from significant decline of estrogen and androgen production to intrapersonal and interpersonal factors[42]. Hormonal changes may impair the normal structure and function of genital tissues by influencing sensation, vasocongestion, lubrication, smooth muscle relaxation and vaginal microbiota. Indeed, especially the absence of estrogen stimulation contributes to the loss of mucosal elasticity by inducing fusion and hyalinization of collagen fibers and fragmentation of elastin fibers. Even mucosal hydration is reduced in the dermal layer with a reduction of intercellular mucopolysaccharide and hyaluronic acid[1,2,11,12,43]. The hemodynamic process of sexual arousal, involving the peripheral neurovascular complex and the pelvic floor muscles, is tightly linked to the biomechanical and viscoelastic properties of the vaginal wall. Women with VVA have difficulties in opening and distending the introitus, are less capable of lubricating in response to sexual stimuli and, as a consequence of the shortening and narrowing of the vaginal vault, experience painful and/or unpleasant intercourse[2,10,12,44]. Over time, postmenopausal women who are sexually active may develop hypertonic pelvic floor with secondary vaginism triggered by avoidance, anxiety and loss of sexual desire because of the anticipation of coital pain[45].

We have previously shown that vaginal laser technology was able to ameliorate elasticity, fluid volume, pH, epithelial integrity and moisture in postmenopausal women with symptoms related to VVA not responding/being unsatisfied with previous local estrogen therapies[27]. The precise mechanisms underlining the normalization of some vaginal properties is not yet completely clear but collagen remodelling and increased vascularization have been documented in *ex vivo* vaginal specimens following laser application[46]. Such an improvement of vaginal receptivity seems to be crucial for resuming sexual activity in women with FSD associated with VVA, as confirmed by the present data. On the other hand,

maintaining an active sexual life is a well-known protective element to counteract the loss of mucosal elasticity and hydration consequent to estrogen deprivation[47].

It is of paramount importance that health-care professionals display a positive attitude in discussing sexual health with postmenopausal women, to recognize signs and symptoms of VVA and, eventually, to look for potential treatment strategies[6,7,10]. Therapeutic options should be tailored for each individual woman in the context of her personal biopsychosocial profile to optimize healthy aging and partnership. Indeed, the interest in continuing sexual activity is a component of life satisfaction and successful aging[48]. Even though a significant proportion of middle-aged and older women still engages in satisfying sexual activity[49], more than one-third reports some problems with sexual function[50] and deserves specialized attention and care.

In conclusion, the present study demonstrated that the fractional microablative CO_2 laser is associated with an improvement of sexual function and satisfaction with sexual life in postmenopausal women by treating symptoms related to VVA. Whether or not such an effect is long lasting and meaningful for sexual intimacy over time remains to be investigated in further studies with a longer follow-up and in comparison with other effective therapies.

Conflict of interest During the past 2 years, Dr Nappi had financial relationships (lecturer, member of advisory boards and/or consultant) with Bayer-Schering Pharma, Eli Lilly, Gedeon-Richter, HRA Pharma, Merck Sharpe & Dohme, Novo Nordisk, Pfizer Inc, Shionogi Limited, Teva/Theramex. Dr Salvatore had financial relationships (lectures, member of advisory boards and/or consultant) with DEKA. The other authors did not report any potential conflicts of interest.

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GYNECOLOGIC ONCOLOGY

Fractional CO_2 laser for vulvovaginal atrophy (VVA) dyspareunia relief in breast cancer survivors

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Abstract
Purpose The aim of this study was to evaluate the efficacy of fractional CO_2 laser therapy in breast cancer survivors as a therapeutic method for vulvovaginal atrophy (VVA) dyspareunia.
Methods 50 patients (mean age 53.3 years) underwent fractional microablative CO_2 laser treatment for dyspareunia in oncological menopause (mean time of menopause 6.6 years). The Gloria Bachmann's Vaginal Health Index (VHI) score was chosen as system to evaluate the presence of VVA and its improvement after the treatment. Intensity of dyspareunia was evaluated using a visual analog scale (VAS).
Results Data indicated a significant improvement in VVA dyspareunia ($p < 1.86e{-}22$) in breast cancer survivors who had undergone 3 sessions of vaginal fractional CO_2 laser treatment. Moreover, VHI scores were significantly higher 30 days post-treatment (T4) ($p < 0.0001$). 76 % of patients were satisfied or very satisfied with the treatment results. The majority (52 %) of patients were satisfied after a long-term follow-up (mean time 11 months). No adverse events due to fractional CO_2 laser treatment occurred.
Conclusions The treatment with fractionated CO_2 laser appeared to be a feasible and effective treatment for VVA dyspareunia in breast cancer survivors with contraindications to hormonal treatments.

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Keywords Vulvovaginal atrophy · Laser · Oncological menopause · Breast cancer survivors

Introduction

Throughout woman's life cycle, the vaginal epithelium undergoes changes in response to the level of circulating estrogen. After menopause, circulating estrogen levels are dramatically reduced. Numerous cytological transformations follow estrogen reduction, including proliferation of connective tissue, fragmentation of elastin and hyalinization of collagen. These changes may result in granulation, fissures, ecchymosis, telangiectasia and ulcerations, resulting in a condition noted as vulvovaginal atrophy (VVA) [1].

The earliest symptoms of VVA are decreased vaginal lubrication, followed by other vaginal and urinary symptoms that may be exacerbated by superimposed infection, such as burning, itching, bleeding, leucorrhea, dyspareunia and dysuria [2, 3]. These symptoms usually appear 4–5 years after menopause.

This condition affects 20–45 % of women [2, 4] and in contrast to post-menopause vasomotor symptoms it becomes progressive over time and less likely to solve without intervention. Various surveys have shown that VVA symptoms have an adverse emotional and physical impact on female patients and on their partners through unsatisfactory sexual relationship [1].

This aspect is much more threatening for women suffering from premature interruption of ovarian production of estrogens. Examples include cancer treatments, such as surgery, pelvic radiation therapy, chemotherapy or endocrine therapy, that remove ovaries or make them inactive, either temporarily or permanently; use of GnRH agonists to

manage conditions such as endometriosis and uterine leiomyomas; hypothalamic amenorrhea caused by excessive exercise, disordered eating or postpartum state [3].

Oncological patients can not find relief in hormonal replacement therapy (HRT) which is considered the gold standard treatment for VVA symptoms [5]. A recent report of The North American Menopause Society was inconclusive on the modality of treatment for women affected by premature menopause after breast, ovarian or endometrial cancer, suggesting that for these women the management depends on individual preference or need and it must follow a consultation with oncologist considering the potential risks of hormonal treatment choices [3].

Approximately 50–75 % of breast cancer survivors suffer from one or more VVA symptoms [6].

VVA dyspareunia is an extremely difficult symptom of postmenopausal state and it is common in breast cancer survivors because of low estrogen.

Authors' aim was to assess the efficacy of fractional CO_2 laser therapy in a post-menopausal group of breast cancer survivors as feasible and not contraindicated therapy for VVA dyspareunia as presented in previous literature [6, 7].

Methods

From June 2013 to June 2015 a prospective descriptive cohort study was conducted at Authors' Centre of Gynecological Laser Surgery and Colposcopy of The University Teaching Hospital of Careggi in Florence. The study protocol was approved by the Hospital Research Review Committee.

The recruited cohort of population was constituted by patients with breast cancer, having completed the treatment protocol and final evaluation within the study time.

Patients were enrolled actively by a proposal by their physician aware of single patient's VVA dyspareunia and referred to the centre on a volunteer basis.

At enrollment all patients were asked to have a negative Pap smear not older than a year, were asked to read and subscribe an information white paper illustrating physical principles of the technique, intraoperative and postoperative complications and were informed on the scarcity of literature supporting this procedure [8].

The type of informed consent used was specifically designed for the treatment of menopausal vaginal atrophy with fractional microablative CO_2 laser in agreement with the Helsinky Declaration. The type of informed consent coupled the one previously used by other researching groups [7]. The patients entered the study after the informed consent was obtained.

Enrolled patients accepted to undergo a gynecological visit before the first treatment application and 4 weeks after the last laser application of the treatment protocol. Gynecological inspection included colorimetric pH evaluation [9, 10], speculum insertion to measure vaginal elasticity and colposcopic inspection of the integrity of the epithelium.

Therefore the patients' inclusion criteria in the study group were: oncological menopause (women with current or previous breast cancer), VVA dyspareunia, negative Pap smear.

Exclusion criteria were considered the use of vaginal moisturizing agents and lubricants within 30 days before the inclusion in the study group; the presence of active genital infection during the visit at the enrollment; prolapse stage $\geq$II according to pelvic organ prolapse quantification system [11]; previous reconstructive pelvic surgery or topical radiotherapy.

Treatment protocol The fractional microablative CO_2 laser (Smart Xide2; V2LR Monnalisa Touch System, DEKA Florence, Italy) was administrated within a protocol providing 3 time points of application at baseline (T1), at 30 days (T2) and at 60 days (T3). The laser energy was set at 30 W power and transmitted through an intravaginal probe with a dwell time of 1000 μs, a dot spacing of 1000 μm and a smart stack parameter of 1 following a protocol of application suggested by previous Authors [7].

Laser Energy was released by 2 single shots oriented at 45 degrees one from the other so to treat the entire circular vaginal surface covered by the probe which is doted of two opposite focusing points.

All laser application were performed in an outpatient setting, they did not require local anesthesia or not other preparation.

Patients were not prohibited to have sexual activity before or after the treatment sections.

Data collection and analysis T1 was the baseline for the collection of the demographic characteristics of patients, verification of inclusion and exclusion criteria and evaluation of VVA presence.

The Gloria Bachmann's Vaginal Health Index (VHI) [12] was chosen by Authors as scoring system to evaluate the presence of VVA and its improvement after the treatment protocol. VHI included five parameters evaluated by colposcopic inspection: elasticity, fluid volume, pH, epithelial integrity and moisture. Each parameter was graded from 1 (worst condition) to 5 (best condition). VVA was defined as a total score at baseline <15 [12]. VHI was repeated 4 weeks after the last laser application (T4) to measure VVA improvement.

Intensity of VVA dyspareunia was evaluated using a visual analog scale (VAS), which is based on a score from

1 to 5, where 1 indicates the absence of symptoms and 5 severe symptoms.

Treatment satisfaction was evaluated at T4 using a 5-point Likert scale (very satisfied, satisfied, uncertain, dissatisfied and very dissatisfied). Treatment was considered satisfactory when patients were very satisfied or satisfied.

Patients were asked to inform physician on intraoperative complications, such as pain at probe insertion, burning, itching, and postoperative ones, such as bleeding, leucorrhea, discomfort.

VHI data reported in the text are expressed as mean with standard deviation of continuous variables and were analyzed by Student's t test considering significant a $p < 0.05$. VAS data were expressed as median and range of values before and after the treatment and were analyzed by Mood's Median Test to determine whether the median of the two populations were equal or statistically different for a $p < 0.05$.

Results

During the period between June 2013 and June 2015 180 patients voluntarily underwent V2LR treatment for VVA dyspareunia at our Centre.

50 patients affected by breast cancer who had completed the treatment protocol within the study time,with at least 3 months of follow-up, and had never been treated with topic or systemic HRT were eligible to constitute the study group.

Among these, 22 patients (44 %) were assuming adjuvant therapy: 2 (9 %) with Aromatase Inhibitors (AI) and 20 (91 %) with Tamoxifen (TMX). The other 28 patients (56 %) were not assuming any adjuvant therapy.

The mean age of patients was 53.3 years (range 41–66) with a mean time of menopause at the enrollment of 6.6 years (range 1–17). All demographic characteristics of the patients are reported in Table 1.

None of the enrolled patients was missed during the treatment protocol. No patient described intraoperative or postoperative complications with the exception of 12 patients (24 %) who complained for pain at the probe insertion.

Table 1 Demographic characteristics of the study population

Age (mean, range)	53.3 (range 41–66)
Time of menopause (mean, range)	6.6 (range 1–17)
Smokers (n, %)	14 (28 %)
Parity (n, %)	30 (60 %)
Adjuvant therapy with Tamoxifene (n, %)	20 (40 %)
Adjuvant therapy with Aromatase Inhibitors (n, %)	2 (4 %)
No adjuvant therapy (n, %)	28 (56 %)

VHI was evaluated only for a sample group constituted by the first 36 patients enrolled in the study.

Each sample group member at the enrollment had a VHI score of VVA (<15) with a mean score of 8.9 ± 1.7 st. dev. and enhanced her VHI score at T4 to a mean of 21.6 ± 1.6 st. dev. resulting in a statistically significant recovery ($p < 0.0001$) (Figs. 1, 2). No statistically significant difference ($p < 0.3$) was found in VHI score at T4 between the AI-treated subgroup of patients, the TMX-treated subgroup of patients and the others.

The median of VAS at T1 was 5 (range 1–5) while the median of VAS at T4 resulted 3 (range 1–5). The difference between this two medians of the population who underwent the treatment resulted statistically significant with a $p < 1.86e{-}22$ (Fig. 3).

No patient required lubricants or other adjuvant therapies after T4 during sexual activity.

Patient satisfaction with the procedure at T4 was as follows: 18 patients (36 %) were very satisfied, 20 (40 %) were satisfied, 6 (12 %) were uncertain, 6 (12 %) were dissatisfied and 0 were very dissatisfied.

Every patient was called in September 2015 to evaluate long-term treatment effects. The mean time of follow-up was 11 months with a range of 3–25 months. 52 % of patients answered they were very satisfied or satisfied of the treatment results; 26 % were dissatisfied and did not want to repeat the treatment; 22 % were dissatisfied but decided to start a new treatment cycle of laser applications.

Discussion

Management of VVA dyspareunia in women who have been treated for no hormone-dependent cancers is similar to that for women without a cancer history.

Because the lack of circulating natural estrogens is the primary cause of atrophic vaginitis, hormone replacement therapy is the most logical choice of treatment and has proved to be effective in the restoration of anatomy and the resolution of symptoms. Estrogen replacement restores normal pH levels and thickens and revascularizes the epithelium, increasing the number of superficial cells. Presently, local estrogen therapy, given as estrogen creams, vaginal estradiol tablets and estrogen rings, is the treatment of choice for women with vaginal atrophy who do not have other menopausal symptoms, according to the 2013 NAMS guidelines [3].

For women suffering for hormone-dependent cancers such as breast cancer concerns about adverse effects of exogenous estrogens are described thus, many of these women suffer diminished quality of life as they currently have no safe and effective treatment for their condition.

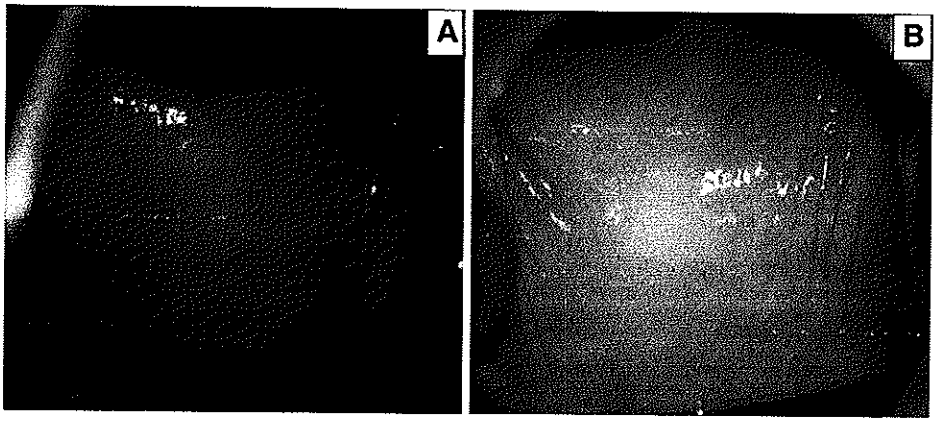

Fig. 1 Vagina mucosa (a) at enrollment: note petechiae (b) after three applications of fractional CO_2 laser: note the disappearance of petechiae

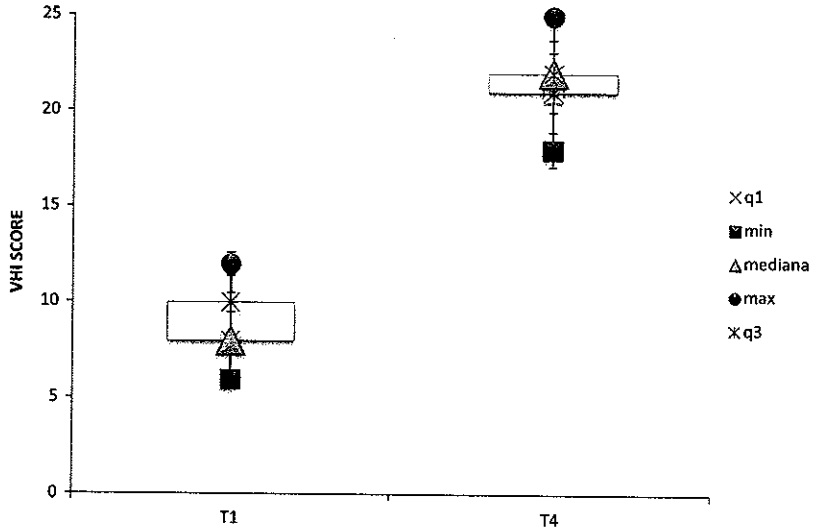

Fig. 2 *Boxes* and *whiskers* represent the medians, 1st and 3rd quartiles, and minimum and maximum values for VHI score. Statistical analysis was performed using Student's *t* test. $p < 0.0001$

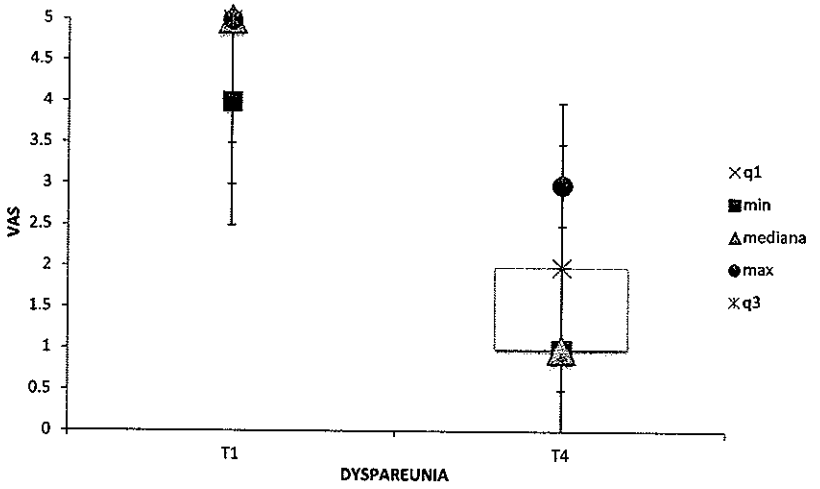

Fig. 3 *Boxes* and *whiskers* represent the medians, 1st and 3rd quartiles, and minimum and maximum values for VAS dyspareunia score. Statistical analysis was performed using Mood's median test. $p < 1.86e{-}22$

Nowadays non-hormonal options are the first line of treatment of VVA dyspareunia in women with breast cancer.

This is the principle reason why there is a clear medical need for an effective "estrogen-free" alternative to treat VVA dyspareunia in this subgroup of postmenopausal women.

VVA dyspareunia is considered by the majority of women as a potentially uncomfortable topic to discuss because of their linkage with sexual health; this is the reason why lubricants an moisturizing agents without pharmacological contents which do not require medical prescription are still the preferred solution [13].

However 40 % of women using lubricants or moisturizing agents as VVA dyspareunia relievers discontinue the treatment because they have concerns about side effects, or find the treatment inconvenient or not having an effect on their symptoms and this behavior often produces a worsening of the VVA dyspareunia [13].

Alternative therapies include selective estrogen receptor modulators (SERMs) and selective tissue estrogenic activity regulators (STEARs).

The most advanced SERM product candidate is ospemifene that acts as an estrogen agonist in the vagina and appears to have no clinically significant estrogenic effect on the endometrium or breast [14, 15]. However the safety of ospemifene has not been demonstrated in women with a prior history or an increased risk of breast cancer.

In this scenario Authors' results show that a physical treatment as the fractional microablative CO_2 laser is safe and produces recovery of VVA dyspareunia.

The induced VVA dyspareunia recovery is statistically significant and independent from the adjuvant use of lubricant agents, qualifying the fractional microablative CO_2 laser as not-chronic method of VVA dyspareunia treatment. Our results agree with other studies present in literature.

In 2011, Gaspar et al. first showed resulting positive changes in biopsy specimens which were treated with fractional microablative CO_2 laser in combination with platelet-rich plasma [16].

Subsequently Zerbinati et al. microscopic and ultrastructural modifications of vaginal mucosa after fractional CO_2 laser treatment. They demonstrated the stimulation of collagen synthesis, the increase of acidic mucopolysaccarides in the ground matrix and the increase of glycogen content in the epithelial cells in the vaginal specimens of patients who underwent fractional CO_2 laser treatment. These findings support the effectiveness of this treatment for the restoration of vaginal mucosa structure and related trophism [17].

In 2014, Salvatore et al. demonstrated for the first time that a treatment with microablative CO_2 laser induced a significant improvement of VVA symptoms. The results of this study showed that laser treatment was feasible, safe, easy to perform and significantly improved both symptoms of VVA and score of vaginal health at 12-week follow-up in women dissatisfied with previous local estrogen therapies [7].

In 2015, Salvatore et al. proved that fractional CO_2 laser treatment is associated with a significant improvement of sexual function and satisfaction with sexual life in postmenopausal women with VVA symptoms [18].

Latest study of Perino et al. confirmed that fractional CO_2 laser could be a safe, feasible and effective option for the treatment of VVA symptoms without adverse events during the study period [6].

The data from our study indicate a significant improvement in VVA dyspareunia and VHI score in breast cancer survivors who underwent a treatment cycle of three laser applications. In the present study, VHI and thus the improvement of the epithelium trophism achieved by the microablative laser treatment were evaluated for the initial 36 patients enrolled, only up to the statistically significance achievement. This Authors' decision was principally due to the request of a complex colposcopic evaluation that VHI requires and that is perceived as painful by the majority of patients, mostly at T1. Authors decided to avoid vaginal biopsies for the same reason and took for granted proofs the results of Zerbinati et al. [17] on the laser-tissue-interaction.

The majority of women (76 %) declared that they were satisfied or very satisfied with the procedure with an improvement in sexual function and quality of life at T4.

Our results suggest that fractional CO_2 laser treatment may be a viable option in women with severe contraindications to hormonal therapy, such as breast cancer survivors, for whom laser treatment may be utilized as "non-hormonal therapy". Moreover we confirmed that the treatment with fractional CO_2 laser of postmenopausal VVA dyspareunia is feasible, safe and effective without adjuvant agents such as non-hormonal vaginal moisturizers and lubricants.

Although the presents results are of uttermost interest, Authors know data are still biased by the small sample size of the studied group. In contrast to previous studies, the absence of a control group of patients is due to the lack of a gold standard therapy for VVA dyspareunia in breast cancer survivors such as traditional local estrogen therapy as estrogen pessaries or SERMs.

At our knowledge, this is the first study evaluating the feasibility and effectiveness of fractional CO_2 laser treatment in breast cancer survivors and it could guide further research in populations of women with contraindications to hormonal therapies. Moreover, differently from the other studies, a long-term follow-up (mean time 11 months) was included in the study.

Authors would like to underline how this protocol of treatment offered the possibility to discuss with women about the importance of proactive behaviors for preserving sexual function in postmenopausal women. Women were taught on how regular sexual activity helps to maintain vaginal health and increases the likelihood that sexual activity will remain comfortable in the future.

In conclusion, our results showed that treatment with fractional microablative CO_2 laser is safe, well-tolerated and effective in breast cancer survivors with VVA dyspareunia and improves the quality of life in majority of patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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An assessment of the safety and efficacy of a fractional CO_2 laser system for the treatment of vulvovaginal atrophy

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Abstract

Objectives: The aim of the study was to assess the safety and efficacy of a novel fractional CO_2 laser for the treatment of genitourinary syndrome of menopause (GSM).

Methods: Women presenting with GSM and meeting study criteria were enrolled. Examinations at baseline and follow-up (3 mo after final treatment) evaluated dilator tolerance and vaginal pH. Visual analog scales were used to assess pain, vaginal burning, vaginal itching, vaginal dryness, dyspareunia, and dysuria; Vaginal Health Index scores were completed before each treatment and at follow-up; Female Sexual Function Index and Short Form 12 questionnaires were also completed. Participant satisfaction was measured on a 5-point Likert scale (1 = very dissatisfied, 5 = very satisfied). Women received three laser treatments, 6 weeks apart.

Results: Thirty women participated (mean age 58.6 ± 8.8 y). None withdrew or were discontinued due to an adverse event; three were lost to follow-up. Average improvement in visual analog scale scoring was 1.7 ± 3.2 for pain, 1.4 ± 2.9 for burning, 1.4 ± 1.9 for itching, 6.1 ± 2.7 for dryness, 5.1 ± 3.0 for dyspareunia, and 1.0 ± 2.4 for dysuria; improvement in average Vaginal Health Index and Female Sexual Function Index scores were statistically significant ($P < 0.001$). Twenty-five of 30 participants (83%) showed increase in comfortable dilator size at 3-month follow up. Before the second and third treatments, 86.6% (26 of 30) of women reported they were better or much better than at the previous treatment; 26 of 27 women (96%) were reportedly satisfied or extremely satisfied at follow-up.

Conclusions: In this sample, the data suggest that the fractional CO_2 laser is effective and safe for treatment of the symptoms associated with GSM.

Key Words: Dyspareunia – Fractional CO_2 laser – Genitourinary syndrome of menopause – Menopause – Vaginal dryness – Vulvovaginal atrophy.

Genitourinary syndrome of menopause (GSM), also known as vulvovaginal atrophy (VVA), affects quality of life (QoL) and sexual function in as many as 50% of postmenopausal women.[1-3] GSM is characterized by atrophy of the mucous membranes and other tissues of the vulva and vagina and occurs as a consequence of reduced circulating estrogen.[4] Constriction of the introitus and vaginal canal is common and is often associated with reduced sexual activity. Other common manifestations include progressive reduced elasticity of vaginal rugae with thinning of the vaginal lining, reduction of lubrication and symptoms of itching, burning, dryness, irritation, dysuria, and dyspareunia.[5] The vagina becomes increasingly prone to trauma and tears, bleeding, and pain as the mucosa thins and becomes more fragile. The prevalence of urogenital infection may also rise as the vaginal secretions became more alkaline, altering the character of vaginal flora.[6]

Many current first-line therapies provide benefit in the treatment of GSM. Vaginal moisturization via topical lubricants may alleviate symptoms. Ospemifene is a well-tolerated synthetic selective estrogen-receptor modulator shown to be somewhat effective for VVA in the treatment of moderate-to-severe dyspareunia.[7-8] When not contraindicated, local estrogen therapies are effective when alternatives fail, but compliance reportedly ranges between 52% and 74%,[9] and some women may not be candidates for estrogen therapy or may decline hormone use.

Device-based options for the treatment of VVA have recently been introduced into the market and have garnered a significant amount of interest. Use of laser for the treatment of VVA was described by Salvatore et al,[10] who recently showed that a fractional CO_2 laser can produce a remodeling of vaginal connective tissue without causing damage to surrounding tissue in an ex vivo study. The fractional CO_2 laser treatment also improved the VVA symptoms of vaginal

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dryness, vaginal burning, vaginal itching, dyspareunia, and dysuria at 12-week follow-up in a clinical pilot study.[11] Other trials have suggested that the laser is feasible, safe, and effective for the treatment of VVA symptoms, and that sexual health and QoL are improved.[12-13]

This is the first US study of the use of the fractional CO_2 laser for the treatment of symptoms of VVA. The purpose of this study is to evaluate the safety and efficacy of a fractional CO_2 laser for the treatment of GSM.

METHODS

This study was performed at two US centers. The research was conducted according to Good Practice Guidelines and was IRB approved. Informed consent was obtained from all participants. Consecutive women who presented with complaints of GSM were approached for the study and invited to participate if they were healthy, nonsmoking postmenopausal women with an absence of menstruation for at least 12 months. Participants had to exhibit bothersome symptoms of VVA, have less than stage 2 prolapse according to the pelvic organ prolapse quantification system and could not have had any procedures in the anatomical area for the previous 6 months. The use of vaginal creams, moisturizers, lubricants, or homeopathic preparations was not permitted for at least 3 months before study commencement and throughout the entire study period. Thirty women were enrolled (15 at each site).

Exclusion criteria included acute or recurrent urinary tract infection or genital infection; history of thrombophlebitis, acute infections, keloid formation or heart failure; use or anticipated use of antiplatelet treatments, anticoagulants, thrombolytics, vitamin E, or anti-inflammatory within 2 weeks before the treatment phase of the study; concurrent use of medications that increase photosensitivity; presence of pelvic organ prolapse greater than stage II by the pelvic organ prolapse quantification system; previous reconstructive pelvic surgery; and the presence of any disease or chronic condition that could interfere with study compliance.

The primary outcome measure was Visual Analog Scale (VAS) change in six categories of symptoms commonly associated with VVA: vaginal pain, burning, itching, dryness, dyspareunia, and dysuria. VAS was scored on an 11-point scale for each symptom (vaginal pain, burning, itching, dryness, dyspareunia, and dysuria) with 0 being the lowest level (none) and 10 being the highest (extreme). Secondary outcome included (1) assessing the effect of treatment on female urogenital health, using the Gloria Bachmann Vaginal Health Index (VHI)[14] score (as shown in Table 1); (2) assessing the effect of treatment on vaginal wall elasticity by tracking the maximal tolerable dilator size; (3) assess the effect of treatment on female sexual function using the Female Sexual Function Index (FSFI) questionnaire; (3) assessing the effect of treatment on general QoL using the Short Form 12 (SF-12)-specific questionnaire; (4) assessing the degree of difficulty encountered by the investigators in performing treatment by means of a 5-point Likert scale; (5) and assessing the rate of participant satisfaction with treatment using the Patient Global Impression of Improvement (PGI) via a 5-point Likert scale.

Baseline screening included measurement of vaginal pH and a gynecological examination to assess the condition of the vaginal wall tissue, including a vaginal calibration to determine the maximal dilator each participant could comfortably tolerate; four sizes—extra small (XS), small (S), medium (M), and large (L)—were available. VVA symptoms were assessed using the VAS and baseline VHI scores were also obtained. Participants completed baseline FSFI, SF-12, and general health questionnaires as well.

Each woman received three laser treatments with a fractional CO_2 laser system (SmartXide^2, MonaLisa Touch, DEKA M.E.L.A. Srl, Florence, Italy) at an interval of 6 weeks (+/−1 wk) between sessions. The procedure was performed in an outpatient setting without any specific preparation such as analgesia or anesthesia required. During treatment, laser energy was transmitted through a tube-shaped vaginal probe with energy deflected at 90° in four directions (the 12, 3, 6, and 9 clock positions) on the vaginal wall tissue. The probe was inserted into the vaginal canal, with two pulses fired; the first at the "noon" position and second at 45° clockwise rotation or "1:30" position, then slowly withdrawn 4 mm (as determined by dedicated markings on the probe) to perform the next treatment similarly until the entire vaginal wall tissue had been treated (Fig. 1). Treatments were performed at 30 W, 1,000 milliseconds dwell time, 1,000 μm spacing, normal scan mode. The Smartstack setting, which controls the number of laser pulses fired at each site, ranged from 1 to 3. Treatment parameters were modulated downward to participant tolerance, if required.

After each session, the participants were evaluated for treatment-related complications and side effects (none, mild, moderate, severe). Participants also assessed their level of discomfort during the treatment using the VAS. Women who were sexually active were instructed to avoid sexual intercourse for 3 days after each treatment, but were otherwise not

TABLE 1. *Vaginal Health Index*

Score	Overall elasticity	Fluid secretion characteristics	Vaginal pH range	Epithelial mucosa	Moisture
1	None	None	≥6.1	Petechiae noted before contact	None, mucosa inflamed
2	Poor	Scant thin yellow	5.6-6.0	Bleeds with light contact	None, mucosa not inflamed
3	Fair	Superficial, thin white	5.1-5.5	Bleeds with scraping	Minimal
4	Good	Moderate, thin white	4.7-5.0	Not friable, thin mucosa	Moderate
5	Excellent	Normal (white flocculent)	≤4.6	Not friable, normal mucosa	Normal

TABLE 2. *Assessment of change in symptoms of VVA*[a]

	Baseline to Tx1		Baseline to Tx2		Baseline to Tx3	
Symptom	Improvement	*P*	Improvement	*P*	Improvement	*P*
Pain	1.1 ± 2.9	0.051	1.6 ± 3.4	0.025	1.7 ± 3.2	0.009
Burning	1.3 ± 2.5	0.015	1.7 ± 2.4	0.001	1.4 ± 2.9	0.018
Itching	1.1 ± 2.0	0.006	1.6 ± 1.9	<0.001	1.3 ± 1.9	0.001
Dryness	4.8 ± 2.9	<0.001	5.1 ± 2.9	<0.001	6.1 ± 2.7	<0.001
Dyspareunia	4.3 ± 2.8	<0.001	5.3 ± 2.8	<0.001	5.4 ± 2.9	<0.001
Dysuria	0.6 ± 2.1	0.154	0.9 ± 2.2	0.049	1.0 ± 2.4	0.035

VVA, vulvovaginal atrophy.
[a]Improvement measured on a Visual Analog Scale (0 to 10, where 0 = none and 10 = extreme), listed as mean ± SD. Cutoff for statistical significance was $P > 0.05$.

given specific activity restrictions. After each treatment, investigators evaluated ease of treatment on a 5-point Likert scale (1 = very difficult, 5 = very easy) and participant satisfaction was evaluated on a similar 5-point Likert scale (1 = very dissatisfied, 5 = very satisfied).

Adverse events were assessed at all visits. At the second and third treatment, participants rated global improvement by completing the PGI on a 5-point scale (1 = much worse, 5 = much better); scores of 4 or 5 indicated perceived improvement.

Participants were required to return for follow-up 3 months after the final treatment. All assessments that were performed pretreatment (vaginal examination, vaginal pH, maximal dilator size, VHI, VAS, PGI, treatment-related discomfort/pain using 5-point Likert Scale, FSFI, and SF-12) were also performed at the follow-up. In addition, women were asked to complete a satisfaction survey regarding the results of the laser treatment using a 5-point Likert scale (1 = very dissatisfied, 5 = very satisfied).

Statistical analysis included appropriate measures for statistical significance (Student's paired two-sample *t* test) using the standard cutoff for significance of $P < 0.05$ via Microsoft Excel.

RESULTS

For the 30 participants, mean age was 58.6 ± 8.8 years, average onset of menopause was 48.9 ± 7.6 years, and the average age of onset of vulvar and vaginal atrophy symptoms was 51.2 ± 8.3 years. Twenty-seven of the 30 women completed the study and three were lost to follow-up; one failed to appear after multiple rescheduling of appointments, one had car trouble and missed follow-up, and one left the state and was unavailable for final follow-up. Two women reported mild-to-moderate pain lasting 2 to 3 days and two reported minor bleeding lasting less than 1 day, but none were discontinued due to the occurrence of adverse events.

The primary outcome measure was VAS change in six categories of symptoms commonly associated with VVA: vaginal pain, burning, itching, dryness, dyspareunia, and dysuria. For all six symptom categories, improvement from baseline to the final (third) treatment was statistically significant. Treatment for dryness and dyspareunia showed the most profound improvement; results for dysuria were more modest but still statistically significant. Table 2 displays the VAS results for VVA in the 27 women who presented for 3-month follow-up. The majority of symptom improvement was noted after the first treatment, though participants continued to experience incremental improvement in their VVA symptoms after the second and third treatments.

For secondary outcome measures, VHI scores for the 27 women completing the evaluation at follow-up ranged from 8 to 20 at baseline (mean 14.4 ± 2.9) and from 16 to 25 (21.4 ± 2.9) after three sessions with an overall mean improvement of 7.0 ± 3.1, which was statistically significant ($P < 0.001$).

Vaginal elasticity was evaluated via dilator insertion. Of the 30 enrolled participants, 24 (80%) could comfortably accept an XS or S dilator at the baseline visit, whereas 23 of 24 women (96%) undergoing dilator testing at follow-up could comfortably accept an M or L dilator. Overall, an

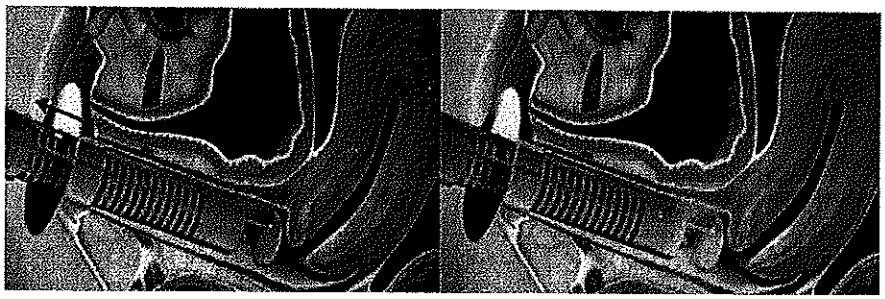

FIG. 1. Diagram of the vaginal probe shown inserted into the vagina (left) with pulse firing (right), deflected in four directions (the 12, 3, 6, and 9 clock positions). Graphics courtesy of DEKA M.E.L.A. Srl, Calenzano, Italy.

increase in dilator size was seen in 25 women (83%) from baseline to follow-up; 5 of 29 (17%) had no change (two women were able to comfortably fit an S-size dilator at all visits, two were able to comfortably fit an M-size dilator at all visits, and one participant was able to comfortably fit an L dilator at all visits). Figure 2 shows dilator size distribution at all visits.

Although part of the overall VHI evaluations, vaginal pH data itself were analyzed separately, and are further detailed in Table 3. Changes were noted but were not statistically significant over the course of the study.

FSFI questionnaires were given at baseline and final follow-up. Of 27 participants undergoing follow-up, 26 completed the FSFI. Scores at baseline ranged from 2 to 25 (mean 11.3 ± 7.3). At follow-up, improvement ranged from -3.7 to 27.2 (mean 8.8 ± 7.3), which was statistically significant ($P < 0.001$).

Of women undergoing 3-month follow-up, 23 completed their second (final) SF-12 questionnaire assessing perceived health. Scores for physical health at baseline averaged 37.2 ± 6.2 with average improvement of 1.6 ± 8.1 (statistically insignificant). Scores for mental health at baseline averaged 43.3 ± 11.9 with average change of -2.6 ± 1.6 (statistically insignificant). Physician investigators rated ease of treatment on a 5-point Likert scale (1 = very difficult, 5 = very easy); scores ranged between 4 and 5 in all cases for all treatments and were virtually unchanged throughout.

Upon study completion, participants were asked to rate satisfaction with treatment on a 5-point Likert scale (1 = very dissatisfied, 5 = very satisfied). Of 27 women at follow-up, 16 were reportedly very satisfied and 10 were reportedly satisfied, with 1 reporting satisfaction as slight, for an overall satisfaction level of 100%. Assessment of global improvement (PGI) on a 5-point Likert scale (1 = much worse, 5 = much better) yielded similar results with approximately 73% of women reporting "much better" (n = 8) or "better" (n = 14); seven reported "same" and one reported as "worse."

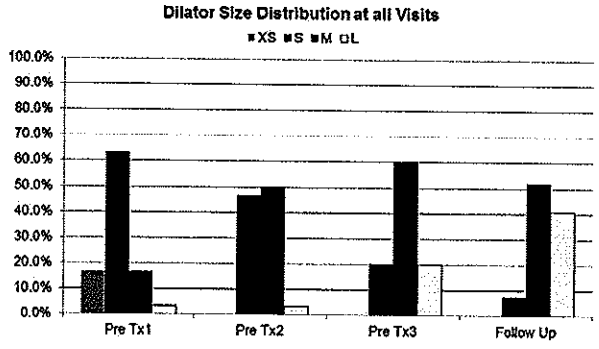

FIG. 2. Distribution of dilator size data for participant population (n = 30), shown as percentages of women able to comfortably accept an XS, S, M, or L vaginal dilator at each of four time points (before treatments 1, 2, and 3 as well as final follow up). Three participants were lost to follow-up. L, large; M, medium; S, small; XS, extra small.

TABLE 3. *Average vaginal pH at baseline and all study visits*[a]

	Baseline	Post 1 Tx	Post 2 Tx	Post 3 Tx
Minimum	4.63	4.03	4.03	3.8
Maximum	7.33	7.57	8.03	7.84
Average	5.519	5.620	5.690	5.655
SD	0.705	1.075	1.188	1.381
P value compared with baseline[b]		0.482	0.340	0.517

[a]Three participants were lost to follow-up; analyzed data include the subset not lost to follow-up (n = 27).
[b]pH changes overall were not statistically significant.

DISCUSSION

This pilot study, which is the first US trial of the fractional CO_2 laser system for the treatment of GSM, suggests that fractional CO_2 laser treatment of the vagina is well tolerated by women and leads to significant improvement in the symptoms of GSM. Limitations of this study include the relatively small number of participants enrolled, lack of a control arm, and the short follow-up. Given the small sample size and lack of placebo control to account for participant or investigator expectations possibly confounding the results, data should be interpreted with caution; nevertheless, findings of improvement in GSM symptoms were highly significant. The participants will be followed out to 1 year, and those results will be reported in the future. Direct comparison to existing therapies via randomized controlled investigation is currently underway.

The rationale behind the use of a CO_2 laser for VVA stems from the nature of vaginal wall tissue, which shares many characteristics with normal surface skin despite being more heavily innervated and vascular. Fractional laser therapy has been a mainstay of aesthetic medicine in recent years because it can be both safe and effective for tissue remodeling.[15] Devices use scanning software and mechanical processes to lay down patterns of microwounds, leaving most of the surrounding epithelium intact, which minimizes downtime and speeds re-epithelialization and healing, yet still provides therapeutically relevant thermal damage. Depending on wavelength and energy level (measured as Joules per cm^2, or J/cm^2) the character of microwounding can be ablative and coagulative in nature at varying levels of aggressiveness, with the goal of stimulating neocollagenesis (along with other beneficial processes associated with the healing process) and the subsequent emergence of new, healthy, more youthful skin.

Mainly absorbed by water, the 10,600 nm wavelength CO_2 laser used in this trial is used for soft tissue applications and has received Food and Drug Administration clearance for incision, excision, ablation, vaporization, and coagulation of soft tissue in a variety of medical specialties. This fractional CO_2 laser system has a maximal power of 60 W and energy is delivered through an articulated arm with a delivery accessory connected to its distal end. The system is equipped with a HiScan Dot scanning system and a 360° probe that allows energy emission at 360°. The scanner use is indicated for precise layer-by-layer

tissue ablation to avoiding charring and delivers energy in a uniform, accurate, and controllable manner. Laser energy penetrates to a maximum depth of 200 μm.

Research has demonstrated the tissue remodeling properties of fractional CO_2 lasers in aesthetic medical applications for body regions such as the skin of the face, neck, and chest, with the effect of stimulating the production of new collagen and elastin, bolstering the extracellular matrix.[16-20] Reports on the use of the fractional Er:YAG laser (similar to a CO_2 laser in its superficial effect) revealed the capability to improve pelvic floor dysfunction (urinary incontinence and vaginal laxity)[21-22]; an ex-vivo study demonstrated connective tissue remodeling in vaginal wall tissue, without collateral tissue damage or side effects, using predetermined parameters.[23] Laser treatment of vaginal wall tissue has been shown to regenerate the connective tissue of vagina lamina propria.[24]

Despite limitations, the results of this study were encouraging, with notable perception of global improvement in most participants and for all symptom categories (pain, burning, itching, dryness, dyspareunia, and dysuria) improving by an average of approximately 48% after a single treatment session, with continued improvement after the second and third treatments. The improvement most participants reported in their GSM symptoms after even a single laser treatment suggests that for some women, a single laser treatment may be all that is required, though further study using a larger sample size would be needed to test this conjecture.

These findings are similar to those of three different prospective observational studies. In 2014, Salvatore et al[11] reported a 12-week evaluation of the use of pulsed CO_2 laser for the treatment of VVA symptoms in 50 postmenopausal women and noted significant improvement compared with baseline in all of the measured symptoms of VVA. The procedure was well tolerated and no complications or side effects were reported.

Similarly, Perino et al[12] treated 48 study participants and noted significant improvement in all VVA symptoms after three sessions of vaginal fractional CO_2 laser treatment. Overall, 91.7% of women in that trial reported that they were satisfied or very satisfied with the procedure and experienced considerable improvement in QoL. No adverse events due to fractional CO_2 laser treatment were reported.

VVA can negatively impact sexual health and sexual intimacy. Nappi et al[25] showed that 58% of women and 61% of men reduced their sexual activity due to VVA, and 35% of women and 14% of men decided to refrain from sex due to VVA. Pain with sexual intercourse associated with VVA was cited as the reason for abstaining from sexual intercourse in 55% of women and 61% of men. The improved FSFI scores we saw in our trial suggest that sexual health improves after treating VVA symptoms with fractional laser. Of note, the FSFI has been shown to be a valid tool regardless of whether a participant engages in sexual intercourse.[26]

Salvatore et al[13] also investigated the effects of the fractional microablative CO_2 laser on sexual function and overall satisfaction with sexual life in postmenopausal women with VVA. Seventy-seven postmenopausal women with VVA symptoms were included and treated with three sessions of a fractional microablative CO_2 laser system (SmartXide^2, MonaLisa Touch, DEKA M.E.L.A. Srl, Florence, Italy) at 30-day intervals. Sexual function and QoL were evaluated with the FSFI and the SF-12 at baseline and at 12-week follow-up. As in our study, a significant improvement in the total FSFI score and in each domain of the FSFI was observed at 12-week follow-up. Seventeen (85%) out of 20 women who were not sexually active because of VVA severity at baseline regained a normal sexual life at the 12-week follow-up.

In this trial, almost all participants were only able to accept an S or XS dilator at baseline, and at follow-up, 83% were able to accept a larger dilator, indicating an improvement in vaginal elasticity. The ability to use a larger dilator suggests that women would be more likely to have successful vaginal intercourse after laser treatment for GSM. Indeed, some of the women in this trial who had not been able to be sexually active for many years due to pain were able to be sexually active after the laser therapy. Although a larger, placebo-controlled study would be needed to confirm this, the procedure was well tolerated by the participants and it was deemed easy to perform by the investigators, so it seems that fractional CO_2 laser therapy could become a popular therapeutic choice for the treatment of GSM in the future if studies continue to suggest good outcomes. Importantly, 96% of the participants were reportedly satisfied or very satisfied with the treatment.

As a safe, well-tolerated therapeutic alternative for GSM, fractional CO_2 laser therapy may provide a number of key advantages to standard treatment. This might be a particularly useful treatment options for those who cannot use hormone therapies, such as those with estrogen-responsive tumors. Some of the participants in this trial were referred from the oncology clinic and were unable to use vaginal estrogens. Also, our outcomes suggest that symptom improvement occurs rapidly and that a single treatment may provide much of the benefit, making further treatment unnecessary in the immediate future. Further treatment is, however, safe and may bolster outcomes for vaginal dryness and dyspareunia. Lastly, this laser therapy is simple and quick to perform (each treatment takes <2 min) and does not require continuous topical application by the participant, which has been shown to lead to poor compliance with topical therapies and vaginal estrogens.[3]

An important factor to consider is out of pocket costs because this novel therapy is not currently covered by insurance. It is impossible to perceive how this treatment would be accepted in the context of the relatively elastic modern era of healthcare in the United States. Currently, the costs vary greatly per region from $1,800 to $3,000 for three treatments. Future investigations should include comparing the associated cost with the continuous use of vaginal estrogen therapy and the costs associated with fractional CO_2 laser therapy over the same time period.

This is a pilot study and should be interpreted as such; although dilator size data is not easily dismissible given the

objective nature of the measurement, caution should still be exercised due to the obvious limitations including a relatively small sample size and potential for placebo effect. Further study of larger, more racially diverse populations may yield additional information. A control arm, possibly rigged with a sham treatment, may be advantageous in future studies. Additional investigation of fractional CO_2 laser therapy versus alternative therapeutic options such as estrogen therapy or lubricant alone may yield useful information.

CONCLUSIONS

The fractional CO_2 laser used in this study seems to be a safe and effective treatment for GSM, also known as VVA. Tolerability and high satisfaction suggest this modality is a viable and promising alternative to existing therapies for GSM, although further investigations will be needed to confirm these results.

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