THE USE OF PULSED CO2 LASERS FOR THE TREATMENT OF VULVOVAGINAL ATROPHY

SALVATORE Stefano

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 CO2 laser for the treatment of 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.

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, chemo- or radiotherapy for oncological pathologies). 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 (ISSWSH) and the Board of Trustees of The North American Menopause Society (NAMS) [4] agreed that the term

genitourinary syndrome of menopause (GSM) 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 VA

International guidelines recommend local estrogens, lubricants, moisturizers and ospemiphene, a new 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 estrgens and with a previous history of TVE. 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 EMA and FDA for VVA symptoms [8,9]. However its effect on breast tissue and lower urinary tract still needs to be clarified.

CO2 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. CO2 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 remodelling.

CO2 pulsed lasers in medicine

Pulsed CO2 lasers are widely used in dermatology for many conditions including scars and atrophic changes caused by different factors (acne, ageing etc.)[10-13]. In 2003 Capon [14] et al reported the mechanism of action of pulsed CO2 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 TGFβ in activating fibrolites to become fibroblast 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 CO2 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 CO2 laser were able to produce remodeling of the affected tissue into healthy tissue.

Ex vivo and feasibility study of pulsed CO2 laser for VVA

The feasibility of using a pulsed CO2 laser to treat VVA symptoms was first explored in an ex-vivo study [16] where 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 papillae, 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, 1,000 Ks of DOT dwell, 1,000 Km of DOT spacing, and smart stack 3), where 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 micro fibrils could be clearly observable, with fine molecular filaments (arrows) in close relationship with collagen micro fibrils in the course of fibrillogenesis.

The microablative pulsed CO2 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, where 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 CO2 laser. These modifications can be interpreted as tissue remodeling in a rejuvenating sense. The most evident effects produced by the microablative fractional CO2 laser are the neocollagenesis with a return of the collagen fibers to a trabecular disposition, which is how the appears in premenopausal women.

Clinical study on the use of pulsed CO2 laser for VVA symptoms and sexual function

The clinical efficacy of the pulsed CO2 laser has been reported in three different prospective observational studies.

In 2014 for the first time Salvatore et al reported a 12-week evaluation of the use of pulsed CO2 laser for the treatment of VVA symptoms in 50 postmenopausal women [18]. The treatment protocol included 3 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 5 different variables (overall elasticity, fluid secretion type, pH, epithelial mucosa and moisture);
- 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 to 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 CO2 laser treatment in 5 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 papillae 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 pulsed CO2 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 3 sessions of vaginal fractional CO2 laser treatment compared to baseline. Objectively the VHI 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 (QoL). No adverse events due to fractional CO2 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 CO2 laser on sexual function and overall satisfaction with sexual life in postmenopausal women with vulvovaginal atrophy (VVA). Seventy-seven postmenopausal women with VVA symptoms were included and treated with 3 sessions of fractional microablative CO2 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 (SF-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 to baseline ($p < 0.001$) as illustrated in Table 3. 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 CO2 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 CO2 laser treatment as well as the amount of placebo effect, with a randomized controlled trial comparing treatment versus sham.

Key points

- VVA is a common condition with great impact on quality of life
- Pulsed CO2 laser is a novel treatment for VVA performed in an outpatient setting
- Histological samples after pulsed CO2 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 papillae and thickness increase
- Vaginal dryness, dyspareunia and all the VVA symptoms significantly improve after a cycle of 3 laser treatment
- Improvement of VVA symptoms after pulsed CO2 laser determines a better sexuality and quality of life

References

1. Nappi RE, Kokot-Kierepa M (2010) Women's voices in the menopause: results from an international survey on vaginal atrophy. Maturitas 67(3):233–238

   This survey reveals the impact that vaginal atrophy can have on quality of life and self esteem of many women. From this study the importance of investigating VVA symptoms in postmenopausal women results quite clearly.

2. Santoro N, Komi J (2009) Prevalence and impact of vaginal symptoms among postmenopausal women. J Sex Med 6(8):2133–2142

3. M. A. Weber & J. Limpens & J. P. W. R. Roovers. Assessment of vaginal atrophy: a review. Int Urogynecol J (2015) 26:15–28

4. **Portman DJ, Gass MLS. Genitourinary syndrome of menopause: New terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and The North American Menopause Society. Maturitas (2014), http://dx.doi.org/10.1016/j.maturitas.2014.07.013

   This paper reports the revision of terminology for VVA from the International Society for the Study of Women's Sexual Health and The North American Menopause Society. Maturitas. The new definition of Genitourinary Syndrome of Menopause better reflects the complaints in such condition related to the lower urinary and genital tract.

5. Brenner PF (1988) The menopausal syndrome. Obstet Gynecol 72(5 Suppl): 6S–11S

6. ACOG Practice Bulletin No. 141: management of menopausal symptoms. Obstet Gynecol. 2014 Jan;123(1):202-16.

7. Management of symptomatic vulvovaginal atrophy: 2013 position statement of The North American Menopause Society Menopause. 2013 Sep;20(9):888-902.

8. Goldstein SR(1), Bachmann GA, Koninckx PR, Lin VH, Portman DJ, Ylikorkala O; Ospemifene Study Group. Ospemifene 12-month safety and efficacy in postmenopausal

women with vulvar and vaginal atrophy. Climacteric. 2014 Apr;17(2):173-82.

9. Nappi RE(1), Panay N, Bruyniks N, Castelo-Branco C, De Villiers TJ, Simon JA. The clinical relevance of the effect of ospemifene on symptoms of vulvar and vaginal atrophy. Climacteric. 2015 Apr;18(2):233-40.

10. Ong MW, Bashir SJ. Fractional laser resurfacing for acne scars: a review. Br J Dermatol. 2012;166:1160-9.
11. Tierney EP, Hanke CW. Fractionated carbon dioxide laser treatment of photoaging: prospective study in 45 patients and review of the literature. Dermatol Surg. 2011;37:1279-90.
12. Tierney EP, Hanke CW. Ablative fractionated CO2, laser resurfacing for the neck: prospective study and review of the literature. J Drugs Dermatol. 2009;8:723-31.
13. Peterson JD, Goldman MP. Rejuvenation of the aging chest: a review and our experience. Dermatol Surg. 2011;37:555-71.
14. Capon, A. and S. Mordon, Can thermal lasers promote skin wound healing? Am J Clin Dermatol, 2003. 4(1): p. 1-12.

    In this paper there is a detailed description of the mechanism of pulsed CO2 laser in regenerating the skin and dermal layers.
15. Tambuwala A, Sangle A, Khan A, Sayed A. Excision of Oral Leukoplakia by CO2 Lasers Versus Traditional Scalpel: A Comparative Study. J Maxillofac Oral Surg. 2014 Sep;13(3):320-7.
16. **Salvatore S, Leone Roberti Maggiore U, Athanasiou S, Origoni M, Candiani M, Calligaro A, Zerbinati N. Histological study on the effects of microablative fractional CO2 laser on atrophic vaginal tissue: an ex vivo study. Menopause. 2015 Jan 20. [Epub ahead of print]

This study represents the feasibility study as well as the proof of concept for the use uf pulsed CO2 laser on the atrophic vaginal tissue. In this study the best laser setting for that specific machine was identified.

17. Xu XG, Luo YJ, Wu Y, Chen JZ, Xu TH, Gao XH, He CD, Geng L, Xiao T, Zhang YQ, Chen HD, Li YH. Immunohistological evaluation of skin responses after treatment using a fractional ultrapulse carbon dioxide laser on back skin. Dermatol Surg. 2011 Aug; 37(8):1141-9.
18. **Salvatore S, Nappi RE, Zerbinati N, Calligaro A, Ferrero S, Origoni M, Candiani M, Leone Roberti Maggiore U. A 12-week treatment with fractional CO2 laser for vulvovaginal atrophy: a pilot study. Climacteric. 2014 Aug; 17(4):363-9.

    This is the first observational study showing subjectively and objectively the safety and the efficacy of pulsed CO2 laser as an office procedure in women with vaginal atrophy.
19. Bachmann G. Urogenital ageing: an old problem newly recognized. Maturitas. 1995;22 Suppl:S1-S5.
20. *Perino A, Calligaro A, Forlani F, Tiberio C, Cucinella G, Svelato A, Saitta S, Calagna G. Vulvo-vaginal atrophy: a new treatment modality using thermo-ablative fractional CO2 laser. Maturitas. 2015 Mar;80(3):296-301.

    This is an observational study confirming the safety and efficacy of pulsed CO2 laser in treating vaginal atrophy in postmenopausal women
21. *Zerbinati N, Serati M, Origoni M, Candiani M, Iannitti T, Salvatore S, Marotta F, Calligaro A. Microscopic and ultrastructural modifications of postmenopausal atrophic vaginal mucosa after fractional carbon dioxide laser treatment. Lasers Med Sci. 2015 Jan; 30(1):429-36.

    This study gives a detailed histological illustration of the effect of pulsed CO2 laser on the vaginal epithelium and lamina propria in postmenopausal women

22. Nappi R, Kingsberg S, Maamari R, Simon J. The CLOSER (CLarifying Vaginal Atrophy's Impact On SEx and Relationships) survey: implications of vaginal discomfort in postmenopausal women and in male partners. J Sex Med. 2013 Sep;10(9):2232-41.

    This survey clearly report the dramatic effect on sexual function, sexuality, intimacy and personal relationship of vaginal atrophy in postmenopausal women

23. *Salvatore S, Nappi RE, Parma M, Chionna R, Lagona F, Zerbinati N, Ferrero S, Origoni M, Candiani M, Leone Roberti Maggiore U. Sexual function after fractional microablative CO_2 laser in women with vulvovaginal atrophy. Climacteric. 2015 Apr;18(2):219-25.

    This study shows the improvement in sexual function in 77 postmenopausal women with vaginal atrophy treated with pulsed CO2 laser

24. Rosen R, Brown C, Heiman J, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther 2000;26:191-208.

25. Ware J, Kosinski M, Keller SD. A 12-item short-form health survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996;34:220–33.

Table 1[Previously published [18]]. Assessment at baseline and at 30 days after each single pulsed CO2 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 ± 2.9	17.9 ± 2.1*	19.3 ± 1.9*, Ω,	21.9 ± 2.4*, Ω, §
Vaginal dryness (n, mean ± SD)	7.2 ± 1.0	2.7 ± 0.7*	1.5 ± 1.0*, Ω	1.7 ± 0.8*, Ω
Vaginal hitching (n, mean ± SD)	6.1 ± 0.9	2.5 ± 0.9*	1.8 ± 0.8*, Ω	1.6 ± 0.6*, Ω
Dyspareunia (n, mean ± SD)	8.7 ± 1.0	3.5 ± 0.8*	2.1 ± 1.0*, Ω	2.2 ± 1.0*, Ω
Dysuria (n, mean ± SD)	5.1 ± 1.0	2.9 ± 0.7*	1.6 ± 0.8*, Ω	0.8 ± 1.0*, Ω, §

* Statistical significant difference with baseline
Ω Statistical significant difference with 1-month follow-up
§ Statistical significant difference with 2-month follow-up

Table 2[Previously published[18]]. 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 Ω	0.4 ± 0.5 Ω, §
Pain experienced due to movements of the probe (mean ± SD)	2.6 ± 1.5	1.0 ± 0.8 Ω	0.2 ± 0.4 Ω, §
Pain experienced during laser application (mean ± SD)	0.6 ± 0.8	0.3 ± 0.5 Ω	0.1 ± 0.4 Ω

Ω Statistical significant difference with first laser application
§ Statistical significant difference with second laser application

Table 3[Previously published[23]]. Sexual function evaluation using the FSFI at baseline and at 12 weeks after pulsed CO2 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