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ORIGINAL ARTICLE

Erectile Dysfunction

Efficacy and safety of low-intensity shockwave therapy plus tadalafil 5 mg once daily in men with type 2 diabetes mellitus and erectile dysfunction: a matched-pair comparison study

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Low-intensity extracorporeal shockwave therapy (LiESWT) represents a promising treatment for patients with erectile dysfunction (ED). We investigated the efficacy of LiESWT combined with tadalafil 5 mg once daily in men with type 2 diabetes mellitus (T2DM) and ED and compared LiESWT protocols administering different number of shockwaves. We performed a retrospective matched-pair comparison using data from a prospectively maintained database. Seventy-eight patients who received tadalafil 5 mg once daily for 12 weeks + LiESWT performed with an electrohydraulic source for 3 weeks (Group A) were matched 1:1 to patients who received tadalafil 5 mg once daily alone for 12 weeks (Group B). A subgroup analysis was performed according to the number of shockwaves delivered during each session (1500, 1800, and 2400 in subgroup A1, A2, and A3, respectively). The mean International Index of Erectile Function-5 (IIEF-5) score variations with respect to baseline recorded at 4, 12, and 24 weeks after the end of the treatment were investigated as treatment outcomes. The mean IIEF-5 scores significantly improved in all groups and subgroups at 4-week follow-up without intergroup differences. At 12- and 24-week follow-up, the mean IIEF-5 improvement was significantly higher among patients in the A3 subgroup (+5.0 ± 2.1 [$P < 0.001$] and +4.7 ± 2.3 [$P < 0.001$], respectively). The combined approach with tadalafil 5 mg once daily and LiESWT with a protocol involving 2400 shockwaves provides significant advantages in terms of IIEF-5 improvement and durability compared to tadalafil 5 mg once daily alone in patients with T2DM and ED.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent chronic disorders in humans. According to the World Health Organization Global Report on Diabetes, the number of patients with DM has risen from 108 million in 1980 to 422 million in 2014.[1] Over the same period, the global prevalence of the disease among adults has risen from 4.7% to 8.5%.[1–5] DM exerts detrimental effects on multiple body systems including blood vessels, heart, eyes, kidneys, hormones, and nerves.[1–5] Moreover, it has been associated with physical, cognitive, and emotional disabilities.[2–6] Erectile dysfunction (ED) represents a well-known complication of DM. The prevalence of ED in men aged 40–70 years was estimated to be 52% in the Massachusetts Male Aging Study.[7] This percentage grows up to 59.1% in men with DM and is significantly higher among patients suffering from type 2 DM (T2DM).[8] Diabetic patients with ED represent a subset of difficult-to-treat ED population because the efficacy of oral therapies is lower if compared to non-diabetic individuals.[4,9] Low-intensity extracorporeal shockwave therapy (LiESWT) represents one of the newest and most promising treatment modalities for patients with ED.[10,11] The rationales behind this treatment involve tissue regeneration and neoangiogenesis.[12] Evidences from *in vitro* experiments demonstrated that vascular shear stress induced by extracorporeal shockwaves stimulates the release of angiogenic factors such as vascular endothelial growth factor, endothelial nitric oxide synthase, and proliferating cell nuclear antigen, thus ameliorating local blood supply and hemodynamics.[12] The controlled generated microtrauma can also increase macrophage activity and synthesis of cellular proteins and may potentially enhance the recruitment and differentiation of stem cells.[12] From a clinical point of view, the efficacy of extracorporeal shockwaves in the field of andrology has been demonstrated by several studies.[13–16] Moreover, this treatment is well tolerated and can be performed without anesthesia in an outpatient setting.[14–16] Despite the positive results of LiESWT in the general ED population, to date, there are very few evidences about its efficacy in combination with type 5 phosphodiesterase inhibitors (PDE5Is) in more difficult cases of ED. Nowadays, various LiESWT devices are available in the market based on electrohydraulic,

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electromagnetic, and piezoelectric generators. Moreover, although different LiESWT setup parameters and treatment protocols have been published, to date, the optimal treatment strategy remains to be defined.[17,18] We aimed to investigate the efficacy of the combined approach involving LiESWT performed with an electrohydraulic source and tadalafil 5 mg once daily in patients with T2DM and ED and to compare LiESWT protocols characterized by different numbers of shockwaves administered during each session.

MATERIALS AND METHODS

Study design

We performed a retrospective matched-pair comparison analysis using a prospectively maintained database (T2DM-ED Dataset, University of Naples "Federico II," Naples, Italy). The sample sizes were calculated fixing $\alpha = 0.05$ as the probability of rejecting the null hypothesis, $\beta = 0.2$ as the probability of failing to reject the null hypothesis, and expecting a mean difference between groups of 1 with a standard deviation (s.d.) of 2 points of International Index of Erectile Function (IIEF) score. A minimum number of 63 patients for each group would have been enough; however, 15 patients for each group have been added to minimize the approximation effect. The medical charts of 78 men with T2DM and ED who underwent therapy with tadalafil 5 mg once daily bedtime for 12 weeks and LiESWT performed with an electrohydraulic source between December 2015 and September 2018 were recorded (Group A). Control group consisted of 78 patients with T2DM and ED who received therapy with tadalafil 5 mg once daily bedtime alone for 12 weeks (Group B). Patients were matched in a one-to-one fashion according to age, baseline IIEF-5 score, and smoking (pack-years) status. Exclusion criteria were previous therapy for ED, incomplete data, follow-up <24 weeks, hypogonadism, and previous pelvic surgery. LiESWT was performed using an electrohydraulic unit with a focused shockwave source (Omnispec ED1000, Medispec Ltd., Yehud, Israel). Shockwaves were delivered to the distal, mid, and proximal penile shaft, as well as to the left and right crura. The duration of each LiESWT session was about 20 min. Energy density was set at 0.09 mJ mm^{-2} and frequency at 120 min^{-1}. The number of shockwaves delivered during each session varied from 1500 to 2400. Treatment protocol consisted of two treatment sessions per week for 3 weeks, 3 days apart within the week. Patients in Group A received LiESWT during the first 3 weeks of treatment with tadalafil. The following data were extracted and compared: age, body mass index (BMI), smoking status, duration of DM, hypertension, triglyceride level, glycated hemoglobin (HbA1c) levels, baseline IIEF-5 score, number of shockwaves delivered during each LiESWT session, mean IIEF-5 scores recorded at 4, 12, and 24 weeks after the end of the treatment, and complications. A subgroup analysis was performed according to the number of shockwaves delivered during each session: subgroup A1 (1500 shockwaves, $n = 26$), subgroup A2 (1800 shockwaves, $n = 26$), and subgroup A3 (2400 shockwaves, $n = 26$). The man IIEF-5 score variation with respect to baseline was investigated as primary outcome. The secondary outcome was mean IIEF-5 variation according to the number of shockwaves delivered during each session.

Statistical analyses

Descriptive data of continuous variables were expressed as mean and s.d. and were compared using Student's *t*-test or Mann–Whitney U test, as appropriate. Proportions were expressed as absolute numbers and percentages and compared using the Chi-squared test or the Yates' or Fisher's test, as appropriate. The one-way analysis of variance (ANOVA) was performed to compare the mean IIEF-5 score changes with respect to baseline in subgroups A1, A2, A3, and B. $P < 0.05$ was taken to indicate statistical significance. All statistical analyses were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) software.

Ethics code

The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients gave their informed consent to the collection of clinical data in a prospectively maintained database and to the use of these data for research purposes. Formal approval by the Institutional Review Boards was not required as we retrospectively used data that were completely anonymous.

RESULTS

The baseline patients' characteristics are reported in **Table 1**. The mean baseline IIEF-5 scores (mean±s.d.) in Groups A and B were 15.5 ± 2.8 and 15.3 ± 2.2, respectively. The baseline IIEF-5 scores (mean±s.d.) in subgroups A1, A2, and A3 were 15.2 ± 3.7, 16.4 ± 1.5, and 14.9 ± 2.7, respectively ($P = 0.160$). The IIEF-5 scores (mean±s.d.) recorded in the four subgroups at 4, 12, and 24 weeks' intervals after the end of the treatments are shown in **Figure 1**.

A statistically significant improvement of mean IIEF-5 scores with respect to baseline was evident in all groups at 4-week follow-up. In details, the IIEF-5 scores (mean±s.d.) improved by 3.9 ± 1.9 and by 2.9 ± 1.5 in Groups A and B, respectively. The changes (mean±s.d.)

Table 1: Baseline patients' characteristics

Variable	*Group A (n=78)*	*Group B (n=78)*	*P*
Age (year), mean±s.d.	56.0±9.6	58.2±3.2	0.063
Diabetes duration (year), mean±s.d.	9.1±6.0	10.0±4.6	0.327
HbA1c (mg dl^{-1}), mean±s.d.	7.0±1.3	6.7±0.5	0.072
Smokers, *n* (%)	39 (50.0)	45 (57.7)	0.064
Packs per year, mean±s.d.	36.4±14.0	40.9±21.9	0.263
Hypertension, *n* (%)	31 (39.7)	38 (48.7)	0.259
BMI (kg cm^{-2}), mean±s.d.	27.4±4.0	28.3±3.8	0.132
Triglycerides (mg dl^{-1}), mean±s.d.	128.7±35.4	121.1±52.8	0.291
IIEF-5 score, mean±s.d.	15.5±2.8	15.3±2.2	0.621

BMI: body mass index; IIEF: International Index of Erectile Function; s.d.: standard deviation; HbA1c: glycated hemoglobin

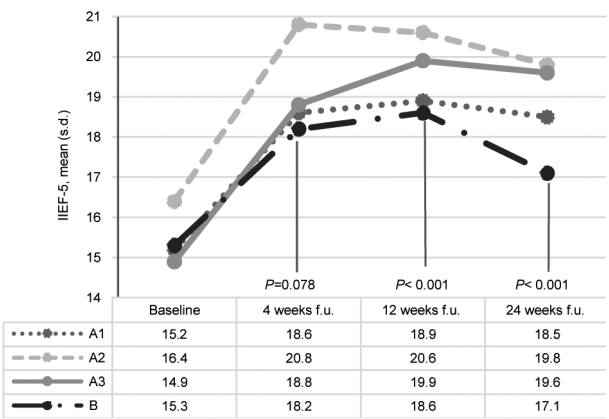

Figure 1: Mean IIEF-5 scores in various subgroups at different follow-ups (f.u.). *P* values refer to the results from the intergroup ANOVA performed to compare mean changes with respect to baseline. IIEF-5: International Index of Erectile Function-5; ANOVA: one-way analysis of variance. Group A1: tadalafil 5 mg once daily + 1500 shockwaves; A2: tadalafil 5 mg once daily + 1800 shockwaves; A3: tadalafil 5 mg once daily + 2400 shockwaves per session; Group B: tadalafil 5 mg once daily.

recorded in the subgroups A1, A2, and A3 were +3.4 ± 2.5, +4.4 ± 1.6, and +3.9 ± 1.3, respectively ($P < 0.001$ with respect to baseline in all groups). The ANOVA analysis failed to find statistically significant intergroup differences in terms of mean IIEF-5 improvement at this follow-up interval ($P = 0.078$).

At 12-week follow-up, the IIEF-5 score (mean±s.d.) variations with respect to baseline were +4.3 ± 2.3 and +3.3 ± 1.6 in Groups A and B, respectively ($P < 0.001$ in both groups). At this follow-up interval, the IIEF-5 score (mean±s.d.) improvement was significantly higher in patients belonging to the A3 subgroup (+5.0 ± 2.1, $P < 0.001$) (**Figure 1**).

At 24-week follow-up, the IIEF-5 (mean±s.d.) variations with respect to baseline in Groups A and B were +3.8 ± 2.4 and +1.8 ± 1.7, respectively ($P < 0.001$ in both groups). The IIEF-5 (mean±s.d.) improvement with respect to baseline was higher among patients in the A3 subgroup (+4.7 ± 2.3) and lower in the Group B (+1.8 ± 1.7) (both $P < 0.001$; **Figure 1**). All patients tolerated the treatments well and there were no serious treatment-related adverse events.

DISCUSSION

To our knowledge, we investigated for the first time the efficacy of the combination of LiESWT and tadalafil 5 mg once daily in patients with T2DM and ED as well as the impact of the number of shockwaves delivered during each session in this clinical scenario. Our results demonstrate that the combined approach provides advantages in terms of both magnitude of mean IIEF-5 score improvement and durability of results if compared to tadalafil 5 mg once daily alone. These results have relevant pathophysiological and clinical implications. The etiology of ED in DM patients is multifactorial.[19] The proposed mechanisms include vasculopathy, neuropathy, visceral adiposity, insulin resistance, and hypogonadism.[20] Microvascular impairment is responsible for ischemic damages involving the peripheral circulation.[20] Moreover, both somatic and autonomic neuropathies may contribute to DM-induced ED due to the impairment of sensory impulses from the penis to the reflexogenic erectile center and to the reduced or absent parasympathetic activity necessary for relaxation of the smooth muscle of the corpus cavernosum.[20] Men with DM tend to present with more severe ED and all PDE5Is are less efficacious in this clinical scenario.[20–22] Although preclinical evidences on diabetic rats demonstrated that chronic low-dose administration of tadalafil is associated with substantial morphological improvement of the structure of penile cavernous tissue with increased smooth muscles and elastic tissue, evidences in humans are controversial and the management of diabetic ED patients remains challenging in many cases.[22] In the last decade, LiESWT has emerged as a potential treatment strategy for patients with severe ED who are inadequate responders or nonresponders to PDE5Is.[18] The current guidelines recommend this treatment modality in patients with mild organic ED of poor responders to PDE5Is.[18] Results from a recently published survey showed that about 91.2% of experts in sexual medicine would consider the treatment specifically for vasculogenic ED and that 81.6% would combine it with PDE5Is.[23] Preclinical studies have demonstrated that LiESWT can improve DM-associated ED by promoting regeneration of neuronal nitric oxide synthase-positive nerves, endothelium, and smooth muscle in the penis.[24] These beneficial effects appear to be in part mediated by recruitment of endogenous mesenchymal stem cells.[24] Results from the present study point out some relevant clinical findings.

First, although both tadalafil 5 mg once daily alone and the combined approach involving tadalafil 5 mg once daily and LiESWT provided statistically significant improvements in terms of mean IIEF-5 score, the magnitude of variation observed in patients receiving the latter treatment modality was significantly greater at 12- and 24-month follow-up in the subgroup of patients who received 2400 shockwaves per session. These results corroborate data from a recently published meta-analysis demonstrating a statistically significant higher improvement of erectile function in patients receiving LiESWT combined with PDE5Is with respect to patients receiving LiESWT alone.[25] The rationales behind these findings are poorly understood. Preclinical evidences deriving from rat models with T2DM have demonstrated a synergistic effect of LiESWT and PDE5Is and that the benefits provided by LiESWT are independent from nitric oxide and cyclic guanosine monophosphate pathways.[26] In humans, both synergistic and/or an additive interaction could be hypothesized and further investigations are needed to elucidate these aspects. Several studies demonstrated that the biological efficacy of LiESWT is dosage dependent. The meta-analysis performed by Man and Li showed that protocols adopting greater number of shockwaves per treatment reported a significant increase of erectile function compared with protocols delivering fewer shockwaves.[25] However, a substantial heterogeneity exists in terms of devices adopted and patients' characteristics, and the optimal treatment protocol remains to be defined.

Durability of the response following cessation of treatments for ED represents a further relevant finding. In their exploratory study, Porst *et al*.[27] investigated the durability of the response following treatment with tadalafil 5 mg once daily. Sixty-three (46.3%) of the 136 patients had a durable response 4 weeks after cessation of the treatment. Interestingly, more patients who experienced durability of response had organic ED than those who did not.[27] To date, however, the issue of durability following ED treatment remains largely underinvestigated.[27] Results from the present study confirm existing evidences about the durability of efficacy of chronic PDE5Is in patients with organic ED and provide novel data about combined treatments involving chronic PDE5Is and LiESWT and longer follow-up. Indeed, the considerable increase in IIEF-5 score we observed in the control group after 4 weeks from the end of treatment was in line with findings from the aforementioned study. Moreover, we confirmed the durability of efficacy with tadalafil 5 mg once daily alone also at 12-week follow-up. Interestingly, at 24-week follow-up, a decline was evident in patients who received tadalafil alone while efficacy remained stable in patients who received the combined approach. Further investigations are needed to elucidate the pathophysiology underlying the slow decline of ED in the long term in patients with organic ED after therapy with tadalafil 5 mg. The proposed mechanisms of action of LiESWT in ED patients may represent the rationale behind the durability of the results observed in patients undergoing the combined approach. Indeed, energy from the acoustic waves delivered by LiESWT has been hypothesized to stimulate cellular and molecular pathways increasing the expression of local growth factors, improving endothelial function, angiogenesis, and regeneration of nerve fibers.[28] Consequently, LiESWT has the potential to restore natural erections and to cure the disease.[28] Based on the results from the present study, diabetic ED patients should be counseled about the potential advantages provided by a combined approach involving LiESWT and daily tadalafil in terms of IIEF-5 improvement and durability of clinical efficacy.

The main limitations of the present study are the retrospective design and the limited number of patients regarding the analysis of the subgroups. The latter aspect impairs the possibility to identify potential predictive factors of treatment efficacy and persistence of the response. Overall, results from the present study should be considered preliminary and further, well designed, prospective randomized studies

with long-term follow-up are needed to confirm the benefits of LiESWT in combination with PDE5Is in diabetic patients with ED, to explore long-term durability of the efficacy, and to elucidate the synergistic and/or additive mechanisms of action.

CONCLUSIONS

The combined approach with tadalafil 5 mg once daily and LiESWT performed with a protocol involving 2400 shockwaves during each session provides significant advantages in terms of IIEF-5 improvement and durability of results with respect to tadalafil 5 mg once daily alone in patients with T2DM and ED.

AUTHOR CONTRIBUTIONS

PV, VM and M Creta contributed to the conception and design of the study. M Capece, RLR, FP and AC acquired all data. M Capece, LS, RLR and FP analysed and interpreted the data. M Capece, M Creta, LS, RLR and FP contributed to draft the article. PV, VM and AC revised the final article for intellectual content.

COMPETING INTERESTS

All authors declared no competing interests.

REFERENCES

1 World Health Organization. Global Report on Diabetes. Geneva: World Health Organization; 2016.
2 Azab SS, El Din Hosni H, El Far TA, Ismail NN, El Bakdady YK, *et al*. The predictive value of arteriogenic erectile dysfunction for coronary artery disease in men. *J Sex Med* 2018; 15: 880–7.
3 Liu Q, Zhang Y, Wang J, Li S, Cheng Y, *et al*. Erectile dysfunction and depression: a systematic review and meta-analysis. *J Sex Med* 2018; 15: 1073–82.
4 Imbimbo C, Creta M, Gacci M, Simonato A, Gontero P, *et al*. Patients' desire to preserve sexual activity and final decision for a nerve-sparing approach: results from the MIRROR (Multicenter Italian Report on Radical Prostatectomy Outcomes and Research) study. *J Sex Med* 2011; 8: 1495–502.
5 Creta M, Riccio R, Chiancone F, Fusco F. Androgens exert direct neuroprotective effects on the brain: a review of pre-clinical evidences. *J Androl Sci* 2010; 17: 49–55.
6 Lizza ER, Rosen RC. Definition and classification of erectile dysfunction: report of the Nomenclature Committee of the International Society of Impotence Research. *Int J Impot Res* 1999; 11: 141–3.
7 Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol* 1994; 151: 54–61.
8 Gentile I, Fusco F, Buonomo AR, Scotto R, Zappulo E, *et al*. Prevalence and risk factors of erectile dysfunction in patients with hepatitis B virus or hepatitis C virus or chronic liver disease: results from a prospective study. *Sex Health* 2018; 15: 408–12.
9 Li X, Zhao Q, Wang J, Wang J, Dai H, *et al*. Efficacy and safety of PDE5 inhibitors in the treatment of diabetes mellitus erectile dysfunction: protocol for a systematic review. *Med (Baltimore)* 2018; 97: e12559.
10 Chung E, De Young L, Brock GB. Investigative models in erectile dysfunction: a state-of-theart review of current animal models. *J Sex Med* 2011; 8: 3291–305.
11 Lue TF. Erectile dysfunction. *N Engl J Med* 2000;342: 1802–13.
12 Chung E, Wang J. A state-of-art review of low intensity extracorporeal shock wave therapy and lithotripter machines for the treatment of erectile dysfunction. *Expert Rev Med Devices* 2017; 14: 929–34.
13 Ruffo A, Capece M, Prezioso D, Romeo G, Illiano E, *et al*. Safety and efficacy of low intensity shockwave (LISW) treatment in patients with erectile dysfunction. *Int Braz J Urol* 2015; 41: 967–74.
14 Palmieri A, Imbimbo C, Creta M, Verze P, Fusco F, *et al*. Tadalafil once daily and extracorporeal shock wave therapy in the management of patients with Peyronie's disease and erectile dysfunction: results from a prospective randomized trial. *Int J Androl* 2012; 35: 190–5.
15 Palmieri A, Imbimbo C, Longo N, Fusco F, Verze P, *et al*. A first prospective, randomized, double-blind, placebo-controlled clinical trial evaluating extracorporeal shock wave therapy for the treatment of Peyronie's disease. *Eur Urol* 2009; 56: 363–9.
16 Clavijo RI, Kohn TP, Kohn JR, Ramasamy R. Effects of low-intensity extracorporeal shockwave therapy on erectile dysfunction: a systematic review and meta-analysis. *J Sex Med* 2017; 14: 27–35.
17 Lu Z, Lin G, Reed-Maldonado A, Wang C, Lue TF. Low-intensity extracorporeal shock wave treatment improves erectile function: a systematic review and meta-analysis. *Eur Urol* 2017; 71: 223–33.
18 Hatzimouratidis K, Giuliano F, Moncada I, Muneer A, Salonia A, *et al*. EAU Guidelines on Erectile Dysfunction, Premature Ejaculation, Penile Curvature and Priapism; 2018. Available from: https://uroweb.org/guideline/male-sexual-dysfunction/. [Last accessed in December 2018].
19 Bolat MS, Cinar O, Akdeniz E, Aşcı R. Low dose daily versus on-demand high dose tadalafil in diabetic patients with erectile and ejaculatory dysfunction. *Int J Impot Res* 2018; 30: 102–7.
20 Maiorino MI, Bellastella G, Esposito K. Diabetes and sexual dysfunction: current perspectives. *Diabetes Metab Syndr Obes* 2014; 7: 95–105.
21 Malavige LS, Levy JC. Erectile dysfunction in diabetes mellitus. *J Sex Med* 2009; 6: 1232–47.
22 Mostafa ME, Senbel AM, Mostafa T. Effect of chronic low-dose tadalafil on penile cavernous tissues in diabetic rats. *Urology* 2013; 81: 1253–9.
23 Fode M, Lowenstein L, Reisman Y. Low-intensity extracorporeal shockwave therapy in sexual medicine: a questionnaire-based assessment of knowledge, clinical practice patterns, and attitudes in sexual medicine practitioners. *Sex Med* 2017; 5: e94–8.
24 Qiu X, Lin G, Xin Z, Ferretti L, Zhang H, *et al*. Effects of low-energy shockwave therapy on the erectile function and tissue of a diabetic rat model. *J Sex Med* 2013; 10: 738–46.
25 Man L, Li G. Low-intensity extracorporeal shock wave therapy for erectile dysfunction: a systematic review and meta-analysis. *Urology* 2018; 119: 97–103.
26 Assaly-Kaddoum R, Giuliano F, Laurin M, Gorny D, Kergoat M, *et al*. Low intensity extracorporeal shock wave therapy improves erectile function in a model of type II diabetes independently of NO/cGMP pathway. *J Urol* 2016; 196: 950–6.
27 Porst H, Glina S, Ralph D, Zeigler H, Wong DG, *et al*. Durability of response following cessation of tadalafil taken once daily as treatment for erectile dysfunction. *J Sex Med* 2010; 7: 3487–94.
28 Young Academic Urologists Men's Health Group, Fode M, Hatzichristodoulou G, Serefoglu EC, Verze P, *et al*. Low-intensity shockwave therapy for erectile dysfunction: Is the evidence strong enough? *Nat Rev Urol* 2017; 14: 593–606.