Research Square

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Improves Symptoms and Urinary Biomarkers in Refractory Interstitial Cystitis/Bladder Pain Syndrome Patients Randomized to Extracorporeal Shock Wave Therapy Versus Placebo

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Abstract

Extracorporeal shock wave therapy (ESWT) has been shown to improve symptoms in patients with interstitial cystitis/bladder pain syndrome (IC/BPS); however, there is a lack of objective evidence. We measured change of urinary biomarker levels in 25 patients with IC/BPS received ESWT or placebo once a week for 4 weeks. Urines were collected from participants at baseline, 4 and 12 weeks post treatment. A representative 41 inflammatory growth factors, cytokines, and chemokines in urine were measured using a MILLIPLEX immunoassay kit. Symptom bother was assessed by O'Leary-Sant symptom scores (OSS), and visual analog scale (VAS) for pain.

The ESWT group exhibited a significant reduction in the OSS and VAS compared to the placebo group 4 weeks post-treatment ($p < 0.05$), and the effects were persistent at 12 weeks. The difference in urinary markers change in ESWT vs placebo was $p= 0.054$ for IL4, $p=0.013$ for VEGF, and $p= 0.039$ for IL9 at 4 weeks. The change of urine biomarker was not significant in other biomarkers or all the measured proteins at 12 weeks. The current data suggest that IL4, IL9, and VEGF mediation may be involved in its pathophysiologic mechanisms and response to LESW treatment.

Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic disease characterized by symptoms of unpleasant sensation, pain, pressure, and discomfort perceived to be related to the urinary bladder, associated with lower urinary tract symptoms, in the absence of infection or other identifiable causes.[1] Treatment of refractory IC/BPS patients includes hydrodistension, oral medications, and intravesical therapy has met with only limited efficacy and there is an unmet need for developing new therapy for IC/BPS.[2]

The etiology and pathogenesis of IC/BPS are multifactorial and several studies have attributed to the exhibited symptoms to an increase in inflammatory cells infiltration, hypoxia-inducible factor-1α, VEGF, and apoptosis[3,4] noted on histopathological and molecular studies. Thus, the treatment of IC/BPS aiming at regulation of inflammatory reaction and regeneration or repair of urothelium defect could be an attractive option for the management of refractory IC/BPS.

Low energy shock wave (LESW), known to exert anti-inflammatory, anti- apoptotic effects, and improve tissue repair, has been applied for the treatment of urological disease, including erectile dysfunction, and chronic prostatitis chronic pelvic pain syndrome.[5, 6] Furthermore, recent publications have been extended into the field of bladder dysfunction and demonstrated therapeutic effects of LESW on overactive bladder and IC/BPS.[7, 8] The approaches used for mechanistic studies on efficacy of LESW in animal model[6,9] need to be modified for human confirmation . Towards that end we assessed the change in urine levels of multiple growth factors, cytokines, chemokines, and clinical symptoms of IC/BPS patients at baseline and after LESW or placebo treatment.

Materials And Methods

Study Design

The Urinary Marker study was a planned exploratory supplementary study to the Low Energy Shock Wave (LESW) for the treatment of IC/BPS — a multicenter, randomized, double-blind, placebo-controlled, prospective study

(ClinicalTrials.gov number, NCT03619486). Details of the trial design and methods have been previously published. [8]

The current study only included patient population from one center for urinary marker analysis. The IC/BPS confirmed patients were randomly assigned to receive (1) LESW or (2) placebo group in a 1:1 ratio after the review and approval by the Institutional Review Board of the hospital (Chang Gung Memorial Hospital IRB 201800525A3), and was in compliance with the ethical principles of Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. Informed consent was obtained from patients before any study procedures were performed.

Study Population

Patients with IC/BPS, who aged 20 years or above and had failed at least 6 months of conventional treatments, were enrolled. The diagnosis of IC/BPS was established based on characteristic symptoms and cystoscopic findings of glomerulations, petechia, or mucosal fissures upon hydrodistention. The inclusion and exclusion criteria are listed in the Appendix of our previous publication.[8]

Treatment

The procedures were done as an office procedure without any anesthesia. Studied patients were placed in a supine position with bladder distended with up to 50 to 100 cc of urine volume as detected by transabdominal ultrasonography. The shock wave applicator (LITEMED LM ESWT mini system, Taiwan) or placebo applicator were gently placed directly on the ultrasound transmission gel over the skin surface of suprapubic region above the urinary bladder once a week for 4 weeks, with 2000 shocks, frequency of 3 pulses per second, and maximum energy flow density 0.25 mJ/mm^2. [8] Follow-up visits were scheduled at one week, four weeks, and three months post-treatment.

Urine Processing

Urine samples were collected at baseline, post treatment 4 weeks and 12 weeks at KCGMH. Specimens were kept on ice or at 4° C for short times until stored at −80° C (within 2-4 hrs). The urine was centrifuged (12000rpmx 15 mins) at 4° C, and the supernatants were directly analyzed.

Multiplex Analysis

The urine sample were stored at -80°C until analysis by MILLIPLEX MAP Human Cytokine/Chemokine Panel (Merck Millipore, Billerica, MA), a magnetic bead-based immunology multiplex assay, which can simultaneously quantify the following 41 human cytokines: sCD40L, EGF, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF, Eotaxin/CCL11, PDGF-AA. The samples were processed in duplicate according to the manufacturer's instructions.[10] Cytokine concentrations were normalized to urine creatinine content.

Outcome Measures

The average changes in O'Leary-Sant symptom scores (OSS), a 3-day voiding diary, Visual Analog Pain Scale (VAS, 0–10), global response assessment (GRA) with categorizations (-3, -2, -1, 0, 1, 2, 3), uroflowmetry, and residual urine detected by ultrasonography at 1, 4, and 12 weeks were compared to baseline and between groups after treatment.

Statistical Methods

The average change in values from baseline at 1, 4, and 12 weeks post-treatment in studied parameters, scores or outcome measures, and net changes of each efficacy item between treatment group and the controlled groups were analyzed using generalized estimating equation. The patient-responded global assessment was analyzed using Fisher's exact test between the treatment and the controlled groups. All statistical assessments were considered significant at $P<0.05$. Statistical analyses were performed using SPSS version 22.0 statistical software (SPSS Inc., Chicago, IL).

Results

Patient disposition

A total of 27 patients were screened and 25 eligible patients were randomly allocated into two subgroups. One patient withdrew consent and thereby did not receive any treatment (Fig. 1). The final intent-to-treat population consisted of 24 subjects, including 13 in ESWT, and 11 in placebo groups of whom all subjects completed the primary endpoint evaluation (i.e. four weeks post-treatment). The baseline characteristics were comparable across the treatment groups except pain and voided volume, which was significant less in the placebo group (Table 1 and 2). None of the participants had Hunner lesions, but all of them showed some evidence of bladder inflammation from pathological findings.

Efficacy and Safety

Statistically significant improvements occurred in the pain scale and OSS, including ICSI and ICPI, from baseline to 1 week, 4 weeks and 12 weeks after treatment in ESWT group (Table 2; Fig.2) compared to an insignificant change in the placebo group for OSS, ICSI, ICPI, and VAS. The difference in improvement

between ESWT vs placebo for OSS was -5.4 (-8.3,- 2.5) vs -0.9 (-4.0, 2.2) (95% CI; p= 0.014) at 4 weeks and the efficacy was maintained till 12 weeks (Fig. 2). The difference in VAS improvement was-2.0 (-3.1, -0.9) vs -0.6 (-1.4, 0.3) (95% CI; p= 0.018) at 4 weeks, and the efficacy lasted till 12 weeks. No patient had urinary incontinence, retention or infection with ESWT or placebo treatment.

Urine biomarkers

Of the 41 candidate markers, 19 markers were measurable in urine and analyzed. Biomarkers levels at baseline, 4 and 12 weeks following treatment are shown in Table 3. No baseline differences were found between ESWT and placebo groups. IL4, IFNα2, and VEGF levels were significantly increased at 4 weeks in placebo group, and IL9, and Flt3 levels were significantly increased at 4 weeks post ESWT treatment. The difference between ESWT vs placebo for the change in urinary markers was -0.0 (-0.3, 0.2) vs 0.4 (-0.0, 0.9) (95% CI; IL4, p= 0.054) and -0.5 (-1.3, 0.7) vs 1.2 (0.3, 2.1) (95% CI; VEGF, p=0.013), and 0.2 (-0.0, 0.4) vs -0.0 (-0.1, 0.0) (95% CI; IL9, p= 0.039) at 4 weeks (Table.4).

Discussions

Similar to previous reports, ESWT was associated with a statistically significant decrease in OSS and VAS pain scale at 4 weeks post-treatment with lower intensity of placebo effect. In contrast to the previous report from our group,[8] placebo group has no statistically significant change in OSS and VAS pain scale at 4 weeks post-treatment, which is reflected in the detection of significant difference in symptomatic improvement between ESWT vs placebo that eluded us in previous study. The lower intensity of placebo effect in this study may be due to a more homogenous patient population in a single study center.

The main goal of the current study was to identify changes in urine biomarkers pre and post-treatment and investigation to the mechanistic understanding of ESWT efficacy on IC/BPS. It has been known that ischemia/hypoxia condition occurs in the bladder mucosa and contributes to IC/BPS symptoms.[11] VEGF is a signal protein that stimulates the formation of blood vessels to restore the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions. Bladder urothelium of IC/BPS patients has been shown to exhibit significantly higher expressions of VEGF, which then induces bladder fibrosis and reduces bladder capacity after chronic inflammation.[11] Furthermore, VEGF expression level was associated with the grade of bladder pain.[12] A previous study showed that intravesical botulinum toxin A injection reduced the expression of VEGF associated with a concomitant decrease in inflammatory marker levels in patients with IC/BPS.[13] Anti-vascular endothelial growth factor treatment has been demonstrated to decrease bladder pain in animal model of cyclophosphamide cystitis.[14] In the present study, urine VEGF level was significantly increased at 4 weeks follow-up in placebo group. However, the ESWT group showed a reduction of VEGF expressions at 4 weeks. Our study suggests that ESWT has the potential to decrease urinary VEGF expression and alleviate IC/BPS symptoms.

Sugaya et al. have reported that about 35% of the patients with interstitial cystitis had some type of allergic or autoimmune disease,[15] which is associated with overproduction of IL-4. IC/BPS is

characterized by an increased number of mast cells in the detrusor and release of cytokines, including IL-4.[16] Another study from rat model demonstrated that intravesical HCl induced an increase of mast cells in the bladder, and ESWT can induce a decrease of mast cells and alleviate the HCl induced inflammatory effects.[17] Our current results showed that IL-4 was significantly increased at 4 weeks in the placebo group, whose increase was suppressed by ESWT. We suggested that ESWT might have effects on immune modulation through mast cells IL4 reaction.

IL-9 is a cytokine secreted by CD4+ helper cells that regulates a variety of hematopoietic cells, including stimulation of cell proliferation and prevention of apoptosis.[18] The current results showed that urinary IL-9 is increased in ESWT group, which was not observed in the placebo group at 4 weeks. This finding might indicate an immune modulation effect of ESWT on IC/BPS patients.

The limitation of this study is the lack of a non-IC/BPS control arm and small sample size. Further control study with larger sample size is necessary to elucidate the actual therapeutic efficacy and urine biomarker change of ESWT.

In conclusion, our clinical study demonstrated that compared to placebo, ESWT in IC/BPS patients improved OSS and pain scale in association with some urine cytokine and chemokine changes. Further study with large patient number is necessary to elucidate the real role of ESWT on urinary biomarker changes.

Declarations

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Author Contributions

Y-C.S. W-C.L. Y-C.C designed the study and all authors interpreted the data and prepared the manuscript and reviewed the manuscript.

References

1. Hanno PM, Erickson D, Moldwin R et al. American Urological Association. Diagnosis and treatment ofinterstitial cystitis/bladder pain syndrome: AUA guideline J Urol 2015;193:1545-53.
2. Meng E, Hsu YC, Chuang YC. Advances in intravesical therapy for bladder pain syndrome (BPS)/interstitial cystitis (IC). Low Urin Tract Symptoms.2018;10:3-11.
3. Jiang, YH., Kuo, YC., Jhang, JF. et al. Repeated intravesical injections of platelet-rich plasma improve symptoms and alter urinary functional proteins in patients with refractory interstitial cystitis. Sci Rep. 2020 ;10 :15218.

4. Shie JH, Liu HT, Kuo HC. Increased cell apoptosis of urothelium mediated by inflammation in interstitial cystitis/painful bladder syndrome. Urology 2012;79:484.e7-13.
5. Liu T, Shindel AW, Lin G,Lue TF. Cellular signaling pathways modulated by low-intensity extracorporeal shock wave Int J Impot Res. 2019;31:170-176.
6. Lin CC, Huang YC, Lee YC, Chuang YC. New Frontiers for the Treatment of Interstitial Cystitis/Bladder Pain Syndrome - Focused on Stem Cells, Platelet-Rich Plasma, and Low-Energy Shock Wave. Int Neurourol J 2020; 24: 211-221.
7. Chen WC, Lu JH, Chueh KS, Shen MC, Liu LW, Long CY, Juan YS.Low-Intensity Extracorporeal ShockWave Therapy Ameliorates the Overactive Bladder: A Prospective Pilot Study. Lee YC, Chuang SM, Lin KL, Biomed Res Int. 2020, 6;2020:9175676
8. Chuang YC, Meng E, Chancellor M, Kuo HC. Pain reduction realized with extracorporeal shock wave therapy for the treatment of symptoms associated with interstitial cystitis/bladder pain syndrome-A prospective, multicenter, randomized, double-blind, placebo-controlled study. Neurourol Urodyn. 2020;39:1505-1514.
9. Wang HJ, Lee WC, Tyagi P, Huang CC, Chuang YC. Effects of low energy shockwave therapy on inflammatory moleculars, bladder pain, and bladder function in a rat cystitis Neurourol Urodyn. 2017;36:1440-1447.
10. Tyagi P, Barclay D, Zamora R, Yoshimura N, Peters K, et al. Urine cytokines suggest an inflammatory response in the overactive bladder: a pilot study. Int Urol Nephrol. 2010;42:629–35.
11. Lee JD, Lee MH. Increased expression of hypoxia-inducible factor-1α and ascular endothelial growth factor associated with glomerulation formation in patients with interstitial cystitis. Urology 2011; 78: 971.e11–15.
12. Kiuchi, H. *et al.* Increased vascular endothelial growth factor expression in patients with bladder pain syndrome/interstitial cystitis:its association with pain severity and glomerulations. *BJU Int.* **104**, 826–831 (2009).
13. Peng, C. H., Jhang, J. F., Shie, J. H. & Kuo, H. C. Down regulation of vascular endothelial growth factor is associated with decreased inflammation after intravesical OnabotulinumtoxinA injections combined with hydrodistention for patients with interstitial cystitis–clinical results and immunohistochemistry analysis. *Urology* **82**(1452), e1-6 (2013).
14. Lai HH, Shen B, Vijairania P, Zhang X, Vogt SK, Gereau RW 4th. Anti-vascular endothelial growth factor treatment decreases bladder pain in cyclophosphamide cystitis: a Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network animal model study. BJU Int. 2017;120:576-583.
15. Sugaya K, Nishijima S, Yamada T, Miyazato M, Hatano T, Ogawa Y. Molecular analysis of adrenergic receptor genes and interleukin-4/interleukin-4 receptor genes in patients with interstitial cystitis. J Urol . 2002;168:2668-71.
16. Bouchelouche K, Andresen L, Alvarez S, Nordling J, Nielsen OH, Bouchelouche P. Interleukin-4 and 13 induce the expression and release of monocyte chemoattractant protein 1, interleukin-6 and stem cell

factor from human detrusor smooth muscle cells: synergy with interleukin-1beta and tumor necrosis factor-alpha. J Urol. 2006;175:760-5.

17. Wang HJ, Tyagi P, Lin TK, Huang CC, Lee WC, Chancellor MB, Chuang TC. Low Energy Shock Wave Therapy Attenuates Mitochondrial Dysfunction and Improves Bladder Function in HCl induced Cystitis in Rats. Paper submitted
18. Perumal NB, Kaplan MH (2011). "Regulating IL9 transcription in T helper cells". Trends in Immunology. **32** (4): 146–50.

Tables

Table 1. Baseline patient characteristics

Variable	**Placebo** (n=12)	**ESWT** (n=13)	
	Mean (95%CI)	Mean (95%CI)	p value [a]
Age	57.8 (49.5, 66.0)	55.7 (48.3, 63.1)	0.687
No. Male/Female	5/6	3/10	0.340
Max bladder capacity (ml) [b]	618.6 (521.0, 716.2)	677.0 (595.6, 758.4)	0.322
Duration of disease (yr)	5.8 (4.2, 7.3)	6.2 (3.6, 8.8)	0.758

Values are presented as mean (95%CI).

*: P < 0.05, **: P < 0.01, ***: P < 0.005, ****: P < 0.0001

[a]: Comparison between ESWT and placebo (Two sample t-test).

[b]: During hydrodistention.

Table 2. Variables at baseline, 1, 4, and 12 weeks in patients in ESWT and placebo

Variable	Placebo (N=12)		ESWT (N=13)		_
	Mean (95% CI)	p value [a]	Mean (95% CI)	p value [a]	p value [b]
O' Leary-Sant score					
Baseline	25.6 (22.5, 28.6)		26.0 (22.6, 29.4)		0.834
Wk 1	23.6 (19.8, 27.3)	0.061	20.4 (16.9, 23.9)	**** < 0.0001	
Wk 4	24.8 (21.3, 28.3)	0.512	20.6 (16.6, 24.6)	**** < 0.0001	
Wk 12	25.1 (20.7, 29.6)	0.061	18.2 (13.2, 23.1)	**** < 0.0001	
ICSI					
Baseline	12.3 (10.1, 14.5)		12.7 (10.6, 14.8)		0.789
Wk 1	10.8 (8.1, 13.6)	0.056	9.3 (7.1, 11.5)	*** 0.001	
Wk 4	11.7 (8.9, 14.6)	0.511	9.2 (6.9, 11.5)	**** < 0.0001	
Wk 12	11.8 (8.4, 15.2)	0.958	7.9 (5.0, 10.9)	**** < 0.0001	
ICPI					
Baseline	13.3 (11.5, 15.0)		13.3 (11.8, 14.8)		0.954
Wk 1	12.7 (11.1, 14.4)	0.518	11.1 (9.4, 12.7)	*** 0.001	
Wk 4	13.1 (11.8, 14.4)	0.795	11.2 (9.4, 13.1)	*** 0.001	
Wk 12	13.3 (11.5, 15.1)	0.567	10.2 (8.0, 12.5)	*** 0.001	
VAS					
Baseline	5.3 (4.2, 6.3)		6.9 (5.8, 8.0)		* 0.017
Wk 1	5.1 (4.0, 6.2)	0.513	4.9 (3.4, 6.3)	**** < 0.0001	
Wk 4	4.8 (4.0, 6.1)	0.192	4.9 (3.3, 6.4)	**** < 0.0001	
Wk 12	5.2 (3.1, 7.3)	0.862	4.7 (3.0, 6.4)	**** < 0.0001	
FBC (ml)					
Baseline	282.1 (197.3, 366.8)		315.4 (258.0, 372.8)		0.456

Wk 1)	238.9 (163.5, 314.3)	* 0.037	287.7 (231.9, 343.5)	0.161	
Wk 4	294.6 (194.9, 394.2)	0.381	296.9 (225.8, 368.0)	0.359	
Wk 12	250.6 (142.0, 359.2)	0.222	293.2 (218.5, 367.8)	0.177	
Frequency					
Baseline	11.6 (7.7, 15.5)		11.1 (8.8, 13.4)		0.793
Wk 1	12.4 (9.0, 15.8)	0.376	10.2 (7.7, 12.7)	0.088	
Wk 4	12.0 (8.5, 15.8)	0.908	10.4 (7.2, 13.6)	0.331	
Wk 12	12.6 (7.2, 18.1)	* 0.022	9.8 (6.7, 13.0)	** 0.005	
Nocturia					
Baseline	2.6 (1.4, 3.8)		1.7 (0.7, 2.8)		0.196
Wk 1	2.9 (1.5, 4.3)	0.243	1.7 (0.7, 2.6)	0.792	
Wk 4	2.3 (1.0, 3.6)	0.380	1.6 (0.6, 2.7)	0.780	
Wk 12	3.9 (1.4, 6.4)	0.125	1.8 (0.7, 0.9)	0.889	
Qmax (ml/s)					
Baseline	14.4 (8.5, 20.2)		16.3 (12.0, 20.7)		0.533
Wk 1	17.5 (9.9, 25.2)	0.373	16.8 (13.4, 20.3)	0.663	
Wk 4 (N=11, 13)	17.6 (10.5, 24.6)	0.329	17.1 (11.9, 22.4)	0.705	
Wk 12 (N=8, 11)	21.1 (11.5, 30.6)	0.290	16.0 (12.9, 19.1)	0.873	
Voided Volume (ml)					
Baseline	172.0 (112.1, 231.8)		256.9 (187.5, 326.4)		* 0.034
Wk 1	185.0 (129.2, 240.8)	0.835	277.0 (199.4, 354.6)	0.316	
Wk 4	238.6 (150.0, 327.1)	0.075	248.8 (163.4, 334.3)	0.792	
Wk 12	243.1 (177.2, 308.9)	0.106	320.6 (210.8, 430.4)	* 0.038	
Residual Urine (ml)					
Baseline	25.0 (7.7, 42.4)		33.8 (17.0, 50.5)		0.408

Wk 1	32.1 (10.6, 53.6)	0.680	25.5 (15.7, 35.2)	0.303	
Wk 4	31.7 (9.1, 54.4)	0.415	28.8 (5.6, 51.9)	0.409	
Wk 12	31.3 (6.1, 56.5)	0.590	38.5 (13.7, 63.3)	0.748	
GRA (<2/≥2)					
Wk 1	9/2 (18.2%)		6/7 (53.8%)		0.105 [c]
Wk 4	8/3 (27.3%)		6/7 (53.8%)		0.240 [c]
Wk 12	8/1 (11.1%)		6/7 (53.8%)		0.074 [c]

Values are presented as mean (95% CI).

*: P < 0.05, **: P < 0.01, ***: P < 0.005, ****: P < 0.0001

[a]: Generalized estimating equation compared with baseline.

[b]: Comparison between corresponding time point of ESWT and placebo (Generalized estimating equation).

[c]: Comparison between corresponding time point of ESWT and placebo (Fisher's Exact Test).

Table 3. Cytokine at baseline, 1, 4, and 12 weeks in patients in ESWT and placebo

Cytokine (pg/mg of Creatinine)	Placebo (N=11)		ESWT (N=13)		-
	Mean (95% CI)	p value [a]	Mean (95% CI)	p value [a]	p value [b]
IL-1RA					
Baseline	7.9 (0.1, 15.7)		14.8 (-4.2, 33.9)		0.493
Wk 1	5.3 (1.1, 9.5)	0.685	35.4 (-26.2, 96.9)	0.305	
Wk 4	8.6 (2.7, 14.5)	0.921	19.0 (-3.3, 41.3)	0.936	
Wk 12	20.4 (-14.7, 55.5)	* 0.015	40.7 (-35.7, 117.0)	0.141	
IL-4					
Baseline	0.5 (0.0, 1.0)		0.5 (0.1, 0.9)		0.972
Wk 1	0.5 (0.0, 1.1)	0.629	0.3 (0.1, 0.5)	0.301	
Wk 4	0.9 (0.1, 1.7)	* 0.028	0.5 (0.1, 1.0)	0.776	
Wk 12	0.8 (-0.2, 1.7)	0.760	0.9 (0.2, 1.6)	**** < 0.0001	
IL-6					
Baseline	0.2 (0.1, 0.2)		0.2 (0.1, 0.2)		0.685
Wk 1	0.2 (0.1, 0.3)	0.834	0.2 (0.1, 0.4)	0.241	
Wk 4	0.3 (-0.1, 0.7)	0.214	0.2 (0.1, 0.3)	0.746	
Wk 12	0.1 (0.0, 0.3)	0.462	0.2 (0.2, 0.3)	0.168	
IL-8					
Baseline	1.3 (-0.4, 2.9)		1.1 (-0.1, 2.2)		0.812
Wk 1	0.3 (0.1, 0.4)	0.775	2.8 (-1.0, 6.6)	* 0.035	
Wk 4	4.9 (-4.5, 14.2)	0.302	1.3 (-0.2, 2.8)	0.970	
Wk 12	1.3 (-1.1, 3.7)	0.988	1.5 (-1.0, 4.0)	0.928	
IL-9					
Baseline	0.2 (0.1, 0.3)		0.4 (0.2, 0.5)		0.179
Wk 1	0.2 (0.1, 0.3)	0.255	0.5 (0.2, 0.7)	** 0.005	
Wk 4	0.2 (0.2, 0.2)	0.322	0.5 (0.3, 0.8)	*** 0.002	
Wk 12	0.2 (0.0, 0.3)	0.070	0.4 (0.3, 0.5)	0.478	

IL-10					
Baseline	0.3 (0.1, 0.4)		0.3 (0.2, 0.4)		0.864
Wk 1	0.3 (0.1, 0.4)	0.930	0.3 (0.1, 0.5)	0.572	
Wk 4	0.3 (0.2, 0.4)	0.221	0.3 (0.2, 0.5)	0.557	
Wk 12	0.3 (0.0, 0.6)	0.896	0.5 (0.3, 0.7)	*** 0.003	
IL-15					
Baseline	0.3 (0.2, 0.5)		0.3 (0.2, 0.4)		0.928
Wk 1	0.3 (0.2, 0.5)	0.902	0.4 (0.2, 0.5)	0.722	
Wk 4	0.4 (0.2, 0.5)	0.543	0.4 (0.2, 0.6)	0.345	
Wk 12	0.3 (0.1, 0.6)	0.402	0.5 (0.3, 0.8)	*** 0.003	
FGF2					
Baseline	3.7 (0.7, 6.8)		3.7 (1.6, 5.7)		0.956
Wk 1	2.5 (1.4, 3.7)	0.126	4.6 (1.6, 7.7)	0.556	
Wk 4	4.3 (2.6, 6.0)	0.275	4.0 (1.8, 6.2)	0.909	
Wk 12	3.3 (0.7, 5.9)	0.476	5.7 (2.2, 9.1)	0.091	
G-CSF					
Baseline	0.8 (0.4, 1.2)		1.0 (0.2, 1.7)		0.781
Wk 1	0.7 (0.2, 1.2)	0.467	0.8 (0.4, 1.2)	0.710	
Wk 4	1.7 (0.3, 3.1)	0.061	1.3 (0.2, 2.4)	0.398	
Wk 12	0.9 (0.1, 1.8)	0.193	1.5 (0.7, 2.3)	0.094	
Flt3					
Baseline	0.8 (0.4, 1.3)		0.8 (0.3, 1.3)		0.963
Wk 1	0.9 (0.4, 1.5)	0.635	0.9 (0.4, 1.3)	0.224	
Wk 4	1.0 (0.3, 1.6)	0.355	1.0 (0.4, 1.6)	**** < 0.0001	
Wk 12	1.3 (0.3, 2.3)	0.313	1.0 (0.4, 1.6)	**0.005	
Fractalkine					
Baseline	8.1 (2.8, 13.4)		7.0 (3.3, 10.7)		0.708
Wk 1	7.9 (4.3, 11.6)	0.705	6.9 (2.5, 11.2)	0.946	
Wk 4	10.0 (5.2,	0.316	6.1 (3.3, 8.9)	0.647	

	14.7)				
Wk 12	6.6 (-0.1, 13.3)	0.115	8.4 (4.1, 12.7)	0.391	
IFNα2					
Baseline	0.9 (0.3, 1.5)		1.2 (0.5, 2.0)		0.512
Wk 1	0.7 (0.4, 1.1)	0.678	1.1 (0.4, 1.8)	0.835	
Wk 4	1.7 (0.7, 2.7)	* 0.046	1.0 (0.5, 1.4)	0.608	
Wk 12	0.9 (0.1, 1.8)	0.561	1.4 (0.6, 2.1)	0.475	
GRO					
Baseline	1.2 (0.3, 2.0)		0.9 (0.4, 1.5)		0.595
Wk 1	0.8 (0.3, 1.4)	0.233	0.8 (0.3, 1.4)	0.842	
Wk 4	1.7 (0.2, 3.2)	0.183	1.4 (0.1, 2.7)	0.214	
Wk 12	1.4 (0.1, 2.6)	0.051	1.5 (0.6, 2.4)	0.156	
MCP-1					
Baseline	23.6 (11.3, 36.0)		26.1 (15.2, 36.9)		0.744
Wk 1	26.1 (11.7, 40.4)	0.693	28.4 (8.4, 48.3)	0.726	
Wk 4	18.1 (11.6, 24.6)	0.216	30.2 (9.6, 50.7)	0.569	
Wk 12	16.8 (3.4, 30.3)	0.718	25.1 (14.1, 36.1)	0.978	
MCP-3					
Baseline	1.3 (0.4, 2.2)		1.3 (0.7, 2.0)		0.873
Wk 1	1.0 (0.6, 1.4)	0.432	1.4 (0.6, 2.3)	0.864	
Wk 4	1.6 (1.0, 2.1)	0.125	1.4 (0.7, 2.2)	0.823	
Wk 12	1.3 (0.3, 2.3)	0.662	2.1 (0.9, 3.2)	* 0.033	
VEGF					
Baseline	1.1 (0.5, 1.7)		1.5 (0.6, 2.3)		0.429
Wk 1	1.5 (0.7, 2.4)	0.519	1.1 (0.7, 1.5)	0.413	
Wk 4	2.3 (1.1, 3.4)	*** 0.004	1.2 (0.4, 2.0)	0.276	
Wk 12	1.6 (0.4, 2.9)	0.960	1.8 (0.9, 2.6)	0.231	

Eotaxin					
Baseline	0.8 (0.4, 1.3)		0.9 (0.6, 1.3)		0.694
Wk 1	0.9 (0.2, 1.5)	0.958	0.9 (0.5, 1.4)	0.990	
Wk 4	1.1 (0.8, 1.4)	0.464	1.1 (0.7, 1.6)	0.421	
Wk 12	1.0 (0.1, 1.9)	0.829	1.4 (0.9, 1.9)	**** < 0.0001	
RANTES					
Baseline	0.6 (0.3, 0.9)		0.7 (0.3, 1.1)		0.619
Wk 1	0.7 (0.3, 1.0)	0.980	0.5 (0.3, 0.7)	0.322	
Wk 4	5.4 (-4.9, 15.7)	0.176	0.8 (0.4, 1.2)	0.471	
Wk 12	0.7 (0.1, 1.4)	0.937	0.9 (0.5, 1.2)	0.624	
TNF-β					
Baseline	0.2 (0.1, 0.3)		0.2 (0.1, 0.3)		0.921
Wk 1	0.1 (0.1, 0.2)	0.532	0.2 (0.1, 0.4)	0.529	
Wk 4	0.2 (0.1, 0.3)	0.504	0.2 (0.1, 0.4)	0.544	
Wk 12	0.2 (0.0, 0.3)	0.543	0.3 (0.2, 0.4)	0.105	

Values are presented as mean (95% CI).

*: P < 0.05, **: P < 0.01, ***: P < 0.005, ****: P < 0.0001

[a]: Generalized estimating equation compared with baseline.

[b]: Comparison between corresponding time point of ESWT and placebo (Generalized estimating equation).

[c]: Comparison between corresponding time point of ESWT and placebo (Fisher's Exact Test).

Table 4. Cytokines at post-treatment 1, 4, and 12 weeks in patients in ESWT and placebo

Cytokine (pg/mg of Creatinine)	Placebo (N=11)		ESWT (N=13)		-
	Mean (95% CI)	p value [a]	Mean (95% CI)	p value [a]	p value [b]
IL-1RA					
Wk 1	-4.3 (-12.0, 3.5)	0.424	20.6 (-22.1, 63.2)	1.000	0.298
Wk 4	0.7 (-5.3, 6.6)	0.898	3.4 (-2.3, 9.1)	1.000	0.458
Wk 12	9.8 (-12.8, 32.4)	0.079	24.8 (-28.6, 78.2)	1.000	0.632
IL-4					
Wk 1	-0.1 (-0.7, 0.6)	0.772	-0.2 (-0.6, 0.1)	0.138	0.577
Wk 4	0.4 (-0.0, 0.9)	0.195	-0.0 (-0.3, 0.2)	0.895	0.054
Wk 12	0.0 (-1.1, 1.1)	0.697	0.3 (0.1, 0.6)	0.079	0.419
IL-6					
Wk 1	-0.0 (-0.1, 0.1)	0.967	0.0 (-0.1, 0.1)	0.461	0.609
Wk 4	0.2 (-0.2, 0.6)	0.223	0.0 (-0.0, 0.1)	0.643	0.418
Wk 12	0.0 (-0.1, 0.1)	0.260	0.1 (0.0, 0.1)	* 0.043	0.087
IL-8					
Wk 1	-1.3 (-3.3, 0.7)	0.662	1.8 (-1.1, 4.6)	0.093	0.087
Wk 4	3.6 (-6.1, 13.3)	0.292	0.2 (-0.1, 0.5)	0.925	0.408
Wk 12	0.6 (-1.6, 2.8)	0.976	0.3 (-1.0, 1.7)	0.978	0.794
IL-9					
Wk 1	-0.0 (-0.2, 0.1)	0.478	0.1 (-0.1, 0.3)	0.304	0.190
Wk 4	-0.0 (-0.1, 0.0)	0.546	0.2 (-0.0, 0.4)	0.054	* 0.039
Wk 12	-0.0 (-0.2, 0.2)	0.409	0.0 (-0.1, 0.2)	0.885	0.635
IL-10					
Wk 1	-0.0 (-0.3, 0.2)	1.000	0.1 (-0.2, 0.3)	0.523	0.591
Wk 4	0.1 (-0.0, 0.2)	1.000	0.1 (-0.0, 0.2)	0.619	0.693
Wk 12	0.0 (-0.3, 0.4)	1.000	0.3 (0.1, 0.4)	* 0.020	0.087
IL-15					
Wk 1	-0.0 (-0.2, 0.2)	0.877	0.0 (-0.1, 0.2)	0.674	0.702

Wk 4	0.0 (-0.1, 0.2)	0.704	0.1 (-0.0, 0.3)	0.372	0.334
Wk 12	-0.0 (-0.3, 0.3)	0.693	0.3 (0.1, 0.5)	* 0.024	0.065
FGF2					
Wk 1	-1.3 (-4.6, 1.9)	0.209	1.0 (-2.6, 4.6)	0.539	0.315
Wk 4	0.5 (-1.4, 2.5)	0.891	1.3 (-0.7, 3.3)	0.840	0.568
Wk 12	-0.4 (-4.4, 3.7)	0.561	3.1 (0.3, 5.9)	0.207	0.092
G-CSF					
Wk 1	-0.3 (-0.8, 0.2)	0.394	-0.2 (-1.0, 0.7)	0.717	0.785
Wk 4	0.8 (-0.4, 2.1)	0.109	0.4 (-0.9, 1.6)	0.554	0.464
Wk 12	-0.1 (-1.2, 0.9)	0.108	0.5 (-0.2, 1.2)	0.510	0.252
Flt3					
Wk 1	-0.1 (-0.5, 0.3)	0.460	0.0 (-0.2, 0.3)	0.761	0.539
Wk 4	0.1 (-0.3, 0.5)	0.789	0.2 (-0.2, 0.5)	0.271	0.814
Wk 12	0.1 (-0.6, 0.8)	0.898	0.1 (-0.3, 0.5)	0.505	0.986
Fractalkine					
Wk 1	-1.0 (-6.8, 4.8)	0.668	-0.2 (-6.0, 5.6)	0.949	0.827
Wk 4	1.9 (-3.7, 7.5)	0.468	0.2 (-3.5, 3.9)	0.634	0.568
Wk 12	-2.3 (-6.5, 2.0)	0.584	2.6 (-2.0, 7.2)	0.582	0.097
IFNα2					
Wk 1	-0.2 (-1.0, 0.5)	0.237	-0.1 (-1.2, 1.0)	1.000	0.866
Wk 4	0.8 (-0.3, 1.9)	0.245	-0.0 (-0.8, 0.7)	1.000	0.171
Wk 12	-0.0 (-1.0, 0.9)	0.312	0.4 (-0.6, 1.3)	1.000	0.533
GRO					
Wk 1	-0.6 (-1.3, 0.1)	0.230	-0.1 (-0.7, 0.5)	0.830	0.236
Wk 4	0.5 (-0.8, 1.9)	0.326	0.5 (-0.7, 1.6)	0.319	0.921
Wk 12	-0.3 (-1.3, 0.7)	0.110	0.5 (-0.1, 1.0)	0.406	0.074
MCP-1					
Wk 1	1.1 (-14.8, 16.9)	0.383	2.3 (-13.5, 18.1)	0.770	0.906

Wk 4	-5.5 (-14.7, 3.7)	0.835	2.3 (-12.4, 17.1)	0.655	0.330
Wk 12	-2.3 (-20.9, 16.3)	0.857	-3.5 (-17.1, 10.2)	0.711	0.906
MCP-3					
Wk 1	-0.3 (-1.4, 0.7)	0.464	0.1 (-1.0, 1.2)	0.853	0.541
Wk 4	0.3 (-0.4, 1.0)	0.466	0.4 (-0.3, 1.0)	0.783	0.892
Wk 12	-0.1 (-1.5, 1.4)	0.866	1.0 (0.1, 1.9)	0.130	0.122
VEGF					
Wk 1	0.3 (-0.4, 1.0)	0.539	-0.4 (-1.3, 0.5)	0.415	0.241
Wk 4	1.2 (0.3, 2.1)	* 0.015	-0.5 (-1.3, 0.7)	0.394	* 0.013
Wk 12	0.3 (-1.3, 1.9)	0.856	0.2 (-0.8, 1.2)	0.744	0.870
Eotaxin					
Wk 1	-0.1 (-1.0, 0.9)	1.000	0.0 (-0.5, 0.5)	0.989	0.896
Wk 4	0.2 (-0.3, 0.8)	1.000	0.3 (-0.1, 0.7)	0.452	0.908
Wk 12	0.3 (-0.8, 1.3)	1.000	0.6 (0.3, 0.9)	* 0.030	0.401
RANTES					
Wk 1	-0.0 (-0.4, 0.4)	0.999	-0.2 (-0.6, 0.2)	0.335	0.493
Wk 4	4.8 (-5.5, 15.1)	0.176	0.1 (-0.3, 0.5)	0.650	0.285
Wk 12	0.3 (-0.4, 0.9)	0.880	0.2 (-0.2, 0.5)	0.722	0.738
TNF-β					
Wk 1	-0.0 (-0.2, 0.1)	0.578	0.1 (-0.1, 0.2)	0.505	0.422
Wk 4	0.0 (-0.1, 0.1)	0.803	0.1 (-0.1, 0.2)	0.570	0.329
Wk 12	-0.0 (-0.2, 0.2)	0.700	0.2 (0.0, 0.3)	0.146	0.082

Values are presented as mean (95% CI).

*: P < 0.05, **: P < 0.01, ***: P < 0.005, ****: P < 0.0001

[a]: Generalized estimating equation compared with baseline.

[b]: Comparison between corresponding time point of ESWT and placebo (Generalized estimating equation).

Figures

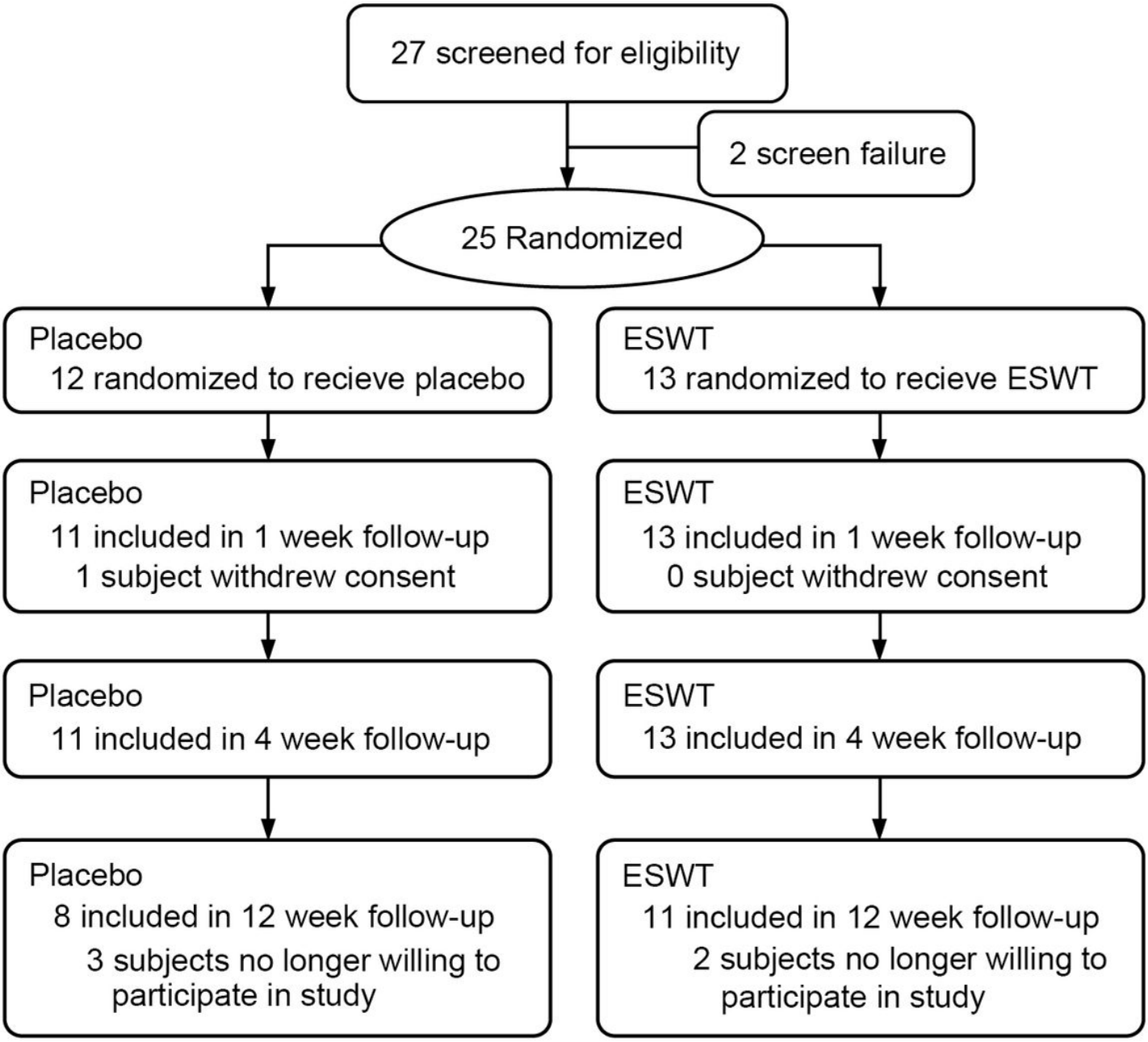

Figure 1

Patient allocation and flow chart of the study.

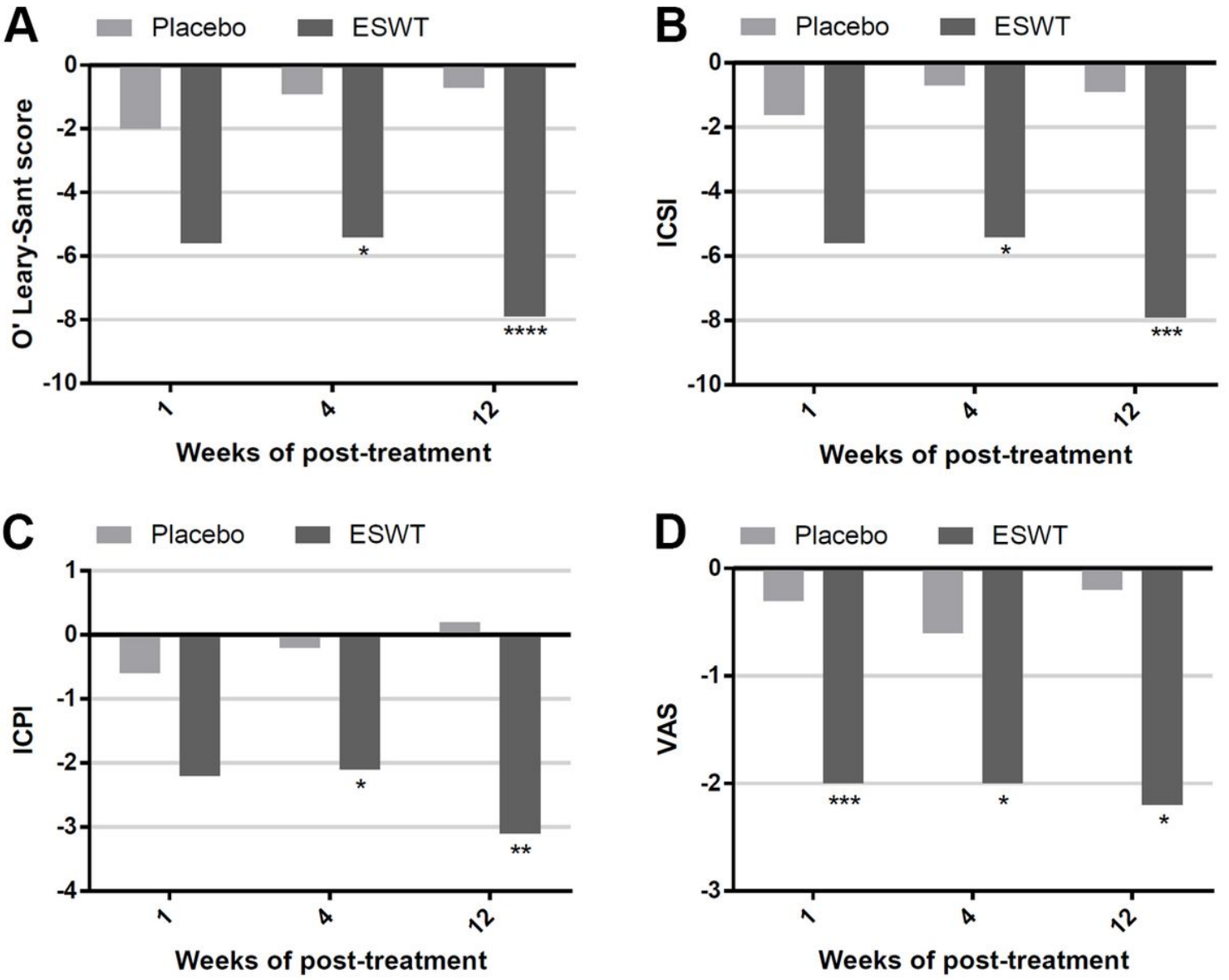

Figure 2

Outcome measures of ESWT group vs placebo group at baseline, week 1, week 4 and week 12 in OSS (A), ICSI (B), ICPI (C) and VAS (D). ESWT, extracorporeal shock wave therapy; ICPI, interstitial cystitis problem indices; ICSI, interstitial cystitis symptom indices; OSS, O'Leary-Sant symptom scores; VAS, visual analog scale. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$, ****: $P < 0.0001$.