

千葉大学学位申請論文

Ultra-pure soft water improves skin barrier function in  
children with atopic dermatitis: A randomized,  
double-blind, placebo-controlled, crossover pilot study

(高純度軟化水は小児のアトピー性皮膚炎において皮膚バ  
リア機能を改善する:無作為化二重盲検プラセボ対照クロス  
オーバーパイロット試験)

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## **Background**

Calcium and magnesium determine water hardness and react with soap to form scum.

Hard water, with or without soap scum, was expected to exacerbate dermatitis affecting daily life. In Chiba prefecture, Japan, where our university hospital is located, the hardness of home tap water is approximately 92 mg/L, the second highest level in Japan.

## **Objective**

Hard water may affect the symptoms of atopic dermatitis (AD). Therefore, we sought to examine the effect of water hardness on AD in Chiba prefecture.

## **Methods**

For this objective, we used ultra-pure soft water (UPSW), in which both mineral components have been almost completely removed, to evaluate clinical and objective improvements in skin barrier function in children with AD via a randomized, double-blind, placebo-controlled, crossover pilot study. We placed a machine in the bathroom of each home, and the child AD patients received shower treatment with UPSW or ordinary tap water (placebo) for 6 weeks. After a 2-week washout period, the treatments were switched between patient groups. Over the course of the study, the water hardness was monitored. We evaluated multiple independent primary outcomes.

Clinical symptoms were assessed by the eczema area and severity index (EASI) score. We measured out-in skin transparency (OIST) using a yellow dye and transepidermal water loss (TEWL). The patients' evaluations of pruritus and satisfaction with therapy were both evaluated using the visual analogue scale (VAS). We also measured serum LDH, TARC, and eosinophil blood counts in addition to bacterial skin cultures specific for *Staphylococcus aureus*.

## **Results**

Eleven AD patients completed the entire study course. Although there were no significant differences in EASI ( $P = 0.21$ ) or TEWL ( $P = 0.14$  and  $0.79$ ), statistically significant differences were detected in OIST ( $P = 0.048$ ), and VAS scores for pruritus ( $P = 0.044$ ) and satisfaction with therapy ( $P = 0.022$ ). We were not able to detect significant differences in LDH or TARC blood levels or in eosinophil counts between UPSW-allocated and tap water-allocated patients. We also failed to show a significant difference in the bacterial cultures of *Staphylococcus aureus* on the skin.

## **Conclusion**

Shower treatment with UPSW is supposedly beneficial in AD patients in terms of OIST and VAS scores for pruritus and satisfaction with therapy.

**Abbreviations:** atopic dermatitis (AD), calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), out-in skin

transparency (OIST), the eczema area and severity index (EASI), transepidermal water loss (TEWL), ultra-pure soft water (UPSW), visual analogue scale (VAS)

## 1. Introduction

Calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ) present in water determine water hardness.

Water hardness is most commonly expressed in milligrams of calcium carbonate equivalent per liter. Water containing  $\text{Ca}^{2+}$  concentrations below 60 mg/L is generally considered soft, 60–120 mg/L as moderately hard, 120–180 mg/L as hard, and more than 180 mg/L as very hard [1].

Exposure to hard water was reported to be a risk factor for atopic dermatitis (AD) in a cohort study of primary school children in Nottinghamshire, UK (where the water hardness is 118–314 mg/L) [2] and also in Osaka, Japan (water hardness of 35.2–100 mg/L) [3].  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  react with soap to form an insoluble precipitate known as “soap scum”. Since invisible soap scum binds tightly to the skin surface and cannot be rinsed away easily, it might exacerbate AD. In Chiba, where our university hospital is located, the  $\text{Ca}^{2+}$  concentration in home tap water is approximately 92 mg/L, the second highest level in Japan.

To evaluate clinical and objective improvements in skin barrier functions by shower treatment using water devoid of these two mineral components, we used ultra-pure soft water (UPSW), in which  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  have been almost completely replaced by sodium ions using a cation-exchange resin, and performed a randomized,

double-blind, placebo-controlled, crossover pilot study. In addition to assessment of the eczema area and severity index (EASI) score [4], we used a closed-chamber instrument for measurement of transepidermal water loss (TEWL) to estimate skin barrier function accurately [5]. We also applied a new method of measuring out-in skin transparency (OIST), using a colorimeter and food dye to evaluate skin barrier function [6]. Pruritus and satisfaction with therapy were also evaluated using the visual analogue scale (VAS). As other factors for objective evaluation, we performed bacterial skin cultures specific for *Staphylococcus aureus* and blood sampling to measure serum LDH, TARC, and eosinophil blood counts.

## **2. Materials and methods**

### **2.1 Patients and clinical study design**

The study was approved by the Institutional Review Board at Chiba University (ID: 1082) and conducted in accordance with the Declaration of Helsinki. It was registered with the UMIN Clinical Trial Registry (ID: 6136, official title: “Effect of ultrapure soft water on skin function of atopic dermatitis”). The trial design was a randomized, double-blind, placebo-controlled, crossover pilot study. The CONSORT 2010 flow diagram for this study and the study design are shown in Fig. 1. Eligibility criteria for the study were presence of mild to moderate AD in child patients between 3 and 6 years of age. AD patients who were administered systemic steroids or immunosuppressive therapy or those who had an EASI score > 20, a malignant disorder, or severe infectious disease were excluded.

Participants were recruited via poster advertisement at the Department of Dermatology or Pediatrics and at clinics and hospitals in Chiba City. Eligibility for interested parents of patients for study participation was assessed in a telephone interview by a pediatrician of Chiba University Hospital. Diagnosis, clinical examinations for assessment of EASI, and measurement of skin barrier functions including TEWL and OIST were performed by a dermatologist of Chiba University

Hospital. Blood tests, including peripheral eosinophil counts and serum TARC and LDH levels, urine tests, and bacterial skin cultures were conducted at the Department of Pediatrics, Chiba University Hospital.

We performed bacterial cultures of *Staphylococcus aureus* sampled from skin on the forearm, chest, and back at each assessment visit according to a procedure described previously [7]. We used stamp-type *Staphylococcus aureus*-selective TGSE agar (Food Stamp<sup>®</sup>; Nissui Pharmaceutical Co., Ltd.) as the culture medium, and the cultures were incubated at 37°C for 24 h under aerobic conditions. A positive culture was defined as the presence of more than one colony of *Staphylococcus aureus* on the agar. We picked the colonies and incubated them in Trypticase Soy II Agar with 5% Sheep Blood<sup>®</sup> (Becton, Dickinson and Company Co., Ltd) at 37°C for 24 h under aerobic conditions. We then identified *Staphylococcus aureus* based on the agglutination test.

Pruritus and therapy satisfaction were evaluated by the mother of the patient using the VAS. All patients and their parents provided informed consent. We informed the patients and their parents that this trial consisted of two cycles of 6-week UPSW treatments separated by a 2-week washout period using tap water. An employee of Miura Co., Ltd. (Matsuyama, Japan) directed the placement of the UPSW units in the



patients' homes. Both the patients and research doctors were blinded to the treatment allocation throughout the study period.

Patients were randomly assigned to two treatment groups on a 1:1 basis by a third party via flipping a coin to allow randomization: 6-week shower treatment with UPSW versus with placebo groups. A machine used to supply either UPSW (Bihadakko; Miura Co., Ltd.) or ordinary tap water without cation-exchange resin (placebo) was set up in the bathroom of each home. The hardness of the UPSW was  $\leq 1.0$  mg/L in this study. After a 2-week washout period using tap water only, the treatment protocols were switched, and a second 6-week treatment was then performed. Shower treatment was not considered a special therapy, simply patients using UPSW once a day during their usual bathing routine. Each patient continued using their normal therapies and medications in the same doses or quantities as used before the study. The baseline and endpoint values of outcomes such as EASI, TEWL, and VAS scores for pruritus and satisfaction with therapy were compared.

## 2.2 Sample size

In general, we calculate the number of subjects required to detect significance at a 5% significance level with 80% power. The original sample size estimate was 30

participants at the time of initial protocol registration. We determined the study period to be 1.5 years and recruited patients from October 2011 to March 2013, excluding the summer season of July and August to avoid excessive sweating, which is a potential bias. Finally, 12 patients (5 females) were entered into the study. Although this number was less than anticipated, we proceeded with the analysis of these patients as a pilot study.

### 2.3 Interventions

The units equipped with a cation-exchange resin for obtaining UPSW, in which  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  have been almost completely replaced by sodium ions, were compared with the placebo unit, which had the same appearance but without the cation-exchange resin, thus releasing ordinary tap water. The subjects on their own could not distinguish whether the unit was the placebo or the UPSW unit. All units were installed in the child AD patient's home and then replaced at the time of crossover by a Miura engineer. The engineer sampled the water upon both installing and removing the units. The hardness ( $\leq 1.0$  mg/L) and quality of both the tap water and UPSW were monitored in the laboratory of Miura Co., Ltd.

Patients received UPSW shower treatment for the first 6 weeks (group 1) or

between weeks 9 and 15 (group 2) of the trial (Fig. 1). Basically, throughout the study period, the patients remained in their homes with the exception of going out during the day.

#### 2.4 Primary outcome

At the time of study registration, the five separate primary outcomes included the mean change in the EASI (as an evaluation of skin conditions), OIST and TEWL (as measures of skin barrier function), the VAS scores for pruritus, and serum TARC levels at 6 weeks compared with baseline. We also compared the mean changes in these five outcomes between the UPSW and placebo intervention groups. At the start of this trial, we added satisfaction with therapy as an additional outcome to evaluate the feelings experienced by the patients in regard to treatment effects. Serum TARC levels ultimately were excluded from among the primary outcomes and were evaluated as a secondary outcome, since we decided to perform blood tests only after treatment crossover to reduce the number of invasive tests.

The EASI score is measured on an objective severity scale ranging from 0 to 72, and it was assessed by the same dermatologist at each follow-up appointment.

OIST is an assessment of out-in skin barrier function [6]. The penetration of

tartrazine (C<sub>16</sub>H<sub>9</sub>N<sub>4</sub>Na<sub>3</sub>O<sub>9</sub>S<sub>2</sub>, molecular weight 534: Wako Chemicals., Ltd., Osaka, Japan) was measured using the photocolormeter model CR-400<sup>®</sup> (Konica Minolta Co., Ltd., Tokyo, Japan). It can record colors in a three-dimensional space known as the CIE 1976 L\*a\*b\* color space based on the CIE XYZ coordinate system. We recorded b\* values from an 11-mm diameter skin area of the forearm near the cubital fossa using the photocolormeter to evaluate initial skin color. Fifty microliters of solution containing the hydrophilic yellow dye tartrazine dissolved in 10 mg/ml saline was applied to a small water-proofed cotton patch (5 × 5 mm). After 30 min, the patch was removed, the solution was flushed with soap, and the b\* values were re-evaluated.

TEWL is an assessment for in-out skin barrier function, measured using the AquaFlux<sup>®</sup> (Biox System, London, UK) condenser-chamber, which is reported to be approximately 40% more sensitive than an open chamber instrument [8]. TEWL values were measured along the same abdominal and upper back regions in each subject. All tests, including TEWL, were performed in the same room with a controlled temperature ranging from 24 to 26°C and a humidity of 20 to 40%. Each measurement was obtained more than 1 h after the patient's arrival at our hospital.

We also evaluated pruritus and satisfaction with therapy using the VAS, which is a symptom subjective severity scale ranging from 0 to 10 and was completed by the

parents of the patients at each assessment visit.

## 2.5 Secondary outcome

At the time of initial protocol registration, the secondary outcomes included bacterial infection in skin, urine levels of allergy-related substances, and the amount of topical agents used. However, we failed to evaluate the amount of topical agents used as a secondary outcome, because we instructed each patient to maintain their same therapies and medications, including ointments, in the same doses or amount during the course of this clinical trial as used prior to the start of this study. We performed bacterial cultures for *Staphylococcus aureus* at each assessment visit in addition to urine tests. In addition to serum TARC levels, we evaluated peripheral eosinophil counts and serum LDH levels as blood sampling data.

## 2.6 Statistical analysis

All data were analyzed according to the intention-to-treat principle. For the baseline characteristics, summary statistics were expressed as frequencies and proportions for categorical data and as means and standard deviations (SD) for continuous variables. We compared patient characteristics using Fisher's exact test for

categorical outcomes and t-tests or the Wilcoxon rank sum test for continuous variables, as appropriate.

The mean changes from baseline were computed for all outcome variables. The mean changes were analyzed using a repeated-measures analysis of variance (ANOVA) based on the Grizzle model, including effects for treatment, period, and subject sequence as a random effect. A  $2 \times 2$  crossover study design model was used to evaluate the carry-over or learning effect [9].

All statistical analyses were performed using SAS software version 9.3 (SAS Institute, USA) and GraphPad Prism (version 6; GraphPad Software). All comparisons were planned, and the tests were two-sided.  $P < 0.05$  was considered to indicate a statistically significant difference.

### 3. Results

A total of 12 patients were assigned randomly to two groups differing only in treatment protocol sequence (Table 1). Group 1 contained six patients (two female, mean age  $\pm$  SD: 63.5  $\pm$  5.7 months, EASI  $\pm$  SD: 10.67  $\pm$  5.35) and was started with a 6-week UPSW shower treatment as the first allocation (water hardness: 91.67  $\pm$  38.25 mg/L), while group 2 contained six patients (three females, mean age  $\pm$  SD: 52.0  $\pm$  14.2 months, EASI  $\pm$  SD: 7.02  $\pm$  5.15) and was started with a 6-week placebo shower treatment (water hardness: 72.5  $\pm$  9.44 mg/L) (Fig. 1). After a 2-week washout period, the treatment protocols were switched between the two patient groups as the second allocation. One patient in group 1 withdrew her consent within the first 3 weeks for personal reasons, while the remaining 11 completed the entire course (Fig. 1).

In the statistical analysis using the Grizzle model for crossover design [9], there were no carry-over, period or sequence effects. There were no adverse events caused from using the installed water softening unit or the placebo unit. Therefore, we performed the following analysis to compare the UPSW versus placebo interventions and also to compare several factors pre-treatment (baseline) versus post-treatment (endpoint) within each treatment group.

With respect to clinical evaluation of the skin as a primary outcome, when

baseline and endpoint values were compared within the UPSW treatment group, the mean EASI showed a tendency to improve; however, this difference did not reach statistical significance ( $P = 0.077$ , Fig. 2A) same as the placebo treatment group which showed no tendency to improve (Fig. 2B) No significant differences were recognized in the mean changes in the EASI between the UPSW and placebo treatments (Fig. 2C).

In terms of skin barrier function, the mean changes in OIST from baseline to endpoint within the UPSW treatment group showed no significant differences ( $P = 0.120$ , Fig. 3A) same as the placebo treatment group (Fig. 3B). On the other hand, the mean change in OIST was significantly greater in the UPSW than the placebo treatment group ( $P = 0.048$ , Fig. 3C).

In contrast, the TEWL in skin from both the abdominal (Fig. 4) and upper back (Fig. 5) regions showed no significant differences between the two water treatments or from baseline to endpoint within the UPSW treatment group.

In the evaluation of subjective symptoms using the VAS, the mean VAS scores for pruritus showed no significant differences from baseline to endpoint within the each water treatment group (Fig. 6A, B). However, there was statistically significant difference between the two water treatments in terms of the mean VAS scores for both pruritus ( $P = 0.044$ , Fig. 6C). The mean VAS scores for satisfaction with therapy also



showed a significant improvement from baseline to endpoint in the UPSW treatment group ( $P = 0.010$ , Fig. 7A), while it did not show improvement in the placebo treatment group (Fig. 7B). In terms of the VAS scores for satisfaction with therapy, there was statistically significant difference between the two water treatments ( $P = 0.022$ , Fig. 7C).

Table 2 summarizes the results shown in Figs. 2-7. There were statistically significant differences between the UPSW and placebo treatments with respect to the following three independent primary outcomes (OIST and VAS scores for pruritus and satisfaction with therapy). In addition, we detected a significant improvement in the VAS scores for satisfaction with therapy within the UPSW treatment period.

We also show a summary of the following secondary outcomes in Table 3: *Staphylococcus aureus* cultures in skin from the three body parts evaluated (Figs. 8-10), serum TARC (Fig. 11) and LDH levels (Fig. 12), and peripheral eosinophil counts (Fig. 13). However, these data indicated no significant improvements with UPSW treatment.

We counted *Staphylococcus aureus* colonies among the bacterial cultures sampled from the right forearm (Fig. 8), back (Fig. 9), and chest (Fig. 10), but there were no significant changes in the number of colonies. The only significant increase was detected in the right forearm during the tap water treatment period (Fig. 8B).

As for the laboratory findings assessed, we could not collect adequate urine data, because some patients were not willing to urinate at the time of assessment. No significant differences in serum TARC (Fig. 11) or LDH levels (Fig. 12) or in peripheral eosinophil counts (Fig. 13) were detected from baseline to endpoint within the UPSW treatment period or between the two treatment groups.

## 4. Discussion

### 4.1 Main findings

In this study, we measured disease severity, which was evaluated by the EASI, as one of the primary outcomes. Other trials investigating water softeners for children with moderate to severe eczema showed no significant improvements in disease severity [10, 11]. However, they discussed the possibility that water softening is beneficial for milder forms of eczema. Therefore, in this study we excluded patients with an EASI score  $> 20$ . Even though UPSW treatment for 6 weeks had a tendency to improve the EASI in children with mild AD, we could not detect a significant difference between the two water treatments. This study was relatively short in duration and potentially insufficient to evaluate the effects of UPSW treatment, and significant improvements might have been detected if the observation period had been increased.

On the other hand, we succeeded in showing that a 6-week shower treatment with UPSW significantly improved OIST, as another independent primary outcome, in child AD patients compared with tap water.

Mochizuki et al. previously showed a significant increase in OIST in patients with AD, which was not found in patients with dry skin or in control subjects, and they proved the efficacy of using OIST for evaluation of out-in skin barrier function [6].

OIST is a direct measurement of tartrazine (molecular weight 534) penetration into the skin, and it may correlate with the large spaces existing between epidermal cells and with barrier dysfunction due to both water loss and nonspecific irritants. On the other hand, TEWL is an indicator only of in-out barrier function and the diffusion of evaporated water molecules through the stratum corneum [12]. In general, small children with high activity levels are prone to sweating, which easily influences the value of TEWL [13]. Our data also suggest that OIST is more accurate than TEWL for evaluation of skin barrier function in children.

We also found statistical significance for subjective symptoms evaluated by the VAS, such as improvement in pruritus and satisfaction with therapy, with the UPSW treatment. These results were supported by the efficacy of UPSW in a previous study in NC/Nga mice, a murine model for AD, and in dogs with pruritus [14].

Previous studies have shown that low extracellular concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the epidermis accelerated skin barrier repair, whereas an increase in the concentration of NaCl did not disturb the recovery of disrupted skin barriers [15, 6]. It was also demonstrated that a low extracellular concentration of  $\text{Ca}^{2+}$  in the epidermis led to exocytosis of lamellar bodies required for skin barrier function [17]. In general,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  react with soap to form an insoluble precipitate known as “soap scum”

that binds tightly to the skin surface and cannot be rinsed away easily; we speculate that soap scum may exacerbate AD, based on the results of our previous and this study [14].

The lesional skin of more than 90% of patients with AD is colonized by *Staphylococcus aureus*, which exacerbates AD, whereas most healthy individuals do not harbor the pathogen [18]. Staphylococcal  $\delta$ -toxin may promote allergic immune responses [19]. Therefore, we also obtained *Staphylococcus aureus* cultures from the skin surface of the forearm, back, and chest. However, we failed to detect any significant differences in the number of colonies between the two different water treatments and during each treatment period, excluding the significant increase in the forearm during the tap water treatment period.

#### 4.2 Limitations

This was a pilot study with only a small number of subjects; however, it was a randomized, double-blind, placebo-controlled, crossover design. The study cost per patient to set up the UPSW machine in each home and to continuously monitor the quality of UPSW prevented us from recruiting a large number of patients. It was also possible that the effects were masked by typical eczema therapy, generally topical steroids, despite the restriction to maintain the same therapies and medications during

the trial as used prior to its start. The start time of the study could also have created bias; for example, the rainy summer season before the dry fall and winter seasons.

We found no significant differences between the two groups with regard to serum TARC (Fig. 11) and LDH levels (Fig. 12) and peripheral eosinophil counts (Fig. 13). Because of the young age of the patients, we took only a small number of blood samples from each patient after the crossover.

#### 4.3 Generalizability

This trial had adequate external validity, since the child AD participants were recruited from the general public and not from a special environment. Indeed, the rate of parents involved who continued to use the UPSW unit after the end of this trial reached 80%, which reflected the high improvement rate of pruritus and satisfaction with therapy. Moreover, the satisfaction of the patients who underwent UPSW shower treatment during the trial period was also high. We believe the results are applicable to not only child AD patients but that UPSW is also beneficial for adults with mild to moderate eczema or with dry skin alone. It is possible that UPSW is more beneficial for European than Japanese AD patients, because the water hardness of home tap water is generally much higher in Europe.

#### 4.4 Interpretation

To our knowledge, this is the first study to investigate the direct effects of UPSW on skin barrier function in child AD patients as a randomized, double-blind, placebo-controlled, crossover study. We also found improvement in pruritus and satisfaction with therapy. It was notable that we could obtain these results in Japan where water hardness is much lower than in European countries. Long-term studies involving larger sample numbers over a wide age group will help confirm the efficacy of this UPSW shower treatment in mild to moderate adult AD patients in addition to child AD patients.

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## References

- [1] Hardness in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Geneva, World Health Organization 2011(WHO/SDE/WSH/03.04/80/Rev/1).  
  
[http://www.who.int/water\\_sanitation\\_health/dwq/chemicals/hardness.pdf](http://www.who.int/water_sanitation_health/dwq/chemicals/hardness.pdf)
- [2] McNally NJ, Williams HC, Phillips DR, Smallman-Raynor M, Lewis S, Venn A, et al. Atopic eczema and domestic water hardness. *Lancet* 1998;352:527-531.
- [3] Miyake Y, Yokoyama T, Yura A, Iki M, Shimizu T. Ecological association of water hardness with prevalence of childhood atopic dermatitis in a Japanese urban area. *Environmental research* 2004;94:33-37.
- [4] Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group. *Exp Dermatol* 2001; 10: 11-18.
- [5] Imhof RE BE, Chilcott RP, Ciordea LI, Pascut FC, Xiao P, et al. New instrument for measuring water vapor flux density from arbitrary surface. *IFSCC Magazine* 2002; 5: 297-301.
- [6] Mochizuki H, Tadaki H, Takami S, Muramatsu R, Hagiwara S, Mizuno T, et al. Evaluation of out-in skin transparency using a colorimeter and food dye in patients with atopic dermatitis. *Br J Dermatol* 2009; 160: 972-979.
- [7] Ozawa N, Shimojo N, Suzuki Y, Ochiai S, Nakano T, Morita Y, et al. Maternal



intake of Natto, a Japan's traditional fermented soybean food, during pregnancy and the risk of eczema in Japanese babies. *Allergol Int.* 2014; 63: 261-6.

[8] Farahmand S, Tien L, Hui X, Maibach HI. Measuring transepidermal water loss: a comparative in vivo study of condenser-chamber, unventilated-chamber and open-chamber systems. *Skin Res Technol* 2009; 15: 392-398.

[9] Grizzle JE. The Two-Period Change-over Design and Its Use in Clinical Trials. *Biometrics* 1965; 21: 467-480.

[10] Thomas KS, Sach TH. A multicentre randomized controlled trial of ion-exchange water softeners for the treatment of eczema in children. protocol for the Softened Water Eczema Trial (SWET) (ISRCTN: 71423189). *Br J Dermatol* 2008; 159: 561-566.

[11] Thomas KS, Dean T, O'Leary C, Sach TH, Koller K, Frost A, et al. A randomised controlled trial of ion-exchange water softeners for the treatment of eczema in children. *PLoS Med* 2011; 8: e1000395.

[12] Hata M, Tokura Y, Takigawa M, Sato M, Shioya Y, Fujikura Y, et al. Assessment of epidermal barrier function by photoacoustic spectrometry in relation to its importance in the pathogenesis of atopic dermatitis. *Lab Invest* 2002; 82: 1451-1461.

[13] Taylor NA, Machado-Moreira CA. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. *Extreme physiology & medicine* 2013; 2: 4.

- [14] Ohmori K, Tanaka A, Makita Y, Takai M, Yoshinari Y, Matsuda H. Pilot evaluation of the efficacy of shampoo treatment with ultrapure soft water for canine pruritus. *Veterinary dermatology* 2010; 21: 477-483.
- [15] Lee SH, Elias PM, Proksch E, Menon GK, Mao-Quiang M, Feingold KR. Calcium and potassium are important regulators of barrier homeostasis in murine epidermis. *J Clin Invest* 1992; 89: 530-538.
- [16] Lee SH, Elias PM, Feingold KR, Mauro T. A role for ions in barrier recovery after acute perturbation. *J Invest Dermatol* 1994; 102: 976-979.
- [17] Menon GK, Price LF, Bommannan B, Elias PM, Feingold KR. Selective obliteration of the epidermal calcium gradient leads to enhanced lamellar body secretion. *J Invest Dermatol* 1994; 102: 789-795.
- [18] Rudikoff, D. & Lebowitz, M. Atopic dermatitis. *Lancet* 1998; 351, 1715–1721.
- [19] Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Muñoz-Planillo R, Hasegawa M, et al. Staphylococcus  $\delta$ -toxin induces allergic skin disease by activating mast cells. *Nature*. 2013; 503: 397-401.

## Figure legends

Fig. 1. CONSORT 2010 flow diagram and study design of the clinical trial evaluating shower treatment with UPSW.

Details are described in Materials and methods. UPSW: ultra-pure soft water, EASI: eczema area and severity index, VAS: visual analogue scale, TEWL: transepidermal water loss, OIST: out-in skin transparency

Fig. 2. UPSW did not improve the EASI.

A-B, EASI scores were plotted pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, the mean changes in the EASI between the UPSW and placebo (tap water) were plotted. None of the data showed significant differences, although the EASI scores within the UPSW treatment period showed a tendency for improvement (A,  $P = 0.077$ ). These data were analyzed using repeated-measures analysis of variance based on the Grizzle model. UPSW: ultra-pure soft water, EASI: eczema area and severity index, n.s.: not significant.

Fig. 3. OIST was significantly lower in the UPSW group than the placebo group.

A, B, OIST was plotted pre-treatment (baseline) and post-treatment (endpoint) from the

first and last allocations. C, the mean changes in OIST between the UPSW and placebo were plotted. The change was significantly lower in the latter. ( $P = 0.048$ ). These data were analyzed using repeated-measures analysis of variance based on the Grizzle model. UPSW: ultra-pure soft water, OIST: out-in skin transparency, n.s.: not significant.

Fig. 4. UPSW did not improve TEWL in the abdominal region.

A, B, TEWL was plotted pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean differences in TEWL between the UPSW and placebo (tap water) were plotted. None of the data showed significant differences. The mean TEWL in the UPSW group, however, showed a tendency to improve (A,  $P = 0.096$ ). These data were analyzed using repeated-measures analysis of variance based on the Grizzle model. UPSW: ultra-pure soft water, TEWL: transepidermal water loss, n.s.: not significant.

Fig. 5. UPSW did not improve TEWL in the upper back region.

A, B, TEWL was plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean changes in TEWL between the UPSW and placebo (tap water) were plotted. None of the data showed significant differences. These

data were analyzed using repeated-measures analysis of variance based on the Grizzle model. UPSW: ultra-pure soft water, TEWL: transepidermal water loss, n.s.: not significant.

Fig 6. The VAS scores for pruritus were significantly lower in the UPSW group than the placebo group.

A, B, The VAS scores for pruritus were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. The VAS scores in the UPSW group showed a tendency to improve (A,  $P = 0.087$ ). C, the mean changes in the VAS scores for pruritus between the UPSW and placebo treatments (tap water) were plotted, and the VAS score was significantly lower in the UPSW group than the placebo group ( $*P = 0.044$ ). These data were analyzed using repeated-measures analysis of variance based on the Grizzle model. UPSW: ultra-pure soft water, VAS: visual analogue scale, n.s: not significant.

Fig 7. UPSW improved the VAS score for satisfaction with therapy.

A, B, The VAS scores for satisfaction with therapy were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. The VAS

scores in the UPSW group improved significantly (A,  $*P = 0.010$ ). C, The mean changes in the VAS scores between the UPSW and placebo treatments (tap water) were plotted, and the score was significantly lower in the UPSW than placebo group ( $*P = 0.022$ ). These data were analyzed using repeated-measures analysis of variance based on the Grizzle model. UPSW: ultra-pure soft water, VAS: visual analogue scale, n.s.: not significant.

Fig 8. The colony counts of *Staphylococcus aureus* on skin from the right forearm did not decrease after UPSW therapy.

A, B, The colony counts of *Staphylococcus aureus* on the skin of the right forearm were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. Colony forming units (CFU) were significantly higher in the placebo (tap water)- than the UPSW-allocated patients (B,  $*P = 0.035$ ). C, The mean changes in CFU between the UPSW and placebo (tap water) treatments were plotted. No significant differences were detected in these data, according to the Wilcoxon signed-rank test for matched pairs.

Fig 9. The colony counts of *Staphylococcus aureus* on skin from the chest region did

not decrease after UPSW therapy.

A, B, The colony counts of *Staphylococcus aureus* on skin from the chest were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean changes in CFU between the UPSW and placebo (tap water) treatments were plotted. No significant differences were detected in these data, according to the Wilcoxon signed-rank test for matched pairs.

Fig 10. The colony counts of *Staphylococcus aureus* on skin from the back did not decrease after UPSW therapy.

A, B, The colony counts of *Staphylococcus aureus* on skin from the back were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean changes in CFU treatments were plotted. No significant differences were detected in these data, according to the Wilcoxon signed-rank test for matched pairs.

Fig 11. Serum TARC levels did not decrease after UPSW therapy.

A, B, Serum TARC levels were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean changes in serum TARC

levels between the UPSW and placebo (tap water) treatments were plotted. None of the data showed significant differences. TARC: thymus and activation-regulated chemokine. These data were analyzed using the Wilcoxon signed-rank test for matched pairs (A, B) and Mann Whitney test (C).

Fig 12. Serum LDH levels did not decrease after UPSW therapy.

A, B, Serum LDH levels were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean change in serum LDH levels between the UPSW and placebo (tap water) treatments were plotted. None of the data showed significant differences. LDH: lactate dehydrogenase. These data were analyzed using the Wilcoxon signed-rank test for matched pairs (A, B) and Mann Whitney test (C).

Fig 13. Peripheral eosinophil counts did not decrease after UPSW therapy.

A, B, Peripheral eosinophil counts were plotted at pre-treatment (baseline) and post-treatment (endpoint) from the first and last allocations. C, The mean changes in peripheral eosinophil counts levels between the UPSW and placebo (tap water) treatments were plotted. None of the data showed significant differences. These data



were analyzed using the Wilcoxon signed-rank test for matched-pairs (A, B) and Mann  
Whitney test (C).

Table 1. Baseline characteristics of the patients.

Characteristics	Group 1 (UPSW/ Tap water)	Group 2 (Tap water/ UPSW)
Number of enrolled patients (male: female)	6 (4:2)	6 (3:3)
Mean age $\pm$ SD, months	63.5 $\pm$ 5.7	52.0 $\pm$ 14.2
Water hardness in tap water $\pm$ SD, mg/L	91.67 $\pm$ 38.25	72.5 $\pm$ 9.44
Baseline EASI scores $\pm$ SD	10.67 $\pm$ 5.35	7.02 $\pm$ 5.15

UPSW: ultra-pure soft water, EASI: eczema area and severity index score

Table 2. Baseline, endpoint and change values of the primary outcomes.

Outcomes		UPSW			Tap water (placebo)			p-value <sup>2</sup>
		LS mean	95% CI	p-value <sup>1</sup>	LS mean	95% CI	p-value <sup>1</sup>	
EASI	Baseline	9.15	5.31 – 12.9	0.077	6.55	3.78 – 9.32	0.92	0.21
	Endpoint	6.68	3.72 – 9.64		6.40	3.45 – 9.36		
	Change	-2.70	-5.77 – 0.35		-0.098	-3.16 – 2.96		
OIST (b* values)	Baseline	6.62	3.29 – 9.94	0.120	4.38	3.17 – 5.58	0.94	0.048*
	Endpoint	4.33	2.49 – 6.17		4.43	2.77 – 6.08		
	Change	-2.42	-4.81 – 0.038		0.055	-2.33 – 2.44		
TEWL (abdominal region, g/m <sup>2</sup> h)	Baseline	33.1	13.3 – 52.8	0.096	26.8	11.6 – 41.8	0.92	0.14
	Endpoint	27.5	8.49 – 46.4		26.5	9.99 – 43.1		
	Change	-5.81	-12.0 – 0.43		-0.037	-6.28 – 6.21		
TEWL (upper back region, g/m <sup>2</sup> h)	Baseline	31.2	10.9 – 51.3	0.60	32.9	12.2 – 53.5	0.54	0.79
	Endpoint	29.2	9.29 – 49.1		28.4	12.3 – 44.4		
	Change	-2.36	-15.8 – 11.1		-4.62	-18.1 – 8.89		
VAS (pruritus)	Baseline	5.19	3.87 – 6.50	0.087	3.61	1.88 – 5.34	0.21	0.044*
	Endpoint	3.61	1.85 – 5.38		4.19	2.31 – 6.06		
	Change	-1.53	-3.10 – 0.037		0.56	-1.00 – 2.13		
VAS (satisfaction with therapy)	Baseline	4.78	3.35 – 6.21	0.010*	6.34	4.51 – 8.17	0.69	0.022*
	Endpoint	6.50	4.80 – 8.19		6.42	4.55 – 8.29		
	Change	1.74	0.78 – 2.71		0.098	-0.86 – 1.06		

CI: confidence interval, LS mean: least-squares mean, UPSW: ultra-pure soft water, EASI: eczema area and severity index score, VAS: visual analogue scale, TEWL: transepidermal water loss, OIST: out-in skin transparency.

p-value<sup>1</sup>: change from baseline to endpoint, p-value<sup>2</sup>: between-treatment comparison of the mean change.

Table 3. Baseline, endpoint and change values of the secondary outcomes.

Outcomes		UPSW			Tap water (placebo)			p-value <sup>2</sup>
		LS mean	95% CI	p-value <sup>1</sup>	LS mean	95% CI	p-value <sup>1</sup>	
Cultures of <i>Staphylococcus aureus</i> (right forearm, CFU)	Baseline	16.50	5.291 – 39.71	0.523	1.000	-3.977 – 30.16	0.035*	0.125
	Endpoint	2.500	1.324 – 29.48		14.00	4.843 – 36.61		
	Change	-2.500	-29.14 – 14.94		5.000	-0.7020 – 15.97		
Cultures of <i>Staphylococcus aureus</i> (chest, CFU)	Baseline	33.00	12.78 – 49.22	0.193	2.000	-7.209 – 38.12	0.555	0.77
	Endpoint	52.50	19.38 – 67.42		6.000	5.873 – 43.40		
	Change	5.00	-8.416 – 33.22		0.0	-14.21 – 32.57		
Cultures of <i>Staphylococcus aureus</i> (back, CFU)	Baseline	36.00	20.05 – 63.35	0.883	5.00	6.294 – 54.07	0.727	0.844
	Endpoint	41.50	22.28 – 59.72		3.00	2.592– 47.23		
	Change	3.00	-17.78 – 16.38		0.0	-35.50 – 24.96		
Serum TARC (pg/ml)	Baseline	2375	1660 – 3773	0.445	1044	461.3 – 2105	0.625	0.90
	Endpoint	1795	1582 – 3279		1096	507.0 – 2051		
	Change	-48.50	-1222 – 649.9		-52.0	-521.3 – 513;7		
Serum LDH (U/L)	Baseline	302.5	261.5 – 352.0	0.313	277.0	253.5 – 304.1	0.75	0.563
	Endpoint	321.5	269.8 – 388.8		278.0	241.5 – 314.9		
	Change	21.50	-34.5– 79.71		-13.0	-22.26 – 21.06		
Peripheral eosinophil counts (/μl)	Baseline	585.0	163.8 – 1046	0.563	330.0	164.7 – 203.2	0.813	0.649
	Endpoint	465.0	65.26 – 1058		522.0	695.7 – 1029		
	Change	-38.0	0.78 – 2.71		-22.0	-420.1 – 791.7		

CI: confidence interval, LS mean: least-squares mean, UPSW: ultra-pure soft water,

TARC: thymus and activation-regulated chemokine, LDH: lactate dehydrogenase,

p-value<sup>1</sup>: change from baseline to endpoint, p-value<sup>2</sup>: between-treatment comparison of the mean change.

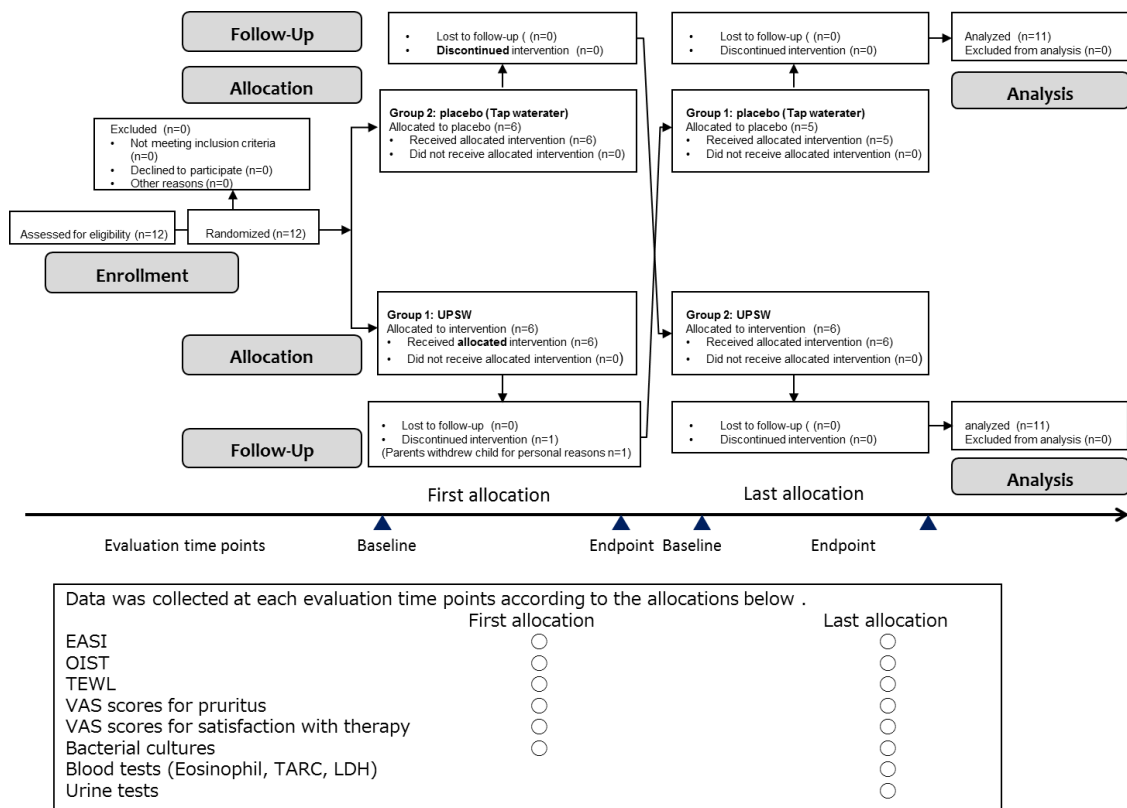


Fig. 1. CONSORT 2010 flow diagram and study design for the clinical trial evaluating shower treatment with UPSW.

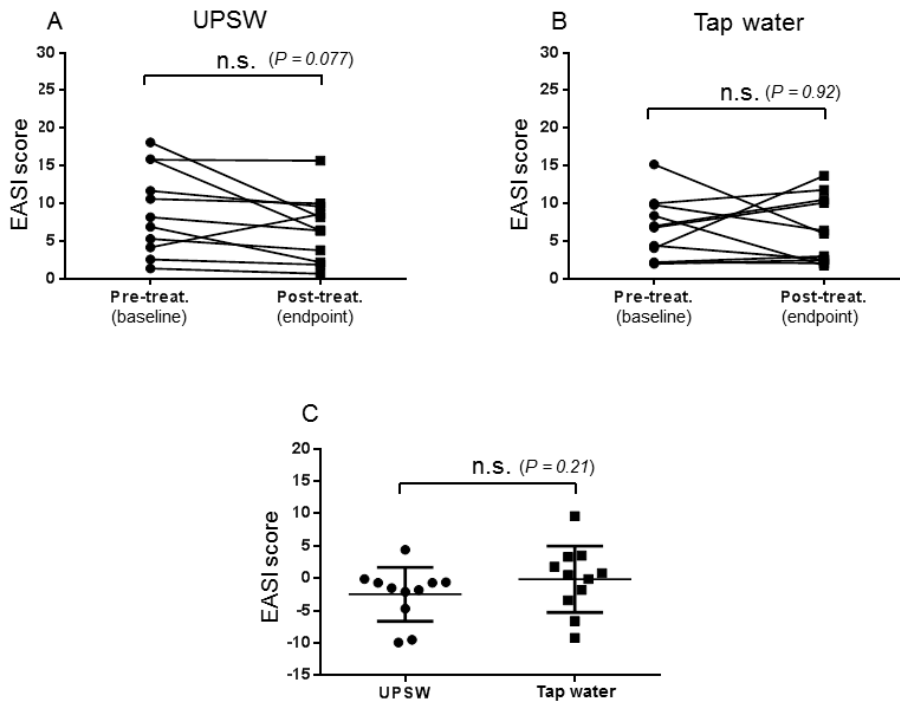


Fig. 2. UPSW did not improve the EASI.

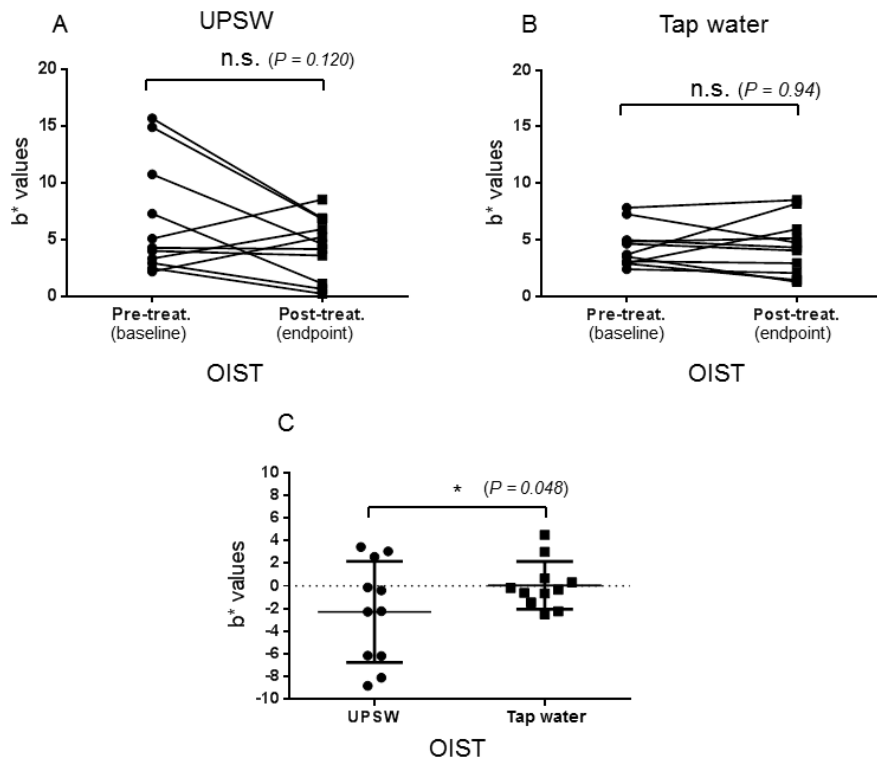


Fig. 3. OIST was significantly lower in the UPSW group than placebo group.

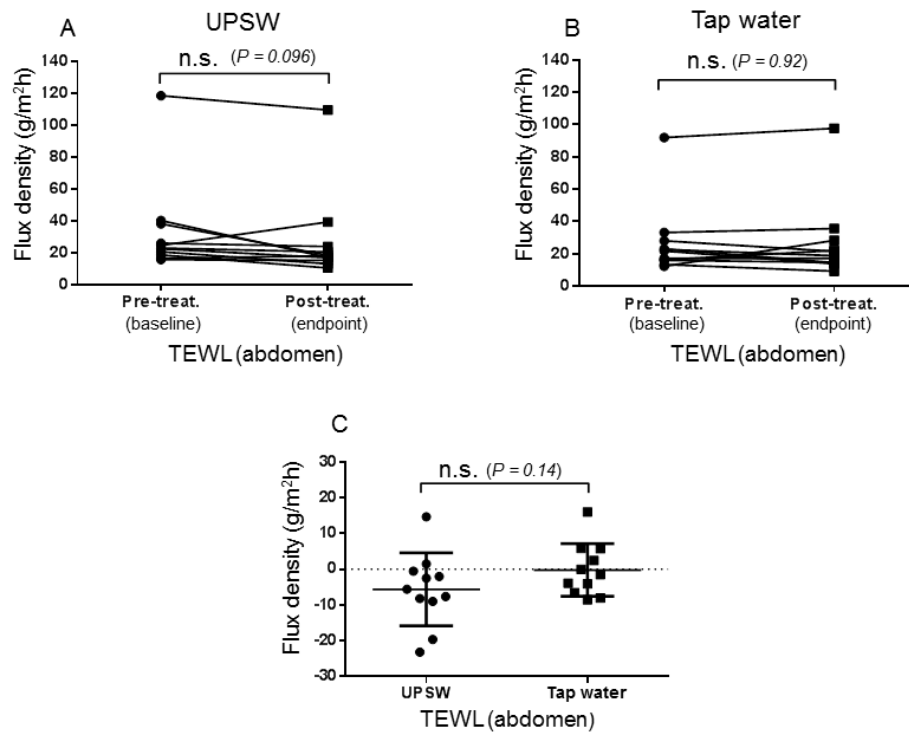


Fig. 4. UPSW did not improve TEWL in skin from the abdominal region.



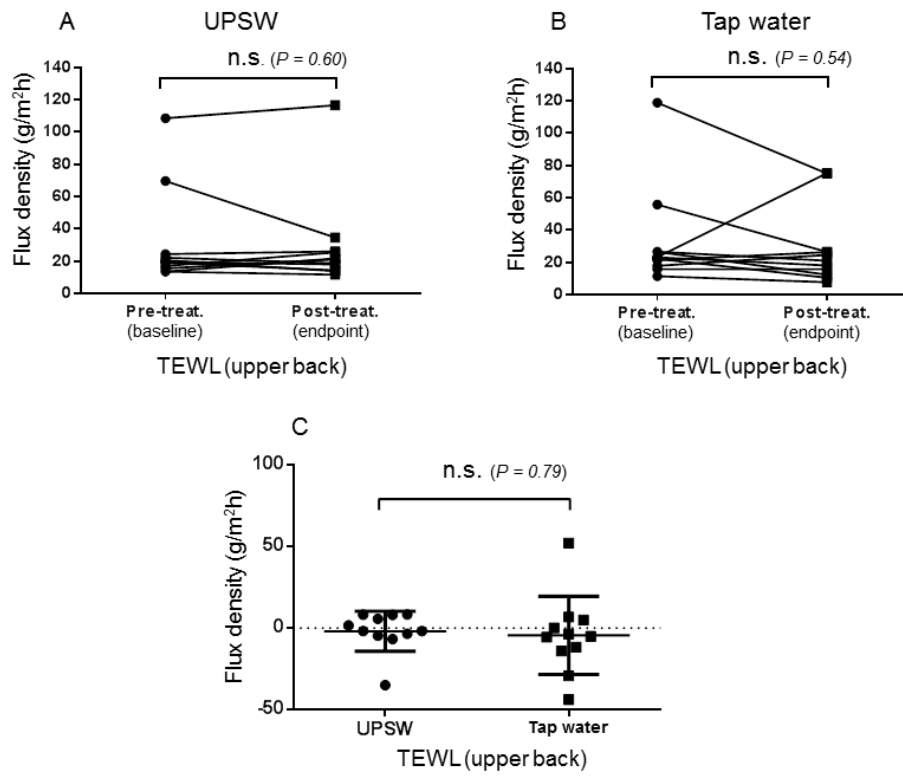


Fig. 5. UPSW did not improve TEWL in skin from the upper back region.

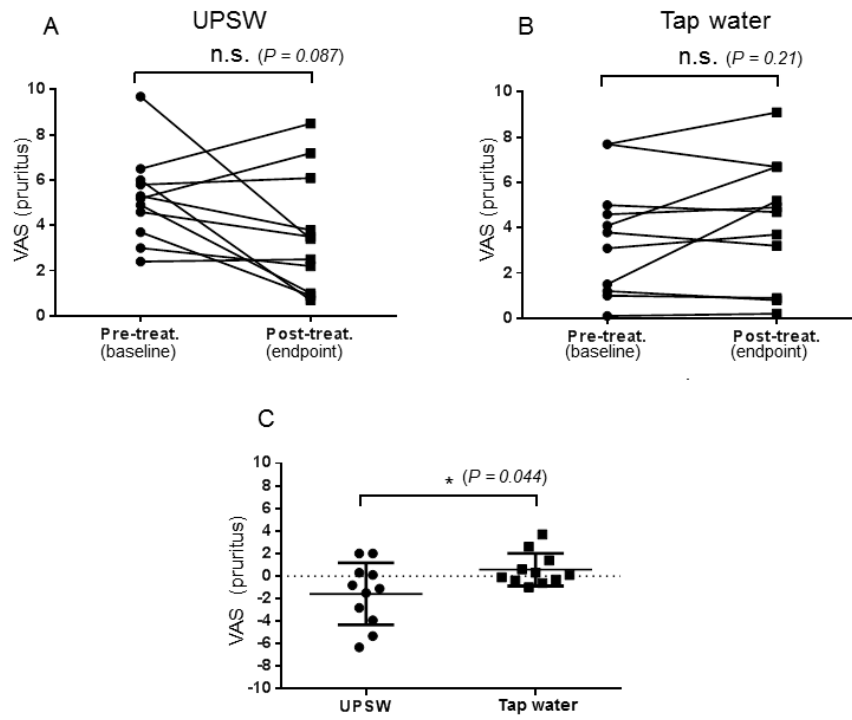


Fig. 6. The VAS scores for pruritus were significantly lower in the UPSW group than the placebo group.

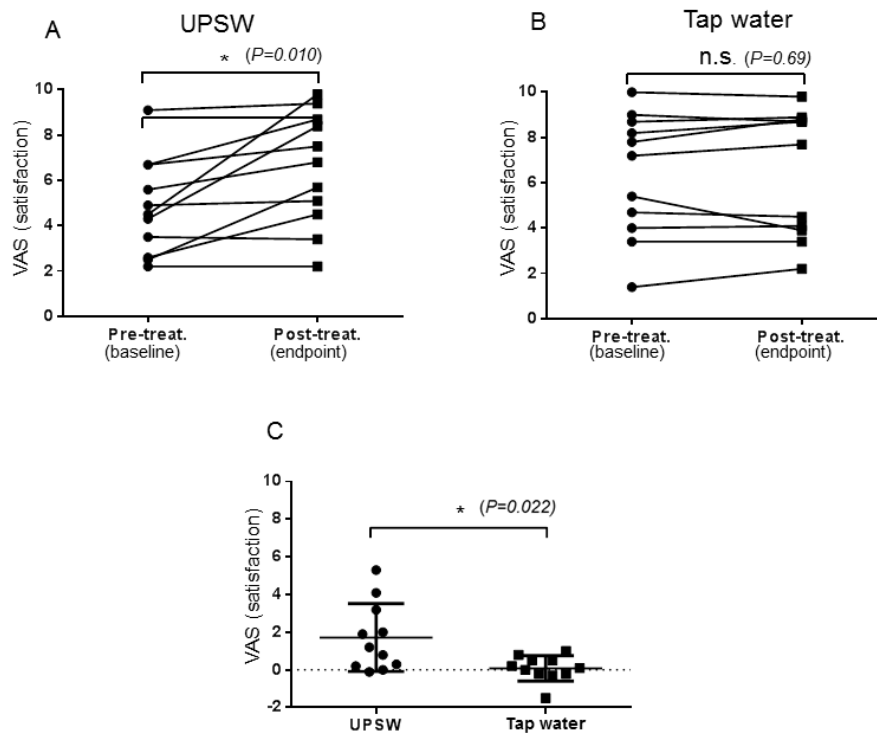


Fig. 7. UPSW improved the VAS scores for satisfaction with therapy.

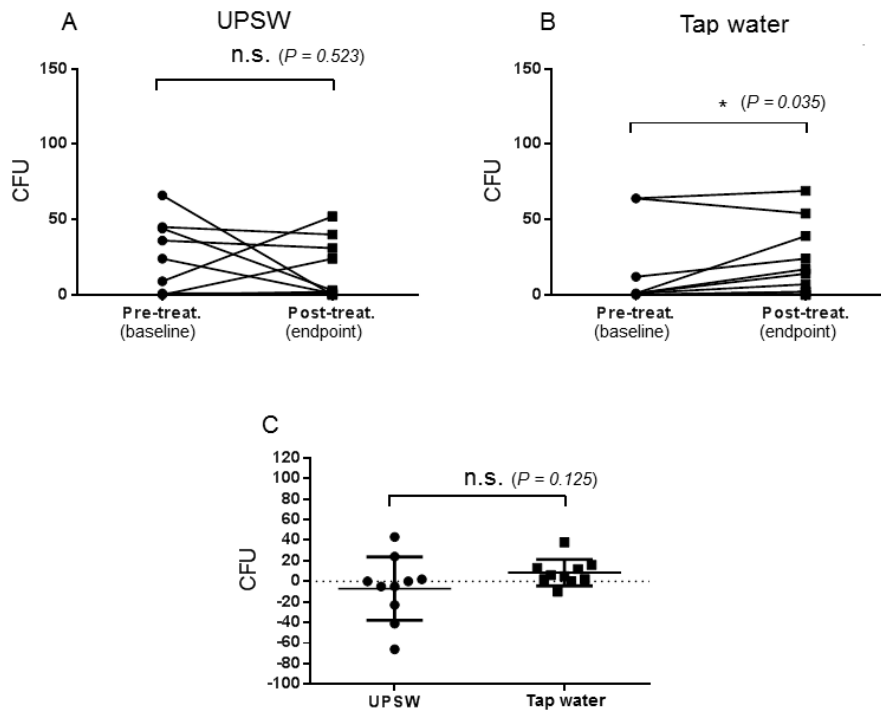


Fig 8. The colony counts of *Staphylococcus aureus* on skin from the right forearm did not decrease after UPSW therapy.

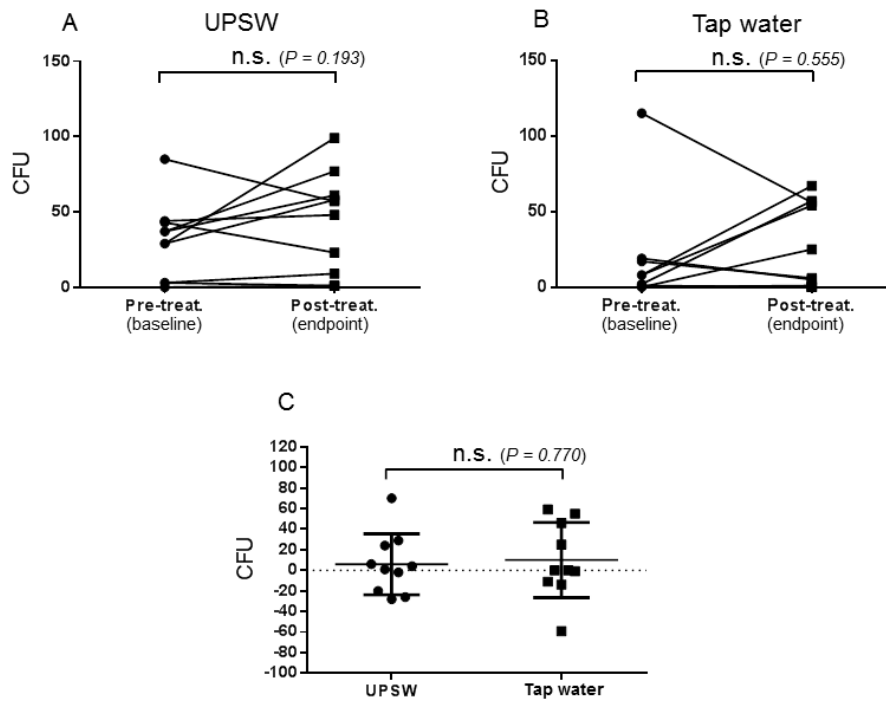


Fig. 9. The colony counts of *Staphylococcus aureus* on skin from the chest did not decrease after UPSW therapy.

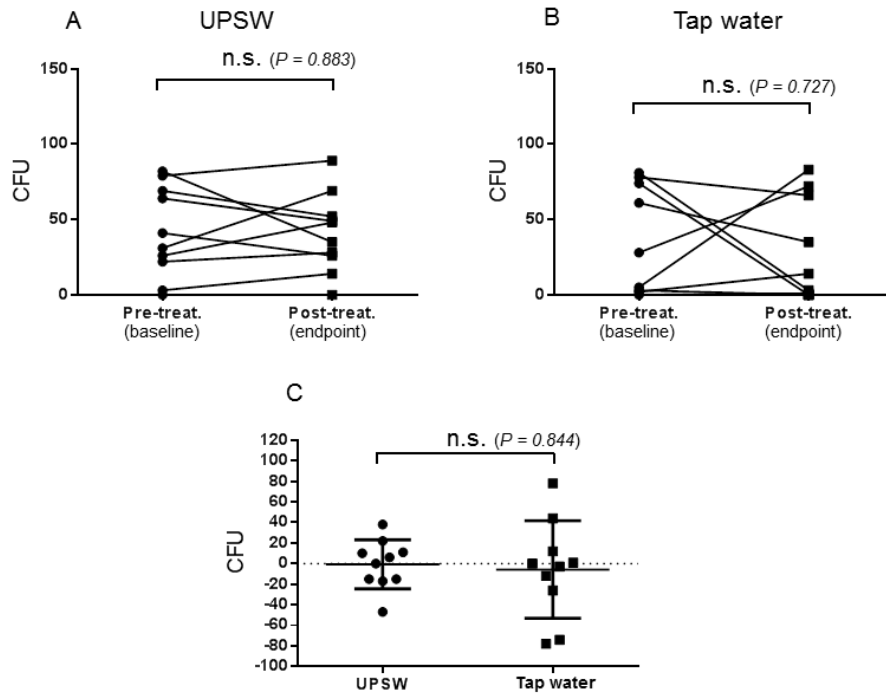


Fig. 10. The colony counts of *Staphylococcus aureus* on skin from the back did not decrease after UPSW therapy.

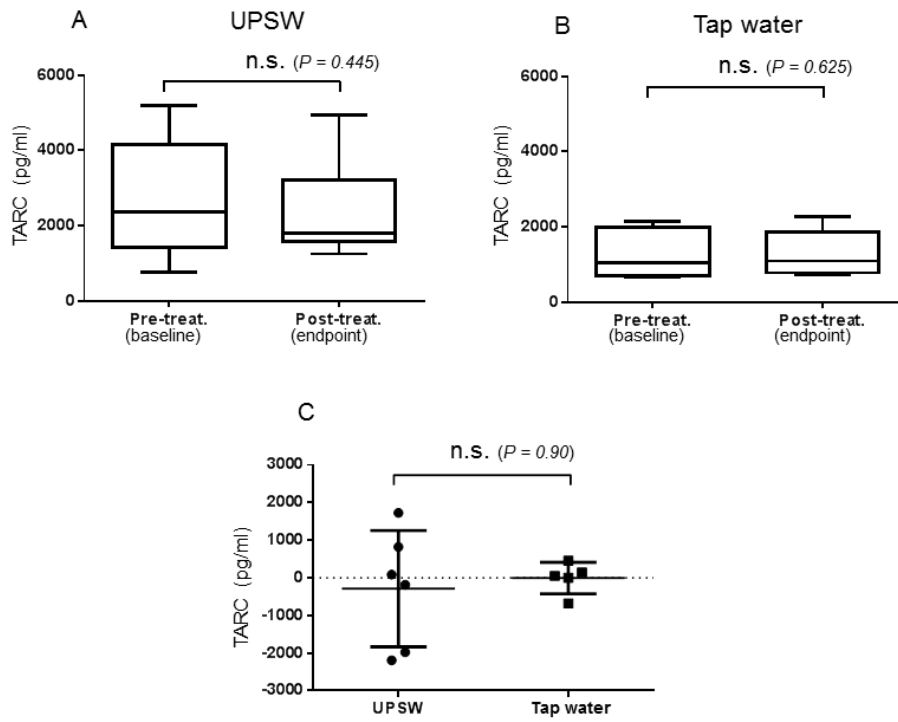


Fig. 11. Serum TARC levels did not decrease after UPSW therapy.

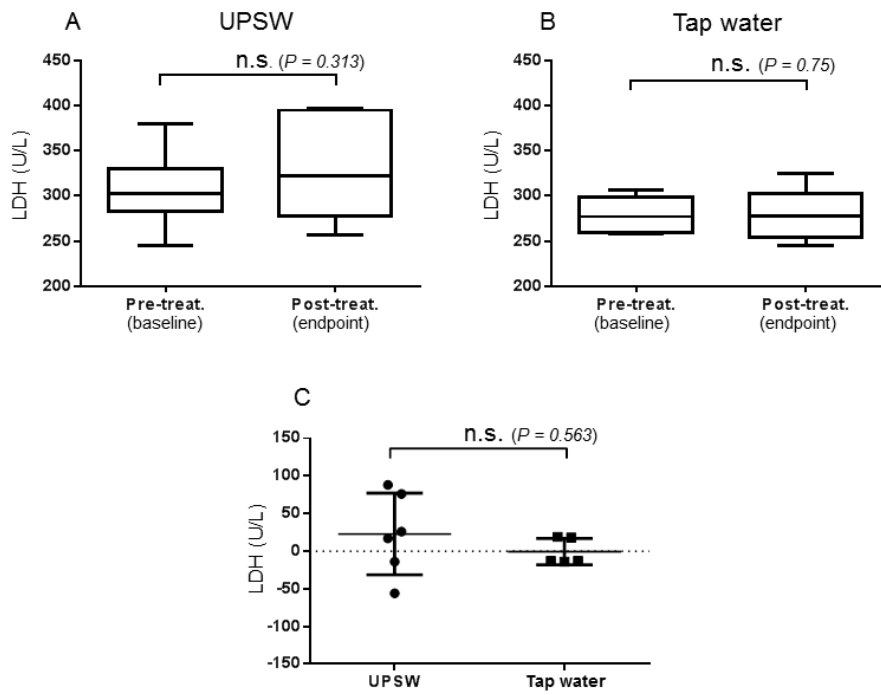


Fig.12. Serum LDH levels did not decrease after UPSW therapy.



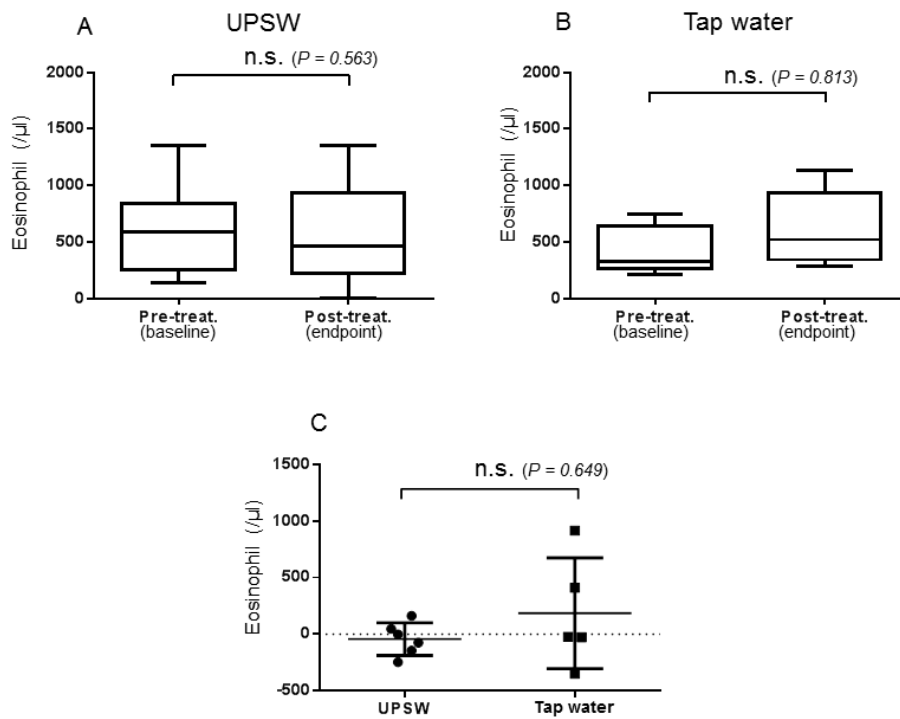


Fig. 13. Peripheral eosinophil counts did not decrease after UPSW therapy.

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