



Clinical efficacy and safety of cervical intralymphatic immunotherapy for house dust mite allergic rhinitis: A pilot study

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ABSTRACT

Background: Previous studies have demonstrated that intralymphatic immunotherapy (ILIT), a less time-consuming alternative to conventional subcutaneous immunotherapy (SCIT), is safe and effective. However, because of the private location of inguinal lymph nodes, inguinal ILIT is relatively inconvenient. We proposed a novel form of ILIT that involves 3 injections of allergen into cervical lymph nodes. The aim of this study is to determine the clinical efficacy and safety of cervical ILIT on house dust mite induced allergic rhinitis (AR) in adults.

Methods: In this study, we performed a prospective cohort study to determine the clinical efficacy and safety of cervical ILIT on house dust mite induced AR in adults, by comparing the symptom scores, quality-of-life scores (QOLS) and drug scores (use of rescue medication) before and after treatment. Meanwhile, side events were also recorded.

Results: Cervical ILIT elicited no moderate-severe adverse events. Patients receiving cervical ILIT experienced a significant improvement in nasal symptoms, eye symptoms and quality of life, as compared to baseline (P all < 0.001). A reduction in the use of rescue medication was also demonstrated ($P < 0.001$).

Conclusions: In this first-in-human clinical study, cervical ILIT was demonstrated safe and induced allergen tolerance after 3 injections.

1. Introduction

Allergic rhinitis (AR) is a growing public health problem [1]. Allergen-specific immunotherapy (AIT) provides sustained benefits of decreased allergy symptoms [2], which may also prevent new sensitizations, improve quality of life and reduce the risk of asthma development [3,4]. However, conventional subcutaneous AIT requires as many as 80 allergen injections for at least 3 years [5,6] and is largely associated with allergic adverse effects [7]. Due to the frequent injections, the long duration of treatment and the side effects, $< 5\%$ of AR patients choose this therapeutic method [3].

Intralymphatic immunotherapy (ILIT) has been introduced for the past few years as a new method of AIT, which is completed after only 3 times of inguinal lymph node injections of allergen extract [8–14]. ILIT could enhance efficacy and safety, as well as speed up relief of symptoms. However, inguinal ILIT is relative inconvenient, because patients have to take off trousers when receiving the allergen injections into

inguinal lymph nodes. Recently, we proposed a novel form of ILIT that involves 3 injections of allergen into cervical lymph nodes instead of inguinal lymph nodes over a period of 3 months. In this study, we performed a prospective cohort study to determine the clinical efficacy and safety of cervical ILIT on house dust mite induced AR in adults, by comparing the symptom scores, quality-of-life scores (QOLS) and drug scores (use of rescue medication) before and after treatment.

2. Methods

2.1. Subjects

The study was approved by the local ethics committee and conducted with written informed consent from each subject. Subjects with house dust mite induced AR were enrolled at The Allergy Department, The First Hospital of Foshan from September 2016 to October 2017.

All subjects met the following enrollment criteria described below.

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1) Patients had allergic house dust mite induced rhinitis and conjunctivitis, with symptoms including itchy nose and eyes, sneezing, nasal congestion and secretion. 2) Allergy verified by skin prick test (SPT) and serum level of allergen specific IgE. Subjects were regarded as being sensitized to an allergen if the allergen/histamine (A/H) ratio in the wheal was ≥ 1 according to the SPT, and serum level of allergen specific IgE was ≥ 0.35 IU/mL. 3) Age ranged between 18 and 65 years. Exclusion criteria included: pregnancy or nursing, planning for pregnancy, autoimmune and collagen diseases, cardiovascular diseases, asthma, sinusitis, nasal polyps, chronic obstructive and restrictive lung disease, cancer, previous immune- or chemotherapy, major metabolic disease, alcohol or drug abuse, mental incapability or medication with a possible adverse effect of interfering with the immune response.

2.2. Study design

Subjects enrolled in the study received cervical ILIT for 3 times and were followed up for one year. Symptom scores, Quality-of-life scores (QOLS) as well as drug scores (use of rescue medication) were evaluated before and after treatment, by a physician who was blinded to the design of the study. Besides, adverse reactions were recorded during treatment.

2.3. Cervical ILIT

Under strict asepsis procedure, 0.1 mL, namely 50 therapeutic units (TU) of standardized house dust mite allergen extracts (Novo-Helisen-Depot, Allergopharma GmbH & Co. KG, Reinbek, Germany) were injected into superficial level II and III cervical lymph nodes (about 1 cm in size) identified with ultrasonographic guidance. In each subject, the same cervical lymph received the allergen injection due to the aid of ultrasonographic pictures saved on the ultrasound machine. To avoid inadvertent intravascular injection, aspiration was performed before each injection. Peak expiratory flow, local reactions at the injection site, systemic reactions and other discomforts were monitored 60 min at the hospital after each injection. The patients were asked to record and report all indications of late reactions for the following 24 h.

2.4. Symptom scores

Subjects were asked to score nasal symptoms (including sneezing, rhinorrhea, itchy nose and nasal congestion), eye symptoms (including itchy/red eyes and lacrimation) and other symptoms (cough or chest tightness), before (baseline) and after (month 1, 2, 3 and 15) treatment. They were assigned scores of 0–3 as follows: 0, no symptoms; 1, mild symptoms occasionally present; 2, moderate symptoms frequently present; and 3, severe symptoms continuously present [8]. Meanwhile, the total symptom scores (range: 0–21), the total nasal symptom scores (range: 0–12) and total eye symptom scores (range: 0–6) were calculated by adding up the individual scores, nasal scores and eye scores, respectively.

2.5. Quality-of-life scores

QOLS was measured by the standardized Rhinoconjunctivitis Quality of life Questionnaire (RQLQ) version [15] before (baseline) and 1 year after treatment. The RQLQ is listed in Table 1. At the first administration subjects were asked to identify three daily activities in which they were limited by rhinoconjunctivitis, and then score the items related to sleep, nonsymptoms of rhinitis or conjunctivitis, rhinitis related behaviours, nasal symptoms, eye symptoms and Emotional behaviours.

2.6. Drug scores

For ethical reasons, all subjects were provided with oral

antihistamines, nasal glucocorticoids and oral glucocorticoids. The patients were asked to record the use of rescue medication for 2 weeks before (baseline) and 1 year after treatment. The “three-step method” was used as follows: oral antihistamines, scoring 1 point per day; nasal glucocorticoids, scoring 2 points per day; oral glucocorticoids, scoring 3 points per day [16]. The drug scores were calculated by adding up all the medication records.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software, San Diego, California, USA) in this study. For normally distributed variables, data are presented as mean with standard deviation (SD). For non-normally distributed variables, data are presented as median with interquartile range (IQR). Continuous variables were analyzed by paired Man-Whitney *U* test, whereas categorical variables were analyzed by Fisher's exact test. Data are represented as mean \pm SEM. The paired *t*-test or Wilcoxon matched-pairs signed rank test was used to compare differences between groups. A *p* value of < 0.05 was considered statistically significant.

3. Results

3.1. Subjects and demographics

A flow-diagram showing enrollment, treatment and follow-up of patients is instructed in Fig. 1. We enrolled 102 patients in total, and 1 year after the completion of cervical ILIT, 21 patients did not complete the treatment or follow-up because of certain reasons. Consequently, the eventual data were analyzed based on 81 (38 female) patients (median age, 36 years; range, 18–48 years), who completed both the treatment and follow-up.

3.2. Cervical ILIT induced allergen tolerance

Study subjects were asked to compare their allergic symptoms including sneezing, rhinorrhea, nasal itching, nasal congestion, itchy/red eyes, lacrimation and other symptoms (cough or chest tightness) 1 month after each injection for short-term therapeutic effect observation, and 1 year after the completion of treatment for long-term observation, with the symptoms they experienced at baseline.

As expected, the total symptom scores, total nasal symptom scores and total eye symptom scores 1 month after each injection and 1 year after completion of treatment were all significantly reduced compared with baseline values (*P* all < 0.001) (Fig. 2A–C). As for individual symptoms, a clear improvement over baseline was seen in sneezing, rhinorrhea, nasal itching, nasal congestion and itchy/red eyes 1 month after each injection and 1 year after completion of treatment (*P* all < 0.001) (Fig. 2D–H). The symptom scores of lacrimation and other symptoms (cough or chest tightness) 1 month after the first injection were not observed significant reduction compared with baseline value (*P* = 0.803 and 0.340, respectively), but they were also significantly decreased 1 month after the second and third injection, and 1 year after completion of treatment (*P* all < 0.001) (Fig. 2I–J).

3.3. Cervical ILIT improved quality of life

The QOLS including daily life, sleep, none symptoms of rhinitis or conjunctivitis, rhinitis related behaviours, nasal symptoms, eye symptoms and emotional behaviours were significantly improved 1 year after cervical ILIT compared with the baseline levels (*P* all < 0.001) (Fig. 3).

3.4. Patients receiving cervical ILIT used less rescue medication

In general, rescue medications were prescribed less frequently after cervical ILIT. The frequency of oral antihistamines, nasal

Table 1
Quality of life Questionnaire.

	No trouble (0 score)	Almost no trouble (1 score)	Mild trouble (2 score)	Moderate trouble (3 score)	Much trouble (4 score)	Severe trouble (5 score)	Extreme trouble (6 score)
Sleep							
Lack of a good night's sleep							
Wake during the night							
Difficulty getting to sleep							
Non symptoms of rhinitis or conjunctivitis							
Tired							
Fatigue							
Worn out							
Reduced productivity							
Poor concentration							
Thirst							
Headache							
Rhinitis related behaviours							
Need to blow nose repeatedly							
Need to rub nose/eyes							
Inconvenience of having to carry tissues or handkerchief							
Nasal symptoms							
Stuffy/blocked							
Sneezing							
Runny							
Itchy							
Eye symptoms							
Itchy							
Watery							
Swollen							
Sore							
Emotional behaviours							
Irritable							
Frustrated							
Embarrassed by nose/eye symptoms							
Restless							

glucocorticoids, and oral glucocorticoids prescription was significantly reduced 1 year after ILIT ($P < 0.001$) (Fig. 4).

3.5. Few mild adverse reactions occurred during cervical ILIT

The adverse events associated with cervical ILIT are listed in Table 2. The 81 study subjects received 243 injections in total. As shown, none moderate or severe adverse events were observed. And 12 cases of local lymph node swelling, 16 local itching, 8 local redness, 7 itching in other location, 3 urticaria and angioedema, and 2 gastrointestinal infection were recorded. Therefore, the clinical safety of cervical ILIT is confirmed.

4. Discussion

Allergic rhinitis (AR) is a highly prevalent disease in industrialized countries and causes a considerable economic burden [17,18]. The efficacy of allergen-specific immunotherapy for the treatment of respiratory allergies has been confirmed in numerous clinical studies. Although it is preferable over symptomatic treatment, conventional subcutaneous immunotherapy could only be accomplished in a few patients due to the frequent injections, long duration of treatment and side effects. In recent years, intralymphatic immunotherapy (ILIT) has been demonstrated effective and safe [8–14]. All previous studies [8–14] of ILIT reported that ILIT involved 3 injections of allergen to inguinal lymph nodes. However, the draining lymph nodes of the regional lymphatic tissues of nasal mucosa, including adenoids, tonsils, lymphatic follicles in the posterior pharyngeal wall, lateral pharyngeal chordae and tonsils of tongue, are anterior cervical lymph nodes. Allergens are mainly drained to internal jugular vein lymph nodes and superficial jugular lymph nodes [19], and it has been suggested that

these regional lymph nodes play an important role in inducing mucosal immune tolerance [20]. Besides, lymph nodes in the cervical regions are readily detected because of their superficial localization, and compared to inguinal lymph nodes, operation on cervical lymph node is more convenient and more conducive to protect patients' privacy. In addition, due to the private location of inguinal lymph nodes, inguinal ILIT is relatively inconvenient, and patients have to take off trousers when receiving the allergen injections into inguinal lymph nodes. Therefore, in this study we proposed a new form of ILIT that involved 3 injections of allergen to cervical lymph nodes instead of inguinal lymph nodes, and explored its clinical efficacy and safety against house dust mite induced rhinoconjunctivitis in adults.

At present, the evaluation of the efficacy of immunotherapy for AR mainly depends on the subjective indicators evaluated by patients after treatment, such as symptom scores, quality of life scores (QOLS), frequency of rescue medication usage, and so on. Our study showed that, 1 month after the first injection, both nasal symptom scores and eye symptom scores were significantly reduced compared with those at baseline, suggesting that cervical ILIT could effectively improve the allergic symptoms of AR patients within a short period of time, and these improvements lasted for 1 year. Furthermore, 1 year after the completion of treatment, the quality of life were also significantly improved compared with baseline, meanwhile the dependence of AR patients on rescue medication was greatly reduced, suggesting that the long-term efficacy of cervical ILIT were also satisfactory. Previous studies had suggested that inguinal ILIT is associated with tolerance induction, including elevated IgG4 levels in serum [14], and increased allergen-specific IL-10-producing Foxp3⁺ T-regulatory cells [21]. As no research about the immunological effects of cervical ILIT has been reported at present, further studies are needed to address these issues.

Adverse reactions, especially serious systemic adverse reactions,

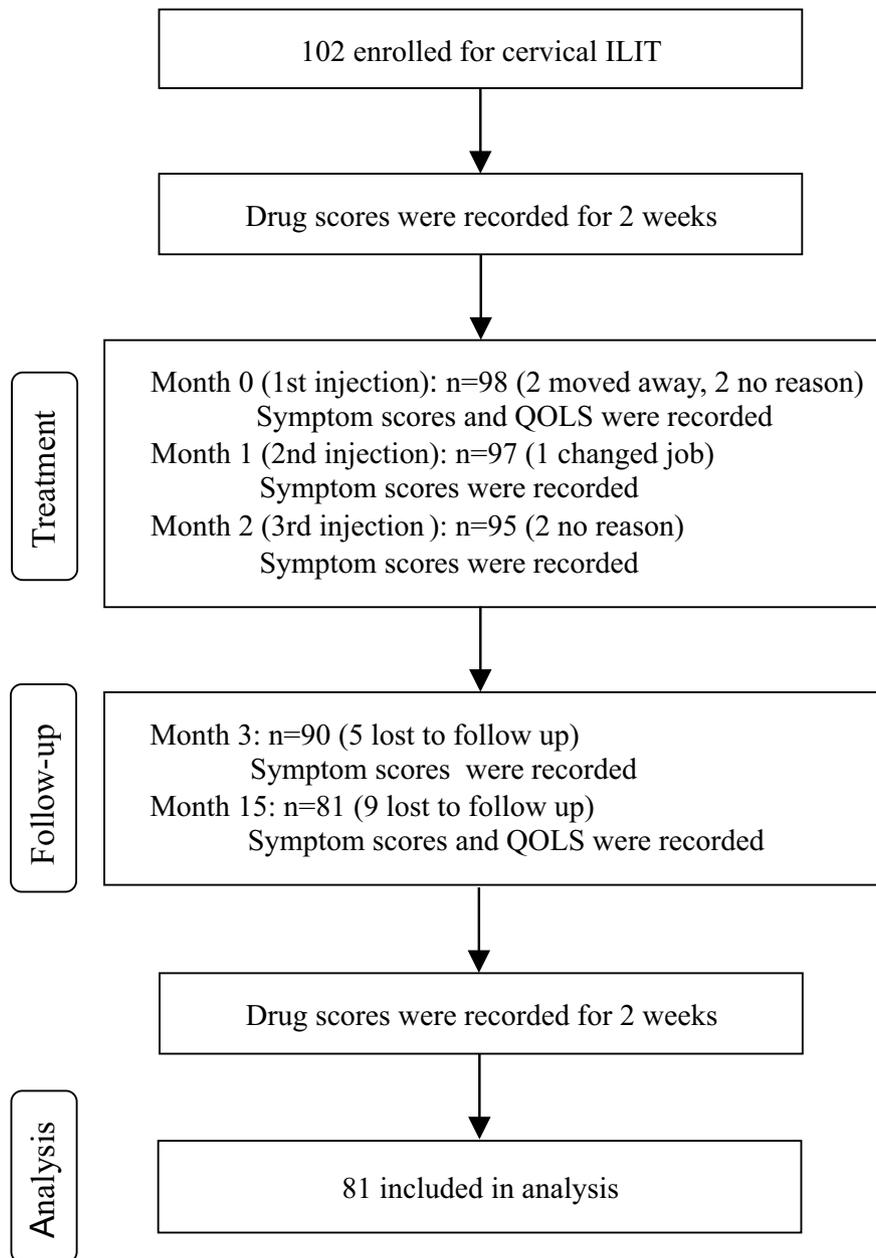


Fig. 1. Flow diagram of study cohort. ILIT: Intralymphatic immunotherapy; QOLS: quality of life score.

occurring in the course of immunotherapy, are the main reasons for the limitation of subcutaneous immunotherapy. The occurrence of adverse reactions of conventional subcutaneous immunotherapy is mainly related to patients' sensitivity to allergens, injection dosage of allergens and physicians' misoperation [6]. In previous studies of inguinal ILIT, adverse reactions rarely occurred due to the significant reduction of allergen dosage and times of injection [9,22]. In this study, 50 TU of standardized house dust mite allergen extracts, which were reduced by as many as about 400 times compared with routine subcutaneous immunization, were accurately injected into superficial lymph nodes of house dust mite sensitized AR patients under the guidance of ultrasound. And the frequency of injections was only 3 times, which was greatly reduced compared with subcutaneous immunization. Expectedly, the results in this study showed that no moderate-severe adverse reactions occurred in the patients during the treatment and 1-year follow-up. Therefore our study suggested that cervical ILIT is a comparatively safe therapy for house dust mite allergy.

This is a preliminary study to determine the safety and efficacy of

cervical ILIT for AR. One of the main limitations of this study is that we used a self-control of patients with AR at the baseline and after cervical ILIT but not a control group. Another limitation is that it is not placebo-controlled, so the effects of other factors, such as pharmacotherapy allergen avoidance and lifestyle modifications were not considered. In addition, the follow-up period is relatively short. These limitations may be addressed in future work through multicenter, double-blind, randomized, placebo-controlled, long-term study.

5. Conclusions

In conclusion, this is the first-in-human study using cervical ILIT as a new type of immunotherapy for AR, which could complete the treatment by injecting allergen extracts into superficial lymph nodes for 3 times. Our pilot study provides the first evidence that cervical ILIT was clinically efficacious and safe and could be applied for the treatment of AR.

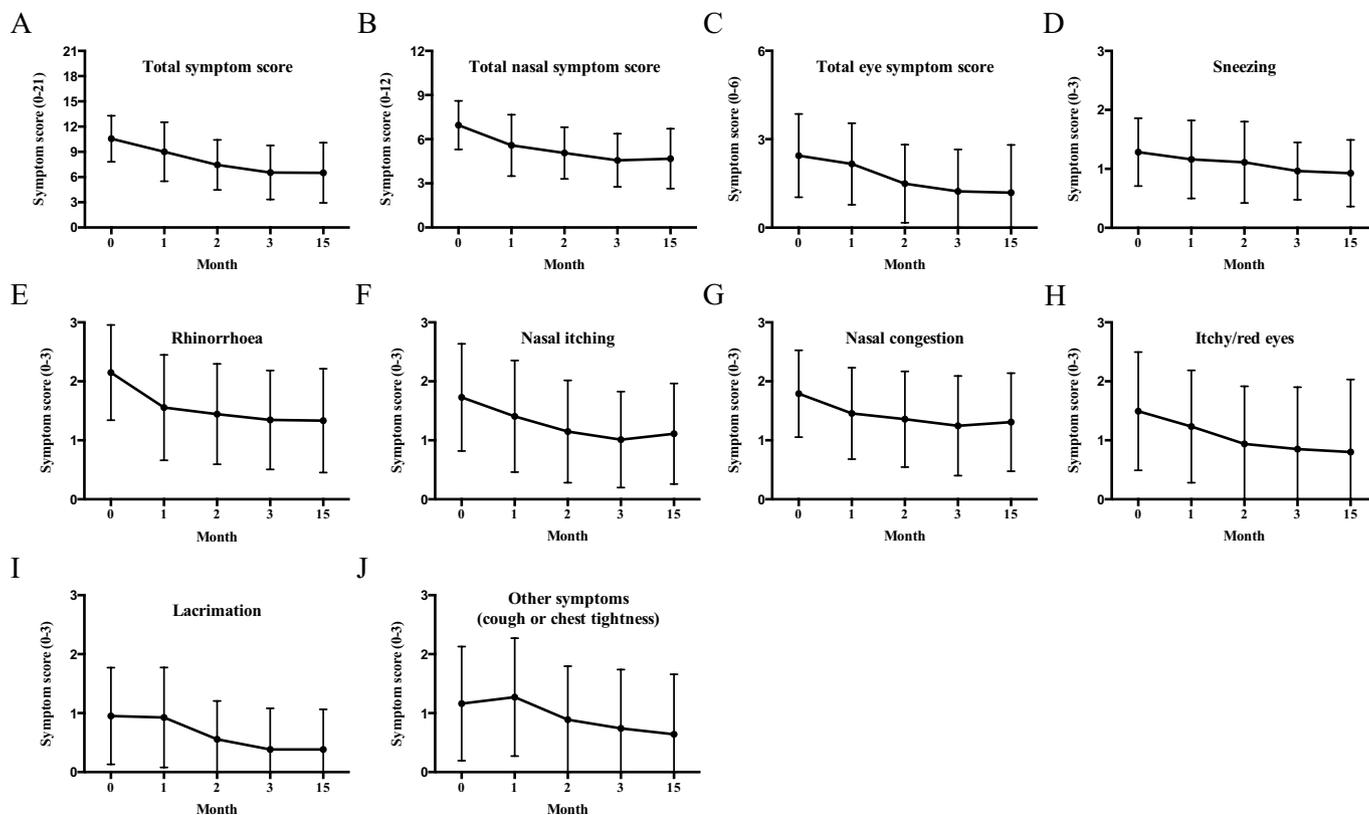


Fig. 2. Symptom scores of study subjects at baseline, 1 month after each injection, and 1 year after treatment.

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Authors' contributions

Dr. Kai Wang, Dr. Rui Zheng, Dr. Youmou Chen, Dr. Qingqing Yu, Dr. Hanrong Zhong, Dr. Ping Xiao, Dr. Yuejian Wang and Dr. Jun Tang

participated in the editing of the manuscript. Dr. Kai Wang and Dr. Jun Tang were involved in the planning, management, and reporting of the study, as well as in the preparation of the manuscript.

Declaration of competing interest

None.

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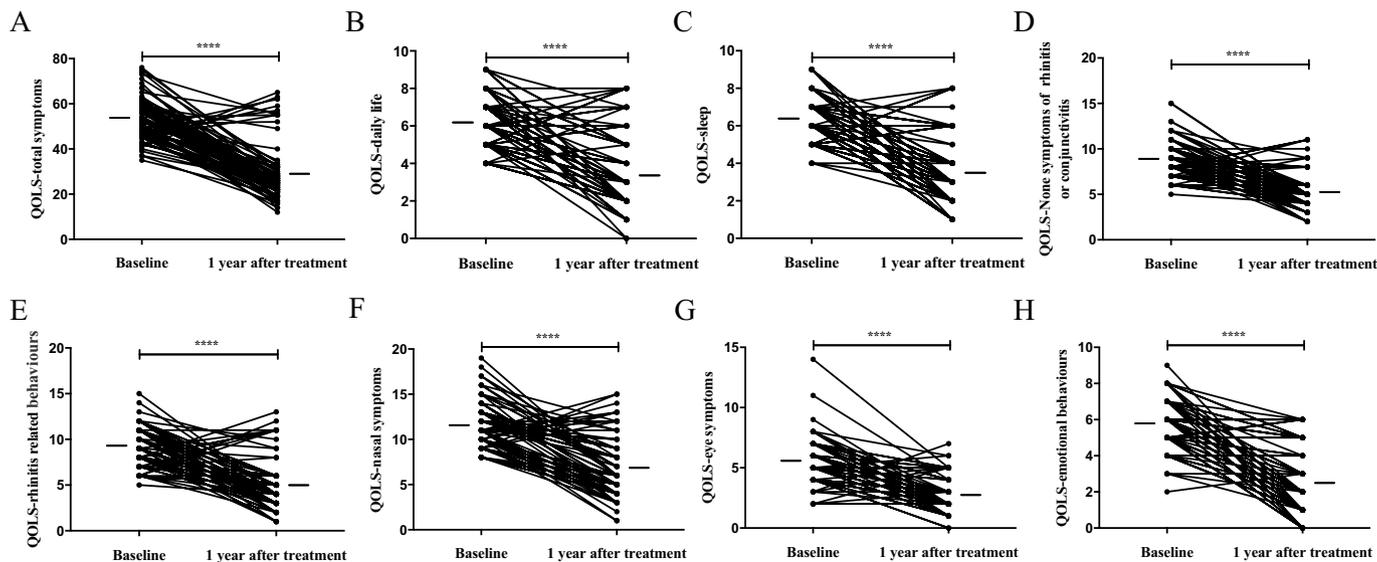


Fig. 3. QOLs of study subjects at baseline and 1 year after treatment. QOLS: quality of life score.

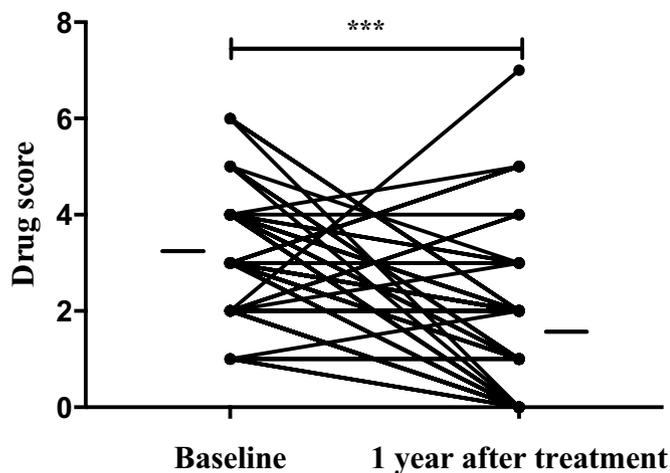


Fig. 4. Drug scores of study subjects at baseline and 1 year after treatment.

Table 2
Adverse events associated with cervical ILIT (243 injections in total).

		Number of injections
Local lymph node swelling	Mild	12
	Moderate	0
	Severe	0
Local itching	Mild	16
	Moderate	0
	Severe	0
Local redness	Mild	8
	Moderate	0
	Severe	0
Itching, other location	Mild	7
	Moderate	0
	Severe	0
Urticaria and angioedema	Mild	3
	Moderate	0
	Severe	0
Gastrointestinal infection	Mild	2
	Moderate	0
	Severe	0

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