

PROF. HANS JÜRGEN HOFFMANN (Orcid ID : 0000-0002-6743-7931)

Article type : Letter to the Editor

The number of successful injections associates with improved clinical effect in intralymphatic immunotherapy

To the editor:

Intralymphatic immunotherapy, ILIT, has emerged as a symptom-reducing immune treatment of allergic rhinoconjunctivitis as most studies find significant efficacy (1)(2). In ILIT, a dose of allergen 100-fold lower than in SCIT, is injected into a lymph node. Precise injection may therefore be essential to give a clinical and immunological response. Conversely, allergic side effects may develop if the allergen is injected extra-nodally. In a multicenter trial (EudraCT (2014-004031-40) we studied the relationship between the number of successful ILIT injections and clinical response and adverse events to ILIT.

175 patients suffering from allergic rhinoconjunctivitis in the grass pollen season and with a positive skin prick test to grass pollen extract were included (Table S1). Three ILIT injections of 1.000 SQU of ALK Alutard, Phleum Pratense (off label), were given with one-month interval. Injections were performed by allergists undergoing a training program in ultrasound guided lymph node injections. Patients served as their own controls; the primary outcome was a combined Symptom and Medication Score (3), cSMS, reported daily in the grass pollen seasons before and after ILIT (Figure S1). Safety was a secondary outcome parameter (see supplementary file for methods). An overall reduction in cSMS of 0.65 (95%CI 0.54-0.76, $p<0.0001$, $n=157$), 39.2% (95%CI 35.3%-42.6%) was found when comparing the pre-ILIT season to the post-ILIT (Figure 1A). As an independent proxy of allergic response, the diameter of the skin prick test was reduced by 5.1 (95%CI 4.4 – 5.7, $p<0.0001$, $n=85$), 43.9% (95%CI 40%-47.7) ($p<0.0001$, $n=85$) (Figure 1B).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14642](https://doi.org/10.1111/ALL.14642)

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All patients received active treatment. Ultrasound film clips were not recorded in all injections. They were only available at all three injections for 52 patients. Six patients received three successful injections, nine received two, 22 received one and 15 unfortunately did not receive any injection into a lymph node (methods in supplement). cSMS was reduced from 1.5 (95%CI 1.3 – 1.8) to 0.8 (95%CI 0.7 – 1.0), $p < 0.0001$ in these 52 patients and there was an association of number of successful injections and clinical effect (Supplementary figure 2B).

However, as median grass pollen counts were significantly lower in the post-ILIT season, $p < 0.0001$, pollen exposures between seasons were adjusted for with a linear regression model of cSMS against Log_{10} transformed grass pollen counts (Supplementary Figure 2B-E). Slopes of the regression lines estimate cSMS response to allergen exposure. The slope of all patients after treatment decreased by 0.54 (95% CI 0.43 - 0.67, $p < 0.0001$). The dose dependent decrease in slope, corresponding to symptoms, with increasing number of successful injections into lymph nodes (Supplementary figure 2B-E, Table 1) suggests that clinical improvement associates with increasing number of successful ILIT injections.

In total 125 (27%) adverse events occurred in the 465 injections. Most, 106 (23%), were local, mild and spontaneously resolving reactions at the injection site. Fourteen (3%) systemic reactions occurred, all mild but one where 10.000 SQU was accidentally injected. Five (1%) unspecific events were registered (see detail in supplementary results). Adverse events were not associated with injection quality ($p = 0.82$). Pain related to the injection was rated to a median of VAS 2 (IQR 1-3).

The non-randomized design of our study limits placebo comparison of effect size. However, the linear regression analyses between grass pollen count and cSMS allows within-patient estimation of effect of ILIT with adjustment of difference in pollen count in the season before and after ILIT.

In conclusion, the clinical response to, but not safety of ILIT seems to depend on successful ILIT injections and low-quality injections must be acknowledged as a reason for treatment failure in ILIT. Training in ILIT injections is required.

Figure legends:

Figure 1:

A: Combined symptom and medication score in the grass pollen season before and after ILIT injections.

B: Diameter of weal in skin prick test to grass before and after ILIT injections.

C: Effect of number of successful ILIT injections on cSMS and sensitivity to grass pollen. Percentage reduction in cSMS before and after ILIT (dot) with 95%CI and left y-axis. Change in value of the slope from a regression analysis between cSMS and grass pollen counts before and after ILIT (presented in supplementary Figure 2C-F) with 95%CI (squares). A dose-response like curve is seen with reduction in cSMS (both as percentages and in slopes) by number of successful injections ($r^2=0.28$ for reduction in cSMS, $r=0.95$ for change in slope of regression analysis).

Number of Successful injections	n	Mean cSMS reduction			Linear regression of cSMS vs $\log_{10}(\text{Pollen})$		
		cSMS reduction	95%CI	p-value	Slope	95%CI	p-value
0	15	0.4	0.11 – 0.75	0.01	-0.07	0.18 to -0.32	0.59
1	22	0.91	0.5 – 1.32	0.002	-0.51	-0.43 to -0.69	0.001
2	9	0.42	0.13 – 0.97	0.1	-0.6	-0.32 to -0.88	<0.001
3	6	1	0.13 – 1.9	0.03	-0.93	-0.56 to -1.3	<0.001

Table 1: Dose response of clinical improvement with increasing number of injections into lymph nodes.

List of authors:

Søren Helbo Skaarup, Department of Respiratory Medicine and Allergy. Aarhus University Hospital. Denmark.

Ole Graumann, Department of Radiology, Odense University Hospital, Denmark. Research and Innovation Unit of Radiology, University of Southern Denmark

Johannes Schmid, Department of Respiratory Medicine and Allergy. Aarhus University Hospital. Denmark.

Anne Sofie Bjerrum, Department of Respiratory Medicine and Allergy. Aarhus University Hospital. Denmark.

Tina Skjold, Department of Respiratory Medicine and Allergy. Aarhus University Hospital. Denmark.

Hans Jürgen Hoffmann, Department of Respiratory Medicine and Allergy. Aarhus University Hospital and Institute for Clinical Medicine, Aarhus University, Denmark.

Corresponding author:

Hans Jürgen Hoffmann, Department of Respiratory Medicine and Allergy. Aarhus University Hospital and Institute for Clinical Medicine, Aarhus University, Denmark.

e-mail: hjh@clin.au.dk

Address: Department of Respiratory Medicine and Allergy. Aarhus University Hospital.
Palle Juul-Jensens Boulevard 99. 8200 Aarhus N. Denmark.

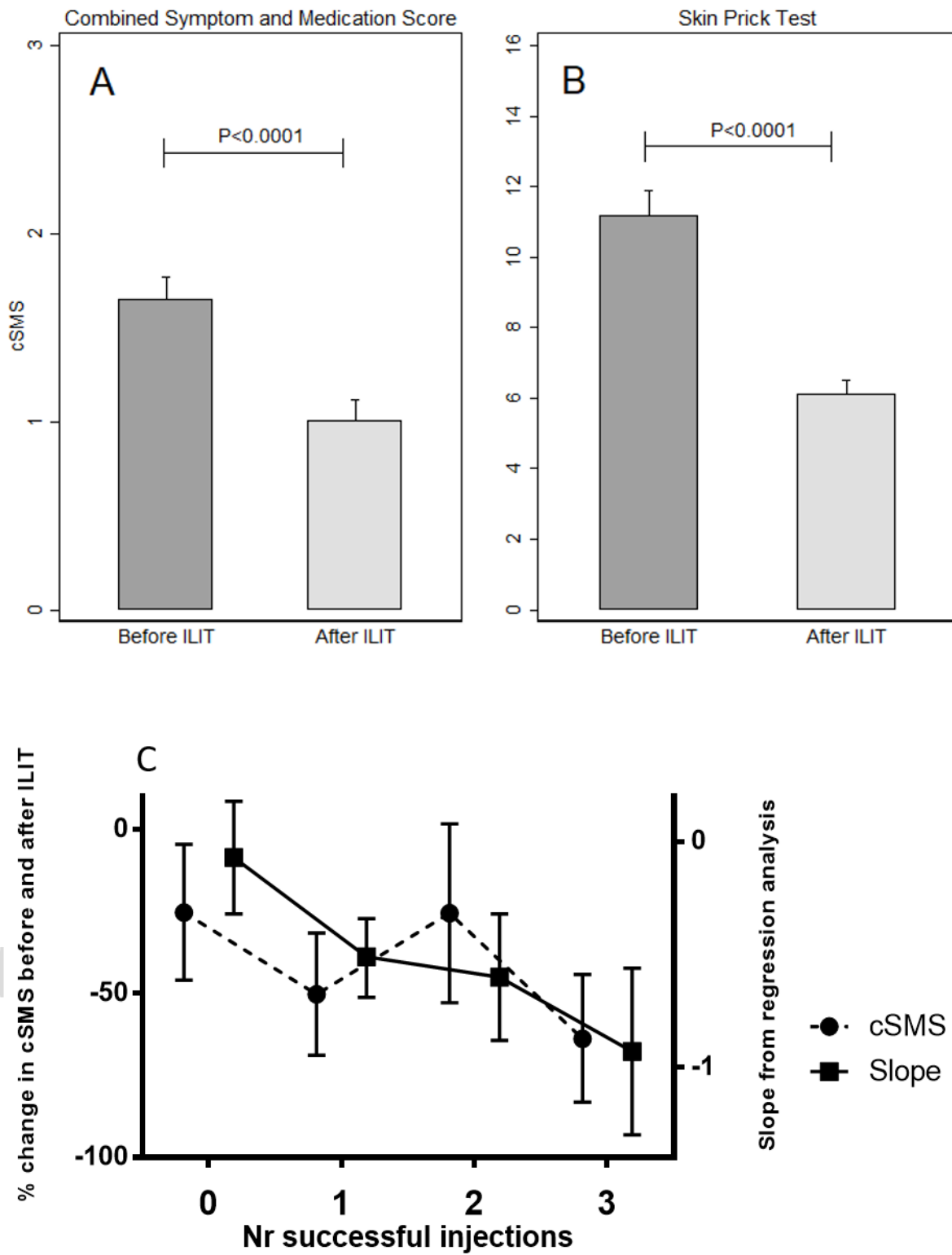
All authors contributed to the manuscript.

Conflicts of interest: The authors have no conflict of interest.

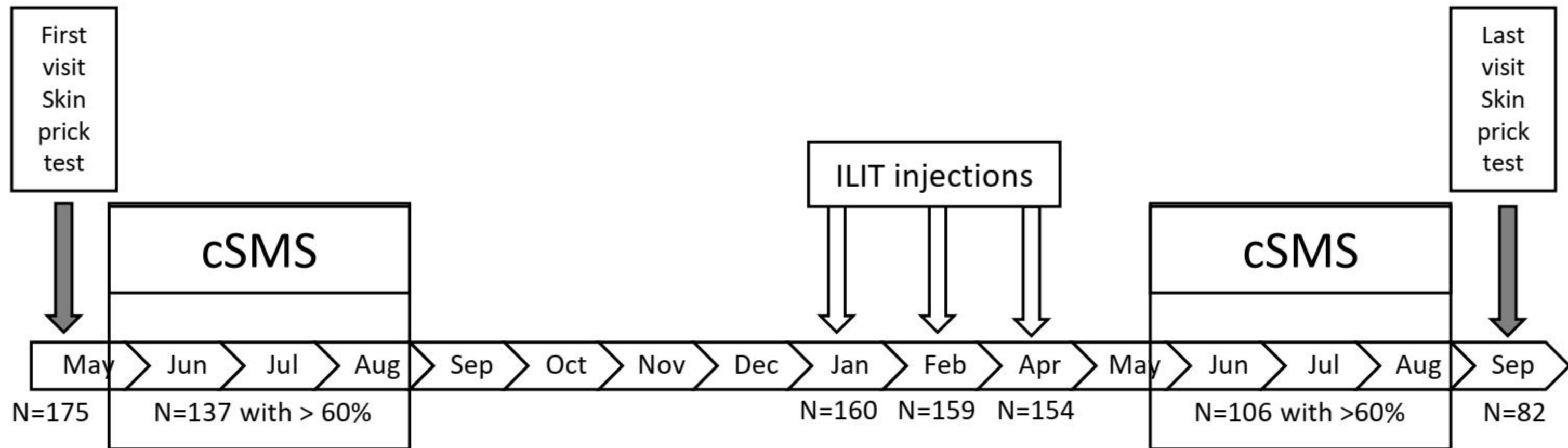
Funding: The project was funded the Central Region Denmark, which is a part of the governmental health care system in Denmark.

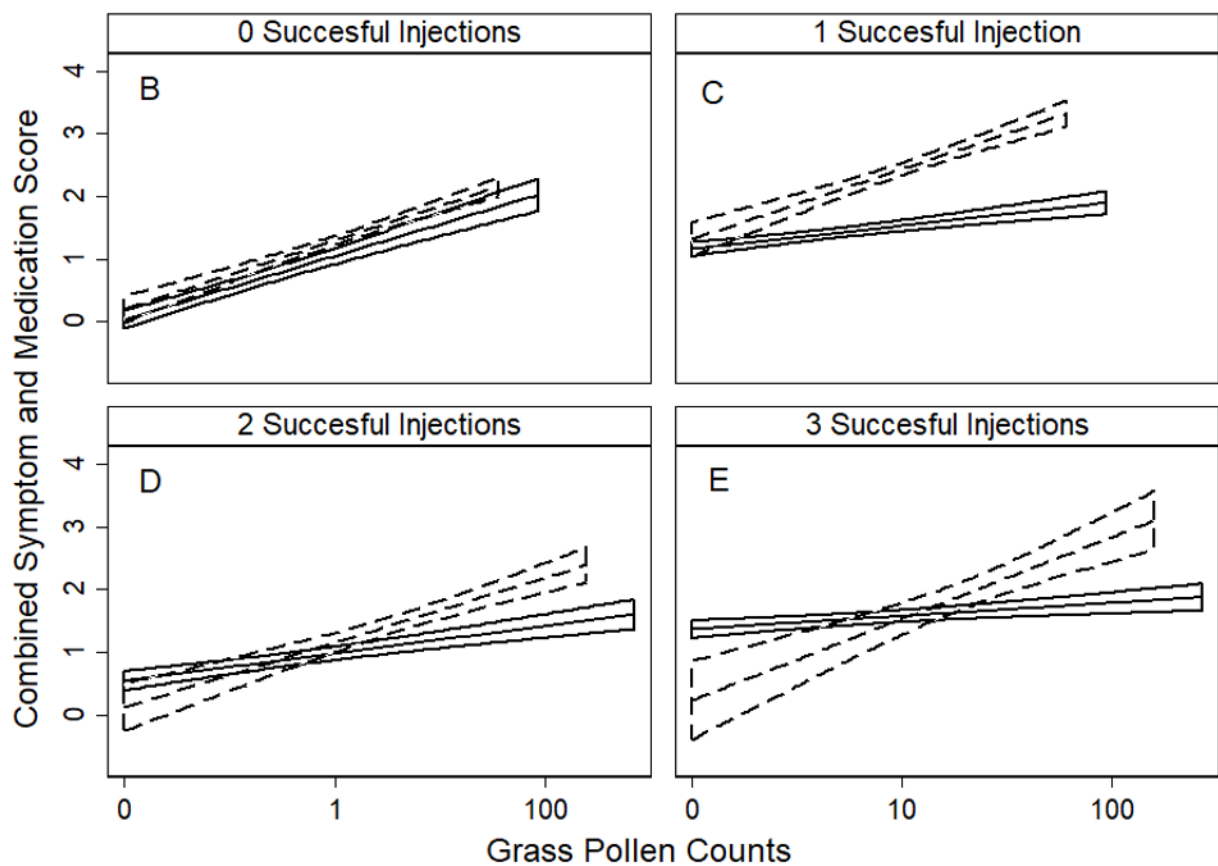
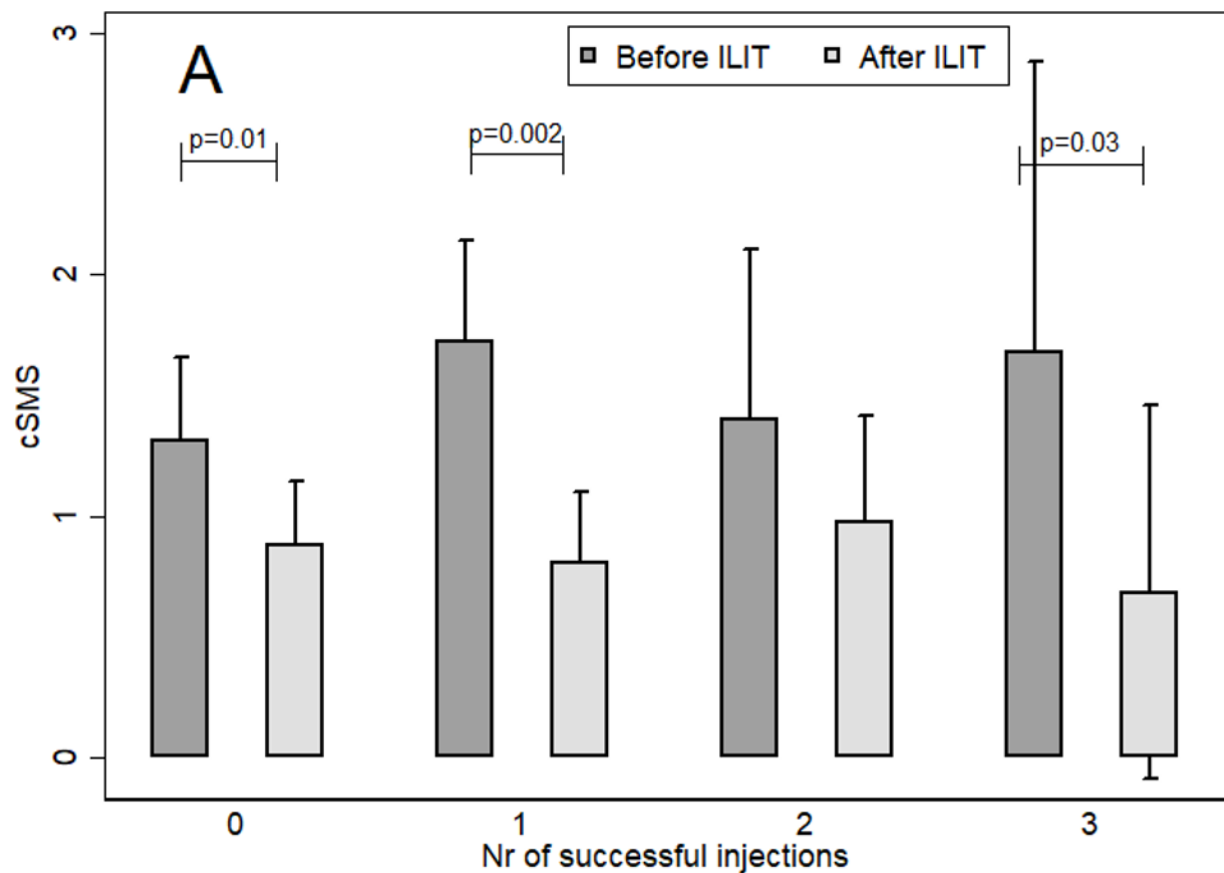
References

1. Skaarup. SH, Schmid. JM, Skjold. T, Graumann. O, Hoffmann. HJ. Intralymphatic Immunotherapy improves grass pollen allergic rhinoconjunctivitis. A three-year randomized placebo-controlled trial. *J Allergy Clin Immunol* 2020;**0**. doi:10.1016/j.jaci.2020.07.002
2. Senti G, Freiburghaus AU, Larenas-Linnemann D, Hoffmann HJ, Patterson AM, Klimek L et al. Intralymphatic Immunotherapy: Update and Unmet Needs. *Int. Arch. Allergy Immunol.* 2019;**178**:141–149.
3. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy* 2014;**69**:854–867.



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Online Supplement:

Supplementary Methods

The Danish scientific ethics committees approved the study protocol (1-10-72-408-14), Good Clinical Practice guidelines were followed and monitored. Participants had given informed consent before inclusion.

The trial also studied the effect of training ILIT procedures. These results will be published elsewhere.

Setting

The Department of Respiratory Medicine and Allergy at Aarhus University Hospital served as the coordinator in a multicenter setup.

Patients

Adult patients with rhinoconjunctivitis symptoms in the grass pollen season and a skin prick test larger than 3 millimeters with ALK 225 Soluprick Grass were eligible for inclusion. Exclusion criteria were malignancy, severe comorbidity, renal failure, severe asthma (FEV1 <70% of expected), autoimmune disease, pregnancy or no anticonception in the injection period, allergy to phenol or aluminum hydroxide, treatment with beta-blocking medication, severe cardiac disease or severe arterial hypertension.

Outcome measures

Injection quality score

An expert in ultrasound guided intervention (OG, 14 years of experience) evaluated all available ultrasound film clips from ILIT injections blinded to all other study information. Quality of injections was scored with five grades where one was given if the allergen was placed inside another anatomical structure, two if allergen was placed far away from the lymph node, three if the allergen was delivered right next to the lymph node, two if allergen was delivered inside the lymph node but not in the cortex and five if allergen was delivered into the cortex of the lymph node. Scores were transformed into binary outcomes of successful injections set to a score equal to or higher than 4 (Injection inside the lymph node) was considered a success. Scores lower than 4 was considered as failures. Patients were divided into groups based on number of successful ILIT injections i.e. 3 successful injections, 2 successful injection and 1 failure, 1 success and 2 failures or 0 successes and 3 failures.

Clinical response to ILIT and pollen counts

Patients registered allergic symptoms and use of rescue medication every day during the grass pollen season before and after ILIT injections with a combined symptom and medication score. Daily grass pollen counts were provided by Astma-Allergi Denmark (the Danish association for allergic patients, www.astma-allergi.dk). Skin prick test using grass pollen extract, ALK 225 Soluprick, was performed before and after ILIT injections. Maximal wheal diameter was measured in millimeters.

Adverse Events

Adverse events to ILIT injections were recorded systematically during a one-hour observation period after ILIT injections and with an interview at the following visit. Procedure pain was evaluated by participants on a Likert scale from 1-10. Pre-ILIT antihistamine was only given if the participant had an adverse event to prior injections.

Statistical methods

Primary outcome measure was evaluated with means and 95% confidence intervals. Significance level was set to 5%. Analysis were performed in Stata vs. 14.2 (Texas, USA). Graphs were made in Stata or in GraphPad Prism (California, USA).

Clinical response to ILIT was assessed by paired T-test of cSMS data and skin prick wheal diameter after evaluation of parametric distribution. Difference in response between groups of injection quality was assessed using Wilcoxon rank-sum test if non-parametric distribution of data.

To reduce impact of different grass pollen count in the two study seasons a linear regression analysis of cSMS response to decadic logarithmic transformed grass pollen count as described recently was done for each of the injection quality groups (1).

Missing data due to incorrect film recording of injections were considered to be missing completely at random and no imputation was done.

Supplementary Results:

Injection quality and clinical efficacy

The effect of injection quality on cSMS was evaluated in the subgroup of patients for whom recorded film clips of all three ILIT injections were available for evaluation, n=52 (32%) (Supplementary Figure 2A). In patients who had three successful injections into the lymph node, n=6, mean reduction in cSMS was 1.0

(95%CI 0.13-1.9), $p=0.03$. In those patients who received two injections into a lymph node and one outside, $n=9$, mean cSMS reduction of 0.42 (95%CI -0.13-0.97), $p=0.1$ was found. The patients receiving only one injection into a lymph node and two outside, $n=22$, had a mean cSMS reduction of 0.91 (95%CI 0.50-1.32), $p=0.002$. Finally, in patients receiving three unplanned injections missing the lymph node, $n=15$, cSMS was reduced by 0.4 (95%CI 0.11-0.75), $p=0.01$. Significant difference in cSMS reduction between no successful injections and one success and two failed injections was found, $p=0.02$ with Wilcoxon Mann-Whitney test. Insignificant but a strong tendency was found in cSMS reduction between no successes and any successful injections, $p=0.06$.

By the linear regression of clinical response (cSMS) to Log_{10} transformed grass pollen counts the response to was studied in different injections quality groups. In the pre-ILIT season no difference between the groups of injection quality was found, maximal slope contrast between-groups 0.33 (95%CI -0.37 – 0.52), $p=0.1$. Contrary, in the post-ILIT season different slopes was seen between the injection quality groups. Contrast between 0 and 1 successful ILIT was 0.56 (95%CI 0.38 – 0.76), $p=0.001$, contrast between 0 and 2 successful ILIT 0.38 (95%CI 0.16 - 0.60), $p<0.001$ and contrast between 0 and 3 successful ILIT 0.64 (95%CI 0.44 – 0.85), $p<0.001$.

The response to ILIT was analyzed with a comparison of slopes before and after ILIT within the groups of injection quality (Supplementary Figure 2B-E). No significant change in the group who received 0 successful injections was found with a contrast of -0.07 (95%CI-0.32 – 0.18, $p=0.59$) Supplementary Figure 2B. All other groups showed significant increasing contrast before and after ILIT. For the group with one successful ILIT; contrast: -0.51 (95%CI -0.34 - -0.69, $p=0.001$) (Supplementary Figure 2C). For the group with 2 successful ILIT; contrast: -0.60 (95%CI -0.32 - -0.88) $p<0.001$ (Supplementary Figure 2D) and for the group with three successful ILIT injections; -0.93 (95%CI -0.56 - -1.3, $p<0.001$) (Supplementary Figure 2E). Percent reduction in cSMS and contrast between slopes before and after ILIT correlate to the number of successful ILIT injections (Figure 1C). This can be seen as a dose-response relationship between injection quality and clinical response. The contrast of the slopes (Supplementary Figure 2B-E) that we have proposed recently (1) scaled more linearly ($r^2=0.95$) with injection quality than difference in cSMS ($r^2=0.28$).

Relation between number of successful injections and change in diameter of reaction to skin prick test with ALK 225 phleum pratense Aquagen is presented in supplementary table 2. Only 29 patients had all three ILIT injection films and pre- and post-ILIT skin prick test available to analysis. In these small numbers a significant and dose-response reduction was found with increasing numbers of successful injections, except in patient receiving three successful injections were $n=3$, may reduce accuracy of the estimate (Supplementary table 2).

Adverse events

In total 125 (27%) adverse events occurred in the 465 injections. Most events, 106 (85% of all adverse events, 23% of all injections), were local reactions with redness, swelling or pain at the injection site. All were mild, not requiring any treatment and all resolved completely. Fourteen (11% of all adverse events, 3% of all injections) mild systemic reactions occurred. Of these three systemic reactions were related to an accidental injection of 10.000 SQU instead of planned 1.000 SQU in six patients. One of these participants (0.8% of all adverse events, 0.2% of all injections) was admitted to hospital for observation for 24 hours and was treated with oral prednisolone and antihistamine. Three patients tolerated a single, accidental treatment with 10.000 SQU. No other systemic reactions required treatment other than oral antihistamine. All reactions resolved completely. Five patients had unspecific reactions (4% of all adverse events, 1% of all injections) including fatigue or vasovagal reaction following the injection. No significant relation between injection quality and adverse event was found with a Fishers Exact test, $p=0.82$.

Age, mean (s.d.)	35.2 (7.5)
Sex, female, n (%)	63 (40)
Skin prick test positive to grass, n (%)	179 (100)
Aria classification of disease severity	
Mild, n (%)	43 (25)
Moderate/severe, n (%)	132 (75)
Years with allergy, mean (s.d.)	19 (10)
Grass Pollen Symptoms at baseline	
Itching nose, n (%)	150 (84)
Runny nose, n (%)	120 (67)
Itching eyes, n (%)	150 (84)
Runny eyes, n (%)	71 (40)
Fatigue, n (%)	22 (12)
Relevant Medication	
Antihistamine, eyedrops, n (%)	118 (66)
Antihistamine, tablets, n (%)	172 (96)
Steroid, nasal, n (%)	128 (72)
Steroid, tablet 5-15 mg daily, n (%)	10 (6)
Steroid, injection of depot, n (%)	77 (43)
Years on medicine, mean (s.d.)	16 (9.3)
Other medications, n (%)	
Beta-2-agonist inhalation, n (%)	37 (21)
Inhaled corticosteroid, n (%)	15 (8.6)
Eltroxin, lamotrigine, betablocker tablets, insulin, metformin, sumatriptan. Each, n (%)	1 (0.6)
Sensitization	
Mono sensitized to grass, n (%)	91 (51)
Two sensitizations	22 (18)
Three sensitizations	15 (8)
Four or more sensitizations	9 (5)
Other allergies	
Birch, n (%)	53 (30)

Ragweed, n (%)	19 (11)
Cat, n (%)	37 (21)
Dog, n (%)	21 (12)
Horse, n (%)	7 (4)
House dust mites, n (%)	21 (12)
Molds, n (%)	1 (0.5)
Other diseases than allergy	
Asthma	46 (26)
Atopic eczema	8 (5)
Recurrent migraine	3 (2)
Morbus Crohn, bipolar affective disorder, chronic pain, depression, diabetes, GI-reflux, hypertension, hypothyroidism, chronic sinusitis. Each, n (%)	1 (0.6)

Table S1: Demographical information of patients.

Number of injections	N	Pre-ILIT		Post-ILIT		Change		
		Diameter, mm.	S.D.	Diameter, mm.	S.D.	Diameter, mm.	95% CI	Change in percent
0	10	10.7	2.3	5.9	1.1	4.8	3.4 – 6.2	44.9%
1	11	11.5	2.3	5.8	1.4	5.7	4.3 – 7.1	50%
2	5	14.4	6.6	5.8	1.9	8.6	2.5 – 14.7	60%
3	3	9	1	6.7	2.1	2.3	-0.5 – 5.2	26%

Table S2: Skin prick test results from the patients who had ultrasound film-clips available for analysis for all three ILIT injections. A dose-response reduction in weal diameter is seen with increasing numbers of successful injections, except in the three patients who received three successful injections, likely a result of small sample size.

Supplementary figure legends:

Figure S1. Flow chart of inclusion and the study. 175 patients with grass pollen rhinitis were recruited and registered Combined Symptom and Medication Score, cSMS, in the grass pollen season before and after ILIT injections. Likewise, a skin prick test was done at first and last study visit. ILIT injections were performed with 1.000 SqU, ALK 225 Phleum Pratense with three injections with one-month interval.

Figure S2.

A: Mean and 95%CI for combined symptom and medication score, cSMS, in the grass pollen season before and after ILIT by number of successful injections. Significant decrease is seen for all injection qualities but for patients with 2 successful and 1 failure.

B,C, D and E: Regression with 95%CI of cSMS and decadic logarithmic transformed grass pollen counts relationship grouped by injection quality. Slopes of data from the season before ILIT (dashed line) were similar between groups, implying equal pre-ILIT sensitivity in all groups. Slopes of data from the season after ILIT (solid line) are less steep in all injection quality groups except the one without any successful injections indicating a change in sensitivity after ILIT injections. Furthermore, post-ILIT slopes tend to flatten out as a result of more successful injections relating drop in sensitivity to grass pollen exposure to a higher number of successful ILIT injections.