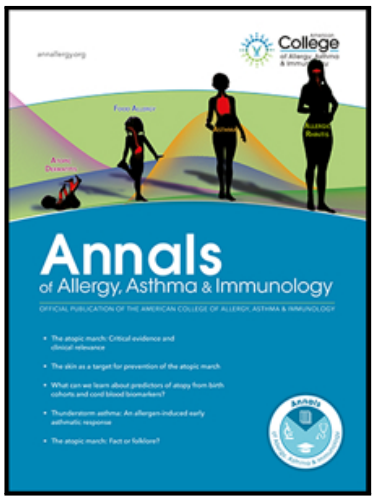
Intralymphatic allergen immunotherapy against pollen allergy. A 3-year open follow-up study of 10 patients

Lars Ahlbeck, Emelie Ahlberg, Ulla Nyström Kronander, Janne Björkander and Maria Jenmalm

Ahlbeck, L., Ahlberg, E., Nyström Kronander, U., Björkander, J., Jenmalm, M., (2018), Intralymphatic allergen immunotherapy against pollen allergy. A 3-year open follow-up study of 10 patients, *Annals of Allergy, Asthma & Immunology*. https://doi.org/10.1016/j.anai.2018.07.010

Original publication available at:
https://doi.org/10.1016/j.anai.2018.07.010



Intralymphatic allergen immunotherapy against pollen allergy. A 3-year open follow-up study of 10 patients

Lars Ahlbeck , Emelie Ahlberg , Ulla Nyström , Janne Björkander , Maria C Jenmalm

PII: S1081-1206(18)30571-4
DOI: 10.1016/j.anai.2018.07.010
Reference: ANAI 2622

To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 23 April 2018
Revised date: 18 May 2018
Accepted date: 6 July 2018

Please cite this article as: Lars Ahlbeck , Emelie Ahlberg , Ulla Nyström , Janne Björkander , Maria C Jenmalm , Intralymphatic allergen immunotherapy against pollen allergy. A 3-year open follow-up study of 10 patients, *Annals of Allergy, Asthma Immunology* (2018), doi: 10.1016/j.anai.2018.07.010

Elsevier Editorial System(tm) for Annals of Allergy, Asthma & Immunology

Manuscript Draft

Manuscript Number: 18-04-0239R1

Title: Intralymphatic allergen immunotherapy against pollen allergy. A 3-year open follow-up study of 10 patients.

Article Type: Letters

Keywords: allergy; immunotheraphy; intralymphatic; rhinoconjunctivitis; T-cells

Corresponding Author: Dr. Lars Erik Ahlbeck, M.D.

Corresponding Author's Institution: Linköping University

First Author: Lars Erik Ahlbeck, M.D.

Order of Authors: Lars Erik Ahlbeck, M.D.; Emelie Ahlberg, BSc; Ulla Nyström, M.D.; Janne Björkander, M.D, PhD; Maria Jenmalm, PhD

Suggested Reviewers:

Opposed Reviewers:

Response to Reviewers: After corespondance with Editor-in-Chief Dr Gailen D Marshall, I wish to resubmit this letter to the editor as a shorter version of the previous submitted original research article entitled “Intralymphatic allergen immunotherapy against pollen allergy. A 3- year open follow-up study of 10 patiens” for consideration by Annals of Allergy, Asthma & Immunology.

Dear Editor,

After corespondance with Editor-in-Chief Dr Gailen D Marshall, I wish to resubmit this letter to the editor as a shorter version of the previous submitted original research article entitled "Intralymphatic allergen immunotherapy against pollen allergy. A 3- year open follow-up study of 10 patiens" for consideration by Annals of Allergy, Asthma & Immunology.

I confirm that this work is original and has not been published elsewhere, nor is it currently under consideratition for publication elsewhere.
The manuscript has been read and approved by all the authors.
The requirements for authorship have been met.
The authors certify that they have (collectively) personally written at least 90 percent of the manuscript.
The manuscript has not been published previously in print/ electronic format (except in the form of an abstract or as part of a published lecture) or in another language and that the manuscript is not under consideration by another publication or electronic media.

In this letter, we report on a novel form of immunotherapy against pollen allergy, intralymphatic immunotherapy (ILIT), meaning only 3 injections with 4 weeks interval. This is significant because nearly 30% of the adult population report allergic rhinitis. Allergen immunotherapy (AIT) is an opportunity to treat these patients. Conventional AIT with subcutaneous injections is effective, but consumes time and resources there is a need for a more convenient, faster and less resource-consuming way to induce tolerance in allergy.

We believe that this manuscript is appropriate for publication by Annals of Allergy, Asthma & Immunology because this pilot study shows, statistically significant clinical efficacy sustained for at least 3 years accompanied by corresponding immunological changes. This study adds to the hitherto positive studies and suggests that ILIT is effective and safe as treatment for pollen allergy of witch one is previously published in Annals of Allergy, Asthma & Immunology

The study was funded by Region Östergötland, the Allergy Center in Linköping, the Medical Research Council of Southeast Sweden (FORSS), the Th Bergh Foundation, and the Asthma and Allergy Association.

Please address all correspondence concerning this manuscript to me at

Lars Ahlbeck
Allergy Center
Linköping University Hospital
58185 Linköping
Sweden

Phone +46-702871309
Fax +46-101034773
lars.ahlbeck@regionostergotland.se; lars.ahlbeck@telia.com

Thank you for your consideration of this manuscript.

Sincerely,

Lars Ahlbeck

Title page:

Title:
Intralymphatic allergen immunotherapy against pollen allergy. A 3-year open follow-up study of 10 patients.

Authors:
Lars Ahlbeck M.D.[1]
Emelie Ahlberg BSc[2]
Ulla Nyström M.D.[1]
Janne Björkander M.D, PhD.[3]
Maria C Jenmalm PhD. [2]

[1]Allergy Center, University Hospital, Linköping, Sweden; [2]Unit of Autoimmunity and Immune Regulation, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden; [3]Futurum, Academy of Health and Care, Jönköping, Sweden

Authors' contribution:

L.A.; study design, data acquisition, clinical data, analysis and manuscript preparation. E.A.; data acquisition, analysis and manuscript preparation. U.N., study and experimental design, clinical data, data interpretation, critically reviewing the manuscript. J.B. and M.C.J.; study and experimental design, data interpretation, critically reviewing the manuscript.

Corresponding author information:
Lars Ahlbeck
Allergy Center,
University Hospital
SE-581 85 Linköping,
Sweden

Tel +46 (0)10 – 103 7812
Fax +46 (0)10 – 103 4773
e-mail: lars.ahlbeck@regionostergotland.se

Conflict of Interest:
Lars Ahlbeck has received honoraria as a speaker and/or adviser from Astra Zeneca, Meda, Takeda, Teva, Boehinger Ingelheim, MSD and Novartis. Janne Björkander has received honoraria as a speaker from ALK

Funding Source:
Region Östergötland, the Allergy Center in Linköping, the Medical Research Council of Southeast Sweden (FORSS), the Th Bergh Foundation, and the Asthma and Allergy Association of Sweden.

Clinical Trial Registration:
EudraCT (2012-004088-38).

Keywords:

immunotherapy, intralymphatic, allergy, rhinoconjunctivitis, T-cells

Abbreviations:

AIT	Allergen-specific immunotherapy
aTreg	Activated T regulatory cell
CCL17	CC motif chemokine ligand 17
CXCL10	CXC motif chemokine ligand 10
FoxP3	Forkhead box P3
GATA3	GATA binding protein 3
ILIT	Intralymphatic immunotherapy
IL-4	Interleukine-4
RORC	Retinoic-acid related orphan receptor C
RQLQ	Rhinoconjunctivitis quality of life questionnaire
RTSS	Rhinoconjunctivitis total symptom score
rTreg	Resting T regulatory cell
SQ-U	Standardized quantified units
Tbet	T-box expressed in T cell
Th	T helper
Treg	T regulatory cell

Word Count : 880

Tables: 1

To date, allergen immunotherapy (AIT) is the only treatment that affects the long-term development of allergic rhinoconjunctivitis and induces clinical tolerance primarily by stimulating regulatory T (Treg) cells, attenuating T helper 2 (Th2) responses and synthesis of blocking antibodies[1]. Conventional AIT with subcutaneous injections, sublingual tablets or drops is effective, but consumes time and resources [2].

In 2008 Swiss researchers published a study in which they gave only three monthly injections directly into the inguinal lymph nodes. The patients were followed 3 seasons after treatment. They found enhanced efficacy and safety, and faster relief of symptoms [3]. This study has been followed by a few smaller studies whereof one with contradictory results, all only one season after treatment [4-8]

We report a 3-year open follow-up study of 10 patients recruited 2012. The patients were 22-47 years old and had seasonal allergic symptoms to grass or birch, verified by skin prick test and elevated specific IgE. The patients received 3 doses of 0.1 ml of ALK-Abello's birch or 5-grass allergen extracts (1,000 standardized quantified units per dose) at 4-week intervals. Ultrasound-led technique was used. The study was approved by the Regional Ethics Committee in Linköping (2012/286-31). Clinical Trial Registration: EudraCT (2012-004088-38). Informed signed consent was obtained from the participants before inclusion in the study.

8 patients were available at follow-up 3 years after treatment, 4 of whom were treated against birch and 4 against grass.

The symptoms were significantly reduced as measured by Rhinoconjunctivitis Total Symptom Score (RTSS) from 14.0 to 7.6 ($p<0.01$) from the beginning to the end of the study

(Table). The minimal important difference (MID) of RTSS is 1.1-1.3 [9]. The use of medication was slightly but not significantly reduced. The impact on quality of life measured by Juniper´s Rhinoconjunctivitis quality of life questionnaire (RQLQ) was reduced from 3.42 before treatment to 1.34 3 years after ($p<0.01$, Table). MID is 0.49[10]. (Table)

Mostly mild/moderate adverse events were noted such as local reactions at the allergen injection site, tiredness after the injections, and mild rhinitis. One patient experienced itch without hives on her neck and trunk and a fall in PEF 40 minutes after her first injection. The reaction was judged as severe but disappeared within 15 minutes after intramuscular epinephrine injection. She had shown poor adherence to her asthma treatment was excluded from more injections.

Levels of allergen-specific IgE were slightly elevated from the first visit to 2 years after treatment ($p<0.01$) and returned to baseline after 3 years (Table). Levels of allergen-specific IgG4 were increased but not significantly. Skin prick tests were slightly, but not significantly, reduced ($p=0.20$). Conjunctival challenge tests showed a trend toward a higher threshold for tolerance, but the number of tests performed was low ($p=0.11$, Table).

Flow cytometry was used to evaluate whether the treatment affected different Th cell subsets in peripheral blood. The proportion of Th2 cells, defined as $CD3^{+}CD4^{+}CD45RA^{-}GATA3^{+}$ cells, decreased in proportion between screening and 1 year after treatment had finished (median and IQR 7.32, 3.8-9.16 and 0.39, 0.06-1.06, respectively, $p<0.001$). The decrease was observed 4 weeks after the first injection. The proportion of activated Treg cells, defined as $CD3^{+}CD4^{+}CD45RA^{-}FoxP3^{++}$ cells, increased between screening and 4 weeks after the second injection (median and IQR 0.77, 0.55-1.03 and 1.18 0.7-1.4, respectively, $p<0.05$).

The proportion of resting Treg cells, defined as $CD3^+CD4^+CD45RA^+Foxp3^+$, increased between screening and 4 weeks after the first injection (median and IQR 0.5 0.4-0.7 and 0.7 0.55-0.9, respectively $p<0.05$). Spontaneous and allergen-induced cytokine and chemokine secretion was analyzed with ELISA or Luminex after 24 hours or 6 days of culture. Spontaneous secretion of IL-10 after 24 hours increased significantly between screening and 4 weeks after the first injection (median and IQR 0.5 0.4-0.7 and 0.7 0.55-0.9, respectively, $p<0.05$). However no significant differences were observed over time for allergen-induced IL-4, IL-5, IL-10, IL-13, IFN-γ, CXCL10 and CCL17 secretion.

The changes in RTSS and Medical Score were calculated with one-way ANOVA, and further comparisons between the visits were calculated with paired Student's T-test. RQLQ scores, IgE and IgG4 levels were not normally distributed and were calculated instead with Friedman's test. Further comparisons between the visits were calculated with Wilcoxon signed rank test. Normally distributed flow cytometry data was calculated with paired student's T-test, otherwise Wilcoxon signed rank test was used for comparisons. The cytokine and chemokine data was not normally distributed. Thus, Kruskal-Wallis test was used to analyze whether the spontaneous and allergen-induced cytokine and chemokine levels changed between the different time points of blood sampling. Data that passed the test was further analyzed with Wilcoxon signed rank test to investigate at what time point the change occurred. GraphPad Prism version 7.03 (GraphPad software, Inc., La Jolla, CA, USA) was used.

This pilot study shows statistically significant clinical efficacy accompanied by corresponding immunological changes. The improvements in symptoms and quality of life measured by RTSS and RQLQ appeared immediately after treatment and were sustained throughout the

following 3 seasons. This suggests that efficacy is not due to placebo effects. The results of this study add to the hitherto positive studies and suggest that ILIT is effective and safe as treatment for pollen allergy. However, an open follow-up study is too small to confirm this hypothesis. Therefore, we are conducting two randomized double-blind placebo controlled studies that will be evaluated during 2018.

We would like to thank Dr Camilla Janefjord and Dr Anna Forsberg for outstanding support and performance of laboratory analyses and Gun Johansson for skillful monitoring. We would also like to thank our research nurses Agneta Carlsson, Ulrica Wikner, Erika Hallberg, Kicki Helander and Totte Togö for excellent assistance, Dr Helene Zachrisson for providing ultrasound and Dr Robert Glavas for performing the injections, as well as Prof Lennart Nilsson, Dr AKM Munir, Dr Pavlos Retsas, Dr Patrik Nordenfelt, Dr Lars Bernfort and Annie Berggren Sjöblom for support and discussions. Region Östergötland, the Allergy Center in Linköping, the Medical Research Council of Southeast Sweden (FORSS), the Th Bergh Foundation, and the Asthma and Allergy Association are acknowledged for financial support.

1. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *The World Allergy Organization journal.* 2015;8:17.
2. Klimek L, Pfaar O, Bousquet J, Senti G, Kündig T. Allergen immunotherapy in allergic rhinitis: current use and future trends. *Expert review of clinical immunology.* 2017;13:897-906.
3. Senti G, Prinz Vavricka BM, Erdmann I, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci U S A.* 2008;105:17908-17912.
4. Witten M, Malling HJ, Blom L, Poulsen BC, Poulsen LK. Is intralymphatic immunotherapy ready for clinical use in patients with grass pollen allergy? *The Journal of allergy and clinical immunology.* 2013;132:1248-1252.
5. Hylander T, Latif L, Petersson-Westin U, Cardell LO. Intralymphatic allergen-specific immunotherapy: an effective and safe alternative treatment route for pollen-induced allergic rhinitis. *The Journal of allergy and clinical immunology.* 2013;131:412-420.
6. Hylander T, Larsson O, Petersson-Westin U, et al. Intralymphatic immunotherapy of pollen-induced rhinoconjunctivitis: a double-blind placebo-controlled trial. *Respir Res.* 2016;17:10.
7. Patterson AM, Bonny AE, Shiels WE, Erwin EA. Three-injection intralymphatic immunotherapy in adolescents and young adults with grass pollen rhinoconjunctivitis. *Ann Allergy Asthma Immunol.* 2016;116:168-170.
8. Schmid JM, Nezam H, Madsen HH, Schmitz A, Hoffmann HJ. Intralymphatic immunotherapy induces allergen specific plasmablasts and increases tolerance to skin prick testing in a pilot study. *Clinical and translational allergy.* 2016;6:19.
9. Devillier P, Chassany O, Vicaut E, et al. The minimally important difference in the Rhinoconjunctivitis Total Symptom Score in grass-pollen-induced allergic rhinoconjunctivitis. *Allergy.* 2014;69:1689-1695.
10. Juniper EF, Thompson AK, Ferrie PJ, Roberts JN. Validation of the standardized version of the Rhinoconjunctivitis Quality of Life Questionnaire. *The Journal of allergy and clinical immunology.* 1999;104:364-369.

Table. Results

	RTSS[a]	MS ARC[b]	RQLQ[c]	CCT[d]	IgE[f]	IgG4[f]	SPT[g]
Before treatment	14.0 13.0 (13-16)	10.0 9.5 (7.2-12.0)	3.42 3.32 (2.8-4.3)	1900 1000 (325-1000)	24.83 7.25 (3.93-44.00)	0.13 0.06 (0.00-0.16)	8.25 8.25 (6.13-9.50)
After 1st pollen season with treatment	8.6 8.5 (6-10)	3.3 2.0 (0.3-5.5)	1.12 0.96 (0.6-1.3)	7750[e] 10000 (3250-10000)	37.48 11.50 (5.00-90.50)	0.27 0.24 (0.12-0.44)	7.25 7.75 (5.75-8.38)
After 2nd pollen season with treatment	9.5 10.0 (8-12)	7.3 7.0 (3.2-10.0)	2.02 1.70 (1.2-3.2)	Not done	36.99 8.20 (5.58-87.25)	0.27 0.25 (0.13-0.43)	6.63 7.25 (4.50-8.25)
After 3rd pollen season with treatment	7.6 7.5 (4-11)	7.6 7.0 (2.5-13.0)	1.34 1.30 (0.7-1.9)	40262 10000 (1000-10000)	29.54 5.90 (4.15-68.50)	0.27 0.25 (0.15-0.44)	7.06 7.50 (6.13-8.00)
	p<0.01*	p=0.32*	p<0.01[#]	p=0.11[**]	p=0.35[#]	p=0.15[#]	p=0.20*

All values presented as mean, median and IQR in parenthesis (Inter Quartile Range, 25-75 percentile)

[a] RTSS=Rhinoconjuntivitis Symptom Score.

[b] MS-ARC=Medical Score excluding ICS and beta-2-antagonists for asthma, but including Montelukast.

[c]RQLQ=Rhinoconjunctivitis Quallity of Life Questionnaire.

[d] Conjunctival Challenge Test. Concentration in SQ-U giving symptoms of birch extract for patient treated with birch or timothy extract for patient treated with 5-grass.

[e] Conjunctival challenge performed with birch only due to lack of timothy extract

[f]Specific IgE and IgG4. Values expressed as kU/L (birch for patient treated with birch and timothy for patient treated with 5-grass)

[g] SPT=Skin Prick Test. Values expressed as millimeter (birch for patient treated with birch and timothy for patient treated with 5-grass)

[*]p values from Students t-test calculated before treatment to after 3rd pollen season with treatment

[#]p values from Wilcoxson signed rank test calculated before treatment to after 3rd pollen season with treatment

[**]p value from Friedman´s test calculated before treatment to after 3rd pollen season with treatment