

Nasal Nitric Oxide and Nasal Cytology as Predictive Markers of Short-Term Sublingual Allergen-Specific Immunotherapy Efficacy in Children with Allergic Rhinitis

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Abstract

Background: Few studies have been conducted on the short-term response to sublingual immunotherapy (SLIT).

Objective: The purpose of our experimental trial was to evaluate if two markers such as nasal nitric oxide (nNO) and nasal cytology could be useful to identify a precocious clinical efficacy of SLIT treatment.

Methods: We enrolled 34 children aged 6 to 14 years old with diagnosis of allergic rhinitis (AR) and documented sensitization towards house dust mites. We started allergoid-monomeric tablets immunotherapy along with any conventional therapy for AR and we evaluated at baseline (T0), after one (T1), two (T2), three (T3), and six months (T6) the effects of the treatment through the study of: i) a visual analogue scale (VAS 1-10); ii) measurement of nNO; iii) measurement of FeNO; iv) nasal cytology; v) spirometry; and vi) evaluation of any conventional therapy.

Results: We observed an improvement in symptoms evaluated by global VAS (T0 vs. T6: 47.13 vs. 17.57; $p < .05$) and a statistically significant reduction of nNO (1035.2 ± 956.08 vs. 139.2 ± 59.01 ; $p < .05$). In this case, significance was reached when the patients completed the 6 months of treatment. Cytological evaluation revealed significant reduction in nasal eosinophils (T0 vs. T6: 87% vs. 16%; $p < .01$). Moreover, at T0, 56% of patients had also neutrophils that were reduced up to the 8% at T6 ($p < .05$).

Conclusions: Our data confirm the effectiveness of SLIT treatment from a clinical perspective and identifies two biomarkers, such as nNO and nasal cytology, as predictive of treatment efficacy in the short term.

Keywords

allergy, immunotherapy, SLIT, children, nitric oxide, NO, nasal cytology, VAS, rhinitis, house dust mites

Introduction

Respiratory allergies are triggered by allergens that are present in domestic environments (dust, mould) as well as in the external environment (pollen). In this context, specific sublingual immunotherapy (SLIT) provides new and important therapeutic perspectives and represents an approach that is currently available for redirecting the inappropriate immune response in atopic patients^{1,2}. SLIT represents an invaluable improvement that can change the clinical history of different types of Immunoglobulin (Ig)E-mediated allergic diseases. It is the only pharmacological treatment that not only alleviates the symptoms of young patients but also modifies the natural history and prognosis of allergic respiratory diseases through an etiological mechanism^{3,4}.

Through a well-defined immunologic mechanism, allergen immunotherapy (AIT) promotes regulatory T cells and cuts down the immune response induced by allergens⁵. According to current guidelines based on the most recent evidence, AIT should be offered to children with moderate to severe allergic rhinitis or controlled asthma starting from 5 years of age after an adequate risk-benefit assessment^{1,6}.

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SLIT is available as an aqueous solution or tablet. According to the directives of the European Medicines Agency (EMA), SLIT tablets for grass pollen and house dust mites (HDMs) have been approved⁷. One of the problems related to SLIT is how to evaluate its effectiveness over time, especially in the short term since the first effects are almost never observed immediately, which can affect patient compliance. From this perspective, few studies have been conducted on the short-term efficacy of SLIT treatment⁸.

Nasal nitric oxide (nNO) and fractional exhaled nitric oxide (FeNO) are considered non-invasive biomarkers to identify and monitor eosinophilic airway inflammation. There is evidence that NO levels increase with airway inflammation and decrease after anti-inflammatory treatments^{9,10}. Few studies have evaluated the efficacy of AIT on FeNO in children with allergic asthma with or without allergic rhinitis^{11,12}; only one study took into consideration the nNO for the evaluation of the effects of AIT after one year from the start of treatment¹³.

The evaluation of nasal cytology is helpful in the differential diagnosis of rhinitis in children, allowing to detect the cellular variations of an epithelium exposed to acute or chronic physico-chemical irritation, or inflammation of a different nature. Allergic rhinitis (AR) is characterized by an abundance of eosinophils and often neutrophils due to the presence of a minimal persistent inflammation¹⁴. Although nasal cytology is cited in the literature for its role in selecting the candidate patient for AIT, little evidence exists on how nasal cytology changes during AIT^{15,16}.

Thus, the purposes of our experimental study were to evaluate the short-term efficacy and safety of SLIT through the evaluation of nNO and nasal cytology.

Materials and Methods

Patients

The inclusion criteria were: (i) age between 6 and 14 years; (ii) diagnosis of allergic rhinitis; and (iii) documented sensitization towards a perennial allergen (HDMs). To homogenize the sample, patients with concomitant bronchial asthma or other chronic airway diseases (eg cystic fibrosis or ciliary dyskinesia) were excluded.

Study Design

This study was a prospective clinical trial that was conducted at the Paediatric Pulmonology and Allergology Unit, Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

Study Interventions

At the time of recruitment (T0), subjects who met the inclusion criteria were evaluated to analyse the clinical

characteristics of the allergic disease, respiratory function, and inflammatory state of the airways. After a complete clinical diagnosis of allergic respiratory disease, a Skin Prick Test (SPT) and Radio Allergosorbent Test (RAST) were carried out. The patients then underwent treatment with SLIT for HDMs based on the IgE values found in RAST and the patient's clinical history. Allergoid-monomeric tablets were used along with any conventional therapy for AR. Patients were administered one tablet per day, twice per week.

Study Endpoints

The clinical-instrumental evaluation was performed at the time of recruitment based on: (i) a visual analogue scale (VAS 1-10); (ii) measurement of nNO; (iii) measurement of FeNO; (iv) nasal cytology; (v) spirometry; and (vi) evaluation of any conventional therapy. A similar evaluation was also performed for the temporal monitoring of therapy at one month (T1), two months (T2), three months (T3), and six months (T6). A data collection form was completed for each patient.

Visual Analogue Scale (VAS). VAS is a tool for measuring the subjective characteristics experienced by a patient. In general, the scale is used for the evaluation of pain, but in our case, a series of subjective symptoms was evaluated. The patient must attribute a value of 0 to 10 to the symptoms according to their intensity. Questions were asked on an outpatient basis or by telephone. The patient provided answers each of the following symptoms: nasal obstruction, runny nose, sneezing, conjunctivitis, dyspnoea, feeling of chest tightness, hisses, and cough¹⁷.

nNO. nNO was measured with constant aspiratory flow using Hypair FeNO + Expair Software (Medisoftware), which is an electrochemical-based analyser. During the procedure, patients connect a catheter to one of their nostrils. Air from the nasal cavity is continuously analysed by an NO electrochemical sensor, and the patient breathes through an expiratory brake so that the velum is closed to prevent any contamination of nasal air with bronchial air. The aspiration flow rate was 350 mL/second through the nasal catheter¹⁰.

FeNO. FeNO was measured at multiple flow rates (50, 100, 150, and 350 mL/second). The measurement was expressed in units of ppb, the diagnostic evaluations were reported in Hypair FeNO + Expair Software (Medisoftware), and the aspiration flow rate was 50 mL/second¹⁸.

Nasal Cytology. The cytological method consists of (i) sampling; (ii) processing (which involves fixing and staining); and (iii) microscopic observation. Cytological sampling consists of harvesting the superficial cells of the nasal mucosa, which can be performed using a sterile tampon (eg the

same type used for pharyngeal sampling), a small plastic curette (Rhino- Probe®), or by scraping the nasal mucosa. Cytology is performed by analysing the nasal scraping obtained from the mucosal surface of the inferior turbinate, and samples are stained using the haematoxylin and eosin stain procedure¹⁹.

Spirometry. Spirometry was performed in the lung function lab using a spirometer (Lungtest 1000; MES Ltd, Kraków, Poland). The forced expiratory volume in 1 s (FEV1) was measured as a standard parameter for airflow obstruction assessment and expressed as a percentage of the predicted value based on the child's age, sex, weight, and height. The best spirometric measure of at least three attempts was recorded for the analysis²⁰. The values represent the percentage increase in FEV1.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (version 8.1.2). The differences between the groups were analysed by ANOVA with Geisser-Greenhouse correction, followed by the application of Tukey's test. A result was considered statistically significant if it was associated with a value of $p \leq .05$.

Informed Consent and Ethics Committee Approval

The study was approved by the local Committee for Clinical Investigation, and informed consent was obtained from both parents of the patients.

Results

The study enrolled 34 children (males 21, females 13) with a mean age of 10.1 ± 3.2 . All underwent HDMs SLIT according to their allergic sensitization.

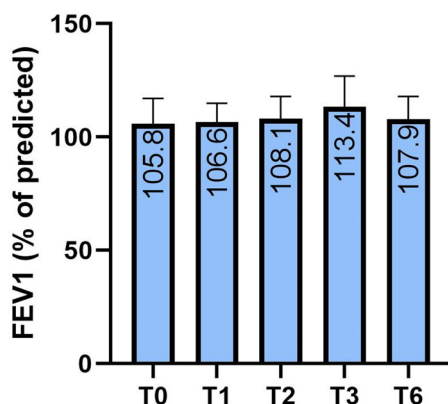


Figure 1. Trend of FEV1 over the months (p: not statistically significant).

We observed an improvement in symptoms evaluated by global VAS (T0 vs. T6: 47.13 vs. 17.57; $p < .05$). Consistent with the fact that none of the patients had a history of bronchial asthma, baseline FEV1 values were on average normal and did not increase statistically significantly after six months (T6) (105.8 ± 11.16 vs. 107.9 ± 10.05 ; p : ns, Figure 1). Also for FeNO, baseline values were normal and there was no significant reduction (18.00 ± 13.67 vs. 13.9 ± 10.72 ; p : ns).

At the beginning of treatment (T0), nasal eosinophilia was found in 87% of patients. Cytological evaluation revealed significant reduction in nasal eosinophils after six months (T6) of AIT, with 16% of patients showing eosinophils ($p < .01$). Moreover, at T0, 56% of patients had also neutrophils that were reduced up to the 8% at T6 ($p < .05$) (Figure 2).

The most relevant result is for nNO, that was statistically significantly reduced (1035.2 ± 956.08 vs. 139.2 ± 59.01 ; $p < .05$, Figure 3). In this case, significance was reached when the patients completed the 6 months of treatment (Figure 4).

With regard to conventional therapy, 88% of the patients were receiving treatment with antihistamines at the beginning of AIT (T0), and 29% received other drugs (nasal corticosteroid or montelukast). After three months (T3), 35% were still using antihistamines, and 14% were using other drugs. At T6, these percentages were reduced to 18% for antihistamines and 9% for the other drugs. No adverse effects were reported.

Discussion

SLIT has been considered useful in treatment of AR in paediatric patients. It is applied in clinical practice in many countries and has an advantage of being quite safe and somewhat effective in the long-term treatment of allergic respiratory diseases^{21,22}. Our data confirm the effectiveness of SLIT treatment administered via allergoid from a clinical perspective (in terms of the reductions of VAS and drug consumption) but above all identified biomarkers that can be used in the short-term monitoring of treatment efficacy such as nNO and nasal cytology.

As for NO, a marker of eosinophilic inflammation, most studies have focused on the evaluation of the exhaled fraction (FeNO) in patients with allergic bronchial asthma and it has been shown that this biomarker is reduced in children undergoing AIT^{11,12}. Ours is a deliberately more selected population since they are all affected only by AR and with allergic sensitization towards HDMs. In this sense, the evaluation of FeNO is less indicated since the bronchial component is less. Indeed, in our study, the average values of this biomarker are normal. We therefore decided to evaluate the nNO which is a more faithful index of nasal eosinophilic inflammation although it is less standardized since even today, unlike FeNO, there are no cut-off values that indicate or not the presence of allergic pathology¹⁰.

Our study first shows that nNO values are elevated in patients with AR confirming the results of other similar studies^{23–25}. Regarding its usefulness as a treatment response

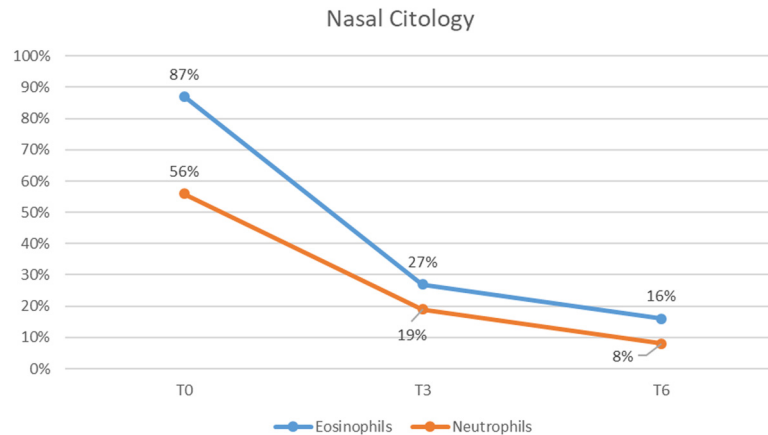


Figure 2. Reduction in nasal neutrophils and eosinophils after three (T3) and six months (T6) of SLIT.

biomarker, Wang et al evaluated its trend in children with AR undergoing therapy with antihistamines or nasal steroids. They demonstrated that the nNO can be useful in assessing the response to treatment, while it does not seem to discriminate well the severity of the disease^{26,27}. As for the use of nNO in monitoring the efficacy of AIT, we found only the study of Tsai et al who demonstrated the reduction in nNO after one year of treatment in a cohort of 40 patients (aged 10-20 years) with perennial AR¹³. However, differently from our study, the children underwent subcutaneous immunotherapy (SCIT) and not SLIT. Furthermore, the reduction in nNO appeared to be less evident (from 772.6 to 548.9 ppb) than in our study. It could be speculated in this sense that SLIT would seem more effective in reducing inflammation than SCIT but other studies are needed to corroborate this thesis.

Once again, in our study, the Tukey test demonstrated a significant improvement in nNO from T0 to T6 if patients complied with therapy. This reduction demonstrates an

important correlation between successful therapeutic response and a decrease in nNO levels, which is consistently observed whenever patients complete a series of at least six months of treatment. This consideration is obviously reduced by the limits of the study, such as the small sample and lack of controls.

However, from the analysis of the concomitant drugs administered to the patient, the results seem to show that the effects of immunotherapy last well beyond three months, at which point conventional therapy has been interrupted for most patients. This means that in the case of AR, antihistamines continue and be the cornerstone of treatment with known methods^{28,29} and must be accompanied by immunotherapy at the beginning, but as the months pass, it is necessary to try to stop them if the immunotherapy is taken correctly. In this sense, supplementation with vitamin D could be useful since it seems to enhance the effects of SLIT^{30,31}. SLIT has proven to be effective in controlling long-term symptoms in the treatment of respiratory allergies,

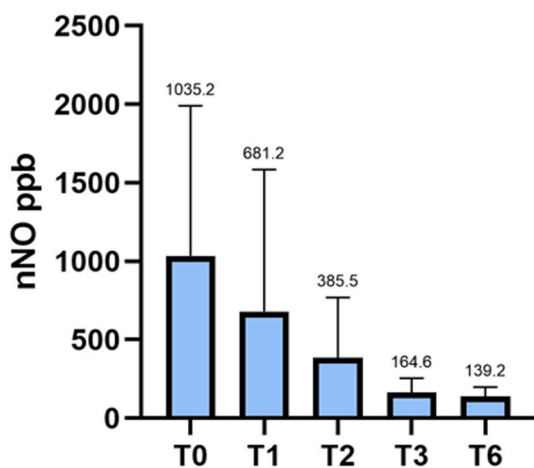


Figure 3. Trend of nNO over the months ($p < .05$).

95% Confidence Intervals (Tukey)

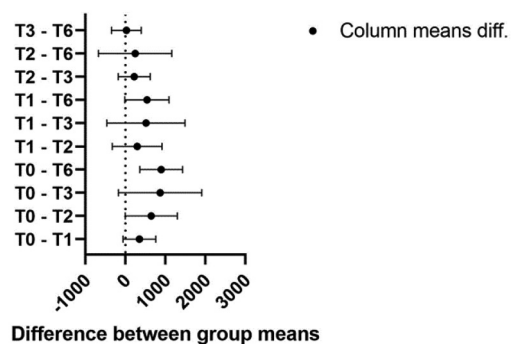


Figure 4. Tukey test for nNO shows statistical significance in the comparison between groups only between T0 and T6, that is, in the comparison between the start of treatment and the end of observation.

unlike the most common drugs (antihistamines, corticosteroids), which only act on symptoms instead. This shows that SLIT has an effect on the underlying causes of these pathologies, which is the reason why it remains effective even many years after stopping treatment^{32,33}.

The VAS represents an extremely useful tool in these cases, and the results improved at T1, T2, T3, and T6. Most patients also showed an improvement in general conditions and wellbeing in the first months of treatment. Moreover, all 34 patients were examined using nasal cytology, and the improvement of symptoms coincided with the reduction of inflammatory cells present in the nasal mucosa. The study of nasal cytology is very useful in the differential diagnosis of rhinitis, especially non-allergic ones, as it allows to discriminate cellularity and identifying the most appropriate treatment. Moreover, it is an easy to perform and minimally invasive method that can be easily proposed to children³⁴. Some studies have evaluated how nasal cytology changes in patients with AR undergoing conventional therapy^{35,36} but we have not found any studies that assessed how it changes during AIT in a pediatric population. In this sense, our study would appear to be the first to demonstrate that SLIT SLIT could also contribute to the change in the state of inflammation in the nasal mucosa.

In terms of prognosis, it is very important to be able to evaluate and predict the efficacy of SLIT according to pre-treatment parameters. In this regard, a long-term study of 517 patients found that the serum levels of specific IgE (s-IgE), s-IgE/t-IgE ratio, IL-10, and IL-35 and air conditioner use were significantly correlated with short-term efficacy of SLIT in children, which may be helpful for patient selection before SLIT⁸. With this knowledge, SLIT's effectiveness and efficacy could be enhanced even further and appropriately targeted to those who would benefit more from it. From this point of view, even the use of nNO could find its place³⁷.

Patient selection seems to be a cornerstone for both the effectiveness and efficiency of SLIT in the long term². However, SLIT has a significantly low risk profile overall. This enables us to gather more data and conduct more research to establish safer results. We have already established that it is a safe option when we want to reduce symptoms in patients with AR caused by perennial allergens. SLIT can also reduce the risk of progression from AR and asthma in children and prevent the onset of new sensitizations, thus representing a powerful preventive treatment method⁷.

Conclusions

Our study once again proves that SLIT is effective in improving symptoms in patients with AR. What characterizes our study is the characterization of two biomarkers, nNO and nasal cytology, which can be used not only to select patients but above all to monitor the effectiveness of the treatment even in the short term. SLIT represents a fundamental

element in the area of allergic diseases and can have a strong impact on the quality of life of patients. This is because SLIT acts at the etiological level by changing the natural history of respiratory diseases and allergic respiratory problems.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article. The authors did not receive any funding for the research. Lofarma® (Milano, Italy) covers the article-processing charge.

Institution at Which the Work was Performed

This study was a prospective clinical trial that was conducted at the Paediatric Pulmonology and Allergology Unit, Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy.

Author Disclosure Statement

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Declaration of Conflicting Interests

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

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Trial Registration

Not applicable, because this article does not contain any clinical trials.

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