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# Effect of two doses of carbamylated allergoid extract of house dust mite on nasal reactivity.

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**Key words**
*Carbamylated-monomeric-allergoid, nasal provocation test, dose-response effect, house-dust-mites, sublingual allergen immunotherapy.*

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**Summary**

***Background and Objective.*** *Single SLIT studies with native allergen extracts support a dose-response effect for clinical and immunological outcomes. Conversely for carbamylated allergoids this dose-response effects is less evident, likely because the threshold for efficacy is more easily reached through the enhanced bioavailability of the extract consequent to the selective chemical modification. Thus this pilot study investigates the dose-response effect on nasal specific reactivity and safety of two unusual doses of carbamylated allergoid in patients mono-sensitized to house dust mites.* ***Methods.*** *A prospective open randomized study involved 6-65 year-old Italian patients with clinically relevant sensitization to house dust mites and positive response to nasal provocation challenge. Monomeric carbamylated allergoid was delivered once daily at the dose of 1000 AU or 2000 AU from June to September 2009, during the lowest level of mites exposure. Primary outcomes were the change of the threshold of allergen concentration for a positive nasal provocation test (NPT) before and after the treatment and the product safety. Secondary outcome was the change in the mean percentage fall of peak nasal inspiratory flow (PNIF) following nasal challenge.* ***Results****. Thirty-four patients were enrolled. Fifteen in group 1 and 14 in group 2 concluded the study. After 12 weeks all patients treated in group 1 and all but one in group 2 showed an increase in the threshold dose provoking a positive NPT. Those with no symptoms onset with the highest dose delivered were 80% in group 1 and 78.6% in group 2 (p=0.92). From first to second challenge, the mean percentage fall of PNIF was reduced with no statistical difference between groups (p=0.95), and with no difference between the final mean percentage falls (p=0.65). No serious adverse reactions occurred and the frequency of events, all mild, was similar in the two groups.* ***Conclusions.*** *Twelve weeks of carbamylated sublingual allergoid delivered at 1000AU or 2000AU once daily appear equally safe and show comparable effect in increasing the threshold of allergen concentration for a positive nasal provocation test, confirming the apparent absence of a dose response effect for the used doses.*

## Background

In the past clinical experience the doses of allergen immunotherapy (SIT) were frequently adapted to the individual patient, thus few data are currently available on dose-response relationships for many SIT preparations (1). More recently an increased attention has been devoted to the appropriate dose administration, required to achieve clinical benefit in full compliance with an acceptable safety profile (2).

Due to the use of different reference materials and methodologies for determining the allergen content in the extracts, together with the heterogeneity of study designs and end-points, no comparison can be made between available studies and, as a consequence, no general dose-tuning recommendations can currently be made for SIT (2). Additional sources of variability that further hamper proper considerations about the dose–response relationships are given by the large difference in the qualitative composition of the marketed products and by the eventual presence and quantity of different adjuvant molecules, able to enhance the immunological stimulation provided by the allergen extract. However investigating the relationship between allergen dose and adjuvant concentration is a matter of debate because it does not seem feasible to test several allergen/adjuvant ratios in human studies.

Finally, the efficacy of an allergen extract may also depend on factors that influence the bioavailability, such as the volume in which the allergen is dissolved and, for sublingual immunotherapy (SLIT), the formulation and modality of administration. As a result, direct comparisons cannot be made between studies using products from different manufacturers to establish an universal dose–response relationship for a particular allergen extract (2).

Allergen products for SIT are being increasingly required to conform to regulatory requirements for human medicines and the recently introduced EMA guidelines on the clinical development of products for SIT states that after establishing a tolerated dose range, studies should be performed to establish a dose–response relationship for clinical efficacy (3). Thus in some recent studies different doses of the same preparation have been compared. The outcomes used in these dose–response studies varied widely and sometimes included surrogate end-points, such as titrated skin prick tests, nasal and bronchial challenge, measurements of blood and intranasal cytokines. A clear evidence of a dose-dependent response, for clinical and immunological effects, has been variably observed for extract containing traditional native allergens in individual SCIT and SLIT studies (4-17). So far this phenomenon has never been observed for chemically modified allergens for sublingual administration with the doses commonly used in clinical practice.

The purpose of the present pilot study was to investigate the clinical effects of two different doses of carbamylated monomeric allergoid on the nasal reactivity, assessed by specific nasal provocation test (NPT) and nasal peak inspiratory flow (PNIF) and the safety, in patients mono-sensitized to *dermatophagoides* in order to get preliminary information for successive phase- two dose-finding studies.

## Material and methods

This was a single-center prospective open randomized study carried out in Italy at the Allergy and Respiratory Physiopathology of Catanzaro local health service, Italy. All subjects gave written informed consent to participate in the study.

*Subjects and Study Protocol.*

Male and female subjects aged 6–65 years who were mono-sensitized to house dust mites (HDMs) and reporting allergy symptoms (rhino-conjunctivitis with/without mild asthma) during acute exposure for at least 2 years, or deterioration of clinical condition during winter months, were included in the study. Further inclusion criteria were: a positive skin prick test wheal larger than 4 mm produced by a mixture of mites extract; a positive CAP-test ( ≥ class 2) to HDM; positive response to specific NPT (total symptoms scores were at least 5).

Exclusion criteria included sensitization to other allergens which might have interfered with the clinical trial protocol (assessed by prick test) and/or allergy symptoms during screening or history of symptoms in the same period throughout the previous years. Patients were excluded if they had contraindications to SLIT, had received any vaccinations within the prior 3 years, were participating to other clinical trials, were not expected to be compliant or reluctant to avoid pregnancy during the study, had not controlled asthma or forced expiratory volume in one second (FEV1) >80% at first visit, lactose intolerance, sinusitis, polyposis or other morphological abnormalities, pregnancy or lactation.

After completing screening, patients were randomized into two groups receiving different dosing regimens of carbamylated monomeric allergoid tablets (Lais® Lofarma S.p.A., Milan) for sublingual-swallow immunotherapy,

standardized with an in-house-reference.The first group received 1 tablet of 1000 AU daily and the second one 2 tablets of 1000 AU daily for 7 days a week for three consecutive months, in accordance with the recommendations of the manufacturer and without build-up phase. The treatment period was between June 2009 to September 2009, that is out of the maximal natural exposure to HDMs, and treatment duration for each patient was of 12 weeks (**figure 1**). The first dose of trial medication was administered in the trial unit, all other doses were daily self-administered at home. Patients were not allowed to receive drugs interfering with the outcomes, but were permitted to receive rescue medications for a short period to manage occurring treatment side effects. Despite that a low environmental exposure was expected in the time of the study, no specific environmental intervention were recommended to limit HDM levels. Patients were informed to avoid place or activities involving a maximal exposure to house dust.

*Evaluation criteria*

The primary endpoints of the study concerned the effect on nasal specific reactivity and the safety of two different cumulative SLIT dosages.

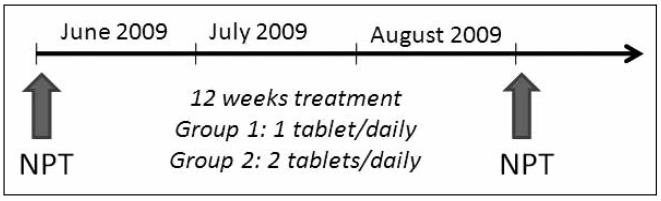
The nasal specific reactivity was evaluated with the comparative assessment of individual changes in symptoms occurrence, following the NPT conducted with specifically prepared solutions. The number of patients per treatment group who showed a change of the response threshold to induce a positive NPT between visit 1 and visit 2 was the primary efficacy endpoint. There were three categories to detect the change in response threshold: Improved (a higher allergen concentration was required to induce a positive NPT), unchanged (the allergen concentration for a positive NPT was unchanged), worsened (a lower allergen concentration induced a positive NPT response). A dose was considered more favorable if the number of patients with a higher response threshold for the NPT was higher than that of the other dose and the number of AEs under the treatment was not higher than that of the other dose. A protection of 60% or more of the patients under treatment was regarded as meaningful.

Secondary outcome to detect the protection was the intergroup comparison between the change in mean PNIF percentage fall following NPT from beginning to end of the study.

The judgment on compliance to SLIT was based on checking returned blisters.

All adverse events (AEs) were recorded throughout the study on a diary card, and the causality was assessed by the investigator. AEs with a causal relationship with the treatment were categorized on each administration day, including interval onset, duration and cessation time, site (local and systemic) and intensity (mild, moderate, severe, life-threatening).

*Figure 1* - Study design




*Nasal Challenge Tests*

Nasal provocation tests were completed as an inclusion criterion on day 1 prior to the beginning of the SLIT course and again after completion of the 12-week therapy course. A positive NPT was required at inclusion in order to ensure that subjects were sensitive to the tested allergen in the applied dose. Anterior rhinoscopy was performed prior to the procedures to exclude abnormalities and to detect the nostril less congested for conducing the test. To assess not specific hyper-reactivity, initially a saline solution (0.9% w/v) was applied into the nose. If a response was elicited after 10 minutes, that patient was excluded. If no response was elicited, an HDM solution at different concentration of Der p1 major allergen (0.02 mcg/ml, 0.2 mcg/ml and 2 mcg/ml) was applied to the same nostril every 10 minutes in order to find the dose eliciting symptoms, up to a maximal dose equivalent to 0.66 mcg of major allergen.

A positive test result was considered if the total symptoms score was at least 5, after allergen provocation with each dose. Symptoms were scored as: itching (nasal =1 point, eye =1 point, palate/ears =1 point), rhinorrhea (moderate =1point, abundant =2points), nasal obstruction (mild =1point; moderate/monolateral =2points; severe/bilateral =3points), sneezing (3-4 sneeze =1; >5 sneezes =2),watering eyes and/or dyspnea and/or urticaria(= 2 points). Late phase reactions were not documented.

*Nasal peak inspiratory flow.*

Measurement of PNIF was performed with In-check® device at baseline and at the end of each allergen specific challenge (**figure 2**). The In-check® with a face mask is a

portable inspiratory flow meter for measuring either oral or nasal inspiratory flow. It is intended for patients' use as a simple way to monitor flow rates providing valuable information about the degree of obstruction within air passage. For peak nasal inspiratory flow the patient should be asked to exhale fully and subsequently to inhale forcefully through the nose with a sharp, short action of about one second duration, maintaining the mouth closed. The peak test should be repeated three times and the highest result recorded. The performance accuracy reported is ±10% or 10 ml/min and repeatability is 6% or ±5 l/min. Details of a simplified allergen provocation test using PNIF measurement have been published (18).

*Statistical analyses*

All measured variables were tabulated using descriptive statistics, including the number of observations and absolute/relative frequency of categorical variables. For the continuous variables, the number of observations, arithmetic mean, standard deviation (SD), coefficient of variation (if appropriate), median, minimum and maximum were calculated. Being a pilot study the sample size was not calculated but around 30 patients were planned to be overall recruited. Intra-group and inter-group analyses were carried out with Wilcoxon paired two-tailed test and Mann-Whitney test respectively. The efficacy and safety analysis were based respectively on the number of patients who concluded the study and who received at least one dose of the study medication.

## Results

A total of 34 patients (18 males, 16 females, age range 10-52 years) were recruited and entered the study. The groups were well balanced in terms of demographic variables and sensitivity to mites at study beginning. Twenty-nine subjects (15 randomized to group 1 and 14 to group2) concluded the treatment and entered the efficacy assessment; all patients assuming at least one study medication were evaluated for safety.

*Efficacy*

All patients in group 1 (100%) and 13 in group 2 (93%) showed an increase in the threshold dose provoking symptoms during the NPT from the first to the last visit, thus were considered 'improved' (**figure 3**). After the second NPT none reacted to a lower dose, so none was considered 'worsened', but one patient in group 2 (7%) had no improvement in the threshold dose and was considered 'unchanged'. Further details including the relative improvement are described in **table 1**.Twelve out of 15 (80%) patients in group 1 and eleven out of 14 (78.6%) in group 2 had no symptoms occurrence with the highest dose delivered (p = 0.92) (**table 2**).

From the first to the second NPT the mean percentage fall in PNIF was reduced of 11.63 (SEM 4.92; $p < 0.05$) in group 1 and 12.23 (SEM 9.26; p = 0.21) in group 2. The mean percentage decrease during NPT was not statistically different (p = 0.65) between the two groups before (-41,47 [SD 8.64] and -37,92 [SD 9.82]) and after the treatment (-29.84 [SD20.85] and -25.69[27.10]). Individual changes in mean percentage fall in PNIF are given for both groups in **figure 4a-4b**.

*Safety and Tolerability*

No serious AEs occurred during the study. Two patients from group 1, receiving 1000 AU daily, abandoned treatment owing to consent withdrawal and nasal symptoms

***Figure 2*** - In-check® devices.

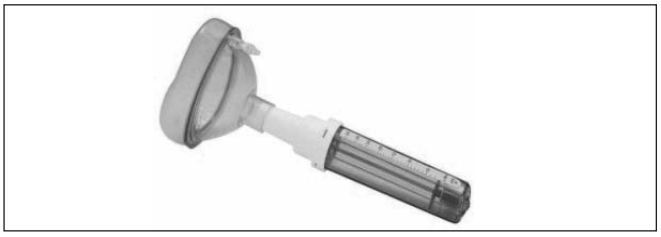


***Figure 3*** - Percentage of patients with improvement in threshold allergen concentration eliciting a positive NPT ( blue) and with no symptoms occurrence with the highest dose delivered (orange).

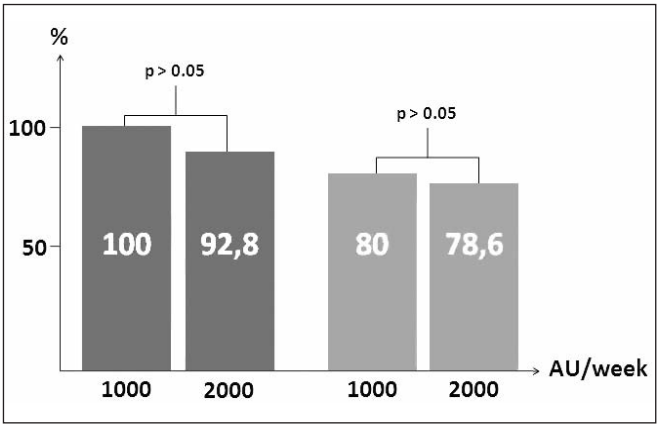


***Table 1*** - Summary of the changes in response threshold to NPT in both groups. Levels of improvement are reported also individually depending on the increase of dose required to elicit symptoms up to the maximum allowed dose.

| Improvement | Group 1 (1000 AU) Frequency (percentage) | Group 2 (2000 AU) Frequency (percentage) |
|---|---|---|
| worse | 0 (0) | 0 (0) |
| unchanged | 0 (0) | 1 (7) |
| improved | 15 (100) | 13 (92) |
| improved (+1) | 5 (33) | 0 (0) |
| improved (+2) | 2 (13) | 4 (28) |
| improved (+3) | 4 (27) | 7 (50) |
| improved (+4) | 0 (0) | 0 (0) |
| improved (+5) | 1 (7) | 0 (0) |
| improved (+6) | 2 (13) | 2 (14) |
| improved (+7) | 1 (7) | 0 (0) |

respectively. The second reason was considered treatment-related by investigators. Three patients from group 2, receiving 2000 AU daily, discontinued treatment for occurrence of pregnancy, nasal symptoms, asthma deterioration respectively. The last two events were considered treatment-related.

Concerning the treatment-related adverse events the occurrence of nasal symptoms was reported by 10 patients in group 1 and by 6 patients in group 2. Asthma symptoms occurred in 4 patients and in 2 patients respectively. These symptoms, developing few hours after administration, were mild in severity and did not require medications but were cause of drop outs in 3 patients. In group 1 an episode of skin diffuse itching afflicted one patient two hours after administration and solved spontaneously in 1 hour. Overall 16 AEs in group 1 and 18 in group 2 were reported. No other adverse reactions were reported. Further details are described in **table 3**.

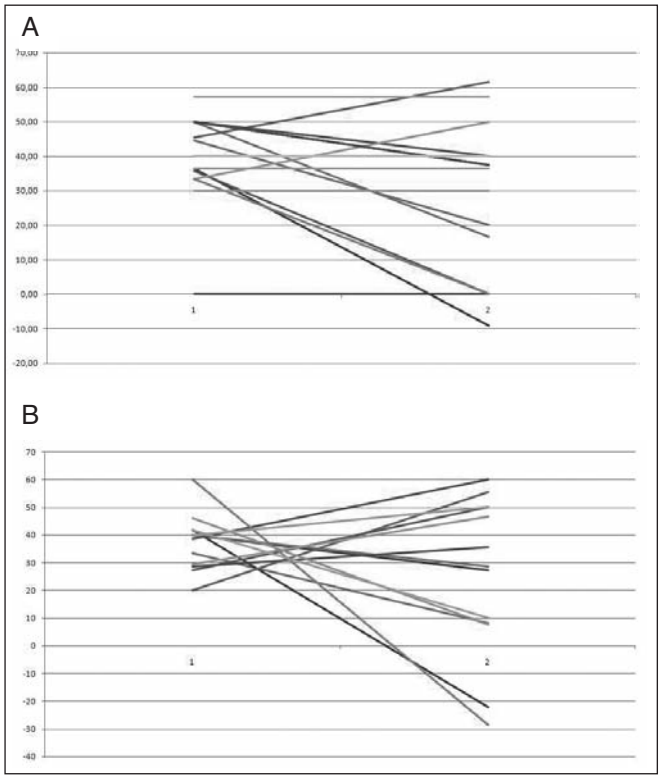
## Discussion

It was the aim of this trial to show that a large proportion of patients can be protected from reacting to house dust mites (HDMs) allergens by a 12 weeks course of SLIT. This was to be documented by an improved response threshold for a positive NPT, with meaningful protection provided by the treatment regimen, superior to 60%. In both groups, receiving daily 1000 AU or 2000 AU of HDM carbamylated allergoid respectively, the percentage of protected patients resulted superior to 90%. Moreover no positive response at all, neither at the highest dose delivered, occurred in a large proportion of patients in both groups. The apparent equivalence of the two dosing regimens in protecting patients during the nasal challenge was consistent with the data on the change of the average percentage fall in PNIF before and after the treatment. Both dosing regimens showed a comparable safety profile.

***Table 2*** - Frequency of patients respondent to the NPT divided by supplied allergen dose.

| Der p1 Allergen dose (mcg/ml) (1 puff corresponds to 120 ul of HDM extract) | | Group 1 (1000 AU) Frequency (percentage) | | Group 2 (2000 AU) Frequency (percentage) | |
|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post |
| 0.02% | 1 puff (0.0022mcg) | 2 (13,3) | 0 (0) | 0 (0) | 0 (0) |
| 0.02% | 2 puff (0.0044 mcg) | 2 (13,3) | 1 (6,6) | 1 (7,1) | 0 (0) |
| 0.2% | 1 puff (0.022mcg) | 1 (6,6) | 0 (0) | 1 (7,1) | 0 (0) |
| 0.2% | 2 puff (0.044mcg) | 0 (0) | 0 (0) | 2 (14,3) | 0 (0) |
| 2% | 1 puff (0.22mcg) | 6 (40) | 0 (0) | 7 (50) | 3 (21,4) |
| 2% | 2 puff (0.44 mcg) | 2 (13,3) | 2 (13,3) | 3 (21,4) | 0 (0) |
| 2% | 3 puff (0.66 mcg) | 2 (13,3) | 0 (0) | 0 (0) | 0 (0) |
| No response | | 0 (0) | 12 (80) | 0 (0) | 11 (78,6) |
| Total | | 15 (100) | 15 (100) | 14 | 14 |

***Figure 4*** - A) Individual percentage fall in PNIF during NPT in group 1 before (1) and after (2) the treatment. B) Individual percentage fall in PNIF during NPT in group 2 before (1) and after (2) the treatment.




No serious adverse events were reported along the study and a similar frequency of treatment-related adverse reactions, all of mild intensity, occurred.

The results of this trial confirm the findings of previous studies which, administering a carbamylated allergoid of HDM extract, showed clinical benefit over a wide range of doses, likely because the threshold dose for efficacy is easily reached through the enhanced bioavailability of the extract consequent to the selective chemical modification (19-21). We wish to remark, in fact, that the chemical modification of an allergen through carbamylation at alkaline pH permits to obtain an allergoid of preserved molecular size, thus suitable for sublingual route of administration, able to partially resist to the enzymatic digestion after swallowing. This peculiar property of the extract provides a systemic enhanced immunological stimulation by acting on the gastro-enteric lymphoid tissue and being absorbed partially intact (22-25). This aspect can justify the achievement of an appreciable clinical benefit following the administration of a relatively low dose in respect to vaccines based on native allergens. Traditional vaccines in fact result largely degraded after swallowing and are likely to require a critical dose sufficient to express their immunological effect mainly at the level of oral mucosa. As a consequence, with these preparations it is more easy to demonstrate a clear dose-response behavior by bridging over doses around the expected threshold.

Conversely between the two dosing regimens adopted in this study (7000 AU and 14000 AU weekly) we could not observe a dose-response effect for efficacy. On the other hand, albeit it is still not clear whether the cumulative dose of allergen in a given period or the frequency of administration of an appropriate dose are important in determining the effects, we can observe that the lower cumulative dosage used in this studys (7000 AU weekly used for 12 weeks of treatment, equivalent to 84000 AU) is not far from that commonly used in current clinical practice (2000 AU weekly in continuous regimen for 52 weeks, equivalent to 104000 AU) which demonstrated to be more effective than placebo in randomized controlled trials, with an extent in line with that observed in recent phase III large trial (more than 20% compared to placebo in symptom and medication scores reduction) (20, 26). This finding suggests the interesting research hypothesis of investigating the potential effect and the appropriate dose of a shortened treatment course limited to anticipate the period of highest mite exposition.

A limitation of this small open pilot study is that the included population was probably too small in order to appropriately distinguish between the effects of two active and effective dosages. In a recent study a dose-dependent clinical efficacy and immunological effect of sublingual immunotherapy with mite monomeric allergoid has been shown between patients receiving 1000 AU or 3000 AU weekly during one-year maintenance phase, thus it is likely that also for carbamylated allergoids a certain dose-response relationship exists, but we can speculate that the cut-off threshold dose for effect is largely lower in respect

***Table 3*** - Frequency of treatment-related adverse events.

| Adverse event | Group 1 (1000 AU) | | Group 2 (2000 AU) | |
|---|---|---|---|---|
| | events | patients | events | patients |
| Rhinitis | 10 | 10 | 12 | 6 |
| Asthma | 5 | 4 | 6 | 2 |
| Cutaneous itching | 1 | 1 | 0 | 0 |
| Total | 16 | 15 | 18 | 8 |

to SLIT preparations with native allergens (27).

For what concerns a dose-response effect for safety, the relatively higher dosage we used in group 2 was not apparently associated with a substantial higher frequency of adverse events. Despite that no build-up phase was carried out, both treatments resulted well tolerated by patients and no serious adverse reactions occurred, confirming previous findings (28, 29). The overall frequency of patients referring side effects was apparently superior to that observed in clinical practice and in post-marketing surveillance studies, however a higher weekly cumulative dose was delivered in both groups and the risk of nocebo effect could not be excluded for the absence of an untreated control group (30). On the other hand, referring to the whole treatment course, 16 adverse reactions per 1260 doses administered (1 dose daily for 12 weeks for 15patients) occurred in group 1 (1,27%) and 18 per 1176 doses (1 dose daily for 12 weeks for 14 patients) in group 2 (1,53%). All reactions were of mild extent, self-reported and self-resolving, also in those three patients who abandoned the study.

We can conclude that the optimal safety profile of the carbamylated allergoid, due to the reduced affinity to IgE consequent to the substitution of -aminogroups of the allergen lysines, is probably maintained also at this dose range (310).

In conclusion twelve weeks of carbamylated monomeric sublingual allergoid delivered at 1000 AU or 2000 AU once daily show comparable effect in increasing the threshold of allergen concentration for a positive nasal provocation test and appear equally safe. The amplified features of this peculiar allergoid suggest that an adjusted dose-tuning and dose-response effect evaluation is specifically required in respect to traditional SLIT preparations.

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## Erratum Corrige

In the issue 5-2013 of the Journal European Annals of Allergy and Clinical Immunology was published the article "Improvement of quality of life in allergi rhinoconjunctivitis patients using nasal filters, a preliminary study" with an error in the authors, the following is the correct indication:

D'Amato G1, Rumi G.2, Cantera E.3, Cortes M.3, Dattilo R.3, D'Amato M.4
Improvement of quality of life in allergi rhinoconjunctivitis patients using nasal filters, a preliminary study. Eur Ann Allergy Clin Immunol 2013, 45 (5): 167-175

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