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# Role of nasal challenge and local eosinophilia in indirect exposure to cat in allergic rhinitis patients

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## KEY WORDS

cat; sensitization; nasal challenge; nasal eosinophil; allergic rhinitis

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10.23822/EurAnnACI.1764-1489.50

## Summary

**Introduction.** Sensitization to cat allergens is common worldwide. Currently, there is a trend towards costly and often unavailable diagnostic analysis. **Objectives.** The aim is to assess the reliability of skin prick test (SPT) and serum specific IgE (ssIgE) to cat sensitization, by performing nasal challenge test (NCT) in a community with low cat ownership but common presence of stray cats. **Patients and methods.** Forty-one patients with perennial allergic rhinitis (AR) who were mono or polysensitized (including cat) were included. We had 31 cat non-owners and 10 present cat owners. SPT (> 5 mm / diameter), ssIgE ( $\geq 0.70$  IU/ml), nasal smear for eosinophil (Eo) and NCT were compared between groups. Outcomes included nasal challenge score, nasal Eo positivity, peak inspiratory and expiratory flow (PIF and PEF) 2 and 8 hours after the NCT, and were compared to baseline. **Results.** Baseline SPT wheal size and ssIgE level were similar in both groups. NCT positivity was more frequent in cat owners. The strongest nasal reaction was on the top concentration in both groups. Nasal Eo positivity in cat owners was higher before and 2 hours after NCT, but similar to non-owners at last measurement. NCT positive cat non-owners had bigger SPT wheal size than NCT negative non-owners, but smaller than NCT positive cat owners. In contrast to PEF, a significant fall in PIF was noticed in both groups. Mono and polysensitized patients showed similar NCT positivity. **Conclusion.** Stray cats may pose a relevant risk of developing perennial AR. Regardless of cat ownership status, SPT and ssIgE should be the first diagnostic tool. Nasal Eo and NCT seem to be good diagnostic tools in cat non-owners if diagnosis is elusive.

## Introduction

Studies have shown that the prevalence of pet sensitization is increasing over the past decades not only in western countries, but also all over the World (1). Together with house dust mites, cat allergens represent the major indoor allergens, and are known risk factors for rhinitis and asthma (2,3). Exposure and sensitization primarily depend on the prevalence of cat ownership, but is still considered ubiquitous (4). The sensitization prevalence varies among countries according to different cultures, climate, environmental factors, traditional and religious beliefs (5-10). Distribution of the main cat allergens indoors depends on their

aero-dynamic diameter and on passive transport by clothes, shoes, or by human hair (11-17) from cat owning home to the environment that has never been occupied by the cat. A study by Woodfolk et al. (18) stressed out the importance of type of vacuum cleaner, which emits cat allergens with a mean of 90% on particles < 2.5 $\mu$ m/diameter. Similarly, Chapman et al. (19) have documented a wide variation of pet indoor allergens, from less than 1  $\mu$ g to greater than 3000  $\mu$ g/g of dust, clearly being the highest in pet-owners homes than in non-owners homes. However, cat ownership should not be considered as the only index of exposure to cat allergens (4). Liccardi had suggested several modalities of exposure to pet allergens and possible con-

sequences in a “real life” condition, which exclude that any indirect and no apparent contact (d and e modalities) should be considered at lower risk of exposure.

Furthermore, Chen et al. (20) documented that exposure to cat allergen concentration as low as 0.24 - 0.63 µg/g could be positively associated with reported asthmatic respiratory symptoms in subjects who have experienced allergic symptoms when near animals (20). It means that in a community with low cat ownership and common presence of stray cats, the low concentration of cat allergens may be of sufficient magnitude to induce sensitization in susceptible people (10,19,21), and to develop respiratory symptoms after occasional animal contacts (5).

Although cat allergen is the third common allergen in the Middle East countries (22), exposure to cat allergen, sensitization, and its impact on developing allergic rhinitis (AR) and asthma is significant (23). Furthermore, available literature regarding sensitization to stray cats is conflicting (24-27). A study from Kuwait showed that despite low rate of cat ownership (4.1%) (28), the presence of cat allergens in public buildings is high (29). In contrast to low rate of cat ownership, the sensitization to cat was relatively high (27%) (30). This was similar to reports from the region (8,10,22). This could be related to numerous stray cats in Kuwait streets and gardens. Therefore, the diagnosis of sensitization to cats is important, irrespective of cat ownership. In up to 95% cases, the sensitizing allergen to cat (31-33) is Fel d 1, a glycoprotein which is produced by the sebaceous and salivary glands and transferred to cat fur (34). So cat fur is considered the primary source of cat allergens (35,36). Exposure occurs in public places mostly in countries with high rate of ownership (32,37-39), but also in countries with common stray cats (10,22,29,40). Recently there is a trend toward costly component resolved analysis (41-43) instead of standard diagnostic approaches, such as the extract based SPT and serology against native extract (44).

### Study design and objectives

A randomized, controlled, prospective, experimental study was done on allergic rhinitis adult patients with indirect exposure to cat allergen in Al Rashed Allergy Center in Kuwait. The primary objective was to determine diagnostic reliability of SPT wheal size (mm/diameter) and level of cat serum specific in cat non-owners by performing NCT with cat allergen fur extract. The secondary objective was to determinate the role of NCT. Nasal smear for Eosinophil, PIFR, PEFR, were used as objective measurements of NCT outcome.

### Materials and methods

Forty-one randomly selected adult patients with perennial AR as defined by ARIA guidelines (45) sensitized to cat only or poly-sensitized to cat and at least one more common inhalant allergen, were included and divided into cat owners (n = 10) and cat

non-owners (n = 31). Cat owners required a confirmed current direct domestic contact ( $\geq 5$  years). Non-owners never kept cat at home and denied any known direct or indirect exposure to cat. The inclusion criteria included the following: 1, a positive skin prick test with a wheal size of (SPT  $\geq 5$  mm) in diameter, and serum specific IgE  $\geq 0.7$  IU/ml, to cat only or to cat and at least one common inhalant allergens using a battery of local inhalant allergens (with a long, almost-perennial, pollination); 2, baseline nasal PIFR and PEFR (Clement-Clarke International Ltd, Harlow, Essex UK) within a normal range. The exclusion criteria included: patients with allergic rhinitis and associated asthma.

SPT was performed with a battery of inhalant allergens (Staller-Genes, France), including local pollens and cat. Normal saline and histamine were used as negative and positive controls. Skin wheal size (diameter/mm) was recorded after 15 minutes as the mean of 2 perpendicular measurement and was considered as positive as wheal diameter was  $\geq 3$ mm. SSigE was determined by CAP (Phadia, Pharmacia Sweden).

NCT with cut fur allergen extract (Staller-Genes, France) was done at least 3 weeks after acute episode of rhinitis, 1 week after discontinuation of oral antihistamine, nasal cortisone and decongestant, and 2 weeks after antidepressant and oral cortisone ( $\geq 10$  mg/day). The NCT was performed out of the main pollination peaks following manufacturer recommendation. Frozen dried cat allergen extract (100 IR/ml), as an active substance, was freshly prepared by reconstitution with 9% diluent in different concentration starting from 0.1 to maximum 10 IR/ml. After patient's acclimatization ( $\approx 10$  min) to the physician office condition, the NCT was performed by placing progressive doses of allergen in contact with a patient's nasal mucous membrane, using nebulized cap to deliver **100 µl/1 puff of allergen solution in each nostril. Nasal reaction was assessed 20 minutes after each dose of allergen, keeping 10 minutes pinched and 10 minutes non-pinched nose as follows: sneezing: 0 = 0 - 2; 1 = 3 - 4; 3 =  $\geq 5$  sneezes; rhinorrhoea and nasal obstruction: 0 = absence, 1 = mild, 2 = moderate; 3 = severe; nasal palate, eyes and/or ears pruritus: 0 = absence, 1 = presence.** The test was considered as positive when the total score was  $\geq 5$ .

Eosinophil nasal smear, as wells as PIFR and PEFR (the best of three measurements was recorded) and compared at three steps: baseline, 2 hours and 8 hours after the challenge.

Nasal samples for Eosinophil positivity were collected by passing a sterile swab, from each nasal cavity, along the medial surface of the inferior turbinate 2 to 3 times, and the specimen smeared on a clear glass slide. Nasal smears were examined by light microscopy after staining with haematoxylin and eosin stain. Eosinophil positivity in nasal smear were calculated in the same time measurement points and compared with a baseline value. Results were interpreted by scale: weak positive (4 - 10 Eo/hpf), moderate (11 - 30 Eo/hpf), strong positive ( $> 30$  Eo/hpf). Non-parametric and parametric methods are used to calculate

statistical significance. The distribution value is determined by D'Agostino and Pearson omnibus test normality. Student's t-test, Mann-Whitney test, Fisher's test and  $\chi^2$  test were used for calculating the difference between the groups. ANOVA test was used to calculate the relative difference distribution variance between variables. The statistical hypotheses were tested at the level of  $\alpha = 0.05$ , and the difference between the groups in the sample was considered significant when  $p < 0.05$  or less. Statistical significance was depicted as:  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ . All data were analysed using GraphPad Prism version 7 (San Diego, California, USA).

This study was approved by the Ministry of Health ethics approval committee, number 2/2016.

## Results

Cat owners and non-owners showed similar age and gender distribution ( $p > 0.05$  for both measurements). The youngest cat owner was 24 years old (female), and the oldest was 54 years old (male). The youngest cat non-owners were 16 years old (male), and the oldest was 57 years old (male).

Mean wheal / diameter (mm) SPT for cat or pollens, as well as ssIgE antibodies level showed similar distribution in cat owners and non-owners ( $p > 0.05$  for both measurements).

NCT positivity was more common in cat owners than in cat non-owners ( $p < 0.05$ ).

Cat owners with positive NCT showed bigger wheal compared to cat non-owners with positive NCT ( $p < 0.05$ ). However, in cat non-owners, wheal on SPT was bigger in NCT positive in comparison to NCT negative patients ( $p < 0.05$ ).

PIF showed similar distribution in both groups compared to baseline levels, as well as 2 and 8 hours after NCT ( $p > 0.05$  for all measurements). In cat owners, PIF decreased significantly 2 hours after NCT ( $p < 0.01$ ), but despite recovering 8 hours after NCT it was still lower in comparison to baseline ( $p < 0.05$ ). However, similarly to cat owners in cat non-owners PIF decreased significantly 2 hours after NCT ( $p < 0.0001$ ), but 8 hours after NCT PIF recovered completely showing no difference in comparison to baseline ( $p > 0.05$ ). Meanwhile, PEF remained the same during the challenge in either group ( $p > 0.05$ ) (**table 1**).

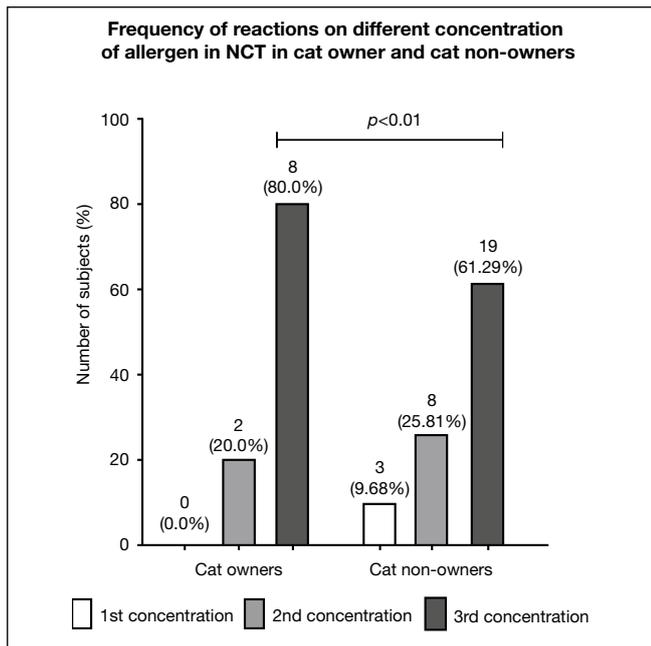
In both cat-owners and non-owners, most patients reacted on 3rd concentration ( $p < 0.01$ ), and then on 2nd concentration ( $p < 0.05$ ). However, more frequent reactions on top concentration was observed in cat owners compared to non-owners ( $p < 0.01$ ). On the other side, similar frequencies were observed among cat-owners and non-owners, on 1st, as well as on 2nd

**Table 1** - Patients' baseline and follow up characteristics.

| Patients  | Cat owners            | Cat non-owners        | p value             |
|---|-----------------------|-----------------------|---------------------|
| number of patients                                    | 10                    | 31                    |                     |
| age (years)   | 37.90 ± 13.36         | 31.94 ± 12.18         | 0.1959              |
| gender f/m (number)                                   | 8/2                   | 12/19                 | 0.0564              |
| SPT positive (cat only)                               | 4 (40.0%)             | 12 (38.71%)           | 0.7642              |
| SPT cat (mean wheal size in mm)                       | 9.4 ± 2.38            | 8.42 ± 2.38           | 0.2673              |
| SPT pollens (mean wheal/mm)                           | 6.90 ± 6.08           | 7.13 ± 5.55           | 0.9122              |
| cat ssIgE (IU/ml: mean ± SD) level                    | 3 [0, 5]              | 3 [0, 5]              | 0.9934              |
| positivity of NCT                                     | 10 (100%)             | 19 (61.29)            | 0.0179 <sup>1</sup> |
| SPT in NCT positive patients (mean wheal diameter/mm) | 10.0 ± 2.75           | 8.37 ± 2.06           | 0.0411 <sup>1</sup> |
| SPT in NCT negative patients (mean wheal diameter/mm) | -                     | 7.083 ± 1.38          | -                   |
| p value   | -                     | 0.0118 <sup>1</sup>   |                     |
| PIFR before NCT                                       | 67.50 ± 8.58          | 65.58 ± 9.3           | 0.5669              |
| PIFR 2 hours after NCT                                | 29.50 ± 7.98          | 40.48 ± 18.04         | 0.0712              |
| PIFR 8 hours after NCT                                | 64.00 ± 7.38          | 64.32 ± 7.45          | 0.9056              |
| p value   | < 0.0001 <sup>1</sup> | < 0.0001 <sup>1</sup> |                     |
| PEFR before NCT                                       | 469.0 ± 62.8          | 459.4 ± 55.91         | 0.6476              |
| PEFR 2 hours after NCT                                | 459.0 ± 60.64         | 457.7 ± 45.51         | 0.9445              |
| PEFR 8 hours after NCT                                | 483.0 ± 51.43         | 488.1 ± 51.41         | 0.7879              |
| p value   | 0.6582                | 0.2143                |                     |

<sup>1</sup>Difference is statistically significant.

**Figure 1** - Frequency of reactions on different concentration of allergen in NCT in cat owner and cat non-owners.



concentration ( $p > 0.05$  for both measurements) (**figure 1**).

Cat owners showed similar Eo positivity in nasal smear before NCT, 2 and 8 hours after NCT ( $p > 0.05$ ). On the other side, cat non-owners showed more significant Eo positivity in nasal smear 8 hours after NCT compared to positivity before and 2 hours after NCT ( $p < 0.01$  for both measurements). Eo positivity in cat non-owners was similar before and 2 hours after NCT ( $p > 0.05$ ). However, in comparison to cat non-owners, Eo positivity in cat owners was more pronounced before NCT and 2 hours after NCT ( $p < 0.05$  for both measurements), but 8 hours after NCT no significant difference was observed ( $p > 0.05$ ) between cat owners and cat non-owners (**figure 2**).

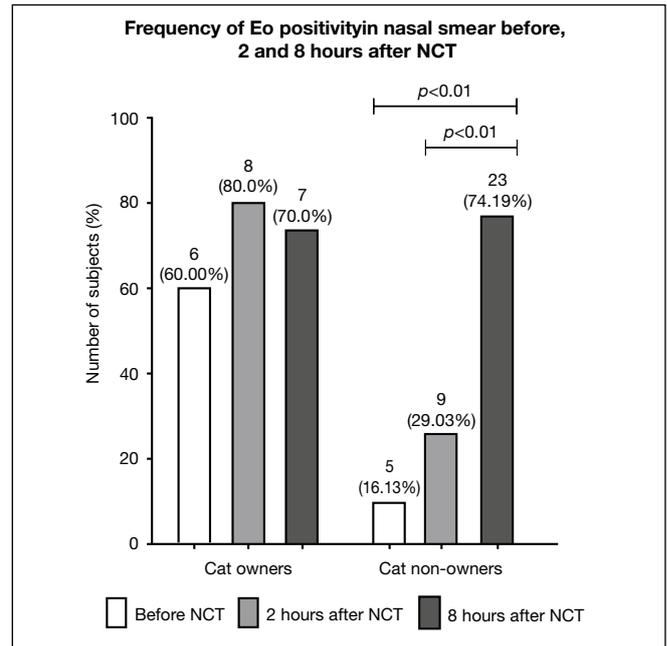
NCT showed similar positivity between patients sensitised on cat only and patients sensitised on cat and pollen ( $p > 0.05$ ).

## Discussion

In contrast of dog allergy, the role of cat allergens delivered from stray cats in the development of perennial AR is less investigated, both in children and adults. However, the dose of allergen exposure is not always linear (15). So, the relationship between exposure and sensitization to cat allergen is complex and still inconsistent, based on difficulties to classify subjects to direct or indirect allergen exposure (5).

In a study performed in Turkey (10), where cat ownership is low but street cats are common, sensitivity to cat allergen by SPT in adults ranged from 11% to 44.7%. Our results have shown SPT

**Figure 2** - Frequency of Eo positivity of nasal smear before NCT, after NCT and 8 hours after NCT in cat owners and non-owners.



positivity in 40% of cat owners and 38.71% of cat non-owners with no significant difference (**table 1**), which was higher than reported results by others (40).

Furthermore, in 2003 Linneberg A et al. (46) have shown that exposed to cat at home, in adult increased the risk of developing IgE sensitization to cat.

However, our results have shown similar distribution of ssIgE concentration in cat owners and non-owners (**table 1**). These findings may point out stray cats as a possible risk factor in the development of perennial AR in adults, regardless on cat owning. On the other side, advanced component based diagnostic testing could not replace SPT and native cat extract serology in the detection of sensitization to cats and differentiation between allergy and sensitization without clinical relevance (47). Complementary with positive ssIgE antibodies, SPT to natural allergen extracts is highly predictive of symptoms development upon allergen exposure (48,49). However, atopy quantification using specific SPT wheal diameter (50) and IgE level may better predict the expression of rhinitis than using atopy as a dichotomous variable (presence / absence of sensitization) (51-53).

NCT may be helpful as additional measurements when the SPT results are not clinically informative enough regarding exposure. However, NCT has not yet been widely accepted in clinical practice (54). Despite of significant difference in NCT positivity in cat owners and non-owners (100% vs. 61.29%,  $p < 0.05$ ) in our study, high percentage of NCT positivity in cat

non-owners could be related to the exposure from stray cats. We observed bigger SPT wheal size in NCT positive cat owners in comparison to NCT negative cat non-owners ( $p < 0.05$ ) (**table 1**), which could be explained by permanent exposure to higher doses of cat allergens in cat owners than in cat non-owners. Performing a conjunctival challenge with cat allergen extract to determine importance of unnoticed exposure, Braso et al. (55) found positive challenge outcome in 15/20 SPT positive non-cat owners with a history of respiratory allergy and exposed to low level (mean of 0.4 microgram/g of dust) of cat allergen. Our results have also shown the bigger SPT wheal size in NCT positive cat non-owners than in NCT negative cat non-owners ( $p < 0.05$ ) (**table 1**). All of these subjects had markedly positive SPT ( $> 5$  mm/diameter) and sIgE  $\geq 0.70$  IU/ml. So, being consistent with literature (55), results from our study support diagnostic importance of wheal SPT size and sIgE antibodies level. Although (56) Scadding et al. consider NCT as a recognized model that can help to understand the effect of challenging the upper airways on systemic or lower airway inflammation, these authors observed no significant change of PEF during up-dosing in NCT. Also in our study, similar PEF was noticed before, 2 and 8 hours after NCT (**table 1**) (56). As objective measurement of upper airway obstruction using PIF similar distribution in both group is shown when compared to baseline level, as well as 2 and 8 hours later ( $p > 0.05$ ). We observed a significant fall in PIF value in the first measurement (2 hours) in both groups (cat owners:  $p < 0.001$ ; cat non-owners: 0.0001) if compared with baseline value. We found recovering after 8 hours, seen by others (56), in non-owner group only ( $p < 0.05$ ) (**table 1**). Our results might be explained with higher sensitivity, but lower specificity of PIF over PEF in detecting of obstruction (57). In the Scadding et al. (56) study conducted on cat owners, significant increase in nasal response between second and highest concentration was absent during NCT. On the contrary, our results have shown that the total nasal score had increased with increasing concentration of cat allergen in both groups ( $p < 0.05$ ) (**figure 1**). We speculate that other factors such as life style and climate may influence this phenomenon. The Kuwaiti dwellings, as well as all public buildings, are well ventilated by air conditioning system, which transfer cat allergens indoor. The harsh climate (high temperature, low humidity, and frequent presence of dry dusty wind (58), may increase dispersion and sedimentation of airborne allergens including cat allergen indoors without cats. Eo in the nasal smear have been reported to display the best correlation with all the clinical and immunological parameters in allergic rhinitis (59). The sensitivity for nasal smear eosinophilia in the diagnosis of allergic rhinitis varies in different studies from 51.3% to 74%, with a specificity of 88.5% to 90% (60,61). However, Eo nasal smears are not necessary for routine use in diagnosing of AR, when the diagnosis is clearly supported by the history, physical examination, SPT and specific IgE diagnostic

findings, but may be a useful adjunct when the diagnosis is questionable (62). In our study, Eo positivity in nasal smear was used to evaluate its importance in overall AR diagnostic approach, to evaluate difference between cat owners and non-owners and to estimate local reactivity after NCT as well in both groups. We have shown that cat owners have had significantly higher frequency of Eo positive in nasal smear before NCT, comparing to cat non-owners ( $p < 0.05$ ). However, cat owners showed similar Eo positivity in nasal smear before NCT and 2 and 8 hours after NCT ( $p > 0.05$ ). On the other side, cat non-owners showed more significant Eo positivity in nasal smear 8 hours after NCT compared to positivity before and 2 hours after NCT ( $p < 0.01$ ), but in those patients Eo positivity was similar before and 2 hours after NCT ( $p > 0.05$ ). However, Eo positivity in cat owners was more pronounced 2 hours after NCT ( $p < 0.05$ ) than in cat non-owners, but 8 hours after NCT no significant difference was observed ( $p > 0.05$ ) between owners and non-owners (**figure 2**). Regarding results on increased of Eo in nasal smear in cat owners, it seems that Eo nasal smear could be a helping tool in making a diagnosis of AR. On the other side, in cat non owners, elevated Eo in nasal smear 8 hours after NCT show on possibility of NCT using as decisive tool in making diagnostic of AR when the diagnose is indeterminate.

The evidence suggests that simultaneous exposure to more than one allergen might modify the effect of individual allergen (15). The same author (24) reported increased prevalence of sensitization to dust mites and pollens in adult pet owners in case of combined exposure. On the contrary, in our study, NCT showed similar positivity between mono (cat) and poly sensitised (cat and pollen) ( $p > 0.05$ ) (**figure 3**). Such results suggest that cat allergy could be an independent risk factor for respiratory symptoms in our environment, where prevalence of sensitization to HDM in general is not high (30).

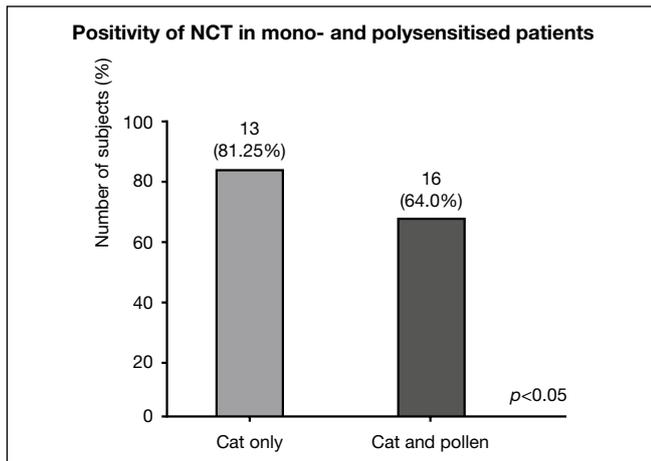
NCT is a safe and helpful procedure in allergy diagnostic. None of patients in either group during up dosing challenge withdrew from further procedure due to clinically significant lower airways symptoms or any other adverse reaction. Similar results are documented by other authors (54,56,63).

In conclusion, in an environment with common presence of stray cats, allergic sensitization to cat without direct exposure may be a relevant risk for developing perennial AR. Regardless of cat owning, SPT wheal size and level of sIgE  $\geq 0.70$  IU/ml should be the first diagnostic tool. NCT and Eo nasal smear seem to be good further method in diagnostic of cat sensitization, especially in cat non-owners.

#### Patients consent

All patients were informed about the risk and outcomes of the procedure and provided informed consent.

**Figure 3** - shows positivity of NCT in mono- and poly-sensitised patients.



### Conflict of interest

The authors declare that they have no conflict of interest.

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