Clinical and immunologic changes due to subcutaneous immunotherapy with cat and dog extracts using an ultrarush up-dosing phase: a real-life study

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Abstract

Objective: We aimed to evaluate the efficacy of and immunologic changes caused by subcutaneous immunotherapy (SCIT) in patients with allergy to cat and dog.

Methods: Patients presenting rhinitis and/or asthma with allergy to cat or dog from a previous safety study were included. All had specific IgE to cat and/or dog. Using an infusion pump (IP), SCIT maintenance dose was administered over one 4-hour session, followed by monthly administration over 6 months. Data were gathered on clinical outcomes, pulmonary function, FeNO, rhinitis and asthma symptoms, quality of life (QoL), asthma control test (ACT), and symptom visual analog scale (VAS) at baseline and then at 1, 3, and 6 months. Specific IgE and IgG antibody responses to different cat and dog allergens were determined.

Results: Sixty-one patients having a mean age of 35.6 ± 9.7 years were included, 40 of whom underwent cat SCIT. A significant improvement was observed in rhinitis and asthma symptoms and in QoL, use of medication, VAS, and ACT at 1 month; these improvements persisted at month 6. Clinical improvement with cat extract was significantly higher than with dog. An increase of >0.9 in ESPRINT-15 health-related quality of life in allergic rhinitis) was observed in 49.09% of patients, and 58.18% showed an increase of >0.5 in AQLQ(asthma quality of life questionnaire), at month 6, both differences indicating the minimal important difference. A significant increase was observed in specific IgG and IgE to different allergens at 3 and/or 6 months.

Conclusions: Ultrarush SCIT with cat and dog extracts has substantial clinical value in many patients.

Key words: Ultrarush subcutaneous immunotherapy. Cat. Dog. Allergy.

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Resumen

Objetivo: Nuestro objetivo fue evaluar la eficacia y los cambios inmunológicos causados por la inmunoterapia subcutánea (SCIT) en pacientes con alergia a perro y gato.

Métodos: se incluyeron pacientes que presentaban rinitis y / o asma con alergia al gato o al perro de un estudio de seguridad previo. Todos tenían IgE específica para gato y/o perro. Usando una bomba de infusión (IP), la dosis de mantenimiento de SCIT se administró durante una sesión de 4 horas, seguida de la administración mensual durante 6 meses. Se recopilaron datos de función pulmonar, FeNO, síntomas de rinitis y asma, calidad de vida (QoL), control del asma (ACT) y escala analógica visual de síntomas (VAS) al inicio y a los 1, 3 y 6 meses. Se determinaron las respuestas específicas de anticuerpos IgE e IgG a diferentes alérgenos de perro y gato.

Resultados: se incluyeron 61 pacientes con una edad media de 35,6 ± 9,7 años, 40 de los cuales se sometieron a SCIT de gato. Se observó una mejora significativa en los síntomas de rinitis y asma, calidad de vida, el uso de medicamentos, VAS y ACT en el primer mes. Estas mejorías se mantuvieron en el mes 6. La mejoría clínica con el extracto de gato fue significativamente mayor que con el de perro. Se observó un aumento de >0.9 en ESPRINT-15 en el 49.09% de los pacientes, y el 58.18% mostró un aumento de >0.5 en AQLQ en el mes 6, ambas diferencias indican la mínima diferencia importante. Se observó un aumento significativo en IgG e IgE específicas a diferentes alérgenos a los 3 y/o 6 meses.

Conclusiones: La SCIT ultrarápida con extractos de perro y gato induce una mejoría clínica relevante rápida y mantenida en muchos pacientes.

SAU declare no conflicts of interest.

Palabras clave: Inmunoterapia subcutánea ultrarápida. Gato. Perro. Alergia.

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INTRODUCTION

Previous research [1-9] had demonstrated the clinical efficacy of SCIT with extracts of cat and dog using a standard administration route, and also with Fel d 1-derived synthetic peptide immuno-regulatory epitopes [10]. Thus, immunotherapy can an alternative to manage patients with cat and dog allergy [11]. However, there have been few studies on the immune response to cat and dog during SCIT [12-16]. A few years ago, we conducted a real-life observational study on the safety and efficacy of subcutaneous immunotherapy (SCIT) with cat and dog extracts. In this earlier research, high doses of extracts were administered during three days through an infusion pump (IP) during the rush up-dosing phase in patients with rhinitis and allergic asthma due to cat or dog, demonstrating a good safety profile and clinical efficacy [17-19]. Having demonstrated the safety of an ultrarush up-dosing schedule [20], in this real-life study we set out to assess the clinical efficacy in terms of symptoms, quality of life, asthma control and pulmonary function of SCIT with ultrarush up-dosing with the same dog and cat extracts used in our previous studies, and its comparison together with the immunologic changes caused by this therapy approach.

METHODS

In a prospective study, patients with rhinitis and/or allergic asthma due to cat or dog were included, all of whom were prescribed SCIT with cat or dog extract (Alutard® SQ, Hørsholm, Denmark, Can f 1:3.21 μg/ml; Fel d 1: 15 μg/ml) and monitored during the first 6 months of treatment. The patients studied here are

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those included in a previously published study on the safety profile of SCIT for whom serum samples were available [20]. SCIT indications were based on EAACI guidelines [21]. All patients or their parents or legal guardians provided signed informed consent. The study was approved by the local ethics committee. No control group was included.

The up-dosing phase of SCIT was initiated (Alutard SQ, cat and dog, Hørsholm, Denmark) with the injection of 1.2 ml of the maintenance vial over 4 hours on the first visit, using a portable subcutaneous IP (Medis Infusa T, Italy) and an infusion set (Accu-Chek TenderLink, Roche, Swiztzerland) as previously described [20]. Subsequent doses were administered monthly by direct subcutaneous injection (1 ml).

Skin tests with cat and dog extracts (ALK, Hørsholm, Denmark) were performed at 3 concentrations (1/1, 1/10, and 1/100); these tests were conducted at diagnosis, at baseline (day 0) and 24 hours (day 1), and then at month 1, 3, and 6 of SCIT. Wheal contours were painted and transferred to the case report form. The contours were scanned and the wheal areas were measured. Changes in wheal area (geometric mean) and skin tolerance index (STI, indicating the difference in allergen concentration required to elicit the same skin response at different times) were analyzed at different times, using baseline values as a reference (day 0).

IgE responses to the dog allergens Can f 1, 2, 3, 4, 5 and 6 and cat allergens Fel d 1 and 7 were analyzed by ELISA as previously described [20]. rFel d 1 and rFel d 7 were produced as described [22,23]. Total IgG, and subclass IgG 1, 2, 3 and 4 allergen-specific reactivity were measured using the IgE ELISA protocol, however diluting serum 50 times for total IgG and 10 times for IgG

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subclasses. Secondary HRP-conjugated goat anti-human IgG (Thermo Fisher, Uppsala, Sweden) specific for total IgG and respective subclass were used. Immunological assessments were performed at baseline and subsequently at month 3 and 6.

At baseline and then at month 1, 3, and 6 of SCIT, all respiratory tests and questionnaires were performed as in our previous study [2]. In brief, values for spirometry and bronchodilation testing and fractional exhaled nitric oxide (FeNO) concentration were obtained. In addition, the following questionnaires validated for the Spanish population were administered: rhinitis quality of life (ESPRINT-15, health-related quality of life in allergic rhinitis), AQLQ (asthma quality of life questionnaire), ACT (asthma control test), and VAS (10-cm visual analog scale, including nasal, ocular, and bronchial symptoms). Scores for nasal (itching, congestion, rhinorrhea, sneezing), ocular (tearing, itching, gritty feeling), and pulmonary (cough, wheezing, dyspnea, exercise asthma) symptoms (0: no symptoms, 1: mild, 2: moderate, 3: severe) and a medication score were applied. To measure the response to SCIT, the minimum clinically important differences (MID) for questionnaire responses were considered [2]. Associations between different immunological parameters and safety and efficacy measures were analyzed in addition to the difference in clinical efficacy between SCIT with cat and dog extracts. Safety monitoring has been described previously [20].

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Statistical analysis

Fisher exact test, Wilcoxon test, Friedman test, Kruskal-Wallis test, and a mixed-effects model were used for statistical analysis. *P* <0.05 was considered significant.

RESULTS

We included 61 patients (36 women and 25 men), of whom 40 were allergic to cat and 21 to dog; their mean age was 35.6 ± 9.7 years. The percentage of patients sensitized to pollens was 62.3%, 23% were sensitized to mites, 11.5% to molds, 93.4% to cat, and 78.7% to dog. Allergic rhinitis was present in 90.2% of patients (n=55), 62.3% presented allergic conjunctivitis (n=38), and 82% allergic asthma (n=50). Most patients had persistent symptoms, the two most prevalent being moderate/severe persistent rhinitis (43.6%) and mild persistent asthma (46%). Eighty-two percent of patients (n=50) had either a dog or cat in the home (direct daily contact was maintained throughout the study), 14.8% had occasional direct contact with these animals (n=9), and 3.2% remained in indirect contact (n=2).

Fifty-four patients concluded the study (36 cat- and 18 dog-allergic patients). Before the third month of SCIT, 3 cat-allergic patients discontinued SCIT (due to pregnancy, work-related reasons, and systemic reactions of SCIT, respectively). Before the sixth month, another cat-allergic patient withdrew from SCIT due to poor SCIT tolerance, while 3 dog-allergic patients suspended therapy due to travel abroad, discontinuation of contact with the dog at home, and 1 was lost to follow-up (Figure 1 supplement).

A skin test revealed an increased STI on all visits after the start of SCIT compared to day 0 (Table 1 supplement and Figure 1). Of note, the STI decreased the day following administration of the ultrarush protocol.

Ninety-two percent of cat-allergic patients had IgE positive results to Fel d 1 (n=37), 10% to Fel d 2 (n=4), 55% to Fel d 4 (n=22), and 42% to Fel d 7 (n=17); 52.4% of dog-allergic patients had positive results to Can f 1 (n=11), 33.3% to Can f 2 (7), 19% to Can f 3 (n=4), 57.2% to Can f 5 (n=12), and 47% to Can f 6 (n=10) (Table 2 supplement). In the group of dog-allergic patients, 25% were monosensitized to Can f 5.

The quantification of the different IgG antibodies (average mean) and IgE are shown in Table 3 supplement and Figure 2 and 3. In some cases, a significant increase was observed in the third month and sixth month, but in others, the only change was recorded in the third month.

Spirometric values and the results of a bronchodilation test did not show significant changes between different assessments; the only significant decrease was observed in FeNO values ($P = 0.015$). In the first month of SCIT, mean FeNO decreased from 66.3 to 49.4 ppb ($P = 0.027$), and in the third month this decreased to 48.8 ppb ($P = 0.016$). No significant difference was observed between the third and the sixth month (Figure 4).

The quality of life questionnaires in rhinitis (ESPRINT-15) and asthma (AQLQ) showed highly significant changes in all dimensions (ESPRINT-15: symptoms, daily activities, sleep, psychological impact; AQLQ: activity limitation, symptoms, emotional function, and environmental stimuli) as well as the scales overall. The significant improvement in patient quality of life was seen in the first, third, and sixth month ($P < 0.001$ in both questionnaires). No significant

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differences were observed between the assessments made in the third and sixth month ($P > 0.05$) in both questionnaires. Improvement across all dimensions of ESPRINT-15 and AQLQ was seen until the third month of SCIT, with no further improvement in the sixth (Figure 4). Nearly half (49.09%) of the patients had an increase of >0.9 (MID) in ESPRINT-15 and 58.18% >0.5 (MID) in AQLQ at month 6.

The ACT and VAS showed statistically significant differences between the baseline evaluation at month 1, 3, and 6 of SCIT ($P < 0.001$ in all cases), though no significant difference was observed between the first and third month and third and sixth month. The highest percentage of patients with controlled symptoms was observed in the third month (from 65% to 87.7% patients with controlled asthma). We observed that 49.09% of patients had an ACT score ≥ 3 at month 6. The VAS decreased in comparison to the baseline, showing a positive effect of SCIT (Figure 4).

The symptom score showed marked improvement on the third month after SCIT, including rhinorrhea ($P = 0.000$), sneezing ($P = 0.001$), cough ($P = 0.003$), and dyspnea ($P = 0.009$), while nasal congestion improved in the first month ($P = 0.02$, intensity decreased by 35.8%). Regarding ocular symptoms, there were no significant differences, as was the case for nasal itching, although in the 3rd month, symptom intensity was reduced by 33.3%. No significant differences were observed regarding the symptom score between the third and sixth month (Figure 5).

A statistically significant decrease in the use of medications was observed, with use of antihistamines ($P = 0.034$) decreasing at month 1, while inhaled beta-2-agonists ($P = 0.008$), nasal corticosteroids ($P = 0.003$), and inhaled

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corticosteroid (*P* = 0.008) were lowered at the third month of SCIT. No differences were observed between the third and sixth month (Figure 5).

The response to SCIT, according to the scale used in our previous study, was very good in the third and sixth month. When analyzing the response to SCIT separately, we found that the patients who received cat extract had a very good response in the third month, while with dog extract this was true only in the sixth month. Significant differences in favour of cat extract were observed in AQLQ (*P* = 0.009) and ACT (*P* = 0.023) in the third month, and for symptom score (*P* = 0.039) in first month to the SCIT.

The cat-allergic patients not sensitized to Fel d 2 presented better quality of life regarding asthma (AQLQ) as measured by limitation of activity (*P* = 0.044), symptoms (*P* = 0.034), and total score (*P* = 0.044) on the AQLQ; better asthma control (ACT) (*P* = 0.037), a greater decrease in some symptoms, such as runny nose (*P* = 0.035), tearing (*P* = 0.043), feeling of grittiness in the eyes (*P* = 0.032), and asthma due to exercise (*P* = 0.035); as well as a decrease in the use of inhaled beta-2- agonists (*P* = 0.03). For their part, those who were not sensitized to Fel d 4 presented a higher decrease in symptoms, such as nasal itching (*P* = 0.017) and a gritty feeling in the eyes (*P* = 0.002). Dog-allergic patients not sensitized to Can f 2 presented less exercise asthma (*P* = 0.029) as well as a decrease in the use of nasal corticosteroids (*P* = 0.016). All other clinical efficacy data, safety profile (previous study), and response to SCIT did not show a significant association with the IgE molecular profile. An increase in IgG4 was not associated with clinical improvement. Adverse reactions (AR) recorded during the study were described in detail in our previous publication [18].

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DISCUSSION

A significant decrease in skin-test reactivity was observed as a result of SCIT with both cat and dog extract, as described in other studies in which these data were assessed. Skin reactivity generally decreases 12 months after SCIT [4, 6-9, 13, 15, 16]. However, in 2 studies in which cat and dog SCIT was administered at different concentrations of the major allergen (0.6, 3, and 15 μg of Fel d 1 and Can f 1), a decrease in skin reactivity was observed following 5 weeks of SCIT at doses of 3 μg and 15 μg of Fel d 1 and Can f 1 [13, 16]. In our study, a noticeable decrease in the wheal area was observed within the first 24 hours of the first dose (15 μg Fel d 1/ml for the cat extract and 3.21 μg/ml Can f 1 for the dog extract), with a more pronounced decrease seen on subsequent visits, possibly owing to the use of an ultrarush schedule.

As in our two previous studies [18, 26], we observed similar sensitization profiles to cat and dog allergens; Fel d 1 was the major allergen in allergy to cat and Can f 1 and Can f 5 in dog-allergic patients. The prevalence of sensitization to Can f 5 exceeded that of Can f 1, and we observed high monosensitization to Can f 5 in dog allergy, affecting up to 25%. For the first time in our country, we found a high percentage of sensitization to Can f 6 (47%), and 42% showed sensitization to Fel d 7. Nevertheless, these figures are similar to those described in Swedish patients allergic to dog (35%) and cat (46%) [24, 25]. Both lipocalins have cross-reactivity with other lipocalins, Fel d 7 with Can f 1, and Can f 6 with Fel d 4 and horse allergen Equ c 1 [25, 27].

Variations were observed over time in levels of specific immunoglobulins (IgG, IgG subtypes, IgE) to cat or dog allergens. We found a significant increase in the third and/or sixth month of SCIT. Previous studies have also demonstrated

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a significant increase in IgG and IgG4 to cat [4, 8, 13-16] and dog extract [9, 13, 14] in the fifth month or at 1 year of SCIT [8, 16]. In the case of the dog SCIT study, significant immunologic response was observed without clinical improvement, despite the use of high doses of Can f 1 (15 μg/ml) [16]. Contrasting with these results, cat SCIT did result in significant clinical and immunologic improvement at similarly high doses to the ones used in this study (15 μg Fel d 1) [8].

Regarding specific IgE, most studies have not found significant variation at 1 year of SCIT with the exception of one study with cat SCIT in which a significant increase was found at year 1 and 2 of SCIT [13, 14]. In our case, Fel d 1 was the only allergen with a significant increase in total IgG, IgG1, IgG2, IgG3, IgG4, and IgE at 3 and 6 months of SCIT. All other allergens had also significant increases, especially in specific IgG4 and total IgG. The rapid response of specific immunoglobulins is likely due to the ultra-rush protocol used in this study in comparison with the previous ones. The higher immune response to Fel d 1 can be explained by the high content of this allergen in the extract, when comparing with other allergens.

Respiratory function tests showed no changes throughout the therapy, as in the previous study [18]. However, a significant decrease in FeNO value was observed in the first and third month of SCIT, though no further changes were observed between the third and sixth month of SCIT.

Clinical improvement was significant in first month of SCIT, which continued being significant in the third and sixth month with no significant changes were observed between the third and the sixth month. This improvement included quality of life in rhinitis and asthma (ESPRINT-15 and AQLQ), ACT, and VASs.

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Nasal and bronchial symptom scores and use of medication decreased significantly at month 3 of SCIT; an exception to this was seen in nasal congestion and the use of antihistamines, which improved at 1 month of SCIT but did not reveal significant changes between the third and sixth month of SCIT, a similar finding to previous clinical evaluations [18]. No significant improvement in ocular symptoms was observed, which was consistent with our previous results using same extracts [18], in which overall clinical improvement occurred at month 6 of SCIT, maintaining improvement at 12 months though without major changes [18]. Of note, the clinical efficacy and immunologic improvement seen here and in our previous study [18] occurred despite direct daily contact maintained with pets in 80% of patients. Interestingly, cat-allergic patients not sensitized to Fel d 2 had a greater improvement in symptoms, AQLQ, and ACT. This fact may be the result of a lower content of these allergens in the extract used.

Cat-allergic patients had a better response to SCIT than dog-allergic patients, as seen also in our previous study [18] and previously publications [3, 5, 13]. This may be due to the higher concentration of Fel d 1 relative to Can f 1 or other dog allergens not contained in the extracts used. In our study, Can f 1 concentration was 3.21 μg/ml when the recommended dose was 15 μg/ml [16].

In our previous study on efficacy (18), we found no association between efficacy and a specific pattern of sensitization to different allergens. In this study, however, we found that sensitization to Fel d 2 was associated with worse results in asthma quality of life, asthma control, nasal and ocular symptoms, exercise asthma, and in a greater use of rescue medication for asthma. Other allergens such as Fel d 4 were associated with greater intensity

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of nasal and ocular symptoms, while Can f 2 was associated with exercise asthma.

Adverse reactions (AR) recorded during the study were described in detail in our previous publication [20]. In brief, the sample was divided into patients who received pretreatment with 10 mg of cetirizine and those who did not. ARs with premedication were reduced from 21.6% to 6.3% (OR 4.13, 95% IC 0.89-19.19 p=0.06), systemic reactions from 17.5% to 6.3% (OR 3.19, 95% IC 0.67-15.08 p=0.14) and local reactions from 5.4% to 0 (OR 4.75, 42 95% IC 0.24-91.10 p=0.30). Only 2 cat-allergic patients dropped out of the study due to the side effects of SCIT, though the reactions were not severe.

Limitations of the study include the lack of a control group to rule out the placebo effect of allergen immunotherapy. Tough real-life studies are also needed to complete the evidence of placebo control studies [28]. Other limitation is the limited number of patients included, specially in the case of dog-allergic patients.

CONCLUSIONS

In this real-life study using an ultrarush schedule, we demonstrate the clinical efficacy and immunologic changes of SCIT with cat and dog extracts after 1 month of treatment. More remarkable results were obtained in cat-allergic patients than in those allergic to dog, likely due to the higher concentration of the major allergen (Fel d 1) in the allergenic extract used in this SCIT formulation.

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Conflict of interest

JS reports having served as a consultant to Thermofisher, MSD, Novartis, Gennetech, Sanofi, Leti, Roche, ALK, FAES FARMA, Mundipharma, and GSK; having been paid lecture fees by Novartis, GSK, Stallergenes, LETI, and FAES FARMA; as well as having received grant support for research from Thermofisher, ALK, and Sanofi.

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Figure 1. Change in the geometric mean (mm^2) of the skin tests with cat or dog extract and in skin tolerance index (STI) at day 1 and then month 1, 3, and 6 compared to day 0 at different doses.

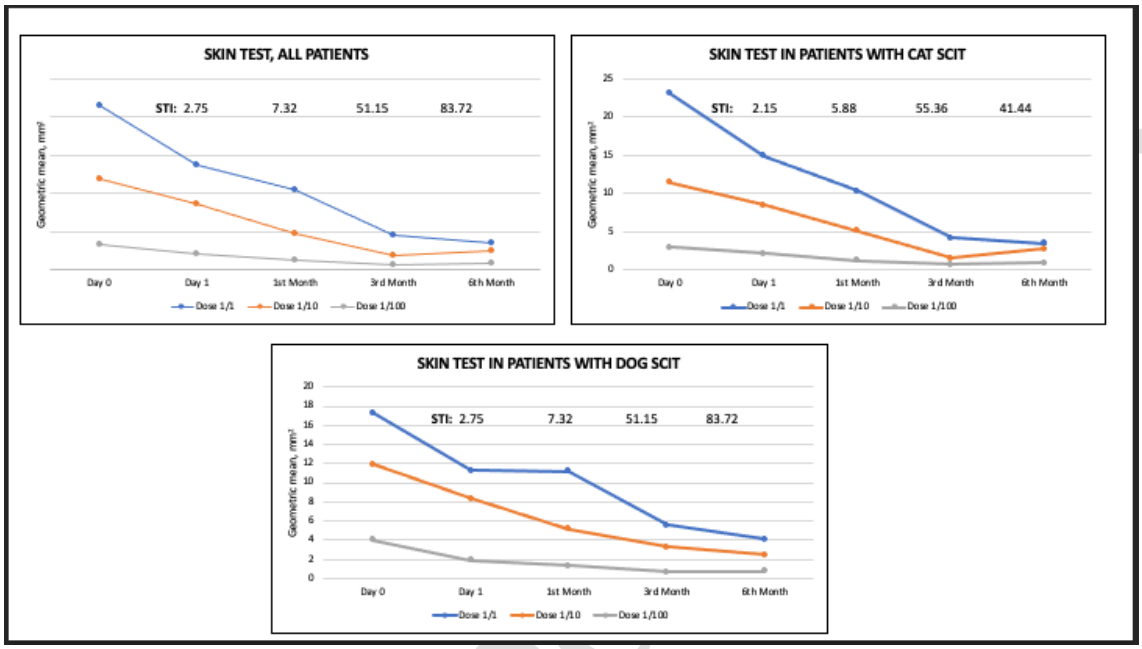

Figure 2. Quantification of immunoglobulin G1, G2, G3, G4, G total, and E to recombinant cat and dog allergens (Can f1, Can f 2, Can f 3, Can f 4, Can f5, Can f 6, Fel d 1, Fel d 7) at baseline and at month 3 and 6 of SCIT. Ig: immunoglobulin. Dots: patients out of range on mean±standard deviation.

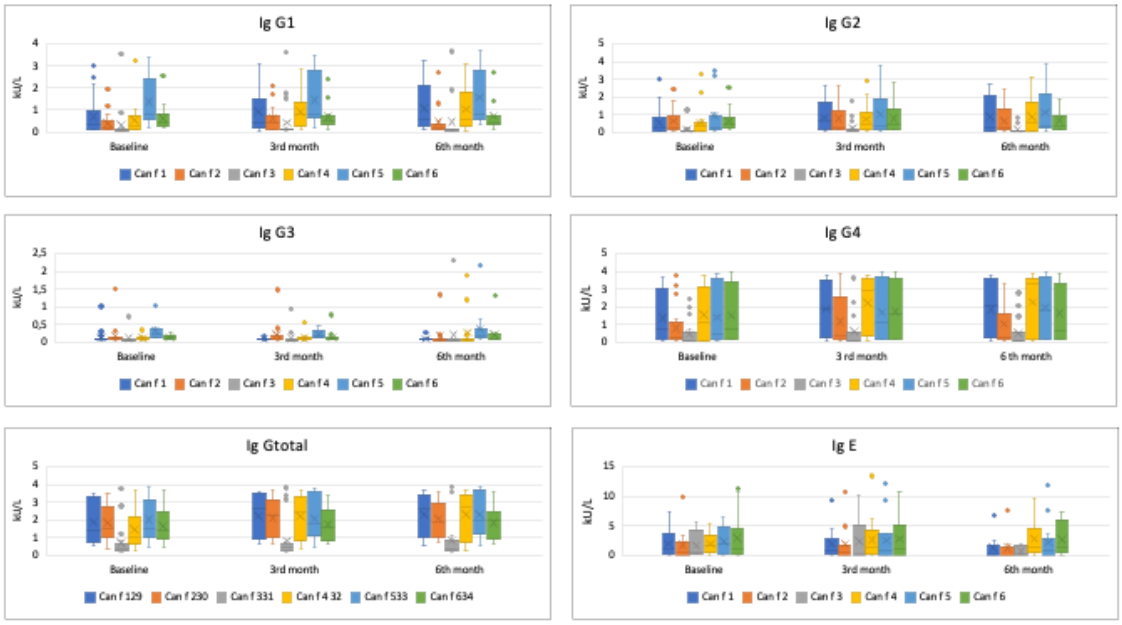

Figure 3. Quantification of immunoglobulin G1, G2, G3, G4, G total, and E to recombinant cat allergens (Fel d 1, Fel d 7) at baseline and at month 3 and 6 of SCIT. Ig: immunoglobulin. Dots: patients out of range on mean±standard deviation.

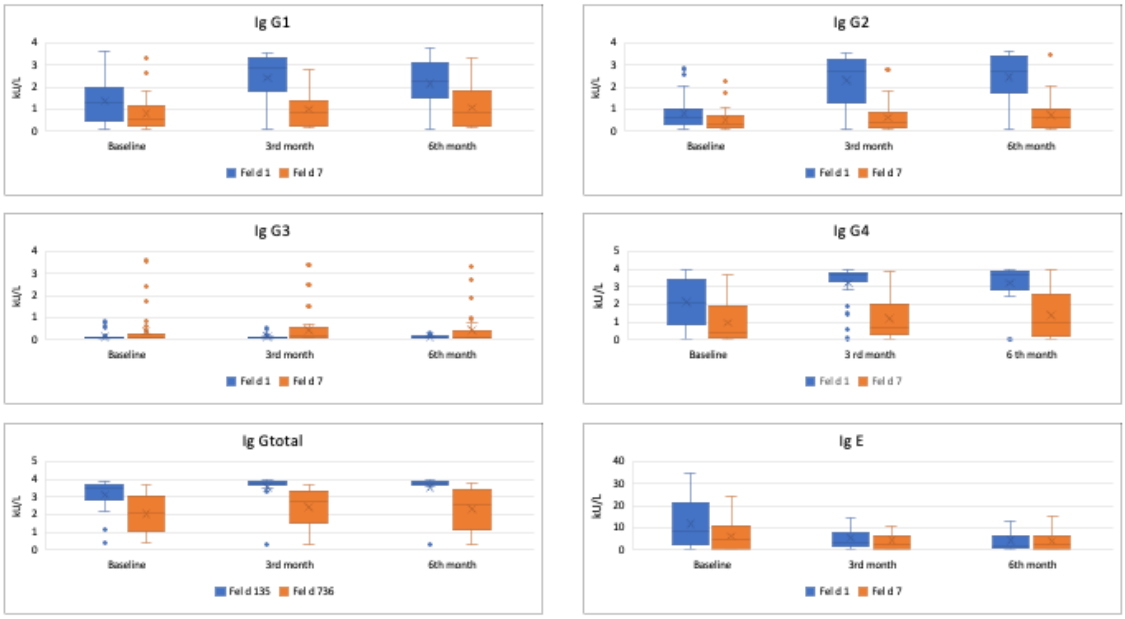

Figure 4. Results of respiratory function test (spirometry: FEV1, FVC, FEV1/FVC), FeNO, ESPRINT-15, AQLQ, ACT, VAS, symptoms and medication score at baseline and subsequently at month 1, 3, and 6.

Figure 5. Results of symptoms and medication scores at baseline and subsequently at month 1, 3, and 6.