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Aqueous intradermal low-dose house dust mite immunotherapy in tropical settings: a valid cost-effective approach for developing nations?

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Abstract
Introduction: Aqueous allergen injections, an effective and century-old technique, is considered a second-line approach in daily clinical practice. Inconveniences still surround conventional subcutaneous immunotherapy (SCIT) administration, such as a need for frequent injections, prolonged up-dosing schedules, elevated costs, and the unlikely possibility of a systemic reaction. The intradermal immunotherapy route (IDR) might favorably impact many of the aforementioned issues (Table 1). House dust mite (HDM) allergens are the main perennial sensitizers in the tropics, and as such, are solely employed in immunotherapy treatments.
Methods: We carried out a year-long real-life study in 25 perennial allergic rhinitis children, symptomatic on exposure to house dust, employing an intradermal low-dose allergen mix consisting of 50 ng of *Dermatophagoides pteronyssinus/Dermatophagoides farinae* and 120 ng of *Blomia tropicalis*, under a unique cost-wise protocol. Basal symptoms/signs and face Visual Analog Scale (fVAS) scores were recorded for 2 weeks and later compared with those registered throughout the 1-year treatment. Serum-specific IgG4 and IL-10 levels were employed in the assessment of the immune responses.
Results: Symptoms/signs and fVAS scores were significantly reduced from days 42 and 49, respectively, and remained so until treatment completion. Increases in specific IgG4's and IL-10 levels reflected significant immune responses. Injections were well tolerated and families reported improved health status (quality of life, QoL).
Conclusions: A unique cost-effective immunotherapy alternative for deprived allergic communities in tropical settings is depicted; further research is needed.

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Introduction

Aqueous allergen immunotherapy, a more than a century-old effective technique and hallmark of the Allergology specialty,[1-3] has been widely employed by allergists worldwide. It has proven its worth by significant alleviation of symptoms and medication usage, among other clinical and more favorable immune effects.[4-11]

Significant progress over the last three decades has been made around its mechanisms of action,[12] for both the subcutaneous (SCIT) and sublingual routes (SLIT). Notwithstanding, some administration issues pertinent to the conventional aqueous subcutaneous administration remain to be addressed as follows:

a. Patients feel discouraged by the frequent injections and lengthy up-dosing regimens needed to reach a maintenance dose, which may take several months and affects compliance.[13] To alleviate this inconvenience, additional protocols (Rush/Cluster) have been developed. However, in a counterintuitive manner, SCIT has been favored against SLIT[14] when adherence issues were considered. Allergoids/depot preparations that space out SCIT injections are most accepted initiatives,[15] widely employed in Europe, but less so in the United States.
b. Individualized immunotherapy allergen vials are worth a comment. As is recommended by Guidelines,[2,13] these are very costly options (four to five vials of different allergens and in pertinent concentrations per patient), something of particular concern for developing world environments.[16] On the other hand, an injection when given from the "board" and allowing for different allergens to be mixed in the syringe, is no longer a recommended technique. However, it was formerly used in North America, turning out to be highly suitable given its easy implementation. Busy allergy clinics handling dozens of polysensitized patients per day, requiring weekly injections, found this extremely useful. In humid tropical environments, where mites are the only major allergens and sensitizers, the above stated is less of a predicament.[17-22]
c. Vaccine costs are impacted by the increased amounts of necessary allergenic materials, more so when the SLIT is compared to SCIT. However, an important practical and related aspect is the reported lack of efficacy of low-dose immunotherapy.[23,24]

Several articles concerning the intradermal route technique administration (IDR) have been published.[25-31] We have reported in a real-life pilot study[32] the effectiveness and tolerance of a particular intradermal technique for *Dermatophagoides* mites and *Blomia tropicalis* allergens. Herein, we are expanding our results, over a year, in a greater number of allergic rhinitis children. A novel cost-effective approach with this IDR major allergens administration is outlined as a possible answer to above-mentioned inconveniences. For Latin America and many other areas of the world,[33,34] the financial burden of immunotherapy remains an issue.[16] If aiming for wider use of allergen immunotherapy in tropical settings, this unique intradermal route approach hopes to encourage further oriented research in such a needed area.

Methods

Immunotherapy-naive patients with perennial allergic rhinitis (PAR) attending a pediatric allergy clinic in a hospital setting (Hospital San Juan de Dios, Caracas-Venezuela), caring for a low-income population were offered this treatment modality. The protocol was approved by the Hospital Institutional Review Board (IRB). The study was carried out from May 2, 2018 until October 28, 2019, with patients and families required to sign an informed consent form.

Patients with perennial symptoms of allergic rhinitis (PAR), such as recurrent sneezing, itching, rhinorrhea, and nasal obstruction, for at least 2-year duration and symptomatic on exposure to house dust (home house dust disturbances), were selected.

Detailed instructions and pertinent guidance were provided by one of the researchers (CR), herself a house dust PAR sufferer, around the recognition of these symptoms/signs. Proficiency in interpretation and reliable translation into a 0–3 number scale score,[35] from patients and families, was aimed for. Baseline daily data were collected for 2 weeks around 8:00 pm (PatientDiaryAL0906rP_Spain_MasterV20_Feb2010translation22Feb2010), and a minimum of 100 score/points were required for inclusion (Total Nasal Symptom Score, TNSS), reflecting moderate rhinitis. Furthermore, proficiency in recognition of a face Visual Analog Scale (fVAS) score was[36,37] an endeavor that had to be mastered. All patients had to have a positive skin test to house dust mites (>3 mm from control solution). Patients and families were discouraged from employing anti-allergic medications, for the above registries to reflect real-life symptoms/signs. The lack of their availability (antihistamines, nasal steroids, Montelukast) due to Venezuela's economic crisis,[38] made us more confident that the TNSS/fVAS accurately detected rhinitis symptoms during this recruitment phase. Out of the 43 patients and families offered this treatment modality, only 25 patients/families were able to satisfactorily fill-out the required TNSS/fVAS recognition registry. When mothers also had PAR, interestingly enough, this process flowed much easier.

Once treatment was started, the TNSS/fVAS diary card was to be filled out at home (around 8:00 pm) the day before each weekly injection (12 injections for the first 3 months), along with a medication usage record (only on a needed basis). After 3 months, injections were spaced out empirically to every 2 weeks for three additional months, then to every 3 weeks for another 3 months and finally to once a month until the 1-year treatment was completed. Furthermore, patients' transportation and easy access to the Hospital was considered an important issue, as well as home/cell phone availability for compliance reasons.

Patients receiving intranasal or oral steroids within 1 month prior to the study and/or immunotherapy ever, as well as passive tobacco exposure, allergic respiratory symptoms triggered by home pets (dogs, cats) regardless of their skin test positivity, were excluded from the study. Also, patients with other chronic diseases such as cystic fibrosis, heart disease, respiratory illnesses (sinusitis, clinical adenoid hypertrophy, symptoms compatible with obstructive sleep apnea, significant septum deviation, or nasal polyps) that might interfere with symptoms' interpretation were disqualified. Researchers were always available

by phone to answer questions. The use of a control group ("histamine sham injections") was ruled out from the beginning, as per our institution's IRB. All patients received oral instructions and written pictorial hand-out material with detailed house dust eviction measures, as part of our clinic work-up routine.

During the pre-treatment baseline data collection phase, weekly patients/families phone calls were made, reinforcing learned abilities. Thereafter, weekly SMS text messages were sent to participants, reminding patients/families to bring their filled TNSS/fVAS diary card to the allergy shot appointments. A new TNSS/fVAS diary card was to be dispensed then. Medication use, like antihistamines, antileukotrienes, and intranasal corticosteroids were discouraged during treatment and allowed on a needed basis only. Allergic Rhinitis & Rhinosinusitis QoL (quality of life) questionnaire was filled out before and following the 1-year treatment.[39]

Treatment compounding

A 50% glycerinated 50 mL extract of a standardized allergen unit (AU) mixture of *Dermatophagoides pteronyssinus*/*Dermatophagoides farinae* (Dp/Df) from Greer Labs, Lenoir, NC, USA, and labeled as "stock solution" (lot number #308809, 10,000 AU/mL: 5000 AU Dp/5000 AU Df, expiration date: 06/2019) and a *Blomia tropicalis* 50% phenol-glycerinated skin test extract, which is also labeled as "stock solution" (Immunotek Laboratories, Alcala de Henares, Spain, 150 mcg/mL, lot A17J6P; expiration date: 09/2020), were used for skin testing as well as for compounding the allergenic material employed for treatment (following the ACAAI's Allergen Immunotherapy Extract Preparation: Physician Instruction Guide[40]). This Dp/Df mixture from Greer Labs contains approximately 62 mcg/mL of major allergens, roughly corresponding to a 1/100 dilution w/v.[13] Likewise, for the *Blomia tropicalis* skin testing extract, a 1/100 w/v dilution was considered and also labeled as "stock solution". A 1.6 mL volume of each of the aforementioned concentrates were added to a 100 mL flask of phenol/saline/albumin (ALK Labs) as dispensed for enhancing the stability[41,42] of very dilute extracts (lot # L2012518, expiration date: 01/2021). Each 0.05 mL volume from this newly prepared flask contained approximately 8 AU of *Dp*/*Df* or 0.05 mcg (50 ng) of HDM major allergens equivalent. For *Blomia tropicalis* allergens, 0.12 mcg (120 ng/0.05 mL) were correspondingly estimated. These concentrations were guided by previous clinical results obtained with this technique[32] and the IgG4 responses resulting from Dp/Df major allergens showed a trend for improvement after 3-month treatment, though found not to be statistically significant. Though increasing the dose of *Dp*/*Df* seemed logical (from 5 ng/0.05 mL to 50 ng/0.05 mL per allergy shot), we still maintained a low-dose range objective (Appendix A). Similar reasoning was entertained for *Blomia tropicalis*, though we could not find a correspondence between the DBU/mL (previously employed) and the mcg/mL of allergens in our present dilution. A recent paper[43] on immunotherapy employed 150 mcg of *Blomia tropicalis* total protein as a maintenance dose; the nanogram (ng) dose herein depicted (120 ng) is well below that range.

This 100 mL multiple-injection flask, properly stored at 4–8°C and taken out of refrigeration only for shot administrations, allowed for the easy weekly operation of our busy clinic. For sake of simplicity and repeatability, a volume of 0.05 mL was adopted for the intradermal injection; this is the minimal volume that can be reliably measured with disposable 0.3 mL/31 G needle syringes (Beckton-Dickinson®). A volume of 0.05 mL, when injected under this technique, makes for a papule of 0.5 cm^2 (Figure 1).

Skin tests

Prick tests were performed using the standardized Hollister-Stier Lancetter® and reactions were considered positive if papules were >3 mm than the negative control. These lancets make for almost no skin irritation; no evidence of dermographism was found in any of the patients. Papules' length and width were read at 15 min and graphically outlined on a scotch tape for transport and proper measurement on patients' charts, as proposed by Dreborg.[44] Besides the Dp/Df and *Blomia tropicalis* ("stock solutions") prick testing, additional prick skin tests with other inhalant extracts (cat, dog, grass mix, mold mix from ALK-Abello®, Madrid, Spain) were also performed, along with histamine 1 mg/mL and a negative glycerosaline control. For better readings, the prick tests were placed in the patient's volar surface of the forearm and read at 15 min. Antihistamines, if any in use, had to be suspended 5 days prior to testing.

Immunotherapy treatment

Patients received 0.05 mL intradermal (ID) injections in the middle and external area of the arms, from the 100 mL ALK phenol/albumin/saline flask referred above. Disposable 0.3 mL/31 G sterile syringes (Beckton-Dickinson®) were employed during our routine clinic operation. A *peau d'orange* papule was to be formed (as performed in routine PPD skin testing) with visualization of small dimples

Figure 1 Outline of intradermal technique. Demonstration of wheal size (0.5 cm^2) and characteristically dimple formation (peau d'orange), lack of bleeding, and surrounding erythema.

and lack of blood drainage (Figure 1); arms sites were alternated. Patients remained at the study site for at least 30 min following injections, with a previous oral antihistamine (cetirizine)-according to weight-administered 1 h prior to shots. To estimate the allergenic stability of the compounded material, 10 patients on a bi-monthly basis were skin tested from the referred 100 mL flask. Allergy treatments were given in a clinical area with full resuscitation facilities. Adverse local or systemic reactions were assessed. The 100 mL compounded flask may dispense up to 2000 injections, hence emphasizing this cost-wise approach.

In vitro tests

Each patient had a 3 mL blood sample drawn from the antecubital fossa in a tube without anticoagulant, before and after the end of the study. The blood sample was centrifuged at 5000 rpm (Labofuge 200 Thermo-Scientific®) and the sera were stored at -20°C until analysis. To determine the total IgE, the automated equipment Minividas (Biomerieux®, France) was used and the values were expressed in IU/mL concentrations, according to the manufacturer›s specifications. This reference values have been adapted to the Venezuelan population according to age and gender as per previous studies carried out by Fabiano et al. For automated allergen-specific IgE and IgG4 determinations, the automated PHADIA CAP 250 Thermo-Scientific® method was used; and to determine the total IgG4, the automated SPA Plus® method (Binding Site) was used, all of them according to the manufacturer's specifications. For IL-10 determinations, the human kit Elisa Kit Abcam® of IL-10 was used; also under the manufacturer›s specifications. Samples from healthy individuals were used as internal controls of known concentrations in each analysis.

Statistical analysis

Comparisons of quantitative variables (mean values) were carried out with the Mann-Whitney test before and after treatment. A P-value of less than 0.05 was considered statistically significant.

Results

Demographic and clinical data

A total of 43 allergic rhinitis children attending the Allergology clinic at Hospital San Juan de Dios, Caracas, were offered this treatment modality. Twenty-five fulfilled the inclusion criteria but only 17, mean age 9.3 ± 3.3 (range 4-18), completed a full year of immunotherapy treatment (68%). The rest were lost to follow-up for different reasons, mostly forced emigration, given Venezuela's present socio-economic crisis.[38] Those remaining patients were the ones considered in our analysis; 12 were females (70.5%) and five were males (29.4%) (Table 1).

All patients had positive skin tests to *Dp/Df* and *Blomia tropicalis* (>3 mm papule greater than control); 35.2% also had positive prick tests to dog and cat without reporting symptoms on pet exposure. The average specific IgE for *Dp/Df* was 21.46 IU/mL and for *Blomia tropicalis* 8.29 IU/mL; for dog and cat, the average specific IgEs were 0.97 and 1.76 IU/mL, respectively. Atopic dermatitis was detected in 10 patients (58.8%), asthma in nine patients (52.9%), and conjunctivitis in four patients (23.5%).

Table 1 Intradermal immunotherapy with mite allergens: demographic and clinical features of studied population.

n	17
Age (years)	9.3 ± 3.3 (range 4-18)
Female	12 (70.5%)
Male	5 (20.4%)
TNSS baseline	118 ± 11.7
Positive prick tests	Mites 17 (100%)
	Dog 6 (35.2%)
	Cat 6 (35.2%)
Comorbidities	Atopic dermatitis 10 (58.8%)
	Asthma 9 (52.9%)
	Conjunctivitis 4 (23.5%)
Baseline total serum IgE	677.55 ± 403.9 IU/mL
Baseline total serum IgG4	0.46 ± 0.31 mg/dL

The TNSS average score points at inclusion were 118 ± 11.7 for the main four symptoms/signs of rhinitis (itching, sneezing, rhinorrhea, and congestion). The daily baseline TNSS was 8.58 ± 0.86 while the fVAS score was 6.0 ± 1.5. As shown in Figures 2a and 2b, results were significantly lower ($P < 0.05$) after 42 and 49 days, respectively.

Patients registered antihistamine use mostly for acute symptoms control, averaging a meager 13.2 days of use for the whole year's treatment.

Injections were well tolerated, with only local minor adverse reactions. QoL significantly improved coinciding with comments brought up by patients and families.

The stability of the compounded preparation (100 mL ALK flask) was evaluated by prick testing in 10 patients, bi-monthly, along with the year's study. No significant wheal size variation (5 mm papules, ± 2mm) was detected.

The baseline total serum IgE was 677.55 ± 403.9 IU/mL and baseline total serum IgG4 was 0.46 ± 0.31 mg/mL. Table 2 depicts results for each patient.

As can be seen in Figure 3 and Table 3, the specific IgG4 (IU/mL) and the IL-10 (pg/mL) serum levels showed a statistically significant increase: $P<0.05$, after IDR treatment. In Figure 3, the emphasis is placed on the specificity of the response by showing a lack of an increase in dog's and cat's specific IgG4.

Discussion

Disparities in healthcare delivery are a significant worldwide issue. Immunotherapy is a hallmark of the allergology specialty and remains the only disease-modifying therapeutic modality available for routine clinical practice. Notwithstanding, it is less employed when compared to pharmacological symptomatic alternatives, sharing a meager 4.46% of the world market.[45,46] In reference to aqueous

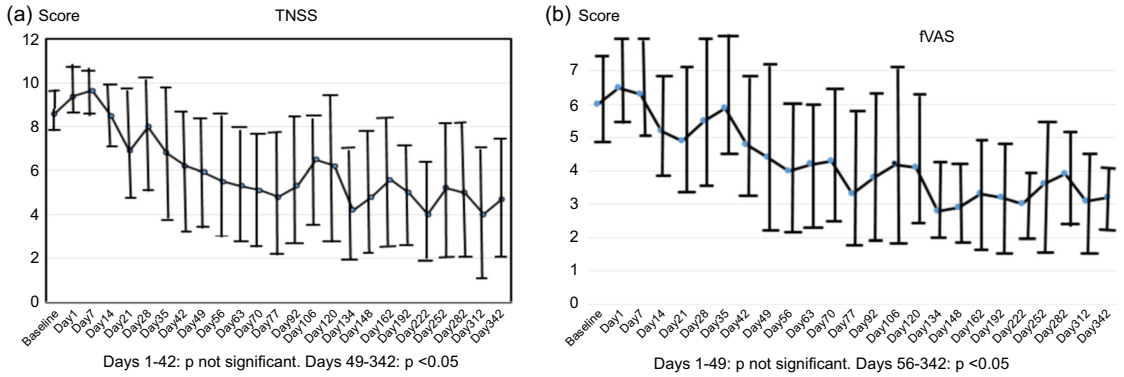

Figure 2 (a) Total nasal symptoms score (TNSS).* (b) Facial visual analogue scale (fVAS).** *Results are expressed as mean values ± 1 standard deviation. Days 1–42: P = not significant. Days 49–342: P < 0.05. **Results are expressed as mean values ± 1 standard deviation. Days 1–49: P = not significant. Days 56–342: P < 0.05.

Table 2 Demographic and clinical features of individual AR patients.*

Patients number	Sex (F/M)	Age (years)	TNSS baseline	Positive prick test	Comorbidities	Base line serum total IgE (IU/mL)	Base line serum total IgG4 (IU/mL)	Base line serum IgG4 Dp/f (IU/mL)	Baseline IgG4 Bt (IU/mL)
1 SL	M	9	9	Df/Dp/Bt	AD	131.3	0.90	0.070	0.034
2 JC	M	18	9.4	Df/Dp/Bt	A	238.4	0.20	1.202	0.033
3 JR	M	7	9.1	Df/Dp/Bt	AD/A	776.7	0.37	2.220	0.210
4 DL	M	15	8.3	Df/Dp/Bt	AD/C	592.1	0.73	1.600	0.123
5 DB	M	10	7.8	Df/Dp/Bt	AD/C	747.3	0.16	4.020	0.010
6 VI	F	9	8.4	Df/Dp/Bt	No	122.3	0.12	0.081	1.264
7 DI	F	9	8	Df/Dp/Bt	No	171.3	0.01	1.400	0.054
8 IV	F	14	8.1	Df/Dp/Bt	AD/C	824.5	0.59	0.737	0.023
9 LG	F	11	9.9	Df/Dp/Bt	A	769.1	0.74	0.023	0.010
10 NV	F	13	9.7	Df/Dp/Bt	No	106.5	0.64	0.280	0.088
11 HR	F	9	8.4	Df/Dp/Bt	No	1213.3	0.92	6.600	0.017
12 EB	F	8	7.9	Df/Dp/Bt	A	1412.8	0.30	4.970	0.020
13 HF	F	9	8.3	Df/Dp/Bt	AD/A/C	879.2	0.48	2.300	0.104
14 KL	F	10	7.1	Df/Dp/Bt	A/AC	574.7	0.03	1.440	0.090
15 SC	F	8	9.7	Df/Dp/Bt	A/AD	1058.5	0.75	2.005	0.303
16 FM	F	15	9.6	Df/Dp/Bt	No	898.3	0.15	3.301	0.400
17 LP	F	4	7.3	Df/Dp/Bt	AD	1001.2	0.79	3.000	0.200

Only 17 patients are included who completed 1 year immunotherapy treatment.
AR: allergic rhinitis; Dp: *Dermatophagoides pteronisynnus*; Df: *Dermatophagoides farinae*; Bt: *Blomia tropicalis*; A: asthma; AD: atopic dermatitis; C: conjunctivitis; M: male; F: female.

SCIT and its rather cumbersome administration issues and cost predicaments, the IDR as depicted here represents a novel initiative. For the significant impoverished majority of people living in tropical settings, where mites stand out as main sensitizers and triggers of disease,[17-22] this approach may improve immunotherapy availability.

First, the cost issue, a matter of importance not only for developing nations, reveals that a single aqueous "allergy shot" (0.05 mL) drawn from the 100 mL ALK flask of our compounded injection material, runs for approximately 3 cents of a dollar, comparing very favorably to any current conventional aqueous SCIT treatment worldwide (Appendix B). The ALK 100 mL albumin/saline/glycerin multiple-dose flask used in the compounding of our allergen preparation allowed sufficient allergenic material for approximately 2000 single immunotherapy treatments (0.05 mL/dose). This multi-dose single concentration injection technique seems quite appropriate for use in high-volume busy allergy clinics, in counter distinction to the usually prescribed four to five different concentration vials of allergens per patient.

Furthermore, another aspect worthy of consideration is the need for a build-up dosing schedule.[13] By employing this IDR technique, a unique volume/dose (0.05 mL) without increments is simpler than any other conventional aqueous SCIT protocol in use today. In fact, larger volumes of intradermal injections may be disturbing to patients' arms. Moreover, in reference to systemic adverse reactions, none were observed; only minor and local ones and no different, in essence, from what is reported during conventional aqueous SCIT. Previous use of an antihistamine, no doubt, helped in allaying such mild post-injection local reactions.

Most of the reported adverse systemic reactions found in the literature tend to occur during the build-up phase. The lack of an incremental dosing schedule coupled with a very low allergen content may have accounted for our results. Patients tolerated the injections well, with almost no pain complaints.

The other significant issue is effectiveness. Earlier studies have suggested that low-dose immunotherapy is devoid of clinical effects.[23,24] In our previous 3-month pilot report,[32] employing TNSS/fVAS as evaluative tools for IDR effectiveness, significant improvement of symptoms/signs were noted commencing on the 5th week of treatment. Moreover, IgG4 measurements before and after treatments demonstrated a trend for improvement, but significant only for *Blomia tropicalis*. The diminished wheal size found after treatment when the Serial Dilution Skin Testing (SDST)[32] was performed, agrees with the above findings. Our present real-life 1-year study showed TNSS and fVAS scores reaching significance at treatment days 42 and 49, respectively. The significant increase of specific IgG4 after the 1-year treatment with Dp/Df and *Blomia tropicalis* (Figure 2) and the lack of response detected to cat and dog allergens, emphasized specificity. The IL-10, a possible biomarker of immunological tolerance,[47] also considerably increased (Table 3). The QoL questionnaire reinforced the improved health status brought up by our patients. Hence, it appears that a clinical response is coupled with the treatment immune effects measured.

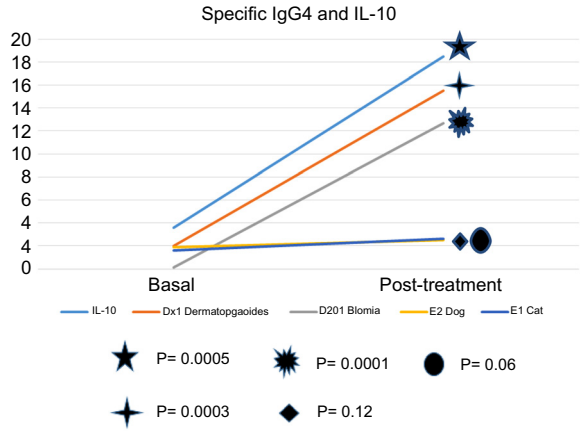

Figure 3 Specific IgG4 (IU/mL). The lack of IgG4 post-treatment responses to dog and cat allergens emphasizes the specificity of immunotherapy treatment *P > 0.05. IL-10 responses before and after treatment are depicted in picograms/mL: P < 0.05.

Notwithstanding, a pertinent set of questions come to the forefront. According to a recent Practice Parameters Guideline,[2,35] the TNSS/fVAS evaluations are currently the best available clinical indications around treatment effectiveness, despite their subjective nature and inherent variability from patients' reporting.[35] The use of frequent reminder phone calls and/or SMS text messages may palliate some of the possible flaws in this regard. Reinforcing in each visit the adequate translation/transcription of symptoms/signs of allergic rhinitis into a numeral score, cannot be overemphasized. However, experimental exposure chambers, by improving the objectivity of assessments, are portrayed as a possible new paradigm in an effort to circumvent some of the above-mentioned treatment evaluation subtleties. Having felt encouraged by the initially favorable clinical response from our previous 3-month pilot study,[32] we decided to pursue this larger study. Our institution IRB, however, did not allow for a control group (placebo) to be employed. We acknowledge the placebo effect that immunotherapy intrinsically carries and hence the note of caution on the interpretation of these results.

Another question refers to humoral and/or cellular immunity response parameters provoked by immunotherapy treatments: Do they have any bearing on clinical symptoms, or do they just represent a possible para-phenomenon? We may only speculate on the possible favorable immune effects of this IDR perennial allergen administration. Are allergens taken up directly from the Langerhans cells in the epidermis or do they gain access from the dermis, because of tissue damage from the injection? (Figure 1). If so, do their better allergen processing capabilities –in spite of a very low antigen dosing–come forward into play?[48] Do IgG4 post-treatment immune responses attest to it? On the other hand, does IDR immunotherapy target other allergen inflammatory pathways asides from IgE,[49] which are not detected by the methodologies herein employed? In any case, the low-cost and betterment of clinical symptoms along with the many practical inconvenience issues, do warrant further consideration.

However, our previous and present findings contrast with those of Slovick et al.,[31,50] employing a seasonal and different allergen (7 ng/dose of Grass *Phl p 5*), with only pre-seasonal treatments. Surprisingly, despite detecting a significant impact on the delayed cellular immune skin response[28] to grass pollen major allergen (*Phl p 5*), patients fared worse in the treatment arm,[31,50] suggesting

Table 3 IL-10 (pg/mL), specific IgG4 (IU/mL), and quality of life (QoL) before and after treatment.

	Baseline	Post-treatment	P-value
IL-10 (pg/mL)	3.61 ± 4.2	18.5 ± 15.1	<0.05
Dermatophagoides IgG4 (IU/mL)	2.07 ± 1.8	16.2 ± 13.4	<0.05
Blomia tropicalis IgG4 (IU/mL)	0.11 ± 0.11	12.69 ± 13.0	<0.05
Dog IgG4 (IU/mL)	1.92 ± 1.2	2.51 ± 1.6	n.s.
Cat IgG4 (IU/mL)	1.63 ± 0.5	5.5 ± 10.0	n.s.
Quality of life score	35.0 ± 11.4	8.3 ± 4.8	<0.05

n.s.: not significant.

that a seasonal clinical deterioration was induced by treatments. Different methodologies impede us from a proper comparison between the latter[50] and our study (perennial vs seasonal allergies/allergens; 50 ng vs 7 ng/dose of major allergens; 20 mL vs 50 mL of injected material; IgG4 responses vs no IgG4 titers detected, among others).

Healthcare disparities need a constant and pertinent look around cost and treatment inconveniences. Henceforth, existing challenges demand prompt and much-needed answers.[51] No doubt, if our findings are confirmed in a greater number of subjects, this particular cost-effective low-dose IDR technique may imply a step forward in allaying the significant healthcare disparities surrounding allergic diseases; an area of prime research interest to allergologists.

Acknowledgments

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Appendix A

In our previous report (Rev. ALERGIA, Mexico, 2018, 65: 41–51), a transcription error was detected. Though 5 ng of HDM major allergens were injected/per each 0.05 mL in weekly allergy shots (as stated in the paper), the transcription calculations erroneously stated 0.05 mcg or 50 ng. This was notified to the Editor of Revista ALERGIA, Mexico and properly acknowledged from the Editorial and author's side.

Appendix B

Approximate cost calculations were rounded to the closest multiple of 10.

The cost of a 50 mL stock solution of *Dp*/*Df* 50% glycerin solution with 10.000 AU from Greer Labs, North Carolina, USA (as per catalog Stallergenes-Greer, effective year 2019), is approximately USD$ 1,261.67. The estimated cost of a 100 mL HAS/phenol dilution flask from ALK is $USD 20.00. A 1.6 mL from this *Dp*/*Df* "stock solution" costs around USD$ 53.00; the *Blomia tropicalis* skin test material "stock solution" (3 mL) cost is around USD$ 20.00. 1.6 mL from each of these stock solutions were added to the 100 cc phenol/albumin solution from ALK Labs. Final major allergen concentrations from this preparation are 50 ng/0.05 mL of *Dp*/*Df* and 120 ng of *Blomia tropicalis*, which overall corresponds to an approximate cost of USD$ 3 cents per allergy shot (0.05 mL). This is no doubt significantly cost savings by any standard.

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