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ALLERGEN-SPECIFIC IMMUNOTHERAPY AS A MODERN METHOD IN THE THERAPY OF ATOPIC DERMATITIS WITHOUT ADDED RISK OF DELETERIOUS HAEMATOLOGICAL AND BIOCHEMICAL EFFECTS

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ABSTRACT

Atopic dermatitis is a complex, multifactorial disease. Treatment could be extremely long–term, even lifelong, and presents a real challenge for the veterinarians. Usually, atopic dermatitis involves a combination of approaches, including reactive therapy for acute conditions and proactive therapy for long–term management. A modern method is the allergen–specific immunotherapy (ASIT). The purpose of the present study is to prove that subcutaneously ad-ministered ASIT is a successful treatment that does not lead to any deleterious hematological and biochemical effects in dogs as opposed to long–term systemic glucocorticoids.

Allergen–specific immunotherapy (ASIT) is considered to be the only treatment that can affect the course of the disease and not just suppress the symptoms.

Key words: Atopic dermatitis, ASIT – allergen–specific immunotherapy, haematology, biochemistry.

Introduction

Atopic dermatitis is a complex, multifactorial disease. Treatment usually is challenging and lifelong and involves many therapeutical methods. Acute conditions are targeted with reactive therapy, whereas post–acute or chronic conditions are handled by a proactive therapy. The ultimate aim is to relieve the patient from pain and discomfort long–term and to avoid relapse for as long as possible. (Olivry *et al.* 2015)

Genetic and environmental factors play a fundamental role in the development of atopic dermatitis and the specific clinical picture. Diagnosis is usually achieved by ruling out all other differential diagnosis. (Saridomichelakis *et al.* 2016)

One of the main differential diagnoses of atopic dermatitis is food allergy. Modern dermatology suggests dividing atopic dermatitis into two categories: food–induced and non food–induced. 40% of dogs with chronic itching respond positively to a change in the diet. Half of these patients have proven concomitant atopic dermatitis and secondary food allergies. (Griffin 1993, Tarpataki *et al.* 2006, Bizikova *et al.* 2015, Olivry *et al* 2015, Nuttall *et al.* 2019). Dermatitis caused by flea allergy can also resemble or occur simultaneously with atopic dermatitis. 79% of dogs with atopic dermatitis also have a flea allergy. Sarcoptosis causes itching similar to atopic dermatitis, but there are some distinct differences. In scabies, an eruption with papules is observed. When bacterial pyoderma and Malassezia dermatitis are suspected, cytological examination of the skin is of the greatest importance for diagnosis and subsequent treatment. At each follow–up examination, it is key to note the degree of change in pruritus and skin lesions. It is necessary to carry out multiple follow–up control cytologies to check the effectiveness of the therapy. (Griffin 1993, Tarpataki *et al.* 2006, Miller *et al.* 2012, Bizikova *et al.* 2015, Saridomichelakis *et al.* 2016). Following a concomitant skin infection, allergic dermatitis usually is favorably affected. Itching due to a bacterial infection is observed in one of the main areas typically affected by atopic dermatitis (paws, especially the ventral

interdigital space, the concave surface of the pinna, the flexor surface of the metacarpus, metatarsus and elbow, abdominal and inguinal area, around the mouth and eyes). (Miller *et al.* 2012, Griffin 2013).

Very often in atopic animals, itching is associated with going outside. Owners report that the animal gets worse while outside or shortly after. This correlation can be observed in a certain season or after placing the animal in certain environmental conditions. It is also important to note that environmental contact can also mean contact with foods, ectoparasites, etc., which can trigger atopic dermatitis. (Zur *et al.* 2002, Griffin 2013)

Today ASIT has been shown to be extremely promising not only for the management but also for the long–term treatment of atopic dermatitis. By modifying the immune response, it can actually heal the animal. (Han *et al.* 2019, Muller 2019)

The purpose of the present study is to prove that subcutaneously administered ASIT is a successful treatment that does not lead to any deleterious hematological and biochemical effects in dogs as opposed to long–term systemic glucocorticoids.

Materials and methods

Animals

The animals included are 12 dogs of various breeds, 7 female and 5 male, aged between 7 months and 6 years. All of them were examined and diagnosed at the Multidisciplinary Veterinary Clinic Bulgaria between August 2022 and March 2023 presenting with symptoms of a skin problems and atopic dermatitis. All manipulations were carried out with the written consent of the owners, as well as in accordance with the standards for animal welfare.

Dermatological examination

Comprehensive dermatological examination was conducted on all 12 dogs, according to standard dermatological protocol. In 8/12 dogs some inaccuracies in the deworming plan were found, the owners were consulted, and the necessary changes were implemented. Ultimately, 12/12 dogs are dewormed monthly against external parasites. 9/12 dogs take a monthly tablet form against external parasites (Next Gard Spectra, Simparica Trio), 5/12 dogs are dewormed with pipette forms monthly (Vectra 3D, Advantix). 2/12 dogs combine more than one external deworming product.

Otoscopy

The otoscope used in this study is the Heine beta vet Otoscope set 2.5 V. Otoscopy is the visual examination of the ear canal and tympanic membrane. In case of external auditory canal infection without involvement of the middle ear, the tympanic membrane is shiny, smooth, translucent, and blood vessels and manubrium are clearly visible. With increased middle ear pressure, the Pars flaccida appears swollen and not always easily and clearly visible. All dogs in this study were examined using an otoscope.

Cytological examination of the ear canal and the surface of the skin

Clinically significant changes observed during the otoscopic examination (erythema, erosions of the ear canal, the presence of a discharge different from normal, etc.), require additional testing. Samples should be taken from the border between the vertical and horizontal ear canal for cytology. Ear canal cytology was performed with a non–sterile swab on all dogs in this study.

Cytological examination of skin lesions with erythema, hypotrichosis, hyperpigmentation, collarettes and/or lichenification was taken as an impression preparation on a glass slide or "scotch

tape". Diff Quick staining type with Hemacolor Rapid kit, 111674001 (Merck) and Microscope Euromex Bio Blue (Netherlands) were used. Samples were examined under immersion and objective SP 100/1.25 oil 160/0.17.

Complete blood count and Biochemical profile

The complete blood count was done at the Multidisciplinary Veterinary Clinic Bulgaria on a Mindray BC–5120 machine. Proteins (total protein, albumin), liver enzymes (ALT, ALP), bilirubin, kidney tests (urea, creatinine and P) and Ca were done at the Multidisciplinary Veterinary Clinic Bulgaria with a Mindray BS–200 analyser.

Intradermal allergy test and serological allergy test

An intradermal allergy test was performed at the Multidisciplinary Veterinary Clinic Bulgaria with the Nexmune Artuvetrin® intradermal set, without anaesthesia.

The serological allergy test was performed in the Nextmune laboratory (Next+ Serum test) in the Netherlands. It is an ELISA test that detects IgE in the serum of cats and dogs and uses a new generation of CCD blockers (carbohydrate cross–reactive determinants) for high specificity and sensitivity. Based on the analysis of the results of these tests, allergen–specific immunotherapy (ASIT) was prepared and applied. (Carlotti et Costargent 1994, Saridomichelakis *et al.* 1999, Tarpataki *et al.* 2006, Vogelnest et Mueller 2008, Miller *et al.* 2012, Hensel *et al.* 2015)

Concomitant therapy

Allergen–specific immunotherapy (ASIT) (Netherlands, Next Mune) is an individually prepared therapy specific for a patient and is applied according to the scheme: first application 0.2 ml/ subcutaneously; second application 0.4 ml/ subcutaneously, 14 days after the first; third application 0.6 ml/ subcutaneously, 14 days after the second; fourth application 0.8 ml/ subcutaneously, 14 days after the third; fifth application 1 ml/ subcutaneously, 14 days after the fourth; sixth application 1 ml/ subcutaneously, 21 days after the fifth; seventh application 1 ml/ subcutaneously 28 days after the sixth; Each subsequent application is 28 days after the previous one, 1 ml/subcutaneously.(DeBoer *et al.* 2017, Flanagan *et al.* 2017, Pali–Scholl *et al.* 2020, Ramio– Lluch *et al.* 2020)

Oclacitinib (Apoquel) is a Janus kinase inhibitor that is used to suppress itching and other symptoms in animals with atopic dermatitis. It is used in a dose of 0.6 mg/kg/12 hours for 14 days, and from the 15th day 0.6 mg/kg/24 hours per os.

Therapeutic bathing with a shampoo containing Chlorhexidine, as well as other local products in the form of wipes (CLX wipes, ICF) and tampons (Douxo Pyo Pads, Ceva) that contain Chlorhexidine.

Ear drops with Dexamethasone 1% locally in the ear canal 1 ml/ once a week.

Hydrocortisone aceponate (Cortavance spray) topical treatment.

Local preparations in spot–on forms containing essential oils and/or ceramides and/or phytosphingosine (Dermoscent spot on pipettes (Dermoscent), Allerderm (Virbac), Douxo seb (Ceva)) – 2 times a week for 8 weeks. After that, a break of 4–8 weeks and again a cycle of application. (Angarano et MacDonald 1991, Griffin 1993, Miller *et al.* 2012, Olivry *et al.* 2015)

Results

Following dermatological examination and putting all 12 dogs on a proper deworming plan, 9/12 dogs suggested to might have food–related symptoms and therefore underwent 2 months of therapeutic nutrition with a hydrolyzed protein diet (Annallergenic RC, Hypoallergenic Purina, Z/d

Hills). 8/9 dogs showed no significant improvement in their skin signs. 1/9 dogs had partial improvement in gastrointestinal signs but no improvement in their otitis and skin lesions. Therefore, they were diagnosed with atopic dermatitis.

During otoscopic examination 5/12 dogs were found to have a history of ear problems (recurrent otitis media). 4/5 dogs underwent a course of therapy for otitis externa and then proactive therapy with ear drops with Dexamethasone 1%, once a week for 3 months. In all of them there was no recurrence of otitis externa during the following 4 months.

6/12 dogs were intradermally tested for allergies. 6/12 dogs were dismissed from this test for various reasons. Of significance were the following results: 4/6 Acarus siro, 4/6 English plantain, 4/6 Kentucky bluegrass, 4/6 L. destructor, 4/6 Perennial ryegrass, 3/6 Malassezia, 3/6 Sycamore, 3/6 T. putrescentiae, 2/6 Common mugwort, 2/6 Tree pollen mix (oak, beech, elm), 1/6 Cat epithelia, 1/6 Orchard grass, 1/6 Timothy grass.

12/12 dogs were serologically tested for allergies at Next Mune Netherlands. All allergens with an Elisa Absorbance Units (EAU) value above 250 EAU were marked a significant allergens and included in the prepared immunotherapy. Based on serological testing results were as following: 7/12 English plantain, 7/12 House dust mite, 7/12 Kentucky bluegrass, 7/12 Malassezia, 7/12 Perennial ryegrass, 6/12 Copra mite, 6/12 Grain mite, 4/12 Common mugwort, 4/12 Elm, 4/12 Sycamore, 3/12 Alternaria alternata, 3/12 Timothy grass, 1/12 Cat Epithelia, 1/12 Flea, 1/12 Orchard grass.

For those 6/12 dogs that had also an intradermal test, the allergen–specific immunotherapy (ASIT) was created combining the results of both tests. All therapies were applied according to a scheme in Multidisciplinary Veterinary Clinic Bulgaria.

Each application of the therapy in the clinic is a control examination for the patient. 12/12 dogs were consulted for a specific complaint a minimum of 2 additional times, with an average of 4 superficial cytologies of skin lesions performed on these dogs. 2/12 dogs demonstrated more than 8 *Malassezia pachydermatis* in the visual field under a SP100/1.25 Oil immersion objective on superficial skin cytology with skin lesions; 4/12 dogs on surface skin cytology showed association between cocci and *Malassezia pachydermatis*. 2/12 dogs had cytology taken from collarettes– type skin lesions that demonstrated single neutrophils, disrupted neutrophil nuclei and single cocci bacteria under the microscope.

5/12 dogs were further consulted about their ears, and after starting ASIT each of these 5 dogs had an average of 3 cytologies per ear canal. In 4/5 dogs, an overgrowth of *Malassezia pachydermatis* was proven on cytology of the ear canal (over 5 / in one field of view under an immersion objective SP100/1.25 Oil). In 1/5 dogs in cytology of the ear canal, it was proven below 5 *Malassezia pachydematis* in plain view under immersion.

7/12 dogs were prescribed Apoqel before the start of ASIT. In 5/12 dogs, Apoquel was continued after initiation of ASIT and stopped 2 months after initiation of ASIT. In 1/12 dogs, Apoquel was continued after the start of ASIT and stopped 2 months and 3 weeks after the start of ASIT. In 1/12 dogs, Apoquel was stopped 1 month after the start of ASIT, but after 3 weeks it was started again, and after 4 weeks it was stopped.

At different stages of the disease and its therapy in 12/12 animals, after cytological examination of skin lesions with evidence of Malassezia pachydermatis of the patients and/or neutrophils with single bacteria and ruptured neutrophil nuclei, local therapy with products containing Chlorhexidine was prescribed. Shampoos (Chlorhexiderm 4%, ICF; Douxo Pyo, Ceva; Pyoderm, Virbac)

were used 2 to 3 times a week for 2 weeks. Products in the form of wipes and tampons with Chlorhexidine were also used locally. This application was followed by control cytological studies and a control examination. Each patient was individually given guidelines for therapeutic bathing.

In 11/12 animals, local spot–on forms – Dermoscent spot on (Dermoscent), Allerderm (Virbac), Douxo Seb (Ceva) pipettes were used as supportive therapy for the skin, to increase the effectiveness of the skin barrier. These products are a valuable helper in the long–term management of patients with atopic dermatitis. In combination with the overall therapy plan, they support the skin barrier, skin integrity, hydration, reduce transepidermal water loss and make the skin a significantly "harder barrier" to the penetration of various irritants. In 7/11 dogs significant increase in hydration was observed. In 8/11 dogs there was a reduction in itching. Skin integrity was improved, and epithelial cell shedding was reduced.

In 5/17 animals, Cortavance (virbac) was applied locally to reduce itching and local anti–inflammatory effect. Hydrocortisone aceponate is a diester dermocorticoid with a highly pronounced action, covering both inflammation and itching. It is applied twice a day for 1 week (reactive therapy), gradually reducing the dose to every other day / 1 week, every 2 days the third week, reducing to pulse maintenance therapy 1 time a week for 4 weeks (Proactive therapy). Within the first 2 weeks, 5/5 animals experienced a significant reduction in itching and inflammation.

12/12 patients who took part in the present study underwent a complete blood count and biochemistry testing at three points: 1. before the start of ASIT and the application of concomitant therapy; 2. between 2^{nd} and 3^{rd} month after starting of ASIT therapy; and 3. between the 5^{th} and 7^{th} month after beginning of ASIT therapy. Results are presented in Table 1 and Table 2.

In 12/12 patients, we have no significant deviations in the indicators of white and red blood cells, there is no evidence of significant changes in the values of Haemoglobin and Hematocrits.

2/12 dogs showed baseline WBC values above the reference value of 6–17.10^9/L, which may be related to the fact that pediatric patients have higher WBC values. In 2/12 patients, we have higher than reference baseline ALAT and ALP values before the start of ASIT. In both patients, we have a history of long–term use of oral corticosteroids, used in one case 6 weeks before, and in the other 8 weeks before the Intradermal and Serological Test. In these patients, at the second testing point there was a decrease and at the third testing point values were within reference values.

5/12 animals were treated with a local steroid Hydrocortisone aceponate (Cortavance spray), and in all of them no changes were registered in the complete blood count and biochemical profile indicators. 4/12 animals underwent pulse therapy with once–weekly 1% Dexamethasone ear drops, with no evidence of changes in complete blood counts and biochemistry.

12/12 clearly showed dissipation of atopic dermatitis symptoms as well as CBC and Biochemistry profile within reference value for a long–term period.

Table 1: Evaluation of CBC in all patients

PATIENT	CBC	WBC (10^9/L) 6.00-17.00	Neu (10^9/L) 3.62-12.30	Lym (10^9/L) 0.83-4.91	Mon (10^9/L) 0.14-1.97	Eos (10^9/L) 0.04-1.62	Bas (10^9/L) 0.00-0.12	RBC (10^{12}/L) 5.10-8.50	HGB (g/L) 110-190	HCT 0.330-0.560	PLT (10^9/L) 117-490
Patient 1	Before starting ASIT therapy	13.81	11.22	0.93	0.21	1.44	0.01	5.05	111	0.34	127
	Between the second and third month of starting ASIT therapy	14.41	11.31	1.23	0.72	1.12	0.03	5.94	128	0.41	217
	Between 5 and 7 months from the beginning of ASIT therapy	13.92	10.12	2.65	0.66	0.48	0.01	7.6	136	0.49	199
Patient 2	Before starting ASIT therapy	14.05	8.09	3.71	1.22	1.01	0.02	4.98	123	0.35	238
	Between the second and third month of starting ASIT therapy	15.11	10.18	3.12	0.82	0.98	0.01	5.01	130	0.36	198
	Between 5 and 7 months from the beginning of ASIT therapy	11.91	9.53	0.98	0.87	0.52	0.01	6.01	121	0.43	189
Patient 3	Before starting ASIT therapy	5.91	3.53	0.98	0.87	0.52	0.01	6.21	122	0.43	189
	Between the second and third month of starting ASIT therapy	7.9	4.22	2.1	0.98	0.58	0.02	8.1	180	0.56	401
	Between 5 and 7 months from the beginning of ASIT therapy	7.4	5.12	1.15	0.81	0.31	0.01	7.9	160	0.54	189
Patient 4	Before starting ASIT therapy	12.78	6.39	4.11	0.54	1.72	0.02	7.3	145	0.52	289
	Between the second and third month of starting ASIT therapy	12.07	5.84	3.96	0.69	1.56	0.02	6.9	132	0.49	176
	Between 5 and 7 months from the beginning of ASIT therapy	12.98	56.35	4.28	0.94	1.41	0.01	7.7	149	0.51	206
Patient 5	Before starting ASIT therapy	18.95	14.88	2.12	0.83	1.11	0.01	4.11	109	0.34	189
	Between the second and third month of starting ASIT therapy	18.05	13.11	3.34	0.77	0.81	0.02	4.59	148	0.31	210
	Between 5 and 7 months from the beginning of ASIT therapy	15.23	10.53	2.92	0.85	0.92	0.01	6.7	161	0.45	205
Patient 6	Before starting ASIT therapy	20.11	14.62	3.23	1.23	1.01	0.02	4.19	112	0.26	189
	Between the second and third month of starting ASIT therapy	18.17	12.44	3.78	1.02	0.92	0.01	3.33	135	0.21	228
	Between 5 and 7 months from the beginning of ASIT therapy	16.11	11.02	3.28	0.91	0.88	0.02	6.19	168	0.49	201
Patient 7	Before starting ASIT therapy	16.09	10.34	3.82	0.32	1.6	0.01	7.88	181	0.56	171
	Between the second and third month of starting ASIT therapy	11.38	6.92	2.41	0.61	1.42	0.02	9.12	185	0.57	312
	Between 5 and 7 months from the beginning of ASIT therapy	12.52	8.43	2.61	0.49	0.97	0.02	7.91	156	0.51	211
Patient 8	Before starting ASIT therapy	10.91	6.22	2.91	0.59	1.11	0.08	7.89	181	0.49	189
	Between the second and third month of starting ASIT therapy	11.04	6.82	3.11	0.39	0.71	0.01	7.11	178	0.45	229
	Between 5 and 7 months from the beginning of ASIT therapy	11.02	6.89	3.15	0.61	0.31	0.06	6.81	155	0.41	218
Patient 9	Before starting ASIT therapy	9.82	5.89	1.98	0.91	1.04	0	7.22	132	0.46	217
	Between the second and third month of starting ASIT therapy	9.42	5.98	2.09	0.19	1.12	0.04	8.1	182	0.56	292
	Between 5 and 7 months from the beginning of ASIT therapy	9.55	6.12	2.22	0.22	0.91	0.08	7.9	186	0.52	227
Patient 10	Before starting ASIT therapy	15.29	10.72	3.12	0.89	0.55	0.01	7.2	137	0.48	217
	Between the second and third month of starting ASIT therapy	12.25	6.98	3.29	1.31	0.65	0.02	7.6	135	0.51	302
	Between 5 and 7 months from the beginning of ASIT therapy	10.25	5.71	3.19	1.22	0.12	0.01	8.1	145	0.54	145
Patient 11	Before starting ASIT therapy	10.99	6.22	2.78	1.01	0.91	0.07	5.9	113	0.39	121
	Between the second and third month of starting ASIT therapy	9.11	5.44	2.19	0.59	0.88	0.01	6.21	127	0.41	138
	Between 5 and 7 months from the beginning of ASIT therapy	8.62	5.19	2.21	0.81	0.39	0.02	5.82	123	0.39	129
Patient 12	Before starting ASIT therapy	12.24	7.98	1.91	0.91	1.43	0.01	7.29	139	0.49	301
	Between the second and third month of starting ASIT therapy	12.03	8.23	1.32	1.25	1.21	0.02	8.11	171	0.56	276
	Between 5 and 7 months from the beginning of ASIT therapy	14.12	11.01	1.07	1.12	0.89	0.03	6.82	176	0.44	171

Table 2: Evaluation of biochemical profile in all patients

PATIENT	Biochemical profile	GLU (mmol/L) 3.3-6.9	UREA (mmol/L) 2.5-10.0	CREAT (45.0-155.0	ALAT (U/L) <=90.0	ALP (U/L) <=230.0	GGT (U/L) <=6.4	ALB (g/L) 26.0-43.0	TP (g/L) 50.0-78.0	BIL T (µmol/L) <=7.0	Ca (mmol/L) 1.87-3.00	P (mmol/L) 0.50-2.60
Patient 1	Before starting ASIT therapy	4.5	3.1	132.1	76.1	220.2	4.5	29.8	56.1	6.1	1.94	0.57
	Between the second and third month of starting ASIT therapy	4.9	3.9	141.4	88	201.4	5.3	27.4	50.3	5.5	2.01	0.62
	Between 5 and 7 months from the beginning of ASIT therapy	5.5	5.7	144.1	81	145.5	4.9	30.1	52.3	5.1	2.11	0.73
Patient 2	Before starting ASIT therapy	3.1	2.2	56.2	117	350.2	6.9	25.1	51.9	7.2	3	0.98
	Between the second and third month of starting ASIT therapy	3.8	3.2	87.1	100.3	311.1	3.7	29.9	57.3	4.9	2.21	0.78
	Between 5 and 7 months from the beginning of ASIT therapy	5.1	7.1	130.1	81.1	211.1	3.9	36.1	69.1	4.5	2.27	0.79
Patient 3	Before starting ASIT therapy	4.5	2.1	78.8	145.8	298.1	6.1	33.4	62.2	6.6	2.21	0.77
	Between the second and third month of starting ASIT therapy	3.9	3.2	89.3	131.1	228.4	5.8	38.2	64.2	6.4	2.41	0.83
	Between 5 and 7 months from the beginning of ASIT therapy	4.8	3.9	86.5	87.2	184.1	5.3	39.2	65.3	6.1	1.98	0.59
Patient 4	Before starting ASIT therapy	3.9	2.7	90.2	161	289.3	6.1	30.1	51.1	4.2	2.41	0.87
	Between the second and third month of starting ASIT therapy	4.9	2.9	79.2	129.1	210.1	5.8	34.1	58.6	4.3	2.23	0.76
	Between 5 and 7 months from the beginning of ASIT therapy	4.6	3.9	78.3	76.6	175.4	5.1	39.1	59.3	3.6	2.41	0.81
Patient 5	Before starting ASIT therapy	3.2	7.1	91.5	67.2	292.5	5.1	29.9	55.2	2.2	1.91	0.51
	Between the second and third month of starting ASIT therapy	2.9	5.3	104.5	54.4	270.3	3.3	39.7	59.2	2.9	2.49	1.41
	Between 5 and 7 months from the beginning of ASIT therapy	4.1	7.2	111.4	76.6	195.3	3.9	44.7	71.3	1.7	2.81	1.41
Patient 6	Before starting ASIT therapy	4.5	6.1	147.1	89.1	283.2	2.9	31	57	4.1	2.21	1.12
	Between the second and third month of starting ASIT therapy	3.9	5.9	101.6	81.6	262.3	3.9	39	61	2.9	1.91	0.89
	Between 5 and 7 months from the beginning of ASIT therapy	3.6	7.9	105.4	71.4	176.6	3.2	46	74	3.7	2.81	1.01
Patient 7	Before starting ASIT therapy	5.6	6.9	126.1	67.2	89.5	1.9	38.1	61.5	4.9	2.29	0.76
	Between the second and third month of starting ASIT therapy	4.9	8.1	111.9	37.3	121.1	4.3	41.2	71.2	3.2	1.99	0.83
	Between 5 and 7 months from the beginning of ASIT therapy	6.1	7.1	131.6	45.3	105.1	2.8	37.2	60.1	3.8	2.56	1.07
Patient 8	Before starting ASIT therapy	3.9	6.1	131.1	61.1	89.3	5.3	26.4	51.2	3.3	1.99	0.63
	Between the second and third month of starting ASIT therapy	6.1	7.8	147.3	59.3	110.3	4.9	40.1	70.2	3.9	1.88	0.62
	Between 5 and 7 months from the beginning of ASIT therapy	3.8	5.3	98.3	70.4	107.1	4.1	41.3	73.1	3.1	2.05	0.79
Patient 9	Before starting ASIT therapy	6.1	5.3	79.6	74.4	90.9	3.9	39.2	67.7	6.1	2.22	0.78
	Between the second and third month of starting ASIT therapy	3.9	7.1	121.4	81.2	79.2	4.8	40.2	71.1	5.2	1.91	0.61
	Between 5 and 7 months from the beginning of ASIT therapy	4.5	3.9	65.9	68.2	101.1	3.1	33.1	59.6	4.5	2.91	1.49
Patient 10	Before starting ASIT therapy	5.1	5.4	121.8	49.2	59.5	6.1	39.1	70.2	4.9	2.12	0.75
	Between the second and third month of starting ASIT therapy	3.9	4.8	141.4	47.6	68.5	4.2	37.2	69.1	6.1	2.22	1.29
	Between 5 and 7 months from the beginning of ASIT therapy	4.1	6.1	105.1	59.3	43.9	5.9	39.9	71.2	3.2	2.06	0.71
Patient 11	Before starting ASIT therapy	5.2	5.8	121.2	78.2	129.1	4.1	40.1	71.2	5.5	2.11	0.76
	Between the second and third month of starting ASIT therapy	2.9	5.1	136.2	67.2	139.1	4.4	39.8	73.7	5.1	1.87	0.64
	Between 5 and 7 months from the beginning of ASIT therapy	3.1	4.7	118.1	65.9	100.3	2.9	38.9	63.9	3.2	1.92	0.63
Patient 12	Before starting ASIT therapy	3.2	8.1	101.9	71.2	89.2	3.8	36.1	60.2	3.9	2.91	1.06
	Between the second and third month of starting ASIT therapy	3.9	9.1	76.9	69.1	99.2	3.9	40.8	64.2	3.2	2.11	0.71
	Between 5 and 7 months from the beginning of ASIT therapy	4.1	7.6	87.3	73.1	71.1	2.7	39.8	65.9	3.7	1.96	0.69

Discussion

Analyzing the dynamics of CBC and basic biochemical indicators in all 12 dogs proves that ASIT does not deleteriously affect them. We would like to suggest that the protocol for the management of atopic dermatitis should always include serological tests, if possible, also intradermal tests, in order to create and perform allergen–specific immunotherapy. Thus, the goal of treating the animal is achieved in one of the best possible ways avoiding negative effects.

The current study and literature data confirm that desensitizing immunotherapy (ASIT – allergen–specific immunotherapy) is harmless to the patient and without negative side effects. By stimulating Regulatory T–cells immune response is modulated and the animal gradually becomes less sensitive to the allergens in a completely natural way. Reduction of IgE is part of the immune response modulation process. Desensitization can occur with prolonged exposure to gradually increasing doses of the allergen. Identifying the specific allergens is crucial for managing the animals, its environment and therapy. (Angarano et MacDonald 1991, DeBoer *et al.* 2017, Miller *et al.* 2012). Combining ASIT with other nonsteroidal forms of therapy allows most patients to be controlled without long–term systemic glucocorticoids.

Conclusion and recommendations

In conclusion, we would like to share our recommendation about owner education. In regards to the treatment of such complex conditions as atopic dermatitis it is crucial that there is good communication with the owners. They should comprehend every step of the process and learn to observe their pets. During our study most owners shared that ASIT has been a "life–saving" tool.

ASIT is a reliable, safe and modern approach to the long–term management of patients with atopic dermatitis. It should be considered as the better alternative to depot injectable and oral corticosteroids whenever possible.

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