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Investigation of the effects of oclacitinib maleate on clinical improvement and serum cytokine levels in dogs with atopic dermatitis

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Summary

The aim of this study was to present allergens involved in the etiology of atopic dermatitis (AD) in dogs, to determine and classify the clinical severity of the disease using the CADESI-4 and pruritus scores, to investigate changes in serum cytokine levels in dogs with AD and their relationship with clinical findings, and to investigate the effects of oclacitinib maleate on clinical improvement and serum cytokine levels. The material of the study consisted of 20 dogs diagnosed with AD and 10 healthy dogs. CADESI and pVAS scores were obtained from the patients, and blood samples were taken. Hematological analyses, specific allergen tests (polycheck), and ELISA analyses were performed. Oclacitinib maleate (Apoquel, Zoetis) was administered orally to the patient dogs at a dose of 0.5 mg/kg twice a day for 7 days. It was found that the most common allergens involved in the etiology of AD in dogs were house dust mites type 1, house dust mites type 2, and mold fungi, and the mean CADESI and pVAS scores for clinical findings such as pruritus, alopecia, erythema, and lichenification in dogs with AD were 48 and 5.15 respectively. When compared to the control group, an increase in the percentage of eosinophils ($p < 0.05$) and a decrease in serum IL-2 and IL-4 levels ($p < 0.05$) were detected. When compared to other groups, it was found that the mean serum IL-4 levels ($p < 0.05$) were low and IL-13 levels ($p < 0.05$) were high in the group with severe clinical findings. It was determined that the use of oclacitinib maleate resulted in clinical improvement and caused an increase in the mean serum IL-2 levels ($p < 0.05$) and a decrease in IL-31 and IL-33 levels ($p < 0.05$). Oclacitinib maleate (Apoquel, Zoetis) was found to be an effective and reliable drug that can be used in the treatment of the disease, but it was concluded that the duration of use should be extended.

Keywords: atopic dermatitis, cytokines, dog, oclacitinib maleate

Canine atopic dermatitis (CAD) is a chronic inflammatory skin disease that typically occurs as a result of hypersensitivity reactions in response to environmental allergens and an abnormal skin barrier (3, 6, 7, 13, 19, 46, 55).

Hypersensitization to environmental allergens and individual susceptibility are considered predisposing factors for atopic dermatitis (AD). Mites found in houses and shelters (*Acarus siro*, *Tyrophagus putresceantiae*, *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus*), pollens (*Senecio vulgaris* and grass pollen), molds, and feathers are environmental allergens that are frequently involved in the etiology of the disease (24, 30, 59, 66, 67).

Although the pathogenesis of AD in dogs has not yet been completely understood, typical reports in the relevant literature suggest that AD occurs as a result of type I hypersensitivity reaction to allergens entering the body via inhalation and increased immunoglobulin E (IgE) production (13, 25, 38). Epidermal antigen-presenting cells trigger the production of specific IgE against allergens, which then migrate to the dermis and regional lymph nodes. Allergen-specific IgE molecules bind to tissue mast cells or basophils. As a result, immune cell-derived inflammatory mediators activate keratinocytes and induce chemokine and cytokines release (19, 36, 46, 55, 59). The released cytokines activate Janus kinase (JAK) proteins, which in turn activate Janus kinase/signal transducers and activators of transcription proteins. Consequently, the impaired epidermal barrier induces chronic and recurrent inflammation characterized by epidermal hyperplasia and

increased cell infiltration (T cells, dendritic cells, and eosinophils), leading to clinical manifestation of AD (10, 12, 13, 59).

AD in dogs is characterized by pruritus with concomitant primary (erythema, papules, and pustules) and secondary skin lesions (epidermal collarettes, crusting, peeling, alopecia, hyperpigmentation, and lichenification). Pruritus and lesions commonly occur on the head, neck, chest, genital areas, and extremities (6, 66).

Clinical findings and an interpretation thereof according to Favrot's criteria and allergy tests (to determine the allergen-specific IgE levels) are used in the diagnosis of AD in dogs (30, 67). Canine Atopic Dermatitis Extent and Severity Index (CADESI-4) and Pruritus Visual Analog Scale (pVAS) are used to determine the clinical severity of the disease.

Canine AD is treated with topical glucocorticoids (triamcinolone acetonide 0.015%, hydrocortisone acetonate, betamethasone valerate etc.), oral glucocorticoids (prednisone or prednisolone), calcineurin inhibitors (cyclosporine), antihistamines (hydroxyzine, cetirizine, etc.), JAK inhibitors (oclacitinib maleate), prostaglandin E1 analogs (misoprostol), essential fatty acids, and immunotherapy (interferon alpha, gamma, and omega) (24, 25, 36, 38, 55).

Oclacitinib maleate, a JAK inhibitor, is used in the treatment of allergic inflammation and pruritus in dogs > 12 months of age (55, 67). It produces effects within a short period of time by selective inhibition of JAK-1-dependent cytokines, especially interleukin (IL)-31, and is considered more effective and safer for long-term use than glucocorticoids or cyclosporins (13, 24, 55, 67).

This study aimed to investigate allergens involved in the etiology of canine AD, to determine and classify the clinical severity of the disease based on CADESI-4 and pVAS scores, to investigate changes in serum cytokine levels in dogs with AD and the relationship of these changes with the severity of clinical findings, and to discern the effects of oclacitinib maleate on clinical improvement and serum cytokine levels.

Material and methods

Ethics statement. Dicle University Animal Experiments Local Ethics Committee decided that the local ethics committee approval is not required with a letter dated 10/06/2021 and numbered 85038.

The animals used in the study included 20 dogs (1-3 years of age) of different breeds and sexes that presented to Dicle University Veterinary Faculty Animal Hospital with recurrent chronic dermatologic problems, were diagnosed with AD according to Favrot's criteria, and had not undergone treatment during the previous 1 month. Ten healthy dogs of different breeds and both sexes between 1 and 3 years of age with no superficial or deep pyoderma, systemic infections (fever, nasal and ocular discharges, vomiting, diarrhea, etc.), or clinical signs of dermatologic (alopecia, dandruff, erythema, lichenification, etc.) or systemic diseases (fever, nasal and ocular discharge, vomiting, diarrhea, etc.) upon clinical examination were also included. The dog owners were provided with information about the study, and they signed informed consent forms. All animals received the same diet throughout the study.

Clinical examination. Routine physical and dermatologic examinations were performed subsequent to taking anamnesis. AD diagnosis was made on the bases of Favrot's criteria. CADESI-4 and pVAS were used to score the clinical severity of AD in the dogs included in the study. CADESI-4 and pVAS reviews were repeated on Days 7 and 14. The dogs with AD were categorized into three groups based on the severity of clinical findings. Those with a CADESI-4 score of 60-100 were classified as severe (n = 6), those with a score of 40-60 (n = 7) as moderate, and those with a score of < 40 (n = 7) as mild.

Collection of samples. Blood samples were collected via vena cephalica antebrachii into anticoagulated and nonanticoagulated tubes during routine clinical examinations of the dogs included in the control group and before and after treatment on Days 7 and 14 from the dogs with AD.

Blood analyses. Blood samples collected into anticoagulant tubes were hematologically analyzed without delay with a Mindray BC-2800Vet Auto Hematology Analyzer (Hasvet-Turkey). The samples in tubes without anticoagulant were allowed to clot at room temperature and centrifuged at 3000 rpm for 10 min, and the resultant sera were tested for serum-specific allergens. Furthermore, the sera were stored at –20°C for the measurement of cytokine levels until enzyme-linked immunosorbent assay (ELISA) was performed.

Parasitological and microbiological analyses. Skin scraping and sterile swabs were used to collect samples from the lesions of the dogs with suspected AD. Scraping samples were studied in potassium hydroxide under a microscope to detect *Demodex* spp. and *Sarcoptes scabiei*.

Each swab sample intended for microbiological analysis was incubated according to the required conditions. Twenty dogs with AD without bacteriologic and mycologic growth upon incubation were included in the study (2).

Allergen detection using serum-specific IgE. As a result of hematological, parasitological, and microbiological analyses, the pretreatment sera from 20 dogs diagnosed with AD were examined using a Polycheck® Canis (Münster, Germany) allergy diagnostic kit.

Hill's Atopy Index. Pruritus scores in dogs were measured with CADESI-4 using Hill's Atopy Index in the dogs with AD. This procedure was repeated on Days 7 and 14. The results were tabulated and recorded.

Measurement of serum mean cytokine levels. Serum IL-2, IL-4, IL-6, IL-13, IL-31, IL-33, CD4, and CD8 levels were measured in blood samples by the ELISA method. ELISA assays were performed as per instructions (SunRed Biotechnology) specified in the test kit manufacturer's procedure, and the results were tabulated and statistically analyzed thereafter.

Statistical assessment. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 24.0 (SPSS Inc., Chicago, USA) software. Analysis of variance was used to compare the pretreatment and

Tab. 1. Hematological examination findings in patient and control dogs

	Patient Day 0 (n = 20) Mean ± standard deviation	Patient Day 7 (n = 20) Mean ± standard deviation	Patient Day 14 (n = 20) Mean ± standard deviation	Control Grup (n = 10) Mean ± standard deviation	P
Eos (%)	9.64 ± 7.72[a]	6.48 ± 4.18[a]	7.65 ± 3.88[a]	1.92 ± 0.86[b]	0.000*

Explanations: *p < 0.001; a, b – the difference between superscripts in the same line was found to be significant (p < 0.000)

post-treatment hematology data of the dogs with AD and the control group, and the Kruskal-Wallis test was used to compare mean serum cytokine levels, CADESI-4, and pVAS scores between the days. Furthermore, the Mann-Whitney U test was used to compare pretreatment data with those for the control group. Mean (minimum–maximum) values of the related parameters were tabulated, and a p value of < 0.05 was considered statistically significant.

Results and discussion

Clinical examination. Redness, alopecia, and lichenification were seen along with itching during the clinical examinations of 20 dogs that presented to Dicle University Faculty of Veterinary Medicine Animal Hospital with complaints of skin lesions and itching.

Hematologic examination. The percentage of eosinophils was significantly (p < 0.05) higher in the dogs with AD, but changes during the treatment period were not significant (p > 0.05) (Tab. 1).

Allergen testing with serum-specific IgE. Upon the testing of serum samples from the dogs with AD for allergens based on the concentration of serum-specific IgE, all 20 dogs were found allergic to house mite type 1 and type 2, 95% were allergic to mold fungi (*Malassezia*) and fleas, 90% to plants and flour mites, 65% to pollen of hornbeam family trees, 60% to food parasites (*Lepidoglyphus*), 55% to rye pollen, 45% to *Cladosporium* mold and pollen of lawn/grass species, 40% to nettle pollen and sorrel pollen, 35% to *Aspergillus/Penicillium* and had hay fever, 25% to pollen of willow family trees, 20% to album grass pollen, banana family plant pollen, and wormwood pollen, and 15% to woodruff pollen.

CADESI-4 and pVAS results. CADESI-4 and pVAS values decreased significantly during the treatment period. The decrease between the start of treatment and Day 7 was significant (p < 0.05), but the change between Days 7 and 14 was not significant (p > 0.05) (Fig. 1).

Serum mean cytokine levels. Upon comparison of mean serum cytokine levels between the dogs with AD and the control group, there were statistically significant (p < 0.05) differences in the mean value of IL-2 on Days 0, 7, and 14, IL-4 on Days 0 and 7, IL-6 and CD8 on Days 7 and 14, and IL-13, IL-33, and CD4 on Day 7 (Fig. 2).

There were intergroup differences upon the review of serum cytokine levels of the dogs with AD in the mild, moderate, and severe AD groups based on CADESI-4 scores.

Comparison of the pretreatment serum cytokine levels of the mild, moderate, and severe AD groups revealed that the elevation in serum IL-4 and the decrease in IL-13 in the severe AD group were significant compared with those in the moderate and mild groups (Fig. 3).

CAD is a prevalent chronic inflammatory skin disease associated with abnormal skin barrier function,

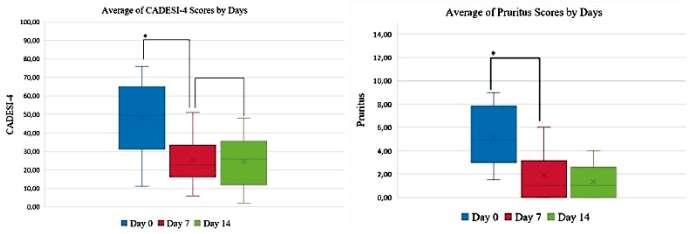

Fig. 1. Averages of CADESI-4 and pruritus scores by days
Explanation: * p < 0.001

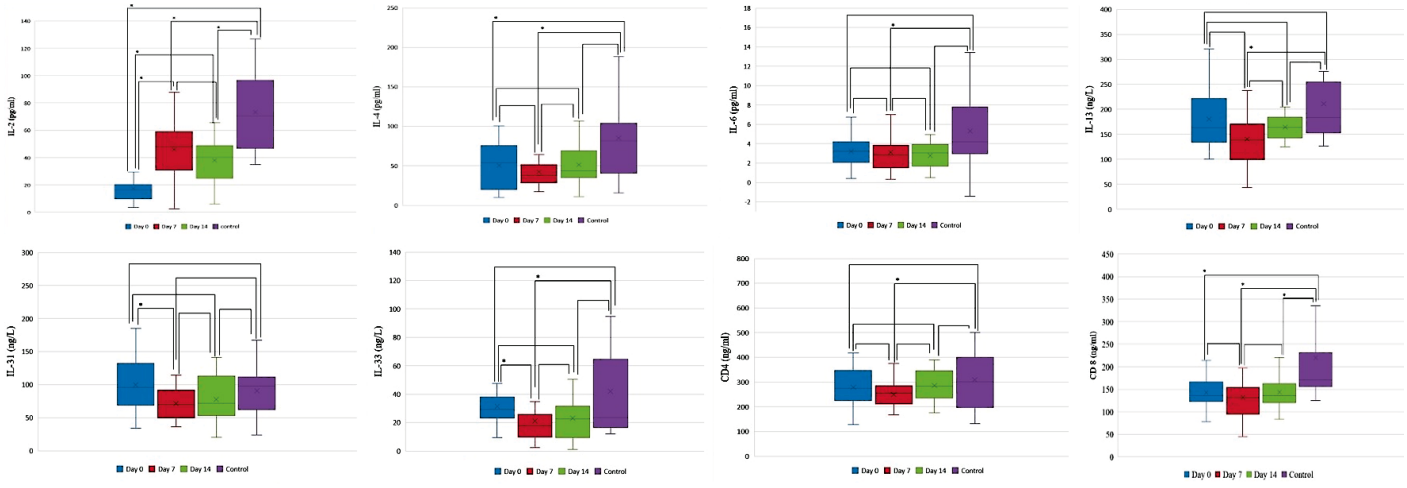

Fig. 2. Averages of serum IL-2, IL-4, IL-6, IL-13, IL-31, IL-33, CD4 and CD8 levels
Explanation: * p < 0.05

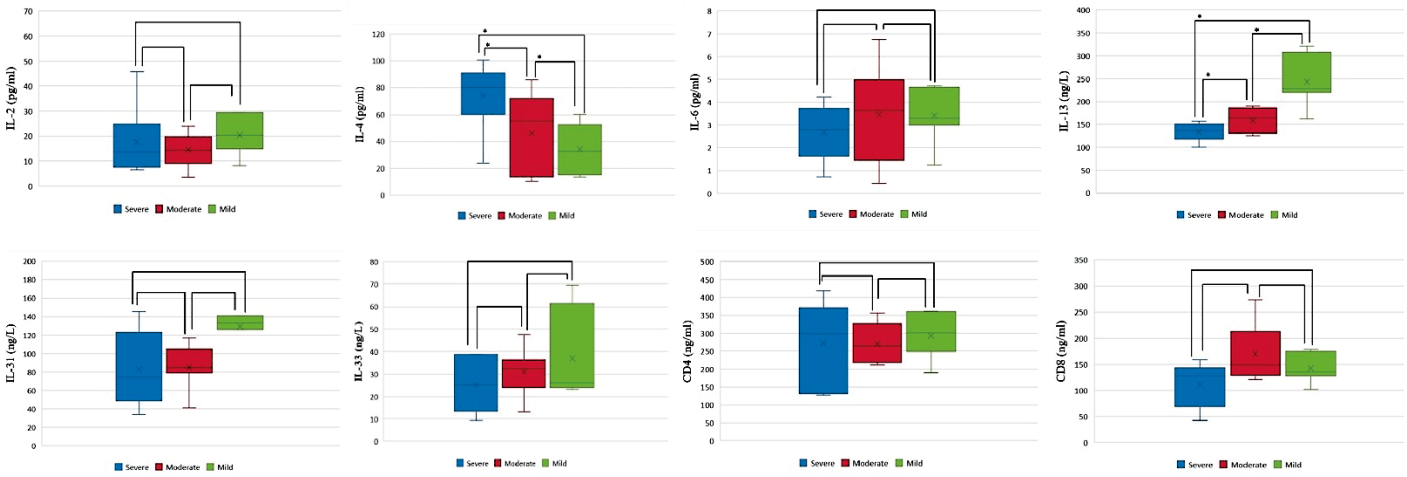

Fig. 3. Averages of serum IL-2, IL-4, IL-6, IL-13, IL-31, IL-33, CD4 and CD8 levels
Explanation: * $p < 0.05$

cutaneous inflammation, secondary *Staphylococcus* and *Malassezia* skin and ear infections, and hypersensitivity to a range of allergens (environmental allergens, food allergens, and *Staphylococcus* and *Malassezia* allergens) (47). The severity of pruritus in skin allergic reactions is important with regard to the occurrence of primary skin lesions (erythema, papule, and pustule) and secondary skin lesions (epidermal collarettes, crusting, peeling, alopecia, hyperpigmentation, and lichenification) during the course of the disease (13, 19).

Mites, lice, and flea infestations and allergens, such as lawn and grass pollen, have been reported to play a significant role in the etiology of AD (6, 19, 30, 41, 66, 67). Various tests (allergen testing with serum-specific IgE and intradermal skin testing) can identify causative allergens involved in the etiology of the disease.

Previous studies on canines with AD (6, 40, 43, 44) have reported that the causative allergens associated with the etiology of the disease include house dust mite 1, house dust mite 2 (6, 40, 43, 44), flea mite (40), food mite (6, 44), flour mite, plant mite, rye pollen (6), grass pollen (6, 43), and banana pollen (43). Upon allergen testing with serum-specific IgE, the present study identified the most prevalent allergens to be house mites type 1, house mites type 2, flea mites, plant and flour mites, pollen of hornbeam family trees, and food parasites (*Lepidoglyphus*). The results of this study agree with previous reports (6, 40, 43, 44), and similar allergens were identified although different testing methods were used across the studies.

Previous studies (15, 18, 20, 23, 29, 31, 45, 53, 61) have observed that primary and secondary skin lesions might occur concomitantly with pruritus in dogs with AD. In the present study, clinical examination results for the dogs with AD were indicative of pruritus with concomitant redness, alopecia, and lichenification.

CADESI-4 and pVAS were used to score the clinical severity in the dogs with AD (9, 31, 32, 49, 50, 60, 64). In animals with AD, CADESI-4 scores have been reported to range from 35.1 to 57.3 (9, 31, 38, 49, 50, 54, 60, 67), while pVAS scores vary from 5.5 to 8 (7, 9, 11, 12, 31, 32, 41, 60, 64). In the present study, the mean CADESI and pVAS scores were 48 and 5.15, respectively, and these results were consistent with previous reports (7, 9, 11, 12, 31, 32, 50, 60, 64).

CADESI-4 and pVAS scores were used to investigate the effects of drugs on clinical improvement in studies involving various drugs used for the treatment of dogs with AD (7, 18, 50, 56, 61). Olivry et al. (49) investigated the effects of misoprostol on clinical improvement and reported that CADESI-4 and pVAS scores decreased by 61% and 50%, respectively, which was statistically significant ($p < 0.05$). Bensignor et al. (7) investigated the effects of different doses (1.28 mg/kg and 1.34 mg/kg) of lokivetmab, which was indicated for pruritus and skin lesions in dogs with AD, and reported that the CADESI-4 and pVAS scores decreased after the treatment, but not in a statistically significant manner.

Saridomichelakis et al. (56) investigated the long-term effect of oral prednisolone in dogs diagnosed with AD and administered the animals with prednisolone (0.5-1.0 mg/kg QD) for 12 weeks. At the end of the 12-week treatment, they reported that the mean CADESI-4 scores decreased from 38 to 12, and the mean pVAS scores from 6.2 to 2.7.

Tamamoto-Mochizuki et al. (61) investigated the effect of lokivetmab on pruritus and skin lesions in dogs diagnosed with AD and found that lokivetmab prevented pruritus in the first 24 h, but failed to inhibit the occurrence of skin lesions.

Lee et al. (31) examined the effects of oclacitinib and lokivetmab on CADESI-4 and pVAS scores in dogs with AD and, accordingly, administered oclacitinib at a dose of 0.4 mg/kg BID for 2 weeks and 0.4 mg/kg QD for the remaining 6 weeks and lokivetmab at a dose of 2 mg/kg for 8 weeks. They reported that there was no significant difference between the oclacitinib

and lokivetmab groups in terms of pVAS scores at the beginning of the study (Day 0). Nevertheless, a statistically significant ($p < 0.05$) decrease in pVAS scores was recorded in dogs treated with both oclacitinib and lokivetmab at weeks 4 and 8 compared with scores before the treatment, and the decrease in the CADESI-4 score was higher in the oclacitinib group than that in the lokivetmab group.

Little et al. (32) investigated the effects of oclacitinib and cyclosporine on CADESI-4 scores in dogs with AD and observed a 65.4% decrease in the cyclosporine group and a 66.9% decrease in the oclacitinib group.

Takahashi et al. (60) introduced a combined treatment protocol with oclacitinib maleate and hydrocortisone aceponate spray (oclacitinib maleate in all patients and topical hydrocortisone aceponate spray QD for the first week in addition to oclacitinib maleate in nine patients) for 28 days in dogs with AD and reported a significant decrease in CADESI-4 and pVAS scores in both groups on Days 7 and 14.

Olivry et al. (50) reported that treatment with oclacitinib (0.5 mg/kg BID for the first 14 days, followed by 0.5 mg/kg QD for 14 days) resulted in a significant decrease in CADESI-4 and pVAS scores in dogs with AD, and the decrease was greater in dogs that received additional doses of prednisolone (0.5-1.0 mg/kg BID for the first 4 days).

De Caro Martins et al. (9) reported an 86.7% decrease in the CADESI-4 score in a study in which oclacitinib was administered to dogs with AD for 12 months at a dose of 0.4-0.6 mg/kg.

Cosgrove et al. (12) investigated the effects of oclacitinib on pruritus and lesions in dogs with AD and reported a statistically significant ($p < 0.05$) decrease in pVAS scores of the group treated with oclacitinib at a dose of 0.4-0.6 mg/kg BID for 7 days compared with the placebo group.

In the present dissertation study, the dogs with AD were orally administered oclacitinib maleate at a dose of 0.5 mg/kg BID. CADESI-4 and pVAS scores were calculated before and after the treatment. There was a 49.06% decrease in the CADESI-4 score and a 73.39% decrease in the pVAS score, and the decrease was statistically significant ($p < 0.05$). A comparison of the treatment efficacy of oclacitinib maleate with that of other drugs (prostaglandin analogs, lokivetmab, prednisolone, and cyclosporine), as reported by previous studies (7, 9, 12, 18, 31, 32, 50, 56, 60, 61), suggested that its effects on clinical improvement were similar. Moreover, there were no drug-related allergic reactions, vomiting, diarrhea, or similar side effects.

Some previous studies (11, 45) have reported the absence of a significant difference between dogs with AD and healthy dogs in terms of hematologic parameters. However, Martins et al. (39) reported that neutrophil and platelet values were higher ($p < 0.05$) and lymphocyte values were lower ($p < 0.05$) than they were in the control group. Gadeyne et al. (17) reported that oclacitinib treatment was not associated with changes in hematologic parameters, whereas Little et al. (32) suggested a decrease in white blood cell (WBC), neutrophil, eosinophil, and monocyte levels. Cosgrove et al. (11) reported transient reductions in leukocyte, neutrophil, eosinophil, monocyte, and platelet levels in some animals individually, and Denti et al. (14) noted a significant decrease ($p < 0.05$) in leukocyte, neutrophil, eosinophil, and monocyte levels.

The present study compared hematologic parameters between dogs with AD and healthy dogs, and eosinophil percentages were found to be higher in the dogs with AD on Day 0 compared with the control group. However, there were no significant changes in WBCs, lymphocytes, monocytes, granulocytes, lymphocyte percentage, monocyte percentage, granulocyte percentage, red blood cells, hematocrit, hemoglobin, mean cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelets, mean platelet volume, plateletcrit, or platelet distribution width. In this study, the increase in the eosinophil levels was similar to the results of Martins et al. (39). The present study found that the use of oclacitinib did not induce significant changes in leukocyte, neutrophil, eosinophil, monocyte, or platelet values, which differed from the results reported by Cosgrove et al. (11) and Denti et al. (14). The dose regimen and duration of use might account for the aforementioned difference.

It is well established that IL-2, a protein released by helper T cells, is associated with changes in serum levels (22, 58) in inflammatory reactions, infectious diseases, autoimmune diseases, and different types of cancer (59). Calvalido et al. (18) reported lower serum IL-2 levels in dogs with lymphoma compared with the control group, albeit not significantly so ($p > 0.05$). Singh et al. (58) observed higher serum IL-2 levels in dogs with scabies compared with the controls, but the difference was no statistically significant either ($p > 0.05$). Mazrier et al. (42) recorded higher serum IL-2 levels in dogs with AD compared with healthy dogs, and Majewska et al. (34) reported a decrease that was not statistically significant ($p > 0.05$). In the present study, upon comparison between the dogs with AD and the healthy group based on mean serum IL-2 levels, there was a significant decrease ($p < 0.05$). A comparison of IL-2 levels based on the clinical severity of the disease did not reveal any statistically significant ($p > 0.05$) intergroup difference. A comparison of the results for the dogs with AD with those reported by previous studies suggested a parallelism with findings of Majewska et al. (34), but the decrease was not consistent with the reports of Mazrier et al. (42). The decrease in IL-2 levels in this study might be related to the inverse correlation between IgE and IL-2 (68).

In studies involving different drugs administered to dogs with AD, Farrell et al. (16) suggested that cyclosporine treatment in dogs with AD was associated

with a 30% decrease in IL-2 levels compared with the pretreatment levels. Banovic et al. (5) administered cyclosporine to one group and oclacitinib to another group of dogs with AD and compared the pretreatment and post-treatment IL-2 levels, reporting that IL-2 levels decreased in both groups; however, the decrease in the oclacitinib group was greater. In the present study, there was a statistically significant increase in IL-2 levels compared with the pretreatment levels for oclacitinib, which was consistent with findings of other researchers (5, 16). IL-2 has been reported to be secreted by activated CD4 cells (34). In the present study, the increase in serum IL-2 levels might have been related to the increase in CD4 levels.

IL-4, a cytokine antagonizing immune responses and supporting tissue repair mechanisms, is also known to play a critical role in IgE and eosinophil-mediated inflammatory reactions (14). Quinnell et al. (52) and Singh et al. (58) reported significantly higher ($p < 0.05$) serum IL-4 levels, compared with those for healthy animals, in dogs with leishmaniasis and in dogs with scabies, respectively. Shida et al. (57) reported that serum IL-4 levels in dogs with AD were lower than they were in healthy dogs, whereas Majewska et al. (35) noted no significant difference between dogs with AD and healthy dogs. In the present study, the mean serum IL-4 level in the dogs with AD was lower ($p < 0.05$) than that in the control group. These results agree with findings of other researchers (5). Furthermore, mean serum IL-4 levels in the severe group were higher than those in the other patient groups, but lower than that in the control group ($p < 0.05$) in animals classified according to CADESI-4 findings.

In studies on different drugs administered to dogs with AD, Farrell et al. (16) and Mikolajczyk et al. (26) reported that cyclosporine and oclacitinib, respectively, were associated with a decrease in IL-4 levels. Similar to results reported by other investigators (16, 68), oclacitinib induced a decrease in IL-4 levels in the dogs with AD in the present study.

IL-6 is released by activated T cells and macrophages when the immune system is suppressed (4). Calvalido et al. (8) and Zygner et al. (69) observed that IL-6 levels in dogs with lymphoma and in those with babesias, respectively, were statistically significantly ($p < 0.05$) higher compared with the levels in healthy dogs. Previous studies (33, 42) found a lack of significant change in serum IL-6 levels in dogs with AD. Similarly, in the present study, there was no significant difference between the dogs with AD and the control group and between the patient groups ($p > 0.05$).

In studies on different drugs administered to dogs with AD for therapeutic purposes, Banovic et al. (5) compared pretreatment and post-treatment IL-6 levels upon administration of cyclosporine in one group and oclacitinib in another group of dogs with AD. The researchers reported that there was a decrease in IL-6 levels in both groups compared with the pretreatment levels, but the decrease in the oclacitinib group was higher. Similar to the results reported by Banovic et al. (5), the IL-6 levels decreased upon oclacitinib administration compared with the pretreatment levels in the present study, but there was no statistically significant difference.

IL-13 is an important cytokine released during the course of allergic inflammatory diseases, such as allergic asthma, eosinophilic esophagitis, and ulcerative colitis (37). Unlike Viljanen et al. (65), Majewska et al. (35) reported that serum IL-13 levels were lower in dogs with AD compared with healthy dogs. In the present study, serum IL-13 levels of dogs with AD were lower than those of healthy dogs, but not statistically significantly so ($p > 0.05$). This agrees with the results of Majewska et al. (35), but is contrary to those of Viljanen et al. (65). Furthermore, there were significant ($p < 0.05$) differences between the patient groups.

In studies on different drugs administered to dogs with AD for therapeutic purposes, Farrel et al. (16) and Kanwal et al. (28) reported that cyclosporine and oclacitinib, respectively, caused a decrease in IL-13 levels. In the present study, serum IL-13 levels of the dogs with AD decreased upon oclacitinib administration, but the decrease was not statistically significant.

Loewinger et al. (33) reported that IL-31 levels were lower in dogs with AD compared with healthy dogs, whereas Verde et al. (64) and Viljanen et al. (65) reported higher levels. In the present study, serum IL-31 levels were lower compared with those in healthy dogs, but this difference was not statistically significant ($p > 0.05$). This result agrees with the findings of Loewinger et al. (33) but contradicts those of Verde et al. (64) and Viljanen et al. (65). Furthermore, a statistical comparison of mean serum IL-31 levels in the patient groups revealed no significant changes ($p > 0.05$).

Kanwal et al. (28) reported that the administration of oclacitinib to dogs with AD was associated with a decrease in serum IL-31 levels. Consistent with the results of the above study (28), IL-31 levels in dogs with AD decreased upon oclacitinib administration in the present study, but the decrease was not statistically significant ($p > 0.05$).

IL-33 is a key cytokine released when the immune system is affected or stressed (59). Sun et al. (59) reported that the increase in serum IL-33 levels in humans with gastric cancer was significant ($p < 0.05$) compared with healthy individuals. Older et al. (48) observed that the increase in serum IL-33 levels in cats with asthma was significant ($p < 0.05$) compared with healthy cats, whereas Asahina et al. (1) reported that the levels in dogs with AD were comparable to those of healthy animals. In the present study, serum IL-33 levels in the dogs with AD were lower compared with the healthy dogs, but this was not statistically significant ($p > 0.05$), which contradicts the results of Asahina et al. (1). Moreover, there were no significant

differences between the patient groups in terms of mean serum IL-33 levels ($p > 0.05$).

Gugliandolo et al. (21) reported that luteolin administration in dogs with AD was associated with a decrease in serum IL-33 levels. Similarly, IL-33 levels decreased from the pretreatment levels upon oclacitinib administration in the present study, but the decrease was not statistically significant ($p > 0.05$).

Laura et al. (36) found that the serum CD4 level was significantly ($p < 0.05$) lower and the serum CD8 level was significantly ($p < 0.05$) higher in dogs with leishmaniasis compared with healthy dogs. Majewska et al. (34) and Verde et al. (64) reported that serum CD4 and CD8 levels were lower in dogs with AD compared with healthy dogs, whereas Taszkun et al. (62) observed no significant difference. The results of the present study are consistent with those of Majewska et al. (34) and Verde et al. (64), but differ from those of Taszkun et al. (62). There were also no significant differences between the patient groups in terms of mean serum CD4 and CD8 levels ($p > 0.05$).

In studies on different drugs administered to dogs with AD for therapeutic purposes, Martins et al. (44) reported that oclacitinib increased the CD4 and CD8 levels in dogs with AD, which is contrary to results reported by Mikolajczyk et al. (27). Again, Farrell (16) reported that cyclosporine was associated with a decrease in CD4 and CD8 levels. In addition, Mikolajczyk et al. (26) observed that oclacitinib affected CD4 and CD8 levels in dogs with AD, but there was no decrease in terms of absolute numbers.

In the present study, CD4 levels decreased upon treatment with oclacitinib, but this decrease was not statistically significant ($p > 0.05$), and the increase in CD8 levels was not significant either ($p > 0.05$). The change in the mean serum CD4 level was consistent with the results of Mikolajczyk et al. (26), but contradicted those of de Caro Martins et al. (9).

In conclusion, based on the results, it could be concluded that oclacitinib maleate (Apoquel, Zoetis) is an effective and safe drug for the treatment of the disease; however, prolonged administration should be considered.

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