Kreuzreagierende Kohlenhydrat Bestandteile und deren Einfluss auf IgE-Serumallergietests bei atopischen Hunden

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Für meine Familie

INHALTSVERZEICHNIS

ABKÜRZUNGSVERZEICHNIS

AD	Atopische Dermatitis
AIT	Allergen-Immuntherapie
Anti-CCD-IgE	IgE Antikörper gegen kreuzreagierende Kohlenhydratbestandteile
bzw.	beziehungsweise
CCD	Cross-reactive carbohydrate determinants (kreuzreagierende Kohlenhydratbestandteile)
Der f	*Dermatophagoides farinae* (Hausstaubmilbe)
Der p	*Dermatophagoides pteronyssinus* (Hausstaubmilbe)
et al.	et alii (und andere)
IgA	Immunglobulin A
IgE	Immunglobulin E
IgG	Immunglobulin G
IKT	Intrakutantest
kg	Kilogramm
Lep d	*Lepidoglyphus destructor* (Heu-/Vorratsmilbe)
µg	Mikrogramm
mg	Milligramm
SAT	Serumallergietest
vs.	versus
z.B.	zum Beispiel

I. EINLEITUNG

Die canine atopische Dermatitis (AD) ist in der Kleintierpraxis eine häufige Hauterkrankung mit steigender Prävalenz (HILLIER und GRIFFIN, 2001). Die Pathogenese ist nicht vollständig geklärt und kein Testverfahren kann bislang zuverlässig zwischen einer AD und anderen Juckreiz verursachenden und entzündlichen Hautkrankheiten unterscheiden. Klinische Symptome können aufgrund von genetischen Faktoren (WILHEM et al., 2011; NUTTALL, 2013), Ausdehnung der Läsionen, Stadium der Allergie (akut/chronisch) und Sekundärinfektionen stark variieren, weshalb eine Verwechslung mit anderen Krankheiten nicht auszuschließen ist (HENSEL et al., 2015). Zwar existieren bestimmte Prädispositionsstellen und typische klinische Merkmale, welche auf eine zugrunde liegende Allergie hinweisen, jedoch gibt es kein pathognomonisches Symptom (DEBOER und HILLIER, 2001a; FAVROT et al., 2010). Die Diagnose einer Allergie basiert somit auf der Historie des Patienten, der klinischen Untersuchung, sowie dem Ausschluss anderer Differentialdiagnosen (DEBOER und HILLIER, 2001a). Grundsätzlich gibt es zwei Behandlungsansätze: einerseits symptomatisch, andererseits spezifisch mittels Allergen-Immuntherapie (AIT). Hierfür erfolgt die Auswahl der Allergene basierend auf einem Intrakutan- beziehungsweise (bzw.) Serumallergietest, dessen Ergebnisse mit der individuellen Geschichte und klinischen Symptomatik des Patienten korreliert werden (DEBOER und HILLIER, 2001a; HENSEL et al., 2015). Die Schwierigkeit hierbei ist, dass eine große Diskrepanz zwischen unterschiedlichen Testergebnissen (Intrakutan- versus (vs.) Serumallergietest) vorliegen kann (FOSTER et al., 2003). Darüber hinaus können bei Serumtests auf Allergen-spezifisches Immunglobulin E (IgE) die Identifikation der auslösenden Allergene durch die niedrige Spezifität (LIAN und HALLIWELL, 1998; DEBOER und HILLIER, 2001b; HENSEL et al., 2015), Inter- und Intralabor Variabilität (HNILICA, 2006) und in-vitro Kreuzreaktionen (SARIDOMICHELAKIS et al., 2008) erschwert werden. Somit werden möglicherweise irrelevante Allergene in den Allergenextrakt der AIT eingeschlossen. Des Weiteren gibt es einige Patienten, die eine sehr hohe Anzahl an positiven Testreaktionen aufweisen und dementsprechend nur schwer relevante Allergene bestimmt werden können.

In der Humanmedizin wurden Antikörper gegen kreuzreagierende Kohlenhydratbestandteile (Anti-CCD-IgE) als eine Ursache für irrelevant positive

bzw. falsch erhöhte in-vitro IgE-Testergebnisse in Relation zum tatsächlichen IgE-Spiegel festgestellt (ALTMANN, 2016; GRZYWNOWICZ et al., 2018). Kreuzreagierende Kohlenhydratbestandteile (CCD) sind Epitope an Glykoproteinen von Pflanzen und Insekten (ALTMANN, 2016). Die meisten Anti-CCD-IgE gegen CCDs in Pflanzen und Insekten scheinen keine bzw. eine sehr limitierte klinische Relevanz zu haben (VAN DER VEEN et al., 1997; MARI, 2002; EBO et al., 2004; MALANDAIN et al., 2007; MARI et al., 2008; HEMMER, 2012; HOLZWEBER et al., 2013), obwohl von Ausnahmen wie etwa Galaktose-α-1,3-galactose in rotem Fleisch und Glykan in Weizen berichtet wurde (COMMINS und PLATTS-MILLS, 2009; COMMINS et al., 2009; SONG et al., 2015). Eine mögliche Erklärung dafür, dass Anti-CCD-IgE keine klinischen Symptome auslösen, ist die monovalente Struktur der CCDs, welche eine Kreuzbindung verhindert und somit keine Degranulation von Mastzellen zur Folge hat (AALBERSE und VAN REE, 1997; FOETISCH und VIETHS, 2001; COMMINS und PLATTS-MILLS, 2009; SOH et al., 2015; ALTMANN, 2016). In der Veterinärmedizin wurde gezeigt, dass Anti-CCD-IgE im Serum von 24 % der untersuchten atopischen Hunden existierte (LEVY und DEBOER, 2018). Jedoch gibt es keine Erkenntnisse über deren Auswirkung auf Allergietestergebnisse.

Das Ziel dieser Arbeit ist es, die Ergebnisse eines Intrakutantests und eines Fc-ε-Rezeptor basierten Serumallergietests zu vergleichen. Darüber hinaus wird evaluiert, inwieweit sich die Hemmung von existierenden Anti-CCD-IgE Antikörpern vor Durchführung des Serumallergietests auf die Übereinstimmung zwischen Serum- und Intrakutantestergebnissen auswirkt. Auch wird der Einfluss von Anti-CCD-IgE auf die Anzahl von positiven Serumallergietestergebnissen in den einzelnen Allergenuntergruppen wie zum Beispiel (z.B.) Milben, Gräser und Bäume analysiert.

II. LITERATURÜBERSICHT

1. Atopische Dermatitis

Die canine AD ist eine sehr facettenreiche Hautkrankheit, deren klinischer Phänotyp von zahlreichen Faktoren wie z.B. Umgebung, auslösendes Allergen, genetische Abstammung und rassebedingte Unterschiede beeinflusst wird (OLIVRY et al., 2007; WILHEM et al., 2011). Akute Allergieschübe können unter anderem saisonabhängig auftreten und durch Sekundärinfektionen und eine geschwächte Hautbarriere begünstigt werden. Im folgenden Artikel wird auf die Pathogenese, die klinische Symptomatik, Diagnostik, Therapie und die jeweiligen Gemeinsamkeiten bzw. Unterschiede zwischen Hund, Katze und Mensch eingegangen.

1.1. Publikation 1

Atopic dermatitis in cats and dogs – a difficult disease for animals and owners

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Atopic dermatitis in cats and dogs – a difficult disease for animals and owners

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Abstract

The purpose of this review article is to give an overview of atopic dermatitis in companion animals and of recent developments including knowledge on immunological background, novel treatment options and difficulties in disease management. The prevalence of hypersensitivities seems to be increasing. The pathogenetic mechanisms are not fully understood, yet multiple gene abnormalities and altered immunological processes are involved. In dogs and cats, the diagnosis of atopic dermatitis is based on history, clinical examination and exclusion of other differential diagnoses. Intradermal testing or testing for serum allergen-specific Immunoglobulin E is only used to identify allergens for inclusion in the extract for allergen immunotherapy. Symptomatic therapy includes glucocorticoids, cyclosporine, essential fatty acids and antihistamines. A selective janus kinase 1 inhibitor and a caninized monoclonal interleukin-31 antibody are the newest options for symptomatic treatment, although longterm effects still need to be assessed. The chronic and often severe nature of the disease, the costly diagnostic workup, frequent clinical flares and lifelong treatment are challenging for owners, pets and veterinarians. Patience and excellent communication skills are needed to achieve a good owner compliance and satisfactory clinical outcome for the animal.

Keywords

Allergy, canine, feline, atopy-like dermatitis, adverse food reaction, IL-31, lokivetmab, immunotherapy

Background

Atopic dermatitis (AD) is a common skin disease in dogs and cats. Its clinical, immunological, histological and pathological features in dogs are so similar to the human counterpart, that canine atopic dermatitis has been suggested as an animal model for human AD (1, 2). In **table 1** some of the similarities and differences are summarized. Much less is known on the pathogenesis in cats, but the clinical findings are different to those seen in humans and dogs.

Table 1: Similarities and Differences of AD in dogs and humans

	Dogs	**Humans**
Pathogenesis	Th2 immune response Skin barrier damage Allergic inflammation (18, 19, 153)	Th2 immune response Skin barrier damage Allergic inflammation (154)
IL-4 and IL-13	Pruritus, acute inflammation (155)	Pruritus, acute inflammation (156, 157)
Periostin (PO) expression	Increased expression, related to the chronicity of skin lesions (158)	Increased expression, related to the chronicity of skin lesions (159, 160)
Histologic pattern	Spongiotic, hyperplastic dermatitis with mononuclear infiltrate; predominantely T-lymphocytes (153, 161)	Spongiotic, hyperplastic dermatitis with mononuclear infiltrate; predominantely T-lymphocytes (162, 163)
Dysbiosis	Reduced microbiome diversity (164) and fungal dysbiosis (165)	Reduced microbiome diversity and fungal dysbiosis (166)
Clinical signs	Eczematous skin lesions with no progression of clinical signs e.g. no development of asthma (2, 44)	Atopic march
Allergy testing	Intradermal testing without high risk of anaphylactic reactions (69)	Skin prick testing
Immunotherapy	Accelerated immunotherapy without increased risk for anaphylactic reactions (76, 78, 79)	Standard AIT

Canine atopic dermatitis

Canine AD is a multifactorial disease process. It is defined as a “genetically predisposed inflammatory and pruritic allergic skin disease often associated with a production of immunoglobulin (Ig) E against environmental allergens” (3). The estimated prevalence of AD in the dog is approximately 10-15 % (4). Although the pathogenesis is not completely understood, there is evidence for genetic abnormalities, an altered immune system with cutaneous inflammation and a skin barrier defect (5, 6).

Genetic background

Multiple gene expressions involved in skin barrier function and cutaneous inflammation have been described as down- or upregulated in the skin of privately owned atopic dogs (7-9) as well as in a canine model of AD (10). In the latter study, 361 genes relevant for inflammation, wound healing or immune response processes showed an increased expression, whereas 226 genes associated with differentiation and skin barrier function showed decreased mRNA concentrations in allergen-treated skin of sensitized dogs (10). In atopic German shepherds a significant association with chromosome 27 was determined, especially with genes that had a connection to plakophilin 2 production (11). Plakophilin 2 is an important structural protein, which is expressed in epithelial and immune cells (11, 12). The predisposition of German shepherds for AD is likely due to a risk haplotype in combination with multiple variants resulting in a changed expression of the plakophilin 2 gene and nearby genes (11). In the United Kingdom the risk of Labrador and Golden retrievers to develop AD was almost 50 % due to the genetic background (13, 14). Multiple breeds including Boxer, Westhighland White Terrier, French bulldog, Bullterrier, American cocker spaniel, English springer spaniel, Poodle, Chinese Sharpei, Dachshund, Collie, Miniature schnauzer, Lhasa apso, Pug and Rhodesian ridgeback are also predisposed (15, 16) and breed predispositions vary with geographic location (17).

Immunologic alterations

In acute lesions, allergic inflammation triggers the release of cytokines such as interleukin (IL-) 4 and IL-13, which induce a T helper 2 (Th2) response (1, 18, 19). In more chronic skin lesions, CD4+ and CD8+ skin-associated T lymphocytes additionally stimulate the production of various cytokines such as IL-13, IL-22 and IFN-γ (20). Recent findings on cytokines and specific cell types in atopic dogs are listed in **table 2**.

Table 2: Recent findings on T cells and cytokines in canine atopic dermatitis

Cytokine/cell	Function
IL-31	Important role in atopic pruritus (167). Its serum concentration correlates with the severity of active skin lesions (168).
IL-13	Induces production of PO in keratinocytes and fibroblasts, associated with chronicity of skin lesions and their deterioration (1).
IL-25	Increased in PO-stimulated keratinocytes (1), clinical relevance unclear. In a murine asthma model relevant for Th2-mediated immunity, contributes to a decreased epidermal barrier function in human AD (169-171).
IL-33	Upregulated in chronic lesional skin, similar to atopic humans (172).
CD 34+ cells	Increase in peripheral blood, unclear clinical relevance (173).
CD4+ CD25+ FoxP3+ cells	Significantly higher percentage in peripheral blood and correlated with severity of AD (174).

Skin barrier defects

According to the "outside-in" theory an impaired epidermis leads to an increased allergen penetration and hence a higher allergen exposure of epidermal immune cells (21). This skin barrier defect may be due to decreased filaggrin concentrations (22). Caspase 14 is involved in the breakdown of filaggrin into natural moisturizing factors such as free amino acids and small peptides and altered concentrations might influence the skin barrier function and hydration of the stratum corneum (23, 24). Conflicting results regarding the filaggrin metabolism in atopic dogs have been published with lower (22) and higher caspase 14 concentrations (24). Changes in the ceramide composition of lesional canine atopic skin have been described (25, 26) contributing to disorganisation of the lipid envelope and hence disruption of the epidermal barrier. Ceramide profiles of atopic dog skin contained lower amounts of CER [EOS], CER[EOP] and CER[NP] (27), similar to what is seen in humans. A decreased relative content of ceramides in atopic dogs might be one reason for the increased transepithelial water loss observed in both lesional and non-lesional skin (28). Moreover, house dust mite allergens can alter the expression and possibly also the function of corneodesmosomal and tight junction proteins through proteolytic digestion and/or allergic inflammation, facilitating a higher allergen penetration through the epidermis (29).

Feline atopy-like dermatitis

The function of IgE in the cat is not completely clarified, consequently the term “feline atopic dermatitis” is not ideal (30, 31), but rather it is referred to as “feline atopy-like dermatitis”. The pathogenesis of feline atopy-like dermatitis is not completely elucidated. Data on genetic alterations and skin barrier abnormalities as reported in human and canine AD are rare.

Genetic background

In a large study evaluating allergic cats, pure-bred cats were overrepresented in the group of cats with atopy-like dermatitis compared to cats with flea allergy, but the study lacked a non-allergic control group (32). In this study, Abyssinians were only affected by atopy-like dermatitis and not flea allergy. A predisposition for Devon rex, Abyssinian and domestic shorthaired cats was reported in another study (33). A case report of three littermates with clinical signs and history consistent with atopy was described implying a heritable factor (34), however more detailed genetic studies are lacking (31).

Immunologic and skin barrier alterations

In cats, histopathologic features of atopy-like dermatitis include perivascular to diffuse dermal infiltration of T lymphocytes, activated antigen presenting cells, eosinophils, macrophages and high numbers of mast cells (35). A significant increase of CD4+ T cells, IL-4 and CD1a+ dentritic cells was found in the skin of cats with atopy-like dermatitis, pointing to a Th2-mediated immune dysfunction (33, 36), although cytokine pathways have not been investigated (37). Comparable to humans and dogs (38) a fungal dysbiosis was found with next generation sequencing of skin swabs taken from healthy and allergic cats (39). Skin hydration as a measure of the skin barrier did not always correlate with clinical scoring indicating that a barrier defect may not be as relevant in cats (40).

Practical approach

Clinical features

The following three main allergy categories can be distinguished in cats and dogs: flea (and other insect bite) hypersensitivities, cutaneous adverse food reaction (AFR) and AD due to environmental allergens. The clinical signs in the atopic dog are mostly distinct when compared to the atopic cat. A short overview of the main clinical features, diagnosis and treatment options in companion animals is given in **table 3**.

Table 3: Clinical Features, diagnosis and treatments of atopic dermatitis for small animals

	Dog	**References**	**Cat**	**References**
Age	Commonly 6 months to 3 years	(41)	Commonly < 3 years	(31, 32)
Clinical Symptoms	Pruritus		Eosinophilic granuloma complex (indolent eosinophilic ulcer, eosinophilic granulomas, eosinophilic plaques)	(32, 46, 47)
	Inflammation (Erythema, self-induced alopecia, excoriation) secondary infection	(41, 42)	Head and neck pruritus Miliary dermatitis Self-induced alopecia	
Affected body part	Ear pinnae, axillae, ventral abdomen, extremities, paws, inguinal, lips, perianal region	(42, 43)	Head, mouth, neck, abdomen, trunk	
Diagnosis	Exclusion diagnosis (rule out differential diagnosis, compatible history and clinical signs		Exclusion diagnosis (rule out differential diagnosis, compatible history and clinical signs	
Therapy	Allergen contact avoidance	(71)	Allergen contact avoidance	
	Specific targeted: Allergen-specific immunotherapy	(70, 72-79, 81, 82)	**Specific targeted:** Allergen specific immunotherapy	(33)
	Untargeted, symptomatic: Glucocorticoids Ciclosporin A Oclacitinib Lokivetmab Antihistamines	 (85) (86, 87, 89) (92-95) (83, 84) (97-100, 103-105)	**Untargeted, symptomatic:** Glucocorticoids Ciclosporin A Oclacitinib Antihistamines	 (88, 90, 91) (96) (33, 106)
	Topical: Shampoos Hydrocortisone-aceponate Tacrolimus	 (113, 114) (108, 109) (111, 112)	**Topical:** Hydrocortisone-aceponate	 (110)
Supportive dietary interventions	Essential fatty acids Probiotics Cholecalciferol	(116-119) (124, 125) (129)	Essential fatty acids	(115)

Clinical features of canine AD

In dogs, clinical signs of an environmental allergy mainly develop between 6 months and 3 years of age (41). Erythema is a primary lesion of canine AD; pruritus and inflammation can result in self-induced alopecia, excoriation and secondary infections with papules, pustules and crusts (41, 42). Axillae, ventral abdomen, distal extremities, inner pinnae and periocular, perioral and perianal regions are commonly affected (42). Otitis externa is present in half of the dogs with AD. Predilection sites differ from breed to breed (43). Even though dogs can have multiple target organs for hypersensitivities (including gut and respiratory) (44), the contact with environmental allergens predominantly induces skin lesions in this species (45). There is no evidence for the progression of initially exclusive cutaneous lesions to respiratory signs and systemic hypersensitivities comparable to the "atopic march" in humans (44). In contrast to the cat, clinical examination in the dog frequently provides clues on the pathogenesis of the pruritus as to the presence of flea bite hypersensitivity versus environmentally-induced atopy or AFR. The former is characterized by pruritus focused on the dorsal lumbosacral area, ventral abdomen, tailbase and thighs.

Clinical features of feline atopy-like dermatitis

The manifestation of specific cutaneous reaction patterns (46) can indicate an allergic primary cause in cats. These involve head and neck pruritus, miliary dermatitis characterised by small crusted papules, self-induced alopecia without any other clinical lesions and eosinophilic lesions such as eosinophilic indolent ulcers, eosinophilic granulomas and eosinophilic plaques (32, 47). In rare cases, untypical AD symptoms such as plasma-cell pododermatitis, seborrhoea, ceruminous otitis, facial erythema and exfoliative dermatitis were reported (31, 48). Additionally noncutaneous signs such as sneezing, coughing, conjunctivitis, diarrhoea or vomiting can be presented in affected cats (32). The disease onset can vary, but commonly it is under three years (31, 32), whereas the mean age for AFR is slightly higher (approximately 4-5 years) with a range from 3 months to 11 years (48). In contrast to the dog, flea-bite hypersensitivity and environmentally induced and AFR look much more similar in the cat (32).

Diagnosis

A differential diagnosis of AD is based on age of onset, breed and clinical signs. Other differential diagnoses such as ectoparasites and flea bite hypersensitivity must be ruled out by a consequent ectoparasite control. There is no single test differentiating the atopic from the non-atopic dog or cat (49).

It is not possible to distinguish clinical signs of AD caused by perennial environmental allergens from AFR (16, 50, 51). Hence an elimination diet followed by a provocation with the original food should be performed in any dog or cat with non-seasonal AD (52), particularly those with a long history of pruritus and/or gastrointestinal signs (51, 53). A diet length of 6-8 weeks is recommended, as 90 % of the dogs with AFR show some improvement during this time period (54). Every food can potentially result in an AFR (55). The most common reported causative allergens for canine AFR are beef, dairy products, chicken, wheat, and lamb (56). However, soy, corn, egg, pork, fish and rice have also been reported as offending allergens (56). The food sources most frequently causing AFR in cats were beef, fish, and chicken (58). Wheat, corn, dairy products, lamb, egg, barley and rabbit were also reported as offending allergens in individual cats. The selection of appropriate protein and carbohydrate sources for an elimination diet can be challenging. It is important to use a protein and carbohydrate source, which the dog or cat has never received before (52), thus a detailed food history needs to be obtained by the veterinarian. Multiple studies have shown that various commercial special diets with only one protein source on their label were contaminated and contained substances not listed on the label (57-60). Highly hydrolysed food is an alternative, but some dogs allergic to chicken also react to diets containing hydrolysed chicken protein (61). Therefore a home cooked diet by the owner is considered as diagnostic gold standard (52), where instead of commercial dry or canned food the owner purchases one type of meat and one carbohydrate source and prepares those him-/herself for the pet. As cats are obligate carnivores, the use of a carbohydrate source is optional in the short term and indeed may reduce palatability. Currently there is no reliable alternative test for diagnosing food allergy (62). There is only poor correlation between IgE- and IgG-antibodies in the serum and clinical food reactions (53, 63). A patch test can be used for the selection of the elimination diet food source if the food history is unknown. This test has a poor positive predictability, but a high negative predictability (53). A lymphocyte

proliferation test was able to detect a type IV hypersensitivity in the blood (64-66) by measuring activated T-helper lymphocytes under food allergen stimulation with flow-cytometry (66). In 49 of 54 AFR dogs this test accurately provided positive reactions against one or more food allergens (66), however this test is not commercially available at this time.

AD in animals is diagnosed by history, clinical examination and exclusion of all differential diagnoses. Positive reactions are frequently seen in healthy dogs on both intradermal tests (67) and serum tests for allergen-specific IgE (68). The total serum IgE concentrations seem to have no clinical relevance in the dog (44). Once AD is diagnosed in an animal, testing can be used in combination with clinical historical information to choose which allergens should be selected for allergen immunotherapy. Serum tests for allergen-specific IgE and intradermal tests are equally useful and both are still performed with allergen extracts in animals, in contrast to component-resolved tests such as single molecule CAP testing or ImmunoCAP ISAC 112 microarray in human medicine (45). Prick puncture testing is not performed routinely in veterinary medicine, as intradermal testing is an established and safe diagnostic tool with a very low risk of adverse effects (69).

Treatment of atopic dermatitis in small animals

Therapy selection depends on the pet's condition, especially the severity of the lesions and degree of pruritus and owner preference and especially in cats – on the ability to medicate. The therapy needs to be reassessed regularly and adapted to the individual (70). With the exception of avoidance of the causative allergen (71), in general there are two different treatment approaches: specific with allergen immunotherapy or symptomatic with a variety of drugs. The combination of various drugs can increase the chance of remission (70).

Specific allergen-targeted therapy

Allergen immunotherapy (AIT) is the only possibly curative treatment option (70). In approximately 50-75 % of the atopic animals desensitization is effective (72-76). In those animals, it is often recommended to continue the treatment lifelong (70, 77). In contrast to human medicine where accelerated immunotherapy ("rush") is only advised in selected patients, due to the high frequency of systemic adverse reactions, in dogs rush-immunotherapy is effective and safe with no reported increased risk of adverse reactions (76, 78, 79).

Intralymphatic desensitization (ILIT) in humans was reported to reduce the therapeutic interval from 3 years to 8 weeks with less severe adverse effects (80). ILIT is also used in veterinary medicine, but with less predictable success than in humans and a recent report showed the need for ongoing immunotherapy at regular intervals (81). Sublingual immunotherapy (SLIT) was introduced to veterinary medicine some years ago, but so far limited published data is available (82).

Biologicals

Monoclonal antibodies are a focus of research in human medicine. They target specific receptors or cytokines and are highly specific and effective in blocking their target molecule. Lokivetmab is a monoclonal caninised anti-IL-31 antibody, that was recently approved for the use in atopic dogs. It significantly decreased pruritus for at least four weeks (83). Its efficacy is comparable to oral prednisolone. Lokivetmab is regarded as safe without any immediate hypersensitivity reactions. Adverse reactions were similar in dogs treated with lokivetmab to those treated with placebo (84). In the treatment group, 2.5 % of the dogs produced antibodies against lokivetmab (84) but their clinical significance is unclear at this point. To date no other therapeutic monoclonal antibody exists in veterinary medicine.

General anti-inflammatory and anti-pruritic treatment

In severely affected dogs and cats, glucocorticoids, cyclosporine, oclacitinib or lokivetmab are used for symptomatic therapy due to their clinical efficacy and high success rates of 70-80 % (85). Glucocorticoids are inexpensive, universally available and have been the mainstay of treatment for allergic pets for many years. However, the potentially severe adverse effects of oral and particularly injectable depot glucocorticoids such as polyuria and polydipsia, polyphagia, muscle atrophy, secondary skin infections, calcinosis cutis and others have led to the development of alternative drugs for dogs and cats.

Cyclosporine A, a calcineurin inhibitor, is highly effective in dogs and cats with comparable results to glucocorticoids (86, 87, 88). The initial daily dosage can be reduced in the majority of animals to every other day or twice weekly (86, 87). Mild gastrointestinal symptoms (e.g. diarrhoea and vomiting) frequently occur at the beginning of treatment but usually resolve during continued administration (89). Hirsutism, gingival hyperplasia and hyperplastic dermatitis are reported adverse

effects which typically resolve with dose reduction or discontinuation (87). Sporadic case reports exist of immunologically naive cats newly infected with *Toxoplasma gondii*, developing systemic and even fatal clinical signs (90, 91). It is recommended to evaluate anti-toxoplasma antibodies in outdoor cats and cats fed raw meat prior to initiating cyclosporine therapy.

Oclacitinib is a selective inhibitor of janus kinase 1. Janus kinase 1 is involved in the signaling pathways of the receptors for IL-2, IL-4, IL-6, IL-13 and IL-31 (92), and thus aims at blocking the Th2 pathway. It is administered to dogs at a dose of 0.4-0.6 mg/kg twice daily for two weeks and then daily at that dose is reported to be as effective as glucocorticoids (93, 94). In comparison to cyclosporine, oclacitinib has a more rapid effect and gastrointestinal adverse effects are less frequently observed (95). Skin infections and histiocytomas were reported with increased frequency in dogs on longer term oclacitinib therapy (93). Oclacitinib given to a small number of cats with atopy-like dermatitis over a 4 week period was effective (96), however the dose required was higher than for dogs, the period of monitoring was short and both more and larger studies are needed before it can be recommended as standard therapy.

Different *antihistamines* are associated anecdotally with individual responses, therefore a trial therapy with various antihistamines over 7-14 days is recommended (97, 98). Histamine binds to four receptor subtypes (H1 to H4) which are expressed in different tissues (99). Its interaction with the high-affinity H1 receptor is known to cause cutaneous vasodilatation, oedema, and wheal formation. Histamine can also attract effector cells such as eosinophils to the region of inflammation (99). Antihistamines targeting the cutaneous H1 receptors block the binding of histamine and are used most frequently in order to reduce the pruritus in atopic dogs (100). Antihistamines binding to the H4 receptor showed an anti-inflammatory and anti-pruritic effect in mice (101, 102). However, they did not prevent the development of acute skin lesions in a canine atopic model (103). A double blinded, placebo-controlled, cross-over study evaluated the efficacy of dimetindene and a combination of hydroxyzine and chlorpheniramine in 19 atopic dogs and concluded that in both groups a limited, but significant improvement on pruritus was achieved, nevertheless other drugs might additionally be needed (104). Many owners consider antihistamines useful therapeutic agents for their pets' allergy (105). The recommended dosage of antihistamines is much higher in cats

and dogs than in humans. Dogs can rapidly metabolise hydroxyzine to cetirizine and need twice daily hydroxyzine orally at 2.0 mg/kg (99). In one study a positive effect of antihistamines, mainly loratidine and cetirizine, was shown in 67 % of 31 atopic cats (33). In contrast, in another study, cats with allergic dermatitis treated with cetirizine hydrochloride showed no significant differences in lesion- and pruritus-scores to those treated with placebo (106).

A future non-specific treatment alternative might be the subcutaneous injection of *cytosine-phosphate guanine oligodeoxynucleotides* bound to gelatine nanoparticles (CpG GNPs). This therapy resulted in decreased lesions and pruritus in ≥50 % of atopic dogs, similar to what is seen with AIT and the mRNA expression of IL-4 was also decreased in those dogs (107). However, this treatment is currently not commercially available.

Due to their hair coat and compliance issues, *topical treatment* of dogs and cats can be difficult for owners and therefore it is less frequently used than in humans (44). Topical glucocorticoid ointments can be used for localised skin lesions in sparsely haired areas, but prolonged application may result in skin atrophy (98). Topical hydrocortisone aceponate was effective for canine AD (108, 109) and feline atopy-like dermatitis (110). Topical calcineurin inhibitors such as tacrolimus have been used successfully in localized lesions of canine AD (111, 112). Atopic dogs may benefit from shampoo therapy (113, 114).

Adding *dietary supplementations* such as essential fatty acids (EFA), probiotics or vitamins can have a positive benefit for atopic animals. EFA are used to treat AD in cats (115) and dogs (116). Oral EFA can improve the coat quality, strengthen the skin barrier and reduce the transepidermal water loss (117). Moreover EFA can lower the amount of glucocorticoids and cyclosporine needed to control clinical signs of canine AD (118, 119).

Probiotics are microorganisms that are claimed to provide health benefits when consumed (120, 121). Their mechanism is not completely elucidated, but may involve binding Toll-like receptors and downregulate the allergic predominately TH2-mediated response (122, 123). *Lactobacillus paracasei K71* given orally to atopic dogs led only to a slight improvement of lesion- and pruritus-score (124). However, the medication score was reduced significantly indicating a potential benefit as a complementary therapy (124). *Lactobacillus rhamnosus GG* given to

puppies led to a reduction of immunologic indicators of AD, even though no significant clinical improvement was observed (125).

In human studies a positive impact of *cholecalciferol* on AD was detected (126-128). Similarly, systemic cholecalciferol reduced pruritus and lesion scores in dogs with AD (129).

How to diagnose and manage AD in the difficult animal and its owner

Both diagnosis and therapy of AD in cats and dogs requires patience, time and effort. An appropriate diagnostic work-up will ensure the correct diagnosis of the disease and concurrent flare factors and usually includes an elimination diet and ectoparasite control as well as cutaneous cytology to rule out secondary infections. It is not uncommon for dogs and cats with environmental allergies to be affected by flea bite hypersensitivity or AFR concurrently (32, 50) and it can be difficult to determine how much of the symptomatology is due to which type of antigen. In those animals, the diagnostic work-up may require an elimination diet with several provocation trials and an extensive flea control in addition to repeated examinations of the animal in order to ensure adequate resolution of secondary infections and concurrent flea bite hypersensitivity. Many owners do not believe that their dog or cats' problem is food triggered and are reluctant to limit their pet's food intake to one protein and one carbohydrate source. AFR is not necessarily related to a recent diet change and in one report most of the dogs with AFR received the same food for two years or longer before symptoms arose (130). An elimination diet with restriction to one food source in outdoor or free-roaming cats, dogs living on a farm or in a household with small children is difficult to impossible. Cats should ideally be kept inside for the diet period (131) and some dogs need to wear a muzzle during walks to prevent the rapid gobbling down of potentially allergenic food stuff (51, 132). Throughout the diagnostic process owner incompliance can be an issue, because of high costs, continuous drug administration and the organisational and emotional problems associated with feeding a limited elimination diet. Thorough and repeated client education and support contribute to good owner compliance (133). A diary for the owners to record the daily pruritus, drug side effects or pitfalls during the elimination diet can increase their motivation (131). Low palatability, refusal of the diet (particularly in cats) or gastrointestinal symptoms such as diarrhoea or constipation can decrease compliance (134). A gradual change to the

"new" food can minimise those problems. In contrast to dogs it is not an option to allow cats to "starve for a few days" while offering the new diet, as a negative energy balance due to anorexia can initiate hepatic lipidosis (135). Owners may need to be made aware of the "traps" of an elimination diet (131), for example tooth paste and medications for pets are frequently flavoured with animal proteins and thus will interfere with the elimination diet. Chewable drugs or drugs in gelatin capsules need to be avoided (131) as it was shown that dogs allergic to corn and soy showed cutaneous flares after receiving chewable capsules containing pig protein, soy and milbemycin (132). Similarly many owners do not consider treats "food" and rely on those for dog training. Those treats need to be replaced with one made of the protein used in the diet to optimise outcome. Secondary infections, most often Malassezia spp. in dogs (117, 136) and staphylococci in dogs and cats (137-140) may mimic the clinical signs of allergy and require investigation of other possible causes for the infection. After establishing the diagnosis, it is important to explain to the owner that an allergy is a lifelong disease and thus will usually require lifelong management. Multiple adaptations of therapy may be needed depending on the individual animal's condition and flare factors. Treatment options, their costs, efficacy and safety need to be discussed with the owners in detail. Some may prefer a rapid clinical improvement with a potent systemic drug, whereas others may not want to risk this drug's side effects. Short-term relief can lead to a higher owner compliance. The emotional relationship between owner and animal should not be underestimated. Often owners suffer with their animal and sleepless nights of the owners are the consequence of a highly pruritic animal.

Unmet needs and research

At this point, the pathogenesis of AD in dogs and cats is not fully elucidated. Multiple genes are implicated (14). However, further genomic studies and investigations on breed differences may allow a better understanding of the heritability. Research on the role of CD25+ FoxP3+ T cells is ongoing (20). In human medicine the hygiene hypothesis ascribes the increasing allergy risk to a modern environment and life style with less pathogen exposure (141, 142). This might apply to animals in the same way as the prevalence of AD seems to be lower in dogs living in rural areas (143). More studies are needed to evaluate environmental influence on AD in dogs and cats, possibly enabling prophylactic measures in the future. Allergen-specific IgE can be measured, but a correlation of

the results with clinical signs is not always present (144). Multiple serum allergy tests are offered, but cannot be used to diagnose AD. Additionally, inter- and intralaboratory variability of some of those tests is high (145-148). With regard to treatment for AD the first monoclonal antibody for atopic dogs, an anti-IL-31-antibody, is available with promising clinical results, but the consequences of a long-term blockade of IL-31 are unknown at this point (84). Individual phenotypes of AD in dogs and cats may respond better to specific drugs than others. More studies and pooling of data to obtain numbers to achieve significance are needed to evaluate the efficacy of specific drugs in specific breeds and pheno- as well as genotypes to allow tailored patient-oriented therapy in veterinary medicine. AIT is typically administered via subcutaneous injections in both dogs and cats, there is however a lack of well-powered dose-finding studies in animals. Further and comparative studies are also needed to investigate which alternative application route is most suitable in which clinical situation. Using recombinant allergens such as *Dermatophagoides farinae* allergen (Der f 2) (149, 150) may result in more reproducible results and a higher success rate compared to standard AIT and ILIT (151). Modified allergen preparations such as allergoids, allergen peptides as well as alteration with adjuvants may decrease the risk of adverse effects and increase efficacy (152). First studies evaluated bacterial oligodeoxynucleotides in canine AD (79, 107) with promising results.

Conclusion

AD in pets is diagnosed by history, clinical signs and the ruling out of differential diagnoses. Allergy tests (intradermal tests and serum tests for allergen-specific IgE) cannot be used as a diagnostic tool for AD, but rather in association with clinical history permit the selection of relevant allergens for immunotherapy. Multiple flare factors such as additional flea-bite hypersensitivity and AFR and secondary bacterial or yeast infections can complicate AD in the dog and cat and need to be identified, prevented and/or treated. Intensive and regular communication with the pet owner and a diagnostic work-up and treatment tailored to the individual pet and owner’s needs is essential for a good compliance and optimal outcome.

List of abbreviations

AD: atopic dermatitis

Ig: immunoglobulin

IL: interleukin

Th2: T helper 2

PO: periostin

AFR: adverse food reaction

AIT: allergen specific immunotherapy

ILIT: intralmyphatic immunotherapy

SLIT: sublingual immunotherapy

EFA: essential fatty acids

CpG GNPs: cytosine-phosphateguanine oligodeoxynucleotides bound to gelatine nanoparticles

Der f 2: *Dermatophagoides farinae* allergen

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1.2. Allergietests

Allergietests beruhen auf dem Nachweis einer humoralen Immunreaktion gegen bestimmte Allergene wie etwa Pollen, Schimmelsporen und Hausstaubmilben und dienen als Grundlage zur Auswahl relevanter Allergene für eine individuell an den Patienten angepasste AIT. Es gibt keine Screeningtests zur Diagnostik einer Allergie, da die Sensitivität und Spezifität limitiert ist und dementsprechend auch gesunde Hunde positive Ergebnisse bzw. atopische Hunde negative Testergebnisse aufweisen können (LIAN und HALLIWELL, 1998; DEBOER und HILLIER, 2001a). Standardmäßig wird der Nachweis von IgE-mediierten Sensibilisierungen gegen Umweltallergene mittels allergiespezifischer Intrakutantests (IKT) und IgE-Serumallergietests (SAT) eingesetzt (DEBOER und HILLIER, 2001b). Jedoch kann Allergen-spezifisches IgE je nach Expositionszeit des auslösenden Allergens variieren, auch in Hunden die bekanntermaßen hypersensitiv auf ein bestimmtes Allergen sind (OLIVRY et al., 2006; OLIVRY und PAPS, 2011). Auch bei gesunden Hunden wurden hohe Allergen-spezifische IgE Serumlevel nachgewiesen, weshalb positive Allergen-spezifische IgE Testergebnisse nicht spezifisch für eine CAD sind (ROQUE et al., 2011). Der Erfolg einer AIT ist von verschiedenen Faktoren wie etwa der Allergenzusammensetzung, Intra-/Interlabor-Zuverlässigkeit und Test-Interpretation abhängig (PLANT et al., 2014). Die Ansprechrate auf eine AIT war in mehreren Studien unabhängig von dem verwendeten Allergietestverfahren (SAT vs. IKT) (PARK et al., 2000; ZUR et al., 2002; LOEWENSTEIN und MUELLER, 2009).

Aufgrund der komplexen und nicht vollständig geklärten Pathogenese der CAD ist unklar, inwieweit die Messung anderer Immunglobuline aussagekräftig ist. Sowohl in der Human- als auch in der Tiermedizin kann nicht basierend auf dem Gesamt-IgE Serumspiegel zwischen allergischen und gesunden Patienten unterschieden werden, da dieser von anderen Erkrankungen, Saison und Alter beeinflusst wird (DEBOER und HILLIER, 2001b). Ein weiterer Faktor ist die genetische Abstammung, so wiesen z.B. Labrador Retriever im Vergleich zu Golden Retrievern häufiger Gesamt-IgE und spezifische IgE-Werte über dem Schwellenwert auf (DEBOER und HILLIER, 2001b; LAUBER et al., 2012). Kein signifikanter Unterschied konnte hinsichtlich der durchschnittlichen IgA Serumkonzentration bei gesunden vs. atopischen Hunden beobachtet werden (MUELLER et al., 1997). In einer Studie waren Allergen-spezifische IgG

Konzentrationen bei atopischen Hunden erhöht und stiegen bei einer AIT Behandlung weiter an (HITES et al., 1989). Jedoch gibt es keine Korrelation zwischen erhöhten IgG1 Konzentrationen und dem Grad der klinischen Verbesserung eines individuellen Hundes (LOEWENSTEIN und MUELLER, 2009). Eine Studie von Lauber et al. (2012) hat gezeigt, dass atopische Hunde, welche mit einer AIT gegen *Dermatophagoides farinae* (Der f) behandelt wurden, hohe Der f-spezifische IgG1, aber nicht Der f-spezifische IgG4 aufwiesen (LAUBER et al., 2012). Bisherige Untersuchungen führten hingegen zu der Schlussfolgerung, dass keine Produktion von blockierenden Antikörpern für ein gutes Ansprechen auf eine AIT notwendig sei, da kein signifikanter Anstieg des Gesamt-IgG und IgG Unterklassen bei Patienten mit erfolgreicher AIT nachweisbar waren (LOEWENSTEIN und MUELLER, 2009).

Inzwischen werden viele verschiedene kommerzielle Testverfahren angeboten, welche nicht zwingenderweise validiert sein müssen. So wurde z.B. festgestellt, dass kommerzielle Haar- und Speichelallergietests nicht reproduzierbar sind und nur zufallsbasiert richtige Ergebnisse liefern (BERNSTEIN et al., 2019). Des Weiteren kann nicht zwischen echten Hundehaaren und Kuscheltierhaaren unterschieden werden (COYNER und SCHICK, 2019). Die fehlende Standardisierung ist ein erhebliches Problem für IKT und SAT, so kann z.B. die enthaltende Allergenmenge im Testextrakt variieren, womit es zu einer Diskrepanz des Testergebnisses bei Patienten kommen kann (TURNER et al., 1980). In einer Studie von Abrams et al. (2018) wurde gezeigt, dass die Zusammensetzung und Potenz bei veterinärmedizinischen Allergenextrakten von Labor zu Labor unterschiedlich war, weshalb gegebenenfalls eine Anpassung der Konzentration zur Verwendung bei einem IKT benötigt wird (ABRAMS et al., 2018). Eine weitere Limitierung ist, dass häufig die Übereinstimmung der Testergebnisse von IKT und SAT für den gleichen Patienten nur sehr gering ist und somit zu Verwirrung führt (CODNER und LESSARD, 1993; HÄMMERLING und DE WECK, 1998; DEBOER und HILLIER, 2001b). Inwieweit dies jedoch signifikant ist und die Ursache hierfür ist nicht bekannt (DEBOER und HILLIER, 2001b).

1.2.1. Serumallergietest

Früher variierte die Reproduzierbarkeit und Zuverlässigkeit der SATs stark, weshalb lange der IKT als “Goldstandard“ angesehen wurde. Inzwischen haben

sich die Testverfahren jedoch verbessert und es kann zwischen einem SAT oder einem IKT gewählt bzw. die beiden in Kombination durchgeführt werden. Die Vorteile bestehen darin, dass der SAT universal erhältlich ist und leicht und ohne großen Aufwand von praktizierenden Tierärzten durchgeführt werden kann. Gerade bei Patienten mit großflächigen, schweren Hautläsionen wird dieser Test bevorzugt verwendet, da der Einfluss von Medikamenten im Vergleich zum IKT auf die Testergebnisse geringer ist und daher die Absetzfristen kürzer sind (OLIVRY et al., 2013).

Die Zuverlässigkeit eines Serumallergietests in drei verschiedenen europäischen Laboren wurde ermittelt, wobei 3 % und respektive 9 % Intra- und Interlabor-Unterschiede in Bezug auf alle positiven und negativen Reaktionen nachweisbar waren (THOM et al., 2010). Kürzlich wurde erneut die Reproduzierbarkeit der Ergebnisse von drei europäischen SATs untersucht. Hierfür wurde randomisiert Serum von 28 Hunden aufgeteilt in drei Proben, zwei davon am gleichen Tag und eine am Tag darauf jeweils vom selben Labor getestet (BAUMANN et al., 2019). Die Intra- und Inter-Assay Übereinstimmung war bei zwei der untersuchten SATs gut, trotzdem müssen die Testergebnisse im Zusammenhang mit der klinischen Historie des Patienten beurteilt werden (BAUMANN et al., 2019). Im Gegensatz dazu war bei einem Vergleich von vier in USA erhältlichen SATs verschiedener Laboratorien die Übereinstimmung der positiven respektive negativen Ergebnisse der einzelnen Allergene niedrig und dementsprechend die Empfehlungen zur Allergen-zusammensetzung für eine AIT sehr verschieden (PLANT et al., 2014). Eine weitere Studie hat gezeigt, dass die Testergebnisse von Serumproben welche in drei Portionen unterteilt waren, wovon zwei zum gleichen Zeitpunkt und eine Probe einen Monat später vom gleichen Labor ausgewertet wurden, große Unterschiede aufwiesen und dabei mindestens ein Allergen bei jedem Hund anders ausgewertet wurde (ZHOU et al., 2019). Diese Interpretationsunterschiede können das Ansprechen einer AIT wesentlich beeinflussen (ZHOU et al., 2019).

Allergen-spezifische IgE Serumlevel werden als „positiv“ gewertet, wenn die gemessene optische Dichte während der Untersuchung über einem bestimmten, eigens vom jeweiligen Labor etablierten Grenzwert liegt (DEBOER und HILLIER, 2001b). Auch hier mangelt es an einer Standardisierung und somit ist aufgrund der unterschiedlichen Testverfahren der verschiedenen Laboratorien kein direkter Vergleich von Studien möglich (DEBOER und HILLIER, 2001b).

1.2.2. Intrakutantest

Die Injektion von Allergenextrakten in die Haut kann bei atopischen Hunden zu einer IgE-mediierten Degranulation von Mastzellen führen und wird als Nachweis einer Typ-I-Hypersensitivitätsreaktion gewertet (HILLIER und DEBOER, 2001). Reaktionen werden anhand von verschiedenen Kriterien etwa Quaddelgröße, Rötung, Schwellung und Konsistenz subjektiv von dem durchführenden Tierarzt ausgewertet und in fünf Ergebnisklassen von 0 = negativ bis 4 = hoch positiv eingeteilt. Jedoch gibt es kein standardisiertes Auswertungsschema (HUBBARD und WHITE, 2011). Das objektive Ausmessen des Durchmessers der Quaddel kann gerade unerfahrenen Tierärzten beim Erlernen der Auswertung helfen, jedoch war nur eine moderate Korrelation zwischen der akkurateren, subjektiven und der objektiven Einschätzung der Reaktionen gegeben (HUBBARD und WHITE, 2011). Da der IKT direkt am Tier nach 15 und 25 Minuten ausgewertet wird, liegt das Ergebnis direkt vor und es kann eine viel höhere Anzahl an Allergenen getestet werden als in den meisten SATs. Probleme, welche bei SATs eine Bedeutung haben wie etwa Lagerung, Transport, Qualität der Serumprobe und Verwechslungsgefahr, können vermieden werden. In der Regel werden IKTs nur von speziell ausgebildeten Dermatologen durchgeführt und daher nur in bestimmten Tierkliniken angeboten. Die dafür benötigten Allergenextrakte sind teuer in der Anschaffung und haben eine kurze Haltbarkeit, weshalb es sich nur lohnt, wenn mehrere Tiere in kurzer Zeit IKTs benötigen. Da die Hunde still liegen müssen, wird häufig eine kurze Sedierung benötigt, dabei ist zu beachten, dass bestimmte Medikamente einen Einfluss auf IKT Ergebnisse haben können. Butorphanol (0,4 mg/kg) führte im Vergleich zu Dexmedetomidine (5 µg/kg) zu einer signifikant kleineren Quaddelgröße, aber die subjektive Auswertung des IKTs wurde nicht beeinflusst (MILOSEVIC et al., 2013). Eine Sedierung mit Propofol führte zu einer höheren Anzahl an Hunden mit stärkeren Reaktionen (GRAHAM et al., 2003). Es empfiehlt sich, orale und topische Glukokortikoide 14 Tage und Antihistaminika 7 Tage vor einem IKT abzusetzen (OLIVRY et al., 2013). Über den Einfluss einer langfristigen Gabe von Immunsuppressiva wie Ciclosporin oder Oclacitinib gibt es keine wissenschaftlichen Erhebungen.

2. Kreuzreagierende Kohlenhydratbestandteile

An Zelloberflächen gebundene IgE Antikörper nehmen eine wichtige Rolle in der Allergiediagnostik ein, da sie eosinophile und basophile Granulozyten, dendritische Zellen und Mastzellen aktivieren können und dadurch z.B. Mastzellen sensibilisieren, bei spezifischem Antigenkontakt biologisch aktive Stoffe freizusetzen (GALLI und TSAI, 2012). Es gibt jedoch bestimmte IgE Antikörper, denen diese Eigenschaft fehlt, wodurch eine Mastzelle, an der derartige IgE gebunden sind, bei Kontakt mit einem passendem Antigen nicht aktiviert wird (AALBERSE, 1998). Ein Beispiel hierfür sind hochallergene Glykoproteine mit kreuzreagierenden Kohlenhydratepitopen (CCDs), welche bei vielen Insekten und Pflanzenarten vorkommen, aber nicht im Gewebe von Säugetieren existieren (AALBERSE und VAN REE, 1997; LEVY und DEBOER, 2018). Es gibt verschiedene CCD Epitope, wobei die relevante Struktur bei Pflanzen und Insektenallergenen die α1,3 gebundene Fukose an Asn-verknüpften Oligosacchariden von sogenannten N-Glykanen ist (HOLZWEBER et al., 2013; ALTMANN, 2016). IgE Antikörper gegen diese Kohlenhydratbestandteile (Anti-CCD-IgE) sind hoch kreuzreaktiv, es wird demnach nicht zwischen ähnlichen Glykanen an sehr verschiedenen Proteinrückgraten unterschieden (AALBERSE, 1998). Dahingegen sind IgE Antikörper besonders gegen von Säugetieren produzierte Glykane sehr spezifisch (AALBERSE, 1998).

2.1. Humanmedizin

2.1.1. Anti-CCD-IgE

Bereits 1981 wurde eine auffällige Kreuzreaktivität in manchen Patientensera nachgewiesen, welche mit IgE Antikörpern gegen ein Allergen, welches in vielen verschiedenen Nahrungsbestandteilen, wie etwa Buchweizen, Spinat, Honig, Kartoffel, als auch in Pollen vorhanden sind, reagierten (AALBERSE et al., 1981). Multiple Reaktionen in Serumallergietests können hierbei auf verschiedene Ursachen zurückgeführt werden (AALBERSE und VAN REE, 1997; CHARDIN et al., 2008):

a) Unabhängige Sensibilisierung gegen viele unterschiedliche Allergene
b) Kreuzreaktivität zwischen (Glyko-)Proteinen aufgrund von Strukturgleichheiten
c) Nicht-spezifische Bindung der IgE Antikörper an Testsubstanzen

d) Existenz von Anti-CCD-IgE

In einer Studie wurden Anti-CCD IgE Antikörper in 22 % der 6000 untersuchten Serumproben gefunden, wobei in der Teenager Gruppe sogar 35 % der Proben Anti-CCD-IgE enthielten (HOLZWEBER et al., 2013). Bisher ist der Grund, warum nur bestimmte Menschen Anti-CCD-IgE aufweisen, unbekannt (ALTMANN, 2016). Bei Imkern und Pollen allergischen Menschen wurden Antikörper nachgewiesen, weshalb angenommen wird, dass eine Sensibilisierung durch die Inhalation von Pollen und durch Bienen- oder Wespenstiche ausgelöst werden kann (AALBERSE et al., 1981; WEBER et al., 1987; TRETTER et al., 1993; VAN DER VEEN et al., 1997; VIDAL et al., 2012). Vermehrt Anti-CCD-IgE wurde bei schweren Alkoholikern (VIDAL et al., 2009) und bei Menschen nach einem Parasitenbefall festgestellt (AMOAH et al., 2013). Generell ist die Prävalenz der Anti-CCD-IgE bei atopischen Patienten höher, jedoch insbesondere bei polysensibilisierten Individuen nochmals gesteigert (MARI, 2002). Mehrere Studien haben gezeigt, dass die Mehrheit der CCDs klinisch irrelevant sind, da CCDs monovalent sind und dementsprechend nur ein einzelnes IgE binden können (AALBERSE und VAN REE, 1997; LEVY und DEBOER, 2018). Damit aber eine Vernetzung (“cross-linking“) und folglich eine Mastzelldegranulation ausgelöst werden kann, benötigt es mindestens zwei IgE Bindungsstellen (FOETISCH et al., 1999; FOETISCH und VIETHS, 2001).

2.1.2. Problematik der Anti-CCD-IgE

Diagnostische Tests werden verwendet, um den Zustand eines Patienten möglichst genau einschätzen zu können. In der Allergiediagnostik, die zum Teil auf der in-vitro Bestimmung von spezifischen IgE Antikörpern gegen Allergenextrakte beruht, ist es dementsprechend wichtig, tatsächlich verursachende Allergene zu identifizieren und keine harmlosen Allergene zu verdächtigen (ALTMANN, 2016). Die klinische Signifikanz der Anti-CCD-IgE besteht nicht darin, dass jene klinische Allergiesymptome bewirken, sondern vielmehr, dass sie die Interpretation der in-vitro Testergebnisse, speziell bei vorliegender Polysensibilisierung, erschweren (LEVY und DEBOER, 2018). Die Mehrheit der Reaktionen, die durch Anti-CCD-IgE hervorgerufen werden, sind als falsch positiv anzusehen (ALTMANN, 2016). Durch die Hemmung der Anti-CCD-IgE wurde eine deutlich reduzierte Anzahl an falsch-positiven in-vitro Testergebnissen erreicht, ohne dabei die Sensitivität gegenüber relevanten Sensibilisierungen zu verringern (HOLZWEBER et al.,

2013). In vielen Fällen korrelierten die Serum-Testergebnisse deutlich besser mit der Klinik und Anamnese des Patienten, sowie mit den Ergebnissen eines Hauttests (HOLZWEBER et al., 2013). Die Testergebnisse von Serumproben, welche keine Anti-CCD-IgE enthielten, wurden nicht von dem CCD Blocker beeinflusst (HOLZWEBER et al., 2013). Daher ist nach aktuellem Wissensstand die Verwendung von CCD Inhibitoren bei Allergietests, die auf natürlichen Pflanzen-Allergenextrakten basieren, empfehlenswert (HOLZWEBER et al., 2013). Eine andere Möglichkeit wäre es, die konventionellen Allergenextrakte mit nicht-glykosylierten rekombinanten Allergenbestandteilen zu ersetzen (ALTMANN, 2016). Allerdings dürfen diese keinerlei CCD Strukturen enthalten, spezifisch technische Fachkenntnis muss vorhanden sein und geographische Unterschiede hinsichtlich Allergenreaktionen müssen in Betracht gezogen werden (SOH et al., 2015). In der Forschung werden häufig noch Periodate zur Entfernung von CCDs eingesetzt, jedoch reduzieren diese möglicherweise den Antigen-Effekt der gebundenen Proteine (AALBERSE und VAN REE, 1997; LEONARD et al., 2005). Neuere Methoden wie etwa CCD-reduzierte Pflanzen und Oberflächen Plasmon Resonanz bildgebende Mikroarrays mit Peptid und Kohlenhydratepitopen sind vielversprechende Möglichkeiten, um die Genauigkeit der in-vitro IgE Tests zu verbessern (KAULFURST-SOBOLL et al., 2011; JOSHI et al., 2014).

2.2. Veterinärmedizin

In der Veterinärmedizin bestehen bei der Verwendung von Multi-Allergen Serum Allergen Panels häufig ähnliche diagnostische Unstimmigkeiten wie in der Humanmedizin (LEVY und DEBOER, 2018). Bisher gibt es nur sehr limitierte Daten zu Anti-CCD-IgE und deren Auswirkungen bei Tieren. In 9 von 38 getesteten Serumproben atopischer Hunde wurden Anti-CCD-IgE nachgewiesen, wobei speziell in diesen Proben starke serologische Reaktionen gegen Gräserpollen zu erkennen waren (LEVY und DEBOER, 2018). Glykoproteine z.B. Askorbinsäure, Bromelain und Meerrettichperoxidase weisen vergleichbare Strukturen wie die CCDs an Pollenantigenen auf und haben somit die Fähigkeit Anti-CCD-IgE zu hemmen (BEXLEY et al., 2018). Diese Glykoproteine wurden in Blutproben von 95 Hunden getestet, welche mindestens auf ein Umweltallergen positiv reagierten (BEXLEY et al., 2018). Dabei banden IgE Antikörper in 73 % der Proben an mindestens ein CCD Glykoprotein, erhöhte Reaktionen gegen CCDs waren in 92 % der Proben zu erkennen, welche bei mehreren Grasantigenen positive Ergebnisse

zeigten (BEXLEY et al., 2018). In einer darauffolgenden Pilotstudie wurde bei 31 Sera der Einfluss der Anti-CCD-IgE Hemmung untersucht, wobei eine deutliche Reduktion der positiven Reaktionen vor respektive nach der Inhibition bei Gräsern zu beobachten war, wie in **Tabelle 1** dargestellt (BEXLEY et al., 2018). Bei Allergenen aus der Kräuter-/Milbengruppe hingegen hatte die Hemmung der Anti-CCD-IgE eine geringere Auswirkung auf die Testergebnisse (BEXLEY et al., 2018).

Tabelle 1: Auswirkung der Inhibition von Anti-CCD-IgE auf Allergen-Testergebnisse (Basierend auf den Daten von (BEXLEY et al., 2018))

Allergen	Vor Inhibition der Anti-CCD-IgE	Nach Inhibition der Anti-CCD-IgE
Wiesenrispengras	33 %	7 %
Wiesenlieschgras	71 %	48 %
Beifuß	67 %	61 %
Dermatophagoides farinae	81 %	77 %

Seit kurzer Zeit werden kommerzielle Serumallergietests angeboten, welche Inhibitoren gegen existierende Anti-CCD-IgE einsetzen. Jedoch gibt es noch keine Erkenntnisse darüber, inwieweit diese die Testergebnisse bzw. die Testspezifität beeinflussen, zumal nicht erforscht ist, ob Anti-CCD-IgE bei Tieren zu klinischen Reaktionen führen können.

III. PUBLIKATION 2

Agreement of serum allergen test results with unblocked and blocked IgE against cross-reactive carbohydrate determinants (CCD) and intradermal test results in atopic dogs

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Conflicts of interest

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Short running title Anti-CCD IgE and serum IgE testing

Abstract

Background – Tests for allergen-specific IgE are used to select allergens for immunotherapy in atopic dogs. Antibodies against cross-reactive carbohydrate determinants (anti-CCD IgE) have been identified in serum samples of atopic dogs. Their presence in humans is a known cause of clinically irrelevant polysensitization to plant allergens.

Objectives – To compare the results of an intradermal test (IDT) and a serum test for allergen-specific IgE, with and without blocking anti-CCD IgE, before testing in dogs.

Animals – Thirty-one privately owned dogs with atopic dermatitis.

Methods – Dogs were prospectively skin tested and their serum samples were analysed for anti-CCD IgE. An Fc-ε receptor-based serum test for allergen-specific IgE was performed with and without blocking anti-CCD IgE.

Results – In dogs with negative anti-CCD IgE samples, the agreement between the results of the serum test and the IDT was substantial ($\kappa = 0.71$). Dogs with positive anti-CCD IgE samples (38.7 %) showed no agreement between serum and skin testing ($\kappa = -0.35$), blocking anti-CCD IgE in those samples resulted in a moderate agreement ($\kappa = 0.43$). Anti-CCD IgE positive sera had multiple positive results for grass and weed allergens, blocking decreased them markedly.

Conclusion and clinical importance – Intradermal testing agreed best with serum testing in dogs with no detectable anti-CCD IgE. Sera containing anti-CCD IgE had no agreement with IDT. Test agreement was improved by blocking the anti-CCD IgE. Apparent serum test polysensitization to plant allergens was associated with anti-CCD IgE.

Introduction

Canine atopic dermatitis is a common skin disease in small animal practice.[1] There is no single reliable diagnostic test that could differentiate between atopic dermatitis and other inflammatory or pruritic skin diseases; consequently, the diagnosis is based on history, clinical examination and the exclusion of other differential diagnoses.[2] Allergen testing is not recommended as a diagnostic tool but rather (in combination with the individual dog's history) to identify offending allergens for inclusion in the extract used for allergen immunotherapy (AIT).[3] A major concern of serum tests for canine allergen-specific IgE is their low specificity,[2,4,5] inter-/intralaboratory variability[6] and in vitro crossreactivity,[7] which increases the chance of including irrelevant allergens in the AIT extract. Moreover, a marked discrepancy between intradermal and in-vitro test results has been observed in the past.[8]

Cross-reactive carbohydrate determinants (CCDs) are epitope structures such as the 1,3-fucose on asparagine-linked oligosaccharides of plant and insect glycoproteins.[9] In humans, specific IgE against these glycoproteins has been reported and these anti-CCD IgE antibodies are believed to be a cause of positive in-vitro test results.[9] Anti-CCD IgE antibodies against CCDs in plants and insects for the most part do not seem to have clinical relevance,[10-15] although notable exceptions such as galactose-α-1,3-galactose in red meat and glycan in wheat have been reported.[16-18] One possible reason for the inability to cause clinical symptoms is the monovalent structure of the CCDs, preventing cross-linking and mast cell degranulation.[9,16,19-21]

In veterinary medicine, little is known about the effect of CCDs on serum allergen testing. One previous study reported anti-CCD IgE in the sera of 9/38 atopic dogs.[22] However, neither the influence of those anti-CCD antibodies on test results nor their clinical relevance has been elucidated in dogs. This study aimed to 1.) compare the results of intradermal testing to an in-vitro serum test using the Fc-Ɛ receptor, 2.) evaluate the impact of blocking anti-CCD IgE antibodies, prior to IgE testing, on the agreement between serum and intradermal test results in dogs with such anti-CCD IgE antibodies and 3.) assess the influence of anti-CCD IgE antibodies on the number of positive results against pollen allergens.

Methods and materials

This prospective study was approved by the Ethics Committee of the LMU Munich. Thirty-one client-owned dogs with atopic dermatitis presented to the dermatology service were included.

Patient inclusion criteria

The diagnosis of atopic dermatitis was based on compatible history, physical examination and ruling out potential differential diagnoses including ectoparasites, flea bite hypersensitivity and adverse food reaction. Every patient was clinically examined and the mean pruritus was recorded on a validated visual analog scale.[23] Oral or injectable glucocorticoids and ciclosporin had to be withdrawn at least six weeks prior to intradermal testing. Oral oclacitinib, antihistamines and topical glucocorticoids had to be withdrawn one week prior to intradermal testing.

Intradermal testing

Forty allergen extracts (Artu Biologicals Europe B.V., Lelystad, Netherlands) were administered intradermally. The concentration of the allergens used was 200 Noon Units (NU) for pollen antigens, 100 NU for mite antigens, 1,000 NU/mL for flea antigen and 100 µg/mL for the Malassezia antigen. The amount of allergen extract obtained from 1 gram of raw material is defined as equivalent to 106 Noon Units. Histamine phosphate and the dilution solution of the allergens (phosphate buffered saline solution with 0.47 % Phenol) served as positive and negative controls respectively. If necessary, the dog was sedated with 0.04-0.08 mg/kg of dexmedetomidine (Dexdomitor®, Zoetis GmbH, Berlin, Germany). After 15 and 25 min the test was evaluated subjectively based on erythema, wheal size formation, turgidity and slope of the reaction ranging from 0 (= negative) to 4 (= high reactivity) as previously reported.[24] Reactions graded as ≥ 2 were graded as positive and those graded as ≤ 1 as negative.

Serum testing

Prior to intradermal testing, approximately 10 ml of blood was collected by venipuncture and spun down at 4000 revolutions/min (24900 RCF) for five minutes (centrifuge universal 320 R; Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany). The serum samples were submitted to the Heska diagnostic laboratory (Fribourg, Switzerland) and tested for the presence of anti-CCD IgE (Heska CHO

ELISA test). Briefly, the ELISA well was coated at 4 µg/ml with a combination of plant glycoproteins containing the N-glycan structures (CCDs). The target of the biotinylated recombinant alpha chain of the human high affinity IgE receptor (B-FcεR1α) in the CHO test was the IgE anti-CCD. Samples were diluted 1/6 in the sample diluent buffer (TRIS-saline 0.05 M, pH 7.5 containing 1 % bovine serum albumin). Subsequently, 100 µl of diluted serum was incubated for 30 min at room temperature (RT) and washed; 100 µl B-FcεR1α reagent was used at 1/100 dilution for 15 min at RT and washed. Thereafter, 100 µl of a 1/100 dilution of streptavidin-alkaline phosphatase (Moss Inc., Pasadena, MD, USA) were added and incubated for 15 min at room temperature. After extensive washing (four cycles), 100 µl of para-nitrophenyl phosphate (pNPP) (Moss Inc., Pasadena, MD, USA) was added for 30 min. The enzymatic reaction was stopped with 50 µl of 20 mM L-cysteine and read at 405 nm. Optical densities higher than 0.15 OD were considered positive for the presence of IgE antibodies that bound to CCD epitopes. The OD cut off value was established by associating the OD values with the appearance of multi-positive plant results after running the samples on the panel test.

When sera were tested negative for CCD antibodies, a commercial allergen-specific IgE Fc-ε receptor ELISA with 24 allergens (Heska Allercept panel, Heska AG; Fribourg, Switzerland) was performed. Samples were diluted 1/10 in the sample diluent buffer and 100 µl incubated overnight at 4°C in allergen-coated ELISA wells and washed. One hundred microlitres of B-FcεR1α reagent was used at 1/250 dilution for 1 h at RT and washed; then 100 µl of 1/250 dilution of streptavidin-alkaline phosphatase (Moss Inc., Pasadena, MD, USA) was added and incubated for 30 min at RT. After extensive washing (four cycles) the reaction was revealed with 100 µl of pNPP (Moss Inc., Pasadena, MD, USA) for 45 min. The enzymatic reaction was stopped with 50 µl of 50 mM L-Cysteine. The reaction was read at 405 nm. Optical densities for each allergen were converted to HERBU (Heska Epsilon Receptor Binding Unit). HERBU values for each allergen were extrapolated from an IgE standard curve which was run for each panel test. The results were reported in five classes (negative to class 4) and classes 2-4 were considered positive.

When sera were positive for CCD antibodies, they were divided in two aliquots. One aliquot of the anti-CCD IgE positive sera was tested without blocking anti-CCD antibodies. The other aliquot was tested after it was mixed with a Heska

proprietary blocking solution (CHO-blocker) which inhibits binding of anti-CCD IgE to the plant allergens used in the test. The CHO-blocker reagent was specifically designed to be used in veterinary samples and contained a mix of plant glycoproteins which are not derived from any of the allergens or allergen families tested in the panel.

Statistical analysis

Statistical analysis was performed using commercial statistics software (GraphPad prism 6.0, GraphPad Software Inc., San Diego, CA, USA). Descriptive data was summarized. Allergens were grouped into seasonal allergens (grasses, weeds and tree pollen), perennial allergens (mites) and others. The allergens tested with both methods (serum and intradermal testing) are listed in **table 1**.

Table 1. Allergens tested with both tests (intradermal and serum panel test)

Mites	
Dermatophagoides pteronyssinus	House dust mite
Dermatophagoides farinae	House dust mite
Tyrophagus putrescentiae	Mold mite
Acarus siro	Grain/flour mite
Grasses	
Dactylis glomerata	Orchard grass
Cynodon dactylon	Bermuda grass
Poa pratensis	Kentucky bluegrass
Lolium perenne	Perennial ryegrass
Holcus lanatus	Velvet grass
Weeds	
Artemisia vulgaris	Mugwort
Chenopodium album	White goosefoot
Plantago lanceolata	Buckhorn plantain/ ribwort
Rumex acetosella	Field sorrel/common sheep sorrel
Ambrosia artemisifolia	Common ragweed
Trees	
Corylus avellana	Hazel
Others	
Flea saliva	
Malassezia	

The reactions to each allergen in both tests were compared (discrepancy versus match) and rated as follows:

(1) Positive disagreement: serum allergen testing was positive, IDT negative.

(2) Negative disagreement: serum allergen testing was negative, IDT positive.

(3) Concordant positive: both tests were positive.

(4) Concordant negative: both tests were negative.

A dog was considered to be polysensitized, when the majority of reactions in each subgroup (at least three of four mites, three of five grasses or three of five weeds) were positive. The number of allergens in the groups “Trees” and “Others” measured in both tests was too low for this analysis, they were therefore not investigated.

Agreement between the two tests was measured with Cohen’s kappa, values < 0 indicating no agreement, 0–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–1 almost perfect agreement.[25] The impact of anti-CCD IgE on allergen reactions in serum testing was evaluated using a two tailed Fisher exact test and p=0.05 was set as significance level. For this purpose the reactions of one representative allergen in each allergen subgroup were analyzed in the anti-CCD IgE positive sera in comparison to the inhibited sera.

Results

A total of, 31 dogs, 17 female (seven intact, ten spayed) and 14 male (nine intact, five neutered) were included. The mean age was four years (range 1-11 years), 13 different breeds and mixed breeds were represented. In 26 dogs, the clinical signs developed during the first 24 months of life; the onset of disease in the other five dogs was unknown. On the day of sampling and intradermal testing, the mean pruritus was 7 ± 3. In 12/31 (38.7 %) of the dog sera anti-CCD antibodies were present, whereas the other 19 (61.3 %) had no detectable anti-CCD IgE. There was no obvious seasonal difference between the number of dogs with and without present anti-CCD IgE. The discrepancies and matches for all evaluated allergens in both tests in each of the three aliquots (anti-CCD IgE negative, anti-CCD IgE positive and anti-CCD IgE inhibited) are illustrated in **figure 1**.

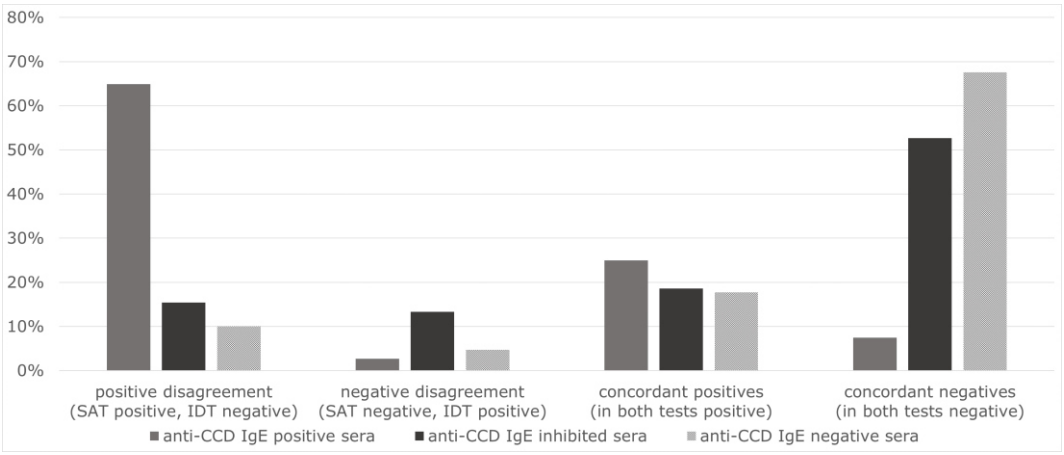

Figure 1. Total amount of test result discrepancies and matches of all allergens included in both serum allergen testing (SAT) and intradermal testing (IDT) of samples with anti-CCD IgE prior to (positive) and after blocking (inhibited), as well as sera without anti-CCD IgE (negative).

Anti-CCD IgE negative dogs

In the 19 dogs with no anti-CCD IgE, a total of 299 comparable test results of the intradermal and Fc-ε receptor tests could be evaluated. The majority (255 reactions, 85.3 %) were concordantly positive or negative in both tests, whereas 44 reactions (14.7 %) showed differing results in the two tests. The Cohen's kappa test demonstrated a substantial agreement (κ = 0.71). The subgroups of the Cohen's kappa test results of each of the specimens are summarized in **table 2**. The agreement of the two tests was substantial with grass allergens and almost perfect with weed allergens, while mite allergens had a fair agreement.

Table 2. Cohen Kappa (κ) agreement for each subgroup of allergens*

Subgroup	Anti-CCD IgE negative samples	Anti-CCD IgE positive samples	Anti-CCD IgE inhibited samples
Mites	0.39	0.17	0.25
Grasses	0.71	- 0.67	0.37
Weeds	0.87	- 0.05	0.53

* Values < 0 indicate no agreement, 0–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–1 almost perfect agreement.

Anti-CCD IgE positive dogs

In the 12 serum samples containing anti-CCD IgE, 188 reactions could be compared to the intradermal test. Sixty-one reactions (32.4 %) were in agreement for both tests, 122 (64.9 %) of positive reactions in the serum test showed a lower or no reactivity on intradermal testing, whereas 5 (2.7 %) had higher reactivity on the IDT. There was no agreement between the two tests (κ = -0.35).

Anti-CCD IgE positive samples tested after addition of blocking solution

The 12 serum samples described above were treated with an anti-CCD IgE blocking solution before testing was repeated. Concordant results were observed with 134 reactions (71.2 %). The percentage of positive serum test reactions in the face of lower or negative intradermal test reactions decreased to 15.4 %, leading to a moderate agreement (κ = 0.43), although an increase of lower or negative reactions (to 13.3 %) in comparison to higher reactions on IDT was observed. The agreement of the two tests was moderate with weed, but only fair with grass and mite allergens.

Evaluation of all samples

In total, 487 reactions were evaluated. The Cohens Kappa between serum and intradermal testing in all samples (negative and anti-CCD IgE positive sera), without blocking the anti-CCD IgE, showed only fair agreement (κ = 0.28). When combining the results of samples after blocking, i.e. those samples with anti-CCD antibodies with results of samples from dogs without anti-CCD antibodies, there was a moderate agreement (κ = 0.59).

Multiple positive test results

Serum test results were analyzed for polysensitization as demonstrated in **table 3**. Anti-CCD IgE negative sera showed multiple positive reactions only with mite allergens, whereas grass and weed allergens had no polysensitization. Dog sera with anti-CCD IgE had a high percentage of polysensitization in all subgroups. In contrast, sera treated with the blocking solution had a much lower rate of such multiple positive reactions. Dogs without anti-CCD IgE in serum revealed multiple

positive reactions on IDT in 11/19 (57.9 %) for mite allergens and none in the other subgroups. Of the dogs with anti-CCD IgE, 6/12 had multiple positive reactions on IDT in the mite allergen group, one dog with grass allergens and 2/12 showed polysensitization with weed allergens.

Table 3. Polysensitization with serum testing for allergen-specific IgE of atopic dogs*

Subgroup	Anti-CCD IgE negative samples	Anti-CCD IgE positive samples	Anti-CCD IgE inhibited samples
Mites	68.4%	91.7%	66.7%
Grasses	0.0%	100.0%	25.0%
Weeds	0.0%	75.0%	0.0%

* Values ≥ 2 were considered positive test results. If the majority of reactions in each subgroup (at least three of four mites, three of five grasses or three of five weeds) were positive, these were rated as multiple positive reactions.

Agreement between blocked and unblocked serum

The evaluation of the serum allergen test prior and post blocking the anti-CCD IgE antibodies, showed no agreement (κ = -0,208); 288 reactions in five classes (four mites, two others, seven grasses, six weeds and five trees) were analyzed and the effect of the anti-CCD IgE was especially seen with grass, weed and tree allergens. There was no significant difference in positive/negative reactions for Dermatophagoides farinae after inhibition of anti-CCD IgE compared to initial testing (p= 1.0000). In contrast, a highly significant difference in positive reactions for plant antigens (grasses, weeds and trees) was observed after blocking of anti-CCD IgE in comparison to the test results without blocking (p= 0.0046 for Dactylis glomerata, p= 0.0003 for Rumex acetosella and p= 0.0001 for Fraxinus sp.).

Discussion

To the best of the authors' knowledge, this is the first study evaluating the impact of blocking anti-CCD IgE antibodies on the results of serum tests for allergen-specific IgE compared to intradermal test results. It showed a much better agreement of the two tests after blocking the anti-CCD IgE and decreased the number of polysensitized animals markedly with this procedure.

Both tests, IDT and serum testing for allergen-specific IgE, are not reliable for diagnosing atopic dermatitis and differentiating dogs with this disease from normal dogs. Their interpretation is difficult as the reactivity does not necessarily correlate with the clinical severity.[26] Furthermore, polysensitization renders the correct selection of relevant allergens for immunotherapy difficult, particularly in Europe, where only a small number of allergens is typically included in a vial of allergen extract. The plant allergens used in the ELISA plate coating contain CCD epitopes. When a serum sample positive for anti-CCD IgE is tested, the binding of those anti-CCD IgE antibodies to CCD epitopes could lead to positive reactions. In these cases, polysensitization to plant allergens is observed. As a result, it is more difficult to identify the "true" offending allergens. In this study the inhibition of antibodies against CCDs markedly decreased the number of polysensitized dogs to plant allergens and also markedly increased the agreement between intradermal and serum allergen testing. Similar results were seen in humans where the application of a CCD blocker also resulted in much lower read-out-values and the correlation of skin tests, history and laboratory results was much better.[10] Even after blocking of anti-CCD antibodies, positive reactions to some plant pollens were still present. In addition the correlation with intradermal testing improved, indicating that blocking did not eliminate all IgE directed against plant antigens.

In this study, 38.7 % of the dogs' sera had anti-CCD IgE antibodies. In a previous study anti-CCD IgE was detected in only 24 % of the examined dogs.[22] Those numbers coincide with the prevalence in humans, where approximately 22-35 % of allergic patients possess IgE against CCD.[10,14] Differences between blood sample collection dates were not observed and too few dogs were included in winter compared to other seasons to perform a statistical evaluation. More research on the prevalence of such antibodies in atopic dogs, as well as their prevalence in healthy dogs and dogs with non-atopic skin diseases, such as parasite infestations, is needed and may shed more light on predisposing factors for and the pathogenetic mechanisms of the production of anti-CCD IgE.

The agreement of the serum test for allergen-specific IgE and the intradermal test was the highest in dogs whose sera had no anti-CCD IgE, followed by those where the antibodies against CCDs were blocked prior to serum testing. The least agreement was found in the sera positive for anti-CCD IgE which were processed without blocking those antibodies. For grass and weed pollens,

correlation after blocking anti-CCD IgE was high and thus the measurement of allergen specific IgE is a good alternative to IDT in those dogs. With mite antigens, blocking of anti-CCD IgE did not result in a better correlation.

Despite the low number of dogs with circulating anti-CCD IgE, the evaluation of all samples (negative and positive sera together) showed their marked influence on the test results, if these antibodies are not blocked. Therefore an inhibitor substance should be used in serum allergen tests that depend on natural derived allergen extracts or components, comparable to human allergy diagnostic tests,[10] although the agreement of the blocked sera was not perfect. Possibly anti-CCD IgE are only one reason for clinically irrelevant sensitization. Another aspect could be that the technique of the test needs to be further improved. Finally, non-IgE-based immunological mechanisms could lead to clinical atopic dermatitis independent of IgE production.

Polysensitization was investigated in each subgroup. For mites, positive reactions only showed minor changes after blocking anti-CCD IgE, indicating that the reason for polysensitization to mite allergens in dogs was not predominantly due to anti-CCD IgE. Similarly, in humans blocking anti-CCD IgE reduced the majority of multiple positive reactions, but not those to mite antigens.[10] Arthropods contain few or no CCDs and thus are not associated with anti-CCD IgE.[10,22,27,28] With grass and weed allergens, the inhibition of anti-CCD IgE led to a marked decrease of polysensitization. As grasses and weeds share partially identical carbohydrate structural units of their glycoproteins (which are not present in mammals), anti-CCD IgE can be produced against those antigens.[29] In human medicine, anti-CCD IgE antibodies were assumed to not contribute to clinical signs of hypersensitivity diseases.[10-15] CCDs are monovalent and thus cannot crosslink IgE antibodies and subsequently cannot lead to mast cell degranulation.[9,19-21] In contrast to the previous findings, in humans the presence of IgE against galactose-α-1,3-galactose in red meat was reported to result in severe clinical reactions.[16,17] Another study showed that IgE against gliadin in wheat led to greater allergenicity in wheat-allergic symptomatic children compared to non-exposed or asymptomatic individuals,[18] implying that the assumption that those antibodies have no clinical impact is not applicable for all CCDs. In the dog, the presence of anti-CCD IgE did not lead to polysensitization with intradermal testing, which may indicate they are clinically irrelevant in canine atopic dermatitis. If CCDs are involved in the

pathogenesis of atopic dermatitis via other mechanisms then this needs to be further elucidated.

A limitation of this study was the small number of concordant allergens in both tests. For this reason, some allergens such as for example tree allergens were not evaluated. In addition, test results need to be correlated to the clinical history of the animal. In this study, this was not always possible as the extended history of some of the cases was unknown, seasonality of clinical signs could sometimes not be determined due to the young age and recent onset of signs in some of the dogs, some owners were unaware of the change of clinical signs during the year and finally in some dogs constant drug administration complicated judging seasonality.

This preliminary study has shown that the high percentage of positive reactions in the evaluated serum test for allergen-specific IgE was associated with anti-CCD IgE antibodies and that agreement with intradermal testing could be markedly enhanced by blocking those antibodies. Moreover it facilitates selection of allergens for AIT and is more reliable than routine serum testing with no inhibition of anti-CCD IgE. However, for both testing methods, results should be interpreted in the light of the dog's clinical history.

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IV. DISKUSSION

Tierärzte stehen häufig vor dem Problem, wie Testergebnisse zu interpretieren sind, besonders wenn diese stark variieren. Ähnlich wie in der Humanmedizin (MARI et al., 1999; EBO et al., 2004; HOLZWEBER et al., 2013) konnte in dieser Studie gezeigt werden, dass Anti-CCD-IgE ein Grund für die große Diskrepanz zwischen Intrakutan- und Serumallergietestergebnissen sind.

Säugetiere erkennen CCD Epitope an Pollenallergenen als “Fremd-Antigen“ und können daher mit einer humoralen Immunantwort reagieren (LEVY und DEBOER, 2018). Warum jedoch bestimmte Individuen im Gegensatz zu anderen Anti-CCD-IgE entwickeln ist bisher ungeklärt. Die Prävalenz von Anti-CCD-IgE bei atopischen Hunden betrug in dieser Studie 38,7 % und war damit höher als in der Studie von Levy und DeBoer (2018), bei der 24 % der untersuchten atopischen Hunde Anti-CCD-IgE aufwiesen. Dies kann daran liegen, dass unterschiedliche Tests und dementsprechend unterschiedliche Nachweisverfahren verwendet wurden. Eine noch nicht veröffentlichte Studie hat in Serumproben bei 14,5 % (7/48) der gesunden Hunde und 16,8 % (17/101) der atopischen Hunde Anti-CCD-IgE festgestellt (PICCIONE und DEBOER, 2019). Es werden weitere Studien mit einer größeren Anzahl an gesunden Hunden, Atopikern und Hunden mit anderen Krankheiten benötigt, um herauszufinden, womit die Entstehung von Anti-CCD-IgE zusammenhängt.

Die Übereinstimmung der Testergebnisse war am besten bei Anti-CCD-IgE negativen Proben, wobei die Hemmung von Anti-CCD-IgE positiven Proben nur zu einer moderaten Übereinstimmung zwischen Intrakutan- und Serumallergietestergebnissen führte. Es gibt einige Substanzen, welche Anti-CCD-IgE binden können, aber nicht alle sind dafür geeignet, da sie mit unterschiedlicher Affinität Anti-CCD-IgE hemmen. Die Lösung, die in dem Serumallergietest dieser Studie verwendet wurde, wurde eigens für veterinärmedizinische Proben entwickelt und enthält eine Mischung aus verschiedenen Substanzen. An der optimalen Substanz bzw. Mischverhältnis zur Hemmung von Anti-CCD-IgE wird weiterhin geforscht.

In der Humanmedizin waren sich Wissenschaftler lange Zeit uneinig, inwieweit Anti-CCD-IgE eine klinische Bedeutung haben, jedoch gibt es bisher keinen

einzigen Bericht über eine klinische Reaktion ausgelöst durch Anti-CCD-IgE gegen Pollenantigene. In dieser Arbeit bewirkten die auf Anti-CCD-IgE zurückzuführenden positiven Ergebnisse im Serumallergietest keine Hautreaktionen im IKT. Auch beim Menschen konnten die durch Anti-CCD-IgE bedingten positiven Ergebnisse im Serumallergietest nicht im Haut-Pricktest repliziert werden (MARI, 2002). Da das Prinzip des IKT auf einer Allergen-spezifischen IgE-mediierten Mastzelldegranulation beruht, welche als klinischer Beweis einer Typ-I-Hypersensitivität angesehen wird, führt dies zu der Annahme, dass Anti-CCD-IgE gegen Pollenantigene bei Hunden keine klinische Relevanz haben. Jedoch ist nicht auszuschließen, dass Anti-CCD-IgE gegen bestimmte Allergene zu klinischen Reaktionen führen können, die nicht auf einer Mastzelldegranulation beruhen.

Die Inhibition der Anti-CCD-IgE hatte keine signifikante Auswirkung auf die positiven Ergebnisse in der Milben-Untergruppe im SAT. Milben haben nur wenige bis keine CCD Epitope und somit kann z.B. bei einer Population mit hoher Milbensensibilisierung und sehr geringer Sensibilisierung gegen Pollen die Häufigkeit der Anti-CCD-IgE nur bei 4,7 % sein (VIDAL et al., 2012). Andererseits ist es möglich, dass Milben andere Epitopstrukturen enthalten und daran bindende IgE nicht mit der Inhibitionslösung blockiert wurden. Ein weiterer Grund kann die häufige Co-Sensibilisierung gegen verschiedene Milben bzw. die hohe Kreuzreaktivität der Milbenbestandteile sein. Spezifische IgE gegen *Dermatophagoides pteronyssinus* (Der p) 2 und Der f 2 sind fast vollständig kreuzreaktiv, wohingegen bei *Lepidoglyphus destructor* (Lep d) 2 keine Kreuzreaktion beschrieben ist (BARBER et al., 2012). In einer Untersuchung bei Menschen waren 32,7 % der Patienten gegen mindestens ein Milben-spezifisches Molekül (Der p 1,2, Der f 1,2) sensibilisiert (PANZNER et al., 2018). Die Mehrheit der Patienten wiesen Co-Sensibilisierungen gegen verschiedene Moleküle des betreffenden Allergenursprungs auf, was darauf hindeutet, dass Co-Sensibilisierungen bei Milben eine große Bedeutung haben (PANZNER et al., 2018).

Grundsätzlich muss zwischen einer primären Sensibilisierung und einer immunologischen Kreuzreaktivität bei multiplen Sensibilisierungen unterschieden werden (PANZNER et al., 2018). Kreuzreaktionen aufgrund von Strukturgleichheiten der Proteine benötigen eine mindestens zu 70 % identische

Sequenz, wohingegen bei weniger als 50 % identischen Sequenzen Kreuzreaktionen sehr selten auftreten (AALBERSE et al., 2001; FERREIRA et al., 2004). Da auslösende Allergene gemieden werden sollten, wäre es unbedingt nötig zu wissen, welche Allergene miteinander kreuzreagieren (PANZNER et al., 2018).

Inwieweit eine Hemmung von Anti-CCD-IgE zu einer besseren Korrelation zwischen Serumallergietestergebnissen und der Klinik des jeweiligen Patienten führt, ist unbekannt. Des Weiteren muss untersucht werden, ob die Auswahl relevanter Allergene basierend auf den Ergebnissen eines Serumallergietests mit Hemmung von Anti-CCD-IgE das Ansprechen auf eine AIT verändert. Die Korrelation zwischen Intrakutantestergebnissen und der Klinik des Patienten ist nur gering (MALLMANN, 2017). Es ist jedoch davon auszugehen, dass dies gleichermaßen auf die Serumallergietestergebnisse zutrifft, da die canine AD auf verschiedenen immunologischen Reaktionen beruht, wie etwa eine Lymphozyten-abhängige Immunantwort (MARSELLA et al., 2012; PUCHEU-HASTON et al., 2015b). Dementsprechend sind Tests zum Nachweis einer IgE-mediierten Immunantwort nur bedingt geeignet, weil sie nur einen Teil des allergischen Geschehens widerspiegeln.

Zudem zweifeln einige Humandermatologen die Notwendigkeit an, eine AIT exakt auf den individuell betroffenen Patienten anzupassen (THOMAS, 2012). Auch in der Veterinärmedizin zeigte z.B. eine Studie, dass 59 von 103 atopischen Hunden auf eine regional spezifische Immuntherapie hervorragend oder gut ansprachen; Nebenwirkungen wurden bei 7 von 286 behandelten Hunden festgestellt (PLANT und NERADILEK, 2017). Jedoch ist noch nicht geklärt, inwieweit klinisch relevante Sensibilisierungen durch eine Desensibilisierung mit Allergenen, welche für den Patienten kein Problem darstellen, entwickelt werden können. Daher ist die aktuelle Überzeugung, dass eine an den jeweiligen Patienten individuell angepasste AIT am effektivsten und sichersten ist.

Eine Limitierung dieser Studie war, dass im IKT Allergenextrakte verwendet wurden, welche eine andere Ursprungsquelle hatten, als jene, welche im ELISA eingesetzt wurden. Generell stellt die mangelnde Standardisierung von Allergenextrakten ein Problem dar, weil beim Testen nicht sichergestellt ist, dass jeweils die gleiche Allergenkonzentration bzw. der gleiche Allergengehalt verwendet wird. Dies ist sowohl von der natürlichen Variabilität der

Allergenquelle, als auch vom Herstellungsprozess abhängig (PANZNER et al., 2018), wie bereits bei Hausstaubmilbenallergenen festgestellt wurde (BRUNETTO et al., 2010; CASSET et al., 2012; TAKAI et al., 2015). Nicht nur für die Diagnostik, sondern auch für den therapeutischen Nutzen wäre es wichtig, den genauen Allergengehalt zu kennen (PANZNER et al., 2018).

Zusammenfassend hat diese Studie gezeigt, dass die Diskrepanz zwischen Serumallergietest und Intrakutantestergebnissen durch die Inhibition von Anti-CCD-IgE signifikant reduziert wurde. Bei Patienten mit hochpositiven Serumtestergebnissen bedeutet dies, dass sofern Anti-CCD-IgE vorhanden sind, eine Testwiederholung mit einem CCD Inhibitor sinnvoll ist. Gerade für praktizierende Tierärzte, welche nicht die Möglichkeit haben, einen Intrakutantest durchzuführen bzw. Patienten dafür an einen Spezialisten zu überweisen, stellt der in dieser Studie verwendete Serumallergietest mit CCD Inhibition eine gute Alternative dar. Die Zuverlässigkeit von weiteren Allergietests mit anderen Anti-CCD-IgE Blocksystemen muss in klinischen Studien evaluiert werden, bevor darüber eine Aussage getroffen werden kann. Da jedoch beide Testverfahren (sowohl IKT, als auch SAT) nur eingeschränkt aussagekräftig (spezifisch/sensitiv) sind, sollte die Interpretation der Testergebnisse auch weiterhin nur im Zusammenhang mit der Historie und Klinik des Patienten erfolgen und nicht zur Diagnostik einer Allergie verwendet werden.

V. ZUSAMMENFASSUNG

Kreuzreagierende Kohlenhydrat Bestandteile und deren Einfluss auf IgE Serumallergietests bei atopischen Hunden

Tests zum Nachweis von Allergen-spezifischen IgE Antikörpern dienen als Grundlage zur Auswahl relevanter Allergene für eine Immuntherapie bei atopischen Hunden. Kürzlich wurden in Serumproben von atopischen Hunden IgE Antikörper gegen kreuzreaktive Kohlenhydratbestandteile (Anti-CCD-IgE) gefunden. Deren Existenz bei Menschen ist eine bekannte Ursache für klinisch irrelevante Polysensibilisierung. Das Ziel dieser Arbeit war die Evaluierung der Ergebnisse eines Serumallergietests vor und nach Hemmung von Anti-CCD-IgE im Vergleich zu den Ergebnissen eines Intrakutantests (IKT). Bei 31 atopischen Hunden wurde prospektiv ein IKT durchgeführt, Blut entnommen und ein Fc-ε-Rezeptor basierter Serumtest für Allergen-spezifisches IgE durchgeführt. Die Serumproben wurden zusätzlich auf Anti-CCD-IgE analysiert und bei deren Vorhandensein wurde der Serumallergietest nach Blocken der Anti-CCD-IgE wiederholt. Die Übereinstimmung zwischen den Serum- und Intrakutantestergebnissen wurde mithilfe des Cohen-Kappa Tests ausgewertet. Bei Hunden ohne nachgewiesenes Anti-CCD-IgE war die Übereinstimmung zwischen den Haut- und Serumallergietestergebnissen substantiell ($\kappa = 0{,}71$). Tiere mit Anti-CCD-IgE (38,7 %) zeigten keine Übereinstimmung ($\kappa = -0{,}35$); die Hemmung der Anti-CCD-IgE in diesen Proben führte zu einer moderaten Übereinstimmung ($\kappa = 0{,}43$). Anti-CCD-IgE positive Sera hatten multiple positive Ergebnisse bei Gräser- und Kräuterallergenen, die Reaktionen waren nach der Hemmung von Anti-CCD-IgE deutlich reduziert. Intrakutantest- und Serumallergietestergebnisse korrelierten am besten bei Proben ohne Anti-CCD-IgE. In positiven Sera bewirkten Anti-CCD-IgE multiple positive Reaktionen im Serumallergietest, die durch den IKT nicht bestätigt wurden. Durch eine Hemmung der Anti-CCD-IgE wurde eine bessere Übereinstimmung erreicht. Polysensibilisierungen auf Pflanzenallergene wurden zum Großteil durch Anti-CCD-IgE verursacht.

VI. SUMMARY

Cross-reactive carbohydrate determinants and their influence on IgE-serum allergy testing in atopic dogs

Tests for allergen-specific IgE are used to select allergens for immunotherapy in atopic dogs. Recently, antibodies against cross-reactive carbohydrate determinants (anti-CCD IgE) were identified in serum samples of atopic dogs. Their presence in humans is a known cause of clinically irrelevant polysensitization. This study aimed to compare the results of an intradermal test (IDT) and a serum test for allergen-specific IgE with and without inhibited anti-CCD IgE. Thirty-one privately owned dogs with atopic dermatitis prospectively underwent intradermal allergy testing and had their serum samples analysed for anti-CCD IgE. An Fc-ε receptor-based serum test for allergen-specific IgE was performed with and without blocking anti-CCD IgE. The agreement between the different tests was analysed with Cohen's Kappa. In dogs with negative anti-CCD IgE samples, the agreement between the results of the serum test and the IDT was substantial ($\kappa = 0.71$). Dogs with positive anti-CCD IgE samples (38.7 %) showed no agreement between serum and skin testing ($\kappa = -0.35$), blocking anti-CCD IgE in those samples resulted in a moderate agreement ($\kappa = 0.43$). Anti-CCD IgE positive sera had multiple positive results for grass and weed allergens, blocking decreased these markedly. These results indicated that intradermal testing correlated best with serum testing in dogs with no detectable anti-CCD IgE. Sera containing anti-CCD IgE had multiple positive reactions on serum testing and no agreement with IDT. This was improved by blocking the anti-CCD IgE. Apparent serum test polysensitization to plant allergens was caused by anti-CCD IgE.

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VIII. ANHANG

Übersicht der Studienpatienten

Patient	Rasse	Geschlecht	Alter in Jahren	Saisonalität	Anti-CCD-IgE
1	Chihuahua	weiblich	2	im Winter schlechter	negativ
2	Pinscher	männlich kastriert	4	im Winter schlechter	negativ
3	Schäferhund	männlich	4	nicht saisonal, schubweise	negativ
4	Bracco Italiano	weiblich kastriert	5	im Winter schlechter	negativ
5	Labrador	männlich	8	nicht saisonal, schubweise	negativ
6	Terrier Mischling	weiblich kastriert	1	nicht saisonal, schubweise	positiv
7	Schäferhund	männlich	3	im Winter schlechter	positiv
8	Mischling	weiblich	3	nicht saisonal, schubweise	positiv
9	Golden Retriever	weiblich	4	nicht saisonal, schubweise	positiv
10	Labrador	männlich	1	saisonal (März - Juli)	negativ
11	Mischling	weiblich kastriert	3	saisonal (März - Oktober)	positiv
12	Französische Bulldogge	männlich kastriert	1	saisonal (März - August)	positiv
13	Border Collie	weiblich	3	saisonal (Juli - Oktober)	positiv
14	Mischling	männlich kastriert	9	Verschlechterung im Frühling - Herbst	negativ
15	Französische Bulldogge	weiblich	1	Verschlechterung im Frühling - Herbst	negativ
16	Rhodesian Ridgeback	weiblich	2	Verschlechterung im Sommer	negativ
17	Labrador	männlich	2	Verschlechterung im Sommer	negativ
18	Terrier Mischling	männlich kastriert	3	Verschlechterung im Frühling - Sommer	negativ
19	Dackel	weiblich kastriert	9	Verschlechterung im Herbst	negativ
20	Französische Bulldogge	weiblich	1	Verschlechterung im Frühling - Herbst	negativ
21	Mischling	weiblich kastriert	4	Verschlechterung im Frühling - Sommer	negativ
22	Labrador	männlich	6	Verschlechterung im Frühling - Herbst	positiv
23	Retriever Mischling	männlich	6	Verschlechterung im Sommer - Herbst	positiv
24	Weißer Schäferhund	männlich kastriert	11	ganzjährig ohne saisonale Verschlechterung	negativ
25	Labrador Mischling	männlich	6	ganzjährig ohne saisonale Verschlechterung	negativ
26	Mops	weiblich kastriert	2	ganzjährig ohne saisonale Verschlechterung	negativ
27	Cavalier King Charles Spaniel	weiblich kastriert	5	ganzjährig ohne saisonale Verschlechterung	negativ
28	Golden Retriever	männlich	7	ganzjährig ohne saisonale Verschlechterung	negativ
29	Mischling	weiblich kastriert	2	ganzjährig ohne saisonale Verschlechterung	positiv
30	Mischling	weiblich kastriert	6	ganzjährig ohne saisonale Verschlechterung	positiv
31	West Highland White Terrier	weiblich kastriert	4	ganzjährig ohne saisonale Verschlechterung	positiv

Es konnte kein saisonaler Unterschied hinsichtlich des Zeitpunktes der Serum-Probenentnahme zwischen den Hunden mit und ohne Anti-CCD-IgE festgestellt werden.

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