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ORIGINAL RESEARCH

Cross-reactive carbohydrate determinants in atopic and healthy dogs and their influence on allergy test specificity

Bettina Kasper[1] | **Teresa Boehm**[1] | **Nadine Wittenstein**[2] | **Ralf S. Mueller**[1]

[1]Center of Clinical Veterinary Medicine, LMU Munich, Munich, Germany

[2]Tierklinik Germering, Germering, Germany

Correspondence
Ralf Mueller, Centre of Clinical Veterinary Medicine, LMU Munich, Veterinaerstr. 13, Munich 80539, Germany.
Email: R.Mueller@medizinische-kleintierklinik.de

Present address
Teresa Boehm, Tierklinik Stuttgart Plieningen, Hermann-Fein-Str. 15, 70599, Stuttgart-Plieningen, Germany
Nadine Wittenstein, filu Tierarztpraxis, Munich, Germany

Abstract

Background: The selection of allergens for immunotherapy in atopic dogs is often based on serum allergy testing. Cross-reactive carbohydrate determinants (CCDs) are common structures in plant and insect allergens that reportedly induce polysensitisation, reduce agreement between intradermal and serum tests and complicate allergen selection.

Methods: Thirty-four dogs with diagnosed atopic dermatitis and 10 healthy dogs were included in the study. An intradermal test was conducted in atopic dogs, and serum samples from allergic and healthy dogs were analysed for allergen-specific immunoglobulin E (IgE) before and after inhibition of detectable anti-CCD-IgE antibodies.

Results: Anti-CCD-IgE antibodies were not found in any of the healthy dogs and no polysensitisation to plant and insect allergens was detected. The agreement between intradermal and serum allergy test results in the atopic dogs with anti-CCD-IgE antibodies improved from slight to fair after blocking the anti-CCD-IgE antibodies. In addition, blocking clearly reduced polysensitisation to plant allergens but not to acarid allergens.

Limitations: Only a limited number of healthy dogs were tested in this study. A gold standard for determining the clinical relevance of IgE sensitisation does not exist.

Conclusion: Inhibition of anti-CCD-IgE antibodies seems to be of importance to improve serum test specificity for allergen-specific IgE in atopic dogs in relation to intradermal allergy testing.

INTRODUCTION

Atopic dermatitis (AD) caused by environmental allergens is a common skin disease in small animal practice.[1,2] Once the diagnosis of canine AD is confirmed, treatment can be either symptomatic or causal. The only option for causative therapy is allergen-specific immunotherapy (AIT).[3–5] Appropriate allergens for AIT are selected based on history and skin or serum testing for allergen-specific immunoglobulin E (IgE).[6] Serum-based IgE testing is widely used, but a challenging problem arises when the positive test reactions do not correlate with the patient's history.[7] The low specificity and sensitivity of in vitro IgE tests in relation to clinical relevance are recognised worldwide,[6,8] as is the fact that serum test results are often inconsistent with those of intradermal testing.[9] These discrepancies may be partly explained by the presence of cross-reactive carbohydrate determinants (CCDs).

CCDs are carbohydrate components of glycoproteins commonly found on the cell surface of different plants and insects, such as β-1,2-xylose or α-1,3-fucose bound to asparagine moieties of a protein. These specific N-glycans do not occur in mammals and form common antigenic epitopes. Consequently, polysensitisation is induced in some patients.[10–13]

In human medicine, anti-CCD-IgE antibodies typically do not cause clinical reactions but lead to false-positive results in serum allergy tests.[14–18] One possible explanation for their clinical irrelevance is their monovalent character, which does not enable cross-linking of IgE on mast cells with subsequent degranulation.[13,19,20]

Little is established about the role of CCDs in dogs[7,21–25] and cats.[23,26] Recently, anti-CCD-IgE antibodies were detected in 17%–39% of atopic dogs,[7,21,22] and in 13% of healthy dogs.[7] Blocking anti-CCD-IgE in the serum of atopic dogs resulted in a reduction of the multiple positive pollen test results.[22–25] Two

recent studies in veterinary medicine looked at the agreement between skin and serum tests before and after inhibition of anti-CCD-IgE antibodies in sera from atopic dogs.[22,25] In the first study, blocking of anti-CCD-IgE prior to serum testing clearly improved the correlation between skin and serum results.[22] In contrast, another study with a larger number of cases using a different serum IgE test and different anti-CCD-IgE-blockers could not detect any improvement in the correlation between testing procedures before and after blocking IgE antibodies specific for CCDs.[25]

The main purpose of this study was to evaluate the agreement between intradermal and serum IgE test results in atopic dogs before and after blocking IgE directed against CCDs using a third serum test and blocking agent known as NEXT+ (Nextmune AB). This second-generation blocker is composed of a purified CCD linked to human serum albumin for stabilisation. The secondary aim was to find out whether polysensitisation to plant and insect allergens in CCD-positive sera from AD dogs is reduced by inhibition. In addition, normal dog sera were also evaluated for anti-CCD-IgE.

MATERIALS AND METHODS

This prospective and blinded study was approved by the Ethics Committee of the University of Munich, part 1 (atopic dogs) under the number 204-04-03-2020 and part 2 (normal dogs) under the number 257-26-02-2021. The study protocol was completed before data collection started. Owners were informed about and consented to the study prior to inclusion. Patients meeting the study criteria were enrolled and samples were obtained and tested in the Dermatological Departments of the Center of Clinical Veterinary Medicine, LMU Munich and the Veterinary Clinic in Germering from February 2020 to August 2021. Thirty-four dogs with diagnosed AD and 10 healthy dogs without a history of allergic disease were included in the study.

Study subjects

The diagnosis of patients with AD associated with environmental allergens was based on a combination of history, clinical presentation and exclusion of other differential diagnoses, such as flea bite sensitivity, food-induced AD, ectoparasite infestation and/or secondary infection. Most of the differential diagnoses were ruled out by simple diagnostic tests and methods, including flea control, skin scrapings, cytology and/or trichograms. Food-induced AD was determined by following a strict elimination diet for 6–8 weeks, which was either home-cooked (consisting of ingredients the patient had never been exposed to before) or a commercially available diet containing fully hydrolysed proteins (such as Anallergenic, Royal Canin or z/d, Hill's). If a complete resolution of symptoms was achieved in this food trial, provocation by the previous food either confirmed or ruled out the diagnosis of food-induced AD. If partial improvement was observed with the implemented dietary change and subsequent provocation with the previously fed diet again resulted in partial deterioration, AD caused by food as well as environmental allergens was diagnosed.[6] To rule out an influence of drugs on the test results, injectable glucocorticoids were withdrawn 6 weeks prior to testing. Oral glucocorticoids and ciclosporin had to be discontinued for 4 weeks, and oclacitinib and antihistamines had to be discontinued for 2 weeks. Topical glucocorticoids were discontinued 1 week before the appointment. To obtain additional relevant information, a detailed questionnaire was completed by the owners. Along with signalment, it included questions about age of onset, presence of concomitant food-induced AD, seasonality of clinical signs, last given medication and pruritus at the time of testing measured on a validated pruritus visual analogue scale (pVAS).[27,28] The authors decided to use a case-by-case approach for minor variations in medication use previously excluded because of symptom onset.

Healthy dogs were at least 12 months of age. They had no historical evidence of skin disease and showed no pathologic findings on physical examination.

Intradermal testing

Intradermal testing was performed exclusively on the atopic dogs. A total of 41 allergens from Nextmune B.V. were injected intradermally into the skin of the atopic patients. The concentrations depended on the individual allergen groups: 200 Noon Units (NU) for pollens, 100 NU for mites, 10 μg/mL for moulds, 1000 NU/mL for fleas and 100 μg/mL for *Malassezia*. Histamine phosphate at a dilution of 1:10,000 served as a positive control, and the diluent, phosphate-buffered saline with 0.47% phenol, was used as a negative control. If required, the dogs were sedated intravenously with dexmedetomidine (Sedadex, Dechra) before the intradermal test, and the dosage ranged from 3 to 8 μg/kg. The intradermal reactivity was evaluated subjectively from 0 (no reaction) to 4 (strong reaction) after 15 and 25 minutes based on erythema, wheal size, turgidity and borders of each reaction site, as previously reported.[6,29] Rarely, intermediate values were assigned, such as 2–3 or 3–4. In these cases, the mean value was taken in order to reflect the evaluation truthfully. Values greater than or equal to 2 in at least one of the readings were considered as positive.

Serum testing

Immediately before the intradermal test in the atopic dogs was performed, 5–10 mL of whole blood was drawn by venipuncture from all dogs with AD and centrifuged. Serum samples from healthy dogs were obtained using surplus blood from health screening consultations. The serum was submitted to Nextmune,

and a serum test was performed at their laboratory in triplicates to detect specific IgE antibodies against CCDs as a first step and, if the three values obtained for the CCD-coated wells were positive, in duplicates against 34 individual allergens.

Samples positive for specific IgE against CCDs were analysed with and without blocking of anti-CCD-IgE, which was achieved by incubating serum with the CCD-blocker for 1 hour at room temperature before further testing. Samples without specific IgE against CCDs were tested without blocking anti-CCD-IgE, since no significant differences have been observed so far for negative samples after blocking in the quality control department of Nextmune.

Dog sera were diluted one-sixth in dilution buffer, regardless of whether they had been blocked with CCD-blocker before, and added to 96-well plates containing the coated allergens. After an overnight incubation at 4°C, plates were washed four times with washing buffer and a dog-specific anti-IgE monoclonal antibody labelled with alkaline phosphatase (targeting the CH_4 domain of dog IgE) was added and incubated for 2 hours. Afterwards, plates were washed again six times, and p-nitrophenyl phosphate substrate (Moss) was added and incubated for 30 minutes. The reaction was stopped with 1 N NaOH and absorbances were read at 405 nm using a spectrophotometer. Throughout the study duration, minor adjustments to improve specificity were included in the NEXT+ assay, although results underwent data curation to ensure consistency over time.

All results were expressed in arbitrary ELISA absorbance units (EAUs) after applying a multiplying factor of 1000. A dog IgE standard curve was included for internal control to assess the interassay reproducibility. Samples were tested in duplicates whenever the sample volume allowed it, and duplicate extracts in different plates were included for each sample. A positive control pool for CCDs with known values (pooled sera known to contain anti-CCD-IgE), negative controls (results below our threshold values) and background controls (coated wells without serum but with buffers and all subsequent reagents) were also included. Two thresholds for serum evaluation were used: 200 and 250 EAU. Values between 200 and 250 EAU are considered inconclusive, while values greater than 250 EAU are classified as positive reactions by the laboratory. The agreement between different test procedures was calculated for both thresholds before and after inhibition of anti-CCD-IgEs.

Allergen selection

Immune responses to 20 allergen extracts tested in both the intradermal and serum tests were investigated. A list of those allergens, including the number of dogs with positive test results for each allergen, is provided in Table 1. Allergenic extracts used for the intradermal testing and those used in the serological test NEXT+ were supplied by the same company (Nextmune). However, not all allergens necessarily originated from the same manufacturer, even when referring to the same allergen. The availability of lyophilised extracts is limited, and thus, the acquisition from different manufacturers was unavoidable. The reason for some discrepancies between the two testing procedures is that requirements for extracts used for intradermal testing are different to those for extracts used for serological testing, as phenol content and glycerol must be avoided for serological testing.

Data accessibility

Blinding of the data was ensured by delaying the analysis of the study results until after data collection was completed. Nextmune did not have access to clinical information, including the results of the intradermal tests, until the completion of the first draft of the manuscript. The serum test results were retained by the study coordinator until the end of data collection.

Statistical analysis

The results of serum and intradermal tests were compared for each allergen, and the agreement was evaluated with Cohen's kappa test. The values were interpreted as follows: less than 0, no agreement; 0–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1, almost perfect agreement.[30]

To assess the influence of inhibition of anti-CCD-IgE antibodies, a two-tailed Fisher's exact test was used to compare all serum reactions before and after inhibition of anti-CCD-IgEs. The two-tailed Fisher's exact test was also used to compare the number of polysensitised dogs before and after inhibition of anti-CCD-IgE antibodies. The significance level was set at a p-value of less than 0.05. The statistical analysis was conducted using GraphPad Prism software (GraphPad Prism 6.0, GraphPad Software).

Similar to the two previously published studies, polysensitisation was defined as the majority of reactions within an allergen subgroup being positive, that is, a minimum of three out of four grasses, three out of five mites or four out of six weeds.[22,25] The number of allergens in the subgroups 'moulds' and 'others' were too small to be evaluated in this fashion.

RESULTS

Study objects

The age and sex of the included dogs are shown in Table 2. The atopic dogs first displayed allergic symptoms at an average age of 1.6 years (range from 0 to 7 years), and 14 of them were additionally diagnosed with food-based AD. On the day of testing, the mean pVAS score was 6.1 (range from 0 to 10). A single dog had a pVAS score of 0 because of treatment

TABLE 1 Number and percentage of positive test results[a] to allergens evaluated by intradermal test (IDT) and serum allergy test (SAT)

		SAT		
	IDT ($n = 34$)	CCD negative ($n = 17$)	CCD positive ($n = 17$)	
			Non-blocked	Blocked
Grasses				
Dactylis glomerata (Orchard grass)	13 (38%)	2 (12%)	9 (53%)	5 (29%)
Lolium perenne (Perennial ryegrass)	13 (38%)	1 (6%)	9 (53%)	1 (6%)
Cynodon dactylon (Bermuda grass)	6 (18%)	5 (29%)	12 (71%)	7 (41%)
Poa pratensis (Kentucky bluegrass)	9 (26%)	3 (18%)	14 (82%)	6 (35%)
Weeds				
Chenopodium album (White goosefoot)	10 (29%)	0	5 (29%)	1 (6%)
Ambrosia elatior (Common ragweed)	8 (24%)	0	5 (29%)	6 (35%)
Rumex acetosella (Red sorrel/field sorrel)	4 (12%)	0	7 (41%)	2 (12%)
Artemisia vulgaris (Mugwort)	4 (12%)	1 (6%)	5 (29%)	0
Plantago lanceolate (Ribwort plantain)	13 (38%)	1 (6%)	5 (29%)	2 (12%)
Brassica napus (Oilseed rape)	9 (26%)	1 (6%)	9 (53%)	3 (18%)
Mites				
Dermatophagoides farinae (House dust mite)	29 (85%)	8 (47%)	11 (65%)	11 (65%)
Dermatophagoides pteronyssinus (House dust mite)	18 (53%)	3 (18%)	8 (47%)	8 (47%)
Tyrophagus putrescentiae (Mould mite)	31 (91%)	1 (6%)	12 (71%)	10 (59%)
Lepidoglyphus destructor (Storage mite)	25 (74%)	1 (6%)	3 (18%)	3 (18%)
Acarus siro (Flour mite)	28 (82%)	4 (24%)	7 (41%)	9 (53%)
Moulds				
Alternaria alternata	5 (15%)	4 (24%)	2 (12%)	3 (18%)
Aspergillus fumigatus	5 (15%)	2 (12%)	4 (24%)	4 (24%)
Cladosporium herbarum	2 (6%)	3 (18%)	5 (29%)	5 (29%)
Others				
Malassezia	8 (24%)	2 (12%)	3 (18%)	1 (6%)
Ctenocephalides spp. (Flea)	3 (9%)	2 (12%)	9 (53%)	7 (41%)

Abbreviation: CCD, cross-reactive carbohydrate determinant.
[a]IDT values ≥2 were considered positive. SAT values ≥250 EAU were considered positive.

TABLE 2 Age and sex of the dogs included in the study

	AD ($n = 34$)	Healthy ($n = 10$)
Sex		
Female	5	5
Female spayed	12	1
Male	10	1
Male castrated	7	3
Age at time of test (years)		
Mean	4.1	7.4
Range	1–11	2–12

Abbreviation: AD, atopic dermatitis.

with lokivetmab. Three of the atopic dogs received medication based on allergy flare-ups that had been previously excluded. One patient received oclacitinib 3 weeks prior to testing, another owner discontinued oclacitinib for only 3 days after 2.5 months of use and treated one small, circumscribed wound on each of his dog's front paws with cortisone ointment 3 days before testing, and the last dog was given a combination of glucocorticoids and antibiotics locally in the ears 5 days prior to testing due to acute otitis externa. After discussion with the owners, it was decided that an intradermal test would be performed to evaluate skin test reactivity. With strong positive reactions, a serum test would also be evaluated, and if both tests showed strong positive reactions, results would be considered interpretable. As all three dogs exhibited strong test reactions, the authors decided to include these patients despite the medication.

Prevalence of anti-CCD-IgE antibodies

Anti-CCD-IgE antibodies were detected in 17 of the 34 atopic dog sera (50%). There were no differences in the seasonality of clinical signs between the groups with and without antibodies against CCDs (Fisher's exact test, $p > 0.999$). Five of the participating patient owners could not report on the seasonality as their dogs were too young and/or the duration of the disease was less than 1 year.

None of the 10 healthy dog sera showed any specific anti-CCD-IgE antibodies, so all subsequent analyses

TABLE 3 Cohen's kappa agreement[a] for intradermal and corresponding serum test results

CCD negative	0.08
CCD positive non-blocked	0.15
CCD positive blocked	0.25

Note: Data are shown for serum threshold of 250 EAU.
Abbreviation: CCD, cross-reactive carbohydrate determinant.
[a]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.

were only performed with the results from the atopic dogs.

Agreement between test procedures

The agreements between the different test procedures for both serum thresholds (200 and 250 EAU) were very similar; hence, only the results for the serum threshold of 250 EAU are presented (the data for the threshold of 200 EAU are shown in Tables S1 and S2).

A total of 680 reactions were investigated in both the intradermal and serum tests in atopic dogs. All Cohen's kappa values are summarised in Table 3. Overall, the agreement between the two procedures was slight. Blocking the anti-CCD-IgE antibodies increased the agreement from slight to fair.

The agreement between the intradermal test and all three variants of the serum allergy test (CCD negative, CCD positive with and without inhibition of anti-CCD-IgE antibodies) is illustrated in Table 4 with each of the four possible outcomes. In the dogs with anti-CCD antibodies, there was a significantly higher number of concordant results after blocking those antibodies compared to before blocking them (Fisher's exact test, $p = 0.031$).

To evaluate the degree of change in serum test results after blocking CCD-positive dog sera, a total of 578 reactions to 34 allergens were available. The number of positive reactions before blocking anti-CCD-IgE was significantly higher than the number of positive reactions after blocking those antibodies in the group of grass pollens, tree pollens and weed pollens (two-tailed Fisher's exact test, $p < 0.0001$ for each comparison). There was no significant difference for the group of mite allergens (two-tailed Fisher's exact test, $p = 1.0$).

Polysensitisation in dogs with AD and healthy dogs

Serum samples from healthy (200 reactions) and atopic dogs (680 reactions) were assessed for polysensitisation. Among the healthy dogs, there were no multiple positives in the groups of grass, weed and mite allergens. The polysensitisation detected in serum allergy tests of atopic dogs is presented in Table 5. In the atopic dogs without anti-CCD-IgE antibodies, the proportion of dogs that were polysensitised ranged from none to 12% between allergen groups. In the atopic dogs with non-inhibited anti-CCD-IgEs, numerous multiple positive test reactions were present. Inhibition of anti-CCD-IgE antibodies significantly reduced polysensitisation for grasses (two-tailed Fisher's exact test, $p = 0.0014$) but not for weeds (two-tailed Fisher's exact test, $p = 0.102$) and mites (two-tailed Fisher's exact test, $p > 0.999$).

DISCUSSION

In this study, blocking the anti-CCD-IgE antibodies of dogs with AD increased the correlation of the intradermal test results with the results of allergen-specific IgE testing from slight to fair. Polysensitisation seen on serum IgE testing decreased significantly with blocking of those antibodies. Healthy control dogs did not show any anti-CCD-IgE antibodies or polysensitisation.

The prevalence of atopic dogs with anti-CCD-IgE antibodies in our study mirrors data from human medicine, where a prevalence of 18–71% was reported in allergic patients.[17,18,31,32] In veterinary medicine, a prevalence of 17–39 % has been reported for atopic dogs,[7,21,22] with a single abstract reporting a prevalence of 73%.[33] There is only one study to date that detected specific IgE antibodies to CCDs in healthy dogs (present in 13% of the population tested).[7] In contrast, we could not identify anti-CCD-IgE antibodies in the 10 healthy dogs included in our study. The prevalence range reported may be due to patient selection, different geographical locations with different genetic backgrounds of the patients or differences in methodology. In addition, it is possible that there is no significant difference at all between the results of all those studies, supported by the fact that the 95% confidence intervals for the studies overlap. Another explanation could be that healthy dogs do not form antibodies against CCD structures and that the study identifying apparently healthy subjects with anti-CCD-IgE antibodies involved dogs that were in fact in an early, subclinical stage of AD. Further longitudinal investigations are needed to elucidate this.

This study confirmed that marked polysensitisation was present in sera from atopic dogs with anti-CCD-IgE antibodies compared to dogs without those antibodies and that those multiple positive test results were significantly reduced by inhibition of the anti-CCD-IgE antibodies. This reduction was particularly evident in the allergen subgroup of grasses and weeds—although the maximum reduction from four to zero dogs polysensitised to weeds was not sufficient for statistical significance, in contrast to grasses—and not so prominent with mites, as reported in previous veterinary[22–25] and human studies.[17] Arthropods contain no to a few CCDs,[16,31] in contrast to grasses and weeds, which carry more carbohydrate structures leading to the formation of anti-CCD-IgE antibodies.[34] Polysensitisation was rarely seen on intradermal testing, most likely due to the monovalent structure of the anti-CCD-IgE antibodies, which prevents cross-linking and thus degranulation of the mast cells, which

TABLE 4 Agreements between the intradermal test (IDT) and the serum allergy test (SAT) for allergens evaluated in atopic dogs

	Positive disagreement (SAT positive, IDT negative)	Negative disagreement (SAT negative, IDT positive)	Positive agreement (both tests positive)	Negative agreement (both tests negative)
CCD negative	22 (6%)	105 (31%)	22 (6%)	191 (56%)
CCD positive non-blocked	83 (24%)	55 (16%)	61 (18%)	141 (41%)
CCD positive blocked	44 (13%)	66 (19%)	50 (15%)	180 (53%)

Note: Data are shown for serum threshold of 250 EAU.
Abbreviation: CCD, cross-reactive carbohydrate determinant.

TABLE 5 Polysensitisation[a] in serum allergy tests of atopic dogs

	CCD negative ($n = 17$)	CCD positive ($n = 17$)	
		Non-blocked	Blocked
Grasses	1 (6%)	11 (65%)	2 (12%)
Weeds	0	4 (24%)	0
Mites	2 (12%)	9 (53%)	8 (47%)
No. of dogs[b]	2 (12%)	13 (76%)	9 (53%)

Note: Data are shown for the threshold of 250 EAU.
Abbreviation: CCD, cross-reactive carbohydrate determinant.
[a]If the majority of reactions within an allergen subgroup were positive (at least three of four grasses, three of five mites or four of six weeds), the dog was considered as polysensitised.
[b]Number of dogs with polysensitisation in at least one allergen group.

is also a reason for the presumed clinical irrelevance of those antibodies.[13,19,20,35] The exception are the IgE antibodies directed against α-1,3-galactose (alpha-Gal) contained in red meat, which have been reported to cause severe allergic reactions in some human patients.[18,36,37] As a self-antigen of the dog, alpha-Gal does not normally induce an immune response in this species (in contrast to primates).[38] Nevertheless, a more recent study has demonstrated that IgG, IgM and IgE antibody production against alpha-Gal can be found in healthy dogs and can also be induced through sensitisation by tick bites.[39] However, further study is required to determine whether this is of clinical relevance in dogs.

Two previous studies compared the correlation between intradermal and serum testing before and after blocking anti-CCD-IgE antibodies in veterinary medicine with conflicting results.[22,25] One study was able to demonstrate an improvement in correlation after inhibiting the antibodies specific to CCDs,[22] as established in humans.[17] In contrast, another study with a larger number of participants did not show any difference.[25] In the present study, we investigated a third blocking procedure and showed an increase in correlation, but this was less pronounced than that seen in the previous study. The strength of this study compared to the publications mentioned above is that the allergens used for the intradermal and serum tests were both provided by the same company (Nextmune), which improves the comparability between the results of the test methods, even though not all allergens necessarily originated from the same manufacturer. Another explanation for the disparate results reported in the three studies evaluating the use of a blocking agent against anti-CCD-IgE is the fact that each of them used a different blocking agent to inhibit reactions with anti-CCD-IgE. Whereas the Gedon et al.[22] study used a carbohydrate-blocker (proprietary product of Heska, specifically for veterinary use) composed of plant glycoproteins not derived from the allergen test field, the Canning et al.[25] study used a bromelain-CCD inhibitor (proprietary product of Stallergenes Greer) containing the carbohydrate components found in bromelain. In our study, we used the CCD-blocker NEXT+ (proprietary product of Nextmune), which is a semisynthetic blocker consisting of a purified CCD bound to human serum albumin for stabilisation. It seems likely that the efficacy of different blockers varies.[17,18,34] Additionally, methodological variations could also have affected the concordance between intradermal and serum allergy testing, as both German studies utilised a subjective scoring system to assess intradermal reactions, while the Americans referred to the global wheal score (composed of subjective and objective criteria). Different dog populations with different genetic backgrounds and different allergens used for intradermal testing may have further contributed to the difference between the American study and the two German studies.

The fact that, in dogs without anti-CCD-IgE antibodies, the serology is negative for 31% of allergens while the intradermal test is positive is very likely an effect of small numbers. The agreement between the negative intradermal and serum test reactions in our study was most pronounced. Inhibition of anti-CCD-IgE antibodies reduced positive serum test results inconsistent with intradermal testing by half, while negative serum results disagreeing with intradermal testing increased by only 3%, resulting in a decrease in the number of positive test results concordant in both tests. Whether anti-CCD-IgE antibodies are all 'false positives' and clinically irrelevant is still not clear. Even with their monovalent structure and inability to cause mast cell degranulation, a role in T-cell-mediated hypersensitivity reactions cannot be ruled out. Non-IgE-based immunological pathways may also play a role in canine AD.[40]

The measured responses in the allergen-specific IgE test were evaluated using two different thresholds (200 and 250 EAU) to see which of the two achieved better agreement with the intradermal testing. Overall, no difference in agreement was found between the two thresholds using Cohen's kappa scale. Polysensitisation was more frequent in atopic dogs in the lower threshold group, as expected, but inhibition of

anti-CCD-IgE antibodies equally reduced polysensitisation in both groups (Tables S1 and S2).

One limitation of the present study was that the number of healthy dogs was not large, and a larger number of healthy dogs would have possibly identified dogs with anti-CCD-IgE antibodies. Another restriction of this study was the participation of three atopic dogs that had received medication based on the onset of symptoms that had previously been excluded. Short-term use of oclacitinib does not seem to affect intradermal test activity. In one study, intradermal test activity was not significantly affected after 14 days of therapy in dogs experimentally sensitised to house dust mites.[41] All three dogs exhibited strong skin test reactions and each dog served as its own control in this study; consequently, the authors decided to include these patients despite the medication.

In conclusion, the present study confirmed that strong polysensitisation identified in serum allergen-specific IgE testing directed against pollen allergens in dogs with anti-CCD-IgE antibodies could be reduced by a blocking procedure, which increased the agreement between intradermal and serum allergy tests from slight to fair. This shows that inhibition of anti-CCD-IgEs in serological tests with suitable blocking agents is likely to improve test specificity and reliability.

AUTHOR CONTRIBUTIONS

Teresa Boehm made substantial contributions to the acquisition of data. Nadine Wittenstein made substantial contributions to the acquisition of data. Ralf Mueller made substantial contributions to the conception and design, analysis and interpretation of data and was involved in drafting the manuscript by revising it critically and giving final approval of the version to be published. Bettina Kasper made substantial contributions to the acquisition of data, analysis and interpretation of data and wrote the first draft of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Ralf Mueller acted as a consultant or received support for studies or lectures from Artuvet, Bayer Animal Health, Ceva Animal Health, Ecuphar, Elanco Animal Health, Greer Laboratories, Heska Laboratories, Hill's, Royal Canin, MSD Animal Health, Nextmune, Synlab, Virbac Animal Health and Zoetis. Bettina Kasper, Teresa Boehm and Nadine Wittenstein did not report any conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

This prospective and blinded study was approved by the Ethics Committee of the University of Munich, part 1 (atopic dogs) under the number 204-04-03-2020 and part 2 (normal dogs) under the number 257-26-02-2021.

ORCID

Bettina Kasper https://orcid.org/0000-0002-2876-5529

Ralf S. Mueller https://orcid.org/0000-0001-5835-5910

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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