

Basophil activation testing, IgG, and IgG4 in the diagnosis of dog allergy in children with and without a dog at home

To the editor,

Diagnosis of dog allergy in children relies mainly on clinical history and the presence of IgE antibodies (IgE) against dog dander. In recent years, refined molecular diagnostics has enabled the analysis of IgE against specific dog allergen molecules. However, asymptomatic sensitization is common, and there is still a need for more specific diagnostic tools.¹

A nasal provocation test (NPT) can reproduce an allergic reaction in the nose under standardized and controlled conditions but is rarely performed in clinical settings.² Consequently, *in vitro* allergen testing such as the basophil activation test (BAT) could be an attractive alternative to reflect *in vivo* responses to dog allergen exposure. Basophil allergen threshold sensitivity, CD-sens, shows good reproducibility, correlates with response to *in vivo* allergen provocation, and can be used to monitor the course of allergic disease.^{3,4} As the induction of IgG4 toward cat allergens during cat exposure has been suggested to protect against allergic reactions, diagnosis may also be improved by the assessment of allergen-specific IgG antibodies.⁵ Our aim was to investigate the usefulness of the BAT and levels of IgG and IgG4 antibodies in differentiating dog allergy from asymptomatic dog sensitization in relation to the results of NPTs with dog dander extract and dog exposure.

Fifty-eight children sensitized to dog dander (dog dander IgE >0.1 kU_A/L) between 10 and 18 years of age participated.¹ Children and parents were interviewed regarding history of asthma, rhinitis, and exposure to dogs. Sixty-nine percent reported rhinitis, and 52% reported asthma caused by dog exposure. Fifteen of 58 patients were living in a home with at least one dog (Table S1).

Serum samples were analyzed for IgE against dog dander extract and dog allergen molecules by ImmunoCAP (Can f 1, Can f 2, Can f 3, and Can f 5) or Streptavidin ImmunoCAP (Can f 4 and Can f 6), and for IgG and IgG4 antibodies against dog dander extract and Can f 1, Can f 2, and Can f 5 by ImmunoCAP (ImmunoCAP System, Thermo Fisher Scientific) (Data S1).

Basophil activation testing was performed using dog dander and the two dog allergen molecules eliciting the highest IgE levels in each individual, and CD-sens was calculated (Data S1).

The BAT using dog dander gave a positive result in 54 of 58 patients. Among Can f 1-sensitized individuals, 30/34 showed a positive BAT toward Can f 1. Corresponding numbers for Can f 2 were 17/20 and Can f 5 19/23. There were too few positive BAT responses for the minor allergens Can f 3 (*n* = 5), Can f 4 (*n* = 8),

and Can f 6 (*n* = 5) to allow further analysis. We found significant correlations between CD-sens levels and IgE against dog dander, as well as the investigated allergen molecules. IgE levels were low among BAT-negative individuals (Figure S1). All patients reporting rhinitis (*n* = 40) or asthma (*n* = 30) upon exposure to dogs showed positive BAT responses to dog dander. No patients with a negative BAT to dog dander (*n* = 4) reported respiratory symptoms on exposure to dogs (rhinitis *P* = .007, asthma *P* = .048) (Table S2).

Nasal provocation testing (NPT) was performed using a commercially available dog dander extract, Aquagen 100 000 SQ-U/mL (ALK-Abello) according to the protocol of Lebel et al with minor modifications.^{1,6} Twenty-four patients showed a positive NPT toward dog dander extract, and twenty patients had a negative NPT. The results of 14 NPTs were considered inconclusive and therefore not possible to evaluate.^{1,2}

Positive BAT results for dog dander were found in 23 of 24 children with positive NPTs and 17 of 20 children with negative NPTs. Three out of four children with a negative BAT response to dog dander also displayed a negative NPT (Figure 1). Furthermore, CD-sens levels for dog dander were lower in children with a negative NPT (0.10 vs 0.67, *P* = .04) (Figure S2). Among children where the BAT for Can f 1 was performed, all of those with a positive NPT also showed a positive BAT response for Can f 1. The four individuals with a negative BAT for Can f 1 also showed a negative NPT (*P* = .014). Among children with a negative NPT, six showed a positive BAT response for Can f 1, but with significantly lower CD-sens levels than among children with a positive NPT (2.35 vs 34.8, *P* = .025) (Figure S2). Our results agree with previous studies. Nopp et al showed that CD-sens correlates with nasal symptoms and peak nasal inspiratory flow in grass pollen allergic patients,³ and further, a correlation between bronchial response to inhaled allergens and CD-sens has been demonstrated in adult asthmatics.⁴ Moreover, CD-sens levels for dog dander and the dog allergen molecules Can f 1, Can f 2, and Can f 5 were significantly lower in children with a dog at home compared to those without (Table 1).

Interestingly, only one of 15 children exposed to dog at home showed a positive nasal challenge compared with 23/43 among those without a dog at home (*P* = .002). No significant difference in IgE levels for dog dander was seen between these two groups. However, we found higher levels of IgG for dog dander (13 vs 9.9 mg/L, *P* = .019), as well as higher levels of IgG4 for Can f 1 (0.5 vs 0.18, *P* = .039) and Can f 5 (0.13 vs 0, *P* = .025) among exposed compared with nonexposed individuals (Table 1). There were no differences in IgG or IgG4

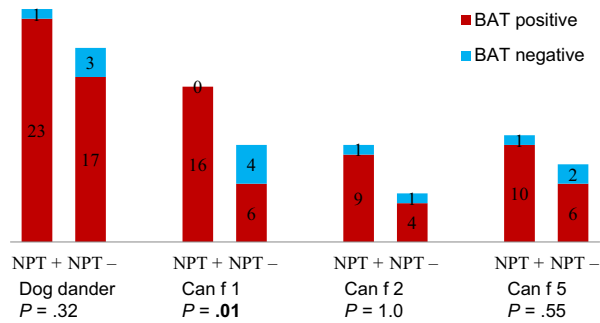


FIGURE 1 Positive and negative BAT results for dog dander and dog allergen molecules in relation to nasal provocation testing (NPT) results. Positive and negative predictive values (PPV/NPV) and positive and negative likelihood ratios (LR) of BAT in relation to NPT with dog dander are presented. Results for individuals with an inconclusive NPT (n = 14) are not evaluated

BAT in relation to NPT	P-value BAT/NPT	PPV % (CI)	NPV % (CI)	Positive LR (CI)	Negative LR (CI)
Dog dander (n = 44)	0.32	58 (41-73)	75 (19-99)	1.13 (0.92- 1.4)	0.28 (0.03- 2.5)
Can f 1 (n = 26)	0.014	73 (50- 89)	100 (40-100)	1.67 (1- 2.8)	0 n.a.
Can f 2 (n = 15)	1.0	64 (50-89)	50 (1.3-99)	1.12 (0.69- 1.8)	0.5 (0.04- 6.4)
Can f 5 (n = 19)	0.55	63.0 (35-85)	67 (9.4-99)	1.21 (0.78- 1.9)	0.36 (0.04- 3.4)

	Dog at home (n = 15) median(IQR)	No dog at home (n = 43) median(IQR)	P-value
Positive NPT—dog dander	1	23	.002
IgE dog dander kU _A /L	11 (4-41)	16 (2.8-47)	.67
IgG dog dander mg/L	13 (9.8-25)	9.9 (8.4-14)	.019
IgG4 dog dander mg/L	2.3 (1.4-4.9)	1.8 (0.81-2.7)	.16
Ratio IgG4/IgE dog dander	97 (38-279)	44 (17-160)	.19
Level CD-sens dog dander ^a (n = 51)	0.11 (0.07-0.44)	0.50 (0.13-2.0)	.038
IgE Can f 1 kU _A /L	2.1 (0.68-5.9)	13 (2.6-33)	.006
IgG Can f 1 mg/L	3.2 (2.1-6.4)	2.0 (0-2.9)	.07
IgG4 Can f 1 mg/L	0.5 (0.2-1.6)	0.18 (0.07-0.3)	.039
Ratio IgG4/IgE Can f 1	141 (36-315)	5.7(1.9-12)	.006
Level CD-sens Can f 1 ^a (n = 26)	1.0 (0-8.8)	23 (4.1-50)	.026
IgE Can f 2 kU _A /L	1.6 (0.42-14)	10 (1.1-34)	.22
IgG Can f 2 mg/L	2.2 (0- 4.3)	0 (0-2.2)	.06
IgG4 Can f 2 mg/L	0.2 (0-0.4)	0 (0-0.1)	.10
Ratio IgG4/IgE Can f 2	13 (0-34)	0 (0-2.5)	.11
Level CD-sens Can f 2 ^a (n = 19)	20 (0-41)	224 (70-271)	.018
IgE Can f 5 kU _A /L	0.61 (0.25-1.5)	5.8 (2.1-16)	.001
IgG Can f 5 mg/L	1.1 (0-5.0)	2.1 (0-3.1)	.62
IgG4 Can f 5 mg/L	0.13 (0-0.31)	0 (0-0.09)	.025
Ratio IgG4/IgE Can f 5	34 (0-247)	0 (0-4.7)	.01
Level CD-sens Can f 5 ^a (n = 20)	0 (0-0.24)	2.5 (1.3-3.6)	.005

TABLE 1 Levels of basophil allergen sensitivity expressed as CD-sens, IgG and IgG4/IgE ratio in children with and without a dog at home

^a1/(100 SQ-U/mL) for dog dander extract and 1/(100 ng/mL) for allergen molecules.

Bold values indicate a p-value < .05.

levels for dog dander or any dog allergen molecule between the NPT positive and the NPT negative children (Table S3).

These findings indicate that allergen-specific IgG and IgG4 responses reflect exposure rather than tolerance and are in line with a study by Burnett et al who showed that teenagers with allergic symptoms toward dogs had similar levels of IgG4 compared with asymptomatic study participants.⁷ Previous studies on IgG and IgG4 cat antibodies have shown that current cat owners had a lower prevalence of IgE and a higher prevalence of IgG and IgG4 for the important cat allergen Fel d 1, although without significant effects on reported asthma.⁸

To our knowledge, this is the first study to evaluate the BAT for dog allergen molecules in relation to clinical symptoms of dog allergy. We show that children sensitized to Can f 1 but with a negative BAT response to Can f 1 seem clinically tolerant to dog. Thus, the BAT may be a useful complementary tool to reduce over-diagnosis of dog allergy, while the presence of IgG and IgG4 antibodies may reflect exposure to dogs, rather than tolerance.

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CONFLICTS OF INTEREST

U. Käck has received lecture fees from Thermo Fisher Scientific. A. Asarnoj has received lecture fees from Thermo Fisher Scientific. M. van Hage has received lecture fees from ALK and Thermo Fisher Scientific (Uppsala, Sweden) and consultancy fees from Biomay AG (Vienna, Austria) and Hycor Biomedical (LLC, CA, US). J. R. Konradsen has received material from Thermo Fisher to perform the IgE analysis in this project and lecture fees from Novartis and Meda AB. No other authors declare any conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary online material:

Table S1. Patient characteristics: Assessment of dog dander sensitized children with negative, indecisive or positive nasal challenge in relation to exposure, asthma, rhinitis and other atopic manifestations.

Parameter	All patients	Negative Challenge	Indecisive Challenge	Positive Challenge
Number of patients	58	20	14	24
Mean age (s.d)	13.1 (2.3)	13.5 (2.6)	12.5 (2.3)	13.2 (2.2)
Female gender, n (%)	20 (34 %)	5 (25 %)	6 (43 %)	9 (38 %)
At least one parent with dog allergy, n (%)	18 (31%)	3 (15 %)	5 (36 %)	10 (42 %)
Exposure, n (%)				
Dog at home	15 (26 %)	8 (40 %)	6 (43 %)	1 (4 %)
Asthma, n (%)				
Asthma diagnosis	50 (86 %)	15 (75 %)	12 (86 %)	23 (96 %)
Reported asthma at dog exposure	30 (52 %)	6 (30 %)	7 (50 %)	17 (71 %)
Rhinitis, n (%)				
Rhinitis	56 (97 %)	18 (90 %)	14 (100 %)	24 (100 %)
Reported rhinitis at dog exposure	40 (69 %)	8 (40 %)	9 (64 %)	23 (96 %)

Table S2: Positive vs. negative basophil activation test (BAT) in relation to reported rhinitis (A) and reported asthma (B) at dog exposure. Positive and negative predictive values (PPV/NPV) of BAT and positive and negative likelihood ratios (LR) of BAT for reported symptoms at dog exposure are shown.

A. Reported rhinitis at dog exposure

	Reported rhinitis	No reported rhinitis	p-value, BAT/ rhinitis	PPV % (CI)	NPV % (CI)	Positive LR (CI)	Negative LR (CI)
Basophil activation test (BAT)							
Dog dander (n=58)	40	18		74	100	1.29	0
Negative	0	4	0.007	(60-85)	(40-100)	(1- 1.7)	n.a
Positive	40	14					
Can f 1 (n=34)	27	7		87	75.0	1.69	0.09
Negative	1	3	0.021	(69-96)	(19-99)	(0.9-3.2)	(0.01- 0.7)
Positive	26	4					
Can f 2 (n=20)	15	5		88	100	2.50	0
Negative	0	3	0.009	(64-99)	(29-100)	(0.9-7.3)	n.a
Positive	15	2					
Can f 5 (n=23)	17	6		84	75	1.88	0.12
Negative	1	3	0.04	(60-97)	(19-99)	(0.8-4.2)	(0.02-0.9)
Positive	16	3					

B Reported asthma at dog exposure

Basophil activation test	Reported asthma	No reported asthma	p-value, BAT/ asthma	PPV % (CI)	NPV % (CI)	Positive LR (CI)	Negative LR (CI)
Dog dander (n=58)	30	28		56	100	1.17	0
Negative	0	4	0.048	(41-69)	(40-100)	(1-1.4)	n.a.
Positive	30	24					
Can f 1(n=34)	18	16		60	100	1.33	0
Negative	0	4	0.039	(41-77)	(40-100)	(1-1.8)	n.a.
Positive	18	12					
Can f 2(n=20)	12	8		64	67	1.22	0.33
Negative	1	2	0.54	(38-86)	(9-99)	(0.8-1.9)	(0.04- 3.1)
Positive	11	6					
Can f 5 (n=23)	14	9		57.9	25.0	0.88	1.93
Negative	3	1	1.0	(34-80)	(1-80)	(0.6-1.3)	(0.2-16)
Positive	11	8					

Table S3. IgE, IgG, IgG4 and ratio IgG4/IgE in children with positive vs negative nasal provocation test.

IgE (kU _A /L) IgG(mg/L) IgG4 (mg/L)	Positive NPT (n=24) Median (IQR)	Negative NPT (n=20) Median (IQR)	p-value- pos vs neg NPT
Dog dander			
IgE	23 (10-72)	4.1 (1.6-12)	0.008
IgG	9.9 (8.8-14)	10.2 (6.8-14)	0.59
IgG	2.0 (0.90-2.8)	1.9 (0.98-2.7)	0.76
IgG4/IgE	37 (13-92)	163 (38-272)	0.05
Can f 1			
IgE	16 (5.7-34)	1.8 (0.68-10)	0.010
IgG	2.3(0- 2.7)	2.5 (0-4.1)	0.46
IgG4	0.21 (0.065-0.33)	0.33 (0.15-0.74)	0.25
IgG4/IgE	6.4 (2.0-12)	38 (4.7 - 312)	0.068
Can f 2			
IgE	14 (4.5-37)	2.2 (1.1-24)	0.22
IgG	0 (0-0)	0 (0- 2.5)	0.16
IgG4	0.08 (0-0.13)	0.05 (0-0.42)	0.87
IgG4/IgE	2.1 (0-4.6)	1.2 (0-34)	0.81
Can f 5			
IgE	5.4 (2.3-21)	3.7 (0.51-9)	0.35
IgG	2.1 (0- 2.8)	0 (0-3.3)	0.63
IgG4	0.05(0-0.09)	0.09 (0-0.31)	0.11
IgG4/IgE	0.57 (0-6.5)	4.7 (0-247)	0.25

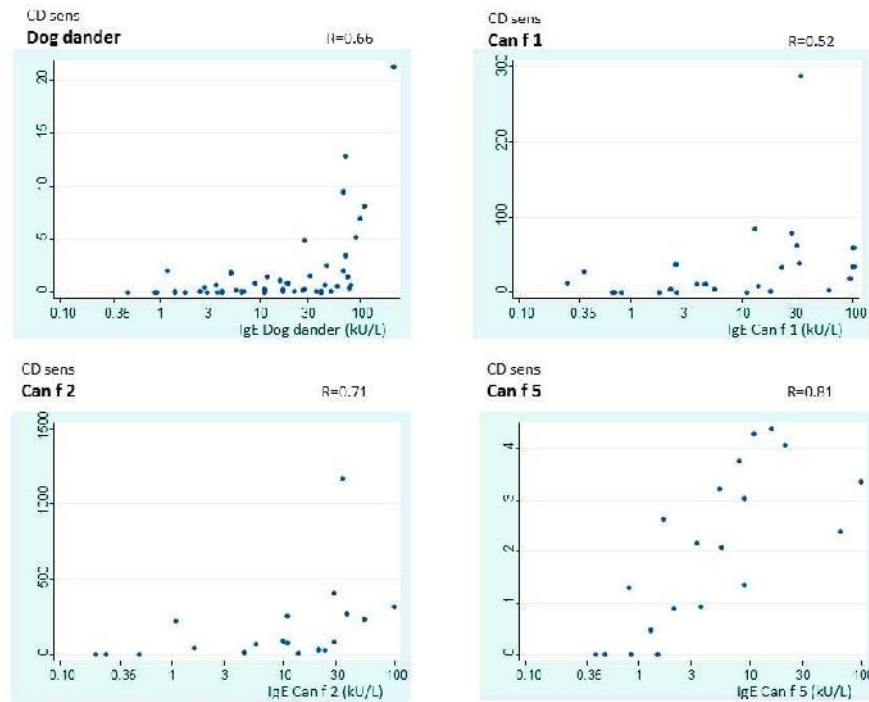


Figure S1. Correlations between CD-sens levels and IgE levels to dog dander and components. IgE above 100 kU_A/L was set at 100 for allergen molecules. Units for CD-sens are 1/(100 SQ-U/ml) for dog dander extract and 1/(100 ng/ml) for allergen molecules.

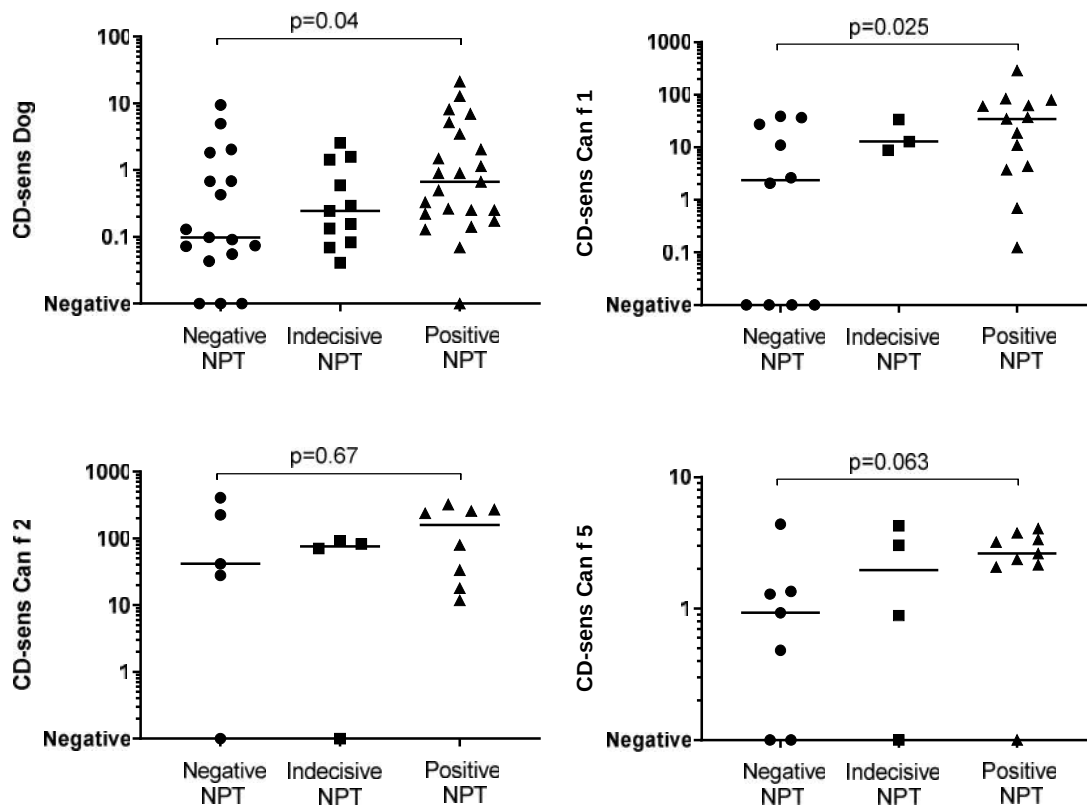


Figure S2: Levels of CD-sens (allergen sensitivity) in patients with a positive/indecisive/negative nasal provocation test with dog dander extract. Units for CD-sens are 1/(100 SQ-U/ml) for dog dander extract and 1/(100 ng/ml) for allergen molecules.

Data S1: Complete methods

Patient population

60 children and adolescents (age 10-18 years) participated in the study. As previously described the main inclusion criteria was confirmed sensitization to dog dander through a positive SPT (wheal size >3mm) and/or serum IgE antibodies to dog dander >0.10 kU_A/L, regardless history of clinical symptoms to dog (1). Written informed consent was obtained from all participants and their parents. The study was approved by the regional board of ethics, Stockholm, Sweden (Dnr 2014/1453-31/4). Children and parents were interviewed according to a modified version of the standardized questionnaire employed in the Environmental and Childhood Asthma study (2).

Nasal provocation testing

Nasal provocation testing (NPT) was performed with a commercially available dog dander extract, Aquagen 100 000 SQ-U/ml (ALK-Abello, Copenhagen, Denmark) according to a modified Lebel protocol. The table below shows the concentrations of dog allergens molecules in the dog dander extract:

Specific component	Content of specific allergen in dog dander extract (ng/ml)
Can f 1	256 ng/ml
Can f 2	10 ng/ml
Can f 3	923 ng/ml
Can f 4	282 ng/ml
Can f 5	255 ng/ml
Can f 6	8 ng/ml

Serological analysis

Samples of venous blood were collected and IgE against dog dander extract (e5) and the dog allergen molecules Can f 1, Can f 2, Can f 3 and Can f 5 were analyzed by ImmunoCAP (ImmunoCAP System, Thermo Fisher Scientific, Uppsala, Sweden). Can f 4 and Can f 6 were produced as recombinant proteins according to previously published data and IgE reactivity against the allergens was analyzed by coupling 5 µg of biotinylated antigen to Streptavidin ImmunoCAP (Thermo Fisher Scientific) according to the manufacturer's instructions (4, 7). The results are presented as kU_A/L, where the cut-off for presence of allergen-specific IgE was ≥0.10 kU_A/L.

Allergen-specific IgG- and IgG4 antibodies against dog dander extract and the dog allergen molecules Can f 1, Can f 2 and Can f 5 were measured by ImmunoCAP (ImmunoCAP System, Thermo Fisher Scientific) according to the manufacturer's instructions. The cut-off for allergen-specific IgG was ≥2 mg/L and for IgG4 ≥ 0.05 mg/L.

Basophil activation test

Basophil activation test was performed to dog dander and to the two dog allergen molecules eliciting the highest IgE-levels in each individual. Basophils were stimulated with increasing concentrations of dog dander extract (final concentration: 0.5-5000 SQ-U/ml) and the allergen molecules Can f 1- Can f 6 (final concentration: 0.05-500 ng/ml) (3, 4). Anti-FcεRI (Bühlmann Laboratories AG, Schönenbuch, Switzerland) was used as positive control and RPMI (cell culture media developed at Roswell Park

Memorial Institute) as negative control. To differentiate the basophils from the leukocyte population they were stained for CD203c. To detect activated basophils, the cells were stained for CD63 (Immunotech, Marseille, France) followed by analysis in a Navios flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA). Cut-off determining a positive test to dog dander/dog allergens was set to 5 % of CD63-positive basophils.

Patients, whose basophils after stimulation with the positive control (anti-FcεRI) responded with less than 5 % CD63 up-regulation, were regarded as non-responders. Individuals with a response between 5 % and 16 % were classified as low responders. The cut-off of 16 % was calculated (mean 76 %; - 3 SD 16 %) from the positive controls of an in-house reference material of 264 allergic children and adults (4). These individuals were excluded from the study (non-responders n=0, low responders n=1).

Definition of CD-sens

To determine the basophil allergen threshold sensitivity, CD-sens, the eliciting allergen concentration giving 50 % (EC50) of maximum CD63 % up-regulation of the individual's dose-response curve was calculated. CD-sens is defined as the inverted value for EC50 multiplied by 100 ($1/EC50 \times 100$), and describes the patient's allergen-specific sensitivity. A high CD-sens indicates high basophil allergen sensitivity (3). When basophils react only at the highest allergen concentration, a CD-sens value cannot be calculated, nor can the test be ruled as negative. In the present study, these test results were regarded as positive, but they were not included in the analysis of CD-sens levels.

Statistics

Statistical analyzes were performed with Stata statistical software (release 14.2, Stata Corp, Texas, USA). IgE-, IgG-, IgG4- and CD-sens levels to dog dander and dog allergen molecules are presented as median and inter-quartile range (IQR).

Categorical data were compared using Fisher's exact test. Wilcoxon rank-sum test was used for group comparisons of IgG-, IgG4- and CD-sens levels (continuous variables). Correlations between levels of CD-sens and levels of IgE were calculated with Spearman rank- order correlation test. Positive and negative predictive values (PPV and NPV) as well as likelihood ratios (LR) for CD-sens positivity as a marker for a positive vs a negative nasal challenge were calculated. A p-value < 0.05 was considered significant.

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