pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 33(5) : 270-273 (2016)
http://dx.doi.org/10.17555/jvc.2016.10.33.5.270

Journal of Veterinary Clinics
www.ksvc.or.kr

Evaluation of the Agreement between Immunodot Assays and Intradermal Skin Testing or Favrot Diagnostic Criteria in Canine Atopic Dermatitis

Sun-Hyung Cho, Ji-Houn Kang, Mhan-Pyo Yang and Byeong-Teck Kang[1]

Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea

(Received: July 14, 2016 / Accepted: August 22, 2016)

Abstract : This study was undertaken to identify differences between atopic and non-atopic dogs in three rapid screening immunodot assays as well as the ability of the assays to predict the results of intradermal skin testing (IDST) or Favrot diagnostic criteria (FDC). Twenty-nine dogs diagnosed with canine atopic dermatitis (CAD) were selected as the atopic group. Twenty-five dogs without CAD were included as the non-atopic group. Three types of immunodot assays were conducted on all serum samples from both groups: Allercept E-screen 2nd generation (ES2G), Canine Allergic Tendency Reference Test (ALERT), and Asan Easy Test Canine IgE (AETC). IDST, which included 39 allergens, and immunodot assays were performed concurrently in 13 dogs from the atopic group and compared. While there were no significant differences in positivity between the two groups in the evaluation of ALERT ($P = 0.435$) and AETC ($P = 0.313$), positivity in ES2G testing was significantly higher in the non-atopic group than the atopic group ($P = 0.038$). The ES2G, ALERT, and AETC results showed fair ($\kappa = 0.235$), slight ($\kappa = 0.133$), and slight ($\kappa = 0.014$) accordance with IDST, respectively. The outcomes of ES2G, ALERT, and AETC indicated poor ($\kappa = -0.211$), slight ($\kappa = 0.106$), and slight ($\kappa = 0.087$) agreement with FDC. In conclusion, rapid screening immunodot assays were not useful for the diagnosis of CAD. These assays may provide a supplementary method for predicting the results of IDST in atopic dogs.

Key words **:** canine atopic dermatitis, immunodot assay, intradermal skin test, Favrot diagnostic criteria.

Introduction

Canine atopic dermatitis (CAD) is defined as a genetically predisposed pruritic and inflammatory dermatitis with characteristic clinical features that include immunoglobulin E (IgE) antibodies against environmental allergens (15). Dogs with atopic-like dermatitis (ALD) have clinical signs of atopic dermatitis but no specific IgE antibodies (6). The diagnosis of CAD depends on the fulfillment of associated clinical criteria along with a ruling out of other pruritic dermatoses. Among several sets of diagnostic criteria, the Favrot diagnostic criteria (FDC) is a tool that has recently emerged in veterinary medicine (3,14).

A diagnosis of CAD may be confirmed by demonstration of the presence of allergen-specific IgE using *in vivo* or *in vitro* tests (4), of which intradermal skin testing (IDST) is considered the 'gold standard' method. However, skin reactivity in IDST may be affected by the age of the patient, season of testing, and administration of anti-allergic drugs. Additionally, it requires sedation and large shaving areas (9).

IgE serum testing (IST) is another method for confirming allergen-specific antibodies from canine serum samples that offers quantitative results, no requirement for sedation, and applicability in patients with widespread cutaneous inflammation. However, its disadvantages include frequent false-positive results, variable reliability and reproducibility, and low sensitivity (1,17). The possible replacement of IST by IDST is controversial (4,17,18).

Recently, inexpensive immunodot assays for IgE screening have been developed. The Allercept E-screen 2nd generation (ES2G) has been beneficial for predicting the results of IDST or IST, but is not useful for the diagnosis of CAD (2,12,16). The Canine Allergic Tendency Reference Test (ALERT) and Asan Easy Test Canine IgE (AETC) have been used in Korea. Their usefulness for diagnosing CAD and detecting IgE antibodies has not yet been evaluated. Therefore, the purpose of this study was twofold: 1) identify any differences between atopic and non-atopic dogs in the results of three rapid screening immunodot assays; and 2) evaluate the ability of these immunodot assays to predict the results of IDST or FDC.

Materials and Methods

Case selection

A total of 54 dogs who presented to Veterinary Medical Center, Chungbuk National University between February 2013 and June 2015 were included in this study and divided into two groups: atopic ($n = 29$) and non-atopic ($n = 25$). Serum samples were collected for the diagnostic procedures, and then stored at −80ºC. CAD was diagnosed in part based on fulfillment of at least five items from a total of eight items of the FDC: 1) onset of signs under 3 years of age; 2) dog living mostly indoors; 3) glucocorticoid-responsive pruritus; 4) pruritus without lesions at onset; 5) affected front feet; 6) affected ear pinnae; 7) nonaffected ear margins; and 8) non-affected dorso-lumbar area. The CAD diagnosis was con-

[1]Corresponding author.
E-mail : kangbt@chungbuk.ac.kr

firmed by ruling out other possible pruritic causes such as microbial and fungal infection, parasite burdens, adverse food reactions, and endocrine diseases (3,14). The non-atopic group included dogs that were free of CAD as well as other dermatologic problems.

Intradermal skin testing

Of the 29 dogs in the atopic group, 13 were evaluated by IDST with 39 allergens (Greer Labs Inc., Lenoir, NC, USA), according to current guidelines (9,10,12). Drugs that may adversely affect the results of IDST, which include glucocorticoids and anti-histamines, were not administered for at least 4 weeks prior to IDST. The dogs were sedated with an intravenous injection of medetomidine (10 μg/kg) (Domitor; Pfizer, Seoul, Korea). The hair coat of the lateral thorax was gently shaved with a clipper without prior scrubbing or washing. Each test site was marked with a waterproof marker. Approximately 0.05 mL of each allergen extract was injected intradermally using an insulin syringe (BD Ultra-Fine; Becton, Dickinson and Company, Seoul, Korea). The negative and positive controls were 0.9% phosphate-buffered saline and histamine phosphate (Histatrol; Alk Abello, Port Washington, NY, USA), respectively. The allergic reactions were evaluated at 15 min after injection and scored from 0 to 4 based on the measurements of diameter or area of erythema or wheal. Zero was equivalent to the reaction of the negative control and 4 was equivalent to that of the positive control. Any reaction of 2 or stronger was classified as positive. After finishing the procedure, sedation was reversed with atipamezole (10 μg/kg) (Antisedan; Pfizer, Seoul, Korea).

Rapid screening immunodot assays

Serum samples from all 54 study dogs were collected after centrifugation of blood at 3500 rpm for 5 min and then stored at −80°C until examination by rapid screening immunodot assays. Prior to testing, samples and reagents for ES2G (Heska, Fribourg, Switzerland), ALERT (Excelsior Bio-System, Taipei, Taiwan), and AETC (Asan Pharmaceutical, Seoul, Korea) were thawed to room temperature. A color formation on the control spot or line in each immunodot assay indicated a valid test. Any visible colored test spot was considered a positive result in all three kits; therefore, none of the tests was quantitative.

ES2G testing

ES2G detects allergen-specific IgE antibodies. Four separate spots on the ES2G membrane displayed three allergen groups containing a proprietary mixture of individual allergens (trees, grasses/weeds, and indoor) and a control spot containing purified IgE. Reagents were sequentially added to the test wells as follows: test serum, biotinylated detection reagent (FcεR1a), streptavidin-alkaline phosphatase, and color development reagent; additionally, the wells were washed with a buffer solution between each step. Color development of any test spot in addition to the control spot was considered positive confirmation of the presence of one or more detectable allergen-specific IgEs against the allergen group in the serum. If only the control spot showed a color change within 120 sec of the test completion, the result was considered negative.

Canine ALERT testing

ALERT combines immunoassay techniques and chromatography principles by fixing the highly specific anti-canine-IgE antibody onto a target test line and using colloidal gold nano particles to indicate detectable total IgE in the patient's serum. Initially, 0.5 mL running buffer solution was mixed with a 10 μL serum sample in a microtube by vortexing. After laying the reaction cassette flat, four drops of mixed sample were added onto the sampling area of the strip using a dropper. When a red line was observed in both the control and test areas within 10 to 15 min, the result was considered positive confirmation of total IgE > the defined cut-off concentration (10 μg/mL). If a red line appeared only in the control area, the result was considered negative.

AETC IgE testing

AETC is another chromatographic immunoassay kit for the detection of total canine IgE in serum. A nitrocellulose membrane was immobilized with anti-dog IgE polyclonal antibody in the test line and anti-mouse IgG monoclonal antibody as a control line. Firstly, 10 μL serum was added to a capillary tube containing assay buffer that was provided in the kit, and the mixture gently stirred. After placing the test device horizontal, four drops of mixed specimen was added in to the test device using the disposable dropper and the results were interpreted within 15-20 min. In cases of a positive reaction, anti-dog IgE monoclonal antibody conjugated to colloidal gold particles reacted with canine IgE in the serum, forming a red or purple test line. If a red or purple line appeared only in the control line, the result was considered negative.

Statistical analysis

Positive rapid screening immunodot assay results were compared between the two groups using a chi-square test. Sensitivity, specificity, positive predictive value, negative predictive value, and the κ (kappa) coefficient were calculated to evaluate the agreement between IDST or FDC and immunodot assays (Microsoft Excel 2013; Microsoft, USA). The κ values were interpreted as follows: > 0.80, almost perfect; 0.61-0.80, substantial; 0.41-0.60, moderate; 0.21-0.40, fair; 0.01-0.20, slight; and ≤ 0, less than chance (11).

Results

Differences in immunodot assays between atopic and non-atopic dogs

Serum samples from all dogs in the two study groups were evaluated by three types of immunodot assays (Table 1). No significant difference in positivity was found between atopic and non-atopic dogs using the ALERT ($P = 0.435$) and AETC ($P = 0.313$) kits. The ES2G test revealed significantly higher positivity in the non-atopic group compared with the atopic group ($P = 0.038$).

Agreement between rapid screening immunodot assays and IDST or FDC

IDST and immunodot assays were performed concurrently

Table 1. Results of three rapid screening immunodot assays in the two study groups

Group		Positive Number of dogs (%)	Negative Number of dogs (%)
Non-atopic ($n = 25$)	ES2G	24 (96.0)*	1 (4.0)
	ALERT	12 (48.0)	13 (52.0)
	AETC	2 (8.0)	23 (92.0)
Atopic ($n = 29$)	ES2G	22 (75.9)	7 (24.1)
	ALERT	17 (58.6)	12 (41.4)
	AETC	5 (17.2)	24 (82.7)

*$P < 0.05$ (chi-square test).
ES2G = Allercept E-screen 2nd generation; ALERT = Canine Allergic Tendency Reference Test; AETC = Asan Easy Test Canine IgE.

Table 2. Comparison of IDST and three immunodot assays in 13 atopic group dogs

Statistical values	ES2G	ALERT	AETC
Sensitivity	66.7%	50.0%	8.3%
Specificity	100.0%	100.0%	100%
Positive predictive value	100.0%	100.0%	100%
Negative predictive value	20.0%	14.3%	8.3%
Observed agreement (OA)	69.2%	53.8%	15.4%
Chance agreement (CA)	59.8%	46.7%	14.2%
Kappa = (OA – CA)/(1 – CA)*	0.235	0.133	0.014
Agreement interpretation	Fair	Slight	Slight

*Kappa coefficient: ≤ 0 = poor; 0.01-0.20 = slight; 0.21-0.4 = fair; 0.41-0.60 = moderate; 0.61-0.80 = substantial; 0.81-1.00 = almost perfect agreement. IDST = intradermal skin testing; ES2G = Allercept E-screen 2nd generation; ALERT = Canine Allergic Tendency Reference Test; AETC = Asan Easy Test Canine IgE.

Table 3. Comparison of FDC and three immunodot assays in 54 dogs from the atopic group and compared

Statistical values	ES2G	ALERT	AETC
Sensitivity	75.9%	58.6%	17.2%
Specificity	4.0%	52.0%	92.0%
Positive predictive value	47.8%	58.6%	71.4%
Negative predictive value	12.5%	52.0%	48.9%
Observed agreement (OA)	42.6%	55.6%	51.8%
Chance agreement (CA)	52.6%	50.3%	47.3%
Kappa = (OA – CA)/(1 – CA)*	–0.211	0.106	0.087
Agreement interpretation	Poor	Slight	Slight

*Kappa coefficient: ≤0 = poor; 0.01-0.20 = slight; 0.21-0.4 = fair; 0.41-0.60 = moderate; 0.61-0.80 = substantial; 0.81-1.00 = almost perfect agreement. FDC = Favrot diagnostic criteria; ES2G = Allercept E-screen 2nd generation; ALERT = Canine Allergic Tendency Reference Test; AETC = Asan Easy Test Canine IgE

in 13 dogs from the atopic group (Table 2). The highest sensitivity (66.7%) was shown in the ES2G test and the lowest sensitivity (8.3%) in the AETC. Interestingly, the specificity and positive predictive values were 100% in all three types of immunodot assays, whereas negative predictive values ranged from 8.3% to 20%. The agreement between IDST results and those for ES2G, ALERT, and AETC were fair ($\kappa = 0.235$), slight ($\kappa = 0.133$), and slight ($\kappa = 0.014$), respectively.

The results of a comparison between immunodot assays and FDC in 54 dogs from both groups are presented in Table 3. The highest sensitivity was observed in ES2G (75.9%), whereas AETC had the highest specificity (92.0%). The highest positive predictive value was shown in AETC (71.4%), followed by ALERT (58.6%), and ES2G (47.8%). Negative predictive values ranged from 12.5% to 52.0%. The outcomes of ES2G, ALERT, and AETC indicated poor ($\kappa = -0.211$), slight ($\kappa = 0.106$), and slight ($\kappa = 0.087$) agreement with FDC, respectively.

Discussion

This study demonstrated that the presence of serum IgE in three rapid screening immunodot assays would not be sufficient for diagnosis of CAD. While these immunodot assays presented a low rate of agreement with FDC, they had a fair to slight degree of agreement with IDST.

The results of ALERT and AETC were not significantly different between atopic and non-atopic dogs. Although a significant difference in the results of ES2G was observed between the two groups, ES2G testing was insufficient for the diagnosis of CAD due to the higher rate of positivity in the non-atopic group. Therefore, this combination of three immunodot assays would not be valuable as a screening test for the diagnosis of CAD. Similarly, previous studies showed that there was no significant difference in total IgE concentration between healthy and atopic dogs (7,8), and a higher rate of positivity of IST in normal dogs (16). Recently, a high IgE level has been regarded as an outcome rather than a cause of AD in both humans and dogs (13).

In this study, the ES2G results were in fair agreement with IDST, whereas the ALERT and AETC had only a slight accordance with IDST. A previous study in the United States showed a moderate agreement between IDST and ES2G (16). Generally, the prevalence of tree, grass and weed pollen allergens is higher in the United States compared with Korea (10,19). Although the exact cause is unknown, differences in allergens and the study population may have had an influence on the relatively lower level of agreement between IDST and ES2G in this study.

All three types of immunodot assays in this study had high positive and low negative predictive values for the findings of IDST. Therefore, a positive initial reaction in the ES2G, ALERT, or AETC, but not a negative reaction on these immunodot assays, may provide cause to perform subsequent IDST.

The outcomes of ES2G, ALERT, and AETC appeared to have poor, slight and slight agreement with those of FDC, respectively. These lower levels of agreement indicate that immunodot assays are insufficient to predict the results of FDC.

In the present study, there were several limitations for generalizing the findings. Firstly, frozen-stored serum were used for the examination and analysis. Although immunodot assays have previously been shown to detect IgE, as well as IgG,

IgM, and IgA from frozen serum in dogs (5,16), the potential influence of freezing on the experimental results is unknown. In addition, frozen feline serum was used to detect IgE at fewer than 20 years after the initiation of storing (2). The other limitation was the small sample size relative to previous studies (2,4,16,18).

In conclusion, a combination of rapid screening immunodot assays was not useful for the diagnosis of CAD. These assays may provide a supplementary method for predicting the results of IDST in atopic dogs.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2014R1A1A1036387).

References

1. DeBoer DJ, Hillier A. The ACVD task force on canine atopic dermatitis (XVI): laboratory evaluation of dogs with atopic dermatitis with serum-based "allergy" tests. Vet Immunol Immunopathol 2001; 81: 277-287.
2. Diesel A, DeBoer DJ. Serum allergen-specific immunoglobulin E in atopic and healthy cats: comparison of a rapid screening immunoassay and complete-panel analysis. Vet Dermatol 2011; 22: 39-45.
3. Favrot C, Steffan J, Seewald W, Picco F. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. Vet Dermatol 2010; 21: 23-31.
4. Foster AP, Littlewood JD, Webb P, Wood JLN, Rogers K, Shaw SE. Comparison of intradermal and serum testing for allergen-specific IgE using a FcεRIα-based assay in atopic dogs in the UK. Vet Immunol Immunopathol 2003; 93: 51-60.
5. German AJ, Hall EJ, Day MJ. Measurement of IgG, IgM and IgA concentrations in canine serum, saliva, tears and bile. Vet Immunol Immunopathol 1998; 64: 107-121.
6. Halliwell R. Revised nomenclature for veterinary allergy. Vet Immunol Immunopathol 2006; 114; 207-208.
7. Halliwell REW, Gilbert SM, Lian TM. Induced and spontaneous IgE antibodies to Dermatophagoides farinae in dogs and cats: evidence of functional heterogeneity of IgE. Vet Dermatol 1998; 9: 179-184.
8. Hill PB, Moriello KA, DeBoer DJ. Concentrations of total serum IgE, IgA, and IgG in atopic and parasitized dogs. Vet Immunol Immunopathol 1995; 44: 105-113.
9. Hillier A, DeBoer DJ. The ACVD task force on canine atopic dermatitis (XVII): intradermal testing. Vet Immunol Immunopathol 2001; 81: 289-304.
10. Kim HJ, Kang MH, Park HM. Common allergens of atopic dermatitis in dogs: comparative findings based on intradermal tests. J Vet Sci 2011; 12: 287-290.
11. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159-174.
12. Lee Y, Kang JH, Jung DI, Jin YB, Lee SR, Yang MP, Kang BT. Comparison of rapid screening immunoassay and intradermal test for canine atopic dermatitis. J Biomed Res 2015; 16: 115-120.
13. Le Roy L, Le Poder S, Desquilbet L, Perrot S, Cavana P, Marignac G. Canine atopic dermatitis diagnostic criteria: evaluation of four sets of published criteria among veterinary students. J Vet Med Educ 2015; 42: 79-84.
14. Olivry T. New diagnostic criteria for canine atopic dermatitis. Vet Dermatol 2010; 21: 124-127.
15. Olivry T, DeBoer DJ, Griffin CE, Halliwell RE, Hill PB, Hillier A, Marsella R, Sousa CA. The ACVD task force on canine atopic dermatitis: forewords and lexicon. Vet Immunol Immunopathol 2001; 81: 143-146.
16. Olivry T, Paps J. Evaluation of the agreement between allergen specific intradermal or IgE serological tests and a point-of-care immunodot assay in dogs with atopic dermatitis. Vet Dermatol 2011; 22: 284-285.
17. Park S, Ohya F, Yamashita K, Nishifuji K, Iwasaki T. Comparison of response to immunotherapy by intradermal skin test and antigen-specific IgE in canine atopy. J Vet Med Sci 2000; 62: 983-988.
18. Zunic M. Comparison between immunodot tests and the intradermal skin test in atopic dogs. Vet Dermatol 1998; 9: 201-206.
19. Zur G, Ihrke PJ, White SD, Kass PH. Canine atopic dermatitis: a retrospective study of 266 cases examined at the University of California, Davis, 1992-1998. Part I. Clinical features and allergy testing results. Vet Dermatol 2002; 13: 89-102.