EVALUATION OF THE CORRELATION OF SEROLOGICAL AND INTRADERMAL ALLERGEN TESTING WITH CLINICAL HISTORY IN 29 DOGS WITH ATOPIC DERMATITIS

by

Eric J Chong

(Under the Direction of FRANE BANOVIC)

ABSTRACT

Environmentally induced canine atopic dermatitis (cAD) is a common genetically predisposed cutaneous inflammatory and pruritic disease typically mediated by immunoglobulin E directed against environmental allergens. Allergen immunotherapy (AIT) is a safe, causative, and long-term therapy for cAD. Intradermal allergy testing (IDAT) and serum allergy testing (SAT), in conjunction with a clinical history that shows the seasonality of cAD, are utilized to formulate AIT. However, there is a need for a better understanding of the correlation between IDAT and SAT and the correlation between clinical history and allergy testing results, as this information may help veterinary dermatologists formulate a more successful AIT. The study presented here provides an in-depth analysis of the correlation between IDAT and SAT and the correlation between clinical history and allergy testing results since there is scarce information about this.

INDEX WORDS: Canine; Atopic Dermatitis; Allergy testing; Clinical history

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DEDICATION

I dedicate the work of this thesis to my girlfriend and family for their encouragement, patience, and emotional support throughout this journey.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Thesis Structure

This thesis follows a manuscript style format and includes an introduction and literature review chapter, an objectives chapter, one article included as a chapter, and discussion and concluding chapters. Each chapter has its own references section with discussion and conclusion references combined. Some material within the introductory and concluding chapters will unavoidably be duplicated as the included articles are reprinted here in their original full versions, either submitted or published proof. The objectives presented are each addressed in turn by the article chapters immediately following.

Pathogenesis of environmentally induced canine atopic dermatitis

Based on the most up-to-date definition proposed by the International Committee on Allergic Diseases of Animals (ICADA) in 2023, environmentally induced canine atopic dermatitis (cAD) is a predominantly T-cell-driven skin disease characterized by inflammation and typically pruritus.[1] Historically, cAD was considered an inflammatory and pruritic skin disease mediated by immunoglobulin (Ig) E antibodies, most commonly directed against environmental allergens.[2] Although IgE levels are elevated in many dogs with cAD, not every dog with cAD has shown elevated IgE levels.[3,4] Approximately 10 to 15% of dogs with cAD, known as "atopic-like dermatitis," do not exhibit elevated serum or intradermal IgE to the tested allergens.[3,4] Therefore, the new definition of cAD includes a dysregulated immune response, typically involving a T-helper 2 (Th2)-dominated immune profile that promotes the production

of allergen-specific IgE by B lymphocytes in most cases, since allergen-specific IgE does not always seem to be involved in the pathogenesis of cAD.[5]

Although the pathogenesis of cAD is not entirely understood, it likely involves a complex interaction between genetic and environmental factors that contribute to skin barrier abnormalities, microbial dysbiosis, and allergen sensitization.[1,6] Historically, the prevalence of cAD was estimated at 3-15%.[7] However, the American College of Veterinary Dermatology (ACVD) task force concluded that the true prevalence of cAD remains unknown due to variability across geographical regions, survey methods, population selection, types of veterinary practices, and the criteria used for cAD diagnosis.[8] Although the age of onset for cAD varies between different breeds, it generally occurs between 4 months and 3 years of age.[9-11] There has been no apparent sex predisposition for the development of cAD.[3,12] The common clinical features of cAD include pruritus, erythematous macules and/or papules, excoriation, self-induced alopecia, hyperpigmentation, and lichenification.[13-15] The commonly affected body sites, although they may vary between breeds, include the paws, axillae, caudal abdomen, inguinal region, face, concave pinnae, and ear canals (Figure 1.1).[11,13-15]

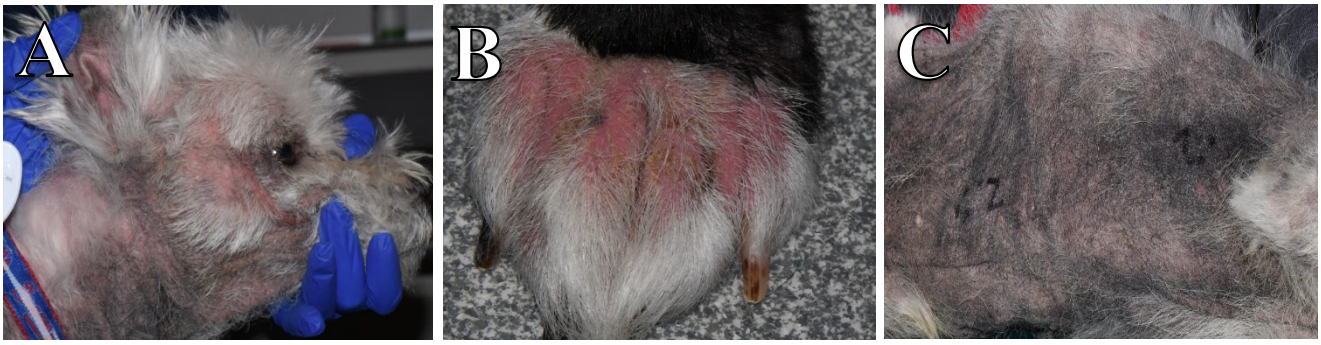

Figure 1.1: Clinical features and distribution of cAD.[11,13-15] Dogs with cAD commonly present with erythema, self-induced alopecia, lichenification, and hyperpigmentation that involve the (A)

face and neck, (B) paw, (C) ventrum and inguinum. (*Courtesy of the Veterinary Dermatology Service of the University of Georgia Veterinary Teaching Hospital*)

Certain breeds are more predisposed to develop cAD, such as Golden Retriever, Labrador Retriever, French Bulldog, West Highland White Terrier, and German Shepherd dog.[11,13] The presence of breed predilections suggests that genetic factors may contribute to the pathogenesis of cAD.[16,17] For example, mutations in filaggrin, an epidermal protein, appear to play a significant role in the development of human AD. Given the many similarities between cAD and its human counterparts, several studies have investigated a potential association between filaggrin gene mutations and cAD.[20] However, the current evidence suggests that filaggrin gene mutation does not appear to be a significant factor in the development of cAD in the majority of predisposed breeds.[19,20] There have been many other candidate genes that are potentially associated with the pathogenesis of cAD, but further research is needed to verify their implications in the pathogenesis of cAD.[6] In summary, the role of the genetic factor in the pathogenesis of cAD is not entirely understood due to the complex, polygenic nature of cAD, which results from diverse genetic mutations that differ across breeds and geographic regions.[6]

Environmental factors are believed to contribute to the development of cAD, as observed in human AD.[6] The potential risk factors include living predominantly indoors with a high standards of cleanliness and frequent contact to upholstered furniture, residing in an urban environment with high population density, exposure to high levels of tobacco smoke, neutering in male dogs, being born during the fall season, and living in regions with high average annual rainfall.[9,21-25] Conversely, several potential protective factors against the development of cAD have been identified, and these include being born and raised in a rural and outdoor environment with lower levels of air pollutants, residing within the household a dog was born in, cohabiting

with other animals, and living in families with more than two children.[23,24,26-28] In summary, these findings may support the "hygiene hypothesis," which proposes that early-life exposure to a diverse range of environmental, microbial, and parasitic stimuli may reduce the risk of developing AD, including in the context of cAD pathogenesis.[29]

Epidermal barrier dysfunction appears to play a role in the development of cAD, as has been demonstrated in human AD.[20] The outermost layer of epidermis is comprised of the stratum corneum, which is embedded in intercellular lipid lamellae that are composed of ceramides, cholesterol, and fatty acids.[30] In dogs with cAD, both lesional and non-lesional skin exhibit alterations in the intercellular lipid lamellae, characterized by abnormal structures that are highly disorganized and discontinuous, and a reduced content of ceramide and fatty acids compared to the skin of healthy dogs.[31-38] While epidermal barrier impairment is recognized as a contributing factor to the development of cAD, it remains unclear whether it represents a primary defect that facilitates increased percutaneous allergen penetration and initiates inflammation (the "outside-inside" hypothesis) or is a secondary consequence of ongoing inflammation (the "inside-outside" hypothesis).[39]

One of the hallmarks of cAD is cutaneous inflammation, which arises from dysregulation of the immune system.[40] The innate immune systems implicated in cAD include host defense peptides, keratinocytes, and white blood cells, such as neutrophils, macrophages (Langerhans cells and dermal dendritic cells), mast cells, and eosinophils.[41] The adaptive immune systems implicated in cAD include T-helper (Th) lymphocytes and B lymphocytes.[41] The pathogenesis of cAD reflects a complex interplay between the innate and adaptive immune system, and the full mechanisms of which remain partially understood.[42] Historically, cAD was thought to be a result of an imbalance between Th1/Th2 lymphocytes with a Th2-skewed cytokine milieu, such as

interleukin (IL)-4, IL-5, IL-13, and IL-31.[42,43] More recent literatures, however, indicates that while Th2-driven immune response remains central to cAD pathogenesis, other types of Th lymphocytes (e.g., Th1, Th17, Th22) with their cytokines, T regulatory lymphocytes with their cytokines, keratinocyte-derived cytokines, and noncytokine factors also contribute to cAD pathogenesis.[42,44-46]

In healthy skin, a rich diversity of commensal bacteria, fungi, protozoa, and their metabolites, collectively referred to as the microbiome, plays a vital role in modulating host immune responses and inhibiting colonization by pathogenic microbes.[47,48] In contrast, the skin of dogs with cAD is frequently associated with microbial dysbiosis, characterized by a reduction in microbial diversity.[40] This shift often involves increased relative abundances of certain bacteria (e.g., *Staphylococcus pseudintermedius*) and fungi (e.g., *Malassezia pachydermatis*).[49-51] Notably, a recent publication demonstrated that reduced bacterial diversity correlates with greater clinical disease severity scores and more pronounced impairment of the epidermal barrier.[49] Although dysbiosis is a recognized feature of cAD, it remains unclear whether cutaneous dysbiosis is a consequence or a driver of epidermal barrier dysfunction and immune dysregulation.[40]

In summary, the pathogenesis of cAD is complex and remains incompletely understood. Advancing our understanding of the underlying mechanism is essential for the development of more effective and safe treatments aimed at alleviating patient discomfort. Continued research is therefore critical to further elucidate the pathogenesis of cAD and improve therapeutic outcomes.

Diagnostics of cAD

Making a definitive diagnosis of cAD can be challenging due to its highly variable clinical presentation.[52] This variability is influenced by several factors, including extent of the lesions (localized versus generalized), genetic factors (breed-associated phenotypes), stage of the disease (acute versus chronic), and the presence of secondary microbial infections (bacteria and/or yeast), or other flare factors that will be discussed below.[11,14,53] To support clinicians in this diagnostic process, a subgroup of ICADA developed consensus guidelines.[14] These guidelines recommend diagnosing cAD based on a thorough clinical history, the presence of characteristic clinical features, and the exclusion of other diseases with a similar clinical presentation.[14,54-57]

The clinical history relevant to diagnosing cAD includes age of onset and seasonality of clinical symptoms (pruritus and/or dermatitis), familial or breed predispositions (e.g., Golden or Labrador Retriever, West Highland White Terrier, German Shepherd, Boxer, Shar-pei, French Bulldog, and Bull Terrier), and the previous response to glucocorticoids.[56,57] The characteristic clinical features are based on "Favrot's Criteria" (Table 1.1), which were developed from a large case series of confirmed cases of cAD.[58] Two sets of criteria are available, allowing clinicians to choose the version that best fits their diagnostic approach.[14,58] The likelihood of accurately diagnosing cAD increases as more criteria are fulfilled, with corresponding improvements in sensitivity and specificity.[14,58] Several diseases that can mimic the clinical presentation of cAD, including food-induced atopic dermatitis, ectoparasitic dermatitis (e.g., flea allergy dermatitis, demodicosis, sarcoptic mange, cheyletiellosis, pediculosis, trombiculiasis, and otoacariasis), secondary microbial skin infections (bacteria and/or yeast), and cutaneous epitheliotropic T-cell lymphoma.[14] A strict elimination diet trial is recommended to rule out food-induced atopic

dermatitis.[59] The identification of flea and/or flea feces on direct examination or brushing of the hair coat, along with the typical initial distribution of lesions in areas such as the lumbosacral area, tail base, and caudomedial thighs, supports a diagnosis of flea allergy dermatitis.[14,60] In cases where flea and/or flea feces are not observed, implementing a rigorous flea control program is advised; clinical improvement following such treatment may aid in distinguishing cAD from flea allergy dermatitis.[14,60] Diagnostic procedures, such as superficial or deep skin scrapings and acetate tape impressions, are helpful for ruling out ectoparasitic dermatoses.[61-66] Skin cytology helps identify secondary microbial infections, while a skin biopsy may be necessary to rule out cutaneous epitheliotropic T-cell lymphoma.[14, 67-68]

Table 1.1: Favrot's Criteria [14,58]

	Use	**Reliability**
Set 1: 1. Age at onset <3 years 2. Mostly indoor 3. Corticosteroid-responsive pruritus 4. Chronic or recurrent yeast infections 5. Affected front feet 6. Affected ear pinnae 7. Non-affected ear margins	- Use for clinical studies and adapt the required criteria based on the goal of the study - If higher specificity is required, 6 criteria should be fulfilled (e.g., drug trials with potential side effects) - If higher sensitivity is required, 5 criteria should be fulfilled (e.g., epidemiological studies) - Use to evaluate the probability of the diagnosis of cAD - 5 criteria should be fulfilled - Do not use alone for the diagnosis of cAD, and rule out resembling diseases	- 5 criteria: Sensitivity: 85.4% Specificity: 79.1% - 6 criteria: Sensitivity: 58.2% Specificity: 88.5%

8. Non-affected dorso-lumbar area		
Set 2: 1. Age at onset <3 years 2. Mostly indoor 3. “Alesional” pruritus at onset 4. Affected front feet 5. Affected ear pinnae 6. Non-affected ear margins 7. Non-affected dorso-lumbar area		- 5 criteria: Sensitivity: 77.2% Specificity: 83% - 6 criteria: Sensitivity: 42% Specificity: 93.7%

Allergy testing commonly used includes intradermal allergen testing (IDAT) and serological allergen testing (SAT).[14] However, these tests should not be used to diagnose cAD, as they lack standardization and may yield false-positive and false-negative results, which will be discussed below.[14] The primary purpose of allergy testing is to identify relevant environmental allergens to formulate allergen-specific immunotherapy (ASIT), which aims to desensitize affected dogs to the offending allergens.[14]

IDAT is an indirect assessment of cutaneous mast cell reactivity, mediated by allergen-specific IgE.[69] The common allergens that are tested include pollen, mites (house dust mites and storage mites), molds, epidermal extracts, insects, and whole flea extract.[14] Regional variation in environmental allergens, particularly pollens, necessitates geographic customization of test panels.[52] Additionally, intradermal allergen concentrations may vary, as different testing concentrations have been proposed over time.[52,70,71] IDAT is typically conducted on the lateral thorax following gentle hair clipping. Each allergen is injected intradermally in a volume of 0.05-0.1 mL, spaced at least 2 cm apart, to elicit a visible IgE-mediated wheal reaction.[14,72] The test site is evaluated 15-20 minutes after injection, with reactions compared to both a positive control (histamine phosphate) and a negative control (saline with phenol).[14] Assessment of wheal formation is performed using subjective and objective scoring methods.[73] The subjective scoring evaluates the diameter, degree of erythema, and turgidity of the wheal, while the objective scoring only evaluates the diameter of the wheal, measured in millimeters.[52,70, 74-76] Both methods typically use a 0 to 4+ grading scale, with a reaction graded ≥2+ considered positive, as outlined in Table 1.2.[52, 73-78] One study reported a moderate correlation between subjective and objective scoring, suggesting that using both methods in combination may yield a more accurate interpretation of IDAT results.[73]

Table 1.2: Subjective and Objective Scoring Parameters.[73] In subjective scoring, a reaction was assigned a score of 2+ when the combination of erythema, turgidity, and wheal diameter was considered midway between those of the positive and negative controls. In objective scoring, a score of 2+ was given when the mean wheal diameter was equal to or greater than the midpoint between the diameters of the positive and negative control.[73]

Subjective/Objective score	Description

1+	A wheal measures at least 25% greater than the negative control
2+	A wheal measures at least 50% greater than the negative control
3+	A wheal measures at least 75% greater than the negative control
4+	A wheal measures the same size or greater than the positive control
Clinically and significantly positive reactions	Any reaction with a score of ≥2+

SAT measures the concentration of allergen-specific IgE in the serum.[14] Among the various assay formats developed, the solid-phase enzyme-linked immunosorbent Assay (ELISA) is the most widely used.[14] This assay detects serum IgE specific to a panel of common indoor and outdoor allergens, including pollen, molds, mites (house dust mites and storage mites), epidermal allergens, flea, and insects.[14] Of the antibody types used for IgE detection, monoclonal, mixed monoclonal, and polyclonal anti-canine IgE, monoclonal anti-canine IgE antibody is the most commonly utilized due to its higher sensitivity and specificity.[14] These monoclonal anti-canine IgE antibodies bind to serum IgE that is attached to allergen-coated surfaces, and the amount of signal generated is proportional to the quantity of monoclonal antibodies bound to allergen-specific IgE.[79] This result is quantified by measuring optical density, with a reaction considered positive when the optical density exceeds a cut-off value established by the testing laboratory.[79] An alternative method involves the use of a recombinant fragment of the extracellular portion of the human high-affinity IgE receptor alpha-subunit (FcεRIα), which exhibits high affinity for canine IgE and minimal cross-reactivity with IgG, thereby improving the specificity of the test.[14,80,81]

IDAT and SAT have advantages and disadvantages. The advantage of IDAT is that it has been considered as a "gold standard," because it provides functional evidence of hypersensitivity reactions in the skin of dogs with cAD.[14,82] However, it has several disadvantages. Sedation is typically required due to discomfort associated with multiple intradermal injections.[14] Various sedative options, such as xylazine hydrochloride, medetomidine (dexmedetomidine), thiamylal, halothane, isoflurane, tiletamine/zolazepam, propofol, and methoxyflurane, have historically been used without affecting IDAT outcomes.[14] A recent publication found that butorphanol reduced wheal size compared to dexmedetomidine, though it did not alter the subjective interpretation of test results.[83] Another recent publication demonstrated that Zenalpha® (a combination of medetomidine and vatinoxan hydrochlorides) did not affect the wheal formation relative to dexmedetomidine, suggesting it may serve as an acceptable alternative sedative option for IDAT.[84] Conversely, certain sedatives, such as oxymorphone, acepromazine, morphine, and ketamine/diazepam, are not recommended, as they may interfere with the test results.[14] In addition to sedative considerations, certain medications must be discontinued prior to the test to reduce the risk of false-negative results.[14,85] These medications include antihistamine (7 days washout period), short-acting oral glucocorticoids (14 days washout period), long-acting injectable glucocorticoids (at least 28 days washout period), and topical glucocorticoids (14 days washout period).[85] This test also requires specialized training for accurate administration and interpretation, and as such, is typically performed by veterinary dermatologists. Consequently, IDAT may not be accessible in regions without specialist care.

SAT offers several advantages. It does not require sedation, is less traumatic since it avoids multiple intradermal injections, can be performed by general practitioners without specialized training, and requires less time to complete.[14] Additionally, unlike IDAT, SAT may

not necessitate withdrawal of certain medications prior to testing.[85] However, a recent publication suggests that modified cyclosporine and lokivetmab (Cytopoint®) may negatively influence SAT results, although further studies are needed to confirm these findings.[86]

Both tests present similar limitations. These tests are not standardized and are conducted without independent oversight of quality control.[87] Both test methods are prone to false-positive and false-negative results.[14] In IDAT, false negative results may arise from several factors, including improper injection technique, suboptimal allergen concentrations, interference from medications, intrinsic host factors (e.g., stress), incorrect allergen selection, testing outside the appropriate window (i.e., >60 days after or during the peak allergy season), and the presence of atopic-like dermatitis.[14,52,70,71,85,88] False positive results in IDAT may arise from excessively high allergen concentrations, allergenic cross-reactivity (e.g., between house dust mite and Sarcoptes spp., or between house dust mite and storage mite), and positive reactions occurring in non-atopic dogs.[14,89,90] SAT faces its own set of challenges, such as low specificity, inter- and intra-laboratory variability, and in vitro cross-reactivity due to cross-reactive carbohydrate determinants (CCD).[14,79,87,90,91-93] CCDs are highly antigenic unique carbohydrate moieties present on various plant and insect allergens.[94] Although they are generally of limited clinical relevance, they can affect sensitivity and specificity of SAT.[95-100] Recent studies reported that the inclusion of anti-CCD IgE blocker, such as pineapple stem bromelain and horseradish peroxidase, reduced the incidence of false positive reactions in SAT.[86,101,102] Another limitation of SAT lies in the use of crude whole allergen extracts, which are derived from natural allergen sources and thus represent undefined mixtures of allergenic and nonallergenic components.[103] This introduces several concerns: 1) Difficulty in standardization, leading to batch-to-batch variability and inconsistent test results 2) Absence of clinically relevant allergens in some

extracts, potentially resulting in false negative results 3) Increased risk of cross-reactivity (e.g., between *Toxocara canis* and *Dermatophagoides farinae* allergens Der f 15 and Zen-1).[103-105] In response to these issues, a recent publication advocated for the incorporation of defined, clinically relevant single molecular components, either alongside or in place of crude extracts, for potentially improved diagnostic accuracy.[104]

Additionally, only a limited number of studies have reported the correlation between IDAT and SAT, with agreement ranging from slight to fair.[86,101] This discrepancy likely reflects fundamental methodological differences: IDAT evaluates cutaneous allergen-specific IgE bound to mast cells and other immune cells (e.g., eosinophils and basophils), whereas SAT measures circulating allergen-specific IgE in the serum.[14,106] Nonetheless, one study suggested that allergen-specific immunotherapy (ASIT) guided by either testing modality yields comparable clinical outcomes.[74] However, further research is warranted to confirm the equivalency of treatment efficacy between IDAT- and SAT-based protocols.

In summary, the diagnosis of cAD remains complex, and neither IDAT nor SAT should be relied upon as a standalone diagnostic tool. A comprehensive clinical history, detailed dermatological examination, and exclusion of other dermatological diseases with similar clinical presentation continue to be the foundation for an accurate diagnosis.

Treatment of cAD

As previously noted, cAD is an inflammatory skin disease characterized primarily by pruritus and driven by a complex interplay among skin barrier dysfunction, dysregulated immune system, and allergen sensitization.[1] Currently, there is no definitive cure for cAD; therefore, a multimodal therapeutic approach is essential to alleviate cutaneous inflammation and pruritus.[6,82] Treatment strategies should aim to address flare factors, such as allergens (e.g., mites, pollens,

molds), food induced cAD, flea bite hypersensitivity, and secondary infections (bacteria and/or yeast), improve skin barrier dysfunction, and modulate dysregulated immune system.[6,82] Therapeutic plans should be individualized, taking into account the chronicity and severity of the disease.[6] Once acute flares are brought under control, it is critical to implement a long-term management strategy to minimize the risk of relapse and maintain clinical remission.[82]

The management of flare factors in cAD begins with their identification.[14,107] Dogs with cAD are predisposed to flea bite hypersensitivity, and year-round flea control using adulticidal products, in combination with environmental decontamination, is strongly recommended.[14]

Microbial dysbiosis is a hallmark of cAD and can significantly exacerbate cutaneous inflammation and pruritus.[6,49] Dogs with cAD have an increased risk of microbial dysbiosis characterized by the overgrowth of bacteria (e.g., *Staphylococcus pseudintermedius*) and/or yeast (e.g., *Malassezia pachydermatis*).[6] Once microbial dysbiosis is confirmed via skin cytology, treatment should be guided by the type of organism (bacteria vs. yeast), the depth of infection (superficial vs. deep), and the extent of the lesion (localized vs. generalized).[108] Topical therapy is the first-line treatment for superficial infections, whether localized or generalized.[6,108] Formulations containing antiseptic, antibacterial, or antifungal agents may be applied as sprays, ointments, wipes, or mousse for localized infections, or as shampoos for generalized involvement.[6,108] Treatment frequency is dictated by the severity and chronicity of the infection.[6] Typically, medicated bathing is recommended twice weekly, while topical applications of ointment, wipes, mousse, and spray products once to twice daily during the initial management of acute flares.[6,108] Systemic therapy (e.g., oral antibiotics and antifungals) is generally reserved for deep or generalized infections.[108] For recurrent bacterial infections that do not respond to empirical systemic antibiotics, bacterial culture and susceptibility testing are advised.[109] Due to

the increasing prevalence of antimicrobial resistance, recent guidelines emphasize the use of topical therapies when feasible.[110] Topical therapies offer several advantages; they can deliver drug concentrations exceeding minimum inhibitory concentrations, target the site of infection directly, and aid in reducing surface microbial load through mechanical cleansing.[6,108,110] Following resolution of infection, once-weekly bathing with a non-irritating shampoo is recommended for long-term maintenance to help prevent recurrence and support skin barrier health.[6]

For dogs with both environmentally- and food-induced AD, it is recommended to avoid dietary components known to trigger clinical flares.[59] Ideally, environmental allergens should be avoided for dogs with cAD. However, complete avoidance is often impractical given the ubiquitous presence of common allergens such as mites and pollens.[82] One uncontrolled study demonstrated potential clinical improvements in mite-hypersensitive dogs following environmental control using an acaricide benzyl benzoate spray (Acarosan Spray).[111] While further evidence is lacking, routine and thorough cleaning of the home environment, including pet bedding, may provide some benefits.[82]

Given the challenges of allergen avoidance, ASIT remains the mainstay for inducing and maintaining clinical tolerance to allergens, thereby reducing the frequency and severity of flares and potentially minimizing the need for pharmacologic treatments, which will be discussed below.[112] Although the precise mechanisms of ASIT are not fully understood, proposed immunological effects include early desensitization of mast cells and basophils, induction of interleukin-10-producing regulatory T and B cells, modulation of IgE and IgG4 production, and inhibition of eosinophils, mast cells, and basophils' activity within the affected tissues.[113]

Several forms of ASIT are available, each with distinct protocols, efficacy profiles, and safety considerations:[114]

1. Subcutaneous immunotherapy (SCIT) – Conventional Protocol: This traditional form of ASIT involves subcutaneous injections of allergen extracts, beginning with a low concentration during an induction phase, with a gradual increase in volume, concentration, and dosing interval.[114] Once the maintenance dose is achieved, injections are typically administered every 7 to 30 days, depending on the protocol and manufacturer, as there is currently no standardized regimen.[8,9] Reported efficacy rates range from 19% to 70%, and the clinical improvement may take up to 12 months.[116-119] Severe adverse reactions have been reported for only 1% of patients.[116-119]

2. Subcutaneous immunotherapy – Rush Protocol: It significantly shortens the induction phase to less than 24 hours and is generally performed in a clinical setting under close veterinary supervision due to the increased risk of adverse reactions.[116,120] The overall efficacy was reported to be comparable between the conventional protocol and the rush protocol.[116,120]

3. Sublingual immunotherapy (SLIT): It involves the administration of allergen extracts onto the oral mucosa once or twice daily.[121,122] It is considered safe, well-tolerated, and non-invasive, with none to minimal reported adverse effects.[121,122] However, a recent study reported a relatively low success rate of approximately 14%.[119]

4. Intralymphatic immunotherapy (ILIT): It delivers allergens directly into peripheral lymph nodes, thereby targeting T cells more efficiently.[123] This approach may shorten the time to clinical improvement, prolong therapeutic efficacy, and reduce adverse reactions, as lymph nodes typically lack mast cells.[123] A recent study demonstrated a high success rate (80%) of intralymphatic immunotherapy, outperforming both SCIT and SLIT.[119] However, ILIT requires

administration by a veterinarian, often under ultrasound guidance, due to the technical difficulty in locating lymph nodes.[124]

5. Epicutaneous immunotherapy (EPIT): It is a novel modality where allergens are delivered via a transdermal patch worn for 12 hours once weekly.[125] Preliminary data from a single study in dogs with cAD demonstrated promising results, with 73.3% and 66.7% reduction in pruritus and skin lesions, respectively.[125] Further studies are needed to validate these findings and establish long-term efficacy.

6. Adjuvanted immunotherapy: Adjuvants may be incorporated into subcutaneous and intralymphatic immunotherapy to induce a quicker, more potent, and longer-lasting immune response to ASIT.[114,126] Therefore, they help to make ASIT more efficacy and even simpler with less frequent injections.[126] However, the efficacy of adjuvants appears to vary depending on the type used.[127] Recent data suggest that Def f 2-pullulan, polymerized allergoids coupled to a nonoxidized mannan, and tyrosine adjuvanted SCIT demonstrated superior efficacy and shorter time to achieve clinical improvement compared to alum-precipitated SCIT.[127]

ASIT formulations are typically based on the combination of the clinical history of the patient (i.e., seasonality of clinical signs) and allergy test results.[128] However, only one study has investigated the correlation between clinical history and IDAT outcomes, reporting a poor correlation between the two.[129] This highlights the need for more research to refine allergen selection criteria and improve predictive value.

For dogs with atopic-like dermatitis that test negative on allergy testing, nonspecific immunotherapy may be considered as a therapeutic option.[114,130] This approach involves the use of a predefined mixture of 20 to 22 allergens considered clinically relevant for the specific geographic region in which the patient resides.[130] It is available in both SCIT and SLIT

formulations.[114] One study reported a good to excellent clinical response in 57% of dogs with cAD following at least nine months of SCIT administration.[130] However, further research is needed to validate these findings and to better define the indications, efficacy, and mechanisms of nonspecific immunotherapy in atopic-like dermatitis dogs.

Although ASIT is a safe and effective long-term management strategy for cAD, it has a delayed onset of action as mentioned above. Clinical improvement may take several months to a year, with approximately 20% of dogs achieving an excellent response and an additional 40-50% showing satisfactory improvement.[6,116,117,131] Given this delay, concurrent use of faster-acting symptomatic therapies is often necessary to manage pruritus and inflammation during the induction phase of ASIT.[114]

Glucocorticoids remain among the most effective and rapidly acting anti-inflammatory agents for managing acute flares of cAD.[82] They exert their effects by suppressing a wide range of inflammatory cells and mediators.[82] Previous data indicated that 50-80% of dogs with cAD experienced ≥ 50% reduction of pruritus and skin lesions.[132] The improvement was observed within a few hours.[132] Both systemic and topical formulations of glucocorticoids are available.[82] Typically, systemic and/or topical glucocorticoids are used during the initial phase to induce clinical remission, after which topical preparations may be continued for maintenance therapy.[82] It is important not to taper or discontinue glucocorticoids until clinical signs are adequately controlled.[82] Prolonged use of systemic glucocorticoids is associated with well-documented adverse reactions, including polyuria, polydipsia, polyphagia, muscle and skin atrophy, increased susceptibility to secondary bacterial and fungal infections, demodicosis, and iatrogenic hyperadrenocorticism.[54,133,134] However, long-term topical glucocorticoids are less likely to induce systemic side effects, especially cutaneous atrophy, when used appropriately.[135-137]

To minimize the adverse reactions associated with long-term systemic glucocorticoid use, a variety of steroid-sparing agents are available for the management of cAD.[82] These agents offer an alternative mechanism to control inflammation and pruritus, providing both short- and long-term relief while reducing reliance on glucocorticoids.[82]

1. Janus Kinase (JAK) – Signal Transducer and Activator of Transcription (STAT) Pathway Inhibitors: Oclacitinib (Apoquel®) was the first JAK inhibitor approved for the treatment of cAD in the United States and Canada.[138] JAKs are non-receptor tyrosine kinases that mediate signaling from various cytokine receptors, playing a key role in inflammatory gene expression.[138] Among four JAK families of enzymes (e.g., JAK1, JAK2, JAK3, and tyrosine kinase 2), oclacitinib selectively inhibits JAK1, thereby modulating immune dysregulation in cAD.[139]

In 2024, ilunocitinib (Zenrelia®) became the second JAK inhibitor approved for the treatment of cAD.[140] Ilunocitinib inhibits JAK1, JAK2, and tyrosine kinase 2.[140] A recent comparative study suggested ilunocitinib may offer improved efficacy, with a 70% reduction in pruritus and 73% reduction in skin lesions at four weeks, compared to 60% and 70%, respectively, for oclacitinib.[140] Both drugs demonstrated a rapid onset of action, often within a few hours.[140] Reported adverse reactions in both medications include vomiting, diarrhea, increased susceptibility to secondary bacterial or fungal infection or opportunistic infection (e.g., viral papilloma), demodicosis, bone marrow suppression, and hepatopathy.[82,140] Further research is warranted to fully establish the long-term safety and efficacy profiles of ilunocitinib.

2. Calcineurin inhibitor: It exerts an anti-inflammatory and immunomodulatory effect by inhibiting T-cell activation.[141] Systemic formulations (e.g., modified cyclosporine) have shown good to excellent efficacy, with ≥ 50% improvement in pruritus and skin lesions in 50-70% of

dogs with cAD.[132] Topical formulations (e.g., tacrolimus) have demonstrated effectiveness in reducing localized pruritus and erythema in one pilot study.[142] The onset of action is slower, typically requiring 4 to 6 weeks to achieve clinical benefit.[141] The most common adverse reactions include vomiting and diarrhea, which occur in approximately 30% of treated dogs but are usually self-limiting within 7-10 days.[82,143] Administering it with food or freezing the capsule may help reduce these adverse reactions.[143] Less frequent adverse effects include lower urinary tract infection, increased susceptibility to opportunistic infections (e.g., fungal infection), gingival hyperplasia, psoriasiform-lichenoid-like dermatitis, and hyperplastic verrucous lesions.[144,145]

3. Monoclonal antibodies: They are highly specific, biologically engineered proteins designed to target defined antigens.[146] Lokivetmab (Cytopoint®) is a caninized monoclonal antibody that binds and neutralizes canine interleukin-31, a key cytokine involved in pruritus in dogs.[146] It provides rapid relief, often within 1 to 3 days, and has a prolonged effect lasting 3 to 4 weeks due to its long half-life.[146] Approximately 50% of treated dogs with cAD showed a reduction in pruritus.[146] It is a well-tolerated medication, with a minimal incidence of adverse reactions.[146]

Other therapeutic options with limited efficacy or supporting data include antihistamines, pentoxifylline, azathioprine, and mycophenolate mofetil.[14,107,147-149] Nutritional interventions, such as prescription diets (e.g., Royal Canin Skintopic™), omega-3 and omega-6 fatty acids supplements, vitamin D, and palmitoylethanolamide (PEA) may provide adjunctive benefits, but further research is necessary to validate their clinical utility.[14,150-153]

In summary, the treatment of cAD requires a multimodal, individualized approach.[6] While symptomatic medications offer rapid relief, they are associated with varying degrees of

undesirable adverse reactions. Client education is essential to emphasize the importance of long-term disease control strategies, including ASIT, which may offer a safer and more sustainable solution over time.

Study Rationale

In summary, cAD is a chronic, multifactorial dermatologic condition that requires lifelong and multimodal management. ASIT is often used as a safe, long-term treatment option for cAD. It is typically formulated by allergy testing. However, the existing literature offers limited data on the correlation between IDAT and SAT results. Clarifying the degree of agreement between these two tests could aid clinicians in selecting or combining tests more effectively when designing ASIT protocols. Furthermore, evaluating the relationship between clinical history and allergy test outcomes may enhance the clinical relevance of test interpretation, ultimately leading to the formulation of more appropriate ASIT.

This study aims to address these knowledge gaps by assessing the correlation between IDAT and SAT results and their relationship with patient clinical histories.

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CHAPTER 2

OBJECTIVES

The two sections below, Objective 1 and Objective 2, correspond to the included article.

Objective 1

Hypothesis: The correlation between IDAT and SAT results will range from fair to moderate overall and within specific allergen categories (mites, molds, grasses, weeds, trees, and flea). Stronger correlations are anticipated among allergens with higher SAT reactivity.

Objective 1: To assess the degree of correlation between IDAT and SAT results for 29 allergens, comprising four mites, six molds, eight grasses, five weeds, five trees, and flea, in 29 dogs diagnosed with atopic dermatitis, using various positive cut-offs.

Objective 2

Hypothesis: There will be no significant correlation between clinical history and the results of IDAT and SAT.

Objective 2: To investigate the correlation between clinical history and the outcomes of IDAT and SAT in dogs with cAD.

CHAPTER 3

EVALUATION OF THE CORRELATION OF SEROLOGICAL AND INTRADERMAL ALLERGEN TESTING WITH CLINICAL HISTORY IN 29 DOGS WITH ATOPIC DERMATITIS[1]

[1] Chong, E., Austel, M., and Banovic, F. 2024. *Veterinary Dermatology*. 35(5): 516-23.
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Abstract

Background –Limited information exists about the correlation between clinical history and positive serum (SAT) and intradermal allergen test (IDAT) results in atopic dogs. **Objectives –** To evaluate the correlation between clinical history and SAT/IDAT results in atopic dogs. **Animals –** Twenty-nine client-owned dogs with nonseasonal atopic dermatitis with or without seasonal exacerbation were enrolled. **Materials and Methods –** IDAT, SAT (immunoglobulin (Ig)M antibody capture enzyme-linked immunosorbent assay [MacELISA] with bromelain CCD inhibitor), and clinical information collected in a questionnaire regarding seasonal variations in pruritus affecting the dogs were performed on the same day. Two independent investigators (Inv A and Inv B) recorded IDAT results. **Results –** The kappa coefficient agreement for positive IDAT scores between Inv A and Inv B was substantial. The agreement between IDAT and SAT was slight and hair for both investigators, respectively. A higher agreement was observed between IDAT and SAT (≥300 EAU) than between IDAT and SAT (>79 EAU), with the exception of mite and flea allergens. There was a statistically significant association between clinical history and positive IDAT results for seasonal allergens (Inv A and Inv B, P=0.016). There was no significance between positive SAT results and clinical history. Five (IDAT) and 12 of 13 (SAT) atopic dogs without clinical seasonal exacerbation showed positive results for seasonal allergens. **Conclusions and Clinical Relevance –** The agreement between IDAT and SAT ≥300 EAU results was fair and the agreement between IDAT and SAT >79 EAU results was slight for all allergens. Only positive IDAT results significantly correlated with clinical history.

Introduction

Canine atopic dermatitis (AD) is a common inflammatory and pruritic skin disease, typically mediated by immunoglobulin (Ig)E directed against environmental allergens.[1] Allergen immunotherapy (AIT) is considered a relatively safe long-term therapeutic option for the management of canine AD.[2] Intradermal allergen testing (IDAT) and serum allergen-specific IgE testing (SAT) are regularly performed to select allergens for the formulation of AIT.[3]

Although IDAT has been considered the preferred diagnostic tool for selecting allergens to formulate AIT for many years, it is not typically performed by veterinary surgeons. In addition, SAT has several advantages over IDAT, including lack of complications associated with sedation, minimal time effort for a one-time blood collection, and overall lower stress levels for the patients involved.[3] However, recent studies have shown conflicting results regarding the correlation between IDAT and SAT in atopic dogs.[4,5] Such variable results may belong to the results of differences in SAT platform testing systems, variable multicentre study designs and multiple evaluators involved in IDAT assessment without accounting for the correlation analysis between investigators.

Pruritus is a main clinical sign associated with canine AD[6], and it can vary seasonally depending on the offending allergens.[1] The clinical history, which relates to the development of pruritus and clinical AD signs, is considered an essential aspect in formulating AIT.[7] Correlating positive IDAT and/or SAT results with the patient's history regarding disease seasonality and presence of allergens in the environment is an important aspect in the decision-making process for the clinician.[7] There has been limited information regarding the association between clinical history and positive IDAT or SAT reactions in atopic dogs.[6] However, a recent study demonstrated a poor correlation between positive IDAT results and clinical history.[6] To the best

of the author's knowledge, no studies have investigated the correlation between positive SAT results and the clinical history of atopic dogs to date.

There were two aims for the current study: (i) to evaluate the correlation between IDAT and SAT results for 29 allergens (four mites, six moulds, eight grasses, five weeds, five trees, and flea) in 29 atopic dogs; and (ii) to investigate the correlation between clinical history, and IDAT and SAT results.

Material and Methods

This prospective study was approved by the Institutional Animal Care and Use Committee of the author's practice (CR-686). Informed consent was obtained from pet owners before each patient's enrollment. The power analysis for the Cohen's kappa (κ) correlation assessment (estimated moderate κ of 0.5) was conducted with an online calculator (https://wnarifin.github.io/ssc/sscorr.html) using a power of 0.8 (two-sided analysis, $p = 0.05$) revealed a minimum sample size of 29 dogs.

Patient inclusion criteria

Patients were included after a clinical diagnosis of environmentally-induced canine AD was made based on compatible history and clinical signs as previously described[8]; all dogs were ruled out from having concurrent flea-bite hypersensitivity or food-induced AD by established standardized criteria including lack of clinical signs, presence of fleas and regular continuous flea preventatives (e.g., isoxazolines) for flea-bite hypersensitivity without clinical improvement and elimination diet trial with novel protein diets or hydrolyzed diets (e.g., Purina Elemental or Royal Canin Ultamino) for a minimum of 8 weeks without clinical improvement. During the elimination diets, all of the flea prevention was changed to nonoral medication, such as topical

isoxazolines or imidacloprid.[3] At the time of examination, the pruritus score was recorded by owners using a Visual Analog Scale (pVAS) scored in respect of the previous 24h.[13] Where possible, dogs were tested during a period of exacerbation of pruritus. To minimize possible effects of pharmaceuticals on IDAT and SAT test results, injectable glucocorticoids, oral/topical glucocorticoids, and oral antihistamines were discontinued ≥ 28, 14, and 14 days, respectively, before testing.[9] Ciclosporin was discontinued for a minimum of 5 days before IDAT and SAT testing. There were no withdrawal times for lokivetmab and oclacitinib.[16] Although no specific recommendation exists regarding AIT washout for IDAT and SAT, dogs that had not AIT for ≥6 months were allowed in the study.

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Serum allergen testing

Before IDAT, 6 mL of blood was collected by venipuncture from each dog, and serum was shipped to Stallergenes Greer Laboratories (Lenoir, NC) immediately for allergen-specific IgE testing via Stallergenes Greer IgM antibody capture enzyme-linked immunosorbent assay (MacELISA).[12] Bromelain cross-reactive carbohydrate determinants (BROM-CCD) inhibitor was added to the diluent buffer at the defined concentration of 2.5 mg/mL before adding the serum sample. All results were expressed as ELISA absorbance units (EAU) per manufacturer.[12] Two cut-off values for positive reactions were used in this study as provided by the manufacturer: SAT allergen values > 79 EAU (all positive) and allergen values ≥ 300 EAU (strongly positive).

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Intradermal allergen testing

All patients were sedated with dexmedetomidine (Dexdomitor, Zoetis) intravenously at 5 µg/kg body weight. An area of approximately 20 x 10 cm was clipped on the right or left lateral thorax for intradermal allergen injections. Twenty-nine allergens used in IDAT and SAT(see Table 3.2 in Supporting information) were selected for this study. The test concentration of allergens that were used for IDAT was provided in Table 3.3. After 15 and 30 min, subjective evaluations of IDAT reactions were performed by two independent investigators: Inv A, a dermatology referral clinician with years of experience; and Inv B, a resident in training after a dermatology-specific internship. Both were blinded to the other's scores which were based on erythema, wheal size, turgidity, and slope of the reaction ranging from 0 (negative) to 4 (high reactivity), as reported previously.[4-6,10,11] The reaction was considered positive if the reaction was graded ≥ 2 at any reading of the two readings. A reaction was considered negative if the reaction was graded ≤ 1 at any of the two readings.[4-6,10,11].

Clinical history questionnaire

All owners were asked to fill out a clinical history questionnaire form at the time of allergen testing (Table 3.4).[6] The questionnaire was utilized in the previous study and contained relevant information regarding the clinical history of patients (e.g., the owner reported severity of pruritus for each calendar month) and the environment in which the patient lives.[6] All patients in this study, per inclusion criteria, had a history of pruritic behaviors, with the intensity graded as mild, moderate, or severe.[6] The investigators reviewed the questionnaire after scoring IDAT.

Statistical analysis

Statistical analysis was performed using Prism 9.0 (GraphPad Software Inc.). Descriptive data were summarized. Allergens were grouped into seasonal allergens (tree, grass, and weed

pollen) and perennial allergens (house dust and storage mites, moulds, and flea). Cohen's kappa (κ) was used to evaluate the agreement between the two tests. 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 0.81-1.0 almost perfect.[14]

In order to evaluate the correlation between clinical history and IDAT and SAT results, allergens were categorized into seasonal allergens and perennial allergens as before. Based on the clinical history, atopic dogs were allocated to one of two groups: patients with nonseasonal pruritus without seasonal exacerbation or patients with nonseasonal pruritus with seasonal exacerbation. Results of IDAT and SAT were correlated with the clinical history of occurrence of pruritus over the calendar year. Fisher's exact test was utilized to calculate statistical significance; P values of <0.05 were considered significant.

Results

A total of 29 dogs, 16 males (3 intact and 13 castrated) and 13 females (all spayed), were included in the study. The mean age was 4.5 years (range: 1-8 years). The mean weight was 21.6 kg (range: 5.5-38 kg). The following breeds were included: mixed breed (n=8), Shih Tzu (n=2), Welsh Terrier, Boykin Spaniel, Rat Terrier, American Bulldog, Dalmatian, Pug, Jack Russell Terrier, Labrador Retriever, Cavalier King Charles Spaniel, Vizsla, French Bulldog, Golden Retriever, German Shepherd, English Bulldog, Boxer, Chinese Crested, Cocker Spaniel, Basset Hound, Husky (n=1 each). The mean age of onset of clinical signs was 1.8 years old (range: 4 months to 6 years). On the day of IDAT and SAT, the mean pVAS was 5.6 (range: 0-10).

Evaluation of all samples for assessing agreement between tests

A total of 1,682 reactions were evaluated to determine the agreement between IDAT and all positive SAT results (>79 EAU) and IDAT and strongly positive SAT results (≥300 EAU).

Agreement between IDAT and SAT for all allergens

Substantial agreement (κ = 0.63) was noted between Inv A and Inv B for IDAT results (Table 3.1). Slight agreement was noted between all positive SAT results (>79 EAU) and IDAT results of Inv A (κ = 0.17) and IDAT results of Inv B (κ = 0.19), respectively (Table 3.1). A fair agreement was noted between strongly positive SAT (≥300 EAU) and IDAT results of Inv A (κ = 0.38) and IDAT results of Inv B (κ = 0.25), respectively (Table 3.1).

Agreement for different allergen subgroups between IDAT and all positive SAT results (>79 EAU)

The results of the correlation assessment between IDAT (Inv A and Inv B) and all positive SAT results (>79 EAU) are summarized in Figure 3.1. Across all comparisons, there were only minor differences between investigators overall; the correlations for threes, grasses, and weeds were very similar for both investigators. Slight (Inv A; k = 0.05) and fair (Inv B; k = 0.29) agreements were noted for flea. Fair (Inv A; k = 0.36) and moderate agreement (Inv B; k = 0.42) were noted for mites.

Agreement for different allergen subgroups between IDAT and strongly positive SAT results (≥300 EAU)

The results of the correlation assessment between IDAT (Inv A and Inv B) and strongly positive SAT results (≥300 EAU) are summarized in Figure 3.1. For Inv A and strongly positive SAT results (≥300 EAU), fair agreement was noted with trees (k=0.38), grasses (k=0.38), and weeds (k=0.35), and moderate agreement was noted with mites (k=0.41). For Inv B and strongly positive SAT results (≥300 EAU), fair agreement was noted with trees (k=0.23), grasses (k=0.22), weeds (k=0.21) and mites (k=0.21).

Correlation between clinical history and IDAT and all positive SAT results (>79 EAU)

Of 29 dogs, 13 showed year-round pruritus without seasonal worsening, and the remaining 16 showed year-round pruritus with seasonal worsening. The distribution of dogs that showed positive reactions to perennial or seasonal allergens regarding IDAT (Inv A and Inv B) and SAT (>79 EAU) compared to clinical history are summarized in Figure 3.2 and Supplementary Table 3.5-3.10.

The correlation between clinical history and IDAT results of Inv A and Inv B showed that 2 out of 16 dogs (12%) with year-round pruritus and seasonal exacerbation exhibited negative IDAT results for seasonal allergens. For both investigators (A and B), 5 out of 13 dogs (38%) with year-round pruritus without seasonal exacerbation exhibited positive IDAT results for seasonal allergens. There was a statistically significant positive correlation (Fischer exact test; $p = 0.016$) between positive IDAT results for seasonal allergens and clinical history, for Inv A and Inv B, respectively.

The correlation between clinical history and all positive SAT results (>79 EAU) revealed that 3 out of 16 (18%) dogs with year-round pruritus and seasonal exacerbation exhibited negative SAT results for seasonal allergens. Furthermore, 12 out of 13 (92%) dogs with year-round pruritus without seasonal exacerbation still exhibited all positive SAT results (>79 EAU) for seasonal allergens; 6 of these 12 dogs (50%) had positive SAT results for all seasonal allergen subgroups (trees, grasses, and weeds). No statistical significance was observed for any comparisons of all positive SAT results (>79 EAU) with clinical history.

Although 12 out of 13 (92%) atopic dogs without seasonal exacerbation showed all positive SAT (>79 EAU) results for seasonal allergens compared to 5 out of 13 (38%) atopic dogs tested via IDAT, this difference was not statistically significant (P = 0.21).

	Inv A (IDAT)	Inv B (IDAT)	SAT (>79 EAU; all positive)	SAT (≥300 EAU; strongly positive)
Inv A (IDAT)		0.63	0.17	0.38
Inv B (IDAT)	0.63		0.19	0.25

Table 3.1: Cohen's kappa (k) agreement between investigator (Inv) A and Inv B, and intradermal allergen testing (IDAT) with subjective scoring and serum allergen testing (SAT) with immunoglobulin (Ig)M antibody capture enzyme-linked immunosorbent assay (MacELISA) with bromelain cross-reactive carbohydrate determinants (BROM-CCD) inhibitor for all allergens. Values <0 indicate no agreement, 0-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.8 substantial and 0.81-1 almost perfect agreement. Abbreviations: EAU, ELISA absorbance units, Inv A, investigator A; Inv B, investigator B; IDAT, intradermal allergen test; SAT, serum allergen-specific IgE testing.

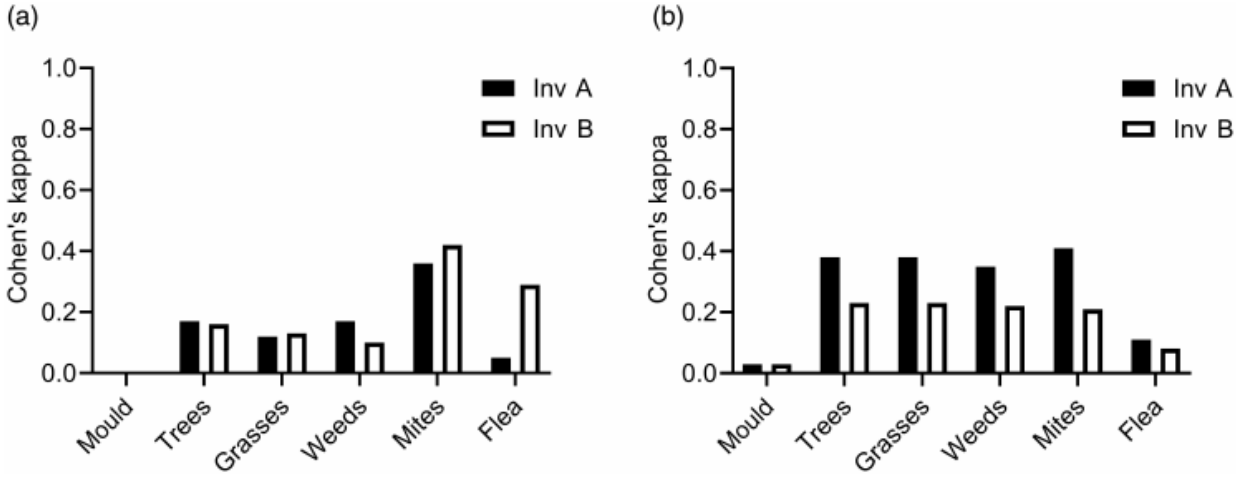

Figure 3.1: Correlation (Cohen's kappa) between intradermal allergen testing (IDAT) results for investigator (Inv) A (a) and Inv B (b), and all positive serum allergen testing (SAT; >79 ELISA absorbance units [EAU]) results and strongly positive SAT (≥300 EAU) results. Values <0 indicate no agreement, 0-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.8 substantial and 0.81-1 almost perfect agreement.

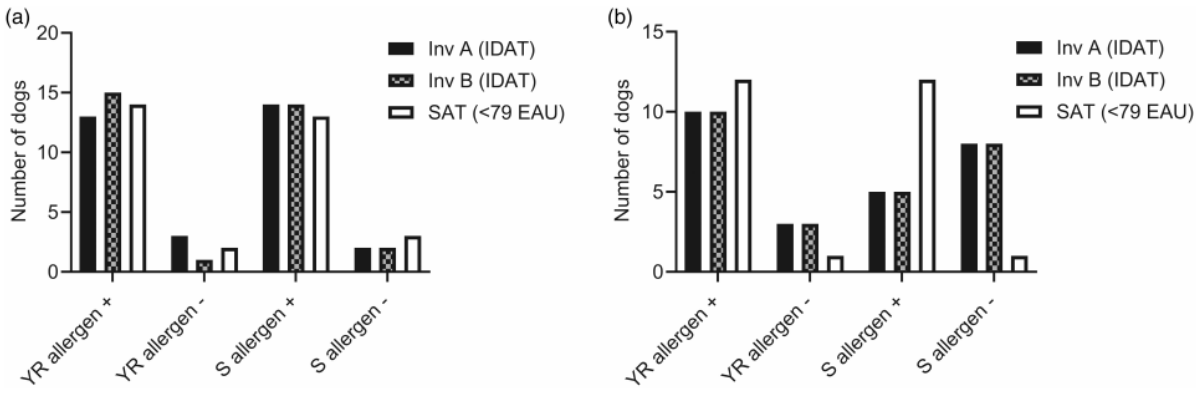

Figure 3.2: Correlation between clinical history of pruritus and intradermal allergen testing (IDAT) results for investigator (Inv) A and Inv B, and all positive serum allergen testing (SAT; >79 ELISA absorbance units).

Discussion

In this study, slight agreement was observed between IDAT and all positive SAT results (>79 EAU), and fair agreement was noted between IDAT and strongly positive SAT results (≥300 EAU) in atopic dogs. Furthermore, the IDAT results were more strongly correlated with the clinical history of seasonal exacerbation than those of SAT. To the best of the author's knowledge, there have been no specific guidelines published on how the specific reference range cut-off values (e.g., EAU and Heska Episolon Receptor Binding Units (HERBU)) for positive test reactions for SAT are determined by the laboratories performing SAT. In this study, the laboratory (Stallergenes Greer Laboratory) provided SAT results with two cut-offs for positive reactions: a cut-off of >79 EAU for all positive allergens and a cut-off of 300 ≥EAU for strongly positive allergens. A higher correlation agreement was observed between IDAT and SAT results at an allergen cut-off value of ≥300 EAU in the study of this report, which may indicate that higher concentration of allergen-specific serum IgE antibodies correlate better with IDAT results in atopic dogs.

Our results support the findings of a previous study correlating IDAT and SAT results in atopic dogs.[5] However, in contrast to our findings, a second study showed moderate agreement between IDAT and SAT results in dogs with AD.[4] A possible explanation for the differences in the study results may be the utilization of different SAT platforms and cut-off values to determine positive reactions compared to previous studies.[4,5] In one study, serum samples were submitted to Heska diagnostic laboratory (Fribourg, Switzerland), which provides results in HERBU and utilizes a commercial allergen-specific IgE Fc-ε receptor ELISA with CHO-blocker used as the IgE anti-CCD blocker.[4] However, our study resembled the methodology by a former study where Stallergenes Greer Laboratory technology was utilized for SAT[5]; this technology

uses a secondary antibody mixture of biotinylated monoclonal anti-IgE antibodies with the BROM-CCD used as the IgE anti-CCD blocker.

IDAT and SAT aim to evaluate the presence of allergen-specific IgE in an individual. However, it is reasonable to expect some variability in the test results, considering that differences exist in how these tests are performed.[15] While IDAT evaluates the reactivity of cutaneous allergen-specific IgE bound to mast cells and other immune cells (e.g., eosinophils and basophils), the SAT measures allergen-specific serum IgE antibodies in the circulation (e.g., serum).[3] Although it is currently unknown for dogs, the presumed half-life of free IgE in the blood is 2-3 days, whereas the cell-bounded IgE through the high-affinity receptor FcεRI on mast cells can be stable in human skin for several weeks.[20]

In the previous publications regarding IDAT testing in atopic dogs, subjective and/or objective scoring with global wheal scores have been utilized. In our study, subjective scoring, which evaluates the size of the wheal and the degree of erythema and turgidity compared to a positive (histamine) and negative (saline) control, was used.[4-6,10,11] Objective scoring requires the reader to measure the diameter of the wheal and compare that to the diameter of positive and negative controls, and then decide on the threshold for positive and negative results.[10] Interestingly, a recent study showed a substantial correlation between subjective and objective IDAT scores in atopic dogs.[5] Generally, subjective scoring methods have been used more frequently for IDAT because they can be performed faster than the objective scoring methods.[10] Considering that we had two blinded evaluators scoring IDAT reactions, we utilized only subjective scoring to be within a reasonable time frame.

No specific guidelines exist regarding the optimal time of the year when IDAT and SAT should be performed in atopic dogs. In our study, allergy testing with IDAT and SAT was

performed in most dogs during the time of the year when they were symptomatic for pruritus and/or atopic skin lesions. Previous correlation studies between IDAT and SAT in atopic dogs did not mention the specific time for allergy testing.[4,5] Limited studies evaluated serum IgE levels in atopic dogs at different times of the year with conflicting results. Two SAT studies supported finding a higher concentration of serum IgE antibodies against Japanese cedar pollen[17] and ragweed[18] during the pollination season in atopic dogs. A previous publication showed even higher positive serum IgE antibodies to botanical aeroallergen groups 60 days after heavy frosting in atopic dogs.[19] In the previously cited IDAT and SAT correlation study,[5] no apparent connection between seasonality and positive reactions to any allergen groups in IDAT and SAT was observed. To the best of the author's knowledge, there has been no prospective study with serial IDAT and/or SAT in atopic dogs during different seasons of the year to evaluate how different seasons may impact the results of SAT and IDAT. Therefore, the appropriate time for allergy testing remains unclear, and different testing times could have yielded different results in our study.

A recent study revealed no association between clinical history (seasonality) of pruritus and IDAT results in atopic dogs questioning the validity of the positive IDAT results and the possible impact on the success of AIT in atopic dogs.[6] In our study, we observed a statistically significant association between positive IDAT reaction to seasonal allergens and clinical history. Interestingly, we observed that 12 out of 13 (92%) atopic dogs without seasonal exacerbation exhibited positive SAT (>79 EAU) results for all seasonal allergens, with 6 of these 12 (50%) dogs being positive for seasonal allergen subgroups of trees, grasses, and weeds. In contrast, 5 out of 13 (38%) atopic dogs without seasonal exacerbation exhibited positive IDAT results for seasonal allergens, with only 2 dogs being positive for all seasonal allergen subgroups (trees,

grasses, and weeds). Although this difference was not statistically significant, all positive SAT (>79 EAU) results showed higher positive seasonal allergen reactions in atopic dogs without seasonal exacerbation based on the clinical history. Unfortunately, one of our study limitations was the lack of a solely seasonal AD group. Further studies should ideally include canine AD patients with purely seasonal symptoms.

Conclusion

In conclusion, this prospective study showed that the agreement between IDAT and SAT is slight to fair, with an increased number of atopic dogs without seasonal exacerbation showing positive results to seasonal allergens on SAT compared to IDAT. Conversely, the positive IDAT results correlated better with the history of seasonal exacerbation. Considering these differences, it is uncertain which allergy testing method is more suitable for the formulation of AIT in canine AD, and further studies should address these questions by prospectively following these patients during AIT clinical efficacy trials.

Several limitations of our study include small sample size from one geographic region, inherent differences between IDAT and SAT, the possibility of pollen allergens causing nonseasonal pruritus or mite allergens causing seasonal worsening of pruritus, lack of objective scoring for IDAT and the accuracy of the owner's memory for the clinical history.

Supplemental

SUPPLEMENTARY TABLE 3.2: Allergens tested with IDAT and SAT

Mites	
Dermatophagoides farinae	House dust mite
Dermatophagoides pteronyssinus	House dust mite
Tyrophagus putrescentiae	Food/storage mite
Acarus siro	Food/storage mite
Trees	
Morus rubra	Red Mulberry
Pinus taeda, Pinus strobus, Pinus echinata	Pine mix
Liquidambar styraciflua	Sweetgum
Platanus racemosa	American/Eastern Sycamore
Salix nigra	Black willow
Weeds	
Xanthium strumarium	Cocklebur
Eupatorium capillifolium	Dog fennel
Plantago lanceolate	English plantain
Chenopodium album	Lamb's quarter
Ambrosia trifida, Ambrosia artemisiifolia	Ragweed mix (giant/short)
Grasses	
Paspalum notatum	Bahia
Cynodon dactylon	Bermuda
Sorghum halapense	Johnson
Poa pratensis	Kentucky/June bluegrass
Festuca pratensis	Meadow fescue grass
Lolium perenne	Perennial ryegrass
Aagrostis gigantea	Red top
Phleum pratense	Timothy
Molds	
Alternaria alternata	
Aspergillus fumigatus	
Cladosporium sphaerospermom	
Drechslera spiceifera	
Penicillum chrysogenum	
Aureobasidium pullulans	
Others	
Ctenocephalides canis/Ctenocephalides felis	Flea

SUPPLEMENTARY TABLE 3.3: Concentration of allergens for IDAT

Allergen	**Diluent concentration (pnu/mL)**
Negative control	Plain diluent
Positive control 1	0.01 mg/mL
Positive control 2	0.1 mg/mL
Mixed grasses	
Bahia grass	5,074
Bermuda grass	7,500
Blue grass, Kentucky/June	7,500
Fescue grass, meadow	7,500
Johnson grass	2,608
Red top grass	1,818
Rye grass, perennial	7,500
Timothy grass	7,500
Mixed weeds	
Cocklebur	7,500
Dog fennel	7,500
Lamb's quarter	7,804
Plantain, English	1,818
GS Ragweed mix	7,500
Mixed trees	
Mulberry, red	1,818
GS pine mix	1,818
Sweet gum	1,818
Sycamore	7,500
Black willow	8,000
Mixed moulds	
Alternaria	1,818
Aspergillus	1,818
Drechslera	1,818
Cladosporium	1,818
Penicillum	1,818
Pullularia	1,818
Mites	
Dermatophagoides farina	476
Dermatophagoides pteronyssinus	503
Acarus siro	576
Tyrophagus putrescentiae	372

SUPPLEMENTARY TABLE 3.4: Clinical history questionnaire

Animal ID:	Age:	Breed:	
Gender:			
□ Female	□ Female neutered	□ Male	□ Male neutered
Was your animal obtained from a breeder? □ Yes □ No			
What do you feed your dog?			
Did your dog undergo an elimination diet? □ No □ Yes Result?			
Besides the skin disease, are there any other known problems?			
When did your dog's skin problems begin?			
Which clinical signs does the dog show? □ Pruritus □ Erythema □ Scales/dandruff □ Crusts □ Pustules □ Papules □ Alopecia/hair loss □ Hyperpigmentation □ Dull coat □ Oily skin □ Lacrimation			
Which body regions are affected? □ Head □ Ears □ Neck □ Back □ Axillae □ Ventrum □ Inguinal area □ Flanks □ Tail (-base) □ Paws			
How severe is the pruritus (itching, scratching, licking, chewing, biting, head shaking, rubbing) today? Use **the owner Pruritus visual analog sheet** attached to this form.			
In which months of the year is pruritus present? Any season variation?			

												Severe pruritus
												Moderate pruritus
												Mild pruritus
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	

Do you currently administer any medications to your dog? If so, which ones and at what dose?			
When did your animal last receive one of the following medications? □ Glucocorticoids (e.g., prednisone) □ Antihistamines □ Oclacitinib (Apoquel) □ Ear medications □ Ciclosporin (e.g., Atopica) □ Topical steroids □ Lokivetmab (Cytopoint)			
Where do you live? □ Urban (City, Town/Suburb) □ Rural (Village/Countryside)			
Where is your dog most of the time? □ House/Apartment □ Garden/Yard			
What flooring do you have in your apartment/house? □ Floorboards □ Tiles □ Carpet □ Linoleum			
Where is the pruritus the most severe? □ Inside □ Outside			

Which trees do you have in your immediate environment? □ Birch □ Beech □ Oak □ Poplar □ Pine □ Maple □ Walnut □ Linden □ Alder □ Willow

SUPPLEMENTARY TABLE 3.5: Correlation between IDAT results of perennial allergens (Investigator A) and clinical history of pruritus

Number of dogs	Perennial allergen positive	Perennial allergen negative	Total
Year-round with seasonal exacerbation	13	3	16
Year-round without seasonal exacerbation	10	3	13

SUPPLEMENTARY TABLE 3.6: Correlation between IDAT results of seasonal allergens (Investigator A) and clinical history of pruritus

Number of dogs	Seasonal allergen positive	Seasonal allergen negative	Total
Year-round with seasonal exacerbation	14	2	16
Year-round without seasonal exacerbation	5	8	13

SUPPLEMENTARY TABLE 3.7: Correlation between IDAT results of perennial allergens (Investigator B) and clinical history of pruritus

Number of dogs	Perennial allergen positive	Perennial allergen negative	Total
Year-round with seasonal exacerbation	15	1	16
Year-round without seasonal exacerbation	10	3	13

SUPPLEMENTARY TABLE 3.8: Correlation between IDAT results of seasonal allergens (Investigator B) and clinical history of pruritus

Number of dogs	Seasonal allergen positive	Seasonal allergen negative	Total
Year-round with seasonal exacerbation	14	2	16
Year-round without seasonal exacerbation	5	8	13

SUPPLEMENTARY TABLE 3.9: Correlation between SAT results of perennial allergens and clinical history of pruritus

Number of dogs	Perennial allergen positive	Perennial allergen negative	Total
Year-round with seasonal exacerbation	14	2	16
Year-round without seasonal exacerbation	12	1	13

SUPPLEMENTARY TABLE 3.10: Correlation between SAT results of seasonal allergens and clinical history of pruritus

Number of dogs	Seasonal allergen positive	Seasonal allergen negative	Total
Year-round with seasonal exacerbation	13	3	16
Year-round without seasonal exacerbation	12	1	13

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CHAPTER 4

DISCUSSION

The findings from this study demonstrated slight to fair agreement between IDAT and SAT, consistent with previous reports. Notably, improved concordance was observed when higher cut-off thresholds of SAT.[1] were used. Most commercial laboratories do not disclose the rationale or methodology used to determine their positive cut-off values or thresholds, complicating the determination of which positive results are true positives. To the best of the author's knowledge, only one laboratory has publicly detailed its method for establishing a positivity threshold.[2] This laboratory established cut-off values using serum from laboratory beagles not previously sensitized to house dust mite allergens.[2] Serum IgE levels for house dust mite allergens were measured, and the mean plus three standard deviations was calculated after excluding mite tropomyosin results, known to cause cross-reactivity even in healthy dogs.[2,3] A similar calculation was performed for the negative control on their test cartridge using a large population of allergy-suspected dogs.[2] The average of these two calculated values was rounded to determine the final positive threshold for house dust mite.[2] Additionally, this laboratory incorporated two anti-CCD IgE blockers into the assay to reduce cross-reactivity.[4]

While these methodological steps might be expected to enhance the specificity and clinical relevance of SAT results, unpublished data from our ongoing research suggest that this particular SAT did not correlate more closely with IDAT than other conventional SAT.[5] This reinforces the fundamental distinction between IDAT and SAT methodologies: IDAT reflects in

vivo mast cell-bound IgE activity in the skin, whereas SAT measures circulating free IgE.[6] This inherent differences supports the view that one test cannot replace the other.

It remains unclear whether this novel SAT protocol shows a stronger correlation with clinical history (e.g., seasonality), an important consideration when selecting allergens to formulate ASIT.[7] While an older study suggested comparable clinical outcomes between ASIT protocols based on either SAT or IDAT, definitive conclusions cannot be drawn due to limited data.[8]

A logical next step in this research would be to conduct a controlled clinical trial enrolling dogs with similar clinical histories and presentations. These dogs would undergo both IDAT and SAT, and subsequently be randomly assigned to one of the three groups: 1) ASIT formulated based on SAT results, 2) ASIT based on IDAT results, 3) ASIT based on the combined results of both tests. Ideally, a placebo control group would be added, but this would be difficult for humane and owner compliance reasons. Clinical outcomes could then be assessed across groups to determine whether using both tests offers any advantage that justifies the additional cost.

Furthermore, the timing of allergy testing may influence the accuracy and relevance of results. Although a few studies have explored the relationship between SAT results and seasonal variation, there is currently no published data on the impact of seasonality on IDAT, or on both IDAT and SAT performed concurrently.[9-11]

Further investigations could address this knowledge gap by enrolling atopic dogs with distinct seasonal patterns: 1) dogs with strictly seasonal clinical signs, 2) dogs with non-seasonal signs without seasonal exacerbation, 3) dogs with non-seasonal signs with seasonal exacerbation. Allergy testing would be conducted at multiple points throughout the year, ideally, spring,

summer, fall, and winter, to evaluate how seasonality affects IDAT and SAT outcomes, and to determine the most appropriate timing for testing.

Collectively, such studies may provide critical insights into optimizing allergen testing protocols and improving the formulation and efficacy of ASIT.

CHAPTER 5

CONCLUSION

In conclusion, this study expands upon existing research by investigating the correlation between allergen testing modalities and clinical history. To our knowledge, this is the first study to assess the relationship between seasonality and SAT results, and more studies are needed to validate our findings. While the precise prevalence of cAD remains undefined, it is a commonly encountered and chronically managed condition in clinical veterinary practice.[12] Understanding the clinical utility and limitations of diagnostic tools, such as IDAT and SAT, is critical for effective case management.

Given the complexity of cAD pathogenesis, there remains a pressing need for continued research. Specifically, future studies should aim to refine our understanding of how to optimize the formulation of ASIT and explore its relationship to both test outcomes and clinical history. Such efforts may ultimately lead to more targeted, effective, and individualized treatment strategies for dogs affected by this lifelong condition.

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