Reactions to environmental allergens in cats with feline lower airway disease

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ABBREVIATIONS

AIT	Allergen immunotherapy
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BWBP	Barometric whole-body plethysmography
CB	Chronic bronchitis
CCD	Cross-reactive carbohydrate determinant
CT	Computed tomography
DNA	Deoxyribonucleic aicd
EA	Equine asthma
EI	Eosinophilic inflammation
ELISA	Enzyme-linked immunosorbent assay
FA	Feline asthma
FASS	Feline atopic skin syndrome
FcεR1	High-affinity mast cell receptor for IgE
FeNO	Fraction of exhaled nitric oxide
FEV1	Forced expiratory volume
FFA	Feline food allergy
FLAD	Feline lower airway disease
FVC	Forced vital capacity
GINA	Global initiative for asthma
HC	Healthy cat
HDMA	House dust mite allergen
IAD	Inflammatory airway disease
IDT	Intradermal test
IgE	Immunoglobulin E
IL	Interleukin
ILC2s	Type 2 innate lymphoid cells
MI	Mixed inflammation
NI	Neutrophilic inflammation
RAO	Recurrent airway obstruction
SAT	Serum allergen-specific IgE test
Spp	Species
Th2	T helper 2 cell

I. INTRODUCTION

Feline lower airway disease (FLAD) is a common respiratory disease affecting approximately 1 to 5% of the cat population (Padrid, 2009). It is often differentiated into feline asthma (FA) or feline chronic bronchitis (CB) (Grotheer et al., 2020). While in FA an allergic etiology is assumed to result in an eosinophilic inflammation (EI) of the lower airways, CB is assumed to develop due to preceding insults such as infections or inhaled irritants, inducing a neutrophilic inflammation (NI) (Reinero, 2011). Nonetheless, a clear distinction between both entities is not possible which poses the question, whether they could arise from the same etiology showing different inflammatory profiles (Kirschvink et al., 2006). In FA, aeroallergens are thought to be potential triggers of inflammation (Reinero, 2011). Recently, studies have reported that allergen immunotherapy (AIT) has been successfully performed as a curative treatment in cats with experimentally induced FA (Lee-Fowler et al., 2009b; Reinero et al., 2006).

Allergen testing is essential for choosing relevant allergens later applied in AIT. For testing, intradermal test (IDT) or serum allergen-specific immunoglobulin E-(IgE) test (SAT) can be performed. To date, only few studies exist on allergen testing in naturally diseased cats with FLAD, and environmental factors have scarcely been evaluated. Therefore, the aim of the study was to compare both forms of allergy testing applied in cats with FLAD, and hence acquire more information about potential environmental allergens as triggers based on results of an owner's questionnaire. In addition, SAT results from cats with FLAD and healthy cats (HC) were compared. In cats suspicious of FLAD, blind bronchoalveolar lavage (BAL) and IDT were performed during anesthesia, and owners were handed an environmental questionnaire. For SAT, leftover serum was taken from cats with FLAD and HC. Owners from HC group filled out the same questionnaire.

II. BIBLIOGRAPHY

1. Feline lower airway disease

A condition later known as "feline lower airway disease" (FLAD) has first been described in cats in 1906 as "asthma" and "bronchitis" (Hill, 1906).

1.1. Definition

The term "FLAD" describes a chronic idiopathic noninfectious inflammatory disorder of the lower airways. Controversy about its exact definition exists, resulting in multiple different terminologies such as "bronchial asthma", "feline bronchial disease", "allergic bronchitis", "feline asthma syndrome", "feline asthma", "chronic bronchitis", "asthmatic bronchitis", and "idiopathic small airway disease" (Nafe et al., 2010; Reinero, 2011). Feline asthma (FA) and chronic bronchitis (CB) depict the two most important phenotypes of FLAD (Adamama-Moraitou et al., 2004). Little is known about its exact etiology, and a clear clinical distinction between the foresaid phenotypes has been failed to be established (Grotheer et al., 2020). Therefore, FLAD is rather used as an umbrella term (Adamama-Moraitou et al., 2004). Commonly, it is thought that FA goes along with an eosinophilic inflammation (EI) and CB with a neutrophilic inflammation (NI) and that they embody two distinct disease entities (Adamama-Moraitou et al., 2004; Nafe et al., 2010; Reinero, 2011). In addition to that, cats with CB are thought not to show reversible bronchoconstriction as it is often seen in cats with FA (Trzil, 2020). Recently, further categorization into mixed airway inflammation (MI) has been established (Buller et al., 2020; Grotheer et al., 2020; Norris et al., 2002), where it is assumed that chronic damage to the airways due to chronic FA might lead to a mixed inflammatory pattern (Moise et al., 1989).

1.2. Epidemiology and risk factors

FLAD belongs to the most prevalent respiratory disorders of the lower respiratory tract of the cat (Venema and Patterson, 2010). It is estimated that around 1 to 5% of the cat population is affected (Padrid, 2009). Prevalence might continuously be rising as it does in human asthma, which is thought to be due to several aspects such as urbanization and concomitant increase of air pollution (Guarnieri and Balmes, 2014). Due to the closely shared environment with their owners, cats are

exposed to the same pollutants as humans, increasing the risk of allergies in the cat as well (Schäfer et al., 2008). One study has found a link between high indoor air pollution and icreased prevalence of respiratory disease in the cat (Lin et al., 2018). Other major risk factors for respiratory disease may be environmental tobacco smoke, ozone or early respiratory infections (Moses and Spaulding, 1985). These are proposed risk factors in human medicine, though no clear interdependence is found in the cat as scarcely any studies have been performed in these fields (Reinero, 2011). Most commonly environmental allergens are assumed as potential triggers, leading to an allergic reaction in the airways (Hirt, 2012; Norris Reinero et al., 2004). For cats with EI, it has also been reported that irritants such as dusty cat litter, feather pillows, aerosol sprays and environmental cigarette smoke can induce acute asthma attacks (Dye et al., 1996; Moses and Spaulding, 1985). Genetic predisposition has been discussed in cats, as some authors assumed an overrepresentation of Siamese cats with FLAD (Adamama-Moraitou et al., 2004; Moise et al., 1989). This claim is seen as controversial, as others could not find a significant overrepresentation of Siamese or other purebred cats (Foster et al., 2004a; Grotheer et al., 2020). Cats with EI are commonly thought to be younger at the time of presentation with a median age of four years compared to cats with NI showing a median age of around eight years in studies (Lee et al., 2020; Trzil and Reinero, 2014). However, other investigations did not find any significant difference in age between the two inflammatory types (Grotheer et al., 2020; Lin et al., 2015).

1.3. Pathogenesis

While for EI it is broadly assumed that the underlying pathology consists of a type-1 hypersensitivity reaction, NI is thought to arise from different causes such as cigarette smoke or preceding infection (Reinero, 2011; Trzil, 2020; Trzil and Reinero, 2014). To date, the exact pathophysiology of NI is still unknown (Nafe et al., 2010). It was found that chronic NI leads to histological changes in the lungs such as hyperplasia of the submucosal glands causing increased mucus accumulation in the lumen, alteration in epithelial cells and smooth muscle hypertrophy of the bronchial wall (Moses and Spaulding, 1985). These changes then lead to airway obstruction, causing typical symptoms such as coughing, labored breathing or dyspnea (Dye, 1992). In cats with EI, those previously described pathological changes appear similarly. Additionally, one of the major

aspects discussed to discriminate between both inflammatory types is spontaneous bronchoconstriction which can be relieved by the administration of bronchodilators (Reinero, 2011). Especially in cats with EI, allergic reactions are suspected to play a major role in the pathogenesis of FLAD, as EI could be experimentally induced via allergen challenge (Kirschvink et al., 2007a; Norris Reinero et al., 2004; Padrid et al., 1995). In experimental cat models it could be demonstrated that the sensitization to *Ascaris suum* via an intramuscular injection and further nebulization challenge with the same antigen leads to a persistent airway hyperreactivity, changes in the respiratory epithelium, smooth muscles, mucus glands, and EI in BALF cytology (Kirschvink et al., 2007b; Padrid et al., 1995). Cats were also experimentally sensitized to ovalbumin using intraperitoneal injections. Consequently, these cats presented elevated total serum IgE levels and respiratory hypersensitivity (Talbott and Strausser, 1977). As ovalbumin is not an allergen cats are exposed to in real life, and as *Ascaris suum* does not cause asthma but gastrointestinal signs in naturally exposed cats, new allergens for experimental sensitization have been selected (Norris Reinero et al., 2004). To induce asthma in cats, the allergens now used in studies are clinically relevant aeroallergens such as Bermuda grass allergen and house dust mite allergen (HDMA), resulting in the same pathophysiological changes seen in naturally diseased cats with airway hyperreactivity, airway eosinophilia, allergen-specific IgE production, and airway remodeling (Norris Reinero et al., 2004).

It must be noted that NI could also be experimentally induced, making it hard to clearly exclude an allergic origin in cats with NI (Nafe et al., 2010). A type-1 hypersensitivity reaction results in the production of eosinophils and subsequently in EI (Reinero, 2011). When an allergen is inhaled, dendritic cells present it to a naïve CD4+ T cell which then switches to a T helper 2 (Th2) cell producing cytokines such as interleukin (IL)-4, IL-5 and IL-13 (Reinero, 2011; Venema and Patterson, 2010). Especially IL-4 causes B lymphocytes to transform into allergen-specific immunoglobulin E (IgE) producing cells (Venema and Patterson, 2010). Mast cells do have a high affinity receptor for IgE (FcεR1), which allows very specific binding, leading to their degranulation and release of leukotrienes, histamine and IL-5 (Grotheer and Schulz, 2019). Leukotrienes and histamine further initiate an increase in vascular permeability and smooth muscle contraction, causing typical symptoms such as coughing and wheezing in affected

cats (Venema and Patterson, 2010). IL-5 on the other hand stimulates the production of eosinophils. They further release inflammatory mediators such as major basic protein, eosinophil-derived neurotoxin and cationic protein resulting in tissue pathology (Bayers, June 2005; Venema and Patterson, 2010). Cationic proteins can cause epithelial damage with resulting epithelial thickening and tendency towards smooth muscle spasm (Padrid, 2000). Additionally, enlargement of goblet cells leads to excessive mucus production, following mucus plug accumulation with the consequence of significant airway narrowing (Bayers, June 2005). MI has been thought to develop from chronic EI, causing damage to the airways and therefore, generating a neutrophilic response with the final consequence of a MI (Moise et al., 1989).

1.4 Clinical signs

Cats with FLAD most commonly show chronic paroxysmal cough as the major clinical sign (Dye et al., 1996). This can be observed together with other symptoms such as wheezing and episodes of dyspnea (Padrid, 2000). Some cats may only show altered respiration such as labored breathing, typically with a prolonged expiration (Dye et al., 1996). Other clinical signs such as sneezing, vomiting/regurgitation, restlessness and nasal discharge have been observed in some cases (Corcoran et al., 1995; Grotheer et al., 2020). A study compared clinical signs in cats with FLAD with either EI or NI and found significantly more cats with NI presenting nasal discharge compared to cats with EI (Grotheer et al., 2020). Overall, it has been shown that no clear differentiation can be made between NI and EI based on clinical signs alone (Grotheer et al., 2020; Lee et al., 2020).

1.5. Diagnostics

There is no specific single parameter that can be used to clearly diagnose FLAD, which makes it very important to rule out other possible causes (pneumonia, neoplasia, respiratory parasites, inhaled foreign body, interstitial lung disease, heart disease, pleural effusion) for the presenting clinical signs such as chronic cough, wheezing or acute dyspnea, and to perform a thorough clinical examination (Hirt et al., 2011; Padrid, 2000). If the cat’s clinical history and signs lead to the assumption of FLAD, for cats with outdoor access it is important to rule out lungworm infection using the Baerman method in addition to flotation for

fecal examination (Barutzki and Schaper, 2013).

1.5.1. Thoracic imaging

To further evaluate clinical signs and localize the problem, thoracic radiography is recommended as a screening tool for imaging in cats with respiratory signs. Cats with FLAD commonly show bronchial or bronchointerstitial, sometimes alveolar or mixed lung patterns and can present with lung hyperinflation or lung lobe atelectasis (Gareis et al., 2023; Venema and Patterson, 2010). Still, lack of radiographic findings cannot rule out FLAD as in some cases radiographs appear normal (Adamama-Moraitou et al., 2004).

Another possibility for imaging of cats with FLAD embodies computed tomography (CT). It defines bronchiectasis, bronchial wall thickening and alveolar infiltrates in more detail and identifies subtle lesions that are not visible on radiography (Trzil, 2020). It was demonstrated that using a plexiglass chamber, CT can generate evaluable images in conscious cats, avoiding general anesthesia and associated risks (Oliveira et al., 2011; Romagnani, 2000; Trzil, 2020). Although CT provides a sensitive diagnostic tool for detection of abnormalities, it is not possible to discriminate FLAD from other lower respiratory tract diseases based on thoracic imaging alone (Trzil, 2020).

1.5.2. Bronchoalveolar lavage

Most important for an accurate diagnosis of FLAD is the evaluation of bronchoalveolar lavage fluid (BALF) cytology, revealing a sterile inflammation (Grotheer and Schulz, 2019). Bacterial cultures from BALF should be taken, although a positive result should always be interpreted in context with BALF cytology, looking for degenerated neutrophils with intracellular bacteria as an indication for an active bacterial infection (Norris et al., 2002). This is important, since some cats with FLAD may have a positive bacterial culture with low-grade bacterial growth as the bronchi of the cat are not considered sterile and bacterial contamination can occur with sampling (Dye et al., 1996). It has generally been proposed that CB generates a NI and FA an EI (Nafe et al., 2010). However, there exist many different references for the definition of those inflammatory types (Johnson and Vernau, 2011; Lee et al., 2020). Furthermore, it was demonstrated that bronchoalveolar lavage (BAL) performed in different lung lobes in the same cat can result in different inflammatory profiles in BALF cytology (Ybarra et al.,

2012). Another controversial point in the diagnostic workup embodies the detection of *Mycoplasma* species (spp) in BALF, as some authors propose these bacterial organisms carry out a pathogenic role in the lower respiratory tract (Foster et al., 2004b), while others were able to demonstrate that *Mycoplasma* spp are also detectable in the lower respiratory tract of healthy cats (HC) (Schulz et al., 2014)

1.5.3. Laboratory findings/biomarkers

Hematology may reveal eosinophilia and polycythemia (Adamama-Moraitou et al., 2004), although cats with EI were more likely to show blood eosinophilia than cats with NI (Grotheer et al., 2020). Still, these parameters remain very unspecific, as cats with FLAD often do not show any changes in their blood work (Grotheer et al., 2020). In cats with experimentally induced FLAD attempts have been made to measure relevant biomarkers such as endothelin-1 concentrations, hydrogen peroxide and acetone to discriminate between cats with FLAD and HC (Fulcher et al., 2016; Kirschvink et al., 2005; Xu and Zhong, 1999). To date, more research is needed to further determine whether biomarkers may be useful tools in the diagnostic workup of naturally diseased cats (Trzil, 2020).

1.5.4. Lung function testing

In human asthma spirometry is used for lung function testing as part of the diagnostic workup and control of the therapeutic success (Global Initiative For Asthma, 2022). This method is not applicable for the cat, as spirometry requires the patient to cooperate and to exhale maximally into a mouthpiece (Trzil, 2020). An alternative method called barometric whole-body plethysmography (BWBP) has found its use in veterinary medicine as it provides a non-invasive, well-tolerated approach to pulmonary function testing in the cat (Lin et al., 2015). For testing, an awake cat is placed into a ventilated, transparent airtight chamber that is able to measure changes in air pressure (Venema and Patterson, 2010). With the use of BWBP it is possible to recognize airflow limitation in the cat (Lin et al., 2014). A previous study found significant differences in BWBP parameters between HC and cats with FLAD (Lin et al., 2014). In addition, BWBP seems to provide a useful method to monitor treatment response in cats with FLAD (Gareis et al., 2022; Lin et al., 2015).

1.6. Allergy testing in cats

Allergy testing is essential for the selection of relevant allergens included in AIT and for establishing allergen avoidance measures (Mueller et al., 2002). It aims to detect relevant IgE that are specific antibodies produced as a result of a hypersensitivity reaction (Hurst and McDaniel, 2021). Generally, allergy tests do not distinguish between healthy and diseased cats. HC may have positive reactions as well, meaning that they have an intrinsic atopy which is clinically not relevant (Bajwa, 2018). Therefore, it is important to know that allergy tests alone are not suitable to establish a diagnosis, but should be seen as an additional tool in the diagnostic workup (Diesel and DeBoer, 2011).

In cats with FASS, intradermal test (IDT) and serum allergen-specific IgE test (SAT) are commonly used for the identification of relevant allergens (Moriello et al., 2007), and a combination of those is recommended for a workup (Bajwa, 2018). Still, both tests demonstrate a poor correlation when applied in cats with FASS (FOSTER and O'DAIR, 1993; Gilbert and Halliwell, 1998). This can be explained by the fact that SAT measures the free circulating IgE in the blood experiencing a short half-life (around 2-3 days) while IgE tested in IDT are bound to FcεR1 on mast cells having a substantially longer half-life (Norris et al., 2003). It has been described that HC do not show less positive reactions on SAT than cats with FASS (Diesel and DeBoer, 2011).

To date, in FLAD allergy tests are not commonly used as an additional diagnostic tool in naturally diseased cats (Moriello et al., 2007). However, experimental studies in FA models showed promising results, as cats developed positive reactions to allergens after allergen sensitization in both tests (Norris Reinero et al., 2004). In cats experimentally sensitized to HDMA and Bermuda grass allergen, SAT and IDT were thoroughly investigated concerning their sensitivity and specificity (Lee-Fowler et al., 2009a). It could be shown that IDT displays higher sensitivity compared to SAT, whereas SAT provides higher sensibility in cats with known allergen exposure (Lee-Fowler et al., 2009a). Another experimental cat model evaluated effects of glucocorticoid therapy in the context of allergy testing under controlled conditions (Chang et al., 2011). It could be demonstrated that glucocorticoid treatment interferes with wheal formation in IDT, which led to the conclusion that therapy should be stopped for at least two weeks before carrying out IDT. Therefore, it has been proposed, that if therapy

cannot be stopped, SAT can be performed, as glucocorticoid treatment does not interfere with SAT results (Chang et al., 2011).

1.6.1. Intradermal testing

In veterinary dermatology IDT is perceived as the "gold standard" method in allergy testing presenting a higher sensitivity and therefore, providing a high chance of detecting relevant allergens (Bajwa, 2021; Wassom and Grieve, 1998). In IDT, allergen concentrations are injected into the skin leading to a mast cell degranulation with consequent wheal formation. This is caused by the binding of injected allergens to mast cell-bound IgE in the skin (FOSTER and O'DAIR, 1993). In the cat however, this test faces some difficulties for correct interpretation. Compared to the dog, it produces weaker reactions with indistinct wheal formations that often disappear rapidly (Diesel and DeBoer, 2011). Some authors propose to additionally use intravenous fluorescein during IDT to facilitate the wheal interpretation (Schleifer and Willemse, 2003). One explanation for weak reactions could be the influence of stress during IDT, as cats had been shown to have elevated cortisol concentrations in the blood during the test which may lead to subtle reactions (Hudec and Griffin, 2020). Another problem is the lack of standardized irritant threshold concentration (highest concentration not causing reactions in at least 90% of normal individuals) of the IDT injections in cats, while in dogs it is well determined (Austel et al., 2006; Scholz et al., 2017). To date, the same concentrations used in canine IDT are applied for feline IDT, though it could be demonstrated that cats might tolerate a higher concentration compared to dogs (Austel et al., 2006; Scholz et al., 2017). Furthermore, cats need to be sedated for testing, and the test can only be performed by an experienced dermatologist (Wassom and Grieve, 1998). On the other hand, results are directly available from IDT and do not need to be referred to a laboratory. Altogether, even though IDT in cats faces many difficulties, it seems to be a valuable diagnostic tool for the detection of relevant allergens (FOSTER and O'DAIR, 1993).

1.6.2. Serum allergen-specific immunoglobulin E testing

SAT, as an in vitro test, can be performed for identification of allergen sensitization (Bell and Jones, 2021). In general, has been recommended that SAT may be applied as an additional diagnostic tool since sensitivity is considered

quite low, meaning that some relevant allergens might be “missed out” (Bajwa, 2018). The great advantages of SAT are that only a small amount of blood is needed for measurements, the fact that the cat does not need to be sedated or shaved, and that no specialized dermatologist is required for test performance (Wassom and Grieve, 1998).

In general, SAT is an enzyme-linked immunosorbent assay (ELISA) test for which different serological techniques exist using either polyclonal or monoclonal anti-IgE methodology, or a more recent one using the α-chain of FcεR1 (Bell and Jones, 2021). For the test, allergens are placed on a plate. Then the serum sample is added and allergen-specific IgE and IgG can bind the allergens. Depending on the methodology, either polyclonal or monoclonal anti-IgE antibodies or IgE receptors are then added to bind IgE. Finally, when the substrate is added and produces a color change, allergen-specific IgE is then labeled (Wassom and Grieve, 1998). The disadvantage of the mono- and polyclonal antibodies is their less specific binding; they might not only bind relevant IgE but additionally IgG antibodies, leading to false positive reactions (Wassom and Grieve, 1998). In contrast to that, the FcεR1-α-methodology allows for exclusive binding to IgE, elevating test specificity (Wassom and Grieve, 1998).

Another cause for a false positive reaction in SAT may be cross-reactive carbohydrate determinants (CCDs) (Gedon et al., 2019). These are epitopes consisting of glycans found on plant or insect glycoproteins that lead to cross-reactivity, but anti-CCD IgE antibodies do not have clinical relevance (Aalberse and van Ree, 1997). Therefore, tests have been established to that synthetic CCD inhibitors are applied. This means that anti-CCD IgE first need to be detected and consequently blocked, that they cannot further bind plant allergens in the test (Gedon et al., 2019). As a result, significantly less false positive allergen reactions appear on SAT (Gedon et al., 2019; Luo and Sun, 2019).

1.7. Therapy

Standard therapy in FLAD consists mainly of the administration of corticosteroids, and it has been shown that either inhaled or orally administered corticosteroids significantly reduce airway inflammation (Kirschvink et al., 2006; Reinero et al., 2005). To minimize side effects such as diabetes mellitus or infections due to immunosuppression, inhaled corticosteroid therapy is

recommended as the standard treatment, if possible (Cohn et al., 2010). However, even with corticosteroid therapy a subclinical inflammation may persist, as the direct trigger is not treated (Cocayne et al., 2011). Bronchodilators can be applied as a supportive treatment in cats with acute dyspnea and in patients that cannot be controlled with corticosteroids alone (Grotheer and Schulz, 2019).

Besides the conventional pharmaceutical therapy for FLAD, a large number of different drugs has been experimentally evaluated regarding their efficacy. One of them is inhaled lidocaine. It was shown in a study that nebulized lidocaine decreased airway hyperresponsiveness, but did not suppress airway eosinophilia in experimentally induced asthmatic cats (Nafe et al., 2013).

Cyclosporine, a T-cell and mast cell inhibitor, generated controversial results on its efficacy in experimental FA (Mitchell et al., 1998; Padrid et al., 1996). It may still be a promising alternative treatment in cats in that glucocorticoids are contraindicated due to medical issues such as diabetes mellitus and/or heart congestive failure, as in a case report clinical signs improved with cyclosporine therapy (Nafe and Leach, 2015).

Tyrosine kinase inhibitors such as masitinib function as blockers in the signaling cascade of the asthmatic immunopathogenesis (Trzil, 2020). Eosinophilic airway inflammation could be reduced with the use of masitinib in experimentally diseased cats (Lee-Fowler et al., 2012).

Other pharmaceutics such as doxycycline, salivary tripeptide, maropitant, cetirizine, leukotriene-receptor antagonist (zafirlukast) and antiserotonergic drugs (cyproheptadine) that were tested in experimental studies did not show any positive effects on airway inflammation or airway hyperresponsiveness (Grobman and Reinero, 2016; Leemans et al., 2012; Reinero et al., 2005; Schooley et al., 2007)

Stem cell therapy showed promising results in experimentally induced asthmatic murine models, but applied in cats with chronic experimentally induced FA failed to reduce airway inflammation and airway hyperresponsiveness (Trzil et al., 2014).

Anti-inflammatory therapies such as the use of omega-3 polyunsaturated fatty acids may be beneficial as adjunctive therapy (Venema and Patterson, 2010). A

study in *Ascaris suum*-sensitized cats found beneficial effects of oral supplementation of omega-3 polyunsaturated fatty acids on airway hyperresponsiveness, but no improvement of BALF cytology (Leemans et al., 2010).

In cats with FLAD, in that allergy is assumed as a potential causative factor for disease development, the best treatment would be allergen avoidance, but this might often not be feasible, especially as it is hard to identify the causative allergen(s) (Reinero et al., 2006). Another hope for potential curative treatment comprises allergen immunotherapy (AIT) (Reinero et al., 2006). In theory, AIT would be the most effective approach to induce an immunological remission by treating the cause of asthma and not just the symptoms, as is it the case with glucocorticoid therapy (Nakagome and Nagata, 2021). AIT is not yet commonly established in cats with FA, and the exact mechanisms behind AIT are not fully understood (Reinero et al., 2006). It is assumed that AIT leads to an immunologic shift towards a Th1 response in conjunction with upregulation of regulatory T cells and IL-10 (Mueller et al., 2018). In an animal model many factors such as the environment, the timing of exposure and types of allergens can be controlled and therefore can be better evaluated regarding the outcome of the AIT (Reinero et al., 2006). In cats that were experimentally sensitized to Bermuda grass allergen, eosinophilic airway inflammation could significantly be decreased with AIT, showing the efficacy of the treatment (Reinero et al., 2006). Promising results were also seen in another study investigating AIT given with adjuvant therapy using immunostimulatory deoxyribonucleic acid (DNA) sequences (Reinero et al., 2008). Both studies investigated the effects of a shortened course of AIT called “rush immunotherapy”. Due to the occurrence of adverse reactions during the trials of the studies (Reinero et al., 2006; Reinero et al., 2008), the safety of an injected rush protocol was questioned and a mucosal approach of rush immunotherapy was evaluated (Lee-Fowler et al., 2009b). It revealed a slightly less effective reduction in EI compared to the subcutaneous approach, but in contrast to the injected protocol (Reinero et al., 2006; (Reinero et al., 2008), no life-threatening adverse effects were detected (Lee-Fowler et al., 2009b).

2. Asthma in humans

2.1 Definition

Asthma in humans is defined as “a common chronic disorder of the airways” characterized by “airflow obstruction, bronchial hyperresponsiveness and underlying inflammation” ((NAEPP), 2020). It is a complex and heterogeneous disease with different underlying disease processes such as the combination of genetic predisposition and environmental exposure (Global Initiative For Asthma, 2022). For a better management of individual disease, asthma is further classified into different phenotypes which often overlap (Handoyo and Rosenwasser, 2009). Phenotypic categorization is based on a combination of different observable aspects such as biological, clinical, and physiological characteristics (Kuruvilla et al., 2019). Main phenotypic distinctions are “allergic” and “non-allergic” asthma (Global Initiative For Asthma, 2022), but other categorizations based on symptom triggers (“aspirin-sensitive asthma”, “smoke-induced asthma”, “obesity-induced asthma”), disease severity (“mild asthma”, “severe asthma”), age of onset (“early-onset asthma”, “late-onset asthma”) and many other observable aspects exist, showing the complexity of the disease (Handoyo and Rosenwasser, 2009; Kuruvilla et al., 2019). People with “allergic” asthma have positive allergy tests, often show a sputum with EI and mostly respond well to inhaled corticosteroid therapy, while “non-allergic” asthma does not go along with reactions to allergens, sputum consists of a neutrophilic or eosinophilic inflammatory profile, and they often lack a response to corticosteroids (Global Initiative For Asthma, 2022). Therapy based on phenotypic differentiation does not coercively attack underlying causes of inflammation, commonly resulting in the absence of treatment responses (Davis and Sheats, 2021). Therefore, new attempts regarding asthma classification have been made in recent years, now proposing to further classify asthma into so-called “endotypes”. They enable a more personalized treatment for specific causative molecular mechanisms (Fahy, 2015). Based on elevated serum periostin, fraction of exhaled nitric oxide (FeNO) and sputum eosinophils classification into “type 2 immune response”, “Th2-high asthma”, or “Th2 asthma” has been established, as this type seems to show a Th2 driven immune response (Akdis et al., 2020; Carr et al., 2018; Fahy, 2015; Wenzel, 2012). On the other hand, “non-type 2 immune response“ and “non-Th2 asthma” or “Th2-low asthma” seems to arise from a different etiology and–in contrast to

"Th2 asthma"–mainly lack of responsiveness to corticosteroid treatment (Wenzel, 2012). For "Th2-low asthma" no clear biomarkers have yet been identified (Kyriakopoulos et al., 2021), though a recent study proposed that serum amyloid A1 might play a role as biomarker for neutrophilic asthma (Bich et al., 2022).

2.2. Epidemiology and risk factors

Asthma is a widespread disease affecting approximately "1 to 18% of the population in different countries" (Global Initiative For Asthma, 2021). It is estimated that 262 million people suffered from the disease in 2019 (World Health Organization, 2020).The prevalence of asthma is rising continuously, varying in different parts of the world (Holgate et al., 2015). For example, in the United States in 1980 3.1% of the population were affected, while in 2016 the number was already reported as high as 8.3% (Gans and Gavrilova, 2020). Children are more commonly affected than adults, and boys predominate in childhood asthma, while in adult-onset asthma women are more often affected compared to men (Stern et al., 2020). According to a survey from the Robert Koch Institute, the 12-month prevalence of asthma in Germany revealed 6.2% among citizens aged 18 years or older, with women presenting a higher prevalence (7.1%) compared to men (5.4%) (Steppuhn et al., 2017).

It is assumed that the rising asthma prevalence is connected to urbanization, which is linked to increasing air pollution (ozone, nitrogen dioxide, diesel-exhaust particles, particulate matter, sulfur dioxide). Several studies emphasized that air pollution is an enormous risk factor for asthma and plays a major role in disease exacerbation, or might even be the cause of new onset asthma (Guarnieri and Balmes, 2014; Stern et al., 2020; Tiotiu et al., 2020). Oxidative stress caused by air pollution leads to increased epithelial permeability and to pro-inflammatory cytokine expression, facilitating the entrance of aeroallergens and therefore triggering sensitization to aeroallergens (Guarnieri and Balmes, 2014; Huff et al., 2019).

About 25% of adults with asthma in developed countries are cigarette smokers (Thomson et al., 2004). In experimental studies with laboratory animals it was shown that cigarette smoke causes histological changes and lesions in the airways (Dye and Adler, 1994). Therefore, it is not surprising that cigarette smoking leads to the development of more severe symptoms in asthma and an accelerated

decline in lung function as well as impaired responses to corticosteroids (Thomson et al., 2004). Maternal cigarette smoking during pregnancy is a great risk factor for childhood asthma in the fetus (Stern et al., 2020). Another cohort study was able to detect a linkage between maternal smoking during pregnancy and the subsequent development of adult-onset asthma (Toppila-Salmi et al., 2020).

Genetics play an important role as a risk factor for asthma, as children from parents that are affected have a higher chance of becoming asthmatic (Thomsen, 2015). More than a hundred different genes associated with asthma have been discovered in multiple studies; however, a lack of universal replication of the results from those studies prevents a confident linkage (Stern et al., 2020; Thomsen, 2015).

Another risk factor and disease modifier in asthma is obesity. The obesity-linked asthma risk can start *in utero*, as an association between maternal obesity during pregnancy and subsequently developed asthma in the offspring was detected (Dumas et al., 2016). Besides lung compression due to accumulated thoracic and abdominal fat, further alterations such as increased adipokines and other inflammatory cytokines produced by adipose tissue can support asthma development and exacerbation (Peters et al., 2018).

2.3. Pathogenesis

Multifactorial influences can elevate the risk of asthma in humans. Asthma embodies a heterogeneous disease in that an interplay of innate and adaptive immune system agitates in the context of a complex pathophysiology leading to chronic airway inflammation (Gans and Gavrilova, 2020).

It was assumed for a long time that asthma is an atopic disorder showing a Th2-driven reaction to aeroallergens with subsequent EI (Wills-Karp, 1999; Woodruff et al., 2009). Recent research categorizes this type of pathophysiology now into “Th2-high” asthma. Th2 cells embody a subpopulation of CD4+ T cells that secrete the cytokines IL-4, IL-5, and IL-13 (Fahy, 2015). They switch from naïve T-helper cells to Th2 cells after contact to allergens presented on dendritic cells in the airways. Furthermore, IL-4 might as well play a major role in the priming of T cells, and research even suggests that in asthmatics altered genes lead to an elevated IL-4 production (Wills-Karp, 1999). Released cytokines such as IL-13

then stimulate B-cells to release IgE (Gans and Gavrilova, 2020). IgE on the other hand crosslinks allergens with the FcεR1 on mast cells, resulting in activation and consequently degranulation. Mast cell-mediators further activate smooth muscle cells, which leads to changes in the lung function (Méndez-Enríquez and Hallgren, 2019). IL-5 is important for the eosinophilopoiesis in the bone marrow, promotes terminal differentiation of eosinophils, prolongs eosinophil survival in tissues, and augments their cytotoxic activity (Wills-Karp, 1999). Eosinophils then migrate to the airways leading directly or indirectly to inflammation and tissue damage as they release mediators such as basic proteins, chemokines, growth factors, enzymes and cytokines (Carr et al., 2018). As an example, profibrotic factors released by eosinophils activate bronchial fibroblasts, which potentially lead to airway remodeling and thickening of the basement membrane (Kuruvilla et al., 2019). Chronic airway inflammation in consequence causes mucus hypersecretion, plugging and airway edema (Gans and Gavrilova, 2020).

In non-atopic EI, studies have found a lack of IgE-mediated signals like increase in IL-4, which in consequence leads to less B-cell class switching and IgE production. It is suggested that irritants can induce airway epithelial injury, leading to a release of IL-25, IL-33 and thymic stromal lymphopoietin. They might stimulate type 2 innate lymphoid cells (ILC2s) to release IL-5. IL-5 again promotes eosinophilopoiesis leading to an EI (Carr et al., 2018). Another common phenotype of non-atopic EI comprises Aspirin-(acetylsalicylic acid) induced asthma, often presenting concurrent nasal polyposis and eosinophilia (Del Giacco et al., 2017; Wenzel, 2012). The pathogenesis seems to be an imbalance of arachidonic acid metabolites and overproduction of cysteinyl leukotrienes, but is not yet fully understood (Obase et al., 2005). Cysteinyl leukotrienes are assumed to cause bronchoconstriction, amplify EI, and increase mucus secretion (Laidlaw and Boyce, 2016). Other theories for development of non-atopic asthma are previous respiratory infections or irritants such as particulate matter (Handoyo and Rosenwasser, 2009; Micillo et al., 2000).

Besides the above-described pathogenesis, evidence has arisen of other Th-cell subsets causing asthma and leading to different forms of inflammation (Durrant and Metzger, 2010; Woodruff et al., 2009). Briefly, all other types of asthma than the “Th2-high” type are summarized in the term “Th2-low” asthma, which seems to be much more complex in its pathophysiology, and so far no clear biomarkers

have been identified (Hammad and Lambrecht, 2021).

However, it is known that NI reflects a “Th2-low” inflammation. Recent studies suggest that the role of neutrophils is far more important in the pathogenesis of asthma than long assumed (Gao et al., 2017). Th17 cells might be a major cause in the development of NI, producing the cytokine IL-17, which is a cause of lung inflammation (Durrant and Metzger, 2010). In NI, a chemokine profile of elevated IL-8 and IL-17 was detected, indicating their role in neutrophil recruitment (Hosoki et al., 2016). Furthermore, neutrophils might even promote allergic inflammation through the production of reactive oxygen species (Hosoki et al., 2016).

Another interesting part in the pathophysiology of asthma involves Th1 cells. For a long period of time, they have been put in context with Th2 cells emphasizing the Th1/Th2 balance in healthy subjects. Thus, in subjects with asthma, it was assumed that the underlying pathophysiology was due to an imbalance with subsequent Th2 dominance (Oosterhout and Motta, 2005). However, recent studies have revealed that Th1 cells might as well play a pathogenic part in the development of asthma (Durrant and Metzger, 2010).

2.4. Clinical signs

Typical symptoms listed in the report of the global initiative for asthma (GINA) are “chest tightness, wheezing, shortness of breath (dyspnea), cough, and reversible airflow limitation” in variable forms. Symptoms can be absent for weeks or months and then suddenly reappear. They can be triggered by exercise, allergens, viral respiratory infections, or irritant exposure (Global Initiative For Asthma, 2022).

2.5. Diagnostics

Generally, diagnosis of asthma is based on a combination of objective lung function testing and typical symptoms (Brigham and West, 2015). It is important to rule out all other differential diagnoses, as so far there is no “gold standard” test for diagnosis (Stern et al., 2020). For that reason, a thorough medical history should be taken and additionally a profound clinical examination needs to be performed (Global Initiative For Asthma, 2022).

2.5.1. Thoracic imaging

Thoracic imaging is commonly not applied as part of the diagnostic workup of asthma in humans ((NAEPP), 2020). However, performing thoracic radiography has been recommended for questionable cases to rule out other diseases (Bundesärztekammer (BÄK), 2018). CT provides more detailed visualization of parenchyma and airways, being able to picture typical changes found in asthma such as bronchiectasis, atelectasis, bronchial wall thickening and air trapping (Khadadah et al., 2012). Additional imaging methods comprise magnetic resonance imaging and positron emission tomography (Trivedi et al., 2017). These imaging techniques together with CT may serve as an assessment of therapeutic response and to further classify asthmatic phenotypes (Trivedi et al., 2017).

2.5.2. Lung function testing

When asthma is suspected based on the description of typical symptoms, lung function testing should be performed (Bundesärztekammer (BÄK), 2018; Global Initiative For Asthma, 2022). The most applied and recommended pulmonary function test in humans is spirometry, which measures the maximal volume of air that is forcibly exhaled from the maximal inhalation point (forced vital capacity - FVC) ((NAEPP), 2020). Additionally, during the first second of exhalation the volume of air is measured (forced expiratory volume - FEV1) ((NAEPP), 2020). Spirometry indicates airflow limitation with reduced FEV1 and FEV1/FVC (Global Initiative For Asthma, 2022). It should be performed repeatedly to detect variability in lung function which makes an asthma diagnosis more confident (Brigham and West, 2015). For further assessment, bronchial provocation testing can be performed, using spirometry before and after exercise (nonpharmacological bronchial provocation), or application of methacholine/histamine (pharmacologic bronchial challenge testing) to induce bronchoconstriction, indicated by a drop in FEV1 in asthmatic patients (Brigham and West, 2015). Spirometry is generally only feasible in children ≥5 years of age as results depend on the patients cooperation ((NAEPP), 2020). For younger children the forced oscillation technique, impulse oscillometry or whole-body plethysmography can be applied as these methods evaluate airflow in passive manners (Jat and Agarwal, 2023).

2.5.3. Sputum analysis

To further classify asthma, sputum cellular profiles can be evaluated, and sputum eosinophil levels of greater than 2-3% reflect eosinophilic asthma, as it is presumed that they indicate eosinophilic lung inflammation (Carr et al., 2018). On the other hand, the definition for cut off values for neutrophilic sputum profiles is less well-established, varying from >40% to >76% neutrophils (Thomson, 2016).

2.5.4. Biomarkers

Different biomarkers have been established to indicate allergic asthma in humans, including FeNO, serum periostin, total serum IgE and blood eosinophilia (Akar-Ghibril et al., 2020). FeNO is often elevated in patients with atopic asthma as production of nitric oxide in the lungs is a result of EI (Brigham and West, 2015). Serum periostin is also a common biomarker for allergic asthma as it agitates as a signature molecule associated to EI, airway hyperresponsiveness, and subepithelial fibrosis (Chiappori et al., 2015). Being inexpensive and widely available, blood eosinophil measurement is considered a good biomarker as it often correlates with sputum eosinophils (Guida et al., 2022). An association between allergic asthma and elevated total IgE has been shown (Akar-Ghibril et al., 2020). Nevertheless, it cannot be applied for differentiation of allergic and non-allergic asthma as high IgE levels have also been reported in non-allergic asthma (Guida et al., 2023). However, measurement of IgE levels seems to be useful for determining the capability of omalizumab as a treatment option in patients with severe asthma (Guida et al., 2023). When allergic asthma is suspected, allergy tests should be performed as part of the diagnostic workup.

2.6. Allergy testing

In the management of allergic asthma, the identification of relevant allergens with the help of allergy tests is crucial to facilitate allergen avoidance and plan possible treatment with AIT (Rodriguez Del Rio et al., 2022). As an in vivo test, the skin prick test remains the “gold standard” in confirming allergen sensitization, showing higher sensitivity compared to in vitro testing (Bignardi et al., 2019) . Therefore, being considered as time- and cost- effective and reliable, the skin prick test is usually used first and foremost for the diagnosis of IgE-mediated allergy (Ansotegui et al., 2020). In most cases serum-specific IgE measurement is recommended as a complementary test (Bignardi et al., 2019). However, it can be

indicated as sole test when skin prick test cannot be performed due to either diffuse dermatological conditions, medication that cannot be stopped, or unstable asthma (Ansotegui et al., 2020). Depending on the allergens tested in both tests, the specific IgE test and the skin prick test resulted in good correlation in several studies (Bignardi et al., 2019; Milavec-Puretić et al., 2004). In contrast to that, another study testing patients with assumed atopy found no significant correlation between skin prick test, allergen-specific IgE test and total IgE test (Blaziene et al., 2004). Interestingly, in young children only a poor to moderate agreement exists between the two test forms. Therefore, allergy testing should only be applied when meaningful clinical signs exist (Schoos et al., 2015). People with atopic asthma might have a higher tendency of sensitization to environmental allergens compared to healthy ones (Kocks et al., 2020). Despite that, it must be emphasized that clinical relevance should at all times be interpreted in accordance with clinical problems and environmental conditions, as sensitization to an allergen leading to positive reactions in either test does not always imply an allergic disease (Supakthanasiri et al., 2014).

2.7. Therapy

Before starting any medication, the most important treatment of asthma is the identification and avoidance of potential triggers such as exposure to allergens (pets, seasonal pollens, house dust mites, molds) or irritants such as environmental tobacco smoke, ozone, and other particle matters (Global Initiative For Asthma, 2022). The most common anti-inflammatory treatment comprises corticosteroids in oral or inhaled form (Global Initiative For Asthma, 2021). Generally, bronchodilators are added to corticosteroid therapy in order to improve the lung function in patients with asthma, as they target the smooth muscles in the bronchioles (Almadhoun and Sharma, 2023).

In addition to the conventional therapy, in “Th2-high” asthma an IgG1-antibody such as Mepolizumab can be used to inhibit the IL-5 pathway (Hammad and Lambrecht, 2021). It binds IL-5 and inhibits its ligation to the specific receptor for IL-5 on eosinophils and basophils (Farne et al., 2017).

Another approach is therapeutic use of a recombinant humanized monoclonal anti-IgE antibody, which can be used as an additional therapy in atopic asthma. It is called rhuMab-E25 and binds circulating IgE, blocks the binding of IgE to the

FcεR1-receptor, and thus inhibits mast cell degranulation (Barnes, 2000).

Other therapeutic options are directed against different pathophysiological pathways such as inhibition of IL-4Rα, inhibition of IL-9 and inhibition of epithelial-derived cytokines such as IL-1, IL-33, and IL-25, and with advancing research identifying specific asthma endotypes more alternative therapies are likely to come (Hammad and Lambrecht, 2021).

Nowadays AIT is considered an important therapeutic option for allergic asthma, as it treats the underlying cause of the disease directly by building up allergen-tolerance, thus stopping further triggering of the inflammatory cascade (Ankermann and Brehler, 2023). AIT is performed with administration of relevant allergens in a subcutaneous or sublingual form, possibly leading to immunological remission (Nakagome and Nagata, 2021). Therefore, it is important to first identify relevant allergens.

3. Allergens and asthma

3.1. Asthma in other animal species

3.1.1. Asthma in laboratory animals

In research, laboratory animals are essential to better examine pathophysiologic mechanisms in detail and evaluate efficacy and safety of asthma therapies (Aun et al., 2017). Therefore, several species including rodents, pigs, guinea pigs, sheep, primates, and even fruit flies have been experimentally sensitized (Aun et al., 2017). One of the most common models used in asthma research is the mouse as it offers a wide availability of transgenic individuals, allowing diverse research (Nials and Uddin, 2008). However, one striking disadvantage of mice as research models is the fact that they do not develop asthma nor spontaneous bronchoconstriction or airway remodeling naturally (Davis and Sheats, 2021). Yet, in an acute allergen challenge model, they develop many features of clinical human asthma such as airway inflammation, epithelial hypertrophy and elevated IgE levels. Furthermore, one frequently used mouse strain (BALB/c) showed great use for detailed immunological research, as it develops a good Th2 response (Nials and Uddin, 2008). To mimic an allergic reaction, sensitization is often carried out through intraperitoneal and subcutaneous injections, but new preferences are intranasal or inhalational challenges, as they resemble the more

natural pathologic way of asthma (Aun et al., 2017). The most frequently used allergen is ovalbumin from chicken eggs, as it is easily produced in large amounts and hence low in costs. Furthermore, it induces an intense allergic pulmonary inflammation (Aun et al., 2017; Nials and Uddin, 2008). However, ovalbumin is not naturally implicated in human asthma; therefore, alternative allergens such as HDMA with more clinical relevance are used progressively (Nials and Uddin, 2008). As mentioned earlier, the acute challenge models in mice are useful for understanding of the direct airway inflammatory process, but they do not generate all features of airway remodeling or persistent airway hyperresponsiveness (Nials and Uddin, 2008). For this reason, chronic allergen challenge models have been established, which consist of repeated exposure of the airways to low levels of an allergen such as HDMA over a period of up to 12 weeks (Nials and Uddin, 2008). These are nowadays the models of choice in research, as they develop more features of chronic airway remodeling such as goblet cell hyperplasia, epithelial hyperthrophy, and subepithelial fibrosis (Nials and Uddin, 2008).

The fruit fly *Drosophila melanogaster* can represent an important model in asthma research as well, as it provides many insights into airway immunity and asthma susceptibility genes (Roeder et al., 2009). Although it does not possess a lung, the fruit fly has an airway system (tracheae consisting of interconnected tubes) with striking similarities to that of mammals regarding its physiology, and its simple structure resembles a cell culture for research (Roeder et al., 2012). As the fly is lacking an adaptive immune system, it makes it easier to exclusively investigate the role of the innate immune system (Roeder et al., 2012).

The oldest animal model for asthma research is the guinea pig which was first introduced in 1937 with the histological investigation of the lungs after aerosol sensitization in this species, showing increased airway responsiveness similar to human asthmatics (Kallós and Kallós, 1984). The guinea pig is commonly used for the development of drugs such as corticosteroids or beta receptor agonists, as it represents a good model for airway responsiveness (Kianmeher et al., 2016).

Sheep would have the advantage of natural susceptibility to allergens and the ability to create an immediate physiological response to inhaled allergens, but for greater use in research they are considered too expensive (Aun et al., 2017; Kianmeher et al., 2016).

3.1.2. Equine asthma

Horses can suffer from an inflammatory lower airway disease summarized under the umbrella term “equine asthma” (EA), showing similar pathogenic mechanisms as human asthma (Bullone and Lavoie, 2015) . Therefore, the horse may embody a valuable model for asthma research, as its lifespan with 25 to 30 years is more similar to that of humans, compared to other animals such as mice with a lifespan around two to three years (Davis and Sheats, 2021). As the horse’s lungs are quite large, they permit harvesting multiple lung biopsies for research for investigation of small airway remodeling over time in the same subject (Bullone and Lavoie, 2015). In reality however, costs for drugs, breeding and ordinary care are too high, compared to other laboratory animals, preventing horses from becoming regular animal models for asthma (Bullone and Lavoie, 2015).

Commonly, EA is defined as mild to moderate asthma named “inflammatory airway disease” (IAD) and “severe asthma”, also called “recurrent airway obstruction” (RAO) or “heaves” (Couëtil et al., 2016). It is not clearly established whether these phenotypes evolve from different etiologies or simply show a variation in severity of clinical signs (Bond et al., 2018). The differentiation between both phenotypes is based on clinical signs and BALF cytology. While IAD implies a mild inflammatory profile with >10% neutrophils, > 5% mast cells and >5% eosinophils as well as intermittent chronic cough and mild exercise intolerance, RAO is characterized by an inflammatory reaction with >25% neutrophils in BALF cytology (Couëtil et al., 2016). In addition to the clinical signs already mentioned, horses with RAO show strong labored breathing at rest, which is not the case in horses with IAD (Couëtil et al., 2016; Davis and Sheats, 2021). Horses with RAO are usually older (>7 years), while horses with IAD tend to be young to middle aged, though EA can appear at any age (Couëtil et al., 2016). Horses with RAO not only present with airway neutrophilia and mucus accumulation, but also show bronchospasm, bronchial hyperreactivity and airway remodeling (Pirie, 2014).

Genetics are thought to play an important role in EA (Scharrenberg et al., 2010), but the exact pathophysiology is unknown and susceptibility for hypersensitivity to aeroallergens is suspected (Pirie, 2014). Molds from hay and straw are assumed to be the main trigger for development of hypersensitivity reactions (Ivester et al., 2014; Pirie, 2014), or allergens associated with stabling environment in general

(White et al., 2017). Interestingly, horses can also develop severe summer pasture associated EA, signifying that additionally different environmental allergens may play a role in the hypersensitivity reaction as well (Simões et al., 2022)

In EA a lot of controversy exists regarding typical changes in cytokine levels (Davis and Sheats, 2021). Some studies support the idea of a Th2-driven response (Lavoie et al., 2001), while others propose a Th1- and/or Th17-mediated response (Murcia et al., 2016; Sage et al., 2023; Simões et al., 2022). It becomes clear that EA embodies a complex and heterogeneous disease which might consist of several different endotypes merging into one clinical phenotype (Simões et al., 2022). While some authors propose that horses with severe EA can act as a good animal model for non-allergic human asthma or late-onset non-atopic asthma (Bullone and Lavoie, 2015), others postulate that the horse might be a good animal model for allergen-induced neutrophilic human asthma (Lavoie et al., 2001). Furthermore, it has been assumed that horses present a type-3 or -4 hypersensitivity reaction rather than a type-1 hypersensitivity due to delayed clinical signs after allergen challenge (Klier et al., 2021).

Diagnosis of mild or severe EA is generally based on thorough clinical examination and typical clinical history (Couetil et al., 2020). In addition, diagnostics reveal mucus accumulation in the airways on endoscopy, inflammation detected in BALF cytology and reduced lung function (Couëtil et al., 2016).

To identify relevant allergens in horses, IDT is regarded as the “gold standard” method (Lorch et al., 2001) and is commonly used to identify hypersensitivity reactions to environmental allergens (Lo Feudo et al., 2021). For implementation of the test, multiple allergen injections are performed on the lateral aspect of the clipped neck (Lo Feudo et al., 2021). Generally, many researchers propose that EA is an IgE-mediated hypersensitivity reaction, as horses with EA showed more positive reactions after 30 minutes in several studies compared to healthy controls (Eder et al., 2000; Evans et al., 1992; Jose-Cunilleras et al., 2001). Yet, some suggest that affected horses might rather present a late phase reaction on IDT (Tahon et al., 2009), and therefore recommend reading results not only after 30 minutes, but additionally after 4 and 24 hours (Lo Feudo et al., 2021; Wong et al., 2005). Due to delayed reactions to an allergen provocation test and the lack of differences found for positive allergen reactions between healthy and affected

horses in other studies, it was doubted on the other hand that horses with EA present IgE-mediated hypersensitivity reactions (Klier et al., 2021; Tahon et al., 2009). So far, IDT in horses lack standardization for the use of allergens and their concentrations (Tahon et al., 2009) as well as lack standardized interpretation times which could explain inconsistencies between studies (Lo Feudo et al., 2021).

As an in vitro test SAT can be applied, but it is known for its low sensitivity in horses (Lorch et al., 2001). One study compared three different in vitro allergy tests (Polyclonal antibody-based ELISA, Radio-allergo-sorbent-test, FcεR1 α-based ELISA) to IDT results in horses with and without atopy and found overall low sensitivity for all three serum tests, whereas the FcεR1 α-based ELISA and polyclonal-based ELISA showed good specificity. Based on that research it was concluded that all three serum tests cannot be recommended for allergen selection and that IDT should be applied (Lorch et al., 2001). Other authors confirmed the statement that in vitro allergy tests represent unreliable screening tests for hypersensitivity reactions in horses, as no difference could be found regarding positive reactions between horses with EA and healthy ones (Klier et al., 2021; Tahon et al., 2009). Furthermore, it has been assumed that free circulating IgE may not play a major role in the pathogenesis of EA (Lorch et al., 2001).

When in vivo and in vitro allergen tests were compared, low agreement was found for most allergens (Lorch et al., 2001). This is in accordance with other studies, in which results obtained by different allergy tests were compared (FcεR1 α-based ELISA, monoclonal antibody ELISA, functional in vitro test and IDT) (Klier et al., 2021).

Standard therapy of EA consists of the use of corticosteroids (systemically administered or in inhaled form), bronchodilators and change of the stable environment (Bullone and Lavoie, 2015; Couëtil et al., 2016). Hence, long-term corticosteroid treatment may provoke adverse effects, and avoidance of allergens is often not completely feasible (Klier et al., 2018)

3.2. Feline atopic syndrome

Cats can develop different allergic disorders that are thought to be associated with the production of IgE antibodies, which are summarized under the term “feline atopic syndrome” (Halliwell et al., 2021a; Halliwell et al., 2021b; Santoro et al.,

2021). Feline atopic syndrome agitates as an umbrella term for feline allergic diseases such as FA, feline atopic skin syndrome (FASS), feline food allergy (FFA) and flea allergy dermatitis (Mueller et al., 2021; Santoro et al., 2021). Atopy is a tendency towards an immune response (elevated production of IgE) against environmental allergens (Justiz Vaillant et al., 2023). An individual can even suffer from two or more atopic diseases at the same time (Justiz Vaillant et al., 2023). Therefore, a possible strong connection among the respiratory system, the gastrointestinal tract and the skin should not be underestimated (Bajwa, 2021). To date there is little information about possible heritability of allergic diseases in the feline species (Santoro et al., 2021). Some studies assume a certain breed predisposition for example in Siamese cats for FA (Adamama-Moraitou et al., 2004) or Abyssinian cats for the FASS (Scott and Miller, 2013). However, those assumptions of predispositions have not been proven so far (Santoro et al., 2021).

FASS comprises an inflammatory and pruritic skin syndrome, which has previously been called “feline atopy” or “non-flea, non-food-induced feline hypersensitivity dermatitis” (Bajwa, 2021). In contrast to dogs, cats do not express an allergic skin syndrome like the so-called “atopic dermatitis” in humans. They show distinct clinical patterns and uncertainty of IgE involvement exists; therefore, this term should not be used in cats (Halliwell et al., 2021b; Older et al., 2021). Cats with FASS express one or more “cutaneous reaction patterns” such as self-inflicted alopecia, head and neck pruritus, miliary dermatitis, and eosinophilic granuloma complex (Santoro et al., 2021). Similar to FLAD, the diagnosis of this syndrome is based on the exclusion of differential diagnoses, the patient’s history and clinical signs (Mueller et al., 2021). Flea allergy dermatitis comprises one of the cardinal differentials for FASS and needs to be ruled out with the help of flea control. Diagnosis of flea allergy dermatitis is made when cats show a resolution of clinical signs due to applied agents against fleas (Santoro et al., 2021). Furthermore, in FASS, allergy tests have been used to identify relevant allergens for possible AIT; thus, positive reactions must always be interpreted together with xythe patient’s history and clinical signs (Santoro et al., 2021). The outcome of AIT has shown to be a promising treatment in cats with FASS (Jones and Bloom, 2021; Trimmer et al., 2005).

FFA expresses striking similarities in the clinic to FASS, and therefore, it is inevitable to perform a strict food trial and previous anti flea treatment prior to

testing (Halliwell et al., 2021b; Santoro et al., 2021). Further reported clinical signs may as well be typical gastrointestinal signs such as vomiting, diarrhea and flatulence, or even just conjunctivitis (Santoro et al., 2021). In contrast to FA and FASS, FFA is easy to diagnose with the help of a food elimination trial. For that the cat simply needs to exclusively be fed a commercial hypoallergenic diet or a home-cooked selected protein diet for six to ten weeks–depending on the resolution of clinical signs–followed by a re-challenge with the previous diet. If clinical signs then relaps, and during second elimination diet re-disappear, FFA can be diagnosed. Treatment consists of maintaining the special diet (Leistra and Willemse, 2002). Allergy tests such as SAT and IDT are not reliable methods for detection of relevant allergens in food allergy (Guilford et al., 2001; Ishida et al., 2012; Mueller and Tsohalis, 1998). One study could demonstrate that the lymphocyte stimulation test might help to detect food allergens (Ishida et al., 2012), although this test is technically difficult to manage and is currently not feasible in clinical practice (Mueller and Olivry, 2017)

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Reactions to environmental allergens in cats with feline lower airway disease

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Objectives: Aeroallergens have been discussed as potential triggers for feline asthma (FA), which can be induced experimentally by allergen sensitization. To date, only few studies have investigated reactions to environmental allergens in cats with naturally occurring feline lower airway disease (FLAD). The aim of the study was to compare results of intradermal testing (IDT) and serum allergen-specific immunoglobulin E-(IgE) testing (SAT) in cats with FLAD, and to investigate possible associations with allergen exposure.

Material and methods: Eight cats with eosinophilic airway inflammation (EI), ten cats with mixed inflammation (MI), six with neutrophilic inflammation (NI), and 24 healthy cats (HC) were included. Cats diagnosed with FLAD were assigned to the different inflammatory groups based on bronchoalveolar lavage fluid (BLAF) cytology. SAT was performed in all cats; IDT was only carried out in cats with FLAD. Information about the cats' environment and potential allergen exposure was obtained using an owner questionnaire.

Results: In comparison to 83% of HC with positive reactions on SAT only 52% of cats with FLAD had positive responses ($p = 0.051$). Significantly more positive reactions per cat were detected on IDT than on SAT ($p = 0.001$). No significant difference was found for positive reactions per cat on SAT when compared between HC, NI, EI, and MI ($p = 0.377$). Only "slight" agreement was found for most allergens when reactions obtained in both tests in cats with FLAD were compared, except for "moderate" agreement for English plantain (k = 0.504) and *Alternaria alternata* (k = 0.488). Overall, no clear association between the cats' environment and allergen reactions were detected.

Conclusions and clinical importance: Interpretation of allergy test results in cats with FLAD should be done in the context of clinical signs and individual factors.

KEYWORDS

immunoglobulin E, intradermal test, feline asthma, chronic bronchitis, feline atopic syndrome

1 Introduction

Feline lower airway disease (FLAD) is a common inflammatory condition in cats. Recent studies support the idea that feline asthma (FA) and feline chronic bronchitis (CB) are the two most common inflammatory diseases falling under this term (1–4). However, a clear discrimination between these two phenotypically similar syndromes and their potential etiologies could not be established (5, 6). That raises the question whether FA and CB are two different disease entities or if they are just arising from the same underlying

cause with variations in their inflammatory profile (6, 7). To date, the only attempt to differentiate between FA and CB is based on cell differentiation of bronchoalveolar lavage fluid (BALF) cytology, suggesting an eosinophilic inflammation (EI) representing FA, and a sterile neutrophilic inflammation (NI) typical for CB (2, 8). Recently, a subdivision of so-called "mixed inflammation" (MI) has been introduced for further categorization (9–13). It is assumed that MI is a consequence of chronic EI, resulting in damage of airway epithelium, and therefore leading to immigration of neutrophils (1, 5, 14). According to recent definitions, FA is assigned to the feline atopic syndrome comprising allergic diseases of the skin, respiratory and gastrointestinal tract in cats (15).

In multiple studies FA could be induced experimentally via parenteral sensitization and aerosol challenge using common environmental allergens. Therefore, it is highly suggestive that an allergic reaction is an underlying major cause for the inflammation seen with FA (16–20). Currently, standard therapy consists of inhaled or systemically administered glucocorticoids, often in combination with bronchodilators (1, 8, 14, 21). Even though it could be demonstrated that clinical signs improve significantly with this therapy (21, 22), subclinical inflammation may continue to damage the lower airways in some cats (23), and side effects associated with long-term use of corticosteroids can pose a risk for patients (24, 25). Therefore, it has been suggested to utilize allergen immunotherapy (AIT) as a causative treatment of a possible allergic etiology, which has been successfully performed in experimental studies using FA models (17, 26, 27). For successful AIT, it is essential to identify patient-relevant allergens which can be supported by serum or skin allergy tests (20). In one study using an experimental model of cats sensitized to Bermuda grass allergen or house dust mite allergen (HDMA), IDT revealed a greater sensitivity compared to serum allergen-specific immunoglobulin E-(IgE) testing (SAT), although SAT showed higher specificity (18). In naturally occurring FA, IDT and SAT results have been compared in a pilot study which showed significantly more individual positive reactions on IDT and SAT in cats with FLAD compared to healthy cats (HC) (28). Recent studies identified responses to house dust mites and storage mites (SM) as common reactions on SAT in cats with FLAD; however, IDT was not performed in these investigations and environmental factors were not specifically assessed (9, 29).

Therefore, the aim of the study was to prospectively investigate agreement between IDT and SAT results in cats with FLAD and to assess possible correlations with environmental factors.

2 Materials and methods

The prospective case-controlled study was approved by the Ethics Committee of the Centre for Clinical Veterinary Medicine of the Ludwig Maximilian University of Munich, Germany (No. 239-16-11-2020) and was conducted between December 2020 and June 2022.

2.1 Study population

All cats with FLAD were privately owned patients of the Small Animal Clinic of the Ludwig Maximilian University of Munich, Germany, presenting with respiratory complaints. HC were presented for health care check-up, vaccination titer check or dental care. Inclusion criteria were clinical signs indicative of FLAD (chronic cough, wheezing, episodes of tachypnea or respiratory distress), radiographic evidence of bronchial or bronchointerstitial lung pattern, elevated total cell count in BALF >400 cells/µl (30, 31), and cytological evidence of sterile airway inflammation. For BALF cytology, 200 cells of a cytospin sample were assessed by the same board-certified clinical pathologist. Inflammation was categorized as eosinophilic (eosinophils >20% with neutrophils <14%, or eosinophils >50%), mixed (eosinophils 20%-50% and neutrophils >14%), or neutrophilic (neutrophils >14% and eosinophils <20%) inflammation as previously described (6). In addition, aerobic bacterial cultures and *Mycoplasma* spp.-PCR were performed on all BALF-samples. Cats with positive *Mycoplasma spp.*-PCR were not excluded, as *Mycoplasma* spp. has been described as a commensal organism in the lower airways of cats (32). Additionally, cats with a positive *Mycoplasma* spp. PCR received oral Doxycycline for 3 weeks, yet symptoms persistet. Deworming status of the cats included in this study could not be determined. In all outdoor cats, Baermann fecal analysis was performed to rule out lungworm disease. Exclusion criteria were the administration of glucocorticoids within 4 weeks or of antihistamines within 2 weeks prior to examination, detection of respiratory disease other than FLAD, presence of severe systemic disease, or unstable patients exhibiting respiratory distress at presentation. Inclusion criteria for HC were an unremarkable clinical examination and the absence of a history of respiratory or dermatological diseases.

2.2 Sample collection

2.2.1 Diagnostic workup

All owners of cats with FLAD and HC filled out a questionnaire on environmental factors of their cats (Supplementary material). In cats suspicious for FLAD, a thorough clinical examination and thoracic radiographs (ventrodorsal and laterolateral right-sided) were carried out. Blood samples were collected for a complete blood cell count and serum biochemistry analysis. In cats with outdoor access, Baerman analysis from a three-day fecal collection was carried out to exclude lung worm infection. As *Dirofilaria immitis* is not endemic in Germany, and none of the patients originated from or had traveled to an endemic area, heart worm testing was not performed. Prior to anesthesia, cats received 0.01 mg/kg terbutaline subcutaneously (Bricanyl®, AstraZeneca, Wedel, Germany) to minimize the risk of bronchoconstriction, and 0.2mg/kg Butorphanol (Butorgesic®,

Abbreviations: AIT, allergen immunotherapy; BALF, bronchoalveolar lavage fluid; CB, chronic bronchitis; CCD, cross-reactive carbohydrate determinant; EI, eosinophilic inflammation; FA, feline asthma; FASS, feline atopic skin syndrome; FLAD, feline lower airway disease; HDMA, house dust mite allergen; HC, healthy cat; IgE, immunoglobulin E; MI, mixed inflammation; NI, neutrophilic inflammation; SAT, serum allergen-specific IgE testing; SM, storage mite.

TABLE 1 Allergens, number of reactions to allergens in cats with FLAD and HC and agreement between tests.

IDT		SAT			Agreement between IDT and SAT (κ-value)
Allergens tested in both tests		Allergens tested in both tests			
Allergen	FLAD (Number of positive reactions)	Allergen	FLAD (Number of positive reactions)	HC (Number of positive reactions)	(kappa could only be calculated when there was at least one positive reaction in each test)
Rapeseed	5	Rapeseed	2	1	0.145
Goosefoot	4	Goosefoot	2	5	0.129
Ragweed	8	Ragweed	0	2	IDT 34% positive results, 0% positive results on SAT
Sheep sorrel	6	Sheep sorrel	0	3	In IDT 24% positive results, 0% positive results on SAT
Mugwort	4	Mugwort	0	1	IDT 25% positive results, 0% positive results on SAT
English plantain	5	English plantain	2	2	0.504
Orchard grass	2	Orchard grass	0	0	IDT 5% positive results, 0% positive results on SAT
Timothy grass	7	Timothy grass	5	4	0.034
Perennial ryegrass	4	Perennial ryegrass	0	2	IDT 15% positive results, 0% positive results on SAT
Bermuda grass	9	Bermuda grass	0	2	IDT 43% positive results, 0% positive results on SAT
Kentucky bluegrass	1	Kentucky bluegrass	5	5	IDT 0% positive result, on SAT 24% positive results
Mold mite *(Tyrophagus putrescentiae)*	3	Mold mite *(Tyrophagus putrescentiae)*	1	6	0.068
American house dust mite *(Dermatophagoides farinae)*	5	American house dust mite *(Dermatophagoides farinae)*	7	12	0.080
Grocers'itch mite *(Lepidoglyphus destructor)*	6	Grocers'itch mite *(Lepidoglyphus destructor)*	0	0	IDT 29% positive results, 0% positive results on SAT
European house dust mite	5	European house dust mite	0	0	IDT 15% positive results, 0% positive results on SAT
Flour mite *(Acarus siro)*	5	Flour mite *(Acarus siro)*	0	4	IDT 24% positive results, 0% positive results on SAT
Flea	3	Flea	1	1	0.050
Alternaria alternata	4	*Alternaria alternata*	3	8	0.488
Aspergillus fumigatus	2	*Aspergillus fumigatus*	2	6	0.105
Cladosporium herbarum	3	*Cladosporium herbarum*	2	5	0.167
Malassezia	7	*Malassezia*	0	2	IDT 34% positive results, 0% positive result on SAT

(Continued)

TABLE 1 (Continued)

IDT		SAT			Agreement between IDT and SAT (κ-value)
Allergens tested on IDT only		Allergens tested on SAT only			
Allergen	FLAD (Number of positive reactions)	Allergen	FLAD (Number of positive reactions)	HC (Number of positive reactions)	(kappa could only be calculated when there was at least one positive reaction in each test)
Upright pellitory	5	Stinging nettle	1	1	
Creeping bentgrass	2	Dandelion	1	2	
Perennial grass	10	Oat	0	2	
Cough grass	4	Rye	0	2	
Goldenrod	6	Birch	2	1	
Beech	1	Olive tree	3	3	
Hazel	6	Plane tree	3	2	
Silver poplar	5	Willow	6	9	
Cockroach	0	Pine	2	1	
House fly	2	Privet	4	5	
Mosquito	4	Cypress	1	1	
Culex	5	Oak	0	1	
Biting midges	5	American elm	0	1	
Horse fly	10				
Sheep's wool	3				
Goose feather	2				

FLAD, feline lower airway disease; HC, healthy cats.

cp pharma, Burgdorf, Germany) was given intravenously for mild sedation. Anesthesia was induced and maintained intravenously with Alfaxan (Alfaxalon®, Jurox, Rutherford, Australia). Blind bronchoalveolar lavage was performed in cats with FLAD according to a previously published protocol (32). Each BALF was submitted for aerobic bacterial culture (Institute for Infectious Diseases and Zoonoses of the Ludwig Maximilian University of Munich, Germany) and *Mycoplasma*-spp.-PCR (Synlab Laboratory, Augsburg, Germany). Total cell count of BALF was determined directly after sample collection, and stained native and cytocentrifuged BALF-smears were evaluated cytologically by a board-certified clinical pathologist (JP). For this purpose, two direct smears and two cytospin preparations were stained with modified Wright's stain. Multiple microscopic fields were examined to obtain a total of 200 cell differential count.

2.2.2 Intradermal testing

During anesthesia for the diagnostic workup, IDT was performed according to a published protocol (33). For this procedure the lateral aspect of the left thorax was carefully clipped, and injection sites were marked with a water-soluble pen. In total, 39 injections were administered intradermally with a volume of 0.08 ml each, including a negative (saline) and a positive control (histamine phosphate) (Table 1). After 15 and 25 min, injection sites were evaluated for diameter of the wheal, turgidity, and erythema in comparison to positive and negative control. The local reactions were graded from 0 (negative control) to 4 (positive control) and results were recorded. Evaluation was carried out by a board-certified dermatologist as well as dermatology residents, who were familiar and experienced with the procedure.

2.2.3 Serum allergen-specific immunoglobulin E-(IgE) testing

In HC, left over material was used for analysis. Serum had been collected for general check-up, vaccination titer check or blood work collected prior to anesthesia for dental care. All serum samples were stored at −80°C immediately after centrifugation. The samples were then sent in groups to the laboratory (Nextmune S.L.U., Madrid, Spain) for specific IgE-antibody detection for 34 different allergens (Table 1). On the way to the external laboratory, one batch of serum samples was lost in mail transit, which reduced the total number of SATs carried out to 21 instead of 24 in the

TABLE 2 Standard interpretations of Cohen's Kappa (34).

Kappa statistic	Strength of agreement
<0	Poor
0.01–0.20	Slight
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–1.00	Almost perfect

group of cats with FLAD. All sera were tested against cross-reactive carbohydrate determinants (CCDs) using indirect ELISA (CCD-screening). Sera then were diluted 1/6 in buffer solution containing 1% bovine serum albumin and 0.1% Tween 20. Samples with positive specific IgE against CCDs were processed after adding a CCD-blocker before dilution. For blocking, serum was incubated for 1 h at room temperature with a CCD-blocker. After that, samples were added to 96-well plates coated with allergens in duplicates. The plates were incubated at 4 °C overnight. Next, plates were washed four times with washing buffer, and monoclonal antibody was added, followed by incubation at 4 °C for 2 h. Thereafter, plates were washed six times with washing buffer, and p-nitrophenyl phosphate substrate (Moss, MD, USA) was added to the wells. After 30 min incubation time at room temperature, 1 N sodium hydroxide (NaOH) was added to stop the reaction. Absorbances were read at 405 nm using a spectrophotometer. Positivity was defined according to a protocol established by Nextmune expressing results in ELISA absorbance units.

2.3 Statistical analysis

All analyses were performed using commercially available software (IBM®, SPSS Statistics version 28.0.1.0). Nonparametric tests were used, as data was not normally distributed. Mann-Whitney U test was used for age comparison between cats with FLAD and HC. In addition, it was used to compare positive reactions per cat in IDT and SAT with allergens appearing in both tests ($n = 21$) as well as positive reactions in indoor- and outdoor cats. Comparison of more than two groups was performed with Kruskal–Wallis test, which was applied for positive reactions on SAT and IDT between the different groups, and for positive reactions between specific allergen groups. When significance was demonstrated, a *post-hoc* Bonferroni correction was performed. Fisher's-exact test was applied for evaluation of an association of SM reactions and dry food diet. In addition, it was used to compare the number of cats with at least one reaction on SAT between groups. Agreement of IDT and SAT results for allergens appearing in both tests was evaluated using Cohen's *kappa*. "Poor" to "almost perfect" agreement was reported as previously defined (Table 2) (34). Eleven allergens were compared only descriptively as *kappa* was by definition zero given the fact that one test showed no positive reactions for a specific allergen. Seasonality was assessed descriptively. For all tests $P < 0.05$ was considered significant.

3 Results

Twenty-four client-owned cats diagnosed with FLAD and 24 HC who served as a control group were included. Four cats suspected of having FLAD were previously excluded due to physiological BALF cytology. Of the remaining 24 cats with FLAD, eight showed EI, ten MI, and six NI on BALF cytology (Table 3). Four cats had positive PCR results for *Mycoplasma spp.* and two cats had low levels of bacterial growth after enrichment on BALF culture: growth of *Pasteurella multocida*, *Neisseria zoodegmatis,* and *Staphylococcus felis* in one cat, and *Pasteurella* spp. in another patient (Table 3).

Of the 24 cats with FLAD ten were female spayed (42%), and 14 male neutered (58%). HC consisted of ten female spayed (42%) and one intact female (4%), and twelve male neutered (50%), and one intact male (4%). Breeds of FLAD cats included Domestic Shorthair ($n = 13$), Siamese ($n = 4$), Maine Coon ($n = 3$), Norwegian Forest ($n = 2$), Russian Blue ($n = 1$), and Oriental Shorthair ($n = 1$) (Table 3). Breeds of HC were Domestic Shorthair ($n = 20$), Siberian ($n = 1$), Russian Blue ($n = 1$), Norwegian Forest ($n = 1$) and British Shorthair ($n = 1$). Median age did not differ significantly between cats with EI (2.5, range: 1–9 years), MI (8.0, range: 1–15 years), NI (7.5, range: 3–13 years) and HC (3.0, range: 1–13 years) ($p = 0.118$). In both groups most cats were kept indoors (63% with FLAD and 83% HC) and lived in an urban environment (79% FLAD and 88% HC). Two cats with FLAD showed additional signs of dermatitis and pruritus, one of them with more severe dermatological and respiratory signs during summer (Table 3). The duration of time since first onset of clinical signs ranged from 3 months to 6 years (median 1.5 years), with cough $n = 22$ (92%) being the most predominant clinical sign besides tachypnea $n = 3$ (13%), episodes of respiratory distress $n = 4$ (17%) and wheezing $n = 2$ (8%). Blood eosinophilia was present in ten cats with FLAD (42%) (Table 3).

More HC 20/24 (83%) showed at least one positive allergen reaction on SAT compared to cats with FLAD 11/21 (52%) ($p = 0.051$). On IDT 22/24 (91%) cats with FLAD showed positive allergen reactions. When results of IDT and SAT for cats with FLAD were compared, significantly more positive reactions per cat were found on IDT ($p = 0.001$). No significant difference could be detected for median positive reactions per cat on SAT between cats with FLAD and HC ($p = 0.185$), nor when compared between NI, EI, MI, and HC ($p = 0.377$) (Tables 4A, B).

When agreement between IDT and SAT results was assessed for the ten allergens evaluated in both tests, "moderate agreement" was observed for the allergens *Alternaria alternata* ($\kappa = 0.488$) and English plantain ($\kappa = 0.504$). For the other eight allergens, agreement was only "slight" (Table 1).

On SAT the most common allergen reaction in cats with FLAD ($n = 7$) and HC ($n = 12$) was American house dust mite (*Dermatophagoides farinae*), followed by fungi in HC with *Alternaria alternata* ($n = 8$) and *Aspergillus fumigatus* ($n = 6$) (Table 1).

When median positive reactions per cat were compared for the three inflammatory types regarding different allergen groups on IDT, reactions to tree pollen were significantly more common in cats with EI than in those with NI and MI. In addition, cats

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TABLE 3 Signalement, clinical, laboratory, and microbiological parameters in cats with FLAD.

Cat no.	Age year	Breed	BALF inflammatory type	Blood Eosinophils* ($10^9/l$)	BALF bacterial culture	BALF total cell count (cells/μL)	*Mycoplasma spp.* PCR	BALF Eosinophils** (%)	BALF Neutrophils** (%)	BALF Macrophages** (%)	Seasonality
1	2	Siamese	EI	0.61	Neg	1,250	Neg	70	8	22	None
2	2	DSH	EI	0.43	Neg	1,330	Neg	26	6	68	None
3	4	DSH	EI	0.53	Neg	3,260	Neg	43	12	45	IU
4	1	Oriental Shorthair	EI	0.49	Neg	1,140	Neg	26	8	66	None
5	9	Siamese	EI	0.23	Neg	870	Neg	35	2	63	Spring
6	3	Siamese	EI	**1.28**	Neg	1,740	Neg	85	6	9	None
7	2	Norweigan Forest	EI	**0.75**	Neg	2,060	Neg	62	7	31	None
8	7	DSH	EI	0.29	Neg	2,030	Neg	38	14	48	None
9	7	Russian Blue	MI	**0.76**	Neg	830	Pos	27	47	26	IU
10	11	DSH	MI	**0.89**	Neg	2,470	Neg	22	66	12	None
11	15	DSH	MI	**0.74**	Neg	2,270	Pos	21	68	11	None
12	10	DSH	MI	0.36	Neg	410	Neg	33	53	14	None
13	9	DSH	MI	**0.63**	Neg	2,710	Neg	39	42	19	Spring
14	3	DSH	MI	**2.35**	Neg	2,210	Neg	32	47	21	Summer
15	11	DSH	MI	0.52	Neg	2,310	Neg	27	64	9	None
16	2	Maine Coon	MI	0.5	Neg	1,030	Neg	41	21	36	None
17	1	Maine Coon	MI	**1.23**	Neg	910	Neg	25	18	57	None
18	2	Maine Coon	MI	0.45	*P. multocida Neisseria zoodegmatis Staphylococcus felis* (AE)	4,950	Neg	20	24	56	None
19	3	DSH	NI	0.35	Neg	11,350	Pos	1	95	8	None
20	11	Siamese	NI	0.5	Neg	2,060	Neg	2	72	26	None
21	11	Norwegian Forest	NI	0.33	Neg	2,560	Pos	2	91	7	None
22	13	DSH	NI	**2.38**	Neg	3,630	Neg	2	82	14	Summer
23	3	DSH	NI	**0.69**	*Pasteurella spp* (AE)	850	Neg	7	21	70	Summer
24	4	DSH	NI	0.23	Neg	3,620	Neg	11	21	68	None

EI, eosinophilic inflammation; MI, mixed inflammation; NI, neutrophilic inflammation; AE, bacterial growth after enrichment; IU, information unavailable. *Values above reference range marked in bold numbers. **Cell distribution in BALF-cytology in %. Cats 23 and 13 had dermatitis and puritus issues. Cat 23 had pruritus only in summer and Cat 13 had puritus the whole year.

TABLE 4A Positive reactions per cat on IDT ($n = 37$) in cats with FLAD.

EI ($n = 8$)		MI ($n = 10$)		NI ($n = 6$)		p-value	FLAD ($n = 24$)	
median	range	median	range	median	range		median	range
8.5	5–14	2.5	0–12	5.0	5–10	0.008*	5.0	0–14

Post-hoc Bonferroni correction: EI has significantly more positive reactions compared to MI ($p = 0.002$), but not when compared to NI ($p = 0.195$).

TABLE 4B Positive reactions per cat on SAT ($n = 34$) in cats with FLAD and HC.

EI ($n = 7$)		MI ($n = 9$)		NI ($n = 5$)		HC ($n = 24$)		p-value	FLAD ($n = 21$)	
median	range	median	range	median	range	median	range		median	range
5.0	0–9	1.0	0–6	0.0	0–10	2.0	0–29	0.377	1.0	0–10

IDT, intradermal test; SAT, serum allergen-specific immunoglobulin E test; EI, eosinophilic inflammation; MI, mixed inflammation; NI, neutrophilic inflammation; HC, healthy cats; FLAD, feline lower airway disease.

with EI and those with NI reacted significantly more commonly against allergens of the insect group than cats with MI (Table 5A). There were no significant differences for the comparison of median positive reactions per cat in allergen groups tested on SAT (Table 5B). Total positive reactions for all groups of cats are listed in Tables 5A, B.

Two cats have had clinical signs of FLAD for <1 year at the time of presentation; therefore, seasonality could be assessed only in the remaining 22 cats. All 22 cats showed clinical signs throughout the year. While in 17 cats the severity of clinical signs did not change throughout the year, five cats had seasonal changes of severity. Of those five, two cats showed more signs in spring and three cats in summer.

Significantly more cats with FLAD (12/24, 50%) lived in a household with smoke exposure compared to HC (4/24, 17%) ($p = 0.030$). No difference was found regarding this factor when cats with EI, MI and NI were compared ($p = 0.641$).

In the FLAD group, no difference could be detected between cats eating mainly dry food and those eating moist food in reactions to SM (*Tyrophagus putrescentiae*, *Acarus siro*, *Lepidoglyphus destructor*) on IDT ($p = 0.629$). No calculation was done on SAT as only one cat showed a reaction to one of the mite allergens.

There was no difference in median positive reactions per cat for "outdoor-allergens" such as weed, grass, and tree pollen in the FLAD group between cats living exclusively indoors (median: 0.00 on SAT, median: 4.00 on IDT) and cats with outdoor access (median: 0.00 on SAT, median: 3.5 on IDT) on SAT ($p = 0.630$) or IDT ($p = 0.252$), respectively.

4 Discussion

Experimentally induced FA has been shown to elicit positive allergy test results (16, 20). In addition, application of AIT resulted in resolution of induced clinical signs and airway inflammation, strongly supporting the hypothesis that aeroallergens can trigger an allergic reaction causing clinical signs of FLAD (17, 26). To date, only few studies have been investigating the role of environmental allergens in cats with naturally occurring inflammatory airway disease.

In the present study HC showed more positive reactions per cat than cats with FLAD on SAT, questioning the diagnostic accuracy of allergy testing in cats with FLAD. In contrast to this finding, two other studies detected significantly more positive reactions in cats with naturally occurring FLAD compared to HC (9, 28). One of these studies that compared results from SAT and IDT in ten HC and ten cats with not further classified FLAD detected more positive reactions in both tests in affected cats compared to HC (28). Similarly, the second study found that 15 cats with EI and MI had more positive allergen reactions on SAT compared to nine HC (9). However, both studies included only small patient groups which might have influenced the statistical power. Another explanation for different findings in the present study could be the measurement methods used for SAT. In the present study, a monoclonal antibody methodology was used where antibodies may falsely bind irrelevant IgG antibodies (35). For higher specificity, the alpha chain of the high-affinity mast cell receptor for IgE (FcεR1α) is recommended as binding site for antibodies used for testing (18, 35). However, only Moriello and coworkers (28) used the FcεR1α-receptor measurement in their study. Buller and coworkers (9) performed SAT using polyclonal antibody measurement which, compared to the FcεR1α-receptor measurement, is more likely to cause false positive results (35). Another possible explanation could be a differing deworming status for cats in different studies. Referring to a study investigating pruritic cats, non-dewormed cats showed significantly more positive reactions for allergen-specific IgE than dewormed cats. The authors assumed that the presence of intestinal parasites could lead to production of IgE cross-reacting against environmental allergens (36). Deworming status was unknown for most cats in the present study as well as in the study performed by Moriello et al. (28), making it impossible to compare allergy test results regarding that point. In the study performed by Buller and coworkers on the other hand, all cats were regularly dewormed. As most cats with FLAD and HC in the present study were indoor only cats, it is unlikely that infestation with parasites could have explained the higher number of positive reactions on SAT in HC compared to cats with FLAD. Moreover, a functional heterogeneity due to different glycosylation of allergen-specific IgE could be the cause for positive reactions in healthy animals leading to the assumption that "pathogenic" and "non-pathogenic" IgE should be considered (37, 38). However, the results of the present study are consistent with findings in cats with feline atopic skin syndrome (FASS), in which no difference in allergen-specific IgE could be detected in HC compared to cats with FASS (37–40). These findings

TABLE 5A Total positive reactions in cats (and median positive reactions per cat) with different type of inflammation on IDT, reactions to groups of allergens.

IDT (35 allergens)	EI ($n = 8$)		MI ($n = 10$)		NI ($n = 6$)		p-value
	Total positive reactions	Median positve reactions (range)	Total positive reactions	Median positve reactions (range)	Total positive reactions	Median positve reactions (range)	
Grasses ($n = 9$)	17	2.00 (0–5)	13	1.00 (0–3)	15	2.50 (1–4)	p = 0.285
Weeds ($n = 7$)	15	2.00 (0–4)	13	1.00 (0–5)	9	1.50 (0–2)	p = 0.416
Trees ($n = 3$)	11	2.00 (0–3)	1	0.00 (0–1)	0	0.00 (0–0)	p = 0.007*
Fungal ($n = 4$)	9	1.00 (0–3)	4	0.00 (0–2)	2	0.00 (0–1)	p = 0.353
Mites ($n = 5$)	11	1.00 (0–3)	6	0.50 (0–2)	7	1.00 (0–3)	p = 0.229
Insects ($n = 7$)	14	1.50 (1–4)	5	0.50 (0–1)	10	2.00 (0–4)	p = 0.021**

**Post-hoc* Bonferroni correction: EI significantly more positive reactions than NI (p = 0.007) and MI (p = 0.007). No significant difference between NI and MI (p = 0.731). ***Post-hoc* Bonferroni correction: EI significantly more positive reactions than MI (p = 0.010). NI significantly more positive reactions than MI (p = 0.044). No significant difference between EI and NI (p = 0.731).

TABLE 5B Total positive reactions in cats (and median positive reactions per cat) with different type of inflammation on SAT, reactions to groups of allergens.

SAT (34 allergens)	EI ($n = 7$)		MI ($n = 9$)		NI ($n = 5$)		HC ($n = 24$)		p-value
	Total positive reactions	Median positve reactions (range)	Total positive reactions	Median positve reactions (range)	Total positive reactions	Median positve reactions (range)	Total positive reactions	Median positve reactions (range)	
Grasses ($n = 7$)	5	0.00 (0–2)	3	0.00 (0–1)	2	0.00 (0–3)	17	0.00 (0–6)	p = 0.733
Weeds ($n = 8$)	4	0.00 (0–3)	1	0.00 (0–1)	3	0.00 (0–2)	17	0.00 (0–6)	p = 0.838
Trees ($n = 9$)	9	0–00 (0–4)	7	0.00 (0–4)	5	0.00 (0–4)	24	1.00 (0–8)	p = 0.808
Fungal ($n = 4$)	4	0.00 (0–3)	3	0.00 (0–3)	0	0.00 (0–0)	21	0.00 (0–4)	p = 0.124
Mites ($n = 5$)	5	1.00 (0–2)	2	0.00 (0–1)	1	0.00 (0–1)	22	1.00 (0–3)	p = 0.114
Insects ($n = 1$)	1	0.00 (0–1)	0	0.00 (0–0)	0	0.00 (0–0)	1	0.00 (0–1)	n. a.

EI, eosinophilic inflammation; MI, mixed inflammation; NI, neutrophilic inflammation; HC, healthy cats; n.a., not applicable.

emphasize that, considering AIT as a potential treatment of FA, SAT should only be used as a guidance for allergen selection and results should always be evaluated in context with clinical signs and environmental factors, as previously recommended for treatment of FASS (33, 41, 42).

Due to ethical reasons, we could not perform IDT in HC, therefore it is not known whether more HC than cats with FLAD would have shown positive reactions on IDT as it was the case on SAT. In one study on cats with FASS it could be seen that more cats with FASS showed positive reactions on IDT than HC (43). This is in concordance with the study from Moriello et al. (28). This leads to the assumption that in our study more cats with FLAD than HC would have shown positive reactions on IDT. These findings could be explained with the high sensitivity of the test. Furthermore, IDT rather faces problems of subtle wheal formation, making it hard to read compared to dogs (44), meaning that rather some relevant allergen reactions might be missed than false positive reactions generated in HC.

Significantly more positive reactions per cat were seen on IDT compared to SAT in the present study. Previously, in a study evaluating allergy test results in cats with induced FA sensitized with bermuda grass allergen or HDMA, IDT showed higher sensitivity compared to SAT (18). In the same study, good agreement was found between allergens tested in both tests; however, because of the experimental study design allergens were known in that investigation limiting the ability to compare the results with patients suffering from naturally acquired disease.

When positive results of allergens evaluated in both tests were compared with cohen's kappa, only *Alternaria alternata* and English Plantain showed "moderate agreement." This is consistent with results from studies on FASS (37, 45). In one study eight cats diagnosed with FASS had positive IDT results but tested negative on SAT (45). In another study comparing allergy test results for HDMA in cats with FASS, only weak correlation was found between results in both tests (37). One reason for the discrepancies between both tests could be different detection methods for allergen-specific IgE. While free circulating IgE is detected in serological assays, IDT reveals IgE bound to the FcεR1α-receptor on dermal mast cells (40). In contrast to IgE circulating in blood, dermal mast cell-bound IgE shows a significantly longer half-life, resulting in positive reactions lasting longer on IDT compared to a shorter period of detection in SAT (46). Furthermore, it can be assumed that irregular

allergen exposure could also lead to the absence of circulating IgE. This might explain a higher sensitivity of IDT compared to SAT, therefore potentially "missing" some relevant allergens when using SAT.

Ten cats with FLAD in the present study showed reactions to SM on IDT, but no association with dry food diet could be found in these patients. A previous study also investigated IDT results in cats with naturally occurring FLAD in the context with environmental factors, using information gained by an owner questionnaire. Three of the six cats that showed reactions to SM reached remission of clinical signs when dry food was changed to moist food diet (47). This was also mentioned in an abstract reporting that removal of dry food led to clinical remission in 2/5 cats with naturally occurring FLAD and positive reactions to SM (48). However, in dry dog food, contamination with SM is usually very low or undetectable and therefore with the correct storage conditions quite unlikely (49). It can be assumed that these findings account for dry cat food as well, although no specific studies on SM contamination of dry cat food exist so far. In the present study, however, cats that showed reactions to SM were not changed to moist food to assess a potential clinical improvement related to a dietary change.

No difference could be detected when indoor cats and cats with outdoor access were compared in the present study regarding positive reactions per cat on environmental allergens in both tests. This finding stands in contrast to results from a study on pruritic cats, suggesting that outdoor cats are consistently challenged by environmental allergens, endo-, and ectoparasites and therefore tend to have more positive reactions on SAT. In that study, outdoor cats showed more positive reactions to food and environmental allergens compared to indoor cats (36).

Seasonality has scarcely been assessed in cats with naturally occurring FLAD. One study mentioned more intense signs and episodes of respiratory distress in five cats during summer, in two during autumn and in two during winter. The remaining 16 cats did not show any seasonal changes (12). In the present investigation, only five cats revealed stronger clinical signs during spring and summer. It can be assumed that seasonality is not common in cats with FLAD.

On SAT the most common positive reactions in cats with FLAD were seen for American house dust mite (*Dermatophagoides farinae*). This is not surprising, as HDMA has been shown to be one of the most prevalent allergens cats are sensitized to in several studies on FASS (37, 45). In addition, cats with FA were commonly sensitized to this allergen on SAT in previous studies (9, 29). One study compared SAT results in cats with FA to those in HC and interestingly, reactions to HDMA were only found in cats with FLAD (9). In contrast to that, in the present study house dust mite was shown to be one of the most common reactions on SAT in HC as well as in affected cats. However, in the previous study only nine cats served as a control group and just one of those showed positive reactions on SAT, which potentially underestimates the most common allergen reactions in HC (9).

While in the present study house dust mite was not one of the most common allergen reactions on IDT in cats with FLAD, in two other studies using IDT for allergen testing, reactions to American house dust mite in 8/15 cats showing positive reactions (47) and 4/9 cats showing positive reactions to this allergen (48), represented the most common positive skin reactions in cats with FLAD (47, 48).

To date, no studies have been investigating allergy test results in the context of different types of airway inflammation. In the present study, for most allergen groups no significant differences could be detected between median positive reactions per cat comparing EI, MI, NI and HC. However, cats with EI showed significantly more positive allergen reactions compared to those with MI for tree pollen and insects, and cats with NI had significantly more positive reactions to insects compared to cats with MI. These findings stand in contrast to the assumption that EI is a consequence of an allergic reaction. Similar to human asthma, FLAD might also have a non-allergic non-IgE-mediated etiology in cats, therefore overestimating the role of allergens in cats with airway disease and potentially explaining the overall low rate of positive reactions in SAT in cats with FLAD in the present study.

In general, categorization into different inflammatory types based on BALF cytology alone remains questionable, since definitions and cytology cut-off values for the different groups vary in many studies (6, 9, 10, 12). Therefore, it would have had an impact on the results of this study, if a different classification scheme had been chosen, emphasizing the problem of non-existing standardized cutoff-values. In addition to that, it has been shown that BALF samples from different lung segments of cats revealed different predominance of inflammatory cells in BALF cytology (30), raising the question whether FA and CB represent different conditions with different etiologies at all. It seems not unlikely that NI can develop as a consequence of an allergic reaction to environmental allergens as well since in one study NI could be induced experimentally by allergen exposure in research cats (2). Likewise, horses with severe equine asthma commonly express a neutrophilic inflammatory response associated with exposure to environmental allergens (50, 51). In humans, allergen-induced severe asthma is commonly associated with a neutrophilic inflammatory pattern as well (52).

CB in cats as a non-septic inflammatory condition of the lower airways with involvement of non-degenerated neutrophils is thought to arise secondarily to insults of the airways induced by multiple possible irritants, one of them being cigarette smoke in humans (1, 53). It could be shown that cigarette smoke can generate various lesions in the epithelial cells of human airways (54). In the present study, significantly more cats with FLAD lived in a smoker-household compared to HC. Nonetheless, cats with NI were not overrepresented in the group of cats from smoker households. However, to investigate the potential role of smoke exposure in the pathogenesis of FLAD, a larger sample size of cats with FLAD and HC would be needed to evaluate this factor.

There are several limitations of this study. One of them was the evaluation of the cats' environment dependent on their owners' subjectivity in the questionnaire. In addition, the impact of seasonality on clinical signs was also assessed by the cat owners only. Allergens tested in this study might not have reflected all relevant allergens present in the cats' environment, but to date, data on relevant allergens inducing sensitization in cats are limited (55). Therefore, some relevant allergens including food allergens might not have been tested in the study population. To evaluate potential differences in allergen profiles between the three types of

airway inflammation, studies with distinctively larger groups are warranted. Since most cats included in this study showed perennial signs and were therefore more likely sensitized against perennial allergens such as HDMA, it would be interesting to collect dust samples in the cats' environment for investigation of mite exposure, since HDMA seems to be a relevant allergen in cats with FLAD (56).

5 Conclusions and relevance

Cats with FLAD do not show more positive reactions to environmental allergens on SAT than HC. In addition, correlation for most allergens is weak between SAT and IDT. Therefore, positive reactions to environmental allergens primarily demonstrate exposure to allergens but need to be interpreted with some caution in patients with FLAD. For selection of allergens for AIT, interpretation of results should always be performed in the context of clinical signs and allergen exposure for the individual cat.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by the Ethics Committee of the Centre for Clinical Veterinary Medicine of the Ludwig Maximilian University of Munich, Germany (No. 239-16-11-2020). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BH: Writing—original draft. RM: Conceptualization, Data curation, Project administration, Supervision, Writing—review & editing. JG: Data curation, Writing—review & editing. TW: Conceptualization, Writing—review & editing. TB: Conceptualization, Writing—review & editing. JP: Conceptualization, Methodology, Writing—review & editing. BS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing—review & editing.

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Conflict of interest

JP is employed by the Vet Med Labor GmbH Division of IDEXX Laboratories, Kornwestheim, Germany.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1267496/full#supplementary-material

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IV. DISCUSSION

It has been discussed that FLAD may be caused by an allergic reaction to environmental allergens (Trzil and Reinero, 2014). For that reason, it has been recommended to avoid relevant allergens and perform AIT. Allergy tests need to be carried out to identify clinically relevant allergens (Rodriguez Del Rio et al., 2022). To date, allergy tests in cats are commonly recommended as part of the diagnostic workup of FASS (Bajwa, 2018; Diesel and DeBoer, 2011). In cats with naturally occurring FLAD, however, allergy tests embody a new field, and so far, few studies have been conducted in cats with this disease. The present study aimed to investigate to which extent the results of two different allergy tests (SAT and IDT) agree in cats with FLAD, to compare SAT results from cats with FLAD with those from HC, and to assess possible correlations with environmental factors.

Most cats included in this study were directly presented to the Small Animal Clinic of the Ludwig Maximilian University of Munich, Germany, by their owners who had recognized respiratory problems in their pets over a certain period. Some patients had been referred to the clinic by their general practitioner for further investigation. Four cats initially suspicious of having FLAD were excluded from participation in the study due to physiological BALF cytology. Finally, 24 cats with FLAD and 24 HC could be included in the investigation. For greater statistical power a larger number of cats would have been needed. Large patient numbers are difficult to achieve, as many cats with FLAD are often directly treated by general practitioner, since BAL workup to establish a definitive diagnosis requires anesthesia and is more expensive than diagnostic treatment. In comparison to previous studies in which allergy tests in cats with naturally occurring FLAD had been performed (Buller et al., 2020; Moriello et al., 2007), the present study involves the largest study population. Additionally, this is the first study including and differentiating three different types of airway inflammation in cats with FLAD for investigation of allergen reactions.

The present study could show that cats with FLAD did not generate more positive allergen reactions compared to HC on SAT. Additionally, only “slight agreement” was found between most allergens tested in both allergy tests. Significantly more

positive reactions were found on IDT compared to SAT. For most allergen groups, no significant differences could be found between median positive reactions per cat comparing MI, EI, NI and HC.

Unlike in two other studies on naturally occurring FLAD, HC did not have less positive reactions on SAT than cats with FLAD in the present study (Buller et al., 2020; Moriello et al., 2007). Those previous investigations compared either results of SAT in HC with those from cats with FLAD (Buller et al., 2020), or results of SAT and IDT in cats with FLAD with those from HC (Moriello et al., 2007) and revealed significantly more positive reactions in cats with FLAD. One explanation for the discrepancies between studies might be different measurement methods in SAT. Moriello and coworkers used the FcεR1α-receptor for IgE-measurement, which has been recommended, as it avoids false positive results due to highly specific binding (Wassom and Grieve, 1998). Therefore, it could be argued that in the present study false positive results might have been generated due to the use of the monoclonal antibody measurement, potentially leading to false positive results, if irrelevant IgG antibodies bind (Wassom and Grieve, 1998). In the study by Buller and coworkers however, polyclonal antibodies were used as well, posing the very same problem as the monoclonal antibodies used in the present study (Buller et al., 2020). Another reason for false positive results may be CCDs, which lead to cross-reactivity, but do not have any clinical relevance (Gedon et al., 2019). For that reason, in the SAT used in the present study CCD blockers were applied. This was also the case in the study by Moriello and coworkers, but not in the other previous study (Buller et al., 2020; Moriello et al., 2007). It must be noted that both studies contained only small patient groups possibly influencing the statistical power, which makes it hard to compare the results. Generally, the findings of the present study are consistent with results from studies on cats with FASS and horses with EA. In those studies, no significant difference between the number of positive reactions could be found between healthy animals and diseased ones (Diesel and DeBoer, 2011; Tahon et al., 2009). Another potential cause for high numbers of positive reactions in healthy animals could be a different functional glycosylation of allergen-specific IgE (Gilbert and Halliwell, 1998; Halliwell et al., 1998). In terms of a functional heterogeneity of IgE, they present different abilities to mediate histamine release, making IgE either “pathogenic” or “non-pathogenic” (Halliwell and DeBoer,

2001). It can be assumed that HC in the present study might have been sensitized to certain allergens but are not presenting clinical signs. Therefore, a distinction between “atopy” and “allergy” is necessary. While atopy simply implies a predisposition to develop high levels of IgE against certain allergens, the term “allergy” is only applied when clinical signs are present (Ali, 2011). This emphasizes the statement that allergy test results should always be interpreted in the context of the clinical findings (Schoos et al., 2015). In humans, sensitization to environmental allergens in healthy subjects is encountered as well (Supakthanasiri et al., 2014). In contrast to allergy test results in cats in the present study, humans with different types of diseases display more positive reactions on SAT compared to healthy ones (Crespo-Lessmann et al., 2020).

In the present study, IDT and SAT only showed “moderate agreement” for two allergens and “slight agreement” for the others tested in both tests. Such lack of agreement between those two test systems has previously been described in allergy tests performed in cats with FASS and horses with EA (FOSTER and O'DAIR, 1993; Lorch et al., 2001). It is assumed that circulating IgE might not play a relevant role in EA, as concentrations in the horse’s blood might be significantly lower than those of tissue-bound IgE, leading to a low sensitivity of SAT (Lorch et al., 2001). In cats with FASS, discrepancies between results from IDT and SAT and a lower sensitivity of SAT might result from a shorter half-life of the free circulating IgE detected in serum (Shade et al., 2019). IgE bound to the FcεR1α-receptor in the tissue lasts markedly longer, leading to more positive reactions on IDT (Taglinger et al., 2005). In atopic humans, the two tests present high concordance when compared to one another (Milavec-Puretić et al., 2004). A potential explanation is that allergy tests for humans are generally more standardized and therefore lack discrepancies. However, it has been shown that results in children do not show strong agreement between the skin prick test and SAT, which might be due to different immune reactions in skin and blood. It is assumed that skin prick-negative and SAT-positive children have an IL-10 cytokine involvement, possibly leading to less mast cell-bound IgE in the tissues (Schoos et al., 2015).

From studies in cats with FASS it is known that IDT lacks standardization in allergen concentration, assuming that concentrations used for testing might frequently be too low and therefore could lead to false negative results (Austel et

al., 2006; Scholz et al., 2017). IDT in horses face the same difficulties as neither allergens nor concentrations used are standardized (Tahon et al., 2009). In the present study, allergen extracts used for IDT contained the same allergen concentrations applied for dogs, which might not be ideal (Scholz et al., 2017). Another potential reason for subtle wheal formation could be elevated cortisol concentrations in the blood of the cats (Hudec and Griffin, 2020). These might be triggered due to shared waiting rooms (for example with dogs) and inadequate stressful handling during clinical examination. In the Small Animal Clinic of Munich however, cats have an extra waiting room and are handled appropriately, reducing stress for the cat as much as possible. For IDT in the cat, it has been recommended to additionally inject fluorescein intravenously–which can facilitate the interpretation of wheal formation–to resolve the problem of interpreting subtle wheal formations (Schleifer and Willemse, 2003). In this study IDT was carried out without the use of fluorescein which could have led to more "false negative" results. Nevertheless, IDT was performed by a board-certified dermatologist and well instructed dermatological residents who were trained to interpret results without fluorescein. According to some authors, intravenous application of fluorescein poses a greater risk for anaphylaxis than benefit for result interpretation (Pérez-Rodríguez et al., 2005). In conjunction with the fact that significantly more positive reactions were found on IDT than on SAT, it is therefore rather unlikely that a relevant number of positive results had been overlooked on IDT. It must be noted that, to the author's knowledge, fluorescein wasn`t applied for IDT interpretation in all other studies in cats with naturally occurring FLAD either (Buller et al., 2020; Moriello et al., 2007; Prost, 2008). In cats and horses, IDT is considered the "gold standard" allergy test, experiencing higher sensitivity than SAT (Bajwa, 2021; Lorch et al., 2001; Wassom and Grieve, 1998).

On IDT, significantly more reactions to tree pollen were found in cats with EI compared to cats with NI and MI. On the one hand, this would reinforce the assumption that EI is the consequence of an allergic reaction to environmental allergens while NI results from a different etiology (Reinero et al., 2009). On the other hand, no significant differences on median positive reactions per cat for most of the allergen groups have been found between different inflammatory types. Given the fact that all cats in the present study did show clinical signs

throughout the year, and only five cats presented stronger season associated signs in either spring or summer, it is most unlikely that seasonal allergens play a greater role in cats with FLAD. Therefore, it can be doubted that the results on tree pollen on IDT play a relevant role. Additionally, for most allergen groups, no significant differences could be found between median positive reactions per cat comparing NI, EI, MI, and HC. To date, no clear BALF cytology cut-off values and definitions exist for differentiation of the different BAL-inflammatory types, as they vary in earlier studies on cats with FLAD (Buller et al., 2020; Johnson and Vernau, 2011; Lee et al., 2020). Even samples taken from different lung segments of the same cat may generate different results for inflammatory cell pattern (Ybarra et al., 2012). It remains questionable if EI and NI can solely be defined as two marked disease entities with distinct etiologies, or if they embody different parts of a heterogeneous disease complex. Horses with severe EA present a predominantly NI, which is thought to be caused by an allergic reaction to environmental allergens (Fairbairn et al., 1993). Still, in EA a lot of controversy exists on the question whether it represents an IgE-mediated disease (Tilley et al., 2012) or rather a late phase allergic reaction (Klier et al., 2021). Some researchers propose that horses with EA may represent a good animal model for human asthma in the context of non-allergic, late-onset and severe asthma phenotypes (Bullone and Lavoie, 2015). Human asthma research focuses more and more on the investigation of endotypes, meaning that different immunologic processes lead to distinct disease phenotypes (Gans and Gavrilova, 2020). In horses it has been suggested that EA may also present a heterogeneous disease with different endotypes, which would explain the diverse findings on cytokine profiles and allergy test results (Simões et al., 2022). Like in human asthma, in cats with FLAD it can be assumed that some patients may be allergic while others may not, proposing that different endotypes might exist in the cat as well and create a heterogeneous disease complex. Therefore, it can be recommended to carry out allergy tests in cats with FLAD regardless of the type of inflammation. Most importantly, it should be evaluated, if atopy could play a role in the individual patient, but test results should always be interpreted in the context of the cat's clinical signs.

In the present study, the most common allergic reaction found on SAT was against American house dust mite (*Dermatophagoides farinae*) in cats with FLAD

and HC. This result is not surprising, as house dust mites in general represent a common allergen causing reactions also in cats with FASS, horses with EA and humans with asthma (Ankermann and Brehler, 2023; Foster et al., 2003; Lo Feudo et al., 2021; Tahon et al., 2009). In another study testing cats with FLAD, the most common allergic reaction was also seen for HDMA (Buller et al., 2020). Conversely to the present study, no HC showed reactions to mites in that study (Buller et al., 2020). This might be explained by the fact that in the previous study only nine cats served as a control group out of which only one cat showed positive reactions on SAT (Buller et al., 2020). In human medicine it has been stated that HDMA embodies the most relevant perennial allergen worldwide (Ankermann and Brehler, 2023). It has been shown that AIT for HDMA led to significant improvement in humans suffering from allergic asthma (Mosbech et al., 2014; Wang et al., 2006). In the present study most of the participating cats lived indoors. This could lead to the assumption that if HDMA is an important indoor allergen, regular exposure might have an impact on sensitization of a cat with the consequence of possible allergy. Even for outdoor cats, this HDMA may be relevant, as many of them spent a meaningful time in the house as well. One study investigated 50 cat-only households and collected dust samples from the cat's resting areas and found high levels of HDMA, emphasizing the possible importance of this allergen in atopic feline conditions such as FASS and FA (Loft and Rosser, 2010). On the contrary, one might argue that HDMA is a less important allergen in atopic cats, as it causes IgE-formation in healthy subjects as well (Taglinger et al., 2005). A study in horses with RAO that compared three allergen tests in diseased horses and healthy controls found no significant difference for HDMA reactions and consequently doubted a clinical relevance of these allergens (Tahon et al., 2009). A study in naturally diseased atopic cats, clinically HC and purpose bred laboratory cats showed that those housed in a laboratory, which possibly never had had any contact to HDMA before, showed undetectable or very low levels of IgE antibodies (Halliwell et al., 1998). In contrast to that, positive reactions to HDMA were commonly found in clinically HC in this study (Halliwell et al., 1998). In humans a study found a protease activity of HDMA cleaving the low-affinity IgE receptor (CD23) from the surface of B lymphocytes, thus possibly promoting an IgE immune response by eroding an inhibitory feedback mechanism that normally restricts any IgE response (Hewitt et al., 1995). Another attempt to explain the common reactions against

HDMA in HC may be the already mentioned possible functional heterogeneity of IgE (Halliwell et al., 1998). Overall, it remains difficult to clearly determine whether HDMA are clinically relevant in the context of FLAD and therefore the role of this allergen requires further investigations.

In horses the stable environment seems to play a major role in the pathogenesis of EA (Ivester et al., 2014). Common irritants found in the stables such as different mold species, bacterial endotoxins, and gases such as ammonia embody potential triggers for disease development (Pirie, 2014). Stable management always needs to be the first attempt in the therapy of EA, as there is evidence that clinical signs already improve by reducing exposure to airborne dust in the stables (Couëtil et al., 2016). In humans with respiratory issues, indoor air pollution has been acknowledged as a global health thread (Mortimer et al., 2012). A study investigating a possible linkage between indoor air pollution and respiratory disorders in cats showed that cats with respiratory diseases were exposed to significantly higher levels of indoor particulate matters compared to HC (Lin et al., 2018). In contrast to these findings, in the present study more HC lived indoors (83%) than cats with FLAD (63%).

Significantly more cats with FLAD lived in a household with smoke exposure compared to HC. Environmental tobacco smoke is regarded as a major risk factor for asthma development in humans (Toppila-Salmi et al., 2020). Cigarette smoke can lead to mucus hypersecretion, pulmonary connective tissue damage and mucus accumulation, leading to chronic airflow obstruction (Dye and Adler, 1994). In an experimental study with mice, smoke exposure lead to various lesions in the respiratory epithelium with the consequence of increased permeability for larger molecules (Dye and Adler, 1994). This is one assumption for the pathogenesis of CB which is thought to represent a sterile NI of the lower airways (Nafe et al., 2010). While it is comprehensible that cigarette smoke might facilitate or even cause CB, cats with NI in the present study were not overrepresented in the group of cats with FLAD from smoker households. To investigate this possible connection to disease development, a larger sample size would be essential. Nevertheless, as environmental tobacco smoke poses a potential trigger in cats with FLAD, it is generally recommended to avoid exposure in terms of disease management (Moses and Spaulding, 1985).

The present study faced some limitations. The study populations of cats with

FLAD and HC were rather small, possibly influencing the statistical power. Especially for further investigation of potential differences in reactions to allergens between the three types of airway inflammation, larger groups are warranted. In addition, the evaluation of environmental factors was based on the subjectivity of the cat's owner. Thus, data on seasonal clinical signs were also gained from the owner's questionnaire. To date, data on relevant allergens inducing sensitization in cats are limited (Mueller et al., 2016); therefore, it might be possible that not all allergens present in the cat's environment were tested. Additionally, allergen concentrations used for IDT may not be ideal for cats, as they were the same used for dogs before (Scholz et al., 2017). To date, only few data exist regarding IDT in cats and further studies are needed to determine the ideal allergen concentrations.

Nevertheless, this was the first study to compare two different allergy tests (SAT and IDT) in the context of environmental factors. In contrast to other studies investigating allergy test results in cats with FLAD, the present study contained a distinctively larger study population (Buller et al., 2020; Moriello et al., 2007; Prost, 2008). To the author's knowledge this is the first study that examined the different lower airway inflammatory types in the context of allergy test results.

V. SUMMARY

The present study investigated allergy test results in cats with FLAD in the context of the cats' environment. Eight cats with EI, ten with MI and six with NI met all the inclusion criteria and were tested with IDT and SAT, in addition, serum of 24 HC was evaluated using SAT as a control group.

When results of SAT were compared between cats with FLAD and HC, it could be demonstrated that more HC 20/24 (83%) showed at least one positive allergen reaction compared to 11/21 (52%) ($p = 0.051$) cats with FLAD. Furthermore, in patients with FLAD significantly more positive reactions per cat were found on IDT when compared to SAT ($p = 0.001$). While "moderate agreement" was seen for two allergens, eight allergens only showed "slight agreement" between both tests. Overall, no significant difference could be found for median positive reactions per cat on SAT when the three different groups of inflammatory types and HC were compared ($p = 0.377$). American house dust mite was the most common allergen reaction on SAT in HC ($n = 12$) and in cats with FLAD ($n = 7$). On IDT no difference was found in reactions to storage mites between cats eating mainly dry food and those eating moist food ($p = 0.629$). Compared to HC 4/24 (17%), significantly more cats with FLAD 12/24 (50%) were exposed to smoke in their environment.

In this study it could be shown that sensitization to environmental allergens is common in HC as well as in cats with FLAD. Therefore, positive reactions in allergy tests should always be interpreted with caution and in the context of the cat's environment, seasonality and clinical signs. IDT and SAT show only low agreement of allergy test results, therefore, tests cannot be used interchangeably. More studies are needed to investigate the role of environmental allergens in the pathogenicity of FLAD.

VI. ZUSAMMENFASSUNG

In der vorliegenden Studie wurden die Ergebnisse von Allergietests bei Katzen mit felinen entzündlichen Bronchialerkrankungen (FLAD) im Zusammenhang mit deren Umgebung untersucht. Acht Katzen mit eosinophiler Entzündung EI, zehn mit gemischter Entzündung (MI) und sechs mit neutrophiler Entzündung (NI) erfüllten alle Einschlusskriterien und wurden mit Intradermaltest (IDT) und Serum-IgE-Test (SAT) getestet. Zusätzlich wurde das Serum von 24 gesunden Katzen (HC) mittels SAT als Kontrollgruppe ausgewertet.

Beim Vergleich der SAT-Ergebnisse von Katzen mit FLAD und HC konnte gezeigt werden, dass mehr HC 20/24 (83%) mindestens eine positive Allergenreaktion zeigten, verglichen mit 11/21 (52%) ($p = 0,051$) Katzen mit FLAD. Darüber hinaus wurden bei Patienten mit FLAD signifikant mehr positive Reaktionen pro Katze im IDT im Vergleich zum SAT gefunden ($p = 0,001$). Während nur bei zwei Allergenen eine „mäßige Übereinstimmung“ festgestellt wurde, zeigten acht Allergene eine „geringe Übereinstimmung“ zwischen den beiden Tests. Insgesamt konnte kein signifikanter Unterschied bei den medianen positiven Reaktionen pro Katze im SAT zwischen den drei verschiedenen Entzündungsarten und HC festgestellt werden ($p = 0,377$). Die amerikanische Hausstaubmilbe war die häufigste Allergenreaktion im SAT bei HC ($n = 12$) und bei Katzen mit FLAD ($n = 7$). Beim IDT wurde kein Unterschied zwischen den Reaktionen auf Vorratsmilben bei Katzen, die hauptsächlich Trockenfutter fressen, und solchen, die Feuchtfutter bekommen, festgestellt ($p = 0,629$). Im Vergleich zu HC 4/24 (17%) waren signifikant mehr Katzen mit FLAD 12/24 (50%) in ihrer Umgebung Rauch ausgesetzt.

In dieser Studie wurde gezeigt, dass eine Sensibilisierung auf Umweltallergene sowohl bei gesunden als auch bei Katzen mit FLAD üblich ist. Daher sollten positive Reaktionen in Allergietests mit Vorsicht, im Zusammenhang mit der Umgebung der Katze, dem saisonalen Auftreten und den klinischen Symptomen interpretiert werden. IDT und SAT zeigen nur eine geringe Übereinstimmung der Allergietestergebnisse, daher können die Tests nicht austauschbar verwendet werden. Es sind weitere Studien erforderlich, um die Rolle von Umweltallergenen in Bezug auf die Pathogenese von FLAD zu untersuchen.

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VIII. SUPPLEMENTARY MATERIAL

Questionnaire on cats with inflammatory bronchial diseases

Label

Date:_____

How long has your cat been showing respiratory problems?

◯ _____Weeks ◯ _____Months ◯ _____Years

How was your cat living at the time the respiratory problems started?

◯ Outdoor cat ◯ Indoor cat ◯ Balcony/terrace only

What kind of environment were you living in at that time?

◯ Big City ◯ Small Town ◯ Village

Are the symptoms: ◯ Seasonal -> ◯ Spring ◯ Summer ◯ Autumn ◯ Winter

◯ First seasonal, now non-seasonal ◯ non-seasonal (all year round)

Where does your cat come from: ◯ Farm ◯Animal welfare/animal shelter ◯ Private offspring

◯ Cat breeder

Is your cat exposed to tobacco smoke in your home? ◯ Yes ◯ No ◯ Occasionally

Do you or other family members smoke outside the house/apartment? ◯ Yes ◯ No

◯ Occasionally

What diet has your cat been fed until the onset of symptoms?

◯ Commercial dry food

◯ Commercial moist food

◯ Moist and dry food

◯ Home-cooked

◯ BARF

◯ Other

Is there an open fireplace/stove in the house? ◯ Yes ◯ No

What cat litter was used at the time of symptom onset?

_____

At the time of the first symptoms, did you have other animals in your household or did your cat have regular contact with other animals? ◯ No

◯ Yes, namely: ◯ Dog

◯ Cat

◯ Small mammals (rabbits, etc.)

◯ Horse

◯ Bird

◯ Other ___

How would you rate the intensity of dust exposure in your environment?

(Assessment in regard of construction sites, particulate matter, factories, etc.)

◯ None
◯ Low
◯ Moderate

◯ High

What kind of floors do you mainly have in your apartment/house?

◯ Carpet ◯ Parquet

◯ Linoleum ◯ Tiles

Do you use fragrance sprays in your home? ◯ No ◯ Yes, namely:_____

Do you have a humidifier? ◯ Yes ◯ No

What types of trees are common in your area? ◯ Birch ◯ Beech ◯ Oak
◯ Poplar ◯ Larch ◯ Maple
◯ Lime ◯ Alder ◯ Willow
◯ Walnut (hazel) ◯ others,
namely_____

Have you had mold infestation in your home? ◯ Yes ◯ no

Do you have cat grass in your home? ◯ Yes ◯ No

Do you live in an old apartment/house? ◯ Yes ◯ No

Do you have goose feathers/down at home? (bed linen, winter down jacket)?

◯ Yes ◯ No

Do you have something made of sheep's wool at home? ◯ Yes ◯ No

Does your pet have skin problems? ◯ No ◯ Yes, since when? ____
(If you have ticked "Yes", please fill in the following pages, if you have ticked "No" the questionnaire ends here)

_____ _____
Location, Date Owner's signature

Did skin problems appear first or respiratory problems first?

◯ Skin problems first

◯ Respiratory problems first

Does your pet also react with gastrointestinal signs to certain diets?

◯ Diarrhea

◯ Vomiting

◯ Not applicable

Have you ever consulted a veterinarian for the skin? ◯ Yes ◯ No

If so, when? _____

What diagnosis has been made in relation to the skin: _____

Does you cat have any other diseases? ◯ No ◯ Yes, namely:_____

What kind of skin problems does your cat show?

◯ Itching ◯ Redness of the skin ◯ Dandruff ◯ Crusts

◯ Nodules ◯ Hair loss ◯ Ocular discharge ◯ Blackening of the skin

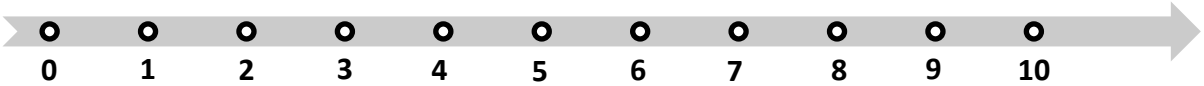
◯ Dull coat ◯ Oily skin

Which parts of the body are affected by skin problems (multiple answers possible)?

◯ Neck/Chest ◯ Back ◯ Head ◯ Ears

◯ Armpits ◯ Abdomen ◯ Groin ◯ Sides ◯ Tail (base) ◯ Paws

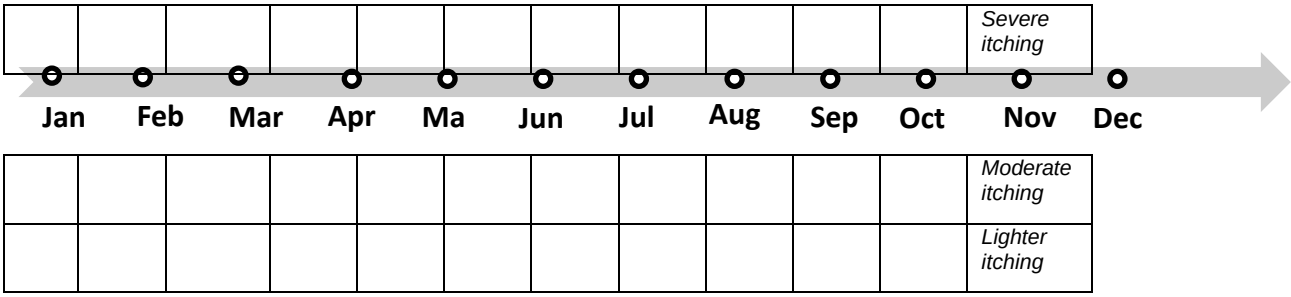
How severe is the itching?



No itching **Extreme itching**

In which months do the symptoms appear and how severe are they?

All year round ◯, or ◯ :



Does your pet have a known food allergy? ◯ No ◯ Yes, against_____

Does your pet need medication at the moment? ◯ No ◯ Yes, namely:_____

When was the last time your pet was given the following medications?

◯ Cortisone: _____

◯ Apoquel: ____

◯ Antihistamines: _____

◯ Ear medication (provide name): _____

Has an allergy test already been performed?◯ No ◯ Yes, namely:
◯ Skin test ◯ Serum allergy test

Results:_____
_____
_____
_____
_____

Has desensitization been performed on your pet? ◯ Yes ◯ No

_____ _____
Location, Date Owner's Signature

Questionnaire on healthy cats

Label

Date:_____

Is your cat showing respiratory problems?

◯ Yes ◯ No

How does your cat live?

◯ Outdoor cat ◯ Indoor cat ◯ Balcony/terrace only

What kind of environment?

◯ Big City ◯ Small Town ◯ Village

Where does your cat come from:

◯ Farm ◯ Animal welfare/animal shelter ◯ Private offspring ◯ Cat breeder

Is your cat exposed to tobacco smoke in your home?

◯ Yes ◯ No ◯ Occasionally

Do you or other family members smoke outside the house/apartment?

◯ Yes ◯ No ◯ Occasionally

What diet is fed?

◯ Commercial dry food

◯ Commercial moist food

◯ Moist and dry food

◯ Home-cooked

◯ BARF

◯ Other

Is there an open fireplace/stove in the house? ◯ Yes ◯ No

What cat litter is used? _____

Do you have other animals in the household or does your cat have regular contact with other animals? ◯ No

◯ Yes, namely:

◯ Dog

◯ Cat

◯ Small mammals (rabbits, etc.)

◯ Horse

◯ Bird

◯ Other ___

How would you rate the intensity of dust exposure in your environment?
(Assessment in regard of construction sites, particulate matter, factories, etc.)

◯ No

◯ Low

◯ Moderate

◯ High

What kind of floors do you mainly have in your apartment/house? ◯ Carpet ◯ Parquet

◯ Linoleum ◯ Tiles

Do you use fragrance sprays in your home? ◯ No ◯ Yes, namely:_____

Do you have a humidifier? ◯ Yes ◯ No

What types of trees are common in your area? ◯ Birch ◯ Beech ◯ Oak
◯ Poplar ◯ Larch ◯ Maple
◯ Lime ◯ Alder ◯ Willow
◯ Walnut (hazel) ◯ others, namely_____

Have you had mould infestation in your home? ◯ Yes ◯ No

Do you have cat grass in your home? ◯ Yes ◯ No

Do you live in an old apartment/house? ◯Yes ◯ No

Do you have goose feathers/down at home? (bed linen, winter down jacket)?

◯Yes ◯ No

Do you have something made of sheep's wool at home? ◯ Yes ◯ No

Does your pet show skin problems? ◯ No ◯ Yes

_____ _____

Location, Date Owner's Signature

Number of positive reactions per cat

EI (Cat No.)	Number of positive reactions (IDT)	Number of positive reactions (SAT)	MI (Cat No.)	Number of positive reactions (IDT)	Number of positive reactions (SAT)	NI (Cat No.)	Number of positive reactions (IDT)	Number of positive reactions (SAT)
1	11	5	9	9	0	19	10	0
2	9	7	10	5	1	20	7	0
3	14	n.a.	11	0	4	21	5	0
4	8	9	12	5	n.a.	22	4	n.a.
5	5	0	13	12	0	23	5	10
6	5	7	14	1	0	24	5	1
7	12	0	15	1	4			
8	6	0	16	5	0			
			17	7	1			
			18	0	6			

EI= Eosinophilic inflammation, NI= Neutrophilic inflammation, MI= Mixed inflammation

n.a.: not applicable (serum sample not available)

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