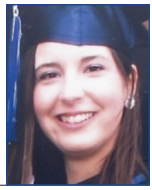
Cytological Characteristics of the Eye Conjunctiva in Dogs with Atopic Dermatitis



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Abstract

Atopic dermatitis is a chronic, pruritic inflammatory skin disease affecting approximately 10% of dogs worldwide. The disease typically manifests at an early age, causing pruritus and secondary skin lesions in specific anatomical regions. The pathophysiology of canine atopic dermatitis (CAD) remains incompletely understood, but recent research suggests a complex interplay between skin barrier dysfunction, allergic sensitisation, and microbial dysbiosis. Despite the well-established association between atopic dermatitis and conjunctivitis, the prevalence of conjunctivitis in dogs with CAD is often underestimated in clinical practice due to inadequate recognition of symptoms and a lack of specific diagnostic protocols. This study aimed to analyse the cytological characteristics of the conjunctiva in dogs affected by atopic dermatitis. Samples were collected from 24 dogs diagnosed with CAD and treated at the University Veterinary Hospital, Faculty of Veterinary Medicine, University of Zagreb. Cytological analysis was performed on samples obtained using a cytobrush. The results revealed the presence of inflammatory cells in all samples, with lymphocytes most prevalent (87.5%). A mild degree of conjunctival metaplasia was observed in all dogs, regardless of the type of inflammatory cells and the severity of conjunctivitis. These findings suggest that atopic dermatitis may induce changes in the conjunctiva that are not necessarily correlated with the intensity of the inflammatory response. The presence of lymphocytes, along with eosinophils in some cases, underscores the diagnostic value of conjunctival cytology in dogs with CAD. Furthermore, the increased density of goblet cells suggests similarities in the pathophysiology of ocular allergies in dogs and humans, opening avenues for further research to better understand and treat these conditions. This study highlights the importance of an interdisciplinary approach in the diagnosis and treatment of atopic dermatitis, including regular ophthalmologic examinations as part of standard clinical practice.

Key words: *atopic dermatitis; allergic conjunctivitis; cytological analysis; dogs*

Introduction

Until 2023, atopic dermatitis (AD) was defined as a chronic and pruritic inflammation of the skin, genetically predisposed, occurring as a result of the immune system's hypersensitivity to environmental substances. Atopic dermati-

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tis is a very common allergic dermatosis in dogs, affecting approximately 10% (3–15%) of the global canine population. The disease typically manifests in dogs between 6 months and 3 years of age, with pruritus and secondary skin lesions resulting from the inflammatory process. Pruritus and characteristic secondary skin lesions appear on the face (eyes and mouth), ears, ventral neck and abdomen, axillary and inguinal regions, interdigital spaces, perineal area, and limb joints. The pathophysiology of canine atopic dermatitis (CAD) is still not fully understood (Olivry et al., 2015).

Based on new research into the pathogenesis of canine atopic dermatitis, in 2023, the International Committee for Allergic Diseases in Animals (ICADA) published a new definition of CAD: "Canine atopic dermatitis is an inherited, typically pruritic, and predominantly T-cell inflammatory skin disease involving interactions between skin barrier dysfunction, allergen sensitisation, and microbial dysbiosis." This new definition is valuable as it encompasses a broader understanding of the disease's pathogenesis (Eisenschenk et al., 2024).

Conjunctivitis in dogs with atopic dermatitis has been described in the literature, but its prevalence is rarely observed in clinical practice. Although relevant reports and studies document this pathology, its true incidence appears to be underestimated. This may be due to inadequate recognition of clinical signs and a lack of specific diagnostic criteria within veterinary practice. The clinical presentation of conjunctivitis in allergic dogs can be ambiguous or masked by symptoms of other ophthalmic diseases, further complicating accurate diagnosis. Therefore, raising awareness among veterinarians about this possibility is crucial, as is the development and implementation of more precise diagnostic protocols that enable etiological diagnosis and optimal treatment. The clinical symptoms of allergic conjunctivitis in dogs include periocular erythema and pruritus, conjunctival hyperaemia, chemosis, and increased tear production and/or the presence of serous to mucous discharge (Almeida, 2012).

Allergic conjunctivitis typically involves a type I hypersensitivity reaction affecting the eyelids, conjunctiva, and/or cornea. Ocular allergies generally consist of three phases: the sensitization phase, early phase, and late phase. The sensitisation phase begins with exposure of the ocular surface to allergens and is asymptomatic. Dendritic cells in the conjunctiva phagocytise the allergens and differentiate into T-helper (Th) cells, predominantly Th2. Cytokines released by Th2 cells stimulate B cells to produce IgE antibodies, promote mast cell development, accumulate eosinophils, and lead to mucus hyperproduction. Mast cells and basophils possess high-affinity IgE receptors. Pruritus, conjunctival hyperaemia, and chemosis characterise the early phase and are triggered by inflammatory mediators, particularly histamine. Mast cells produce platelet-activating factors and cytokines that enhance vascular permeability, induce vasodilation, and promote chemotaxis and activation of neutrophils, eosinophils, and other inflammatory cells, leading to the late phase. The late phase is characterised by T lymphocyte activation through the production of Th2 cytokines and infiltration of the conjunctiva by eosinophils, neutrophils, and basophils (Varandas et al., 2020). The diagnosis of allergic conjunctivitis is based on a clinical ophthalmological examination conducted by a specialist who determines the type of conjunctivitis based on anamnesis and clinical signs. Exfoliative

cytology of the cornea and conjunctiva is an ancillary diagnostic method for anterior segment eye diseases. The most commonly used techniques for obtaining conjunctival samples in dogs include the use of ophthalmic spatulas, blades, cotton swabs or cytology brushes (*Cytobrush*). Sampling with a cytology brush has proven to be a reliable (providing adequate cellularity) and non-invasive method. The presence of even a single eosinophil indicates allergic conjunctivitis. Additionally, plasma cells and lymphocytes are common findings in this diagnosis (Hendrix, 2013). These cells are absent in conjunctival smears of healthy dogs (Athanasiou et al., 2018). The aim of this study was to analyse the cytological characteristics of the conjunctiva in dogs affected by atopic dermatitis.

Materials and methods

The subjects of this study were patients from the University Veterinary Hospital at the Faculty of Veterinary Medicine, University of Zagreb, diagnosed with atopic dermatitis. The study included 24 dogs treated at the request of their owners. For the purpose of this study, conjunctival swab samples from dogs were collected during a non-experimental clinical investigation for diagnostic purposes as part of the patients' clinical evaluation (Figure 1). All procedures were conducted in accordance with the ethical approval obtained from the Ethics Committee of the Faculty of Veterinary Medicine (Class: 640-01/21-02/03, Reg. No.: 251-61-01/139-21-37, February 26, 2021), granted at the 5th regular session of the Faculty Council in the academic year 2020/21, held on 24 February 2021.

The study included dogs diagnosed with atopic dermatitis, which had negative results from an elimination diet, were properly treated for endo- and ectoparasites, and had the consent of their owners to participate in the clinical study. Exclusion criteria were the administration of systemic anti-inflammatory drugs within the past month and/or local ophthalmological treatments within the past 7 days.

Each patient underwent a specialized dermatological and ophthalmological examination, followed by cytological analysis of the conjunctiva. The degree of conjunctivitis was determined for all dogs, and conjunctival swabs were taken using a cytology brush under local anaesthesia. After air drying, cytological samples were stained with May-Grünwald Giemsa for 2 minutes. After rinsing with distilled water, a filtered Giemsa solution diluted with distilled water at a 1:10 ratio was applied to the slides for 20 minutes. The slides were then rinsed with distilled water and air-dried.

For the analysis of cytological samples, a Nikon 2000 Eclipse light microscope with 4x, 10x, 20x, and 40x objectives was used. Microphotographs were taken using an Olympus DP20 camera and the Cell B software (*Olympus*). The slides from the left and right conjunctiva were examined separately by a veterinary pathologist, though grading was based on the combined findings from both conjunctivae. Inflammatory cells were counted across the entire slide. The presence of 1–3 plasma cells or lymphocytes in the slides was classified as grade I, 3–6 cells as grade II, and more than 6 cells as grade III. The presence of a single neutrophil, eosinophil, or mast cell was classified as grade I, 2–3 as grade II, and more than 3 as grade III. In each slide, five fields of view with the highest cellularity were selected, where goblet cells were counted, and the degree of squamous metaplasia was determined.

Table 1. Results of the cytological analysis of conjunctival swabs in 24 dogs diagnosed with atopic dermatitis

Patient Number	Goblet Cells	Eosinophils	Mast Cells	Plasma Cells	Neutrophils	Lymphocytes	Degree of Metaplasia
1	3	0	0	0	0	1	1
2	3	0	0	0	0	1	1
3	3	0	0	1	1	1	1
4	3	0	0	1	2	1	1
5	3	0	0	1	1	1	1
6	3	0	0	1	0	1	1
7	3	0	0	0	1	1	1
8	3	0	0	0	2	1	1
9	3	0	0	0	1	1	1
10	3	1	0	0	0	1	1
11	3	0	0	0	0	1	1
12	3	0	0	0	1	0	1
13	3	0	0	1	1	1	1
14	3	0	0	0	2	1	1
15	3	0	0	0	1	1	1
16	3	1	1	0	1	1	1
17	3	1	0	0	2	1	1
18	3	0	0	0	1	1	1
19	3	0	0	0	1	1	1
20	3	0	0	0	1	0	1
21	3	0	0	0	0	1	1
22	3	0	0	0	1	0	1
23	3	0	0	0	1	1	1
24	3	0	0	0	1	1	1

The degree of metaplasia (0–3) was determined based on cell shape (small, slightly enlarged, enlarged, large), cohesion (strong, some losses, disrupted, isolated cells), nuclear appearance (large and uniform, reduced, small and condensed, pyknotic/absent), cytoplasmic staining (dark, light, variable, pale), and the nucleus-to-cytoplasm ratio (1:2, 1:3, 1:4–1:5, 1:6 or smaller).

Results

The study population consisted of more males (15) than females (9). The average age (mean) was 5 years, with a median age of 4 years. The most repre-

Figure 1. Collection of the conjunctival swab using a *cytobrush*

sented breed was mixed breed (33.33%), while among purebred dogs, Parson Russell Terriers (3) and French Bulldogs (3) were the most common. Only one dog showed no clinical signs of allergic conjunctivitis; however, inflammatory cells were found in the cytological analysis of conjunctival swabs in all 24 dogs. The most common type of cell observed was lymphocytes, which were present in 21 samples (87.5%).

Discussion

Cytological analysis of conjunctival samples in dogs is employed for the diagnosis and treatment of ophthalmic diseases. This method enables the rapid, cost-effective, and accurate identification of inflammatory and pathological changes in the eyes, which is crucial for determining the underlying cause of symptoms and selecting the appropriate therapy. It is most commonly used when neoplasms are suspected (e.g., melanoma). Additionally, it serves as a valuable tool in the diagnosis of infectious diseases (bacteria, viruses, fungi, and parasites). In such cases, cytology is often used in conjunction with other diagnostic methods, such as bacterial culture or parasite testing. Unlike in horses, the cytology of healthy canine conjunctiva has not been extensively described or correlated with histology. Research indicates that conjunctival cytology in dogs reveals layers

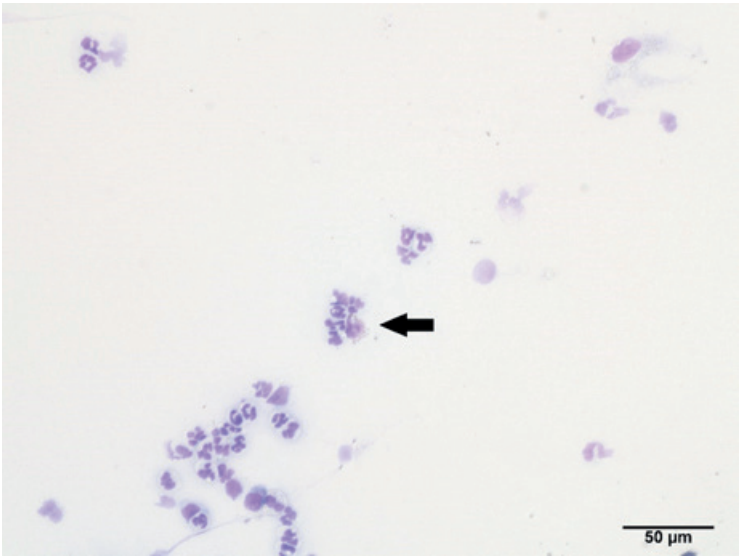

Figure 2. Eosinophil (indicated by arrow) in the cytological findings of the conjunctiva. magnification 40x.

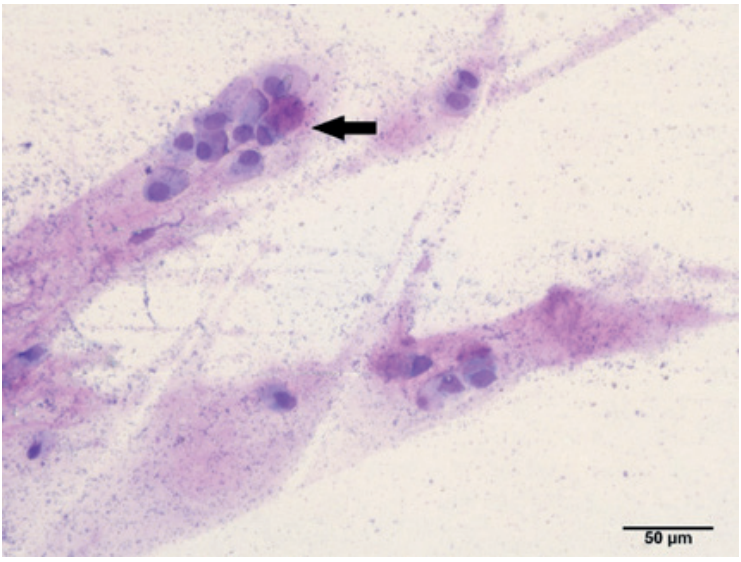

Figure 3. Density of goblet cells (indicated by arrow) in the cytological findings of the conjunctiva. Magnification 40x.

of epithelial cells, including basal, intermediate, and superficial cells. Superficial and intermediate cells often contain brown cytoplasmic melanin granules. In some samples, goblet cells and keratinised cells have been identified, while neutrophils, lymphocytes, and monocytes are present in smaller numbers. Due to the limited knowledge regarding the cytology of healthy canine conjunctiva, conjunctival cytology is not yet routinely performed in the diagnosis of ophthalmic diseases (Athanasiou et al., 2018; Trumel et al., 2020).

Moreover, in human medicine, despite a greater number of studies than in veterinary medicine, conjunctival cytology is not routinely used in the diagnosis

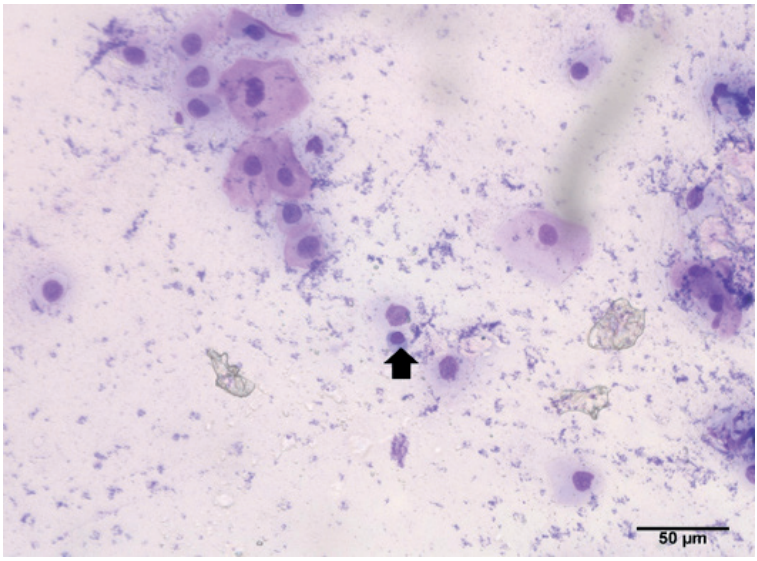

Figure 4. Plasma cells (indicated by arrow) in the cytological findings of the conjunctiva. Magnification 40x.

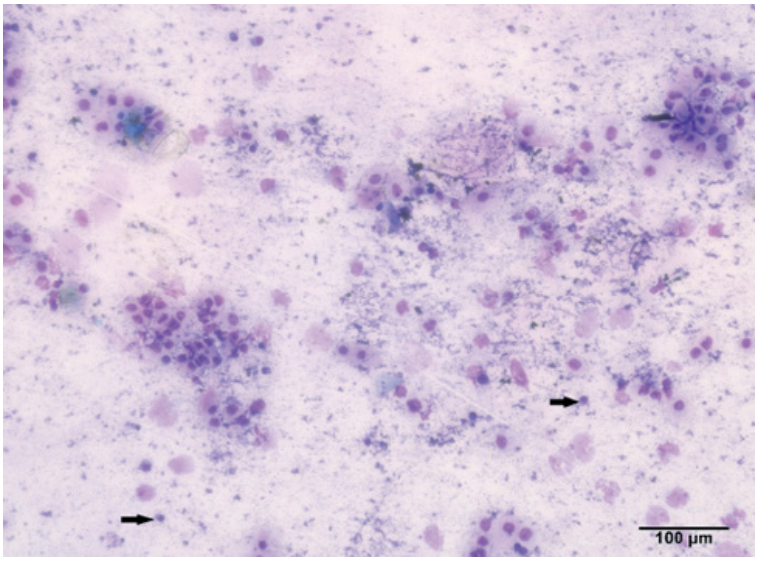

Figure 5. Lymphocytes (indicated by arrow) in the cytological findings of the conjunctiva. Magnification 40x.

of allergic conjunctivitis. Symptoms of allergic conjunctivitis often overlap with those of other ophthalmic conditions (e.g., dry eye syndrome), necessitating the introduction of new diagnostic procedures to ensure more accurate diagnoses and treatments. Cytological examination of conjunctival samples is certainly one of these procedures (Kari et al., 2010; Leonardi et al., 2015; Bielory et al., 2020).

Conjunctivitis without other ocular symptoms is most often of non-infectious aetiology, commonly caused by allergies, dryness, or mechanical irritation. Primary bacterial conjunctivitis is extremely rare in dogs. In cytological preparations for this diagnosis, numerous neutrophils

are visible alongside bacterial findings. Bacterial conjunctivitis is usually secondary to eyelid pathology, dry eye syndrome, or trauma (Furiani et al., 2011; Hendrix, 2013). Among non-infectious causes of conjunctivitis in dogs, allergic conjunctivitis often occurs as part of atopic dermatitis, although it may also present as an isolated manifestation (Lourenço-Martins et al., 2011; Almeida, 2012). The cited studies suggest that, in addition to dermatological examination, every dog diagnosed with atopic dermatitis should also undergo an ophthalmological examination. Our results corroborate this and confirm the importance of an interdisciplinary approach in the diagnosis and treatment of atopic dermatitis in dogs. Cytological preparations frequently reveal eosinophils and lymphocytes, which represent a specific cytological finding. The presence of even a single eosinophil is considered diagnostic (Hendrix, 2013). In the present study, lymphocytes were the most commonly observed inflammatory cells, consistent with the literature. Furiani et al. (2011) found a significant increase in the number of epithelial cells, particularly keratinised epithelial cells, likely due to squamous metaplasia in allergic dogs. In this study, all cytological samples from dogs with atopic dermatitis exhibited a mild degree of metaplasia, regardless of the presence or type of inflammatory cells. These results suggest that atopic dermatitis in dogs may induce changes in conjunctival cells that are not necessarily related to the intensity or type of inflammatory response.

Follicular conjunctivitis occurs as a result of chronic antigenic stimulation without evidence of infectious disease. Conjunctivitis may also be associated with inadequate tear production, and there are variations in the literature regarding the cytological characteristics of dry eye (keratoconjunctivitis sicca - KCS). Scrapes from the ocular mucosa of dogs with chronic untreated KCS are characterised by increased amounts of mucus, goblet cells, and keratinization, while conjunctival samples from dogs with acute KCS reveal bacteria, neutrophils, mucus, and debris. Cytology of the bulbar or palpebral conjunctiva in dogs with immune-mediated KCS confirmed squamous metaplasia with keratinization and possible infiltration of inflammatory cells, predominantly neutrophils, but goblet cells were not present (Bolzan et al., 2005). In human medicine, research has highlighted the key role of conjunctival goblet cells as the primary source of mucus for the ocular surface, in the development of ocular allergies. It has been observed that allergic mediators such as histamine, leukotrienes, and prostaglandins directly stimulate mucus secretion from goblet cells and increase their proliferation (Leonardi et al., 2008; Doughty, 2012; Dartt and Masli, 2014). In the present study, all dogs exhibited an increased density of goblet cells, indicating similar a mechanism of action of allergic mediators as in humans. It would be interesting to conduct a study that includes tear film measurement (Schirmer tear test) and cytological analysis of goblet cells to better understand these processes. In allergic humans (Dartt and Masli, 2014; Hisey et al., 2023) a predisposition to dry eye syndrome is known. In dogs, dry eye syndrome is considered immune-mediated (Ofri et al., 2024), though further research is needed to explore the connection with atopic dermatitis in order to develop adequate therapeutic strategies. These findings lay the groundwork for future research aimed at enhancing the diagnosis and treatment strategies for these conditions in dogs.

Conclusion

This study confirmed the association between canine atopic dermatitis and inflammatory changes in the conjunctiva, as analysed through cytological methods. All 24 dogs included in the study presented with inflammatory cells in conjunctival cytological samples. Although eosinophils are typically associated with allergic reactions, lymphocytes were the most prevalent cell type in this investigation. These findings underscore the need for further research involving a larger cohort of dogs, along with additional ophthalmologic evaluations, such as tear film measurement. Based on the obtained data, it can be concluded that an interdisciplinary approach is necessary for the diagnosis and treatment of canine atopic dermatitis, incorporating ophthalmologic examinations and conjunctival cytology as standard components of the clinical protocol.

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Citološke karakteristike očne spojnice u pasa oboljelih od atopijskog dermatitisa

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Atopijski dermatitis je kronična, pruritična upalna bolest kože koja pogađa oko 10 % pasa diljem svijeta. Bolest često započinje u ranoj dobi, uzrokujući svrbež i sekundarne kožne lezije na specifičnim anatomskim područjima. Patofiziologija psećeg atopijskog dermatitisa (*canine atopic dermatitis* – CAD) nije u potpunosti razjašnjena, no nova istraživanja pokazuju složeniju interakciju između poremećaja kožne barijere, alergijske senzibilizacije i mikrobne disbioze. Unatoč dobro poznatoj povezanosti atopijskog dermatitisa i konjunktivitisa, prevalencija konjunktivitisa u pasa s CAD-om često je podcijenjena u kliničkoj praksi zbog neadekvatnog prepoznavanja simptoma i nedostatka specifičnih dijagnostičkih protokola. Ovo istraživanje imalo je za cilj analizirati citološke karakteristike očne spojnice u pasa oboljelih od atopijskog dermatitisa. Uzorci su prikupljeni od 24 psa s dijagnosticiranim CAD-om koji su liječeni u Sveučilišnoj veterinarskoj bolnici Veterinarskog fakulteta Sveučilišta u Zagrebu. Citološka analiza provedena je na uzorcima dobivenim citološkom četkicom. Rezultati su pokazali prisutnost upalnih stanica u svim uzorcima, pri čemu su limfociti bili najzastupljeniji (87,5 %). Zabilježen je blagi stupanj metaplazije spojnice kod svih pasa, neovisno o tipu upalnih stanica i stupnju konjunktivtisa. Ovi nalazi ukazuju na to da atopijski dermatitis može prouzročiti promjene u očnim spojnicama koje nisu nužno povezane s intenzitetom upalnog odgovora. Prisutnost limfocita, zajedno s eozinofilima u nekim slučajevima, potvrđuje dijagnostičku vrijednost citološke analize očne spojnice u pasa s CAD-om. Nadalje, povećana gustoća vrčastih stanica ukazuje na sličnosti u patofiziologiji očnih alergija u ljudi i pasa, što otvara mogućnosti za daljnja istraživanja u cilju boljeg razumijevanja i liječenja ovih stanja. Ova studija naglašava važnost interdisciplinarnog pristupa u dijagnostici i terapiji atopijskog dermatitisa, uključujući redovite oftalmološke preglede kao dio standardne kliničke prakse.

Ključne riječi: *atopijski dermatitis, alergijski konjunktivitis, citološka analiza, psi*