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Correlations between clinical signs and corneal cytology in feline eosinophilic keratoconjunctivitis

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Abstract

Objective: To assess correlations between clinical and cytological features of feline eosinophilic keratoconjunctivitis at the time of cytological diagnosis.

Animals Studied: Fifteen client-owned, domestic breed cats (18 eyes) examined between 2007 and 2019.

Procedures: An electronic search and medical record review of cats diagnosed with feline eosinophilic keratitis or keratoconjunctivitis (FEK) based on clinical examination findings and eosinophils detected on corneal cytology were conducted. Clinical severity was graded using a modified version of a previously validated semiquantitative preclinical ocular toxicology scoring (SPOTS) system. Clinical grades were assigned following review of clinical images and medical record descriptions, and cytological grades were assigned following review of archived corneal cytology slides. Correlations were analyzed for significance using Spearman’s rank correlation coefficient.

Results: Higher total corneal scores correlated with higher total conjunctival scores, but not with total fluorescein scores. Small lymphocyte scores correlated negatively with scores for collagen degeneration or mineralization. Globule leukocytes, a unique cell type not previously described in ocular cytology, were identified in 4 of 18 cytological samples. Higher globule leukocyte scores were correlated with higher scores for mast cells or plasma cells. Specimens with lower eosinophil scores had higher globule leukocyte scores.

Conclusions: Large variability was detected in the cytological characteristics and clinical features of FEK-affected cats. This is the first report of globule leukocytes being identified in ocular cytology from any species. The role of globule leukocytes in the etiopathogenesis and progression of FEK remains unknown and warrants further investigation.

KEYWORDS

cornea, feline, feline eosinophilic keratitis, feline eosinophilic keratoconjunctivitis, globule leukocyte, hypersensitivity
1 | INTRODUCTION

Feline eosinophilic keratoconjunctivitis (FEK) is a chronic inflammatory disease of the cornea and conjunctiva, with the most pronounced pathology involving the corneal stroma.\(^1\)–\(^3\) One of the defining clinical features of FEK is the prominent white to gray to pink, vascularized, often-raised, and cobblestone-like corneal plaques extending axially from the limbus.\(^1\)–\(^5\) Other common clinical findings include ocular discharge, conjunctival hyperemia, chemosis, corneal vascularization, and corneal ulceration.\(^6\)–\(^7\) The clinical diagnosis is confirmed cytologically by the presence of eosinophils\(^8\)–\(^9\) and/or mast cells.\(^1\)–\(^3\),\(^5\)–\(^10\) The etiopathogenesis of FEK remains unknown; however, an aberrant immune response involving type I or type IV hypersensitivity is proposed.\(^2\)

The cytological features of FEK are well-described as follows:\(^2\),\(^3\),\(^5\)–\(^7\): with eosinophils\(^6\)–\(^7\) and/or mast cells\(^5\) being frequently admixed with neutrophils, lymphocytes, macrophages, plasma cells, and/or epithelial cells.\(^2\),\(^3\),\(^5\)–\(^7\),\(^10\) Considerable variation in cytological findings, however, has been reported between FEK-affected cats,\(^5\) as well as among cytological samples collected from different areas or depths of the same cornea.\(^2\) Prasse (1996) described different cytological patterns in samples collected from the white, exudative material on the corneal surface versus those collected from the raised, pink regions of the affected cornea in the same cat.\(^2\) The white exudate had lower numbers of mast cells\(^5\) than the pink, raised corneal tissue, which contained a mixture of mast cells, eosinophils, neutrophils, lymphocytes, and epithelial cells.\(^2\)

Despite the variability in cytological features, clinical signs, and clinical response to therapy in FEK-affected cats, there are no studies assessing correlations among clinical and cytological features of this disease. It is possible that such correlations would be of great clinical value. The aim of this study, therefore, was to assess correlations among clinical and cytological features of FEK at the time of cytological diagnosis.

In addition, clinical images and medical record descriptions were reviewed, and clinical disease severity was graded by using a modified version of the semiquantitative preclinical ocular toxicity scoring (SPOTS) system.\(^11\) Only elements of the SPOTS system which could be assessed by evaluation of archived digital photographs were scored. These included the presence and severity of conjunctival hyperemia, chemosis, and conjunctival discharge, and the severity and area of corneal opacity and fluorescein staining, with scores ranging from 0 to 2 or from 0 to 4 depending on the category being evaluated (Table S1). At the time of cytological sampling, no cats had undergone surgical intervention for the treatment of FEK and none had clinical evidence of keratomalacia.

Clinical scoring of digital photographs was performed independently by two authors: a board-certified veterinary ophthalmologist (BCL) and a veterinarian trained in the scoring system (DRL). Clinical scores for each reviewer were then compared, and any differences were reassessed by both reviewers together to agree upon a final score.

For all eyes, cytological findings were graded by retrospective review of archived corneal cytology slides by a board-certified veterinary clinical pathologist (WV). Cell types (eosinophils, mast cells, neutrophils, globule leukocytes, small lymphocytes, and plasma cells) and cytological characteristics (collagen degeneration, mineralization, keratinization, and pigmentation) were each graded from 0 to 4 using a published semiquantitative system.\(^12\) Cytologically, collagen degeneration was characterized by the presence of abundant, amorphous, pale pink to pale blue collagen aggregates that stained paler than, and lacked the typical fibrillar pattern of, normal collagen.

Spearman’s rank correlation coefficient was used to identify significant correlations within clinical parameters or cytological findings independently as well as between cytological and clinical scores. All analyses were performed using commercial software (Graphpad Prism v8.4.1 for Macintosh, GraphPad Software), and p-values \(\leq .05\) were considered significant for all comparisons.

2 | MATERIALS AND METHODS

Review of the University of California-Davis Veterinary Medical Teaching Hospital electronic medical record system from 1993 to 2019 identified 39 cats (48 eyes) diagnosed with cytologically confirmed FEK. Eighteen cats (23 eyes) were excluded as clinical photographs of the affected eye at the time of corneal cytology sample collection were unavailable. Six cats (7 eyes) were excluded because archived cytological samples were unavailable for review. Records for the remaining 15 cats (18 eyes) diagnosed between 2007 and 2019 were reviewed; and breed, sex, age at presentation, laterality of disease, and ophthalmic examination findings were retrieved.

3 | RESULTS

3.1 | Clinical findings and correlations

All affected cats were of the domestic breed (13 shorthaired and 2 longhaired) and included 11 castrated males and 4 spayed females. Median (range) age and weight at presentation were 5.1 (2.1–17.3) years and 5.7 (4.2–8.0) kg, respectively.

Multiple significant positive correlations and no significant negative correlations were identified among clinical features from FEK-affected cats. All 18 eyes were assigned the same scores for corneal opacity severity or corneal
vascularization; thus, these categories were excluded from subsequent correlation calculations. The total corneal disease score was comprised only of, and considered equivalent to, the score for corneal opacity area. Clinically, higher total corneal scores correlated significantly with higher total conjunctival scores \( (p = .001, r = .697; \text{Figure 1}) \) as well as increased scores for conjunctival hyperemia \( (p = .007, r = .612) \) and chemosis \( (p = .007, r = .614) \). Higher chemosis scores were also correlated with greater conjunctival hyperemia \( (p < .001, r = .758) \) scores. Increased total fluorescein scores correlated positively with increased scores for conjunctival hyperemia \( (p = .009, r = .626) \) or chemosis \( (p = .008, r = .626) \). Finally, increased fluorescein staining severity scores were correlated with higher scores for conjunctival hyperemia \( (p = .003, r = .723) \) and chemosis \( (p = .011, r = .612) \).

### 3.2 Cytological findings and correlations

Cytological features of samples collected from the cornea of FEK-affected cats varied widely (Figure 2). Of particular note, globule leukocytes were detected in 4/18 corneal cytological samples (Figures 2 and 3) and were scored as either 1 \((n = 3 \text{ samples})\) or 2 \((n = 1 \text{ sample})\) out of 4 (Figure 2). Multiple significant correlations were identified among specific cytological features from FEK-affected cats (Figure 1). There were significant positive correlations between the scores for mineralization and cellular pigmentation \( (p = .001, r = .708) \), mineralization and collagen degeneration \( (p < .001, r = .803) \), and collagen degeneration and pigmentation \( (p = .038, r = .493) \). Regarding cell types, positive and significant correlations were identified between the cytological scores for globule leukocytes and mast cells \( (p = .036, r = .496) \), and globule leukocytes and plasma cells \( (p = .029, r = .515; \text{Figure 1}) \).

Significant negative correlations were identified between various cell types and cytological features. Higher small lymphocyte scores correlated with decreased mineralization \( (p = .033, r = - .504) \) and collagen degeneration scores \( (p < .001, r = - .792) \), while increased plasma cell scores correlated with decreased keratinization scores \( (p = .002, r = - .667) \) (Figure 1). Regarding cell types, higher globule leukocyte scores were significantly correlated with lower eosinophil scores \( (p = .005, r = - .629; \text{Figure 1}) \).

### 3.3 Correlation between cytological findings and clinical appearance

Clinical features and cytological findings varied widely among FEK-affected cats (Figure 4). However, considered collectively, several significant correlations between scores for clinical and cytological features were detected. Higher cytological scores for either eosinophils \( (p = .043, r = .482) \) or neutrophils \( (p = .028, r = .406) \) were correlated with larger conjunctival discharge scores. Additionally, higher neutrophil scores were correlated with increased corneal opacity area scores \( (p = .018, r = .550) \).

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**FIGURE 1** Heat map showing Spearman’s rank correlation coefficients for select cytological (eosinophils, mast cells, neutrophils, globule leukocytes, small lymphocytes, plasma cells, and collagen degeneration) and clinical (total conjunctival, corneal, and fluorescein scores) features of 15 cats (18 eyes) with cytologically proven eosinophilic keratoconjunctivitis. Semiquantitative grading was performed on presentation, and grading criteria are described in the methods. Red represents negative correlations, and blue represents positive correlations. For each, color intensity indicates absolute value of Spearman’s rank correlation coefficient. *Significant correlations \( (p \leq .05) \).
Discussion

In this study, we described a broad spectrum of clinical and cytological findings in a referral population of FEK-affected cats, and identified many significant positive and negative correlations among these findings. In addition, we reported a cell type—the globule leukocyte—that to the authors’ knowledge, has not been previously reported in the cornea of any species. Taken together, our data may suggest that a number of clinical syndromes are currently considered collectively under the umbrella diagnosis of FEK.

A number of clinical, cytological, and clinicocytological correlations identified in the present study warrant further discussion. Firstly, it is not unexpected that multiple significant positive correlations and no significant negative correlations were identified among clinical features from FEK-affected cats. This suggests that the SPOTS scoring system was appropriate for this study and that it was applied consistently by the two trained scorers. Likewise, the finding of significant positive correlations among various cellular cytological features and among various noncellular cytological features helps validate the cytological scoring system and its application in this study. Some of these cytological correlations likely represent evidence of chronicity—for example, the positive correlation seen between mineralization and cellular pigmentation. Given that the clinical and cytological scoring systems appear valid for this study, the relative lack of clinicocytological correlations is of great interest. For example, eosinophil scores did not correlate significantly with total clinical corneal, conjunctival, or fluorescein scores, suggesting that eosinophil abundance was unrelated to the degree of conjunctival or corneal pathology in this population of FEK-affected cats. This is especially intriguing considering that infiltration of eosinophils into conjunctival tissue and release of eosinophil-derived cytotoxic substances are
FIGURE 4  Paired clinical images and corneal cytologic samples of cats with eosinophilic keratoconjunctivitis. Clinical disease severity and cytologic features on presentation were semiquantitatively graded; grading criteria are described in the methods. (A) Clinical image and (B) corneal cytology of the left eye of a 17.3-year-old female spayed domestic shorthaired (DSH) cat. (A) Clinical disease severity was graded as 6/10 for total conjunctival score, 9/10 for total corneal score, and 6/8 for total fluorescein score. (B) Globule leukocytes were graded as 2/4. Wright-Giemsa stain, 75x objective. (C) Clinical image and (D) corneal cytology of the right eye of a 2.8-year-old male castrated DSH cat. (C) Clinical disease severity was graded as 2/10 for total conjunctival score, 7/10 for total corneal score, and 2/8 for total fluorescein score. (D) Small lymphocytes were graded as 2/4. Wright-Giemsa stain, 75x objective. (E) Clinical image and (F) corneal cytology of the right eye of a 2.1-year-old male castrated DSH cat. (E) Clinical disease severity was graded as 1/10 for total conjunctival score and 7/10 for total corneal score. (F) Eosinophils were graded as 4/4, and there was notable collagen degeneration (3/4), mineralization (3/4), and pigmentation (3/4). Wright-Giemsa stain 60x objective. (G) Clinical image and (H) corneal cytology of the left eye of a 5.7-year-old male castrated DSH. (G) Clinical disease severity was graded as 3/10 for total conjunctival score, 8/10 for total corneal score, and 0/8 for total fluorescein score. (H) Eosinophils were graded as 4/4 and neutrophils as 3/4. Wright-Giemsa stain, 75x objective. Eosinophils (e), globule leukocytes (g), neutrophils (n), small lymphocytes (sl)
important in the pathogenesis of the similar human allergic condition—vernal keratoconjunctivitis.\textsuperscript{14}

There are a number of potential explanations for the relative lack of correlations detected between cytological and clinical features in the present population. First, variability in cytological findings in the present study may have been in part due to the topographic area or depth within the cornea from which the sample was collected.\textsuperscript{2} Although it is standard procedure at our institution to collect cytological samples from the plaques and immediate surrounding cornea, the site of sampling could not be assessed retrospectively. It is also likely that some variability in cytological findings within the examined population may be explained by differences in disease chronicity. Serial cytological assessment of affected cats would be required to further investigate this. It is also possible that cats sampled here were manifesting different disease subtypes that are currently all considered under the umbrella diagnosis of FEK, in the same way that multiple distinct diseases such as mucocutaneous indolent/eosinophilic ulcers, eosinophilic granulomas, and eosinophilic plaques of the skin and oral cavity in cats are all considered part of the eosinophilic granuloma complex.\textsuperscript{15} There are already a number of clinically distinct ocular surface diseases which share eosinophilic infiltration as a predominant cytologic or histologic feature, for example—feline epitheliotropic mastocytic conjunctivitis,\textsuperscript{16} bilateral nodular eosinophilic granulomas of the feline third eyelids,\textsuperscript{17} as well as feline eosinophilic conjunctivitis (without corneal involvement),\textsuperscript{18} keratitis (without conjunctival involvement),\textsuperscript{4} and keratoconjunctivitis.\textsuperscript{3,6,9}

Although there is notable overlap in the cytological findings among these conditions, they are recognized as distinct clinical entities. It is likely that ongoing careful correlation of clinical and cytological features will aid in the identification of new syndromes as well as more exact subtyping of these currently identified eosinophilic ocular surface diseases of the cat. Finally, we present data from a relatively small population, and no attempt was made to control for treatments employed prior to cytological sampling of the cornea. Future prospective studies utilizing standardized therapeutic protocols and follow-up times should assess for correlations between cytological or clinical findings and clinical response. This may permit isolation of certain clinical or cytological findings of prognostic value.

Perhaps of most interest in the present study is the detection of globule leukocytes in cytological specimens from about one-quarter of the cats diagnosed with FEK. This is a notable and unexpected finding which, to the authors’ knowledge, represents the first time these cells have been reported in ocular cytological specimens from any species. Globule leukocytes are cells\textsuperscript{19,20} with large, eosinophilic, cytoplasmic granules/globules\textsuperscript{21–24} containing basic proteins,\textsuperscript{22} eccentrically located\textsuperscript{24,25} small round or bilobed nuclei,\textsuperscript{23} and a low nuclear-to-cytoplasmic ratio.\textsuperscript{23} They have been identified in the gastrointestinal, respiratory, urinary, and reproductive tracts of a variety of birds, fish, amphibians, and reptiles, as well as mammals,\textsuperscript{26} including humans, pigs, dogs, cats, sheep, goats, rabbits, mice, and rats.\textsuperscript{27} Globule leukocytes may have a beneficial role in downregulating mucosal immunopathology. For example, increased globule leukocytes were correlated with decreased mucosal inflammation and damage in a murine intestinal helminth model,\textsuperscript{23} and more globule leukocytes were present in minimally inflamed airways, and fewer globule leukocytes in more severely inflamed airways, in a cat with bronchitis and bronchiolitis.\textsuperscript{28} There is evidence suggesting that globule leukocytes are within the same developmental cellular lineage as interepithelial mucosal mast cells (ieMMCs) within the gastrointestinal tract.\textsuperscript{23} It is, therefore, perhaps not surprising that globule leukocyte scores correlated significantly with increased mast cell scores in the present study. They were also positively correlated with plasma cell scores in the present study. The clinical significance of the globule leukocytes is not immediately apparent, but it is possible that their presence represents a further subtype of feline inflammatory ocular surface disease currently considered as FEK. This is supported by the observation in the present study that increased globule leukocyte scores were negatively correlated with increased eosinophil scores.

\section{CONCLUSIONS}

This study reports synchronously obtained clinical and cytological features of 15 cats (18 eyes) with FEK. A broad spectrum of cytological and clinical features was identified in affected cats. A number of expected clinical features were correlated as were a number of cytological features. However, clinicocytological correlates were infrequent, reinforcing the need for further cytologic investigation of this disease. To the authors’ knowledge, globule leukocytes detected in about one-quarter of affected cats represented a novel corneal finding in any species. Globule leukocyte scores were negatively correlated with eosinophil scores and positively correlated with mast cell or plasma cell scores. Their clinical significance remains unknown, and further investigations are required to determine whether their presence indicates a new form of feline inflammatory ocular surface disease or, if not, to better assess their role in the etiopathogenesis of FEK.

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CONFLICT OF INTERESTS
The authors have no conflicts of interest to disclose.

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

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