Review

The Role of In Vivo Confocal Microscopy in Ocular Allergies

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Abstract

In vivo confocal microscopy (IVCM) is a non-invasive imaging technique used to visualize the layers of the cornea and conjunctiva in real time. In patients with atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC), this technology can be useful in diagnosing and monitoring the disease, as well as evaluating the efficacy of treatments. IVCM can reveal subclinical abnormalities in the corneal and conjunctival epithelium such as inflammatory cell infiltrates and tissue damage, which can provide insight into the pathogenesis of AKC. In AKC, IVCM reveals changes around the conjunctival papillae, inflammatory cells around punctate defects in the corneal epithelium, changes in subbasal nerve morphology, and deteriorations in the goblet cells in the meibomian gland are observed. In VKC, alterations can be observed in the diameter, brightness and nucleus/cytoplasm ratio of the superficial epithelial cells in the cornea. The use of IVCM in AKC and VKC can therefore aid in the early detection and management of the disease, as well as contribute to a better understanding of its underlying mechanisms.

Keywords: Atopic keratoconjunctivitis, vernal keratoconjunctivitis, in vivo confocal microscopy, cornea, conjunctiva, meibomian glands

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Introduction

The word atopy describes hypersensitivity to common household or environmental allergens in individuals with a history of hereditary allergic disease. Atopic diseases affect 5% to 20% of the general population.[1] Bronchial asthma, allergic rhinitis, atopic dermatitis, and ocular allergic disorders are among the most common conditions encountered by allergic individuals. Recent research shows that the lifetime prevalence of these atopic diseases in children and adolescents ranges from 24% to 45%.[2] Atopic dermatitis alone affects about 3% of the population in the United States, while in Japan it is seen at a higher rate of about 8%.[3]

Between 25% and 40% of patients with atopic dermatitis have ocular involvement. The most severe ocular surface involvement in this disease is atopic keratoconjunctivitis (AKC). Patients with AKC present with findings ranging from typical eczema and keratinization of the eyelids to cicatricial conjunctivitis, severe superficial punctate keratopathy, and corneal neovascularization, thinning, ulceration, and perforation (Table 1).[4,5,6,7]

Ocular allergy is defined as an inflammatory reaction that occurs on the surface of the eye as a result of a hypersensitivity reaction of the ocular adnexa to environmental allergens. Seasonal and perennial allergic conjunctivitis is the most common form, observed at a rate of 6%-30%.[8] Understanding the pathophysiology of allergy is necessary to enable diagnosis and optimize treatment, and corneal and conjunctival dendritic cells are critical in understanding the pathophysiology of ocular allergy.[8] It is thought that increasing our knowledge of the function of dendritic cells in ocular allergic inflammation will facilitate the development of novel therapeutic approaches. In vivo confocal microscopy (IVCM) studies showed that patients with allergic conjunctivitis had higher dendritic cell density in the cornea and conjunctiva compared to control groups.[8,9] These patients were also shown to have larger dendritic cell bodies as well as cells with longer dendrites, suggesting that the corneal dendritic cells had greater antigen capture capacity.[9] In summary,

alterations in corneal and conjunctival dendritic cell density and morphology have been observed in allergic conjunctivitis.[8,9] These findings indicate that the ocular surface immunological response is heightened in allergic conjunctivitis (Table 2).[8,9]

AKC is a bilateral chronic hypersensitivity disease of the ocular surface associated with systemic atopic dermatitis. The ocular inflammation process and release of allergic mediators into the ocular surface and tear film is thought to be responsible for a wide range of clinical corneal findings including keratoconus, herpetic keratitis, superficial punctate keratitis, macroerosions, corneal ulceration, plaque formation, dry eye disease, corneal neovascularization, and lipid infiltration.[10] In a previous study, it was reported that ocular surface disorder in patients with atopic dermatitis was characterized by marked tear film imbalance, goblet cell loss, conjunctival squamous metaplasia, and reduced corneal sensitivity.[10]

Table 1. Ocular findings in patients with atopic dermatitis

Clinical findings	Number of eyes	%
Eyelids		
Eczema	476	65.7
Trichiasis	14	1.9
Ectropion	2	0.2
Conjunctiva		
Atopic keratoconjunctivitis	489	65.7
Conjunctival papillary reaction		
Upper tarsus	179	24.7
Lower tarsus	310	42.8
Chemosis	489	65.7
Hyperemia	489	65.7
Symblepharon	2	0.2
Limbus		
Trantas dots	2	0.2
Cornea		
Superficial punctate keratopathy	489	65.7
Epithelial defect	9	1.2
Keratoconus	24	3.3
Peripheral neovascularization	16	2.2
Tear film abnormalities		
TBUT <10 s	452	62.4
Schirmer test <5 mm	407	56.2
TBUT time <10 s and Schirmer test <5 mm	242	33.4

TBUT: Tear film break-up time

IVCM is a non-invasive imaging method that allows the examination of the cornea at the cellular level and is frequently used both in healthy corneas and in the differential diagnosis and follow-up of many diseases. IVCM enables the evaluation of pathological changes in dry eye disease, diabetes, *Acanthamoeba* keratitis, infectious corneal ulcers, herpetic keratitis, keratoconus, aging, contact lens use, and refractive surgical procedures.[11,12,13,14,15,16,17,18,19,20] With the increased clinical use of IVCM, high-resolution images of the corneal subbasal nerves and immune/inflammatory (dendritic) cells can be obtained in the healthy and pathological cornea.[12]

Evaluating Corneal Changes in Atopic Keratoconjunctivitis Patients with In Vivo Confocal Microscopy

AKC is reported to be associated with herpetic keratitis, keratoconus, and dry eye.[15,21,22,23] A previous study of AKC patients showed that despite the absence of concomitant keratoconus, some similar morphological changes were observed, such as a decrease in basal epithelial cells and subbasal nerve density.[24]

AKC has been shown to be associated with short tear film break-up time (TBUT)-type dry eye.[10] Patients with severe active atopic dermatitis in childhood are more likely to have the aqueous-deficient type of dry eye disease as adults if active skin disease persists in adulthood.[25] The IVCM findings reported in keratoconjunctivitis sicca are an increase or decrease in superficial epithelial cell density, depending on the condition, a decrease in basal epithelial cell density, reduced subbasal nerve fiber number and density, and an increase in nerve tortuosity compared to healthy controls.[26,27,28,29]

In a study of AKC patients, although dry eye was not of the aqueous-deficient type, a similar decrease in subbasal nerve density and increase in nerve tortuosity (compared to control subjects) were observed.[24] As none of the patients in that study had keratoconus, history of contact lens use, history of herpetic eye disease, keratoconjunctivitis sicca, previous ocular surgery, or

Table 2. Confocal microscopy findings associated with allergic (seasonal and vernal) conjunctivitis

Findings	Description
Changes in corneal epithelium thickness	In allergic reactions, the corneal epithelium may increase in thickness
Cell infiltration	Epithelial and subepithelial cell infiltration (especially eosinophils and lymphocytes) may be observed
Superficial keratopathy	Findings of corneal surface irregularity, erosion, and keratopathy
Meibomian gland status	Enlargement or atrophic changes in the meibomian glands
Papillary hyperplasia	Swelling and hyperplasia of the conjunctival papillae may be seen
Subepithelial cystic formations	Small cystic lesions may be observed under the corneal epithelium
Changes in stromal thickness	Stromal changes such as edema or thickening may be seen
Connection and cell irregularity	Abnormal epithelial cell connections and disorganization

any other ocular disease, the abnormalities observed in subbasal nerve structure were attributed primarily to AKC and its associated pathophysiological mechanisms (Figure 1).[24]

Confocal microscopy in patients with AKC reveals abrupt termination of the subbasal nerves, which may represent perforation sites of nerve fibers through the Bowman layer or sites of nerve degeneration.[24] As previously reported in dry eye and diabetic patients, a higher rate of looping and coiling patterns as well as high metabolic activity or nerve regeneration may be seen in AKC patients. It is thought this high metabolic activity is likely intended to repair the changes observed at the epithelial level (Figure 2).[24]

An abnormal architecture can be seen not only in the subbasal nerve plexus, but also the stromal nerves.[24] Thicker stromal nerves (likely due to edema and increased metabolic activity) with deviation and bifurcation abnormalities are observed, which are considered attempts to restore a healthy stromal environment. This regeneration process is thought to be the reason why the stromal nerves in patients with AKC are relatively thicker, less reflective, and more tortuous compared to those of healthy individuals.[24]

Corneal nerve fibers have trophic effects on the corneal epithelium and are important for the maintenance of a healthy ocular surface.[30,31,32] Corneal nerves have been shown to harbor neuropeptides and neurotransmitters with neurotrophic properties, such as calcitonin gene-related peptide and substance P, as demonstrated in previous experimental studies.[33,34] Corneal nerve fibers release dispersible factors that stimulate epithelial growth, proliferation, differentiation, and type VII collagen production.[12] Epithelial cells produce soluble factors with neurotrophic effect, such as neuronal growth factor and glial cell-derived neurotrophic factor.

Significant positive correlations have been demonstrated between corneal sensitivity and basal epithelial cell density and between subbasal nerve density and basal epithelial cell density, all of which support the theory that the corneal nerves exert a trophic effect on the corneal epithelium.[24] The loss of the trophic effect can lead to decreased basal epithelial cell density, higher ocular surface vital staining scores, and corneal ulceration.

Other important IVCM findings are the presence of inflammatory infiltrates around ulcer margins and near the subbasal and stromal nerves, especially in patients with diffuse superficial punctate keratopathy.[24] Moreover, there is strong evidence that corneal sensitivity is significantly reduced in eyes with higher inflammatory cell densities. The density of corneal long nerve fibers and nerve branches has been reported to be significantly lower in AKC patients compared to healthy control subjects on IVCM scans.[24] Previous studies using IVCM have revealed large numbers of inflammatory cells near or on the corneal subbasal and stromal nerves, which explains the lower corneal sensitivity scores and more severe ocular surface inflammation in patients with AKC.[35]

Evaluation of Conjunctival Changes in Atopic Keratoconjunctivitis Patients with In Vivo Confocal Microscopy

Examining conjunctival changes in patients with AKC at the cellular level may help elucidate the pathogenesis and

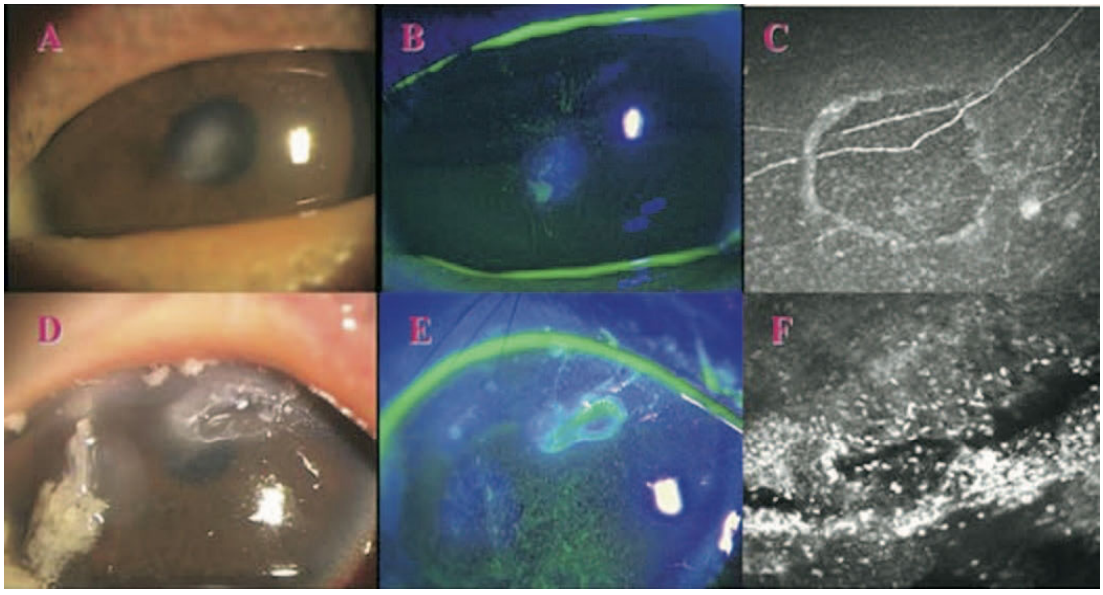

Figure 1. A-C) Anterior segment photographs and in vivo confocal scanning laser microscopy image of a 27-year-old male patient with atopic keratoconjunctivitis (AKC). The images show central corneal opacity (A), extensive superficial punctate keratopathy (B), and the highly reflective irregular borders of the epithelium surrounding the scar tissue with intraepithelial nerve fibers that become apparent on confocal microscopy (C). His Schirmer test value was 9 mm, tear film break-up time was 2 seconds, and corneal sensitivity was 40 mm. D-F) Anterior segment photographs and in vivo confocal scanning laser microscopy image of a 20-year-old male patient with AKC and corneal ulcer. Abundant mucus discharge on the ocular surface (D), positive fluorescent staining of the ulcer, and paracentral ulceration with impaired corneal epithelium (E) are observed. A confocal scanning image taken over the corneal ulcer showed diffuse, round, highly reflective inflammatory infiltrates (F). The patient's Schirmer test value was 7 mm, tear film break-up time was 0 seconds, and corneal sensitivity was 25 mm.[24]

subsequent clinical appearance of atopic ocular allergies, which can be sight-threatening. Histopathologically, tarsal conjunctival changes in AKC have been reported as hyperplasia of the connective tissue, proliferative and degenerative changes in the epithelium, pronounced infiltrations in the epithelium, and an increase in eosinophils, lymphocytes, mast cells, macrophages, basophils, plasma, and dendritic cells in the substantia propria.[36] One of the most important findings in AKC is the overgrowth of conjunctival connective tissue consisting of gelatinous, sessile papillae. In the deep layers of the conjunctiva, collagen fibers form a fibrous structure within the papilla. The proliferation of capillaries and vascular neoformations provides vascular support to the papilla (Figure 3).[36] Hyaline degeneration of the conjunctival stroma has also been observed in papillary lesions. Invasive techniques such as conjunctival biopsies have been the source of such valuable information in many studies. Significant positive correlations have been reported between conjunctival inflammatory cell density and ocular surface vital staining, as well as significant negative correlations between corneal sensitivity, tear stability, and inflammatory cell density, all of which support the assertion that conjunctival inflammation adversely affects tear functions and leads to ocular surface epithelial disease.[36,37] A previous study showed that TBUT was significantly associated with conjunctival goblet cell density in patients with AKC.[38] Goblet cells are known to be very sensitive to inflammation and decline in number at higher grades of inflammation.[39,40] It is thought that patients with higher inflammatory cell density may have lower goblet cell density, leading to lower TBUT.[40] Despite a weak correlation, patients with higher confocal inflammatory cell density were found to have significantly lower TBUT.[35]

In a previous study, diffuse ocular surface inflammatory cell infiltration in AKC was demonstrated with brush cytology samples, and inflammatory cell counts in brush cytology samples were also reported to be associated with the severity of corneal lesions.[41,42,43] IVCM observations have confirmed the role of conjunctival inflammation in the ocular surface disease process and were shown to aid evaluation and comparison of the effects of different treatment protocols on the ocular surface in AKC (Figure 4).[36]

Remarkable observations of the architecture and inflammatory state of tarsal conjunctival papillae have been made with IVCM.[36] AKC patients not using topical cyclosporine were found to have stromal edema along with much more extensive inflammatory cell infiltrates on the surface of the papillae and in the lacunar spaces within the papillae.[36] In patients using topical cyclosporine, a marked reduction of infiltrates in the papillae,

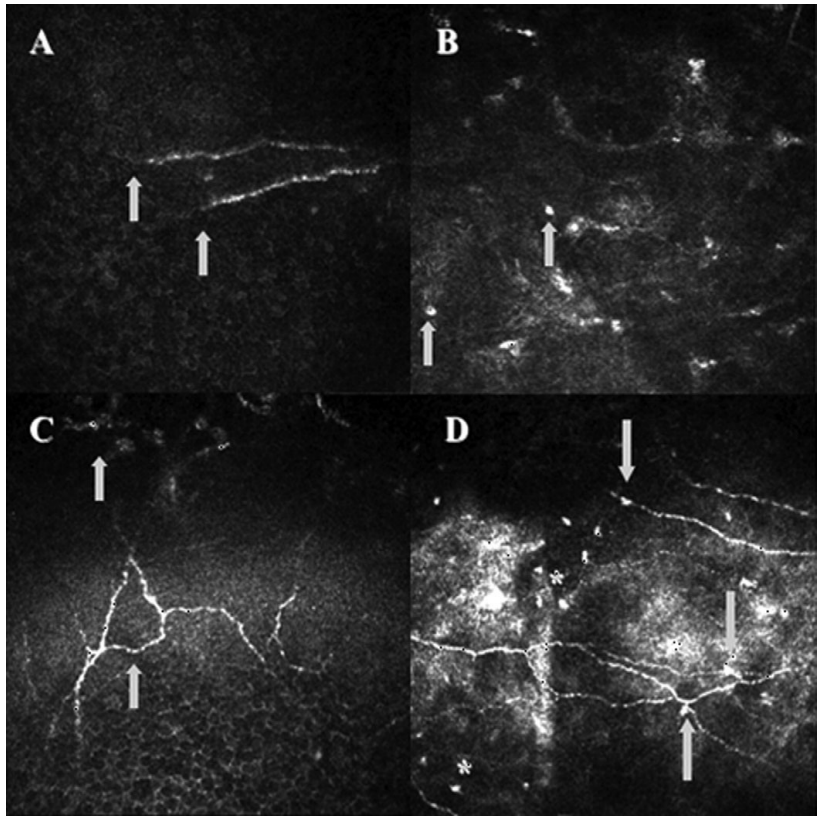

Figure 2. In vivo confocal scanning laser microscopy images of the subbasal nerve plexus in patients with atopic keratoconjunctivitis (AKC). A) White arrows indicate a decrease in the number of long nerve fibers that terminate abruptly. B) Subbasal nerve plexus could not be observed in some patients with AKC. White arrows indicate inflammatory cells. C) Nerve fibers are randomly oriented, wavy, and show frequent looping. D) Inflammatory cells with increased reflectivity on the subbasal nerves (arrows) and numerous inflammatory cells near the subbasal nerves (white asterisks) are seen.[24]

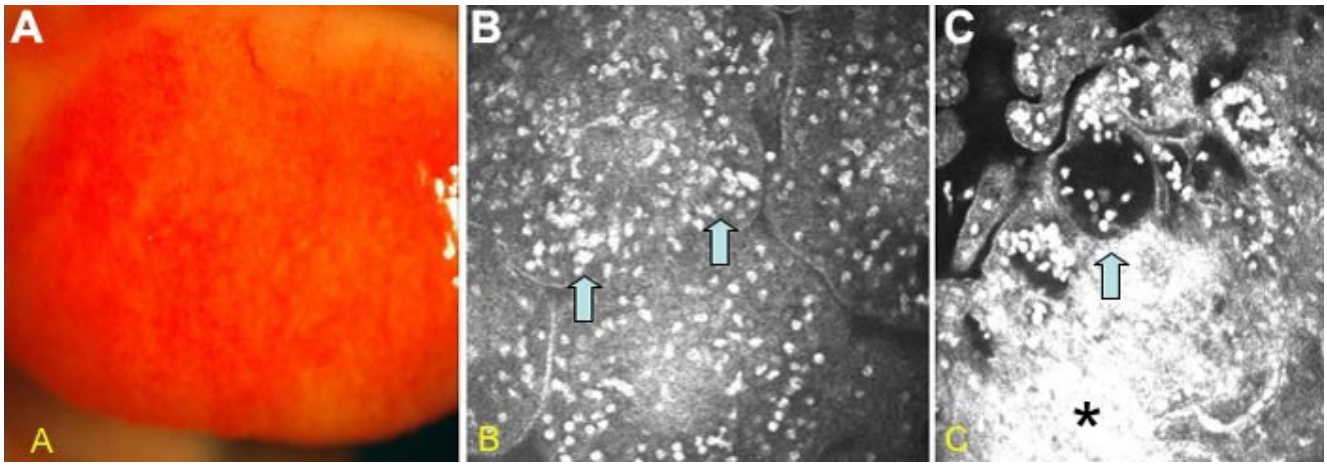

Figure 3. Conjunctival slit-lamp and in vivo confocal microscopy (IVCM) scan images from a patient with atopic keratoconjunctivitis using only topical steroids and topical anti-allergic. A) The slit lamp photo shows cherry red injection of the tarsal conjunctiva. B) IVCM images of intense inflammation on the surface of papillary formations (blue arrows). C) Deeper in the papilla formation, hyperreflective edematous areas (black asterisk) and cavitations (lacunae) surrounded by inflammatory infiltrates (blue arrow) were seen.[36]

dendritic cells, vascular loops, and around lacunar spaces with a fibrotic response were observed.[36] The lacunar morphology in the papillae shows areas of collagen resorption during the remodeling processes or areas of stromal degeneration (Figure 5).[36]

The effects of topical cyclosporine therapy on the corneal microstructure are also being investigated in patients with vernal keratoconjunctivitis (VKC).[38] The conjunctiva and cornea are among the main ocular components affected by VKC, and observations made during the treatment process can provide valuable information about changes in these important structures.[38] Changes in the corneal microstructure observed in patients treated with topical cyclosporine may serve as an important indicator in assessing the response to treatment and the course of the disease. The compilation of such findings will contribute to our understanding of the pathophysiology of VKC and the development of treatment approaches, enabling it to become a focal point of research. In particular, understanding the mechanism of these microstructural changes and integrating this knowledge into the treatment process may pave the way for the development of more effective VKC management strategies in the future.[38]

Evaluation of Meibomian Gland Dysfunction with In Vivo Confocal Microscopy in Atopic Keratoconjunctivitis and Vernal Keratoconjunctivitis Patients

The meibomian glands (MG) secrete lipids on the ocular surface, and these lipids form the outer layer of the tear film. MG secretions prevent rapid evaporation of the tear film, act as a barrier to prevent contamination, and lubricate to reduce friction on the ocular surface from blinking.[45]

With the increase in the clinical use of IVCM, pathophysiological changes on the ocular surface have been investigated in more detail. Studies have reported that IVCM parameters such as acinar unit density and diameter are valuable in understanding histopathological changes such as glandular atrophy and acinar/ductal dilation in obstructive meibomian gland dysfunction (MGD).[46,47] However, periglandular inflammatory cell density has been identified as another IVCM parameter that can distinguish inflammatory obstructive MGD from non-inflammatory subtypes.[46] These parameters were characterized by high sensitivity and specificity in the diagnosis of MGD and showed a good correlation with ocular surface condition.

A decline in the conjunctival goblet cell population and deterioration in tear quality and quantity have been reported in AKC patients.[47] Tear film instability in these patients was attributed in part to disturbances in tear mucins resulting from decreased goblet cell density.[47] In another study, Ibrahim et al.[48] suggested that because AKC patients have greater MG damage and lid margin changes, deterioration in the lipid layer of the tear film leads to tear instability and consequently exacerbates ocular surface epithelial damage. In the same study, the results of IVCM examinations revealed severe fibrosis and atrophy in the MG, as well as a decrease in the size and density of MG acinar units (Figure 6).[48]

In addition, MG acinar unit atrophy is a novel IVCM parameter that can be used in the evaluation of MG damage.[48] MG acinar unit area values were found to be significantly lower in AKC patients compared to obstructive MGD and control subjects.[48] However, there was a significant increase in periglandular inflammatory cell density in AKC patients compared with obstructive MGD and normal controls.[48]

MG status in AKC patients requires careful attention, as it can lead to deterioration in the tear film structure and exacerbate the inflammatory status through a vicious cycle. There is a need for further studies investigating the differences in MG status in AKC and VKC patients using IVCM and infrared meibography technologies.

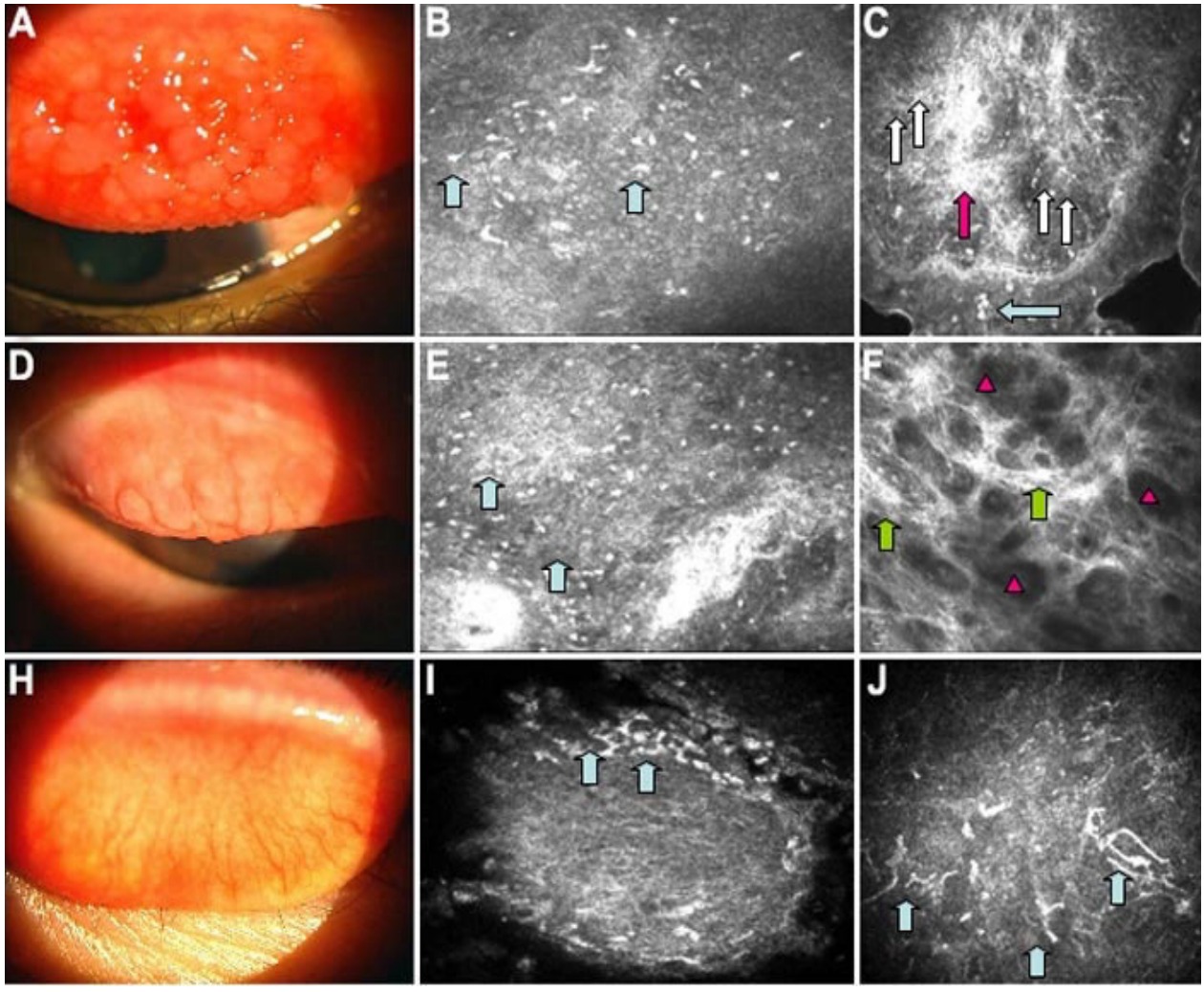

Figure 4. Conjunctival slit-lamp photo and in vivo confocal scan images from patients with atopic keratoconjunctivitis (AKC) using topical cyclosporine. Upper row: A) slit-lamp photo showing injection in the tarsal conjunctiva and papillary hypertrophy. B) Confocal images showed less inflammation on the surface of the papillary formations (blue arrows). C) Deeper images of the papillary formations show fibrosis (pink arrow) and vascular neoformations (white arrows) with inflammatory cells. Middle row: this row shows the conjunctival slit lamp photograph and in vivo confocal scan images of another patient with AKC using topical cyclosporine. D) The slit-lamp image shows papillary hypertrophy and conjunctival hyperemia. E) Confocal images showed less inflammation on the surface of the papillary formations (blue arrows). F) Deeper sections revealed extensive fibrosis (green arrows) with lacunar spaces showing no inflammatory infiltrates (pink triangles). Bottom row: conjunctival slit-lamp photograph and confocal scan images from another AKC patient are shown. H) Notice the conjunctival hyperemia in the slit-lamp photograph. Confocal scans showed less inflammation at the edges of the surface of the papillary formations (blue arrows; I) and dendritic cells (blue arrows; J).[36]

In VKC patients, although obstruction of the MG orifices and metaplasia were not observed, the presence of intraluminal hyperactive solid matter was shown in the MG lumen and contours.[49] In addition, extensive Langerhans cells have been observed surrounding MGs, especially in the tarsal regions. It is thought that these Langerhans cells are closely related to MG acinar unit density and that the immunological inflation initiated by these cells may damage and eliminate the MG.[49]

As a result, the IVCM used in these studies is a non-invasive and effective method that can be used to elucidate the structural and functional changes in the cornea, conjunctiva, and MG that occur in AKC patients (Table 3). IVCM can also provide information about decreased corneal nerve density and distribution in patients with AKC and can be used to evaluate disease severity and treatment response.

Studies have shown that IVCM can help identify infiltrative cells, dendritic cells, and subepithelial fibrosis in patients with AKC and VKC. In addition, they have shown that IVCM can be used to monitor the effects of topical and systemic therapies for AKC.[36,48] This has provided insight into the effectiveness of different approaches and enabled adjustments when necessary.

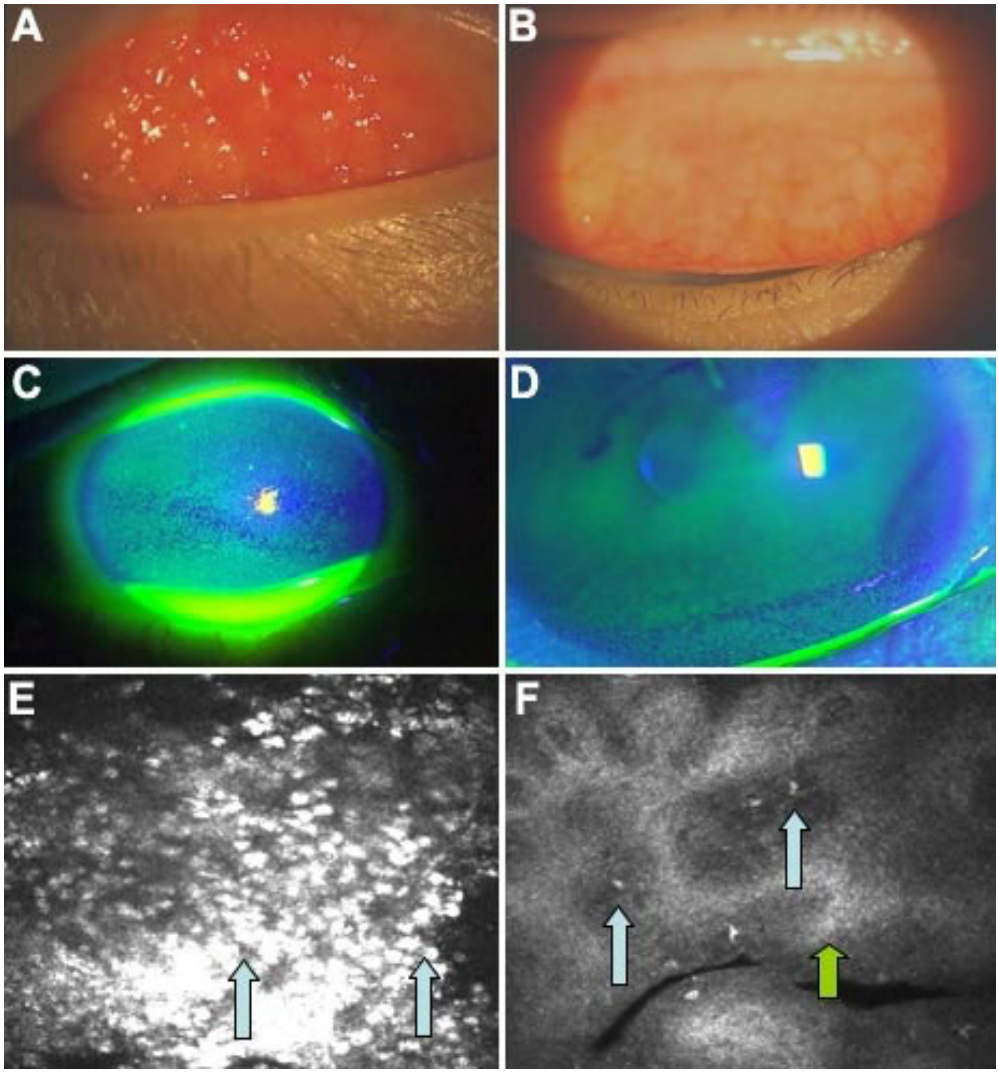

Figure 5. Anterior segment photographs and confocal scan images of a patient with atopic keratoconjunctivitis (refractory to 8 weeks of treatment with steroid and anti-allergic eye drops for eight weeks) before and after treatment with topical cyclosporine drops administered for 8 weeks in addition to the anti-allergic and steroid eye drops. A, B) There was a marked decrease in tarsal conjunctival injection and edema with regression of the papillary formations. C, D) Changes in corneal epithelial damage are observed with cyclosporine treatment. E, F) Dramatic reductions in conjunctival inflammatory cell infiltrates on the surface of the papillary formations (blue arrows) and the fibrotic response in papillary formations (green arrow) are seen.[36]

Evaluation of Vernal Keratoconjunctivitis Patients with In Vivo Confocal Microscopy

VKC is an inflammatory eye disease that particularly affects children and young adults. It is characterized by symptoms such as itching, redness, and excessive tearing.[50] In the context of VKC, corneal IVCM can provide important information when assessing the presence and severity of corneal involvement.[50] It can help visualize structural changes such as significantly increased diameter, reflectivity, and nucleus/cytoplasm ratio of the epithelial cells on the corneal surface; decreased basal epithelial cell density; decreased corneal subbasal nerve density and increased stromal nerve thickness, branching, and tortuosity; and decreased keratocyte density but significantly increased number of active keratocytes and inflammatory cells in the anterior stroma.[50,51,52,53] Csorba et al.[52] showed that Langerhans cells were present at high density and largely showing a mature phenotype in parallel with the severity of papillary hypertrophy, even when VKC was inactive. The alterations in Langerhans cells indicate a subclinical inflammatory process in the absence of ocular symptoms. The IVCM imaging technique is an important tool in establishing a diagnosis, assessing disease severity, and monitoring response to treatment.[53] It can also contribute to an understanding of the pathophysiology underlying VKC, and offers a non-invasive alternative to traditional invasive procedures such as corneal biopsy, which can be uncomfortable for patients.

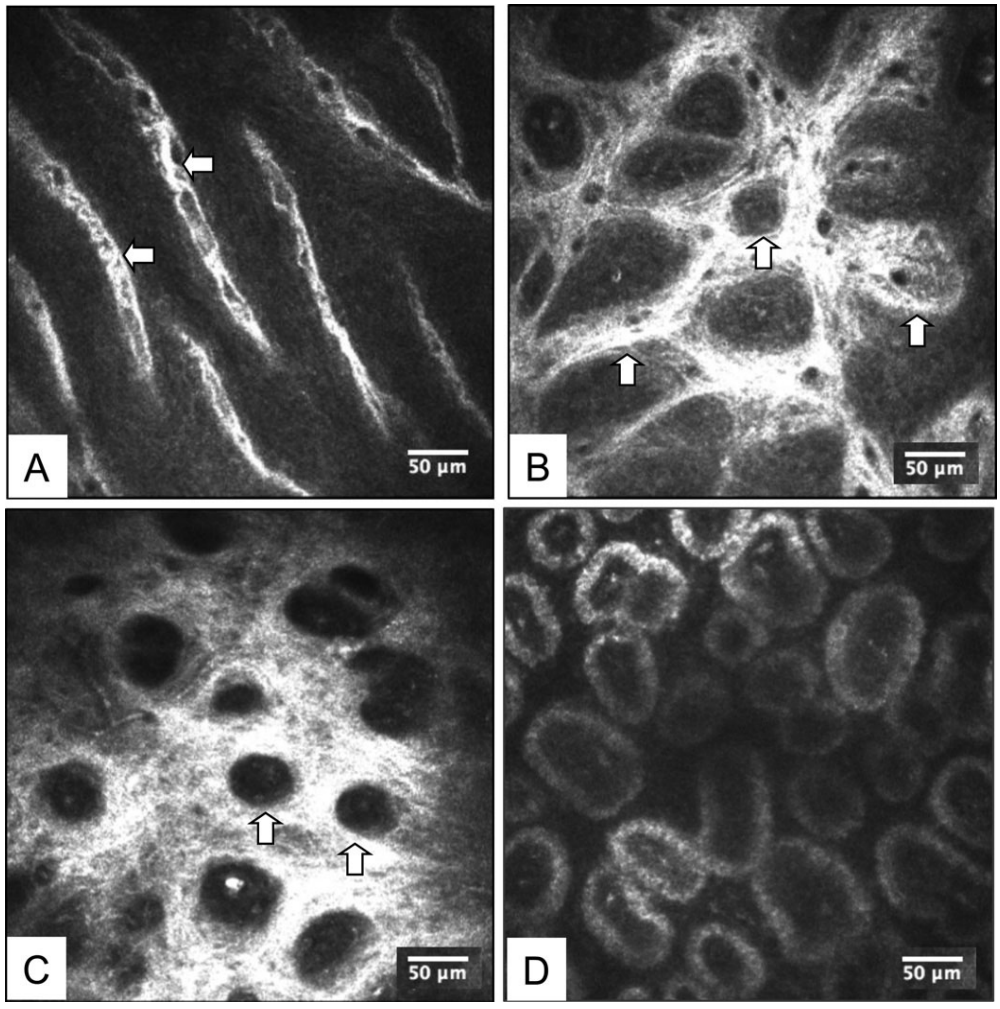

Figure 6. In vivo confocal microscopy images of meibomian gland (MG) changes in patients with atopic keratoconjunctivitis compared to a healthy subject. A) Linear streaks of MG fibrosis. B) Loss of MG architecture with extensive fibrotic tissue surrounding the atrophic remnants of the MG. C) Intense fibrotic changes in the MG and neighboring conjunctival tissues. White arrows indicate areas of fibrosis in the MG. D) Representative image of MG acinar units in a normal position.[48]

Based on the current literature, it is seen that confocal microscopy reveals in detail the effects of ocular allergic diseases, especially VKC. This imaging method provides the opportunity to clearly observe microstructural alterations in the corneal epithelium, cellular infiltration, and degenerative processes. Confocal microscopy has allowed a better understanding of important parameters such as morphological changes in corneal cells, immune responses, and treatment response during the course of allergic diseases, which has in turn provided new perspectives on clinical interventions. As a result, confocal microscopy is considered an important tool in understanding the mechanisms of allergic eye diseases and evaluating treatment processes.

Conclusion

In conclusion, imaging methods, especially IVCM, guide clinicians' treatment approaches by providing more in-depth information about the pathology of AKC and VKC. These methods are regarded as a valuable tool in the diagnosis and treatment of AKC and VKC in particular, contributing to a better understanding of these diseases and allowing more effective care for patients.

Declarations

Authorship Contributions

Concept: C.Ş., T.K., M.D., Design: C.Ş., T.K., M.D., Data Collection or Processing: C.Ş., T.K., M.D., Analysis or Interpretation: C.Ş., T.K., M.D., Literature Search: C.Ş., T.K., M.D., Writing: C.Ş., T.K., M.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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Table 3. Ocular surface in vivo confocal microscopy findings in patients with atopic keratoconjunctivitis
Conjunctival changes
Stromal edema on the surface of the papillae and in the lacunar cavities within the papillae
Dense inflammatory cells on the surface of papillary formations
Hyperreflective edematous areas deep in the papillary formations
Cavitations (lacunes) surrounded by inflammatory infiltrates deep in the papillary formations
Fibrosis and neovascularizations in deeper images of the papillary formations
Decline in goblet cell population
Corneal changes
Epithelium
Presence of inflammatory cells around the ulcer margins in patients with punctate keratopathy
Exposed intraepithelial nerve fibers
Subepithelial fibrosis with epithelial irregularities
Nerves
Decrease in subbasal nerve density
Increased nerve tortuosity
Abrupt termination of subbasal nerves
High prevalence of looping and coiling patterns
Abnormal stromal nerve architecture
Thicker stromal nerves with deviation and bifurcation abnormalities
Stroma
Presence of inflammatory infiltrates adjacent to the stromal nerves
Stromal turbidity
Stromal fibrosis
Meibomian gland (MB) changes
Lid margin changes
Severe fibrosis and atrophy
Reduction in the size and density of MB acinar units
Increase in periglandular inflammatory cell density

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