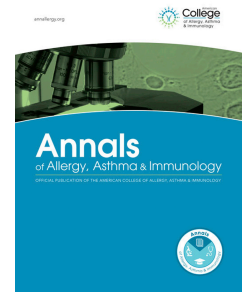


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The Efficacy of 0.1% Tacrolimus Ophthalmic Suspension in the Treatment of Severe Atopic Keratoconjunctivitis

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1 Introduction

2 Allergic conjunctival diseases (ACDs) are defined as conjunctival inflammatory
3 conditions associated with a type I allergy accompanied by some subjective and
4 objective symptoms¹. ACDs are broadly divided into five types according to the
5 presence of proliferative changes, atopic dermatitis (AD), and the presence/absence of
6 mechanical irritation (i.e., seasonal allergic conjunctivitis (SAC), perennial allergic
7 conjunctivitis (PAC), atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis
8 (VKC) and giant papillary conjunctivitis (GPC)). Among them, AKC and VKC are
9 classified as the severe types of ACDs because they can cause keratopathy², such as
10 shield ulcer or corneal plaque leading to visual morbidity in some cases³.

11 Allergen-induced late-phase reactions result in the persistent clinical inflammation of
12 chronic allergic diseases such as AKC and VKC⁴, and are characterized by the mucosal
13 infiltration of eosinophils, neutrophils, basophils and T lymphocytes⁵. Furthermore, the
14 total number of mast cells is increased in the stroma and epithelium of AKC and VKC
15 patients². Various cytotoxic proteins (e.g., eosinophil cationic protein, major basic
16 protein, eosinophil-derived neurotoxin, and eosinophil peroxidase) released from
17 eosinophils and proteases released from mast cells induce corneal epithelial disorders⁶.

18 Moreover, subepithelial fibrosis of the conjunctiva, fornix shortening, and
19 symblepharon may occur in these allergic ocular diseases⁷.

In the treatment of AKC and VKC, topical antihistamines, mast cell stabilizers, non-steroidal anti-inflammatory drugs (NSAIDs) and steroids are usually used. However, we sometimes encounter patients who are refractory to these medications. Although additional steroids may be required to relieve patient symptoms, they can cause adverse effects such as cataract, infection, steroid-induced elevation of intraocular pressure (IOP), glaucoma, eyelid skin atrophy and depigmentation with barrier impairments⁸⁻¹⁰. To overcome these problems, alternative therapeutic medications for AKC/VKC have been required.

Recently, immunosuppressive medications (e.g., cyclosporine ophthalmic suspension and tacrolimus ointment) have been reported to be useful for relieving patient symptoms and can reduce the rate of serious side effects from steroids¹¹⁻¹⁶. Both of these nonsteroidal immunomodulators inhibit calcineurin activity. However, topical cyclosporine can result in an intense stinging sensation leading to poor compliance¹⁷, and tacrolimus ointment also can cause irritation and epithelial keratitis¹⁶.

On the other hand, tacrolimus has been used as a 0.1% ophthalmic suspension (TALYMUS[®], Senju Pharmaceutical Co., Ltd., Osaka, Japan) for treating several ocular diseases including AKC and VKC^{13,18}. In these previous reports, however, the observation period was short and/or mild cases were included. Thus, the therapeutic

effects of 0.1% tacrolimus ophthalmic suspension in longer-term, more severe cases remain poorly understood. The purpose of this study was to examine the efficacy of 0.1% tacrolimus ophthalmic suspension for the treatment of severe AKC throughout a one-year period.

Methods

The present investigation was a single center retrospective clinical study, and was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Institutional Ethics Review Board of Tsurumi University Dental Hospital (Kanagawa Prefecture, Japan; IRB No.1312). The clinical trial registration ID number is UMIN 000034460. Patient data were anonymized before access and/or analysis.

Atopic Keratoconjunctivitis (AKC)

AKC is related to AD in 95% of cases, whereas only 20% to 43% of patients with AD have ocular involvements¹⁹. We diagnosed AKC as chronic keratoconjunctivitis associated with AD on the patient's face according to the following criteria: (1) always associated with other AD; (2) occurred at any time during the associated atopic disease, independent of its severity; and (3) evidence existed for corneal involvement during the

disease course²⁰. The diagnosis of AD was based on previously reported criteria²¹.

Severe AKC was defined as AKC with proliferative changes, corneal involvement, and refractory to conventional anti-allergic agents and/or steroids treatment.

Patients

Sixty eyes from 30 patients with severe AKC who were treated with tacrolimus eye drops alone or in combination with topical anti-allergic agents and/or corticosteroids between April 2011 and October 2016 were enrolled in the current study. All patients were administered one drop of tacrolimus eye drops twice or four times a day at the beginning of the observation.

Clinical signs and criteria

The severity of objective signs was observed at baseline (before treatment) and at 2 weeks, and at 1, 2, 3, 6, and 12 months after initiation of tacrolimus eye drop treatment. Ten objective signs of palpebral conjunctiva (hyperemia, swelling, follicles, papillae, and giant papillae), bulbar conjunctiva (hyperemia and chemosis), limbus (Trantas' dot and swelling), and corneal epithelial disorder were assessed using 4 grades (0 = normal; 1+ = mild; 2+ = moderate; 3+ = severe)¹; these criteria are summarized in Table 1. The

efficacy endpoint was assessed based on the extent of change in the total score of objective signs at the end of treatment. Safety was assessed based on the incidence and the severity of adverse events. Cases in which steroids could be discontinued after the beginning of tacrolimus eye drop treatment were also examined.

Exclusion criteria in this study were as follows: patients with history of allergic hypersensitivity or known hypersensitivity to any compound or diluting agent of tacrolimus; history of using tacrolimus eye drops; presence of any ocular condition that could affect the study parameters and/or patient safety (e.g., glaucoma requiring medication or laser treatment, clinically significant blepharitis, uveitis, scleritis, pterygium); undergone an ocular surgical intervention within 3 months before study and/or a history of refractive surgery within 6 months before study; presence of active ocular infection (bacterial, viral, or fungal), or a positive history of an ocular herpetic infection at initiation of this study; any systemic diseases which may affect the ocular surface (e.g., rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus); any autoimmune disorders (e.g.,); pregnancy, nursing, or planning a pregnancy; smoker; or contact lens wearer.

Statistical analysis

Data were analyzed using Prism version 8.00 (Graphpad Software, Inc.) for MacOS (Apple Inc, Cupertino, CA, USA). Data are expressed as mean $\pm$ standard deviation; P value less than 0.007 was considered statistically significant after Bonferroni correction (i.e., $P = 0.05/7$). The Wilcoxon signed rank test was used to compare the scores of objective clinical signs before and after long-term maintenance treatment.

Results

Characteristics of Patients with severe AKC

Demographic information of the 60 eyes of 30 patients in this study is shown in Table 2. All of patients had AD because these patients had AKC.

Changes in the scores of objective clinical signs

The total score of the 10 clinical signs (range 0 – 30) significantly decreased from baseline 2 weeks after initiation of tacrolimus eye drop treatment, except at 2 months (Figure 1). We also analyzed changes in each score of the objective clinical signs (E-Figure 1). Except palpebral conjunctival hyperemia, papillae and giant papillae, all other clinical signs had significant differences at all points.

Changes in the palpebral conjunctiva

Representative photographs of changes in the palpebral conjunctiva of the right eye after the use of tacrolimus eye drop are shown in Figure 2. A 14-year-old male patient was diagnosed with severe AKC with giant papillae that almost entirely occupied on the palpebral conjunctiva and these diameters had grown to more than 0.6 mm. He had AKC for 7 years. He was treated with 0.1% betamethasone, 0.1% cyclosporine and 0.1% tacrolimus ointment in both eyes at his first visit to our outpatient clinic. A combination of topical 0.1% betamethasone and 0.1% tacrolimus ophthalmic suspension was initiated to control severe AKC. After 1 month, inflammation and proliferative changes began to decrease. **Topical steroid use was discontinued after 6 months, but he continued tacrolimus treatment.** Twelve months after initial treatment, the clinical signs of palpebral conjunctiva drastically improved and elevated papillae became flat.

Changes in bulbar conjunctiva, limbus and cornea

Representative photographs of changes in the bulbar conjunctiva, limbus and cornea of the right eye after using tacrolimus eye drops are shown in Figure 3. An 8-year-old male patient was diagnosed with severe AKC due to shield ulcer with corneal plaque, bulbar

conjunctival hyperemia with dilation of many vessels and thinner diffuse chemosis, four Trantas' dots and swelling of two-thirds of the limbal circumference. He had AKC for 2 years. He was treated with topical antihistamine, 0.1% fluorometholone and 0.1% cyclosporine in both eyes at his first outpatient visit. A combination of 0.1% fluorometholone and 0.1% tacrolimus ophthalmic suspension was started to control severe AKC. Twelve months after initial treatment, the shield ulcer was completely resolved by using combination therapy.

Changes in steroid use in combination with tacrolimus

The percentage of eyes using 0.1% betamethasone and/or oral steroid decreased from baseline 1 month after beginning tacrolimus (Figure 4). Of all eyes, 69.4% that were treated with tacrolimus alone or in combination with topical anti-allergic agents, were successfully weaned off topical steroids into tacrolimus treatment at 12 months.

Adverse reactions

Adverse drug effects related to tacrolimus ophthalmic suspension included herpetic epithelial keratitis by herpes simplex virus in 3 eyes (5.0%) and elevated IOP in 7 eyes (11.6%) (Table 3). However, all adverse events were completely controlled with

medical treatment. When we treated herpetic infection, we stopped tacrolimus and/or steroid eye drops, and then switched to valaciclovir hydrochloride and low-dose oral steroids. Similarly, when we treated elevated IOP, 5 out of 7 eyes were simply treated by stopping steroids, but continued tacrolimus eye drops, whereas 2 out of 7 eyes also needed topical treatment with antiglaucoma agents. Although all patients experienced a burning sensation in the eyelids and conjunctiva, these effects were tolerable. None of the patients discontinued treatment due to adverse effects.

Discussion

AKC is a clinically important disease that causes conjunctival alterations and/or corneal involvement leading to visual morbidity. Ibrahim et al. reported that the patients with severe AKC experienced a reduction in functional visual acuity²². Therefore, appropriate treatment methods have been required to avoid declines in quality of life and vision. Although AKC mechanistically and partially differs from SAC (e.g., pollen) and PAC (e.g., house dust and pollutants), these can be cofactors in deterioration. Most patients with SAC and PAC can be adequately controlled with topical antihistamines, mast cell stabilizers, and/or steroid treatment. Additionally, we reported the effectiveness of an eye wash solution in patients with SAC after conjunctival allergen

challenge test²³. Meanwhile, in the treatment of AKC and VKC, we have been plagued by some cases that were extremely resistant to conventional treatments. Since 2005, 0.1% cyclosporine aqueous ophthalmic solution became available and there were some reports on its therapeutic effects on conventional treatment-resistant disease^{11,17,24}. Similarly, some studies have reported on the efficacy and safety of tacrolimus ointment in the treatment of ACDs^{14-16,25-27}. Cyclosporine binds to cyclophilin and tacrolimus binds to FK506 protein; these agents exert pharmacological activities such as inhibition of cytokine (e.g., interleukin [IL]-2, IL-4, IL-5 and interferon [IFN]- γ) production and suppressing of the degranulation of mast cells by inhibition of calcineurin activation. Tacrolimus has also been reported to inhibit calcineurin approximately 100 times more effectively than cyclosporine²⁸. There are several reports describing the effect on ACDs after the introduction of tacrolimus ophthalmic suspension^{13,29}. Moreover, previous studies have been short-term observations and have usually not covered all seasons. In addition, the definition of severe disease was refractory to the conventional treatment, and did not necessarily consider the presence or absence of the corneal involvement. Therefore, we defined severe AKC as allergic ocular diseases with proliferative changes, corneal involvement, and refractory to conventional anti-allergic agents and/or steroids treatment. Furthermore, we distinguished facial AD of AKC from VKC. When the

epithelial and epidermal cytokines (e.g., IL-25, IL-33, and thymic stromal lymphopoietin [TSLP]) are released in lesional skin of AD, these can more stimulate group 2 innate lymphoid cells (ILC2) to release type 2 cytokines (e.g., IL-5 and IL-13)^{30,31}. Thus, it is necessary to corroborate not only ocular findings but also dermatological findings in the treatment of AKC.

It is noteworthy that the significant efficacy of the treatment in this study was found within 2 weeks of initiation, as well as in previous short-term studies, despite targeting only patients with extremely severe AKC. We don't know why, but there was no significant difference 2 months after beginning tacrolimus eye drop treatment in total scores. However, despite the stringency of the P value (i.e., $P = 0.007$), it is an undeniable fact that the total scores of objective signs decreased. It was very interesting that the effects of tacrolimus eye drops can continue, even considering seasonal fluctuations throughout the course of an entire year.

Decreasing the dose of steroids can prevent several adverse events such as glaucoma, cataract, and infection. In our study, elevated IOP was observed in seven eyes. However, five of those were treated with combined steroid therapy. Because IOP was measured using non-contact tonometry, it may have measured higher due to the poor condition of the eyelid (e.g., lid swelling and hard skin), or secondary to difficulty with lid opening

or closure. However, topical tacrolimus treatment was also confirmed to increase risk of herpetic infection (Table 3).

The cases which kept using steroids after the initiation of tacrolimus were aimed to prevent the reactivation of AKC (Figure 4). It seemed to be no significant difference in the use of steroids during the course of the year because we gradually decreased the dose of oral steroids and switched topical betamethasone to fluorometholone in the most severe cases. Therefore, the number of oral steroids had drastically decreased in one month, whereas the frequency of topical fluorometholone was gradually increased (Figure 4). But no serious systemic adverse events were observed in the current study.

As previously mentioned, the borderline of definitive diagnosis between AKC and VKC is vague in actual clinical practice. According to a Japanese guideline¹, most AKC patients experience no proliferative changes, but most cases of VKC have proliferative changes such as cobblestone papillae of the palpebral conjunctiva accompanied by AD. Nevertheless, only severe AKC with proliferative changes and AD (cf. Figure 2) were considered in this study. Although VKC generally disappears during the end of the second decade of life³², AKC can be prolonged or even life-long. There may be a contraindication in patients who are under the age of 20 diagnosed with VKC with atopic changes on their face, which can become AKC in the transition to adulthood. We

should consider how to distinguish between AKC from VKC more clearly, and the

definition of mild, moderate and severe ACDs again in the near future.

The current study had several limitations. First, this was a retrospective study that presumably covered all seasons and the patients did not all start the medication during the same month. Because most of the patients were adolescents, there was a possibility that eye drop compliance was poor, or clinical deterioration occurred due to sporting activities and/or antigen exposures. Nevertheless, the efficacy and safety of tacrolimus eye drops were positive throughout the year. Although a prospective study design would be better in order to achieve more exact analysis. Second, the number of patients in this study was smaller than in previous investigations^{13,29}. Our results provide the first evidence supporting long-term treatment with tacrolimus eye drop in eyes with severe AKC. In the future, additional and larger comparative studies of tacrolimus eye drop treatment in subjects with severe AKC are needed. Third, we did not evaluate subjective symptoms (i.e., itching, discharge, lacrimation, photophobia, foreign body sensation and eye pain). We should assess the correlation between the severity of subjective and objective symptoms in the future. Fourth, the current study did not exclude patients with dry eye. In fact, some studies have reported that patients with dry eye can experience worsening allergic changes^{33,34}, although few adolescent patients

236 have dry eye inherently^{35,36}.

237 In conclusion, the results of this study suggested that 0.1% tacrolimus ophthalmic

238 suspension is effective in the treatment of severe AKC refractory to standard

239 conventional steroid treatment throughout a one-year period.

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Table 1. Grading scores of ten clinical signs

Palpebral conjunctiva	①Hyperemia	0	None
		1	Dilatation of several vessels
		2	Dilatation of many vessels
		3	Impossible to distinguish individual blood vessels
	②Swelling	0	None
		1	Localized edema
		2	Diffuse mild edema
		3	Diffuse marked edema
	③Follicle	0	None
		1	1-9 follicles
		2	10-19 follicles
		3	20 or more follicles
	④Papillae	0	None
		1	Diameter 0.1-0.2 mm
		2	Diameter 0.3-0.5 mm
		3	Diameter ≥ 0.6 mm
	⑤Giant papillae (papillae size ≥ 1 mm)	0	None
		1	Flat giant papillae
		2	Elevated papillae in less than 1/2 of upper palpebral conjunctiva
		3	Elevated papillae in 1/2 or more of upper palpebral conjunctiva
Bulbar conjunctiva	⑥Hyperemia	0	None
		1	Dilation of several vessels
		2	Dilation of many vessels
		3	Vasodilatation of all vessels
	⑦Chemosis	0	None
		1	Localized chemosis
		2	Thinner diffuse chemosis
Limbus	⑧Swelling	3	Cyst-like chemosis of entire conjunctiva
		0	None
		1	In less than 1/3 of circumference
		2	In 1/3 to 2/3 of circumference
	⑨Trantas' dot	3	In $\geq 2/3$ of circumference
		0	None
		1	1-4 dots
		2	5-8 dots
Cornea	⑩Epithelial disorder	3	≥ 9 dots
		0	None
		1	SPK
		2	SPK with filamentary debris
		3	Shield ulcer or epithelial erosion

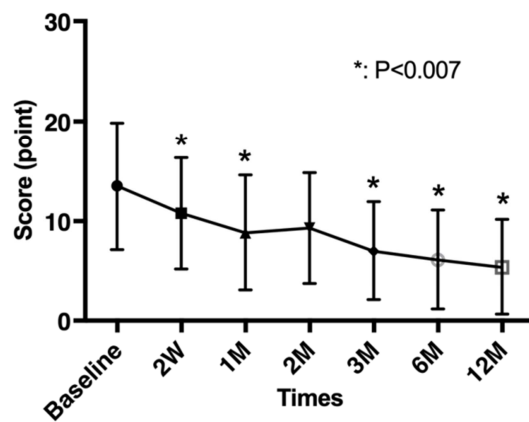
SPK: Superficial punctate keratitis

Table 2. Demographics of patients at baseline

Eyes	60
Sex, n (%)	
Male	26 (86.7)
Female	4 (13.3)
Age (y) $\pm$ SD	21.5 $\pm$ 13.7
Age of Onset (m) $\pm$ SD	43.2 $\pm$ 35.2
Other allergic complications, n (%)	
Asthma	4 (13.3)
Rhinitis	6 (20.0)
Atopic Dermatitis	30 (100)
Pretreatment, eyes (%)	
Topical antihistamine and/or mast cell stabilizer	41 (68.3)
0.1% fluorometholone (FLM)	25 (41.6)
0.1% betamethasone (BEM)	10 (16.6)
Topical cyclosporine	10 (16.6)
Tacrolimus ointment	2 (3.3)
Oral steroid	0 (0)

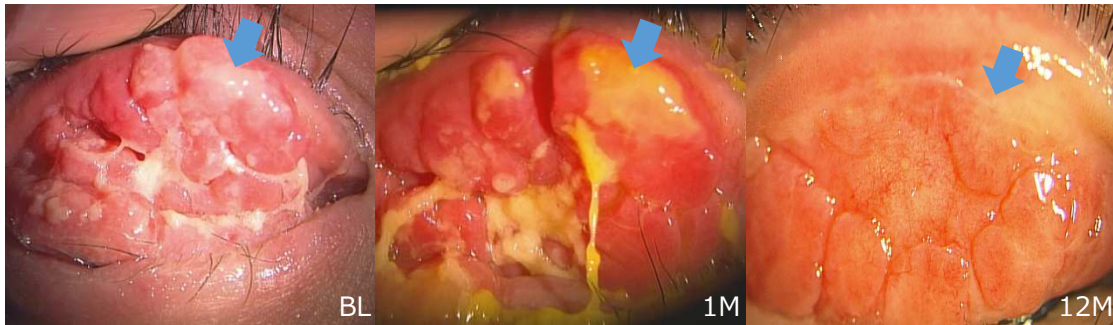
Table 3. Adverse events	Eyes (%)
Elevated IOP	7 (11.6)
Hordeolum	0 (0)
Chalazion	0 (0)
Lid herpes	0 (0)
Herpetic epithelial keratitis	3 (5.0)
Bacterial keratitis	0 (0)
Corneal erosion	0 (0)

Figure 1. Changes in the total scores of objective signs



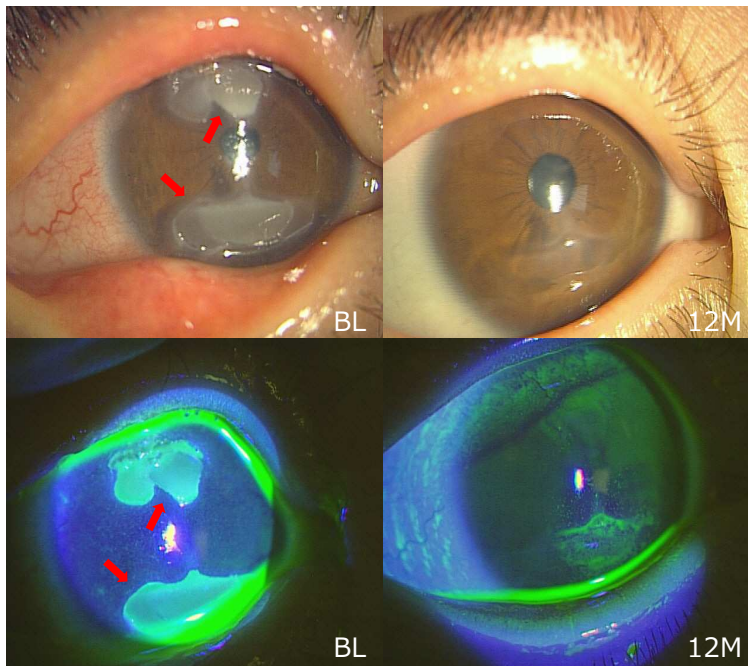
The total scores of clinical objective signs significantly decreased from baseline 2 weeks after beginning tacrolimus, except at 2 months. The mean total score was 13.6 ± 6.6 at the beginning of treatment and decreased to 10.8 ± 5.5 , 8.8 ± 5.8 , 9.3 ± 5.5 , 7.0 ± 4.9 , 6.1 ± 4.9 and 5.4 ± 4.8 at 2 weeks, and 1, 2, 3, 6 and 12 months after initiation.

Figure 2. Changes in the palpebral conjunctiva (14-year-old male, right eye)



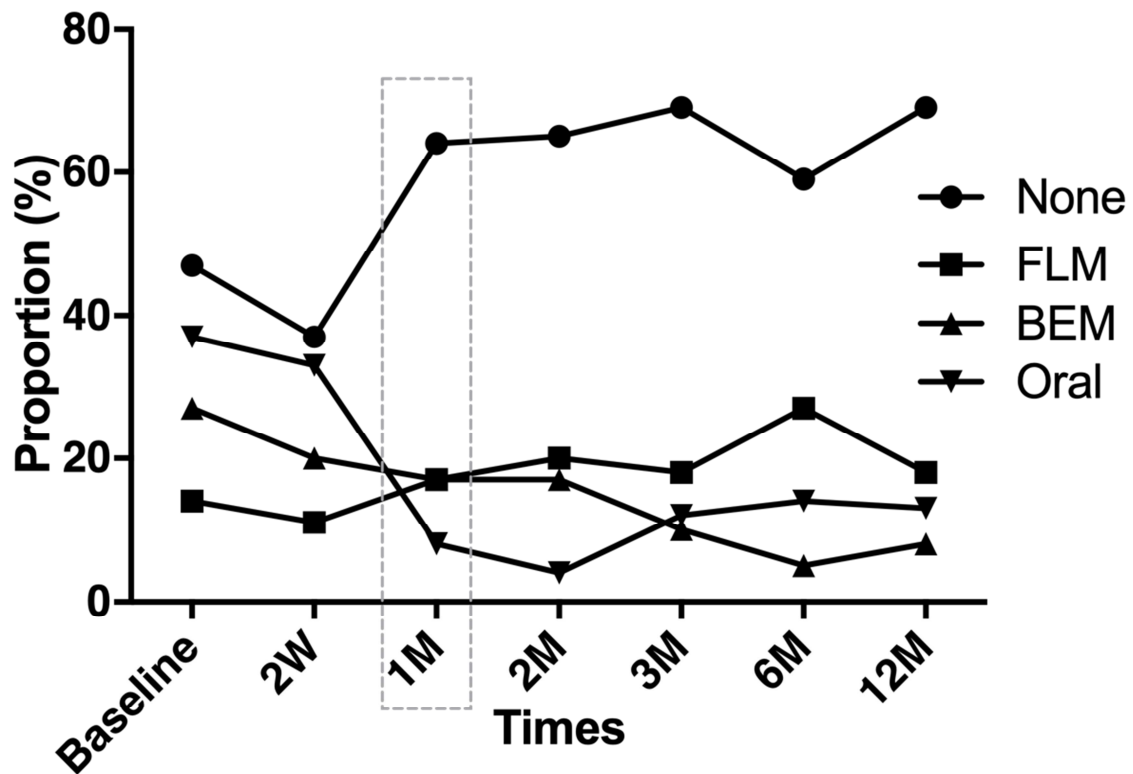
A combination of topical 0.1% betamethasone and 0.1% tacrolimus ophthalmic suspension was started in order to control severe atopic conjunctivitis with giant papillae which were shown by arrows (BL). After 1 month, inflammation started to decrease (1M). Topical steroid use was discontinued and only tacrolimus treatment was started after 6 months. Twelve months after initial treatment, the clinical signs of palpebral conjunctiva drastically improved (12M).

Figure 3. Changes in bulbar conjunctiva & limbus & cornea (8-year-old male, right eye)



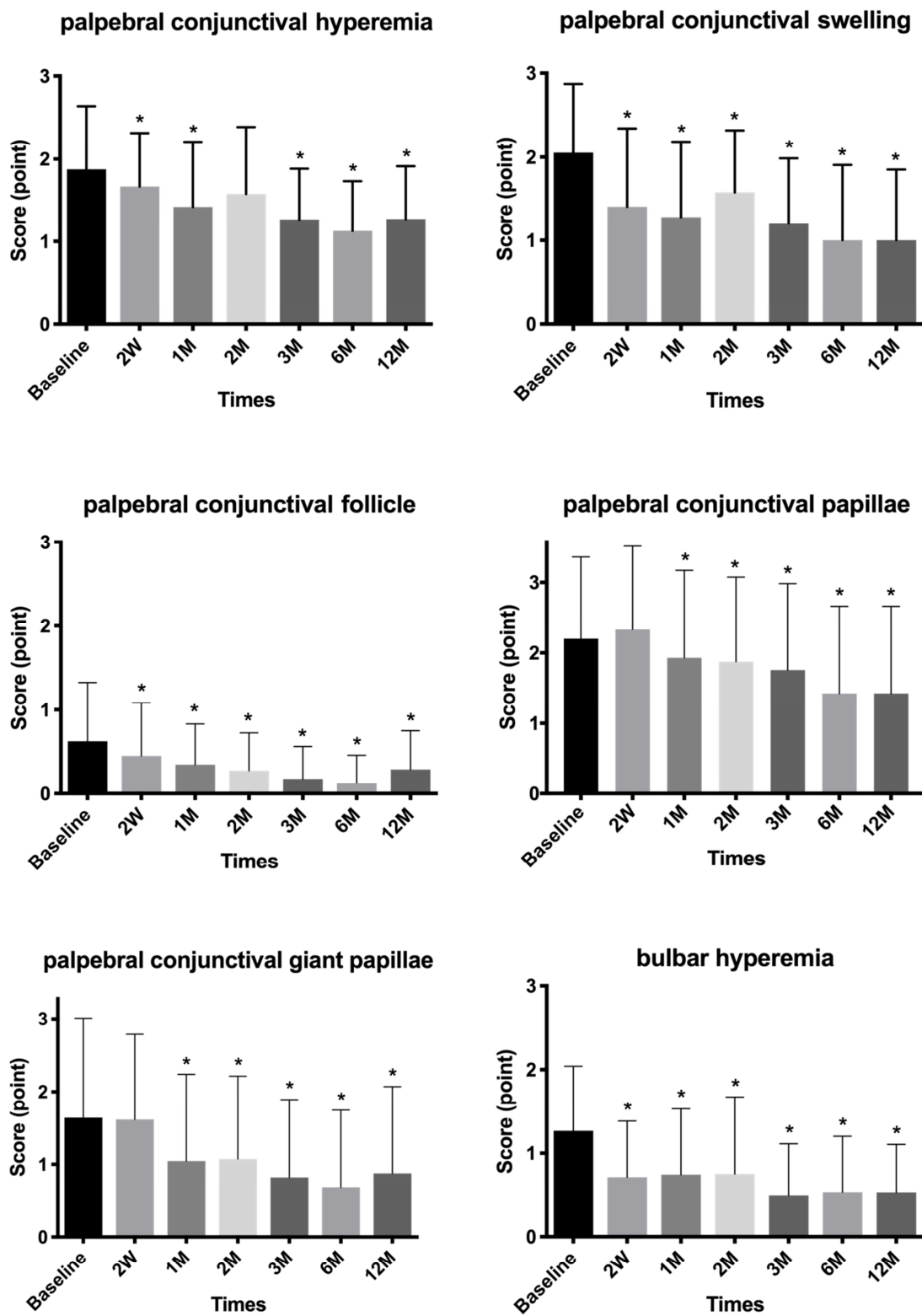
A combination of topical 0.1% fluorometholone and 0.1% tacrolimus ophthalmic suspension was started in order to control severe atopic keratitis with corneal plaques which were shown by arrows (BL). Twelve months after initial treatment, shield ulcer was completely disappeared (12M).

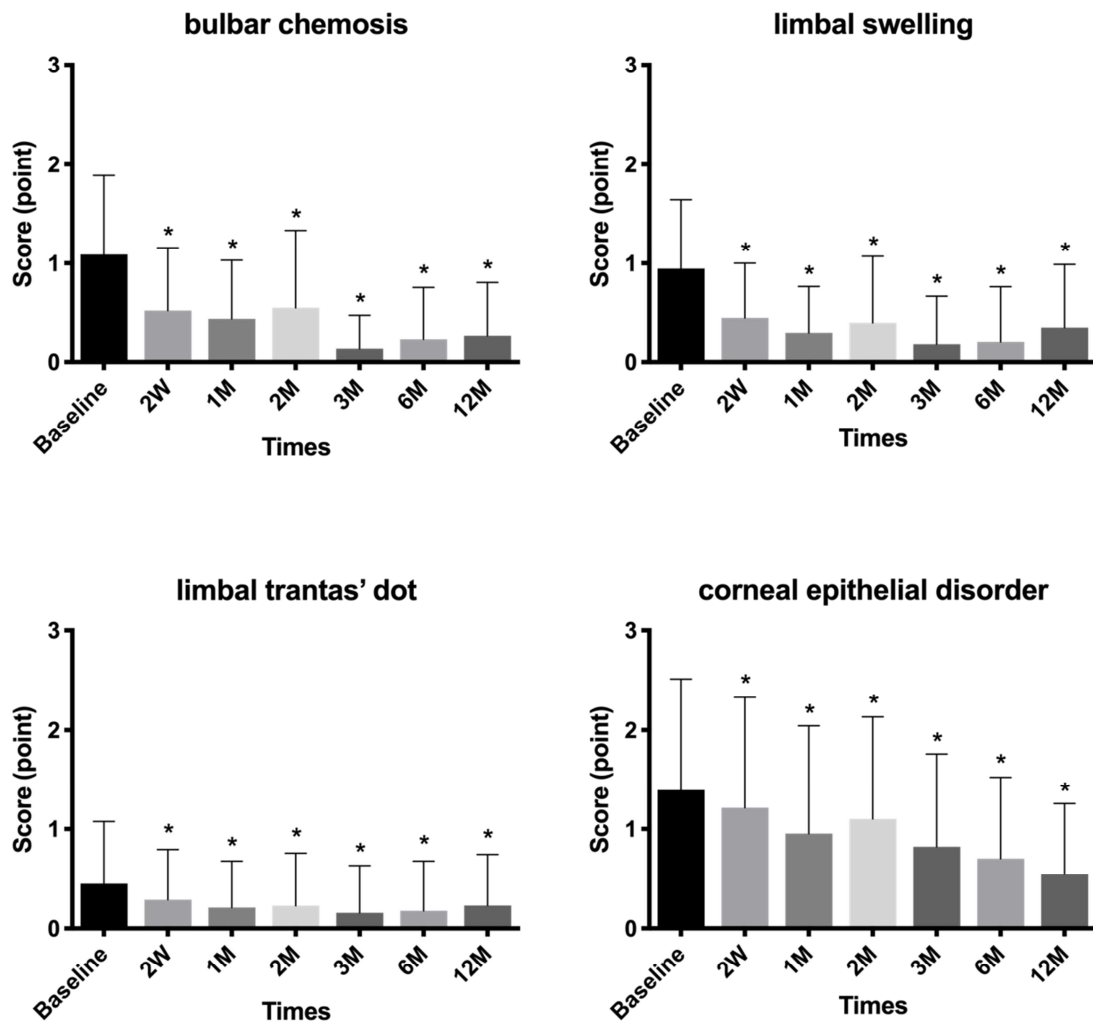
Figure 4. Changes in steroid use in combination with tacrolimus



None: No steroid treatment, FLM: 0.1% fluorometholone,
BEM: 0.1% betamethasone, Oral: Oral steroid treatment

The percentage of eyes using each type of steroid decreased from baseline 1 month after beginning tacrolimus. Of all eyes, 69.4% that were treated with tacrolimus alone or in combination with topical anti-allergic agents, were successfully weaned off topical steroids into tacrolimus treatment at 12 months. The percentage of eyes with 0.1% fluorometholone eye drops was 18.4%, 0.1% betamethasone eye drops was 8.7%, and oral steroids was 12.5% at 12 months.

Supplementary Figure 1 Changes in each score of objective clinical signs

*: $P < 0.007$

Palpebral conjunctival hyperemia had no significant difference 2 months after beginning tacrolimus eye drop treatment as well as changes in the total scores. Palpebral conjunctival papillae and giant papillae had no significant difference 2 weeks after treatment. All other clinical signs had significant differences at all points.