useful for diagnosis of buckle infections. More than one pathogenic strains and unreported environmental strains could be detected if the molecular genetic approach were applied to those cases. Herein, we report the first case of an *Achromobacter* species-associated buckle infection diagnosed by use of a 16S rDNA clone library analysis.

Case presentation

A 56-year-old woman complained of purulent discharge and conjunctival hyperemia in her right eye. These symptoms began several months prior to the first visit to our hospital. Eighteen years prior, she had received an uneventful scleral buckling surgery using a solid silicone tire in her right eye for rhegmatogenous retinal detachment. Thirteen years after the surgery, she was administered oral cephem antibiotics once on suspicion of a buckle infection. Although the symptoms temporarily improved, chronic inflammation persisted for several years. Because subsequent topical quinolone and topical steroid treatments were ineffective, she visited our hospital for rigorous diagnosis and radical treatment. On the first visit, the best-corrected visual acuity was 20/200 in the right eye. Observation by a slit lamp microscope revealed conjunctival hyperemia, purulent discharge, and episcleritis. A conjunctival fistula was also observed in the upper quadrants, and large yellowish conjunctival follicles around the exposed buckle material were present (Figure 1). After examination, we removed the buckle material based on the diagnosis of recurrent buckle infection.

Pre-operatively, *Alcaligenes* and *Corynebacterium* species were isolated from the eye discharge. The bacterial identification and drug susceptibility tests were performed automatically using a MicroScan WalkAway 96 SI (Siemens Healthcare Diagnostics, Tokyo, Japan). During the surgery, a 120° solid silicone tire was removed and the scleral bed was irrigated with 0.5% moxifloxacin ophthalmic solution. Post-operatively, 300 mg/day of oral cefdinir was administered for 3 days, and both 0.5% moxifloxacin ophthalmic solution and 0.1% betamethasone sodium phosphate ophthalmic solution were administered 5 times daily for 2 weeks. After removal of the silicone tire, the symptoms improved rapidly. Retinal detachment had not recurred at this point.

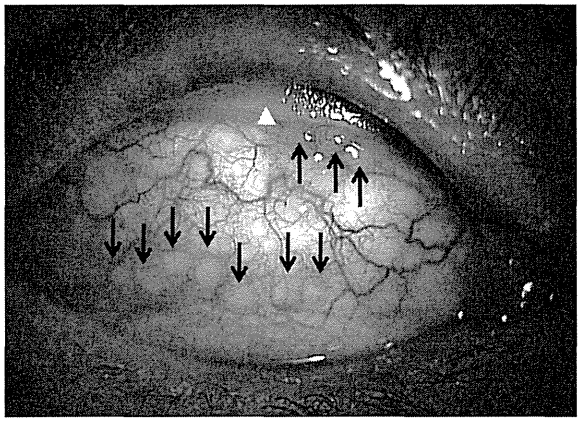

Figure 1 Pre-operative anterior segments photograph. The patient is looking downward. Conjunctival fistula in the upper quadrants and large yellowish conjunctival follicles (black arrows) around the exposed buckle material (white arrowhead) can be observed.

Many small yellowish-white deposits were found on the surface of the removed buckle material (Figure 2A). Gram staining of the deposits showed a large number of gram-negative rods. *Alcaligenes* and *Corynebacterium* species were also isolated from the buckle material. Species identification and drug susceptibility results were obtained through laboratory procedures identical to those performed preoperatively. The drug susceptibility of the *Alcaligenes* strain isolated from the buckle was identical to that of the strain preoperatively isolated from the eye discharge (Table 1). In the case of *Corynebacterium*, there was a definite discrepancy in the drug susceptibilities between the strains obtained pre- and postoperatively; the strain isolated from the eye discharge was resistant to cephalosporin, but the strain isolated from buckle depositions was susceptible to all antibiotics tested (Table 2). Microbiological examination of the removed buckle material indicated that the causative pathogen is a bacterium that belongs to the family Alcaligenaceae. We employed a 16S rDNA clone library analysis to identify the causative bacterium at the species level and to assess the possibility of the involvement of other uncultured species in the buckle infection. Initially, the buckle material was divided into two pieces, and one piece was stained with ruthenium red for examination by scanning electron microscope (SEM) (Figure 2B). The other piece was placed into 15 mL of phosphate-buffered saline (PBS) and sonicated repeatedly using a VialTweeter (Hielscher Ultrasonics Gmbh, Berlin, Germany) at 60 W for 15 min at room temperature. PBS was replaced twice, and the final sonicate was used for DNA extraction.

Bacterial DNA was extracted from 200 μL of the final PBS sonicate using Extrap Soil Kit Plus ver.2 (Nippon Steel Kankyo Engineering Co., Ltd., Tokyo, Japan). The 16S rDNA gene fragments were amplified with the purified DNA as a template and a universal eubacterial 16S rDNA primer set, 27f (5'-AGAGTTTGATCMTGGCT CAG-3') and Bac1392R (5'-ACGGGCGGTGTGAC-3'). After cloning the amplified products, the sequences were obtained from 24 clones using 27f as the sequencing primer. The low-quality sequences (Phred score <15) were trimmed, and the sequences were analysed for homology to NCBI database sequences using the Blast program. Of the 24 clones, high-quality sequences were obtained from 23 clones, but two of these were from the genomic regions other than 16S rDNAs. All of the partial 16S

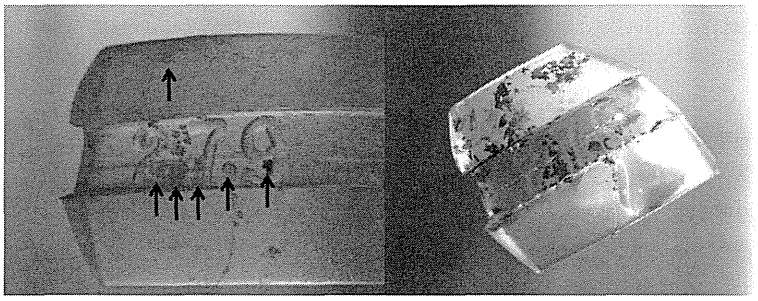

Figure 2 Pictures of the buckle material. (A) Buckle material immediately after the extraction. Many yellowish-white deposits (arrows) on the surface of the buckle material can be observed. **(B)** Ruthenium red staining. Deposits were stained red by ruthenium red for scanning electron microscopy.

rDNA sequences obtained from 21 clones showed the best match to those of *Achromobacter* species (Table 2; identity ranged from 99.4—99.8% over 99% of alignments with query sequences). It is likely that the isolates initially identified as *Alcaligenes* spp. were in fact *Achromobacter* spp. This misidentification was probably due to the low discriminatory power of the biochemical test for the species in the family Alcaligenaceae. Single nucleotide polymorphisms (SNPs) were observed among the sequences (12 sequences were identical). These SNPs might indicate that several different *Achromobacter* strains were present in biofilms on the buckle material, although this was only the sequence diversity among the ribosomal RNA operons in a single *Achromobacter* chromosome. To further refine the identification of causative bacterial species, the most predominant 16S rDNA sequences obtained were aligned with those from 43 reference species (obtained from Ribosomal Database Project ver. 10) in the family Alcaligenaceae. We aligned the 613-bp regions encompassed within well-conserved

Table 1 The drug susceptibilities of the strain of *Alcaligenes* sp. and the strain of *Corynebacterium* sp.

	Alcaligenes		*Corynebacterium*	
Antibiotic	**Discharge**	**Buckle**	**Discharge**	**Buckle**
Ampicillin	S	S	-	-
Penicillin G	R	R	-	-
Cefmenoxime	-	-	R	S
Ceftizoxime	R	R	R	S
Cefroxime	-	-	R	S
Cefepime	-	-	R	S
Cefpodoxime pivoxil	-	-	R	S
Azithromycin	-	-	R	S
Gentamicin	R	R	-	-
Tobramycin	R	R	-	-
Dibekacin	I	I	-	-
Arbekacin	I	I	R	S
Levofloxacin	I	I	S	S
Ciprofloxacin	S	S	S	S
Chloramphenicol	S	S	-	-
Imipenem/cilastatin	S	S	S	S
Meropenem	S	S	S	S

Hyphen: not performed. S: susceptible. I: intermediate. R: resistant.
Although the two strains of *Alcaligenes* sp. show the same profiles, the two strains of *Corynebacterium* sp. show different profiles.

Table 2 Summary of 16S rDNA clone library analysis of the infected buckle material

Sequence type[a)]	No. of clone[b)]	Best match[c)]
ST1	12	*Achromobacter spanius* strain LMG 5911 (631/633; 99.7%)
ST2	1	*Achromobacter spanius* strain LMG 5911 (628/631; 99.5%)
ST3	1	*Achromobacter spanius* strain LMG 5911 (628/632; 99.4%)
ST4	1	*Achromobacter spanius* strain LMG 5911 (627/630; 99.5%)
ST5	1	*Achromobacter spanius* strain LMG 5911 (630/632; 99.7%)
ST6	1	*Achromobacter spanius* strain LMG 5911 (623/625; 99.7%)
ST7	1	*Achromobacter spanius* strain LMG 5911 (627/628; 99.8%)
ST8	1	*Achromobacter spanius* strain LMG 5911 (630/632; 99.7%)
ST9	1	*Achromobacter spanius* strain LMG 5911 (627/628; 99.8%)
ST10	1	*Achromobacter spanius* strain LMG 5911 (624/625; 99.8%)

[a)]Twenty-one 16S rDNA sequences obtained (631 bp of high quality sequence) are classified basing on SNIPs.
[b)]Number of sequence belonging to each sequence type is shown.
[c)]Top hit microorganisms by which Blastn search of each sequence type indicated are listed.
The number in parenthesis shows identical base (bp)/alignment length (bp) to 16S rDNA from indicated species.

regions using the Clustal W program to adjust the positions to be compared. All 21 of the sequences were phylogenetically positioned closely with the sequences from *Achromobacter spanius* (Figure 3). Based on these results, we conclude that an *Achromobacter* sp. closely related to *A. spanius* was the causative agent in this case.

SEM of the buckle material showed numerous rod-shaped bacteria surrounded by a biofilm-like material,

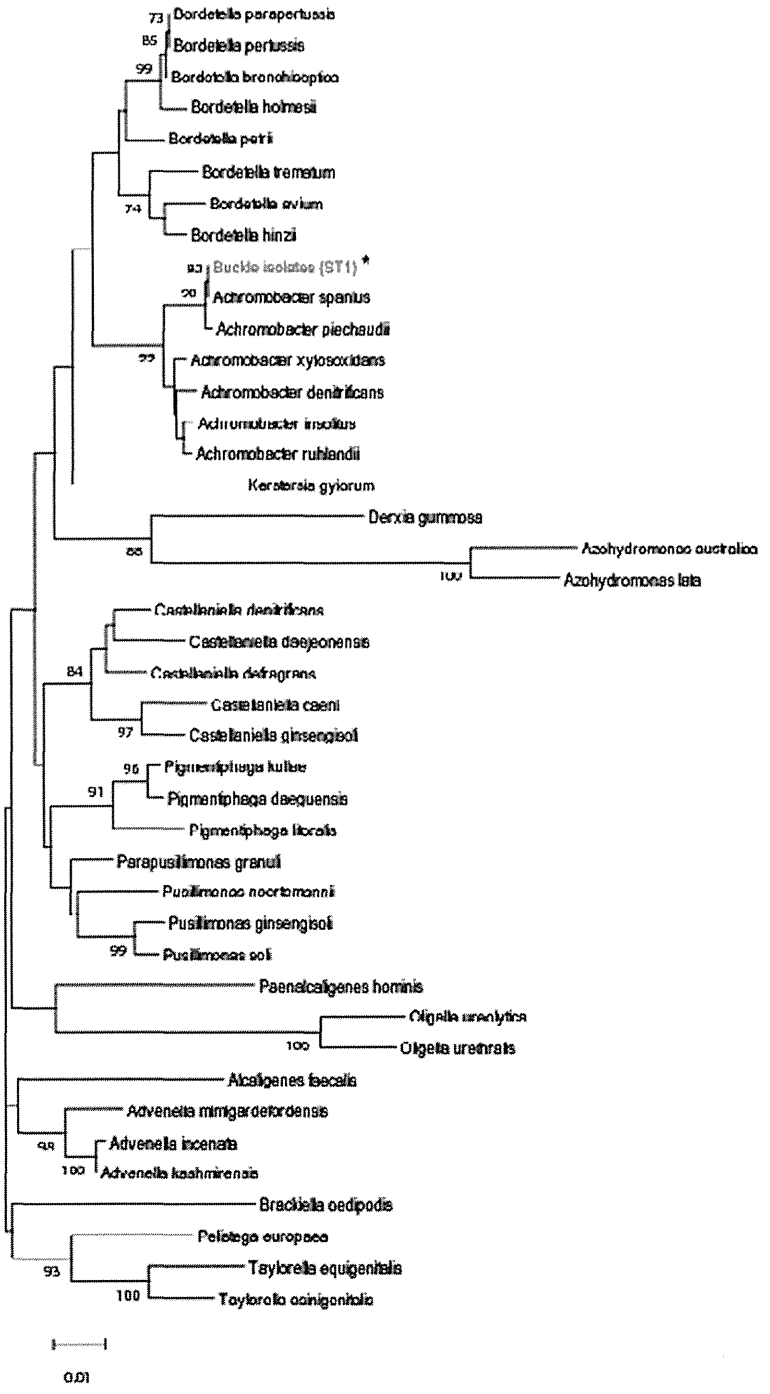

Figure 3 Phylogenetic relationship between the isolate from buckle material and other members of the family *Alcaligenaceae*. Only the most predominant ST1 sequence (indicated by red and asterisks) was analysed. The tree was constructed using the neighbour-joining algorithm. Numbers at nodes are bootstrap percentages based on 1,000 replications; only values >70% are shown. Bar, 0.01 substitutions per nucleotide position.

consistent with our conclusions from the 16S rDNA clone library analysis (Figure 4, A and B).

Conclusions

In clinical settings, cultivation and phenotypic tests of isolated bacteria employing traditional culture techniques is the first step in diagnosis of infectious diseases. In this case, we aimed to identify the causative pathogens for buckle infection by culturing the eye discharge and buckle material. These cultures resulted in the successful isolation of the two candidates, *Alcaligenes* and *Corynebacterium* species. We surmised that *Corynebacterium* spp. were a contaminant as they are one of the resident bacteria on the ocular surface [17], and *Corynebacterium* isolates from the discharge and buckle material showed different antimicrobial susceptibilities. Therefore, these different strains of *Corynebacterium* were most likely from the ocular surface. Correspondingly, the 16S rDNA sequences derived from *Corynebacterium* spp. were not identified in 16S rDNA clone library analysis. We presume that the *Corynebacterium* spp. were washed away by irrigation during surgery and sonication because they only attached to the surface of the buckle material and not embedded within biofilm.

Although *Alcaligenes* spp. were initially considered to be a causative agents, we had doubts about the microbiological identification based on the following observations. First, the isolate in this case showed resistance to aminoglycosides while the majority of *Alcaligenes* species have been reported to be susceptible to gentamicin [18]. Second, the taxonomy of the family Alcaligenaceae is continually revised and updated and the biochemical test is unreliable in discriminating *Alcaligenes* and *Achromobacter* due to their close phylogenetic relationship [19]. Device-related biofilm infections are often caused by opportunistic environmental pathogens and are often polymicrobial. The frequent discrepancy between direct microscopic counts and the number of culturable bacteria from environmental samples is one of several indications that we currently know very little about the diversity of microorganisms in nature [16]. In addition, precise species identification is typically problematic in environmental isolates. Therefore, we employed a 16S rDNA clone library analysis to precisely classify the isolate at the species level and to test the possibility that the biofilm in this case was polymicrobial and contained uncultivable environmental bacteria. Although 16S rDNA clone library analysis using 24 clones is insufficient for excluding the presence of other pathogenic strains, our results show that this case was buckle infection caused by an *Achromobacter* species alone that is closely related to *A. spanius*. To our knowledge, this is the first case report of buckle infection by *Achromobacter* sp. Reliable epidemiological data on bacterial isolates are important for empirical antimicrobial therapy; therefore, precise identification of bacterial species is essential.

Advances in surgery are expected to increase the opportunities for embedding medical devices within the body with a concomitant increase in the risk for device-related infections by opportunistic environmental pathogens. In fact, there are some reports describing *Achromobacter*-related infections from artificial devices such as prosthetic knee joints and contact lenses [20,21]. Clinicians should take into account the inherent limitations of traditional microbiological assays and combine various approaches to obtain precise diagnoses when necessary. These efforts will likely increase the reliability of epidemiological data in the field of infectious diseases.

The taxonomy of the genus *Alcaligenes* is closely intertwined with that of the genus *Achromobacter* and is frequently revised [19]. *Alcaligenes* has also been isolated from clinical specimens, including ophthalmic samples [12,22-29]. Coenye *et al.* reported that several isolates identified phenotypically as *Alcaligenes* species belonged to the genus *Achromobacter* based on genetic analysis, and they proposed two novel *Achromobacter* species from these isolates [30]. It is important clinically to discriminate *Alcaligenes* and *Achromobacter* because epidemiological data demonstrate that 72.7% of clinical *Achromobacter*

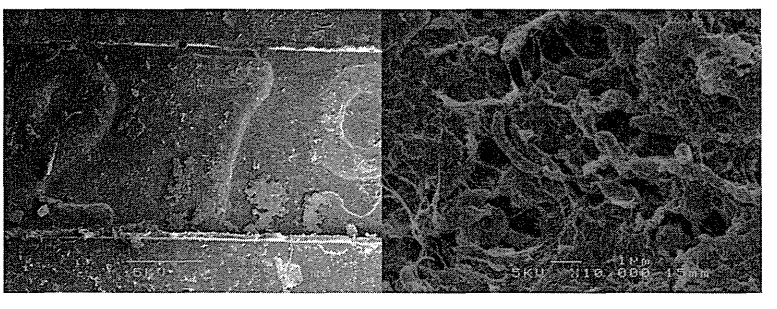

Figure 4 Scanning electron microscopic images of the buckle material. (A) Low magnification. Deposits attached after fixation by glutaraldehyde. **(B)** High magnification. Numerous rod-shaped bacteria surrounded by biofilm-like material are observed.

isolates showed multi-drug resistance while all of the *Alcaligenes* isolates tested were susceptible to imipenem, gentamicin, and ciprofloxacin [18]. With regard to the current clinical case, drugs to which *Achromobacter* spp. are potentially susceptible were initially administered, followed by the administration of drugs to which *Achromobacter* spp. are known to be susceptible. However, inflammation around the buckle material continued for several years. SEM observations were indicative of the long clinical course, recurrent symptoms, and *Achromobacter*'s resistance to antibiotic treatment. Therefore, the *Achromobacter*-associated buckle infection case reported here is valuable for considering the epidemiology and antimicrobial therapy of ophthalmic infections. The emergence of device-related infections caused by *Achromobacter* may be intractable, even when efficacious antibiotics are administered.

In conclusion, *Achromobacter* spp. should be recognized as causative agents for device-related ophthalmic infections. Molecular species identification by 16S rDNA sequence analysis should be combined with conventional cultivation techniques to investigate the significance of *Achromobacter* spp. in ophthalmic infections.

Consent

Written informed consent was obtained from the patient for publication of this case and the accompanying images.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

FK, KK, and TN treated the patient. HE performed molecular genetic investigations, made the final diagnosis, and wrote the manuscript. YM and TK reviewed the manuscript. All authors read and approved the final manuscript.

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ORIGINAL RESEARCH

Clinical Usefulness of Diquafosol for Real-World Dry Eye Patients: A Prospective, Open-Label, Non-Interventional, Observational Study

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ABSTRACT

Introduction: This study was designed to evaluate the efficacy and safety of 3% diquafosol ophthalmic solution in dry eye patients in clinical practice.

Methods: Subjects were dry eye patients who had never used diquafosol, and observation was conducted prospectively over 2 months. The corneal and conjunctival fluorescein staining score, tear film break-up time, 12 dry eye-related subjective symptoms, patient-reported outcomes, and adverse events were investigated.

Results: Data were collected from 465 medical institutions for 3,196 patients. Diquafosol led to significant improvement in all subjective symptoms and objective findings ($P < 0.001$, paired t test). Diquafosol was effective regardless of the degree of severity according to the corneal and conjunctival fluorescein staining score or therapeutic pattern. Overall, 76.0% patients responded that their condition had improved. Adverse reactions were observed in 6.3% of patients. The major adverse reactions were eye discharge, eye irritation, and eye pain.

Conclusion: Diquafosol was effective for various dry eye patients in clinical practice, and no significant safety-related problems occurred.

Keywords: Corneal and conjunctival fluorescein staining score; Diquafosol; Dry eye; Observational post-marketing study; Ophthalmology; Patient-reported outcome; Subjective symptom

Electronic supplementary material The online version of this article (doi:10.1007/s12325-014-0162-4) contains supplementary material, which is available to authorized users.

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INTRODUCTION

Dry eye, a chronic disease of the tear film and corneal and conjunctival epithelium that can be caused by several conditions, is accompanied by eye discomfort and visual function abnormalities [1, 2]. The causes of tear film and corneal and conjunctival epithelial disorders may be intrinsic (e.g., Sjögren's syndrome and meibomian gland dysfunction) or extrinsic [low humidity, contact lens use, and work involving visual display terminals (VDTs)] [2]. In recent years, the prevalence of dry eye disease has risen with increasing levels of environmental pollution, higher numbers of people who work on VDTs, drier indoor environments caused by the use of air-conditioning systems, and the spread of contact lens use, and laser in situ keratomileusis (LASIK) procedures [3, 4]. These trends appear likely to continue in the future.

Before the approval of 3% diquafosol ophthalmic solution (Diquas ophthalmic solution 3%; Santen Pharmaceutical Co., Ltd., Osaka, Japan), the main medications for dry eye in Japan had been artificial tears and sodium hyaluronate. Diquafosol, a dinucleotide derivative, acts on the $P2Y_2$ receptors on the cell membrane of the conjunctival epithelial and goblet cells, promoting water secretion from conjunctival epithelial cells [5] and mucin secretion from conjunctival goblet cells [6]. $P2Y_2$ receptors are a type of nucleotide [adenosine triphosphate (ATP) and uridine triphosphate (UTP)] receptor found throughout the body. On the ocular surface, $P2Y_2$ receptors can be found in the cornea, conjunctiva, and meibomian glands [7]. ATP and UTP promote water and mucin secretion [8–10]. Research has shown that diquafosol was superior to a placebo [11]; in a double-blind clinical trial with sodium hyaluronate, diquafosol exhibited effects similar and superior to those of sodium hyaluronate on corneal and conjunctival disorders, respectively [12]. Diquafosol has also been reported as effective and safe during a 52-week open-label clinical trial [13]. Based on these results, diquafosol was launched in Japan in December 2010.

However, there were many limitations on the patients who participated in these clinical trials. For example, patients aged <20 years, those with no corneal and conjunctival epithelial disorders, those with eye complications, and contact lens wearers were excluded. Furthermore, the use of eye drops other than diquafosol was prohibited during the trial period. Because various types of dry eye patients were excluded in these trials, the efficacy and safety of diquafosol in clinical practice has remained unclear. Here, we evaluated the efficacy and safety of diquafosol in over 3,000 dry eye patients in the real-world setting.

METHODS

Study Design

This study was conducted on the basis of a request from a regulatory agency in Japan. It was a multi-institutional, prospective, open-label, non-interventional, observational study that conformed to the Good Post-Marketing Study Practice (Ministry of Health, Labor and Welfare ordinance 171, December 20, 2004). To remove selection bias, patients were registered by a central registration method. During an observation period covering 2 months after initial diquafosol administration, its efficacy and safety were evaluated. The study protocol

was reviewed and approved in advance by the Pharmaceuticals and Medical Devices Agency, Japan. For this reason, no ethical review by the individual facilities participating in the study was conducted.

Patients

We included dry eye patients who had never used diquafosol before and who were registered within 2 weeks of being prescribed diquafosol. The dry eye diagnosis had been made by different individual physicians. Because informed consent was not required for post-marketing observational studies that were requested by the regulatory agency in Japan, the present study did not solicit informed consent from the patients.

Outcome Measure

Efficacy was evaluated according to subjective symptoms, objective findings, and patient-reported outcomes (PROs). Information related to the subjective symptoms and objective findings was obtained at baseline and 1 and 2 months after the initiation of diquafosol treatment. The subjective symptoms included 12 dry eye-related symptoms (foreign body sensation, photophobia, itchiness, eye pain, dryness, heavy sensation, blurred vision, eye strain, eye discomfort, eye discharge, tearing, and red eye). Each symptom was scored as follows: no symptoms (0 points), mild symptoms (1 point), moderate symptoms (2 points), and severe symptoms (3 points). Objective findings were the corneal and conjunctival fluorescein (FL) staining score and tear film break-up time (TBUT). For the corneal and conjunctival FL staining score, the cornea was divided into three sections: superior, middle, and inferior; the conjunctiva was divided into the nasal and temporal sides. Each of the five areas was scored as follows [12]: no staining (0 points), mild staining (1 point), moderate staining (2 points), and severe staining (3 points). With a blue-free barrier filter, conjunctival disorders can be evaluated by FL staining [14]. The clearing rate was the percentage of patients that had an FL staining score of ≥ 1 in the middle cornea at baseline whose score reached 0 after the initiation of diquafosol treatment. TBUT was the time for a dry spot to appear in the tear film after opening the eyes after normal blinking. PROs were investigated 2 months after the initiation of diquafosol treatment with a five-point scale from 1 to 5 as follows: much better (1 point), better (2 points), unchanged (3 points), worse (4 points), and much worse (5 points). These were compared with PROs before diquafosol treatment. Using information regarding previous and concomitant medications, therapeutic patterns were classified as follows: naïve monotherapy (no previously used medication or concomitant medication), add-on to sodium hyaluronate (added concomitantly to sodium hyaluronate, which had been previously used), switch from sodium hyaluronate (switch from sodium hyaluronate, which had been previously used), naïve combination therapy with sodium hyaluronate (no previously taken medication, concomitant use with sodium hyaluronate), and other (other than those listed above). Safety was evaluated from the data on all adverse events that occurred during diquafosol treatment. Adverse events for which a causal relationship could not be rejected were considered to be associated adverse reactions.

Statistical Analysis

Tabulation and analysis were performed using SAS (Version 9.2, SAS institute, Cary, NC, USA). The eye (left or right) used for determining efficacy was selected according to the following

rules: (1) the eye affected by dry eye and being administered diquafosol was used; (2) if both eyes conformed to rule 1, the eye with the higher baseline corneal and conjunctival FL staining score was used; and (3) if an eye was unable to be selected according to rules 1 and 2, the right eye was used. The corneal and conjunctival FL staining score, subjective symptoms, and TBUT at baseline and after 1 and 2 months were compared using paired *t* test. The level of significance was set at 5% for both sides.

RESULTS

Patient Characteristics

Data for 3,196 patients from 465 medical institutions were analyzed. The registration period of this study was from January 2011 to June 2012. Table 1 shows study population characteristics. Of the 3,196 patients, most were females (2,740 patients; 85.7%). The mean age was 62.4 years (range 7–97 years). Many patients (87.5%) had recorded TBUTs of ≤5 s. Most patients had low corneal and conjunctival FL staining scores (of a possible 15 points), with 0 points in 17.4% of patients and 1–3 points in 39.5% of patients. Concurrent disease included allergic conjunctivitis (16.5%), conjunctivochalasis (5.6%), Sjögren's syndrome (5.3%), and meibomian gland dysfunction (3.8%). During the study period, 195 patients (6.1%) were contact lens wearers. The therapeutic patterns of naïve monotherapy (1,227 patients 38.4%) and as an add-on to sodium hyaluronate (1,008 patients 31.5%) included approximately 70% of patients. Regarding the number of times diquafosol was used per day, 69.9% of patients used the eye drop six times per day and 21.5% of patients used four times per day.

Efficacy Evaluation

All Patients

The mean corneal and conjunctival FL staining scores were: 3.5 points at baseline, 1.9 points at 1 month, and 1.6 points at 2 months. Compared with baseline, scores at 1 and 2 months were significantly decreased (Fig. 1). The mean TBUTs were: 3.6 s at baseline, 4.7 s at 1 month, and 4.9 s at 2 months. Compared with baseline, TBUTs at 1 and 2 months were significantly prolonged (Fig. 1). The mean subjective symptom total scores (of a possible 36 points) for all 12 symptoms were: 7.8 points at baseline, 5.0 points at 1 month, and 4.3 points at 2 months. Compared with baseline, scores at 1 and 2 months were significantly decreased (Fig. 1). Of these 12 symptoms, the scores at baseline were high for dryness (1.3 points), foreign body sensation (1.1 points), eye discomfort (1.0 points), and eye strain (0.8 points). For these four symptoms, scores significantly decreased at 1 and 2 months compared with baseline (Fig. 2). Furthermore, the scores for symptoms other than those listed above significantly decreased in a similar manner (except for eye discharge at 1 month). The clearing rate for the middle cornea was 47% and 54% at 1 and 2 months, respectively, after diquafosol treatment (Fig. 3).

Results According to the Degree of Severity Measured According to the Corneal and Conjunctival Fluorescein Staining Score

Based on the baseline corneal and conjunctival FL staining score, we divided all patients into mild (0–3 points), moderate (4–8 points), and severe (9–15 points) groups, and the changes in the objective findings and subjective symptoms were investigated according to the degree of severity. The corneal and conjunctival FL staining score, TBUT, and subjective symptoms

Table 1 Study population characteristics

Characteristics	Number of patients (%)
Sex	
Male	456 (14.3)
Female	2,740 (85.7)
Age (years)	
<20	25 (0.8)
20–29	90 (2.8)
30–39	220 (6.9)
40–49	330 (10.3)
50–59	450 (14.1)
60–69	822 (25.7)
70–79	921 (28.8)
≥80	338 (10.6)
Shirmer value (mm)[a]	
≤5	557 (49.6)
>5, ≤10	346 (30.8)
>10	219 (19.5)
TBUT (s)[a]	
≤5	2,532 (87.5)
>5	363 (12.5)
Fluorescein staining score[a]	
0	529 (17.4)
1–3	1,199 (39.5)
4–8	1,114 (36.7)
9–15	195 (6.4)
Concurrent disease	
Allergic conjunctivitis	528 (16.5)
Conjunctivochalasis	180 (5.6)
Sjögren's syndrome	162 (5.3)
Meibomian grand dysfunction	122 (3.8)
Past history of LASIK	29 (0.9)
Contact lens wearers	195 (6.1)
Therapeutic pattern	
Naïve monotherapy	1,227 (38.4)
Add-on to SH	1,008 (31.5)
Switch from SH	322 (10.1)
Naïve combination therapy with SH	270 (8.4)
Other	369 (11.5)
Dose (times per day)	
≤3	100 (3.1)
4	688 (21.5)
5	167 (5.2)
6	2,234 (69.9)
8	7 (0.2)

LASIK laser in situ keratomileusis, *SH* sodium hyaluronate, *TBUT* tear film break-up time

[a] Patients with unknown data were excluded; Shirmer value (2,074), TBUT (301), and fluorescein staining score (159)

improved significantly from baseline regardless of the degree of severity (Fig. 4).

Stratification According to the Therapeutic Pattern

Changes in objective findings and subjective symptoms were evaluated according to the therapeutic patterns of naïve monotherapy, add-on to sodium hyaluronate, switch from sodium hyaluronate, and naïve combination therapy with sodium hyaluronate. Regardless of the therapeutic pattern, the corneal and conjunctival FL staining score, TBUT, and subjective symptoms significantly improved at 1 and 2 months compared with baseline (Fig. 5).

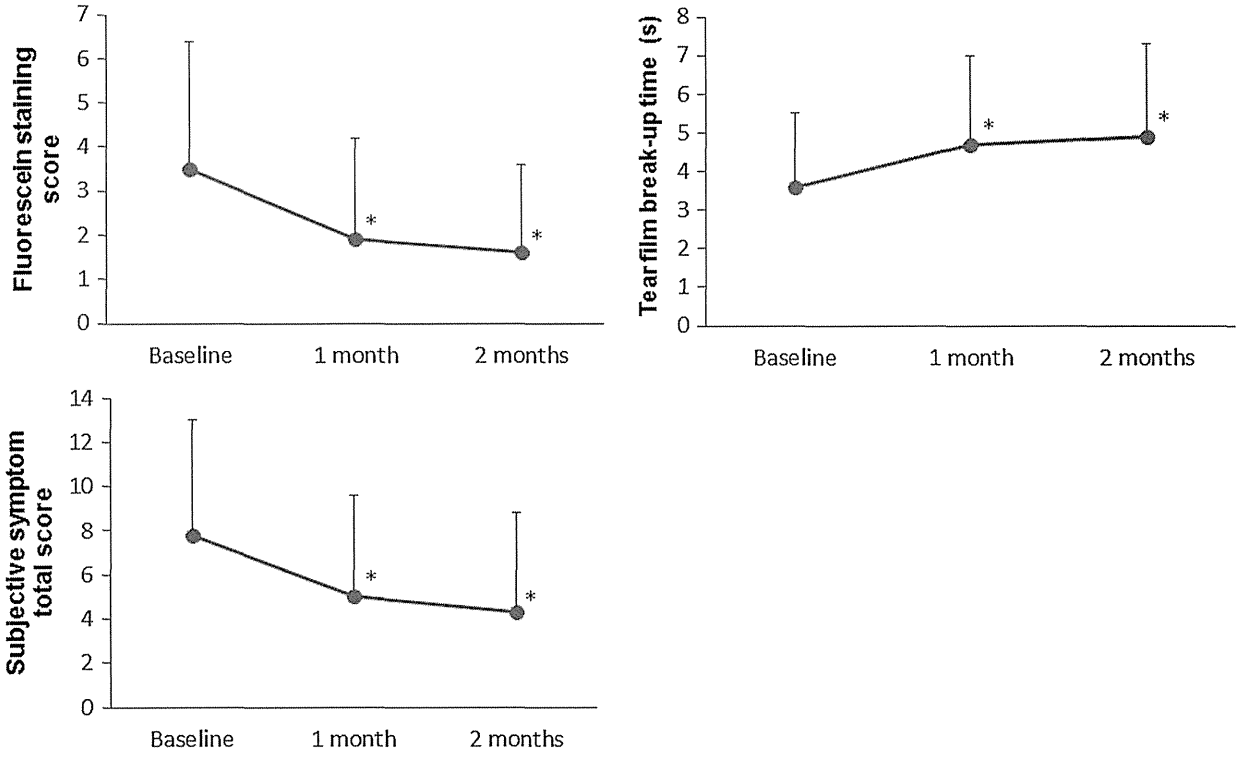

Fig. 1 Change in the fluorescein staining score (*top left*), tear film break-up time (*top right*), and subjective symptom total score (*bottom left*) after diquafosol treatment. Compared with baseline, the fluorescein staining score, total subjective symptoms, and tear film break-up time significantly improved at 1 and 2 months. Each *data point* represents the mean ± standard deviation. $^{*}P < 0.001$ compared with respective baseline, paired *t* test

Patient-Reported Outcomes

Figure 6 shows the percentage of patients who noted that their condition was "much better" or "better" than before diquafosol treatment. Of the 3,020 patients surveyed for PROs, 76.0% of patients responded that their condition had improved. Even in a patient subgroup with Sjögren's syndrome, 67.1% felt that their symptoms were alleviated. Regarding the therapeutic patterns, over 80% of patients who had not been previously treated and over 70% of patients who had been previously treated with sodium hyaluronate responded that their condition had improved compared with that at baseline.

Safety Evaluation

Adverse reactions appeared in 203 (6.3%) of the 3,196 patients surveyed. Table 2 describes the major adverse reactions that occurred at a frequency of >0.5%. Table 3 categorizes the incidence rate of eye irritation and eye pain by patient background. There were no notable differences in incidence rates based on sex or age (non-geriatric, geriatric). Incidence rates tended to be higher in patients with lacrimal disorders. Furthermore, incidence rates tended to increase in patients with higher corneal and conjunctival FL staining scores.

DISCUSSION

This study included patients who were excluded from clinical trials. For all patients diquafosol improved corneal and conjunctival epithelial disorders, TBUT, and subjective symptoms in a

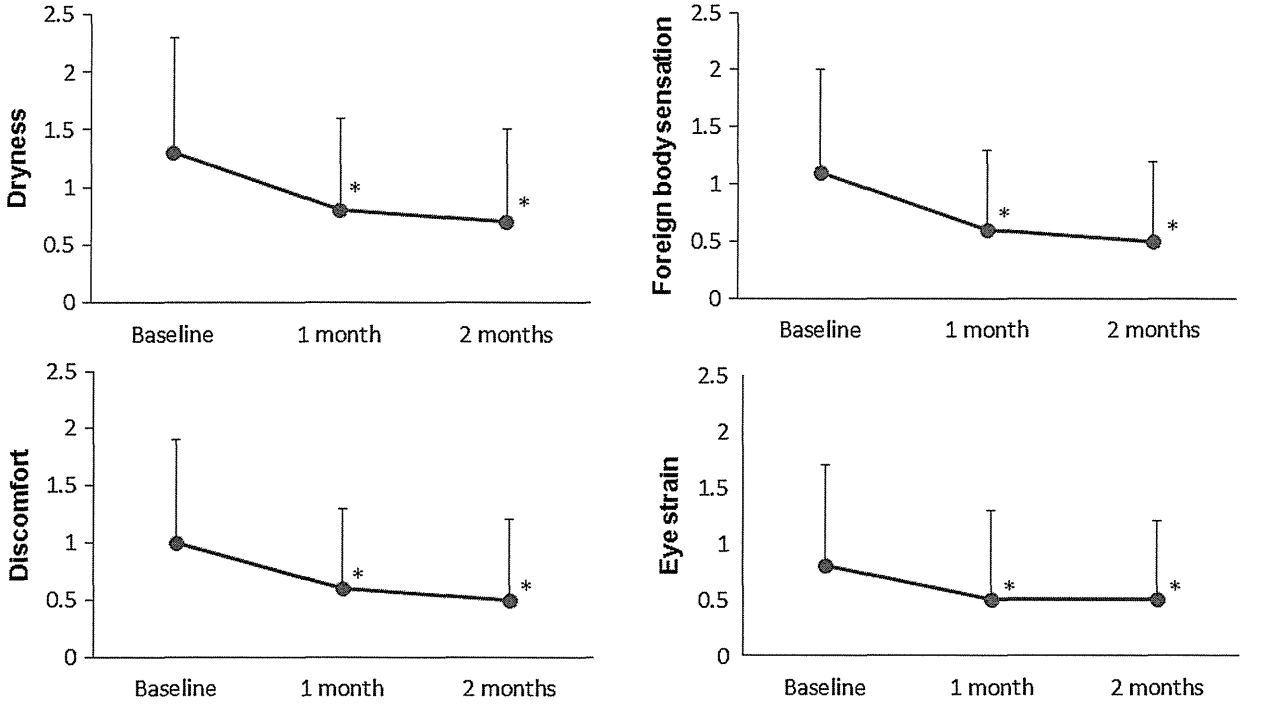

Fig. 2 Change in four major ocular symptoms, dryness (*top left*), foreign body sensation (*top right*), eye discomfort (*bottom left*), and eye strain (*bottom right*), after diquafosol treatment. Compared with baseline, the score of these symptoms significantly improved at 1 and 2 months. Each *data point* represents the mean ± standard deviation. $^{*}P < 0.001$ compared with respective baseline, paired *t* test

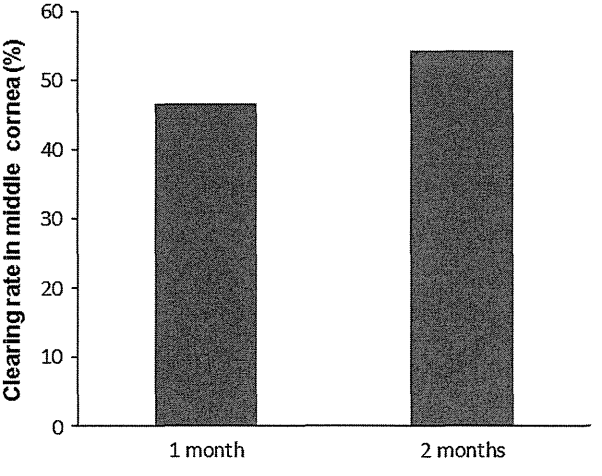

Fig. 3 Change in the clearing rate of the middle cornea after diquafosol treatment

similar fashion as was observed in the clinical trials.

We scored the cornea by dividing it into superior, middle, and inferior sections. Because epithelial disorders of the corneal center directly affect visual function, they can lead to a decreased quality of vision [15]. After 1 month of diquafosol treatment, epithelial disorders in the middle cornea had disappeared in approximately 50% of patients. In a comparison study with sodium hyaluronate, the researchers found that the clearing rates for the middle cornea with diquafosol were superior to those achieved with sodium hyaluronate [12].

We investigated the effect of diquafosol by dividing all patients into mild, moderate, and severe groups according to the corneal and conjunctival FL staining score. We confirmed that diquafosol exhibited effects regardless of the disease severity according to the corneal and conjunctival FL staining score. Diquafosol may improve objective findings and subjective

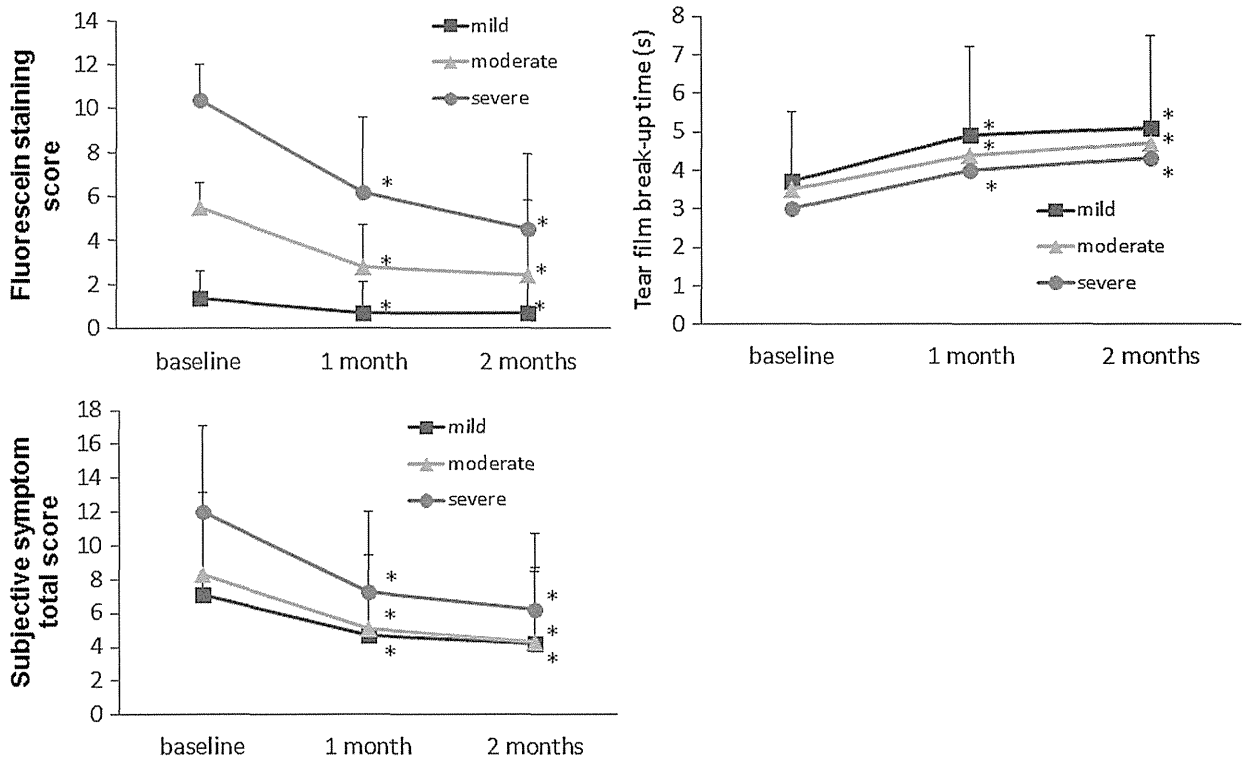

Fig. 4 Each severity group's change in the fluorescein staining score (*top left*), tear film break-up time (*top right*), and subjective symptom total score (*bottom left*) after diquafosol treatment. Regardless of the severity, the fluorescein staining score, total subjective symptoms, and tear film break-up time significantly improved at 1 and 2 months compared with baseline. Each *data point* represents the mean ± standard deviation. $^{*}P < 0.001$ compared with respective baseline, paired *t* test

symptoms of dry eye by regulating the tear film environment through the promotion of water [5] and mucin secretion [6]. Therefore, we believe that diquafosol can be effective for dry eye patients, regardless of the degree of the epithelial disorder.

The most commonly used dry eye medication in Japan is sodium hyaluronate. It promotes improvement in corneal epithelial disorders and stabilization of the tear film with its water retentive properties [16–18]. We found that diquafosol was effective both when used as monotherapy and when additionally administered to patients already using sodium hyaluronate. Diquafosol exhibits a medicinal action different from that of sodium hyaluronate as mentioned above. Water and mucin are important structural components of the tear fluid, and thus increase tear fluid volume and its stability [19, 20]. This may be the reason why additional improvement effects were observed when diquafosol was used as an add-on to sodium hyaluronate.

The goals of dry eye treatment are improvement in ocular symptoms of the patient and maintaining the ocular surface in its normal state [21]. Accordingly, PRO evaluation remains very important. Of the 3,020 patients in whom PROs were surveyed in this study, 76% answered "much better" or "better" than before diquafosol treatment. A high level of patient satisfaction was achieved regardless of sex, age, the degree of severity of objective findings, concurrent disease,

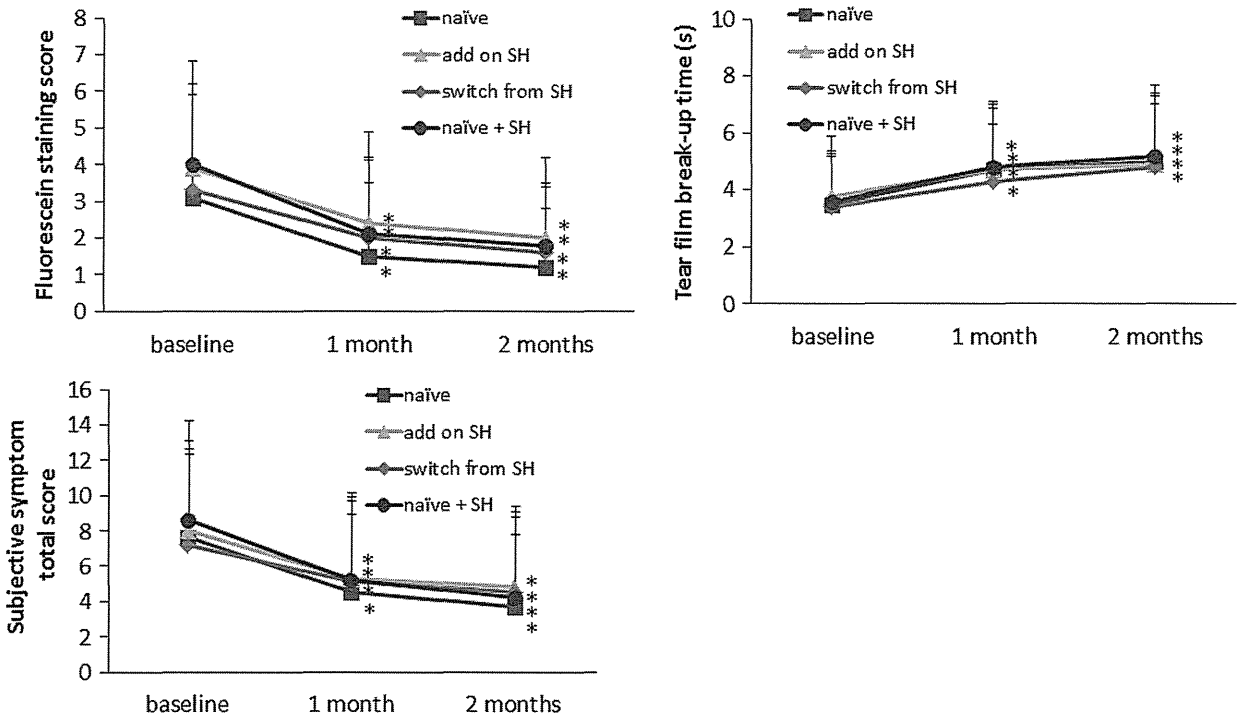

Fig. 5 Changes in the fluorescein staining score (*top left*), tear film break-up time (*top right*), and subjective symptom total score (*bottom left*) according to therapeutic pattern after diquafosol treatment. Regardless of the therapeutic pattern, the fluorescein staining score, total subjective symptoms, and tear film break-up time significantly improved at 1 and 2 months compared with baseline. Each *data point* represents the mean ± standard deviation. $^{*}P < 0.001$ compared with respective baseline, paired *t* test. *SH* Sodium hyaluronate

therapeutic patterns, and the dose. Regarding concurrent disease, meibomian gland dysfunction is a cause of evaporative dry eye [22], and conventional therapy for dry eye with meibomian gland dysfunction is often unsatisfactory [23]. Topical diquafosol therapy was recently reported to be effective for patients with obstructive meibomian gland dysfunction [24]. Using animal experiments, a study revealed that $P2Y_2$ receptors also exist in the meibomian glands [7]. Based on the results of this study and these findings, diquafosol may be effective for dry eye patients with meibomian gland dysfunction. Furthermore, conjunctivochalasis is also a cause of dry eye [1], and the main approach for this condition is surgery. Medical therapy is generally ineffective; however, diquafosol did show high PROs in this study. The same level of satisfaction was also observed in the 179 contact lens wearers. Thus, our results suggest that although contact lenses can be a risk factor for dry eye [4], diquafosol could be just as effective for contact lens wearers as for non-contact lens wearers.

Adverse reactions occurred in 6.3% of patients in this study. The incidence of adverse reactions in this study was lower than that in previous clinical trials [11–13]. It is considered to be caused by difference in the extent of monitoring between clinical trials and clinical practice. Furthermore, the main adverse reactions were eye discharge, eye irritation, and eye pain. These events were also reported as major adverse effects from the clinical trials

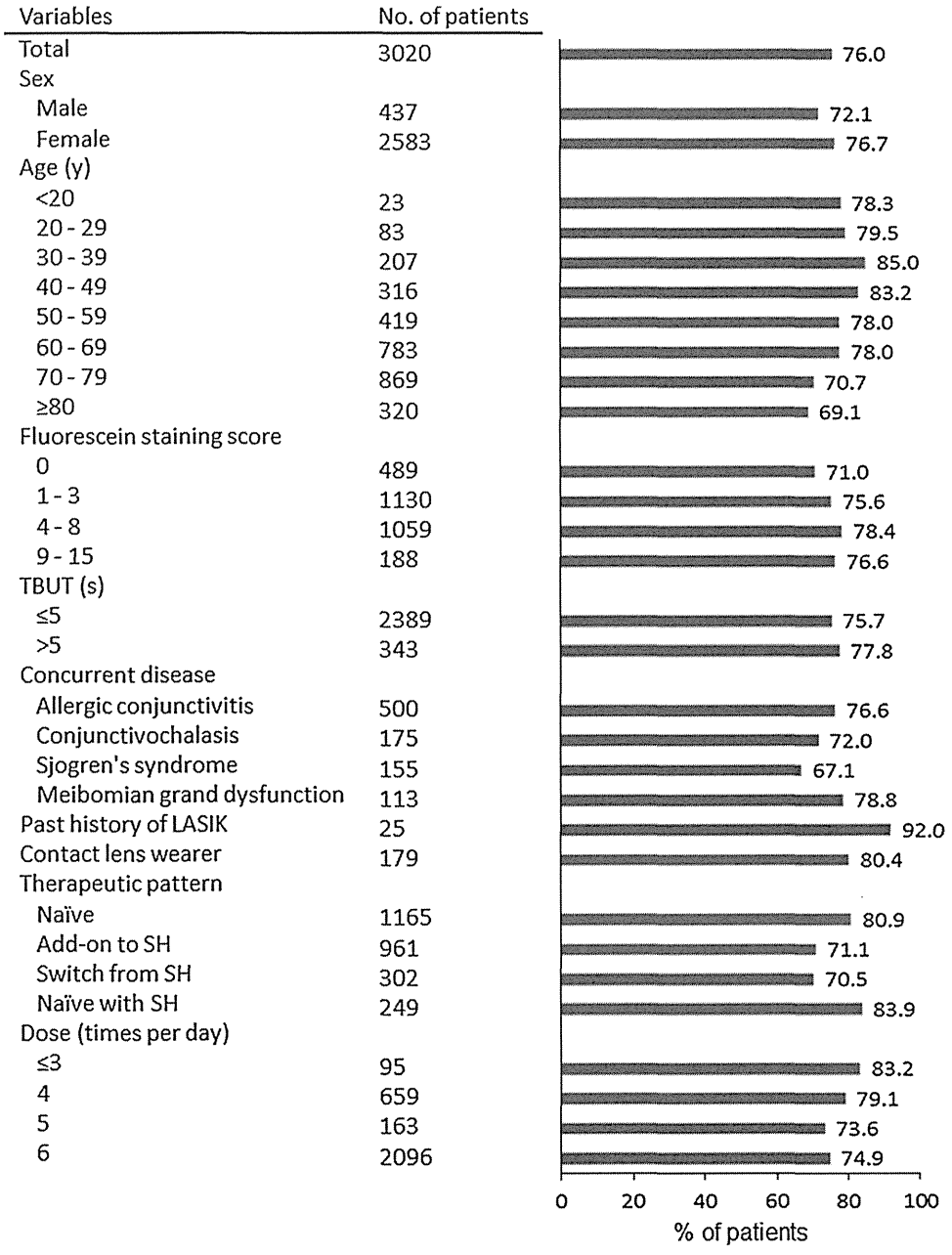

Fig. 6 Percentage of patients who answered "much better" or "better" than before diquafosol treatment. *LASIK* laser in situ keratomileusis, *SH* sodium hyaluronate, *TBUT* tear film break-up time

[11–13]. The incidence rate of eye irritation and eye pain indicated correlation with the severity of lacrimal disorder and the degree of the corneal and conjunctival epithelial disorder. These adverse reactions may develop more frequently in patients with bad ocular surface conditions. All adverse reactions could be easily rectified by discontinuing administration indicated that diquafosol could readily be used for various types of dry eye patients in a real-world setting. Regarding continuation of diquafosol treatment, it is important to provide

Table 2 Major adverse reactions after diquafosol treatment

Event	*n* (%)
Eye discharge	30 (0.9)
Eye irritation	30 (0.9)
Eye pain	22 (0.7)
Lacrimation	20 (0.6)
Blepharitis	19 (0.6)

Table 3 Influence of the characteristics for the incidence of eye irritation and eye pain

Characteristics	Total number of patients	Incidence: number of patients (%)
Sex		
Male	456	8 (1.8)
Female	2,740	43 (1.6)
Age (years)		
<65	1,511	24 (1.6)
≥65	1,685	27 (1.6)
Shirmer value (mm)		
≤5	557	10 (1.8)
>5, ≤10	346	3 (0.9)
>10	219	1 (0.5)
TBUT (s)		
≤5	2,532	45 (1.8)
>5	363	1 (0.3)
Fluorescein staining score		
0–3	1,728	17 (1.0)
4–8	1,114	23 (2.1)
9–15	195	8 (4.1)

TBUT tear film break-up time

patients with information about these possible adverse reactions before the treatment.

We were able to evaluate both the efficacy and safety of diquafosol under a variety of actual use conditions due to the sufficiently large population of 3,196 patients. However, there were several limitations in this study. Because this study is an observational study without a control group, both the observers and patients were aware that diquafosol was used, and therefore, the possibility of bias, such as placebo effect or preconceptions, cannot be denied. In addition, each investigator used the methods they use in the usual clinical practice for the objective findings, so it is highly likely that the criteria for objective findings were not unified.

We investigated PROs 2 months after initiating diquafosol treatment but did not evaluate the quality of life (QOL). In Japan, the Dry Eye-related Quality-of-life Score questionnaire [25] has been developed for the evaluation of QOL in dry eye patients. The question of how diquafosol affects QOL is an issue that warrants future investigation. Moreover, because dry eye is a chronic disease that requires long-term treatment, a more long-term investigation should be conducted in the future. We are currently investigating the long-term efficacy and safety of diquafosol for dry eye patients and the effects of diquafosol on QOL.

CONCLUSION

Results of this multicenter, prospective, non-interventional, observational study demonstrated that diquafosol is highly effective for real-world dry eye patients without particular concerns about the safety of this drug.

ACKNOWLEDGMENTS

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named authors meet the ICMJE criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published. All authors had full access to all of the data in this study and Takeshi Nishijima takes complete responsibility for the integrity of the data and accuracy of the data analysis.

Conflict of interest. Masahiko Yamaguchi has received honoraria for medical advice on this study and honoraria for writing and reviewing this manuscript from Santen. Takeshi Nishijima is an employee of Santen. Jun Shimazaki has received honoraria for medical advice on this study from Santen, and consulting fees from Otsuka and honoraria for lectures from Abbot, Alcon, Novartis, Otsuka, and Santen. Etsuko Takamura has received honoraria for medical advice on this study from Santen, and consulting fees from Alcon, Astellas, GlaxoSmithKline, Kissei, Kyowa Hakko Kirin, Maruho, MSD, Nippon Boehringer Ingelheim, Novartis, Otsuka, Pfizer, Senju, and Wakamoto. Norihiko Yokoi has received honoraria for medical advice on this study from Santen, and consulting fees from Kissei and Rohto. Hitoshi Watanabe has received honoraria for medical advice on this study from Santen. Yuichi Ohashi has received honoraria for medical advice on this study from Santen, and consulting fees from HOYA, Johnson and Johnson, Otsuka, Santen, and Senju.

Compliance with ethics guidelines. This study was conducted on the basis of a request from a regulatory agency in Japan. It was a multi-institutional, prospective, non-interventional, observational study that conformed to the Good Post-Marketing Study Practice (Ministry of Health, Labor and Welfare ordinance 171, December 20, 2004). The study protocol was reviewed and approved in advance by the Pharmaceuticals and Medical Devices Agency, Japan. For this reason, no ethical review by the individual facilities participating in the study was conducted. Because informed consent was not required for post-marketing observational studies that were requested by the regulatory agency in Japan, the present study did not solicit informed consent from the patients.

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Horizontal Intracorneal Swirling Water Migration Indicative of Corneal Endothelial Function

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Purpose. To test our hypothesis about whether there is water migration in the horizontal corneal plane and investigate its developmental mechanism.

Methods. A fluorescein solution was intrastromally injected into normal and edematous corneas of rabbits, and the movement of the fluorescein solution was observed and recorded over time.

Results. In normal corneas, the water flow was characterized by a swirling movement from the center to the periphery in the stroma. The fluorescein solution ultimately spread and occupied the entire cornea, indicating horizontal intracorneal swirling of water. In contrast, when the corneal endothelia were injured by intracameral injection of a preservative to create corneal edema, no water migration occurred, suggesting that the integrity of the corneal endothelial function is essential for water migration. The water migration stopped with injection of a sodium-potassium pump inhibitor, indicating that the enzyme is necessary for physiologic water migration in the cornea. With recovery of corneal endothelial function, the water migration began, and focal edema remained in the periphery with no water migration in this edematous area.

Conclusions. We report for the first time the presence of horizontal water migration in the cornea in a swirling pattern (i.e., intracorneal swirling migration of water, generated by the pump function in the corneal endothelial cells), which may supplement the conventional concept of development of corneal edema in the vertical plane. This dynamic water circulatory system may be involved in increasing the efficiency of the water transfer in the entire cornea.

Keywords: water movement in cornea, corneal hydration, intracorneal water flow

The cornea, the transparent anterior portion in the eye that is highly specialized to refract and transmit light, is comprised of an outer stratified squamous epithelium, inner connective tissue stroma, and the monocellular endothelium on the posterior side that borders the anterior chamber. The corneal tissue component is regular and precisely arranged and light passing through it is bent and transmitted to the retina.[1] The cornea is avascular, with a unique local circulatory system in which essentially all the nutritional needs except oxygen are supplied via the aqueous humor with diffusion of metabolic byproducts into the aqueous humor.[2]

The cornea must maintain its water content to remain transparent; disruption can result in corneal edema.[3] Long-standing edema with irregular fluid accumulation can reduce the corneal transparency and result in bullous keratopathy with severe visual disruption resulting from edema encompassing the entire cornea.[4,5] The basic mechanisms of corneal hydration and transparency between the aqueous humor and ocular surface have been well investigated and depend on the swelling pressure of the corneal stroma, epithelial and endothelial barrier functions, active ion transport, passive transport of water across the endothelium, and evaporation from the corneal surface.[1,2,6] Stromal imbibition pressure, as well as intraocular pressure (IOP) only in the edematous cornea, promote water accumulation in the corneal stroma. However, the transport of ions across the corneal endothelium reduce the osmotic pressure of the stroma such that the semipermeable membrane properties of the endothelium balance the forces promoting corneal edema.[4,5,6–14] The corneal endothelium is principally responsible for active dehydration of the cornea, with the transport system creating an osmotic gradient that prevents the stroma from swelling excessively and becoming cloudy.[1,7,15]

To date, these mechanisms of intracorneal water movement have been reported to occur in the corneal vertical plane, that is, across the endothelium. From our clinical observation of the process of corneal edema, we propose that there exists a pattern shift of the portion of edematous cornea in between the center and periphery of the cornea. We name this phenomenon as water movement within the horizontal plane. Gothard et al.[16] reported the pattern of corneal edema in which focal peripheral corneal edema progresses to diffuse edema years after cataract surgery. The precise water dynamics of the entire cornea in the horizontal direction between the center and periphery remain to be elucidated. We hypothesized that transverse water migration may modulate the corneal water dynamics and contribute to the developmental mechanism of corneal edema. Using fluorescein as a tracing dye, we indirectly

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