D2, or D3 when density was sparse, moderate, or high and the lesions overlapped, respectively. Although Miyata et al used the sum of the grades assigned to the area and density to obtain the final grade for the eye, we simplified their grading system and assigned scores of 0 through 3: A1D1 was scored as 0; A1D2 or A2D1 was scored as 1; A1D3, A2D2, or A3D1 was scored as 2; and A2D3, A3D2, or A3D3 was scored as 3.

Corneal Epithelial Defect. The extent of corneal epithelial defect was scored from 0 through 3, where 0 = no epithelial defect, 1 = epithelial defect involving less than one quarter of the corneal surface, 2 = defect involving one quarter to one half, and 3 = defect involving more than one half of the corneal surface.

Loss of the Palisades of Vogt. The extent of the loss of the limbal POV was graded from 0 through 3, where 0 = presence of the entire POV, 1 = loss of less than half of the entire circumference of POV, 2 = loss of more than half of the entire circumference of POV, and 3 = total loss of POV.

Conjunctivalization. The extent of conjunctivalization was graded clinically from 0 through 3 as follows: 0 = absence of conjunctivalization, 1 = conjunctivalization involving less than one quarter of the corneal surface, 2 = conjunctivalization involving one quarter to one half, and 3 = conjunctivalization involving more than one half of the corneal surface (Fig 1).

Corneal Neovascularization. The extent of corneal neovascularization was scored from 0 through 3, where 0 = no neovascularization, 1 = neovascularization confined to the corneal periphery, 2 = neovascularization extending up to the pupil margin, and 3 = neovascularization extending beyond the pupil margin into the central cornea (Fig 1). In eyes where significant opacification or extensive symblepharon formation made it difficult to evaluate corneal neovascularization, a score of 3 was assigned.

Corneal Opacification. The severity of corneal opacification was graded from 0 through 3, where 0 = clear cornea with iris details clearly visualized, 1 = partial obscuration of the iris details, 2 = iris details poorly seen with pupil margin just visible, and 3 = complete obscuration of iris and pupil details (Fig 1).

Corneal Keratinization. The extent of keratinization was graded from 0 through 3, where 0 = no corneal keratinization, 1 = keratinization involving less than one quarter of the corneal surface, 2 = keratinization involving one quarter to one half, and 3 = keratinization involving more than one half of the corneal surface (Fig 1).

Conjunctival Complications

Conjunctival Hyperemia. Conjunctival hyperemia was graded from 0 through 3 based on the following clinical features: 0 = absence of hyperemia, 1 = mild (mild or sectoral engorgement of the conjunctival vessels), 2 = moderate (diffuse engorgement of the conjunctival vessels), and 3 = severe hyperemia (significant engorgement of the conjunctival vessels).

Symblepharon Formation. The extent of symblepharon formation was scored from 0 through 3, where 0 = no symblepharon, 1 = symblepharon formation involving only the conjunctival surface, 2 = symblepharon formation involving less than half of the corneal surface, and 3 = symblepharon formation involving more than half of the corneal surface (Fig 1).

Eyelid Complications

Trichiasis. The extent of trichiasis for the total area of the upper and lower eyelids combined was scored as 0 through 3, where 0 = no trichiasis, 1 = trichiasis involving less than one quarter of the lid margin, 2 = trichiasis involving one quarter to one half of the lid margin, and 3 = trichiasis involving more than one half of the lid margin.

Mucocutaneous Junction Involvement. The severity of mucocutaneous junction involvement was scored from 0 through 3, where 0 = normal mucocutaneous junction, 1 = mild irregularity of the mucocutaneous junction, 2 = moderate irregularity of the mucocutaneous junction, and 3 = severe irregularity of the mucocutaneous junction (Fig 2). Fluorescein staining of the conjunctiva was helpful in evaluating the involvement of the mucocutaneous junction. Normal mucocutaneous junction showed the linear staining at the end of the conjunctiva, and either mild, moderate, or severe irregularity of this line was observed in the eyes with mucocutaneous junction involvement. In eyes where significant keratinization of the lid margin or extensive symblepharon formation made it difficult to evaluate mucocutaneous junction involvement, a score of 3 was assigned.

Meibomian Gland Involvement. The severity of meibomian gland involvement was determined clinically by the nature of the meibomian gland secretion expressed manually at the center of the upper lid and was scored from 0 through 3, where 0 = clear oily fluid expressed, 1 = yellowish-white oily fluid expressed, 2 = thick cheesy material expressed, and 3 = inability to express any fluid from the meibomian glands.

Punctal Involvement. Punctal damage and occlusion were graded from 0 through 3, where 0 = normal patent puncta, 1 = iatrogenic punctal occlusion (e.g., punctal plugs or suture), 2 = either superior or inferior puncta occluded by scarring, and 3 = both superior and inferior puncta occluded by scarring.

Overall Total Score

Each eye was evaluated and graded by at least 2 trained corneal specialists. When the scores varied from one corneal specialist to another, the scores were averaged or determined after a discussion. The results then were added together to give an overall score from 0 to 39, with 39 representing the most severely affected eyes.

Visual Acuity

We categorized the 138 eyes from the 73 patients according to their visual acuity. In group 1 (n = 28 eyes), visual acuity was 20/20 or better, in group 2 (n = 36 eyes), it was worse than 20/20 and up to and including 20/200, in group 3 (n = 32 eyes), it was worse than 20/200 and up to and including 20/2000, and in group 4 (n = 42 eyes), it was worse than 20/2000.

Eye Complications Independent of Ocular Surface Disorders

Cataract, glaucoma, retinal diseases, or other eye diseases independent of ocular surface disorders also were evaluated and their presence, absence, or the inability to diagnosis because of ocular surface abnormalities was recorded.

Statistical Analysis

Spearman correlation coefficients (2-tailed) were used to evaluate whether the scores of the 13 components were correlated with logarithm of the minimum angle of resolution (logMAR) visual acuity. The correlation between the total score and logMAR visual acuity and the correlations between the subtotal scores of 3 problem categories and the total score also were evaluated. Using a logistic regression model, the scores for each of the 13 components

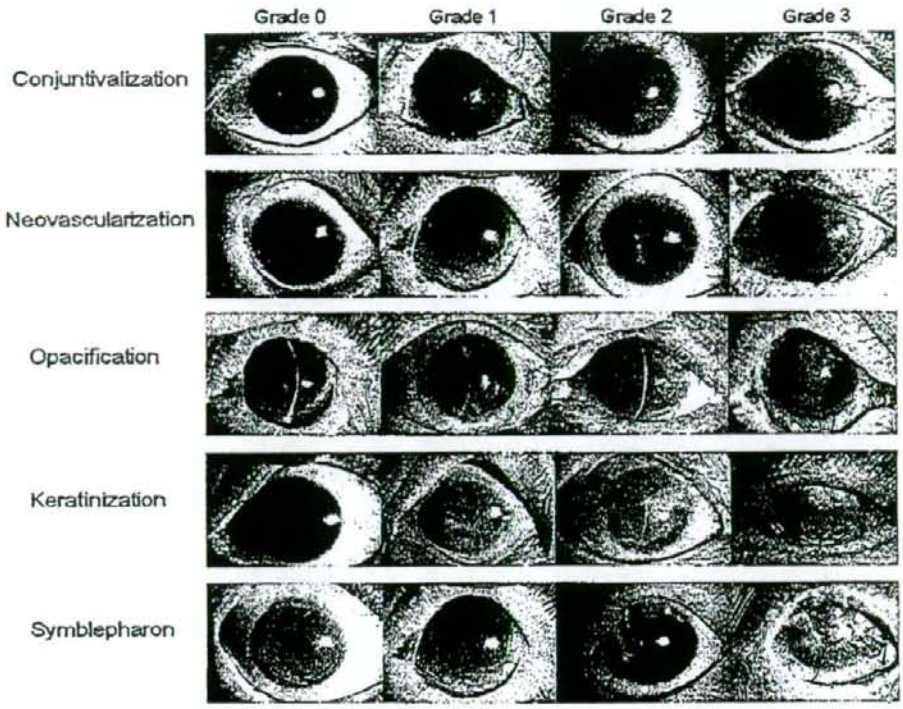

Figure 1. Grading scores of corneal and conjunctival complications.

in eyes with better visual acuity (20/200 or better; i.e., groups 1 and 2) were compared with the scores obtained for eyes with poorer visual acuity (worse than 20/200; i.e., groups 3 and 4). The statistical model for predicting logMAR visual acuity was calculated using a linear model with stepwise variable selection (multivariable regression analysis). In multivariable regression analysis, cataract and glaucoma were graded as follows: with cataract, 1; without cataract or lens invisible, 0; with glaucoma, 1; and without glaucoma or unable to diagnosis glaucoma, 0. All statistical tests were conducted at a 5% level of significance ($P = 0.05$).

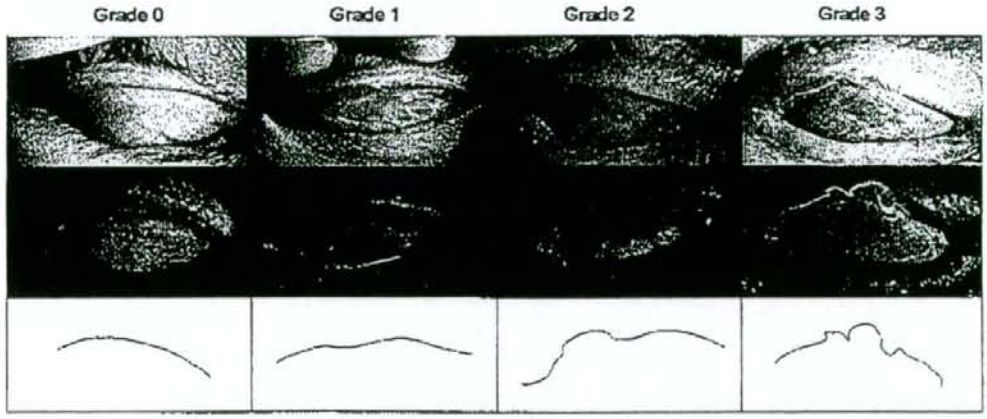

Figure 2. Grading scores of mucocutaneous junction involvement. Top, Grade 1 was assigned for normal mucocutaneous junction, and grades 1, 2, and 3 were assigned for mild, moderate, and severe irregularity of the mucocutaneous junction, respectively. Middle, Bottom, Fluorescein staining of the conjunctiva was helpful in evaluating the severity of the involvement of mucocutaneous junction.

Results

A total of 138 eyes of 73 patients from the 3 institutions were included in this study. There were 33 males and 40 females. Their age ranged from 10 to 83 years (mean±standard deviation, 47.9±18.5 years). At disease onset, the patients' ages ranged from 2 to 69 years (mean±standard deviation, 28.4±18.2 years), and the duration of the illness before seeking consultation at our centers ranged from 1 to 54 years (mean±standard deviation, 18.8±15.5 years). Drugs were the most commonly associated etiologic factor in 47 patients (64.4%). Because 14 of these patients used 2 or 3 types of drugs simultaneously, it was difficult to identify the drug(s) implicated in disease onset; therefore, we considered all their drugs to be causative. The causative drugs were antibiotics in 21 patients, cold remedies in 18 patients, nonsteroidal antiinflammatory drugs in 10 patients, anticonvulsants in 6 patients, and other in 4 patients. The precise history regarding the use of drugs was unclear in 20 patients because of the long interval between disease onset and this study.

Corneal Complications

A detailed summary of the 7 evaluated components comprising corneal complications is shown in Table 1. Among the 138 eyes examined, 114 (82.6%) manifested a total loss of POV (grade 3). Moderate to severe (grade 2 or 3) corneal SPK was present in 93 eyes (67.4%), neovascularization was present in 83 eyes (60.1%), and conjunctivalization was present in 82 eyes (59.4%).

Conjunctival and Eyelid Complications

Among the 6 evaluated components that comprise conjunctival and eyelid complications, the meibomian glands were most frequently and most severely involved; 102 of the 138 eyes (73.9%) manifested grade 3 meibomian gland involvement (Table 2). The scores for punctal damage and mucocutaneous involvement also were high; grade 2 or 3 punctal damage was assigned to 93 eyes (67.4%), and grade 2 or 3 mucocutaneous involvement was assigned to 71 eyes (51.4%).

Eye Complications Independent of Ocular Surface Disorders

Cataract was observed in 11 of 138 eyes. Glaucoma was diagnosed in 4 eyes, none of which had central loss of visual fields. There were no other eye complications independent of ocular surface disorders.

Visual Acuity

The number of eyes in each of the 4 groups was fairly evenly distributed (Table 3). Of the 138 eyes examined, 74 (53.6%) had visual acuity worse than 20/200 (group 3, n = 32; group 4, n = 42). Only 28 eyes (20.3%) had visual acuity equal to 20/20 or better.

Table 1. Summary of Corneal Complications (138 Eyes)

Complication	Grade 0, no. (%)	Grade 1, no. (%)	Grade 2, no. (%)	Grade 3, no. (%)
Superficial punctate keratopathy	22 (15.9)	23 (16.7)	18 (13.0)	75 (54.3)
Epithelial defect	135 (97.8)	2 (1.4)	1 (0.7)	0 (0)
The loss of palisades of Vogt	21 (15.2)	3 (2.1)	0 (0)	114 (82.6)
Conjunctivalization	41 (29.7)	15 (10.9)	10 (7.2)	72 (52.2)
Neovascularization	35 (25.4)	20 (14.5)	22 (15.9)	61 (44.2)
Opacification	43 (31.2)	41 (29.7)	28 (20.3)	26 (18.8)
Keratinization	105 (76.1)	10 (7.2)	5 (3.6)	18 (13.0)

Table 2. Summary of Conjunctival and Eyelid Complications (138 Eyes)

Complications	Grade 0, no. (%)	Grade 1, no. (%)	Grade 2, no. (%)	Grade 3, no. (%)
Conjunctival complications				
Hyperemia	46 (33.3)	61 (44.2)	15 (10.9)	16 (11.6)
Symblepharon formation	40 (29.0)	54 (39.1)	21 (15.2)	23 (16.7)
Eyelid complications				
Trichiasis	42 (30.4)	41 (29.7)	44 (31.9)	11 (8.0)
Mucocutaneous junction involvement	16 (11.6)	51 (37.0)	34 (24.6)	37 (26.8)
Meibomian gland involvement	13 (9.4)	14 (10.1)	9 (6.5)	102 (73.9)
Punctal damage	36 (26.1)	9 (6.5)	15 (10.9)	78 (56.5)

Correlation between Visual Acuity and Grade of Complications

When we compared eyes with better (20/200 or better) and worse (worse than 20/200) visual acuity with respect to the scores obtained for each of the 13 components, we found that with the exception of epithelial defect, the scores differed significantly (Table 4).

We estimated the correlation coefficient between the visual acuity of the 138 eyes and the severity grade, scored from 0 to 3, of each of the 13 evaluated components in the 3 categories of complications. We found that all 13 components were correlated significantly with logMAR visual acuity; the correlation coefficient (R) ranged from 0.359 to 0.810 ($P<0.0001$); for corneal epithelial defects, the value was $R = 0.169$ ($P = 0.0473$; Table 5). Of all the scores, corneal neovascularization, opacification, and conjunctivalization were most highly correlated with poor vision ($R = 0.810$, $P<0.0001$; $R = 0.784$, $P<0.0001$; and $R = 0.726$, $P<0.0001$, respectively).

The statistical model for predicting logMAR visual acuity was calculated using a linear model with stepwise variable selection as follows: logMAR = −0.2573 + cataract × 0.4153 + POV × 0.2814 + SPK × 0.08551 + epithelial defect × 0.3018 + neovascularization × 0.3471 + opacification × 0.3202 + keratinization × 0.1347. This multivariable regression analysis showed that corneal neovascularization, opacification, keratinization, and cataract had a significant effect on logMAR visual acuity (Table 6). The predicted logMAR was correlated significantly with the actual logMAR visual acuity measured ($R = 0.960$, $P<0.0001$).

Overall Total Score

The mean overall total score for the 13 components was 19.3±9.5 (range, 0–35). As shown in Tables 3 and 4 and Figure 3, eyes with a higher total score had poorer vision. The averaged scores for the 4 visual acuity groups were: group 1, 5.86 (range, 0–19); group 2, 16.64 (range, 2–28); group 3, 23.31 (range, 15–33); and group 4, 27.45 (range, 18–35). Pearson's analysis clearly demonstrated that the total score was significantly correlated with logMAR visual acuity ($R = 0.806$, $P<0.0001$; Fig 3). The subtotal scores of 3 problem categories correlated with the overall total score (Fig 4).

Table 3. Ocular Complications and Visual Acuity of Stevens–Johnson Syndrome Patients

Complications	Visual Acuity			
	Group 1, 20/20 or Better, Average Grade	Group 2, 20/20 to 20/200, Average Grade	Group 3, 20/200 to 20/2000, Average Grade	Group 4, Worse than 20/2000, Average Grade
No. of eyes	28	36	32	42
Corneal complications				
SPK	0.82	1.92	2.40	2.78
Epithelial defect	0	0	0.03	0.07
Loss of POV	0.82	2.78	3.00	3.00
Conjunctivalization	0.11	1.36	2.59	2.76
Neovascularization	0.25	1.11	2.38	2.90
Opacification	0.11	0.61	1.66	2.31
Keratinization	0.04	0.11	0.50	1.26
Conjunctival complications				
Hyperemia	0.36	0.89	1.19	1.40
Symblepharon formation	0.18	0.97	1.19	2.07
Eyelid complications				
Trichiasis	0.57	1.08	1.38	1.50
Mucocutaneous junction involvement	0.79	1.56	1.91	2.10
Meibomian gland involvement	1.32	2.50	2.69	2.90
Punctal damage	0.50	1.78	2.65	2.58
Total score	5.86	16.64	23.31	27.45

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Discussion

Severe ocular surface disease arising from SJS or TEN is associated with significant visual morbidity.[1–4] The evaluation of ocular complications in these patients is extremely important, because ocular involvement often represents the only long-term complication of SJS. There is currently no established method for evaluating the spectrum of ocular manifestations arising from these diseases. In this study, we detailed the characteristic ocular complications in the chronic stage of SJS and developed a grading system to assess more objectively the extent and severity of 13 components of these ocular complications. To the best of our knowledge, this is the first study that specifically attempted to improve and standardize the evaluation of ocular complications in SJS.

As we set out to develop a grading system that could be used easily by ophthalmologists, we identified complications that were important and could be evaluated easily by simple slit-lamp examination. After several pilot studies, we eventually settled on 13 components of 3 categories of

Table 4. Comparison between Ocular Complications and Visual Acuity

Complications	Visual Acuity of 20/200 or Better, Average Grade	Visual Acuity Worse than 20/200, Average Grade	P Value
No. of eyes	64	74	
Corneal complications			
SPK	1.44	2.62	<0.0001
Epithelial defect	0	0.05	0.1208
Loss of POV	1.92	3.00	<0.0001
Conjunctivalization	0.81	2.69	<0.0001
Neovascularization	0.73	2.68	<0.0001
Opacification	0.39	2.03	<0.0001
Keratinization	0.08	0.93	<0.0001
Conjunctival complications			
Hyperemia	0.66	1.31	<0.0001
Symblepharon formation	0.63	1.69	<0.0001
Eyelid complications			
Trichiasis	0.86	1.45	0.0002
Mucocutaneous junction involvement	1.23	2.01	<0.0001
Meibomian gland involvement	2.02	2.81	<0.0001
Punctal damage	1.26	2.61	<0.0001
Total score	11.86	25.66	

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Table 5. Correlation Analyses between 13 Complications and Logarithm of the Minimum Angle of Resolution Visual Acuity

Complications	Coefficient	P Value
Neovascularization	0.810	<0.0001
Opacification	0.784	<0.0001
Conjunctivalization	0.726	<0.0001
Symblepharon formation	0.649	<0.0001
SPK	0.601	<0.0001
Loss of POV	0.550	<0.0001
Punctal damage	0.518	<0.0001
Mucocutaneous junction involvement	0.488	<0.0001
Keratinization	0.477	<0.0001
Meibomian gland involvement	0.453	<0.0001
Hyperemia	0.383	<0.0001
Trichiasis	0.359	<0.0001
Epithelial defect	0.169	0.0473

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

Table 6. Multivariable Regression Analysis

Variables	Coefficient	95% Confidence Intervals	P Values
Intercept	−0.2573	−0.5449 to 0.0303	0.0786
Neovascularization	0.3471	0.2113–0.4849	<0.0001
Opacification	0.3203	0.1734–0.4672	<0.0001
Keratinization	0.1347	0.0281–0.2413	0.0142
Cataract	0.4153	0.0249–0.8057	0.0375
Loss of POV	0.2814	−0.0784 to 0.6412	0.1228
SPK	0.0855	−0.0296 to 0.2006	0.1423
Epithelial defect	0.3018	−0.1057 to 0.7093	0.1434

POV = palisades of Vogt; SPK = superficial punctate keratopathy.

complications that we considered important for the assessment of severe or cicatricial ocular surface disorders. We used a simple method for grading the severity of these complications; the components were assigned scores that reflected whether involvement was mild, moderate, or severe. This grading system was judged to be easy and convenient at the 3 participating ophthalmology centers that evaluated 138 eyes from 73 SJS patients. The results obtained at the 3 centers were consistent and comparable. Ours is one of few prospective studies on the ocular complications of SJS, and each patient was evaluated carefully by at least 2 ophthalmologists. To the best of our knowledge, this is the largest such study reported to date.

The initial ocular pathologic process in SJS, inflammation and necrosis of the conjunctiva, often is accompanied by the destruction of goblet cells.[4,13,14] The production of mucin by these cells is vital for maintaining an adequate tear film essential for corneal clarity. Dry eye secondary to goblet cell destruction is the most common long-term ocular complication in patients with various ocular surface diseases.[4,13,14] Cicatricial lid and conjunctival complications include symblepharon formation, forniceal shortening, keratinization, lid malposition (e.g., entropion), and misdirected eyelashes (trichiasis).[4,13,15–22] Limbal stem cell destruction, evidenced by loss of the POV, also may occur at disease onset and may be accompanied by severe inflammation. The combination of these complications may result in recurrent corneal erosion, ulceration, vascularization, stromal scarring, conjunctivalization of the corneal surface, and progressive corneal melting and perforation.[4,13,15–22]

In our study, drugs were the most commonly identified etiologic factor: in 47 patients (64.4%), antibiotics (n = 21 patients), cold remedies (n = 18 patients), or nonsteroidal antiinflammatory drugs (n = 10) were the causative agents. These findings are consistent with previous reports.[15,18,19,23]

Of all the complications, severe (grade 3) meibomian gland involvement and loss of the POV (102 and 114 eyes, respectively) were the most common ocular complications of SJS. We found that the total score for each eye was correlated significantly with its visual acuity; consistently, eyes with higher overall scores had poorer vision. We categorized the complications as those involving predominantly the cornea, the conjunctiva, and the eyelid. As expected, corneal complications were most likely to have a detrimental effect on vision. In particular, corneal neovascularization and opacification were correlated highly with posttreatment visual acuity in the chronic stage. Conjunctivalization, a sequela of limbal stem cell deficiency, also was correlated with poor vision in our series.

In our study, there was a high rate of lid complications in chronic SJS. Of the eyelid complications, meibomian gland involvement was moderate or severe (grade 2 or 3) in 111 of the 138 eyes (80.4%). We found that eyes without apparent corneal complications also manifested cicatricial eyelid changes. As such, the meibomian glands seem to be susceptible to injury after SJS. Because the meibomian glands play a critical role in the stabilization of the tear film, this is likely to contribute to the disruption of the tear film and severe dry eye condition experienced in patients in the chronic stage of SJS.

The use of a standardized method for grading the extent and severity of ocular complications in SJS patients offers

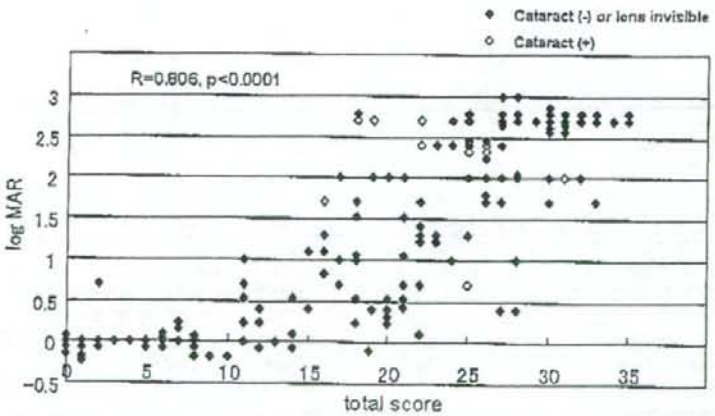

Figure 3. Scatterplot depicting the correlation between the total score and logarithm of the minimum angle of resolution (logMAR) visual acuity. The overall total score of 13 components (0–39) versus logMAR showed a significant positive correlation (Spearman $R = 0.806$, $P<0.0001$).

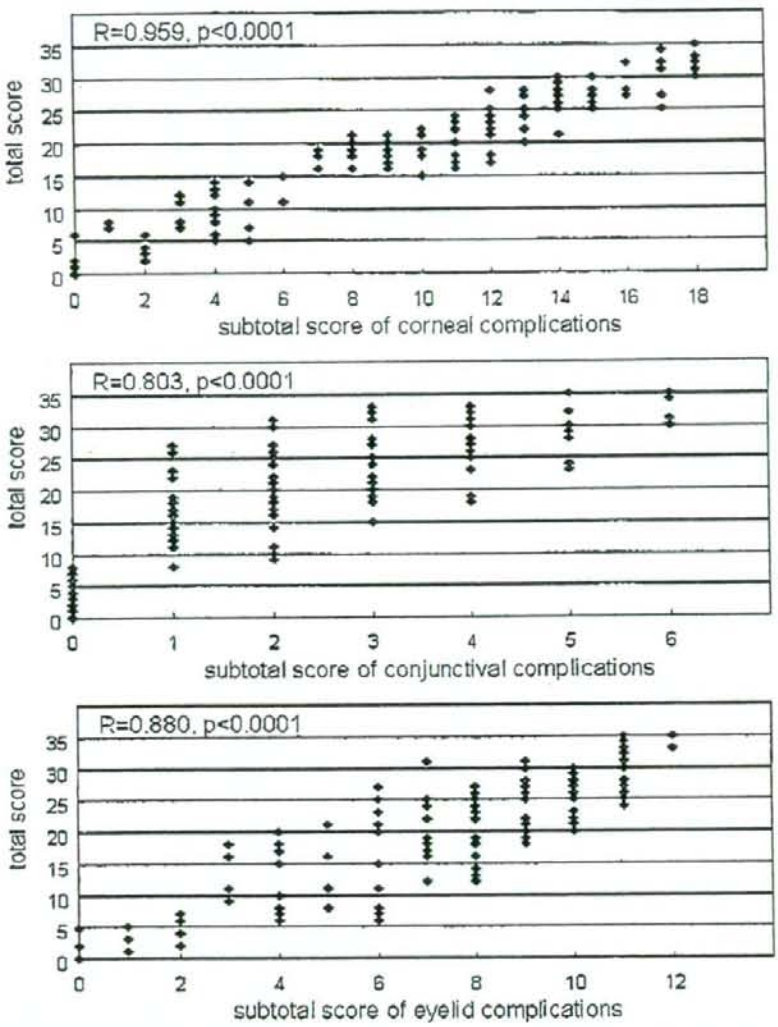

Figure 4. Scatterplots depicting the correlations between subtotal score of 3 categories and overall total score. Subtotal scores of corneal, conjunctival, and eyelid complications versus the total score all showed a significant positive correlation: (top) Spearman $R = 0.959$, $P<0.0001$; (middle) $R = 0.803$, $P<0.0001$; (bottom) $R = 0.880$, $P<0.0001$.

significant advantages. The grading system introduced here can be used in the initial evaluation and the follow-up and monitoring of ocular complications in SJS patients. As documented here, the lid margin is a commonly affected site in the disease process. However, because attention often focuses on the ocular surface, changes in the lid margin may be overlooked. Our grading system ensures that important ocular complications are detected by corneal specialists as well as nonspecialized ophthalmologists.

Ocular surface reconstructive procedures such as limbal and cultivated epithelial stem cell transplantation have been used to treat severe ocular manifestations in SJS patients.[8–11,24] However, because many of the reported studies are nonrandomized case series without control arms and because there is currently no standardized method for grading ocular complications in SJS patients in the acute and chronic stage, it is difficult to compare the treatment outcomes of these studies. Our grading system also provides a standardized method for evaluating patients before corneal and ocular surface transplantation procedures. The use of an objective method of grading the severity of the patient's preoperative condition ultimately may help in prognosticating the long-term clinical outcome of these eyes after surgery.

This is the first study that describes a method for classifying and grading the severity of ocular involvement in SJS patients. Our findings have important clinical implications and facilitate the objective evaluation of patients with ocular complications resulting from SJS. The method presented here may be adapted for use in patients with cicatricial ocular surface diseases arising from other causes such as ocular cicatricial pemphigoid and chemical injury. It also provides a common platform for the discussion and management of patients with ocular surface disorders and may be useful for predicting treatment outcomes. Our method also enables ophthalmologists to monitor more objectively the progression of complications during the follow-up of these patients.

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The Disease Burden of Keratoconus in Patients' Lives: Comparisons to a Japanese Normative Sample

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Purpose. Keratoconus is a chronic, noninflammatory, degenerative disease of the cornea that has an onset in young adulthood. The objective of this study was to evaluate vision-related quality of life (VR-QOL) in patients with keratoconus by using the Japanese version of the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25). **Methods.** Forty-five patients diagnosed with keratoconus at the Keio University School of Medicine were enrolled. Patients were divided into three subgroups according to corrected visual acuity. Group A included patients whose best-corrected visual acuity was at least 20/20 in both eyes. Group B included patients with a best-corrected visual acuity of at least 20/20 in only one eye. Group C included patients whose best-corrected visual acuity was worse than 20/20 in both eyes. Thirty-six age-matched subjects were recruited as control subjects. The Japanese version of the NEI-VFQ-25 was administered to each subject. **Results.** All NEI-VFQ-25 subscale scores were significantly lower ($P<0.05$) in patients with keratoconus than in the control subjects. Subscales evaluating general health, ocular pain, and vision-specific mental health showed particularly low values. Among patients with keratoconus, every subscale score other than color vision correlated with corrected visual acuity. **Conclusions.** The results support that and describe how multidimensional visual function and VR-QOL are impaired in patients with keratoconus, including those with normal visual acuity. Ophthalmologists and other clinicians should carefully evaluate and address the full range of quality of life issues that may affect patients with keratoconus.

Key Words: Contact lens—Keratoconus—Keratoplasty—Quality of life.

Keratoconus is a chronic, noninflammatory, degenerative disease of the cornea that has an onset in young adulthood and is characterized by a cone-shaped bulging of the corneal surface and stromal thinning.[1] Although some patients with keratoconus require keratoplasty to restore visual acuity, most patients are able to maintain normal visual acuity with the aid of contact lenses, although some patients experience contact lens intolerance.

The clinical status of patients with ocular disorders such as keratoconus is often evaluated through tests of objective function, such as visual acuity, refraction, and keratometric analysis. The past few decades have seen an increasing appreciation of the importance of assessing the illness experience in patients with ocular and other diseases in a comprehensive fashion that extends beyond measures of biologic status. Consequently, the concept of health-related quality of life (QOL) has been developed, which encompasses not only the biologic status of disease, but also the full range of complex effects that the illness and its various sequelae have on the life of the patient. Such sequelae may include the practical effects of the disease on the daily life of the patient, the side effects of treatment, issues of identity as the patient reconceives of himself or herself as an individual with a chronic illness, and social challenges involved in managing the disease and meeting the reactions of others to the illness. In this vein, in ophthalmology, concepts such as quality of vision and vision-related quality of life (VR-QOL) have been recently developed and are increasingly used. Several questionnaires have been developed to measure VR-QOL.[2–5]

Vision-related quality-of-life instruments can be divided into two broad categories: generic instruments, which are designed to be used in a broad spectrum of ocular disorders, and disease-specific instruments, which target patients with specific conditions. The National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25), a generic instrument, has been intensively investigated, and its validity has been established for various ocular disorders, including glaucoma, age-related macular degeneration, diabetic retinopathy, cytomegalovirus retinitis, and age-related cataracts.[6–13] The NEI-VFQ-25 evaluates not only visual function and limitations in daily activities related to impaired visual function, but also the impact of ocular disease on patients' lives from various standpoints.[6] The NEI-VFQ-25 has been translated into several languages, including Japanese. The reliability and the validity of the Japanese version of the NEI-VFQ-25 are considered comparable to those of the English version.[14,15]

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Visual acuity is the most common measure of clinical status in patients with keratoconus. It is apparent, however, that normal visual acuity alone cannot ensure good QOV and VR-QOL in patients with keratoconus. In addition to possibly diminished visual acuity, patients with keratoconus also experience the anxiety of knowing that they may someday need to undergo surgery, such as keratoplasty, and of knowing that their condition may progress and possibly lead to low vision. They also endure the burden of frequent hospital visits. Additionally, most patients who depend on contact lenses experience anxiety because of decreased vision when they are not able to wear their lenses. Many patients with keratoconus have less than all-day wear with contact lenses because of irritation and have significantly reduced vision with glasses. Thus, it may be appropriate to use a more comprehensive QOL-related tool in the examination of these patients.[16]

This study sought to evaluate self-reported VR-QOL in patients with keratoconus by using the NEI-VFQ-25. The influence of disease severity on VR-QOL was also examined.

MATERIALS AND METHODS

Subjects

A total of 45 Japanese patients with bilateral keratoconus (12 women and 33 men) were enrolled. The average age was 36.3 ± 9.4 years (range, 20–63 years). All patients had been diagnosed with keratoconus at Keio University Hospital. Keratoconus diagnoses were made based on slitlamp examination findings and videokeratography. The patient sample included contact lens wearers, spectacle wearers, and patients with a history of keratoplasty in one eye. Patients with a history of other ocular diseases, such as retinal disease, cataracts, or glaucoma, were excluded from the study. Patients with a history of keratoplasty complications, such as allograft rejection, infection, and graft failure, were also excluded.

Patients were divided into three subgroups according to corrected visual acuity. Group A included patients whose best-corrected visual acuity in each eye was at least 20/20. Group B included patients with a best-corrected visual acuity of less than 20/20 in one eye. Group C included patients whose best-corrected visual acuity was less than 20/20 in both eyes. Thirty-six age-matched healthy Japanese subjects with no known ocular disorders other than refractive error were recruited as control subjects.

The principles of the Declaration of Helsinki were followed. Each subject received a thorough explanation of the purpose of the study and all procedures involved in the study and provided written informed consent before enrollment. Approval for this investigation was granted by the Committee for the Protection of Human Subjects at the Keio University School of Medicine.

The NEI-VFQ-25

The NEI-VFQ-25 consists of 25 core and 13 optional items. It is divided into the following 12 subscales: general health, general vision, ocular pain, near vision, distance vision, vision-specific social functioning, vision-specific mental health, vision-specific role difficulties, vision-specific dependency, driving, color vision, and peripheral vision. A self-administered Japanese version of the NEI-VFQ-25 was used in this study. The instrument generally took approximately 10 minutes for patients to complete and was administered to patients before the ophthalmologic examination.

TABLE 1. *Characteristics of Patients With Keratoconus and Control Subjects*

Characteristic	Patients with keratoconus (n = 45)	Control subjects (n = 36)
Mean age (years)	36.3 ± 9.4	36.3 ± 13.0
Gender		
Male	33 (73%)	23 (64%)
Female	12 (27%)	13 (36%)
Best-corrected visual acuity		
≥20/20 in both eyes	12 (27%)	36 (100%)
≥20/20 in one eye	26 (58%)	0
<20/20 in both eyes	7 (15%)	0
Means of refractive correction		
None	1 (2%)	10 (28%)
Spectacles	2 (4%)	10 (28%)
Soft contact lenses	1 (2%)	13 (36%)
Hard contact lenses	41 (92%)	3 (8%)
History of keratoplasty	12 (27%)	0

In the NEI-VFQ-25, each of its subscales is scored from 0 to 100, with higher scores representing better function. Clinical data, including patient age, sex, ocular and medical history, and history of ocular surgery, were obtained from medical records and patient interviews.

Statistical Analysis

Descriptive statistics were used and included measurement of means and dispersion. Descriptive data are presented as mean ± standard deviation and percentages. The Mann–Whitney test was used to compare the NEI-VFQ-25 scale scores between groups. To minimize bias, subscale scores were included in analyses only if all questions within the subscale were completed. All statistical analyses were performed with the SAS/STAT module of the SAS statistical software package (SAS Institute, Inc., Cary, NC).

RESULTS

Baseline demographic characteristics of patients with keratoconus and control subjects, including any means of refractive correction used, are shown in Table 1. Most (91%) patients with keratoconus were hard contact lens wearers. There were 12 patients in group A, 26 patients in group B, and seven patients in group C.

The NEI-VFQ-25 subscale scores of patients with keratoconus and control subjects are shown in Table 2. All subscale scores of patients with keratoconus were significantly lower than those of control subjects ($P<0.01$, Mann–Whitney test). Particularly low subscale scores were seen for patients with keratoconus in the general health, general vision, ocular pain, and vision-specific mental health subscale categories.

The NEI-VFQ-25 subscale scores of groups A, B, and C and control subjects are shown in Figure 1. Increasing visual acuity, as assessed by group A through C category membership, correlated with increasing QOL or function scores in all subscales among patients with keratoconus. Even in group A, in which the best-corrected visual acuity was 20/20 or better in both eyes, all subscale scores other than general health and driving were significantly lower than those of age-matched control subjects ($P<0.05$, Mann–Whitney test).

patients with keratoconus. Possible explanations for the reduced VR-QOL observed in patients with keratoconus and normal measured visual acuity may include medical and biologic issues, such as changes in ocular surface characteristics resulting in tear film alterations,[19] which may render traditional measures of visual function incomplete in the examination of patients with keratoconus. Additionally, despite apparently normal visual function, patients with keratoconus may experience other disease-related burdens, such as contact lens intolerance, ocular pain caused by epithelial and stromal damage, the need to seek frequent ophthalmologic care, and anxiety related to disease progression and the possible need for surgery.

An important advantage of using generic instruments to measure VR-QOL, such as the NEI-VFQ-25 used in this study, is that such generic instruments facilitate the comparison of total and subscale scores among different groups of patients with various ocular disorders. When the results from the patients with keratoconus are compared with the findings from patients with other ocular disorders, such as uveitis, diabetic retinopathy, glaucoma, cataracts, and multiple sclerosis,[6–13,20,21] low scores in the ocular pain, general health, and mental health categories appear to be characteristic of patients with keratoconus.[16] The overall NEI-VFQ-25 profile of patients with keratoconus appears similar to that of patients with uveitis.[20] This may be partly related to the fact that both groups of patients experience ocular pain, frequently experience other chronic disorders beginning in young adulthood, require frequent ophthalmologic care, and fear progression to low vision.

The results of this study support the use of the NEI-VFQ-25 in patients with keratoconus and provide further evidence for the general applicability of this scale in various ocular disorders. The results underscore the importance of adopting a comprehensive approach to the examination and care of patients with keratoconus that incorporates but goes beyond measures of biologic disease status, such as visual acuity. Ophthalmologists and practitioners of other clinical specialties alike should be appraised of and bear in mind the full spectrum of QOL issues suffered by patients with keratoconus, such that the medical treatment that these patients receive may respond appropriately to the full range of issues these patients face.

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TABLE 2. *VFQ-25 Subscale Scores of Patients With Keratoconus and Control Subjects*

Subscale	Patients with keratoconus (n = 45)	Control subjects (n = 36)
General health	56.3 ± 18.6[a]	71.2 ± 17.6
General vision	62.3 ± 16.9[a]	81.4 ± 11.7
Ocular pain	57.4 ± 23.6[a]	83.0 ± 13.6
Near vision	80.5 ± 15.8[a]	92.8 ± 9.7
Distance vision	73.7 ± 16.1[a]	89.0 ± 13.3
Vision-specific		
Social functioning	85.9 ± 13.7[a]	97.2 ± 7.04
Mental health	65.9 ± 23.8[a]	90.9 ± 13.8
Role difficulties	77.8 ± 19.6[a]	96.3 ± 9.3
Dependency	88.2 ± 11.9[a]	98.3 ± 5.3
Driving	72.3 ± 20.6[a]	79.1 ± 24.2
Color vision	92.4 ± 11.4[a]	98.6 ± 5.7
Peripheral vision	74.6 ± 23.3[a]	92.4 ± 13.1

[a]P<0.01 for keratoconus–control comparisons by the Mann–Whitney test.

DISCUSSION

All mean NEI-VFQ-25 subscale scores for patients with keratoconus were significantly lower than those of age-matched control subjects (P<0.01, Mann–Whitney test), which was in good accordance with the report of Kymes et al.[16] Additionally, subscale scores correlated with corrected visual acuity as assessed by group A through C category membership. The finding that visual function subscale scores, such as general vision, near vision, distance vision, and driving are decreased in patients with keratoconus is intuitive. Most patients in this study had a corrected visual acuity of less than 20/20 in at least one eye, and many patients additionally may have subtle visual disturbances not evident on standard acuity testing. The low QOL scores observed on the ocular pain subscale may be explained by the need for many patients with keratoconus to wear hard contact lenses. Finally, the notably low mental health subscale scores found among patients with keratoconus warrants attention, suggesting that the various burdens of living with keratoconus may pose a considerable challenge to patient mental health and that practitioners should be attuned to this possible issue when treating keratoconus. These factors may explain the personality trends in keratoconus.[17,18] Through attention to such larger implications of disease, the care of patients with keratoconus may be guided toward helping them gain control of and most productively adapt to the illness experience.

All visual function subscale scores in group A, other than general health and driving, were significantly decreased compared with control subjects (P<0.05, Mann–Whitney test). Such patients notably showed low QOL scores also in the general health and mental health subscales. This supports the hypothesis that apparently normal visual acuity does not necessarily reflect unimpaired VR-QOL in patients with keratoconus.

The results underscore the complex nature of vision- and ocular-related QOL in patients with keratoconus. Patients with keratoconus experience multidimensional visual function impairment and associated QOL issues, and parameters other than traditional measures of visual acuity appear necessary to evaluate VR-QOL in

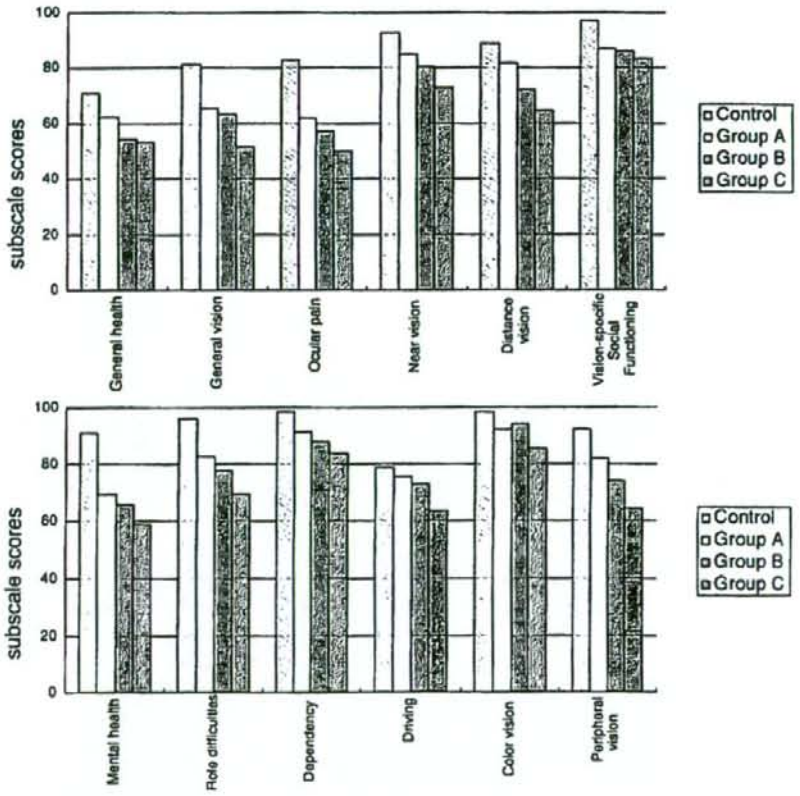

FIG. 1. Mean NEI-VFQ-25 subscale scores in groups A, B, and C and in control subjects. All group A, B, and C subscale scores (other than general health and driving in group A) were lower than those of age-matched control subjects (P<0.05, Mann–Whitney test).

Deposition of Lipid, Protein, and Secretory Phospholipase A_2 on Hydrophilic Contact Lenses

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Purpose. Recent studies have shown that low tear phospholipid levels are associated with tear film instability in hydrophilic contact lens wearers. The concentration of secretory phospholipase A_2 ($sPLA_2$), the enzyme that hydrolyzes phospholipids, in tears is known to exceed the levels found in serum by four orders of magnitude. This study was performed to determine the levels of $sPLA_2$ from the deposition on two different frequent-replacement contact lens materials. **Methods.** Polymacon and etafilcon A contact lenses worn for 2 weeks by 16 experienced contact lens wearers were used for the analysis. Total lipids were determined by the sulfo-phospho-vanillin reaction. Phospholipids in lipid extracts were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion. Total protein was measured by bicinchoninic acid analysis. Double-antibody sandwich enzyme-linked immunosorbent assay was used to determine $sPLA_2$ concentrations. **Results.** Total lipid deposition was found to be greater in the polymacon group (66.3 ± 16.3 μg/lens) than in the etafilcon A group, although phospholipids were not detected in either group. The etafilcon A group had greater deposition of protein (3.7 ± 0.7 mg/lens) than the polymacon group had. The etafilcon A group deposited statistically significantly more group IIa $sPLA_2$ (1.1 ± 0.3 μg /lens) than the polymacon group (0.07 ± 0.04 μg/lens) did ($P<0.001$). **Conclusions.** There was a significant difference in the lipid and protein deposition profiles in the two lenses tested. A significant amount of $sPLA_2$ in the deposition on contact lenses may play a role in tear film instability in hydrophilic contact lens wearers.

Key Words: Contact lens—Dry eye—Phospholipids—Secretory phospholipase A_2—Tears.

Contact lens–induced dry eye is one of the major causes of contact lens intolerance. Tear film stability, which is clinically estimated by tear film breakup time, is compromised in soft contact lens wearers,[1,2] especially in intolerant contact lens wearers.[3]

Recently, the structural and biologic roles of lipids in the tear film have been described in some detail.[4–6] The lipid layer is an essential component of the tear film by providing a smooth optical surface for the cornea and retarding evaporation from the ocular surface. In the current model of the tear film, the aqueous–mucin layer is covered by two thin layers consisting of lipids. Polar lipids, such as phospholipids, lie adjacent to the aqueous layer, and nonpolar lipids are present at the tear–air interface.[7] Recent studies have shown that the level of phospholipids in tears is the most influential factor for tear film stability, which is clinically estimated by tear film breakup time.[8] Thus, phospholipids, which link the nonpolar hydrophobic outer layer and the aqueous layer, are crucial for maintaining a stable tear film. A low level of phospholipids in the tears is associated with a short breakup time in hydrophilic contact lens wearers.[8–10]

Phospholipase A_2 (PLA_2) is a lipolytic enzyme that catalyzes the hydrolysis of phospholipids at the sn-2 position, yielding a free fatty acid and a lysophospholipid. PLA_2 has been categorized into at least 10 groups (I–X) based on amino acid sequence data.[11,12] Many of these enzymes are secreted extracellularly and are commonly referred to as secretory phospholipase A_2 ($sPLA_2$).[13] Of these, group IIa $sPLA_2$ (14 kilodaltons) is the most abundant form of $sPLA_2$ in tears.[14] It was found in concentrations in tears averaging 1.45 μg/mL[14] to 54.5 μg/mL,[15] which exceed the levels found in serum by four orders of magnitude. The presence of group IIa $sPLA_2$ in tears is supposed to be beneficial because of its bactericidal activity.[16,17] However, as suggested by Song et al.,[18] the excess of this enzyme may compromise the tear film stability, because it hydrolyzes phospholipids in tears.

As mentioned earlier, the level of phospholipids in tears of hydrophilic contact lens wearers is lower than that of healthy control subjects, which may be associated with instability of the tear film.[8–10] For the explanation of this phenomenon, two possibilities were hypothesized: Phospholipid deposition occurs on contact lenses, and phospholipids in tears are degraded by group IIa $sPLA_2$ deposited on contact lenses. To test these hypotheses, the levels of lipids, proteins, group IIa $sPLA_2$ content, and $sPLA_2$ activity were determined from the deposition on two different frequent-replacement hydrophilic contact lens materials.

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MATERIALS AND METHODS

Subjects and Contact Lenses

Sixteen experienced, asymptomatic contact lens wearers (six men and 10 women) ranging in age from 21 to 44 years participated in the study. Eight subjects were polymacon lens (Medalist; Bausch & Lomb Japan, Tokyo, Japan; Food and Drug Administration group I, low water content and nonionic) wearers, and eight subjects were etafilcon A lens (2-Week ACUVUE; Johnson & Johnson Japan, Tokyo, Japan; Food and Drug Administration group IV, high water content and ionic) wearers. Lenses worn for 2 weeks were collected and stored at −80°C until analysis. Each lens was cut in half for lipid and protein deposits analysis. All subjects provided written informed consent for participation. The protocol was approved by the institutional review board.

Lipid Analysis

Lipids were extracted by a modification of the Bligh and Dyer procedure.[19] In brief, samples were placed in a test tube with 1.0 mL of an extraction solvent consisting of a 2:1 ratio of chloroform to methanol (Wako, Inc., Osaka, Japan) for 16 hours. After adding 0.2 mL of water, test tubes were vortexed for 30 seconds. The upper aqueous layer was discarded, and the lower organic solvent layer was used for the analysis.

Total lipids were measured by the sulfo-phospho-vanillin reaction. The lipid extracts were evaporated to dryness under nitrogen gas and reconstituted with 50 μL of distilled water. After adding 100 μL of 95% sulfuric acid (Wako, Inc.), the sample was boiled at 100°C for 10 minutes. Then, samples placed in a 96-well microplate were mixed with 150 μL of the working reagent, containing 1.2 mg/mL vanillin (Kokusai Shiyaku Co., Tokyo, Japan). The absorbance of the solution was measured at 655 nm with a spectrophotometer. A standard curve established with lipids solution (Kokusai Shiyaku Co.) was used to quantify the total lipids of the lens extract.

Phospholipids were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion. After lipid extracts were evaporated to dryness under nitrogen gas, 50 μL of 10 mM TRIS hydrochloride (Sigma Chemical Co., St. Louis, MO) buffer (pH 7.8), containing 2.0 U/mL phospholipase C from *Bacillus cereus* (Sigma Chemical Co.), was added and incubated at 37°C for 20 minutes. The samples were incubated at 37°C for an additional 30 minutes after adding 50 μL of 175 mM diethanolamine hydrochloride (Sigma Chemical Co.) buffer (pH 9.6), containing 2.0 U/mL alkaline phosphatase from human placenta (Sigma Chemical Co.). Fifty microliters of samples were placed in a 96-well microplate and mixed with molybdate–malachite green reagent (BIOMOL, Inc., Plymouth Meeting, PA). The absorbance of the solution was measured at 620 nm with a spectrophotometer. A standard curve established with phospholipids mixture (Sigma Chemical Co.) was used to quantify the amounts of phospholipids.

Total Protein and $sPLA_2$ Analysis

A solvent consisting of a 1:1 mixture of 0.2% trifluoroacetic acid and acetonitrile (Wako, Inc.) was used to extract protein.[20] The lenses were placed in the extraction solution for 16 hours, and the extraction solution was subsequently analyzed. This method is a quick, efficient extraction technique for the removal of protein deposits from soft hydrophilic contact lenses. Determination of the total protein deposit from the lenses was carried out by a bicinchoninic acid analysis. The procedure consists of mixing 10 μL of sample solution with 300 μL of protein assay regent (Cytoskeleton, Inc., Denver, CO) comprising bicinchoninic acid and cupric sulfate in a 96-well microplate. Absorbance of the solution was measured at 595 nm with a spectrophotometer. A standard curve established with bovine serum (Sigma Chemical Co.) was used to quantify protein contents of the lens extract.

A double-antibody sandwich enzyme-linked immunosorbent assay was used to determine group IIa $sPLA_2$ concentrations in the protein samples. A commercial enzyme-linked immunosorbent assay kit (Cayman Chemicals, Ann Arbor, MI) was used according to the manufacturer's instructions. Samples were diluted to 1/500 or 1/5,000 concentrations. Absorbance was measured at 420 nm with a spectrophotometer.

Measurement of $sPLA_2$ activity was performed with a commercial $sPLA_2$ activity assay kit (Cayman Chemicals). The assay uses a 1,2-dithio analog of heptanoyl phosphatidylcholine, which serves as a substrate for most PLA_2, with the exception of cytosolic PLA_2. On hydrolysis of the thioester bond at the sn-2 position by PLA_2, free thiols were detected by using 5,5-dithio-bis(2-nitorobenzonic acid). Absorbance was measured at 420 nm with a spectrophotometer.

RESULTS

Total Lipids and Phospholipids

Total lipids and phospholipids deposition are shown in Figure 1. Total lipids were found to be greater in the polymacon group (66.3 ± 16.3 μg/lens) than in the etafilcon A group (44.1 ± 8.2 μg/lens). The difference between the two groups was statistically significant ($P<0.01$, Mann–Whitney test). Phospholipids were not detected in either group.

Total Protein

Results of total protein deposition analysis are shown in Figure 2. The etafilcon A group (3.7 ± 0.7 mg/lens) deposited substantially more protein than the polymacon group (0.03 ± 0.06 mg/lens) did. The difference was statistically significant ($P<0.001$, Mann–Whitney test).

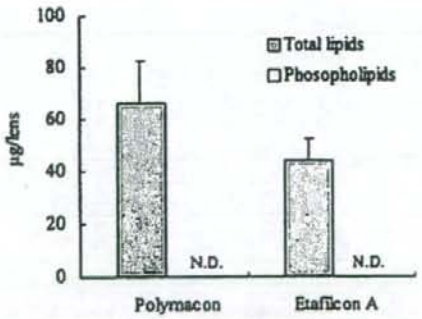

FIG. 1. Total lipids and phospholipids deposited on hydrophilic contact lenses. Total lipids were significantly greater in the polymacon group (66.3 ± 16.3 μg/lens) than in the etafilcon A group (44.1 ± 8.2 μg/lens). Phospholipids were not detected in either group.

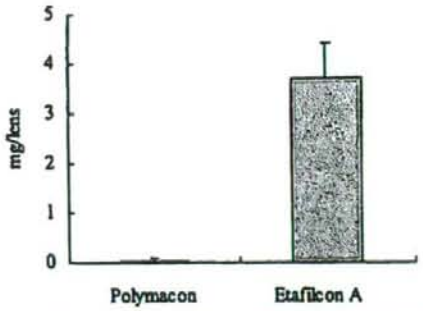

FIG. 2. Total proteins deposited on hydrophilic contact lenses. The etafilcon A group (3.7 ± 0.7 mg/lens) deposited significantly more protein than the polymacon group (0.03 ± 0.06 mg/lens) did.

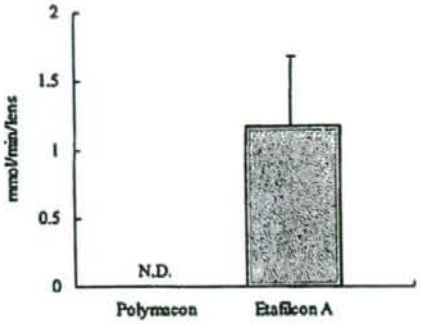

FIG. 4. $sPLA_2$ activity in the etafilcon A group was 1.18 ± 0.59 mmol/minute per lens, whereas $sPLA_2$ activity was not detected in the polymacon group.

Group IIa $sPLA_2$ and $sPLA_2$ Activity

The amount of group IIa $sPLA_2$ and its enzymatic activity deposited on contact lenses are shown in Figures 3 and 4. The etafilcon A group (1.1 ± 0.3 μg /lens) deposited more group IIa $sPLA_2$ than the polymacon group (0.07 ± 0.04 μg/lens) did. The difference was statistically significant ($P<0.001$, Mann–Whitney test). $sPLA_2$ deposited on the etafilcon A lenses retained its enzymatic activity. $sPLA_2$ activity in the etafilcon A group was 1.18 ± 0.59 mmol/minute per lens. In the polymacon group, $sPLA_2$ activity was not detected.

DISCUSSION

It is widely recognized that the adsorption of proteins and lipids on a contact lens is complex and depends on a number of factors. Notable among these are material water content and surface charge.[21–24] There was a significant difference in the lipid and protein deposition profiles between the two lenses tested in the current study. The lipid deposition profiles found in this study are consistent with previously published reports,[23,25] which concluded that hydrogel lenses with nonionic polymer matrices (i.e., groups I and II) deposit more lipids than materials that have ionic matrices (i.e., groups III and IV).[26] In the current study, however, phospholipid depositions were below the detection limit in either lens. Therefore, it is not likely that decreased phospholipid levels in tears of hydrophilic contact lens wearers is the result of the deposition of phospholipids on the lenses.

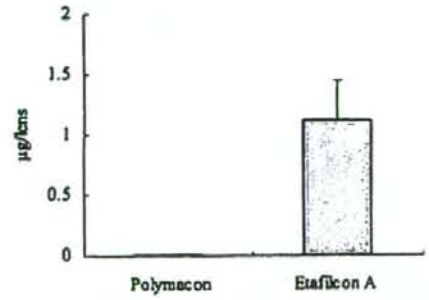

FIG. 3. Group IIa sPLA2 deposited on hydrophilic contact lenses. The etafilcon A group (1.1 ± 0.3 μg /lens) deposited significantly more group IIa $sPLA_2$ than the polymacon group (0.07 ± 0.04 μg/lens) did.

Etafilcon A (group IV material) attracted substantial quantities of protein, which was significantly greater than that measured for polymacon (group I material). Etafilcon A also had significantly more group IIa $sPLA_2$ than polymacon did. Protein deposition was predominantly controlled by the ionic charge of the lens material.[25] Methacrylic acid imparts a negative charge to the material and thus thermodynamically favors the deposition of positively charged species, such as lysozymes. Group IIa $sPLA_2$ is also highly cationic in tears[27] and allows productive electrostatic interactions with the negatively charged contact lens material.

It appears to be important that $sPLA_2$ deposited on etafilcon A lenses retained its enzymatic activity.[8,10] Hume et al.[17] reported that $sPLA_2$ deposited on contact lenses reduced the viable staphylococci adhering to the contact lens, which may be beneficial for the eye to prevent colonization by this pathogen. However, as suggested by Song et al.,[18] the excess of $sPLA_2$ may compromise tear film stability, which results in contact lens intolerance. Aho et al.[28] reported that contact lens wearers had statistically lower group IIa sPLA2 content in their tears at noon and at 4 P.M. than healthy control subjects did. They pointed out that the transient lowering effect on the group IIa sPLA2 content of tears may be the result of the absorption of group IIa sPLA2 onto the contact lenses. The current results appear to support these previous observations. Glasson et al.[29] reported that intolerant contact lens wearers had significantly greater concentration of group IIa $sPLA_2$ and peroxidized lipids in their tear fluids than tolerant subjects did. They suggested that decreased tear phospholipids and tear film instability may be the result of the action of group IIa $sPLA_2$ in the tear fluids. The current study found that a significant amount of group IIa $sPLA_2$ (1.1 ± 0.3 μg/lens) deposited on etafilcon A contact lenses. By assuming that the total tear volume of a healthy subject is 10 μL and that the concentration of group IIa $sPLA_2$ in normal tear fluids is 54.5 μg/mL,[15] 0.55 μg of group IIa $sPLA_2$ is present in the tears of healthy subjects. The amount of group IIa $sPLA_2$ deposited on etafilcon A contact lens is twice that in normal tear fluids. These results suggest an additional mechanism of contact lens–induced dry eye; group IIa $sPLA_2$ deposited on contact lenses may play a role in the development of tear film instability.

In summary, this study found that a significant amount of group IIa $sPLA_2$ deposited on contact lenses, at least on etafilcon A lenses. The enzyme deposited on contact lenses may promote

結膜嚢から分離されたブドウ球菌に対する二変量ノンパラメトリック密度を用いた薬剤感受性分布解析

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Antimicrobial Susceptibility Analysis of *Staphylococcus* Found in the Conjunctival Sacs Using Two-way Nonparametric Density Contours for Scatterplots

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目的：抗菌薬の細菌群に対する抗菌力の指標としては，ディスク法で感受性を示す菌株の割合や，最小発育阻止濃度（MIC）から作成される累積発育阻止曲線およびMIC_{50}，MIC_{90}などが用いられてきた．今回，オキサシリン（MPIPC）と各種フルオロキノロン剤〔FQ剤：ガチフロキサシン（GFLX），レボフロキサシン（LVFX），トスフロキサシン（TFLX），モキシフロキサシン（MFLX）〕のMIC値を用いて新しい三次元的な薬剤感受性の評価を試みた．方法：東京医療センターで術前患者の結膜嚢から分離された56株の黄色ブドウ球菌と140株の表皮ブドウ球菌を対象とした．各FQ剤のMICをX軸，MPIPCのMICをY軸とし，散布図を二変量ノンパラメトリック密度に変換し等高線パターンを描出した．FQ剤同士についても同様の方法で描出した．結果：黄色ブドウ球菌ではどのFQ剤もMPIPCと交差耐性を示した．表皮ブドウ球菌はMPIPCとFQ剤の感受性の差から細菌株が5群に群別され，交差耐性は認められなかった．FQ剤同士での検討では黄色ブドウ球菌，表皮ブドウ球菌ともに交差耐性を示した．結論：本方法は薬剤感受性の分布が三次元的に描出でき，感受性分布の違いや交差耐性の判定に応用できる解析法と考えられた．

Susceptibilties of bacterial species have been determined using the disc diffusion method or minimum inhibitory concentrations (MIC). We demonstrated a new three-dimensional analysis of susceptibilities using the MIC of oxacillin (MPIPC) and fluoroquinolones (gatifloxacin : GFLX, levofloxacin : LVFX, tosufloxacin : TFLX, moxifloxacin : MFLX). We retrospectively reviewed the database on 56 isolates of *Staphylococcus aureus* and 140 isolates of *Staphylococcus epidermidis* found in the conjunctival sacs of patients at National Tokyo Medical Center. Scatterplots were made comparing the MIC of MPIPC (Y-axis) to the MIC of fluoroquinolones (X-axis), and were then represented by two-way nonparametric density contours. The scatterplots comparing each fluoroquinolone were also represented. On *Staphylococcus aureus*, scatterplots revealed cross-resistance between MPIPC and each fluoroquinolone. On *Staphylococcus epidermidis*, isolates distributed into 5 groups, and cross-resistance was not obvious between MPIPC and fluoroquinolones. On both *Staphylococcus aureus* and *Staphylococcus epidermidis*, cross-resistance was confirmed between each fluoroquinolone. This method can be used to analyze three-dimensional susceptibility distribution or cross-resistance.

〔Atarashii Ganka (Journal of the Eye) 24(5)：663～667，2007〕

Key words：結膜，薬剤感受性，フルオロキノロン，黄色ブドウ球菌，表皮ブドウ球菌，conjunctiva, drug susceptibility, fluoroquinolones, *Staphylococcus aureus*, *Staphylococcus epidermidis*.

はじめに

抗菌薬に対する細菌の薬剤感受性検査とその判定法には，おもにディスク法や最小発育阻止濃度（minimum inhibitory concentration：MIC）の測定が用いられている．ディスク法では寒天培地での発育阻止円直径を測定し，sensitive，intermediate，resistantを定性的に判定する[1]．MICは寒天

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平板希釈法や微量液体希釈法により測定され，細菌の薬剤感受性を定量的に表すことができる[2,3]．

個々の細菌株の薬剤感受性ではなく，細菌群の薬剤感受性の指標としては，ディスク法で感受性を示す菌株の割合や，MICから作成される累積発育阻止曲線が用いられている．累積発育阻止曲線は細菌群の薬剤感受性を視覚的に評価することができるだけでなく，MIC_{50}，MIC_{90}などを指標にして抗菌力の強さを示すことも可能である．

しかし，細菌群の薬剤感受性による分布傾向や複数の薬剤に対する薬剤感受性の評価については，従来の方法では表現が困難であった．今回，これらを視覚的に表現する新しい解析方法として，ノンパラメトリック密度を用いた等高線パターンによる薬剤感受性分析の試みを行ったので報告する．

I　対象および方法

国立病院機構東京医療センターで2004年11月から2005年7月に，眼手術の術前検査として結膜嚢細菌培養検査を行った症例のうち，178例196眼（6～91歳）から分離された黄色ブドウ球菌56株，表皮ブドウ球菌140株を対象とした．

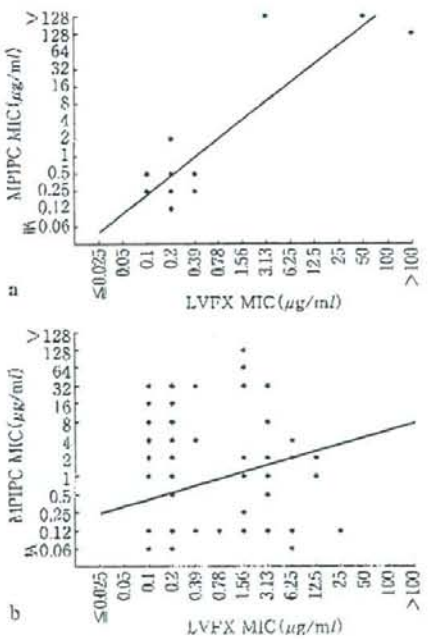

図1　オキサシリン（MPIPC）とフルオロキノロン剤（ここではLVFX）のMICを比較する散布図
a：黄色ブドウ球菌，b：表皮ブドウ球菌．LVFXのMICを対数でX軸に，MPIPCのMICを同じく対数でY軸にとり作成した．グラフ内の直線は回帰直線．相関係数はa：r^2=0.729，b：r^2=0.117．

各菌株を用いて，ガチフロキサシン（GFLX），レボフロキサシン（LVFX），トスフロキサシン（TFLX），モキシフロキサシン（MFLX）の4種のフルオロキノロン剤について，日本化学療法学会標準法に基づき希釈系列0.025～100 μg/ml（TFLXの希釈系列のみ薬剤の溶解性により0.025～25 μg/ml）の寒天平板希釈法によってMICを測定した．また，オキサシリン（MPIPC）のMICはClinical and Laboratory Standards Institute（CLSI）の規定に基づき希釈系列0.06～128 μg/mlで測定した．CLSIの規定に基づき，黄色ブドウ球菌についてはオキサシリンのMICが4 μg/ml以上のものをメチシリン耐性黄色ブドウ球菌（MRSA），2 μg/ml以下のものをメチシリン感受性黄色ブドウ球菌（MSSA）とした[4]．同様に，表皮ブドウ球菌については0.5 μg/ml以上のものをメチシリン耐性表皮ブドウ球菌（MRSE），0.25 μg/ml以下のものをメチシリン感受性表皮ブドウ球菌（MSSE）と判定した[4]．

黄色ブドウ球菌あるいは表皮ブドウ球菌について，比較する薬剤（例：LVFX）のMICを対数でX軸に，指標とする薬剤（例：MPIPC）のMICを同じく対数でY軸にとり散布図を作成した（図1a，b）．この散布図を基に二変量ノンパラメトリック密度の密度分布を等高線パターンで描出した．等高線パターンの描出および統計処理には，SAS社製JMP6を用いた．

II　結　　果

1．黄色ブドウ球菌における薬剤感受性分布

黄色ブドウ球菌に関して，MPIPCのMICをY軸にとり，各フルオロキノロン剤のMICをX軸にとって，二変量ノンパラメトリック密度の等高線パターンを描出した図を示す（図2）．MSSAについては各フルオロキノロン剤の感受性も良好（MIC<0.39 μg/ml）で，MRSAについては各フルオロキノロン剤の感受性も不良（MIC≧0.39 μg/ml）である傾向が明らかに示されている．相関係数を求めるとGFLXでr^2=0.723，LVFXでr^2=0.729，TFLXでr^2=0.769，MFLXでr^2=0.707という値であった．黄色ブドウ球菌に関してMPIPCと各フルオロキノロン剤の感受性には有意の高い正の相関があり，すなわち交差耐性があることが示された．

2．表皮ブドウ球菌における薬剤感受性株分布

表皮ブドウ球菌に関しても同様に，MPIPCのMICをY軸にとり，各フルオロキノロン剤のMICをX軸にとって，二変量ノンパラメトリック密度の等高線パターンを描出した図を示す（図3）．MPIPCと各フルオロキノロン剤の感受性は単純な相関を示さず，図内で計5つのグループに分かれる様子が描出された．

フルオロキノロン剤の感受性が良好（MIC<0.39 μg/ml）

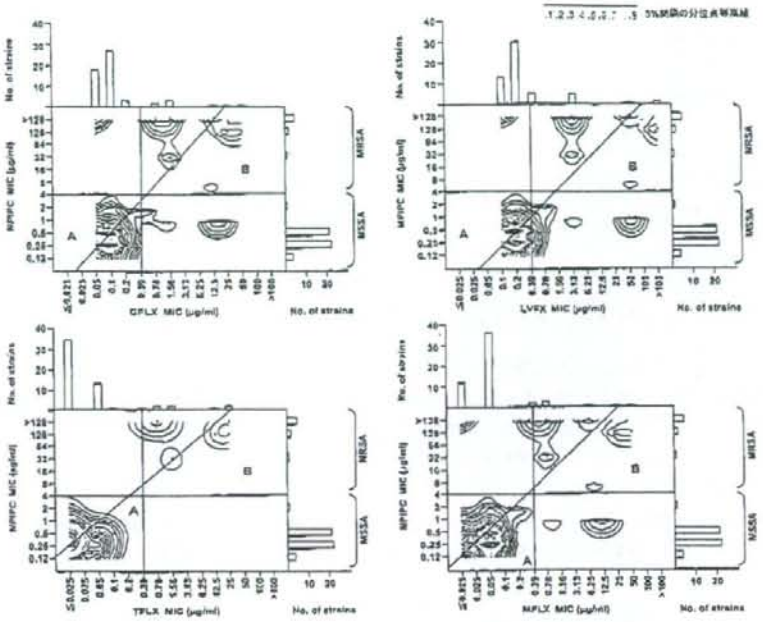

図2　二変量ノンパラメトリック密度の等高線パターンを用いて表した黄色ブドウ球菌のMPIPCとフルオロキノロン剤とのMIC分布の比較

赤線は回帰直線．相関係数はGFLX：r^2=0.723，LVFX：r^2=0.729，TFLX：r^2=0.769，MFLX：r^2=0.707．

黄色ブドウ球菌では，MPIPCとフルオロキノロン剤とは交差耐性を示し，MSSAではフルオロキノロン剤の感受性もよく（A），MRSAではフルオロキノロン剤の感受性も不良である（B）．

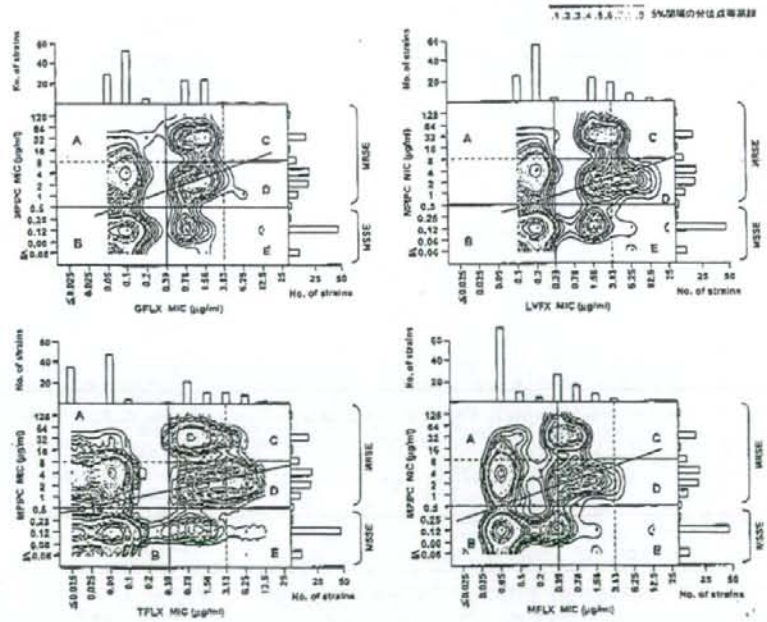

図3　二変量ノンパラメトリック密度の等高線パターンを用いて表した表皮ブドウ球菌のMPIPCとフルオロキノロン剤とのMIC分布の比較

赤線は回帰直線．相関係数はGFLX：r^2=0.135，LVFX：r^2=0.117，TFLX：r^2=0.113，MFLX：r^2=0.108．

表皮ブドウ球菌では，MPIPCとフルオロキノロン剤とは交差耐性を示さず，細菌株はAからEの5群に群別される．MPIPC，フルオロキノロン剤ともに感受性不良であるC，D群についてみるとGFLXとMFLXはMIC<3.13 μg/mlにとどまるが，LVFXとTFLXはMIC≧3.13 μg/mlの領域に分布している．

である領域にはMPIPCの感受性が不良であるMRSE（0.5 μg/ml≧MIC）の群と，良好であるMSSE（MIC<0.5 μg/ml）の群の2群がみられた．フルオロキノロン剤の感受性が不良（MIC≧0.39 μg/ml）である領域には，MRSEのうちMPIPC感受性がきわめて不良（MIC≧8.0 μg/ml）の群と，それ以下（0.5 μg/ml≦MIC<8.0 μg/ml）の群，MSSE（MIC

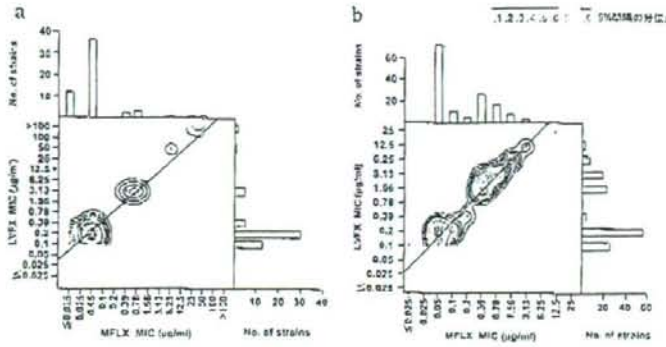

図4　二変量ノンパラメトリック密度の等高線パターンを用いて表したフルオロキノロン剤同士のMIC分布の比較の例（X軸：MFLX，Y軸：LVFX）

a：黄色ブドウ球菌，b：表皮ブドウ球菌．赤線は回帰直線．相関係数は黄色ブドウ球菌：$r^2=0.876$，表皮ブドウ球菌：$r^2=0.946$．フルオロキノロン剤同士では高い交差耐性があることを示している．

$\leq 0.5\,\mu g/ml$）の群の3群がみられた．表皮ブドウ球菌においては黄色ブドウ球菌でみられたようなMPIPCとフルオロキノロン剤との高い正の相関はみられず，交差耐性はないことが示された．MPIPCにもフルオロキノロン剤にも感受性が不良である群について各フルオロキノロン剤で比較すると，そのなかではGFLXとMFLXはMIC$<3.13\,\mu g/ml$にとどまるが，LVFXとTFLXはMIC$\geq 3.13\,\mu g/ml$を超えて分布する菌株がみられた．

3．フルオロキノロン剤同士での感受性の比較

フルオロキノロン剤同士の感受性を比較するためにフルオロキノロン剤同士をX軸とY軸にとって，二変量ノンパラメトリック密度の等高線パターンを描出した．例としてLVFXとMFLXについての黄色ブドウ球菌および表皮ブドウ球菌の感受性の分布を図4に示す．黄色ブドウ球菌，表皮ブドウ球菌いずれの場合でも両者には正の相関がみられ，交差耐性があることを示している．今回検討した4種類のフルオロキノロン剤のいずれの組み合わせの場合も正の相関がみられ，フルオロキノロン剤同士では交差耐性があることが示された．

III　考　　按

細菌に対する薬剤感受性検査とその判定法には，おもにディスク法やMICの測定が用いられてきた．ディスク法はKirby-Bauer法が広く用いられており，これはMueller-Hinton寒天培地を使用し，35℃で24時間培養後，対象薬剤含有のディスク周辺の発育阻止円直径でsensitive，intermediate，resistantを定性的に判定する方法である[1]．個々の細菌株について多種の薬剤感受性を評価するのに簡便かつ有用であり，臨床の現場で広く用いられている．MICの測定はわが国では日本化学療法学会標準法による寒天平板希釈法や微量液体希釈法が用いられている．前者は対象薬剤の濃度を調整した寒天平板培地を用い，後者は液体のMueller-Hinton broth（2% NaCl，Ca^{++} 50 mg/l，Mg^{++} 25 mg/lを含む）を用いる．35℃で24時間培養し発育阻止される最小濃度をMICと測定する[2,3]．

MICの測定は定量的な評価を行うことができるため，個々の細菌についての薬剤感受性の判定のみならず，細菌群に対してMICを測定すれば累積発育阻止曲線を描出することができ，細菌群の薬剤感受性の傾向を視覚的に評価することが可能である．さらに累積発育阻止曲線からMIC_{50}，MIC_{90}を測定することで，その薬剤の対象となる細菌群についての感受性を定量的に示すことも可能である．

しかし，細菌群の感受性による群別化や，分布傾向を調べるには，1種類の薬剤に対する累積発育阻止曲線を描出しただけでは評価が困難である．そこで2種類の薬剤のMICをX軸，Y軸に組み合わせることで細菌群の感受性分布を二次元的な散布図として描出した．散布図の各点においてはMICが同一の複数の菌株が重なっていることがあり，単純な散布図だけではその菌株の「重なり」，すなわち密度の評価ができない．そこで散布図上の点の密度にカーネル平滑化法という統計技法を施し，密度の分布をなだらかに曲線化して，等高線で同じ密度を結んで描出した．この方法を二変量ノンパラメトリック密度の等高線パターン描出とよぶが，本方法を用いることで2種類の薬剤のMICの二次元分布に菌株の密度の一次元を含めた三次元的な散布図として評価することが可能となった．

黄色ブドウ球菌群に関して，MPIPC対各種フルオロキノロン剤のノンパラメトリック密度の等高線パターンを描出したところ，高い相関係数を示し，MPIPCと各フルオロキノロン剤は黄色ブドウ球菌について交差耐性があることが示された．言い換えるとMSSAに対しては各種フルオロキノロン剤の感受性が良好である一方，MRSAにはどのフルオロキノロン剤も感受性が不良であることを示すものである．MRSAはβラクタム系薬剤に耐性を示すだけでなく，フルオロキノロン系を含めた多剤に対して耐性を示すことが問題となっており[5-8]，今回の結果もその傾向に一致するものであった．

MPIPCの作用機序はpenicillin binding protein（PBP）

PBPにはPBP-1, -2, -3, -4の4種類がある）への結合を介した細胞壁阻害であり，MRSAのMPIPC薬剤耐性はMPIPCとの結合の親和性が低いPBP2′というPBP2の代替酵素をつくることによる．PBP2′を産生する遺伝子は*mecA*遺伝子とよばれる．一方でフルオロキノロン剤の作用機序はDNA gyraseとtopoisomerase Ⅳを標的としたDNA複製阻害であり，MPIPCの作用機序とは異なるものである．異なる薬剤作用機序をもつMPIPCとフルオロキノロン剤がなぜ黄色ブドウ球菌に関して交差耐性を有するのかは，不明の点も多いが，フルオロキノロン耐性の主要遺伝子である*gyrA*が，MPIPC耐性の*mec*近傍に局在していることが背景にあると考えられている[9]．今回の方法でグループ分けができた菌株について，これらの遺伝子を標的にした遺伝子解析を行えば，機序の解明に重要な知見が得られる可能性がある．

表皮ブドウ球菌に関してはMPIPCとフルオロキノロン剤の相関は低く，今回は5つのグループに分類された結果となった．これはMRSAと異なり，MRSEでは*gyrA*と*mec*が離れて局在していることが関係するのかもしれない[11]．表皮ブドウ球菌の5つのグループのうち，臨床的に重要と考えられるのはMPIPCにもフルオロキノロン剤にも感受性が不良であるグループであるが，この細菌群に注目して各種フルオロキノロン剤での分布図を比較すると，GFLXとMFLXに関してはMIC値が3.13 μg/mlを超える領域には菌株は分布していない一方で，他の2剤はMIC値が3.13 μg/mlを超える領域にも菌株が分布していた．これまでの報告でも，フルオロキノロン剤の中で比較的新しく開発されたGFLXとMFLXについては，表皮ブドウ球菌に対して耐性菌の出現が少ないことが知られている[10~12]．

また，臨床的に汎用されているディスク法による薬剤感受性試験は全身投与での血中濃度を指標にしているが，点眼された抗菌薬は高濃度であるため薬剤感受性試験で耐性とされても点眼投与が臨床的に効果を示すことがあるという報告もある[13]．今回の分布図で比較したように，どの程度のMIC値まで分布の広がりを示すかを比較して評価することは，抗菌薬の選択に関して一つの参考になると考えられる．また，今後こうした分布の違いを示す菌株においてDNA解析を行うことも薬剤耐性の機序を解明するうえで重要と考えられる．

今回の方法において，フルオロキノロン剤同士で感受性の分布傾向をみてみると，黄色ブドウ球菌，表皮ブドウ球菌ともに高い正の相関を示し，フルオロキノロン剤同士では交差耐性を示すことが確認された．この方法を応用すれば，同系統あるいは異なる系統の薬剤に関して交差耐性の有無の判定をすることも可能である．

今回用いた解析方法を行うためには，統計処理に通常は30株以上の菌株数が必要になること，それぞれの菌株について目的とする抗菌薬のMICを求める必要があることなどの短所はあるが，細菌群の薬剤感受性について，その分布傾向や群別を視覚的に表現できる利点があると考えられた．

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