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Evaluating an impression cytology grading system (IC score) in patients with dry eye syndrome

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CLINICAL STUDY

Abstract

Purpose To evaluate the IC score, a newly developed grading system for the morphological alterations of the ocular surface as a diagnostic tool in relation to type and severity of dry eye disease.

Methods Impression cytological specimens of 309 patients with simple dry eye (S-DE), 50 patients with Sjögren's syndrome and dry eye (SS-DE), and 39 patients with connective tissue disease and dry eye (CTD-DE) were examined. In all, 10 different morphological parameters were judged with points (IC subscores) and summarized to the IC score. IC score and subscores were correlated to tear function test results and with each other.

Results SS-DE patients had the highest IC scores and IC subscores. IC scores and IC subscores correlated significantly with the fluorescein staining of the cornea in all three patient groups. They were correlated to the rose bengal score in SS-DE and S-DE patients. The analysis of the IC subscores showed that there are significant correlations between most of the morphological alterations investigated. This underlines the importance of each parameter for the summarized IC score.

Conclusion The IC score provides detailed information about the morphological alterations of the bulbar conjunctiva. It has proven to be useful for the differentiation of dry eye diseases and might help in the investigation of the pathogenesis of these diseases as well as for follow-up and therapy control.

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Introduction

The diagnosis of dry eye, a disorder of the ocular surface, is commonly based on typical symptoms, and various clinical tests evaluating tear film stability, tear secretion, and integrity of the cornea and conjunctiva by vital staining with fluorescein and rose bengal. Additionally, the morphology of the ocular surface can be investigated by conjunctival impression cytology. As the origin of ocular surface diseases is multifactorial, taking together the results of ocular surface and tear function tests provides a more complete understanding of this complex dynamic process.

Impression cytology is a simple, noninvasive technique that allows the collection of the superficial layers of the conjunctival epithelium for the morphological examination of the ocular surface. It was first described by Larmande and Timsit[1] for diagnosis of squamous neoplasia of the ocular surface in 1954. Egbert *et al*[2] documented the use of impression cytology for examining ocular surface disorders and goblet cell densities in 1977. This technique causes no discomfort for the patient, is repeatable, and offers the possibility of following the course of morphological alterations of the ocular surface.

The normal ocular surface (cornea and conjunctiva) is composed of a stratified nonkeratinizing epithelium, and in case of conjunctiva, different amounts of goblet cells, which produce the mucous part of the tear film. Pathological changes, such as squamous metaplasia of the epithelial cells, keratinization,

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and reduction of goblet cell density up to
total goblet cell loss, occur in numerous disorders
such as dry eye, Sjögren's syndrome,
blepharokonjunctivitis, inflammatory kerato-
konjunctivitis, and vitamin A deficiency.[3–12]

For staging of the morphological changes of the
conjunctiva, several grading systems have been
developed. The first grading system published by Nelson
et al[3] was based on the morphological appearance of the
conjunctival epithelial and goblet cells, which was
completed in 1988[4] by the so-called mean individual
epithelial cell area, according to the degree of squamous
metaplasia, and the number of goblet cells. This grading
system was scaled from 0 to 3, based on the morphology
of the epithelial cells, their staining behaviour, the
nucleoplasmic ratio, as well as the density and the PAS
staining of the goblet cells. This classification system is
still widely used. Tseng[5] published a grading system in
1985, scaled from 0 to 5, which was based on the criteria
described above, and the degree of keratinization of the
epithelial cells in correlation to the three major steps of
squamous metaplasia—loss of goblet cells, increase of
cellular stratification, and keratinization. Adams *et al*[13]
developed a grading system in 1988, scaled from 0 to 3,
taking into consideration the criteria of Tseng together
with the quality of the cell-to-cell cohesion, that is,
confluent or not. Heiligenhaus *et al*[14] added one
morphological parameter to the staging of Tseng, the
condensation of the nuclear chromatin. In the above-
mentioned grading schemes,[3–5,13,14] the alteration of the
epithelial cells and nuclei, goblet cell density, and
morphological changes of goblet cells, as well as other
morphological qualities were judged conjointly.

As we observed that the morphological changes of the
bulbar conjunctiva, seen in ocular surface disorders, do
not always occur in typical combinations, a new grading
system (IC score) was developed. It includes more
morphological parameters (IC subscores) to provide a
complete picture of the conjunctival alterations. The
parameters were separately judged and summarized to a
total score (IC score), as already carried out by Aragona
and co-workers on specimens from contact lens
wearers.[15] The IC score was designed for observing the
course of dry eye patients over a long period, and for the
statistical evaluation of morphological changes.[16,17]

The aim of this study was to evaluate the IC score on
impression cytological specimens of 398 patients with
dry eye syndrome as a diagnostic tool in relation to the
type and severity of disease. The IC scores and the score
points for each morphological parameter (IC subscores)
of patients with simple dry eye (S-DE), patients with
Sjögren's syndrome and dry eye (SS-DE), and patients
with dry eye and connective tissue disease (CTD-DE)
were calculated and compared.

The summarized IC scores and the IC subscores were
correlated to the results of tear function tests such as tear
film break up time (BUT), fluorescein staining of the
corneal surface, Schirmer test without anaesthesia
(Schirmer I), and rose bengal staining.

Finally, the IC subscores were statistically analysed to
find out, if they correlate with the IC score as well as with
each other, as proposed by authors, who graded the
different morphological alterations conjointly.

Patients and methods

Patients' data

A computerized search of medical records from 1
January, 1994, to 31 July, 2003 identified all patients who
presented at the outpatient clinic of the Department of
Ophthalmology of the Medical University, Graz, Austria
with ocular discomfort.

The study was designed in concordance with the
declaration of Helsinki; patients' data were evaluated
anonymously.

The ocular examination included tear film BUT,
fluorescein staining of the corneal surface, Schirmer I,
rose bengal staining, and impression cytology of the
bulbar conjunctiva, as described previously.[17] In brief,
tear film BUT was measured by touching the
inferotemporal bulbar conjunctiva with a fluorescein
sodium strip, wetted with a preservative-free isotonic
sodium chloride solution. The mean value of a total of
three measurements was recorded. The corneal surface
was examined under blue-light illumination with a
biomicroscope after fluorescein instillation into the tear
film. The intensity of fluorescein staining of the cornea
was graded for each eye using a four-point scale (0,
no staining; 1, less than one-third; 2, less than two-thirds;
and 3, more than two-thirds staining of the cornea).
A 5-min Schirmer I test without anaesthesia was
performed on closed eyes using commercially available
5×35-mm paper strips (Clement Clarke International
Ltd, London, UK). Rose bengal staining was performed
by adding one drop (about $20 \mu l$) of 1% rose bengal
solution to the bulbar conjunctiva and the reaction was
classified according to the van Bijsterveld scoring
system.[18] The results of both eyes were averaged.

The criteria for inclusion of patients were typical
symptoms of dry eye such as dryness, burning, itching,
foreign body sensation as well as ocular pain, and red or
easily fatigued eyes, a reduced tear film BUT below 10 s,
and interpretable impression cytological specimens of
the bulbar conjunctiva. A total of 538 patients met the
inclusion criteria.

Contact lens wearers, patients with allergic
conjunctivitis (with the presence of eosinophilic

granulocytes in the inferior tarsal conjunctiva after Giemsa staining), patients with active infectious conjunctivitis, patients after a chemical injury or recent ocular surgery, patients with blepharospasm or with abnormal blinking as well as patients with superior limbal keratoconjunctivitis, and patients with ocular pemphigoid or with graft-versus-host disease (140 in total) were excluded.

The remaining 398 patients were divided into the following groups of dry eye disease:

(1) *S-DE*: patients with simple dry eye without an autoimmune disease: $n = 309$ (77.6%),
(2) *SS-DE*: patients with primary or secondary Sjögren's syndrome who met the criteria based on the classification of Vitali *et al*:[19] $n = 50$ (12.6%), and
(3) *CTD-DE*: patients with dry eye and connective tissue disease who did not match the criteria of Sjögren's syndrome such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis and others: $n = 39$ (9.8%). For demographic and clinical data see Table 1.

Impression cytology

Impression cytology specimens were obtained after topical anaesthesia with 0.4% oxybuprocaine from the superior and inferior bulbar conjunctiva (12 o'clock, 6 o'clock position) of both eyes of each patient. Cellulose acetate filter sheets (type VC, VCWP 04700, Millipore Corp., Bedford, MA, USA) with $0.10\,\mu$m pores were cut into strips of about 3–10 mm, and applied to the bulbar conjunctiva dull side down. With a flat, round-tipped forceps, the strips were gently pressed onto the ocular surface for a few seconds, and removed with a peeling motion. With this method, one to two epithelial cell sheets can be harvested. The filter strips were placed on slides on which double-sided adhesive tape had been applied, and air-dried. The specimens were stained with a combination of Gill's modified Papanicolaou stain and periodic acid-Schiff (PAS) reaction, as described by Tseng,[5] placed in xylene to clear, and permanently mounted on a glass slide in Pertex® xylene-soluble

mounting medium (medite, Germany), covered with a coverslip.

Examination under a light microscope (Zeiss, Axioscop) was always performed by the same researcher (HS-EM) without the knowledge of the clinical status. The following 10 morphological parameters were examined and judged with points (IC subscores) from 0 (normal) to 3 (highly pathological): quality of cell-to-cell adhesion (epithelial cell sheet confluent or not), degree of squamous metaplasia (nucleo/cytoplasmic correlation), tendency for keratinization, nuclear changes: type and frequency, as well as morphology and density of conjunctival goblet cells. According to the findings of Rivas *et al*,[20] the goblet cell densities were judged as normal (abundant), slightly, moderately, and distinctly reduced (single to no goblet cells). Morphology and density of superficial mucus, and the presence or absence of inflammatory cells were also judged. Thus, when summarized to the IC score, a maximum count of 30 points may be reached (Table 2, Figure 1a–f).

Statistical analysis

Data were entered into a computerized database and analysed. Continuous variables are reported as mean ($\pm$standard deviation (SD)) and categorical data are displayed as frequencies with percentages in parentheses. To determine the statistical significance of group differences among the three study groups, the Kruskal–Wallis test was used for continuous data. *Post hoc* tests were performed with the Wilcoxon rank-sum test. Crosstabulations (χ^2 test, two-tailed Fisher's exact test) were used to assess the relationship between categorical variables. For correlations among continuous variables, we calculated the Spearman correlation coefficient.

All computations were carried out using the statistical package SPSS for Windows version 12.0.1®. A *P*-value of 0.05 or less was considered as statistically significant, except for *post hoc* tests where the significance level was corrected according to Bonferroni.

Table 1 Demographic data and clinical picture

	S-DE, n = 309	*SS-DE*, n = 50	*CTD-DE*, n = 39
Age	52.9 ($\pm$15.9)	52.8 ($\pm$13.4)	53.4 ($\pm$12.4)
Gender (female (%))	235 (76.1%)	48 (96.0%)*	35 (89.7%)
Schirmer I (mm)	10.8 ($\pm$9.5)	1.4 ($\pm$1.7)*,†	7.8 ($\pm$7.6)
BUT (s)	5.0 ($\pm$2.4)	2.0 ($\pm$1.8)*,†	4.3 ($\pm$2.7)
Corneal staining score (0–3)	0.4 ($\pm$0.7)	1.5 ($\pm$1.0)*,†	0.7 ($\pm$0.9)
Rose bengal staining score (0–9)	2.6 ($\pm$2.4)	7.5 ($\pm$1.8)*,†	3.7 ($\pm$2.5)‡

Statistically significant differences between groups: *SS-DE and S-DE; †SS-DE and CTD-DE; ‡CTD-DE and S-DE.

Table 2 Morphological parameters and IC points included in the IC score grading system

	IC subscores	0	1	2	3
A	Epithelial cell sheet	Intact, dense	Slightly loosened	Loosened	Single cells
B	Degree of squamous metaplasia = nucleocytoplasmic ratio (N/C)	None 1:1–1:3	Low graded 1:4–1:6	Distinct 1:6–1:10	Massive >1:10
C	Degree of keratinization	None	Low graded	Distinct	Massive
D	Nuclear changes frequency	None	Sporadic	Several	Abundant
E	Nuclear changes type	None	Low graded: pycnosis, dissociation of chromatin, chromatin condensation	1 + snake-like chromatin	2 + nuclear fragmentation
F	Goblet cell density	Normal	Slightly reduced	Distinctly reduced	Sporadic or no goblet cells
G	Goblet cell morphology	Normal	—	—	Pathological
H	Mucus amount	Normal	No mucus	Medium	Abundant
I	Mucus morphology	Granular plaques	0 + exudate	0 or 1 + filaments	1 or 2 + aggregation
K	Inflammatory cells	None	Sporadic	Several	Abundant

Results

A total of 398 patients (318 women and 80 men) of a clinical population suffering from dry eye were retrospectively analysed.

SS-DE patients showed the most pathological tear film and ocular surface parameters compared to S-DE patients ($P < 0.001$ for BUT, corneal staining score, and rose bengal staining) and CTD-DE patients ($P < 0.001$ for BUT, corneal staining score, and rose bengal staining), respectively. In CTD-DE patients, a significantly higher, that is, more pathological rose bengal staining score was found compared to S-DE patients ($P = 0.014$) (Table 1).

Concerning the clinical diagnoses, the most pathological—that is, highest—IC scores (mean = 13.4; SD = 5.4) were found in SS-DE patients. S-DE patients showed a significantly less pathological picture (mean = 10.0; SD = 4.9; $P < 0.001$) as well as CTD-DE patients (mean = 10.1; SD = 4.6; $P = 0.002$) (Table 3).

The IC subscores for each morphological parameter showed that the following features differed significantly in SS-DE patients when compared to S-DE patients. The quality of cell-to-cell adhesion, the degree of squamous metaplasia, the type and frequency of nuclear changes, the morphology of goblet cells, the morphology of the superficial mucus, and the goblet cell density were more pathologic in SS-DE patients. Additionally, the IC subscores for the degree of squamous metaplasia, and the type and frequency of nuclear changes were significantly higher in SS-DE patients than those of CTD-DE patients. There was no statistically significant difference in the tendency towards keratinization, the goblet cell density, and mucus amount, between the three patients groups.

Fluorescein staining of the cornea of each patient group was significantly correlated to the respective IC scores ($r = 0.29$, $r = 0.58$, and $r = 0.47$ for S-DE, SS-DE, and

CTD-DE patients, respectively). The rose bengal score was correlated to the IC score in S-DE and SS-DE patients ($r = 0.30$, $r = 0.32$, respectively). Tear film BUT and Schirmer I test results did not correlate to the IC score in any of the three patients groups.

Concerning the correlations to clinical tear film and ocular surface parameters, the IC subscores, especially quality of cell-to-cell adhesion, degree of squamous metaplasia, nuclear changes, number of goblet cells, mucous aggregation, and presence or absence of inflammatory cells showed the same correlation pattern compared to the IC score. No correlation was observed with tendency towards keratinization, goblet cell morphology, and mucous amount (data not shown).

All IC subscores were well correlated with the IC score in the three patients groups. The analysis of the IC subscores revealed significant correlations between most of the morphological alterations investigated except for the morphology of the superficial mucus. This parameter was only correlated with the presence of inflammatory cells in S-DE and SS-DE patients (data not shown).

Discussion

Impression cytology has become the standard technique to study squamous metaplasia of the conjunctiva and goblet cell loss in ocular surface diseases. Various grading schemes were used, most of them being derived from the schemes proposed by Nelson *et al*,[3] Nelson,[4] or Tseng.[5] Grading schemes provide a quick means of gross assessment of the morphological appearance of the ocular surface, especially the bulbar conjunctiva and are therefore useful for diagnosis and follow-up.

The IC score presented here provides an adoption of the grading scheme published by Aragona *et al*[15] including the following 10 morphological parameters: the quality of cell-to-cell adhesion (epithelial cell sheet
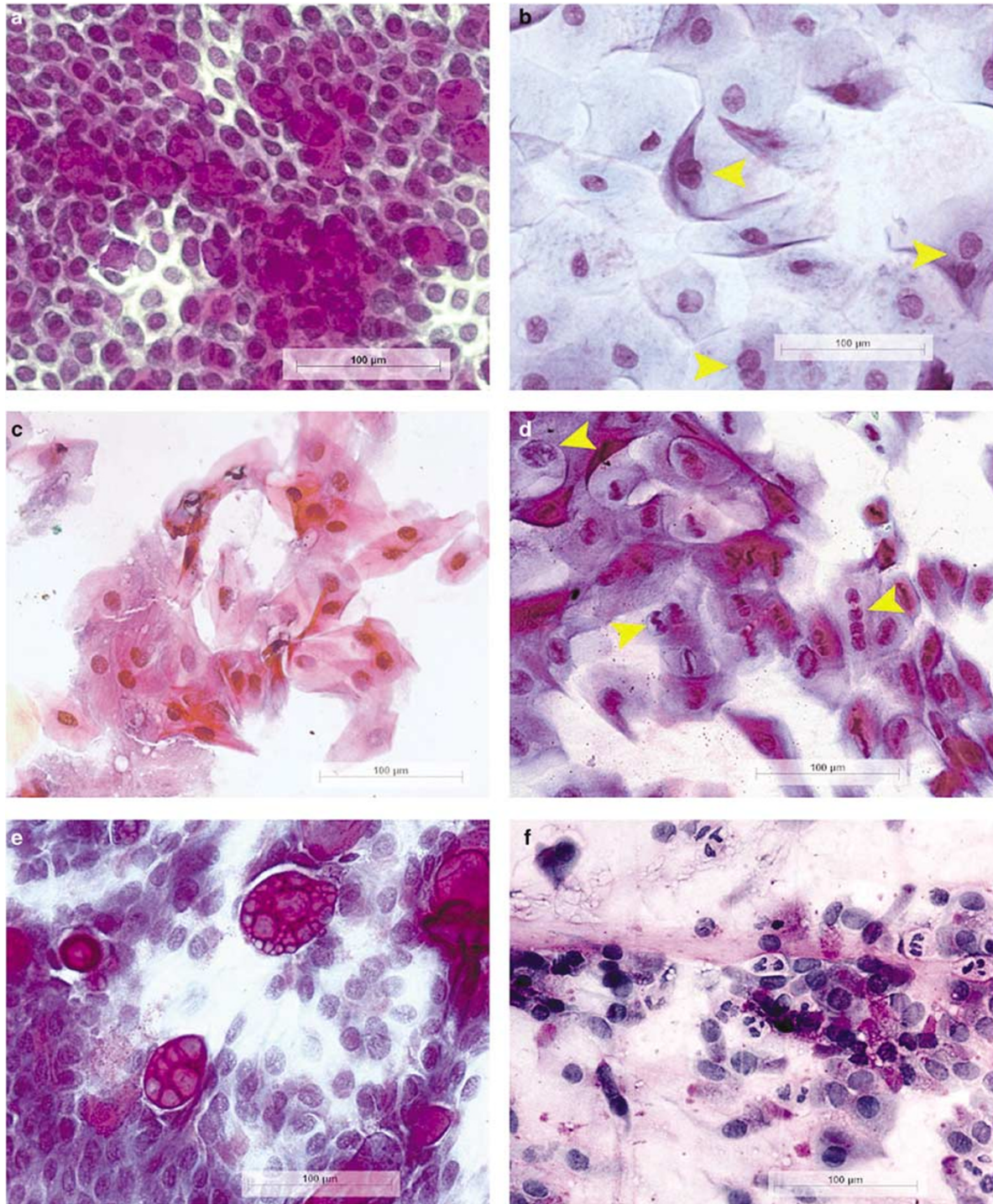
Figure 1 (a–f) Photomicrographs of impression cytology specimens, stained with PAS- and Gill's-modified Papanicolaou stain, microscopic magnification ×40. (a) Normal cytological picture with round-shaped epithelial cells, dense staining round nuclei, and abundant goblet cells. (b) Distinct squamous metaplasia of the epithelial cells (high nucleocytoplasmic ratio) with double nuclei (arrowheads) and absence of goblet cells. (c) Distinct squamous metaplasia of the epithelial cells (high nucleocytoplasmic ratio) and different degrees of keratinization. (d) Distinct squamous metaplasia of the epithelial cells (high nucleocytoplasmic ratio) and different degrees of keratinization. All nuclei pathological altered: double nuclei, snake-like chromatin, and nuclear fragmentations (arrowheads). (e) Normal epithelial cells and pathological altered goblet cells. (f) Slight squamous metaplasia of the epithelial cells with normal nuclei. High mucous amount with mucous strains and polymorphonuclear leucocytes between the epithelial cells. Absence of goblet cells.

Table 3 IC score of the study groups

	S-DE	SS-DE	CTD-DE
IC score	10.0 (±4.9)	13.4 (±5.4)*,†	10.1 (±4.7)

Statistically significant differences between groups: *SS-DE and S-DE; †SS-DE and CTD-DE.

confluent or not), the degree of squamous metaplasia (nucleocytoplasmic ratio), the tendency for keratinization, nuclear changes: type and frequency, the morphology and density of conjunctival goblet cells, as well as the type and amount of superficial mucus, and the presence or absence of inflammatory cells. As performed by Aragona *et al*,[15] but different to the protocols of Nelson *et al*,[3] Nelson,[4] and Tseng,[5] these parameters were judged individually resulting in the so-called IC subscores and summarized to the IC score. This provides the possibility to evaluate each morphological parameter separately, and to inter-relate each result, IC score or IC subscores, with diagnostic or clinical parameters as well. As previously carried out by Nepp *et al*,[21] each IC subscore as well as the IC score may be combined to other morphological or clinical and tear film, and staining scores to obtain some kind of 'overall dry eye score'.

To compare the IC score and IC subscores with the results of diagnostic tests for dry eye disease, we examined patients of a clinical population, who were classified in three different groups: S-DE, SS-DE, and CTD-DE-patients, since they might have a higher risk for a severe course of disease.

As expected, SS-DE patients had the most pathological tear film and ocular surface parameters when compared to the other two groups, and CTD-DE patients showed a more pathological rose bengal staining score than S-DE patients. SS-DE patients presented the highest IC scores reflecting an intensely altered morphology of the ocular surface, as described by other authors,[7,9,22,23] while S-DE- or CTD-DE patients showed similar degrees of conjunctival alteration.

Comparing the IC subscores with the type of dry eye disease, these results were mainly confirmed, but the results for the tendency towards keratinization, the goblet cell density, and the mucus amount were not significantly different between the three patients groups. This might be due to the fact that loss of goblet cells and keratinization occur early after the onset of disease.

To the best of our knowledge most of the researchers investigated the inter-relations of the clinical tear film and ocular surface parameters with single morphological parameters such as degree of squamous metaplasia, goblet cell density, or nuclear changes, while in the study presented, these correlations were calculated for the IC

subscores and the summarized IC score as well. A comparison of our results with previously postulated correlations is difficult, because the patients groups investigated were different over all the studies performed, and there were different localizations of specimen harvesting—12 o'clock, 6 o'clock position *vs* many different parts of the conjunctiva including interpalpebral and palpebral regions.

In concordance to Pflugfelder,[24] we found that in patients with SS-DE, the morphological alterations seen in impression cytology specimens correlated well with clinical findings, such as staining of the cornea and conjunctiva.

The IC subscores for the quality of the cell sheet, the degree of squamous metaplasia, the nuclear changes, the goblet cell density, mucous aggregation, and presence or absence of inflammatory cells were also correlated with tear film and ocular surface tests as described for the IC score. Our results are in concordance with Lee and co-workers,[25] who found that the severity of squamous metaplasia correlated well with the intensity of the rose bengal score, and with Pflugfelder *et al*,[8] who described a correlation between goblet cell density and rose bengal staining. Bjerrum[26] found a correlation between nuclear changes and rose bengal staining, which was also seen in our population. In our opinion, these findings demonstrate and underline that there is a close relationship between ocular surface staining and the IC subscores as well as the summarized IC score.

In our three patients groups, there was no correlation of the IC score and IC subscores with BUT, and Schirmer I test results. This is in contrast to the findings of Pflugfelder *et al*,[8] who observed a correlation of goblet cell density, and an inverse correlation between the degree of squamous metaplasia with Schirmer I test results in patients with ocular irritation.

As expected, all IC subscores were well correlated with the IC score in the three patients groups, and there were significant correlations between most of the morphological alterations investigated. The type and frequency of nuclear changes were moderately (CTD-DE patients) to strongly (S-DE and SS-DE patients) correlated, which demonstrates that nuclear changes such as snake-like chromatin or nuclear fragmentation reflect a highly pathological picture, independent of the frequency of these alterations. In concordance to Rivas and co-workers,[10] we found no correlation between the type of nuclear changes, the degree of squamous metaplasia, and the presence of inflammatory cells in patients with SS-DE. Like Tseng,[5] we found that a decreased goblet cell density was accompanied by an increasing nucleocytoplasmic ratio, but we did not see a distinct correlation of the inflammatory cell infiltrate with the presence of extensive squamous metaplasia in

SS-DE patients, which was observed by Pflugfelder and co-workers.[9]

Overall, these results underline the important contribution of each morphological parameter investigated to the summarized IC score.

In summary, judging conjointly morphological alterations for grading the alterations of the (bulbar) conjunctiva, as carried out by several investigators before, gives a good, reproducible overview, but the IC score provides more detailed information, because each morphological parameter can be judged separately. For an overview, the resulting IC subscores are summarized to the IC score. In the study population presented here, the IC scores of patients with SS-DE are significantly different from the IC scores obtained from patients with S-DE or CTD-DE, thus providing a helpful diagnostic tool for the differentiation of these diseases.

As the specimens were always taken from the same positions, the comparison of the IC subscores obtained during an observation period might be helpful for the follow-up of the alteration of each morphological parameter.

Thus, the IC score provides an extensive evaluation of a lot of morphological alterations of the bulbar conjunctiva, and may therefore also serve as a valuable research tool for the investigation of the pathogenesis of dry eye disease as well as for therapy control.

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