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Diagnostic value of three simple and rapid dry eye tests – lid parallel conjunctival folds, tear meniscus height, and tear ferning

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SUMMARY

Introduction/Objective The objective of this paper was to assess the diagnostic value of three simple dry eye (DE) tests: lid parallel conjunctival folds (LIPCOF), tear meniscus height (TMH), and tear ferning (TF).

Methods LIPCOF, TMH, and TF diagnostic DE tests were performed in 100 patients. Eighty of them were referred to us by rheumatologists and general practitioners either during evaluation for Sjögren's syndrome, or because of DE symptoms. The control group was composed of 20 patients, with no DE-related symptoms. Ocular Surface Disease Index questionnaire was used for DE symptoms' evaluation. Results of LIPCOF, TMH, and TF tests were compared with results of the Copenhagen criteria DE tests i.e., tear fluorescein breakup time, Schirmer I and Rose Bengal tests. Ability of the tests to recognize DE in various grades according to Dry Eye Work Shop (DEWS) report score system was assessed.

Results Compared to the Copenhagen criteria, sensitivity of LIPCOF and TMH was high (92.8% and 83.5%, respectively), while specificity was low (34.4% and 49.2%, respectively). TF had low sensitivity (59.1%) but high specificity (82.7%). Mean values of both LIPCOF and TMH differed significantly ($F = 7.222$, $p < 0.001$ and $F = 11.802$, $p < 0.001$, respectively) between the control group and all DEWS grades, but not among different grades of DE.

Conclusion TMH and LIPCOF diagnostic tests showed high sensitivity, which makes them excellent screening DE tests. Low sensitivity of TF suggests that it is not truly a good screening test on its own, but its high specificity is of definite value.

Keywords: dry eye disease; lid parallel conjunctival folds; tear meniscus height; tear ferning

INTRODUCTION

In the pool of diagnostic tests for dry eye (DE), no test is found to be both sensitive and specific enough on its own [1]. For reaching DE diagnosis in practice, there is a tendency to use a group of clinical tests, chosen at the examiners discretion, to complement overall clinical judgment. To state it otherwise, although there is a consensus of a group of experts on DE definition (Dry Eye Work Shop – DEWS), there is no consensus on a definite set of tests (nor their outcomes) for DE [2]. Also, symptoms often do not correlate with signs of DE nor do they correlate well with the stage of DE [3, 4]. A new report of the DEWS group from 2017 suggests evaluating symptoms with Ocular Surface Disease Index (OSDI) or the Five-Item Dry Eye (DEQ-5) questionnaire. Clinical tests for reaching the DE diagnosis in their opinion are non-invasive breakup time or fluorescein tear breakup time (FTBUT), tear osmolarity, or ocular surface staining. But for grading of the disease and assessing the type of DE they recommend other tests, like non-invasive tear volume measurement, assessing meibomian gland disfunction (MGD), and lipid thickness/dynamics [1].

While searching for any well-defined set of clinical DE tests, commonly used as a whole, rather than as an *ex tempore* formed group of tests, the Copenhagen criteria (CC) tests stand out as a very well defined and time-honored set. These tests combine acceptable levels of both sensitivity and specificity for non-Sjögren's syndrome (SS) DE though they were initially devised for SS-related DE [5]. They were, accordingly, used in our study as the criteria for DE diagnosis and a reference clinical standard for the comparison with single tests that we were interested in: lid parallel conjunctival folds (LIPCOF), tear meniscus height (TMH), and tear ferning (TF).

There is a rising number of people suffering from DE symptoms, seeking help from their eye doctors, who do not always have time or resources to apply sophisticated diagnostic tests. Epidemiological studies have demonstrated that DE has a prevalence of 5–45%, depending on the criteria and location [6–10]. In a study with over 20,000 glaucoma patients, Erb et al. [11] report TMH and LIPCOF as simple and noninvasive tests for DE. TF was suggested by the DEWS group as a potentially good screening test [1].

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Our aim was to compare LIPCOF, TMH, and TF tests with CC DE tests and to analyze their ability to recognize dry eye disease (DED) in it its various stages.

METHODS

Out of 100 subjects we examined for DE (200 eyes) at the Clinic for Eye Diseases, Clinical Centre of Serbia, during 2013 and 2014, 88 were woman. The mean age ± SD was 50.17 ± 16.74 years. Thirty of them were referred to us by rheumatologists during evaluation for SS, and 50 were referred by general practitioners because of DE symptoms. The control group was made up of 20 patients, with no DE-related symptoms, examined during the evaluation for cataract surgery. The two groups were matched for age (no statistically significant difference between groups, $p = 0.21$) and sex ($p = 0.45$). Exclusion criteria in our study were any ocular surgery performed within one year, contact lens wear, topical eye therapy (if the only therapy was tear substitutes, they had to be suspended at least eight hours prior to the examination), entropion, ectropion, or other lid closure problems, ocular allergies, or the presence of anterior blepharitis. The study was approved by the Ethical Committee of the University of Belgrade, Faculty of Medicine. All the patients signed an informed consent form.

We performed the following clinical tests: Schirmer without anesthesia (Schirmer I), FTBUT, Rose Bengal (RB), LIPCOF, TMH, and TF. Eyelids were inspected for MGD. The symptoms were evaluated based on OSDI. Only the patients with OSDI score under 13 were enrolled into the control group.

To confirm DED in our study, we considered results from a group of three clinical tests. These three tests – Schirmer I, FTBUT, and RB – represent the ophthalmological part of testing for SS according to CC but also proved useful in diagnosing DE out of the SS context [5]. In order to be diagnosed with DE, the patient should be positive for two out of three CC tests in one or both eyes. According to CC, a positive result for Schirmer I test is value less than 10 mm, for the FTBUT test the value less than 10 seconds, and for the RB test score equal or greater than 4 according to Van Bijsterveld grading system [12]. Eighty of them had DED, since one or both eyes were positive in two out of three clinical tests. Twenty patients among this symptomatic group had some form of MGD. In the control group, no eye met these criteria. One patient from the control group had MGD, without signs or symptoms of DED. Bearing in mind that we separately analyzed both eyes, we found that 139 eyes were positive for DED. We also graded DE severity from 1 to 4, according to the DEWS report score system, where grade 1 is mild DE and 4 is the most severe form of the disease [13].

The tests were performed during one examination, by two examiners, in the morning. Patients' TMH and the presence of folds for LIPCOF test were examined by slit-lamp. We performed these tests at the beginning of examination to avoid blinking induced by prolonged gaze and also to avoid induced reflex tearing. For TMH, we registered values of 0.3 mm, 0.2 mm, 0.1 mm, and less than 0.1 mm. TMH was compared with variable slit-lamp beam height, which was regulated with a mechanical cylinder attached to the slit lamp. Once we adjusted the beam height, we read the value from the measuring scale connected to the cylinder. The lowest value on the measuring scale at our disposal was 0.2, followed by 0.3. When TMH was half of 0.2 mm beam height, we registered the value as 0.1, and if TMH was lower than half of 0.2 mm beam height, it was registered as lower than 0.1 mm. Measuring of TMH was done at the 6 o'clock position, where lower limbus was in the closest contact with the lid, in order to avoid influence of conjunctival folds on the measurement. For the LIPCOF test, we registered values only in the temporal zone as no folds, half of the fold (if the horizontal fold was not present completely throughout the temporal zone), one fold less than 0.2 mm in height, two folds 0.2 mm heigh, three folds or more of over 0.2 mm. These stages, although similar, are not completely analogous to those most commonly used, described by Höh et al. [14]. Instead of using the term normal meniscus tear height, we used the value of 0.2 mm as a cut-off value between stages. This value was considered as normal height for tear meniscus by other authors as well [15, 16]. In order to form four grades as is the case with the DEWS severity score system, we divided stage 1 by Höh into two stages. Then we performed Schirmer I, FTBUT, and RB tests. The Schirmer I test was performed by hooking the folded end of Schirmer paper over the temporal one-third of the lower lid margin. After a period of five minutes, we measured the length of wetting from the notch. For FTBUT, the dye was applied on the ocular surface with impregnated strips. Looking through cobalt blue filter, we measured the time needed for the dyed tear film to break up. After applying tetracaine eye drops, we instilled RB dye and scored result with the Van Bijsterveld grading system. Collecting tear sample from the inferior tear meniscus, for performing the TF test, was done by Eppendorf (Merck KGaA, Darmstadt, Germany) automatic micropipette with single-use 1–10 µl Eppendorf Tips. Tear sample was pipetted onto a clean microscope slide and allowed to air-dry for 10 minutes. Then it was observed by phase contrast light microscope at magnification levels of 20 × and 40 ×, and quantified according to the Rolando grading scale, based on the level of arborization, where grade 1 is characterized by uniformed large arborization, while in grade 4 there is no ferning [17].

We analyzed sensitivity (ability to recognize the disease), specificity (ability to rule out disease), positive and negative predictive value (PPV and NPV) of all clinical tests used in the study. By using one-way ANOVA and *post-hoc* test, we tested their ability to grade severity of DE according to the severity score system from the DEWS report. The data were statistically evaluated by using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Most of the eyes (37.5%) diagnosed as dry in our study belong to grade 2 according to severity score system from

Table 1. Results of clinical tests from dry eye group and group of normal eyes

Clinical test	Mean value dry eyes	0.95 CI	Mean value normal eyes	0.95 CI	t	p
Schirmer I	15.61	± 1.469	25.125	± 1.989	-7.74	< 0.0001
FTBUT	5.08	± 0.457	10.6	± 0.573	-11.47	< 0.0001
RB	3.38	± 0.385	0.35	± 0.212	13.82	< 0.0001
TMH	0.11	± 0.008	0.165	± 0.019	-5.34	< 0.0001
LIPCOF	1.41	± 0.117	0.625	± 0.222	6.26	< 0.0001
TF	2.52	± 0.137	1.5789	± 0.212	7.52	< 0.0001

CI – confidence interval; t – value of Student's t test; Schirmer I – Schirmer test without anesthesia; FTBUT – fluorescein tear breakup time; RB – Rose Bengal; TMH – tear meniscus height; LIPCOF – lid parallel conjunctival folds; TF – tear ferning;
p is statistically significant at the level < 0.01

the DEWS report. Fifty-four (27%) eyes belong to grade 1, 23 (11.5%) to grade 3, and only 11 eyes (5.5%) to grade 4.

All of the clinical tests that we used in this study were able to distinguish normal from DE. Mean value of parameters measured by these tests and significance of difference between test values for non-DE and DE groups are presented in Table 1.

When tested against the group of DE tests form CC, FTBUT had the highest sensitivity (95%), followed by LIPCOF and TMH (92.8% and 83.5%, respectively). RB and Schirmer I had 100% specificity, but TF also displayed high specificity (82.7%). Sensitivity and specificity of all the tests as well as PPV and NPV are presented in Table 2.

Table 2. Sensitivity, specificity, positive predictive value, and negative predictive value of clinical tests, each against dry eye tests from the Copenhagen criteria

Parameters	FTBUT	RB	Sch I	LIPCOF	TMH	TF
Se (%)	95	48.9	33.1	92.8	83.5	59.1
Sp (%)	80.3	100	100	34.4	49.2	82.7
PPV	0.92	1	1	0.76	0.79	0.89
NPV	0.85	0.46	0.44	0.68	0.57	0.47

Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value; DE – dry eye; FTBUT – fluorescein tear breakup time; RB – Rose Bengal; Sch I – Schirmer I; LIPCOF – lid parallel conjunctival folds; TMH – tear meniscus height; TF – tear ferning

Table 3. Mean fluorescein tear breakup time values in different dry eye severity groups

Groups	n	Mean	SD	SE	95% confidence interval for mean		Min.	Max.
					Lower bound	Upper bound		
0	37	10.59	1.94	0.32	9.95	11.24	4	15
1	54	6.96	2.72	0.37	6.22	7.71	3	11
2	75	5.08	2.3	0.27	4.55	5.61	2	10
3	23	3.48	2.48	0.52	2.40	4.55	0	10
4	11	0.55	0.93	0.28	-0.08	1.17	0	2
Total	200	6.18	3.49	0.25	5.69	6.66	0	15

FTBUT – fluorescein tear breakup time; n – number of eyes; Mean – average parameter value of tested eyes of different grades; SD – standard deviation; SE – standard error

We analyzed mean FTBUT values between different grades of severity according to DEWS (Table 3). By using ANOVA, we found that the average FTBUT value differs between the groups (F = 62.474, p < 0.001). *Post-hoc* test allowed us to establish that this difference was statistically significant for every group compared to all the other groups (Figure 1).

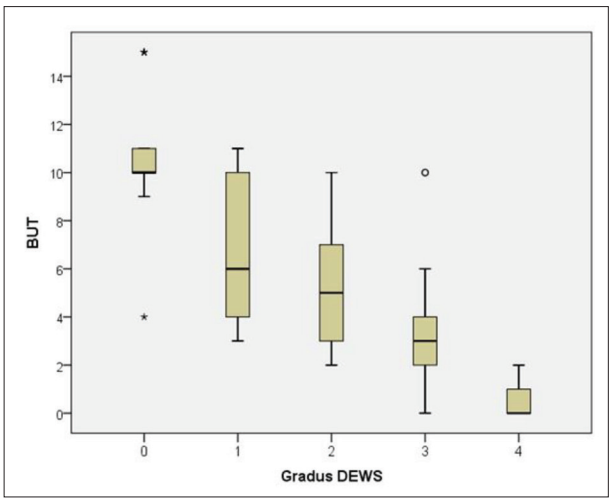

Figure 1. Mean fluorescein tear breakup time values in different dry eye severity groups;
average fluorescein tear breakup time value differs between the groups tested with ANOVA (F = 62,474, p < 0,001); difference is statistically significant for every group compared to all the other groups analyzed with the post-hoc test; the mean difference is significant at the 0.05 level;
FTBUT – fluorescein tear breakup time; Gradus DEWS – grades by the Dry Eye Work Shop report score system [2]

When we analyzed mean values between different DEWS grades with ANOVA (Table 4), we found that there is a statistically significant difference for the TF test (F = 18.192, p < 0.001). Analyzed with the *post-hoc* test, we found a significant difference between all the groups, except between the second and the third, and the third and the fourth grade.

Table 4. Mean tear ferning values in different dry eye severity groups

Groups	n	Mean	SD	SE	95% confidence interval for mean		Min.	Max.
					Lower bound	Upper bound		
0	34	1.59	0.701	0.120	1.34	1.83	1	3
1	53	2.11	0.847	0.116	1.88	2.35	1	4
2	72	2.54	0.786	0.093	2.36	2.73	1	4
3	21	2.81	0.680	0.148	2.5	3.12	1	4
4	10	3.50	0.527	0.167	3.12	3.88	3	4
Total	190	2.33	0.897	0.065	2.2	2.46	1	4

TF – tear ferning; n – number of eyes; Mean – average value of tested eyes of different grades; SD – standard deviation; SE – standard error

LIPCOF and TMH tests' mean values also differed significantly (Table 5 and Table 6) between the groups (respectively F = 7.222, p < 0.001; F = 11.802, p < 0.001). With the *post-hoc* test we established that this was due to the significant difference between the control group and all other severity grade groups, including mild DE grade, for both tests (cut-off value was 0.19 for TMH and 0.97 for LIPCOF). The difference was not significant among different grades of DE.

Table 5. Mean values for tear meniscus height in different dry eye severity groups

Groups	N	Mean	SD	SE	95% confidence interval for mean		Min.	Max.
					Lower bound	Upper bound		
0	37	0.17	0.06	0.01	0.15	0.19	0.1	0.3
1	54	0.12	0.06	0.01	0.10	0.14	0.05	0.3
2	75	0.11	0.05	0.01	0.10	0.13	0.05	0.3
3	23	0.09	0.03	0.01	0.07	0.1	0.05	0.2
4	11	0.09	0.07	0.02	0.05	0.14	0	0.2
Total	200	0.12	0.06	0.00	0.11	0.13	0	0.3

TMH – tear meniscus height; n – number of eyes; Mean – average parameter value of tested eyes of different grades; SD – standard deviation; SE – standard error

Table 6. Mean values for lid parallel conjunctival folds in different dry eye severity groups

Groups	n	Mean	SD	SE	95% confidence interval for mean		Min.	Max.
					Lower bound	Upper bound		
0	37	0.73	0.72	0.12	0.49	0.97	0	2
1	54	1.19	0.82	0.11	0.96	1.41	0	3
2	75	1.39	0.75	0.09	1.21	1.56	0	3
3	23	1.65	0.65	0.13	1.37	1.93	1	3
4	11	1.55	0.69	0.21	1.08	2.01	1	3
Total	200	1.25	0.8	0.06	1.14	1.36	0	3

LIPCOF – lid parallel conjunctival folds; n – number of eyes; Mean – average parameter value of tested eyes of different grades; SD – standard deviation; SE – standard error

We analyzed separately patients with DE who in the course of this study were diagnosed with SS according to revised international criteria [18]. Comparing average values of Schirmer I test between DE of the patients with SS (11.79 mm), and patients without SS (18.23 mm), we found that the first group, expectedly, had significantly lower values ($t = -4.25$, $p < 0.001$). Average FTBUT value of 4.15 seconds in SS patients was also significantly lower than 5.64 in non Sjögren DE ($t = -3.13$, $p = 0.002$), and the RB in average was significantly higher (4.06 in SS group versus 2.98 in non-SS group, $t = 2.64$, $p = 0.009$). Eyes of the patients with SS had in average more folds in LIPCOF test (1.52 in SS group versus 1.33 in non-SS group, $t = 1.57$, $p = 0.06$), but there was no difference between the groups when it comes to TF test and TMH (respectively, $t = 0.27$, $p = 0.39$; $t = -0.39$, $p = 0.35$). Eyes of the patients with SS were statistically more in higher grades of severity ($t = 4.02$, $p < 0.0001$).

DISCUSSION

According to DEWS Diagnostic Methodology Subcommittee we should be aware of the difference between DE tests used for screening, where high sensitivity is preferable and group of diagnostic tests for DED with high overall accuracy along with good sensitivity [1]. Screening tests that the DEWS group suggested are TMH and TF, especially the first one, being rapid and simple, and also with good sensitivity, as confirmed by other studies [11]. In our study, both LIPCOF and TMH had good sensitivity, compared to the CC DED clinical tests group (92.8%, 83.5%). Their ability to distinguish normal from mild DE makes them especially convenient for screening. García-Resúa et al. [19] found that there is a good correlation between osmolarity and subjective grading of TMH as well as measuring of TMH using open-source software (NIH ImageJ) [19]. Both tear osmolarity and tear meniscus optical coherence tomography (OCT) measurements comply with the DEWS grading system as previously reported by Tukenmez-Dikmen et al. [20] Mean values of TMH and LIPCOF between different grades of DE did not show statistically significant difference, so according to our result they are not convenient for grading DE. In our study, the mean value of TMH in the group without DE was 0.17 mm. It is somewhat lower than the one published by Imamura et al. [15] measured on slit-lamp with graticule for three different age groups of patients without DE (from younger to older; group 1: 206 μm; group 2: 209 μm; group 3, 226 μm). One would expect that the average value in the older group would be lower as in their study, but they assumed that the obstruction of lacrimal drainage that occurs with age may have influenced the results in their study. When comparing the average value of TMH measured with slit-lamp and with OCT in normal subjects, Imamura et al. [15] found no statistical differences. Since variability in measurement was less shown with the slit-lamp method, they suggest slit-lamp measuring of TMH may still be one of the most useful clinical methods to evaluate tear meniscus. With the cut off value of 0.19 mm, the sensitivity of TMH in our study was 83.5%. With the cut-off value of 205 μm, Singh et al. [21] found that sensitivity of TMH measured with OCT was 98.3%. As reported in the study by Erb et al. [11], we also found that LIPCOF has high sensitivity with the cut-off value of 0.97, but its ability to rule out diagnosis where DE was not recognized by other clinical tests was low (33.9%). Specificity of TMH compared to CC DE tests was also low (49.2%). TF has been reported previously by Rolando as a valuable test and the grading scale he devised, as the most popular one, has been used by other authors [17, 22]. The TF test shows strong correlation with osmolarity as reported by Versura et al. [23], statistically significant for each DE subgroup. In our study, TF did not have high sensitivity and could not distinguish between all DE subgroups, but had good specificity.

Values of Schirmer I and FTBUT tests of patients with SS were significantly lower than those in the group of patients with no SS. The average value of RB was higher for eyes of the patients with SS, as reported in other studies as well [24]. One would expect that the average value of TMH would be lower in the SS group, but that was not the case in our study. On the other hand, there were more conjunctival folds in the LIPCOF test in eyes of patients with SS. TF showed no difference between the two groups.

New methods of meniscometry have been developed to facilitate simple and dynamic visualization of the tear meniscus. OCT assessment of the tear meniscus and conjunctival folds has been extensively studied in the last decade [25, 26]. Spectral-domain OCT meniscometry has shown

high reproducibility, but can be biased by conjunctivochalasis and LIPCOF in the same way as with slit-lamp measurements of the tear meniscus [1]. Measuring TMH at the 6 o'clock position is, in our opinion, optimal when using slit-lamp, but the same position is suggested by other authors as the preferred one when using swept-source OCT [15]. Whether we observe tear meniscus or the presence of conjunctival folds, analysis of the image acquired with OCT may be complex, time-consuming, and operator-dependent. Therefore, we think that slit-lamp measurements of TMH and LIPCOF are preferred as screening test available in everyday ophthalmology practice.

CONCLUSION

TMH and LIPCOF diagnostic tests are rapid and simple DE tests, whose high sensitivity and ability to recognize even mild cases, in spite of lacking the strength to rule out disease where other tests are negative, makes them excellent screening DE tests. Due to low sensitivity in our study, TF seems not to be a very good screening test. In our study, FTBUT showed remarkably high sensitivity and ability to correctly distinguish between all DED severity groups, which makes it a good screening test, but also a good test for grading and monitoring the effects of therapy for DED.

NOTE

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Conflict of interest: None declared.

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Дијагностичка вредност три једноставна и брза теста за суво око – набори конјунктиве паралелни ивици капка, висина менискуса суза и тест гранања суза

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САЖЕТАК

Увод/Циљ Циљ овог рада је да се процени дијагностичка вредност три једноставна теста за суво око: набори конјунктиве паралелни ивици капка (НКПИК), висина менискуса суза (ВМС) и тест гранања сузе (ГС).

Методе Дијагностички тестови НКПИК, ВМС и ГС су изведени код 100 пацијената, од којих нам је 80 упућено на преглед од стране реуматолога и надлежних офталмолога, током испитивања на Сјогренов синдром или због симптома сувог ока. Контролну групу је чинило 20 пацијената без симптома сувог ока. Симптоми су евалуирани применом упитника о индексу болести површине ока. Резултати тестова НКПИК, ВМС и ГС су упоређени са вредностима резултата тестова за суво око по Копенхашким критеријумима, а то су: време прекида сузног филма обојеног флуоресцеином, мерење секреције суза без анестезије током пет минута Шимеровом траком (*Schirmer I*) и бојење површине ока виталном бојом *Rose Bengal*. Такође је процењена способност тестова да препознају различите стадијуме по систему градирања болести *Dry Eye Work Shop* (*DEWS*).

Резултати Поређењем са групом тестова по Копенхашким критеријумима, НКПИК и ВМС су показали високу сензитивност (92,8% и 83,5%), док им је специфичност била ниска (34,4% и 49,2%). ГС је имао ниску сензитивност (59,1%), али високу специфичност (82,7%). Просечне вредности тестова НКПИК и ВМС се статистички значајно разликују између контролне групе и свих стадијума болести по градацији *DEWS*, али не и између различитих стадијума болести сувог ока.

Закључак Тестови ВМС и НКПИК су показали високу сензитивност, што их чини одличним тестовима за скрининг болести сувог ока. Ниска сензитивност теста ГС га не сврстава у добре скрининг тестове, али његова висока специфичност му даје дијагностичку вредност.

Кључне речи: болест сувог ока; набори конјунктиве паралелни ивици капка; висина менискуса суза; тест гранања сузе