New model to better diagnose dry eye disease integrating OCT corneal epithelial mapping

Norah A Edorh,[1] Adil El Maftouhi,[2] Zoubir Djerada,[1] Carl Arndt,[1,3] Alexandre Denoyer [1,3]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bjophthalmol-2021-318826).

[1]Department of Ophthalmology, CHU Reims, Reims, France
[2]Department 3, National Hospital Centre for Ophthalmology Quinze-Vingts, Paris, France
[3]Reims Champagne-Ardenne University, Reims, France

Correspondence to
Professor Alexandre Denoyer, CHU Reims, Reims 51097, France;
alexandre.denoyer@gmail.com

Received 13 January 2021
Revised 29 March 2021
Accepted 3 May 2021

To cite: Edorh NA, El Maftouhi A, Djerada Z, *et al*. *Br J Ophthalmol* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/bjophthalmol-2021-318826

ABSTRACT

Purpose To optimise the objective diagnosis of dry eye disease (DED), the capabilities of wide corneal epithelial mapping using optical coherence tomography (OCT) were studied and subsequently integrated into a new scoring method.

Methods Fifty-nine patients (118 eyes) with DED and 55 control subjects (110 eyes) were included. All patients underwent a complete ocular surface evaluation. Corneal epithelial thickness was collected using OCT for seven zones. DED and the control group were compared using a t-test, and univariate receiver operating characteristic (ROC) curves were calculated to define the diagnostic ability of OCT epithelial mapping. Multivariate analyses were performed using artificial intelligence (random forest) and logistic regression approaches to define the best way to integrate OCT mapping in the diagnosis of DED. Then, a final multivariable model for diagnosing DED was validated through a bootstrapping method.

Results The DED group had significant epithelial thinning compared with the controls, regardless of location. Superior intermediate epithelial thickness was the best marker for diagnosing DED using OCT (binormal estimated area under ROC: 0.87; best cut-off value: 50 µm thickness). The difference between the inferior and superior peripheral zones was the best marker for grading the severity of DED (analysis of variance, p=0.009). A multivariate approach identified other significant covariables which were integrated into a multivariate model to improve the sensitivity (86.4%) and specificity (91.7%) of this innovative diagnostic method.

Conclusion Including OCT corneal epithelial mapping in a new diagnostic tool for DED could allow optimisation of the screening and staging of the disease in current practice as well as for clinical research purposes.

INTRODUCTION

Dry eye disease (DED) is a common condition that is often underdiagnosed. It is estimated to account for up to 25% of the reasons for seeking eye care. The high prevalence of DED, as well as its financial cost and impact on patient quality of life, makes it a true public health issue. Common symptoms of DED include eye irritation, redness, fluctuating vision and photophobia, which may result from damage at the ocular surface (ie, the tear film and both the conjunctival and corneal epithelia as recently reported by the Dry Eye Workshop (DEWS) II).[1]

A multitude of diagnostic tests are used (patient questionnaires, tear film break-up time (BUT) and ocular surface staining, Schirmer tests, tear film osmolarity and biomarker assessments).[2] The combined use of such a large panel of tests helps to better understand the disease. However, the objective diagnosis of DED, as well as the quantitative evaluation of its severity, remains a crucial issue in current practice, especially since all of these examinations require a large technical platform, are time consuming, correlate poorly with patient symptoms[3–5] and may be influenced by the skill and/or subjectivity of the operator and the evaluation conditions. In addition, the worldwide decrease in medical demography associated with new allocations of paramedical staff should promote the emergence of rapid and objective tests for screening and evaluating frequent diseases such as dry eye.

In this context, the corneal epithelium has been widely investigated at the molecular level ex vivo via impression cytology[6] and the cellular level using in vivo confocal microscopy.[7 8] More recently, the measurement of epithelial thickness using optical coherence tomography (OCT) has been investigated in the field of DED.[9–12] The OCT approach has an advantage over the other tools as it is a non-contact technique for measuring and mapping epithelial thickness over a large corneal area in high resolution in a rapid, objective and reliable way. Nothing has been done, however, to directly assess the performance of OCT in terms of diagnosis and the evaluation of its severity. More precisely, this biomarker could be included into a multivariable model aiming at better diagnosing DED.

The main objective of our study was to study the wide corneal epithelial mapping in DED for integrating the OCT data in a new routine equation to better diagnose and evaluate the disease.

METHODS

This monocentric, prospective and comparative study was conducted in the ocular surface unit of the Department of Ophthalmology, Robert Debré University Hospital (University of Reims Champagne-Ardenne, Reims, France), in accordance with the Declaration of Helsinki, Scotland Amendment, 2000. Permission from the institutional review board was obtained, and all patients gave validated and informed consent.

Subjects and design

From July to December 2019, all patients coming for the first time for specialised consultation in ocular surface disease filled out the Ocular Surface Disease Index (OSDI) questionnaire[13] and underwent a

complete ophthalmological examination including non-invasive tear film BUT, fluorescein ocular surface staining (Oxford scale), meiboscopic evaluation of meibomian gland dysfunction (MGD) in the lower lid, the Schirmer I test and corneal esthesiometry, together with anterior segment examination, intraocular pressure measurement and fundus.

The inclusion criteria were as follows: over 18 years old, best distance corrected visual acuity measured at 0 LogMAR or better and the ability to understand the study consent. The exclusion criteria included: any ocular pathology but DED, eyelid malposition or dynamic disorders, previous ocular/eyelid surgery, contact lens wear, treatment changes within the last 3 months and pregnancy. Also, all patients using any local treatment but tear film substitutes were excluded.

Each eligible subject was consequently included either in the dry eye group or the healthy control group depending on the diagnostic criteria of DED as previously defined by the DEWS II[1]: symptoms of dryness (OSDI) and the presence of at least one ocular surface disorder as assessed by non-invasive BUT and ocular surface staining.

Corneal epithelial OCT

Once accepted for the study, each subject underwent anterior segment OCT followed by a complete ocular examination by the same ophthalmologist (NAE) as detailed above. Fourier-domain OCT device RTVue XR 100 Avanti (Optovue, Fremont, USA) was used in our study. The scan beam wavelength was 840 nm, and the scan speed was 70 000 scans/s with a 5 µm axial resolution. Six individual acquisitions were performed in each eye. Three were taken immediately after a blink, and three were taken 10 s after blinking. A pachymetry scan pattern (namely 'cornea wide') with a 9 mm scan diameter and eight radials was used to map the cornea. Corneal epithelial thickness maps over the 9 mm diameter corneal area were provided by the RTVue corneal adaptor module software via automatic detection. The map is centred on the visual axis. Epithelial thicknesses were averaged from the three captures for each area of interest: superior areas at 3–5, 5–7 and 7–9 mm from the centre (S1, S2 and S3, respectively), the central area and the inferior areas corresponding to the superior ones (I1, I2 and I3) (see online supplemental figure 1).

Statistical analysis

All data are given as the mean±SD. A priori power analysis based on the previously reported DED-related changes in mean corneal epithelial thickness as measured by OCT was conducted to determine the minimum sample size (at least 48 subjects per group given a 2 µm thickness estimated difference (50 vs 52±3), type I and type II error of 0.05 and 0.1, respectively). Data were controlled for normality, homogeneity of variances and sphericity in order to perform the adequate tests, and the probability level of significance was adjusted according to the post hoc Bonferroni procedure in order to maintain an overall type I error equal to 0.05. The DED and control groups were compared using a t-test, Wilcoxon rank test or the χ^2 test, depending on the set of data. Univariate receiver operating characteristic (ROC) curves were calculated to define the diagnostic ability of OCT, and the cut-off values were calculated using the Youden's index. Moreover, in the DED group, patients were divided into three subgroups according to the clinical severity of the disease,[1 14] and the data were compared using one-way analysis of variance (ANOVA). Also, scatter plots and the R^2 correlation coefficient were used to assess the association between pairs of variables. Random forest and logistic regression were applied to assess the ability of a combination of data, including OCT, to diagnose DED. For the multivariable analysis, the a priori power was estimated with the number of events, which was of 118/10 (11.8). This allowed us to include in the multivariable model up to 11 covariates with a power greater than 80%. The post hoc simulations showed a power of all multivariable analyses to be higher than 90%. As the dependent covariates estimated by OCT were correlated, a hierarchical cluster analysis was performed to define the redundant covariates (see online supplemental figure 2) with the same Hoeffding D value. We included the continuous covariate following regression splines (n=3). We used the R^2 and Brier indexes as discrimination and the C-statistics and Dxy indexes as a rank discrimination. Variables included in the full model were selected using a step-down model with a higher significance level for a variable to be included in the model.[15] We also used the bootstrap (n=500) to study the uncertainty in the selection of variables and to penalise this uncertainty when estimating the predictive performance of the model. To assess the model fit, we used the validate function by estimating (bootstrapping resampling validation) the bias-corrected indices that were specific to each type of model. To assess if the observed responses were in agreement with the predicted responses, we used the calibrate function. The model without bias (slope not different from one and the intercept close to zero) was used to draw a nomogram with axes for all predictors.

RESULTS

Population features

One hundred and twenty patients (60 in each group) were selected. One patient in the DED group was excluded after the first visit (due to the inability to obtain accurate OCT epithelial mapping just after a blink and 10 s after), and five were excluded in the control group (two due to the inability to obtain accurate OCT epithelial mapping and three because of changes in general treatment). The mean age and sex ratio of the DED group were in accordance with what is usually observed in the disease[16] and different from those of the controls. All clinical data are detailed in table 1.

Comparative OCT epithelial mapping, and correlations with other clinical data

Zonal epithelial thicknesses were averaged from three consecutive captures after a blink: SD for the entire population was 0.68, 0.81, 0.86, 0.51, 0.49, 0.45 and 0.63 for S1, S2, S3, central, I1, I2 and I3, respectively; the global variation coefficient was 1.24%. Epithelial thickness measured by OCT just after a blink

Table 1 Clinical profile of DED and the control group

	Dry eyes (n=118)	Controls (n=110)	P value
Age (years)	57.18±17.25	47.07±17.60	<0.001
Sex ratio (M/F)	32/86	53/56	<0.001
Patients' symptoms (OSDI)	32.30±19.43	10.10±10.66	<0.001
Clinical data			
Spherical equivalent (D)	0.04±1.97	–0.39±1.96	0.104
Non-invasive BUT (s)	5.91±1.48	13.64±1.97	<0.001
Schirmer I (mm/5')	11.28±4.54	16.51±2.81	<0.001
Oxford (0–5)	0.50±0.71	0.02±0.16	<0.001
MGD (≥2)	60 (51%)	21 (19%)	<0.001
Dry eye severity	Grade 1: 83 (70%) Grade 2: 26 (22%) Grade 3: 9 (8%)		
Tear film substitutes	34 (29%)	0	<0.001

BUT, break-up time; DED, dry eye disease; MGD, meibomian gland dysfunction; OSDI, Ocular Surface Disease Index.

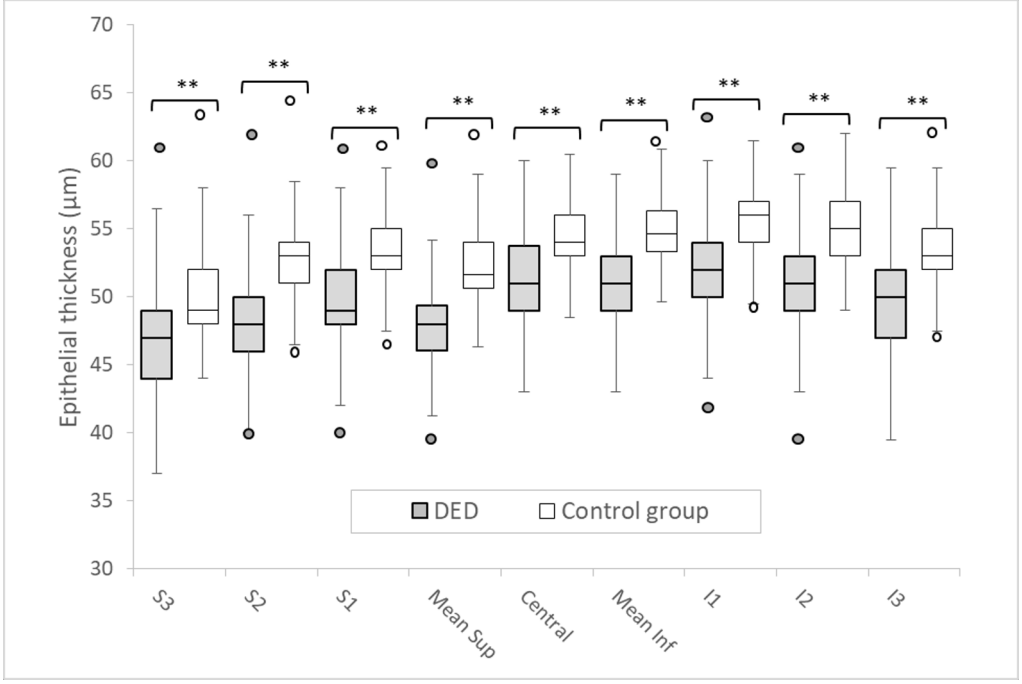

Figure 1 Comparison of epithelial thicknesses between dry eye disease (DED) and the control group. Epithelial thickness averaged 3 mm in the central zone, superior quadrants were 3–5 mm from the centre, 5–7 mm and 7–9 mm (S1, S2 and S3, respectively), and inferior quadrants (I1, I2 and I3). **P<0.001.

was statistically thinner in the DED group than in the control group regardless of location, that is, superior, central or inferior zones (t-test, p<0.001 for each), as detailed in figure 1. Interestingly, the difference between inferior and superior quadrants was not significantly different between groups, regardless of the eccentricity (t-test, p>0.05 for all, see figure 2). All epithelial thicknesses acquired 10 s after a blink did not significantly differ from those reported above, that is, just after a blink in each group and for each zone (repeated measures ANOVA, p>0.05 for all, see online supplemental figure 3).

Neither age nor gender significantly correlated with any epithelial thicknesses as measured by OCT. The average thickness of the peripheral corneal epithelium, regardless of location, was highly correlated with the non-invasive BUT and Schirmer I test. The worse the tear film was, the thinner the peripheral epithelium (table 2). The average thickness of the

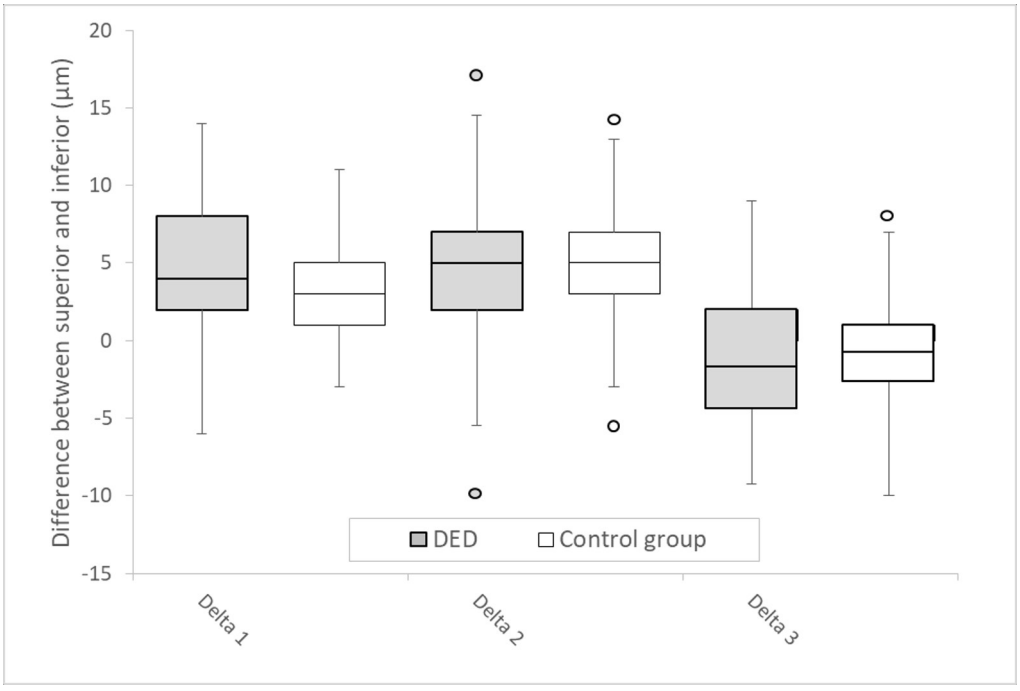

Figure 2 Difference between inferior and superior quadrants (zones 1, 2 and 3) in dry eye disease (DED) and the control group. There was no significant difference between groups (p>0.05).

Table 2 Correlations between the epithelial thickness as measured by OCT and the clinical data

	OSDI	TBUT	Schirmer	Oxford
Mean superior				
R	−0.092	0.412	0.238	0.047
P value	0.321	**<0.001**	**0.009**	0.574
Central				
R	−0.047	0.281	0.151	−0.258
P value	0.616	**0.002**	0.102	**0.005**
Mean inferior				
R	−0.123	0.360	0.278	−0.110
P value	0.186	**<0.001**	**0.002**	0.234

bold value : statistically significant correlation (P<0.05)
OCT, optical coherence tomography; OSDI, Ocular Surface Disease Index; TBUT, tear film break-up time.

central epithelium correlated with BUT and the Oxford score in a weaker manner. In addition, MGD was significantly related to a thinning of the inferior peripheral epithelium (7–9 mm from the centre, namely I3: 49.1±0.53 vs 50.6±0.52 µm, for grading score ≥2, p=0.023).

Diagnostic performance of OCT in DED

ROC curves were drawn to evaluate the diagnostic abilities of OCT epithelial mapping. Mean superior, central and inferior epithelial thicknesses constituted significant criteria to discriminate DED from healthy conditions (area under the curve (AUC)=0.87, 0.77 and 0.83, respectively, as compared with 0.5, p<0.001 for all) as drawn in figure 3. Superior epithelial thickness, and more notably the one from the S2 quadrant, was the best marker for diagnosing DED using OCT. Cut-off thresholds and intrinsic and extrinsic properties (calculated using Bayes theorem) of OCT epithelial mapping in DED are detailed in table 3.

Table 3 Diagnostic abilities of OCT-wide epithelial mapping

Criterion					PPV	NPV
Cut-off value	Sensitivity	Specificity	PPV	NPV	(for 10% prevalence)	
S2 <50 µm	0.81	0.79	0.81	0.80	0.33	0.96
Mean superior <50 µm	0.80	0.84	0.85	0.80	0.30	0.96
Mean inferior <52 µm	0.65	0.89	0.86	0.70	0.40	0.95
Central <53 µm	0.75	0.65	0.70	0.70	0.21	0.95

PPV and NPV were also calculated for a mean prevalence of 10% of dry eye disease (DED), which is closer to what is currently observed in the general population.
NPV, negative predictive value; OCT, optical coherence tomography; PPV, positive predictive value; S2, epithelial thickness in superior zone 2 (5–7 mm diameter).

Grading performance of OCT in DED

The difference between the inferior peripheral zone (7–9 mm from the centre, namely I3) and the superior peripheral zone (7–9 mm from the centre, S3) was the best significant indicator for the severity of DED as detailed in figure 4. Interestingly, patients with low-severity DED (grade 1) presented relative hyperplasia of the superior corneal epithelium, while more severe stages (2 and 3) were related to progressive thinning as compared with the inferior one.

Multivariable model integrating OCT mapping for diagnosing DED

Because of confounding factors, the univariate model's discriminatory performance could be improved with a multivariable model. Therefore, we identified factors that explain the remaining residual predictions. The relevant predictors were selected according to their clinical plausibility and their statistical contribution to the model performance (figure 5). The calibration test with bootstrapping shows that the model correctly predicts all the risk probabilities (mean absolute error: 0.016)

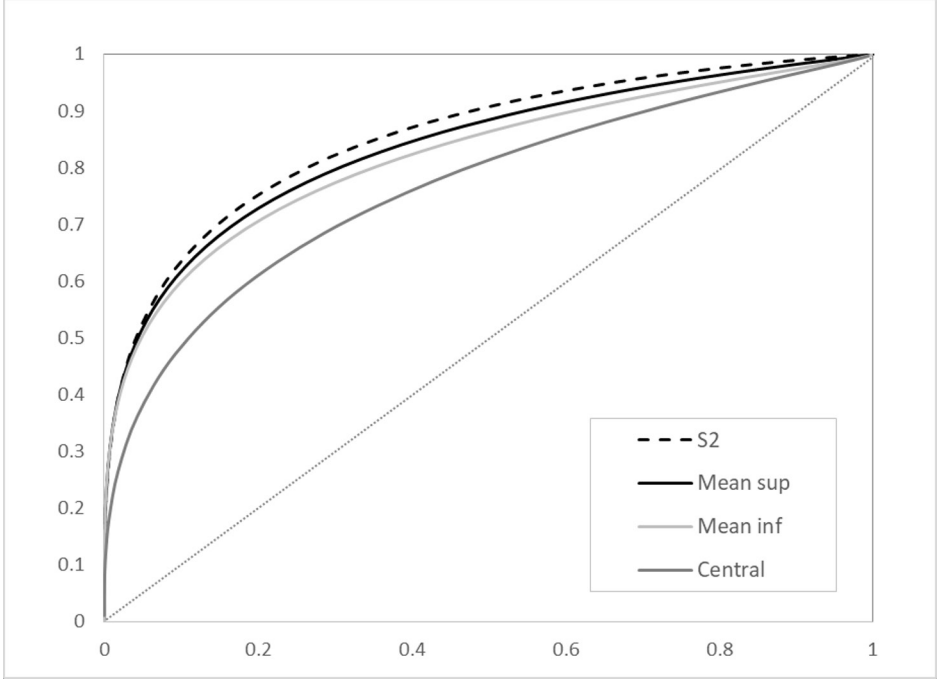

Figure 3 Diagnostic abilities of optical coherence tomography (OCT) epithelial mapping. Receiver operating characteristic (ROC) curves, p<0.001 for area under the curve (AUC) for all the drawn measures.

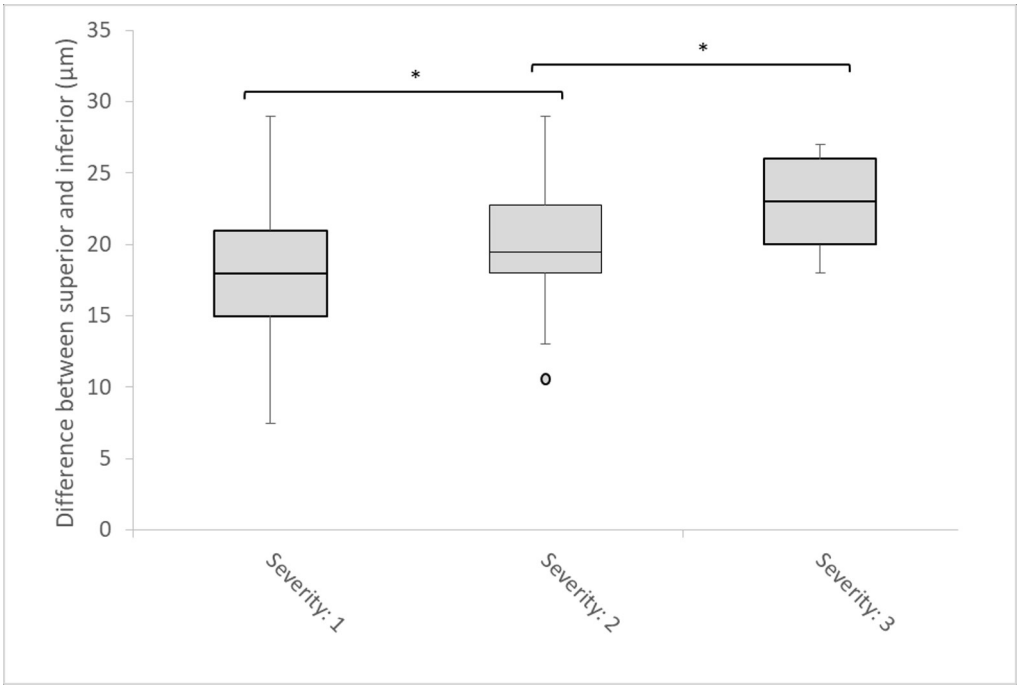

Figure 4 Comparison of differences between the inferior and superior peripheral zones according to dry eye disease (DED) severity. *P<0.05.

of DED with a higher ROC AUC or concordance statistic index (C-index) equal to 0.95 (online supplemental figures 4 and 5). The validated model (C-statistics=0.95, R^2=0.759, Dxy=0.900, Brier=0.085) was used to draw a nomogram for the diagnosis of DED (figure 6), which further improved the intrinsic and extrinsic properties of our diagnostic test (sensitivity 86.4%, specificity 91.7%, see online supplemental figure 5).

DISCUSSION

There is a wide variety of techniques available for evaluating DED.[2] From current practice to laboratory tests, it notably includes patient questionnaires,[13] slit lamp or video assessments of tear film stability, tear secretion and clearance,[17] ocular surface staining,[18] tear osmolarity[19] or biomarker measurements.[20–24] However, the diagnosis and follow-up of dry eye remain difficult in current practice, especially due to the lack of one simple method to objectively embrace the whole disease.[3] As a result, the useful and substantial guidelines currently given to evaluate DED also underscore the need for new additional approaches.[7 8]

The aim of this study was to evaluate the diagnostic performance of corneal epithelial mapping using Fourier-domain OCT, in order to integrate this objective parameter into a new scoring method to detect DED. We reported that OCT mapping of epithelial thickness, especially the mean upper mid-peripheral thickness, could constitute a strong and reliable criterion for diagnosing DED since it differed considerably between patients with DED and healthy subjects. More precisely, we also defined a cut-off value for superior epithelial thickness under 50 µm as a highly sensitive and specific marker to differentiate patients with dry eye from healthy people. These results seem to be consistent with previous publications, which found thinning in the upper area of the corneal epithelium.[7] Cui *et al* explored the characteristics of corneal epithelial thickness topography with Fourier-domain

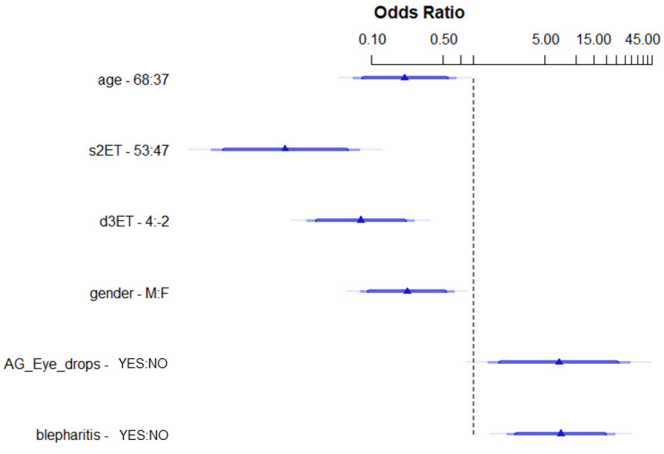

Figure 5 OR of the significant covariate (p<0.05 for each) as defined in a multivariate approach using logistic regression and the random forest approach. AG_Eye_drops, daily use of antiglaucoma eye drops; d3ET, difference between superior epithelial thickness and the inferior one in zone 3; s2ET, superior epithelial thickness in zone 2.

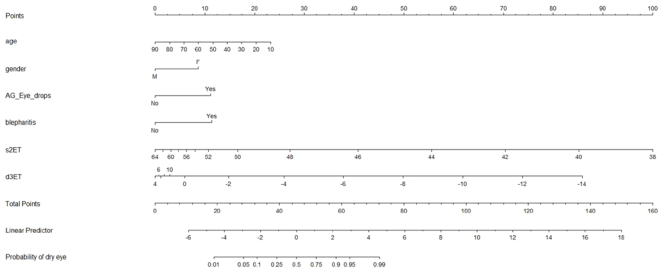

Figure 6 Integrated scale to diagnose dry eye disease (DED). Each subscore of the significant parameters has to be calculated to define the overall score (range: 0–160) and the related probability of having the disease. Interestingly, optical coherence tomography (OCT) epithelial measurements (s2ET: superior epithelial thickness in zone 2 and d3ET: difference between superior and inferior epithelial thicknesses in the peripheral zone 3) are the most important data to predict DED.

OCT in patients with dry eye and demonstrated an average decrease in superior corneal epithelial thickness in 100 patients with DED (average age of 47 years) compared with 35 healthy volunteers (average age of 43 years). They found a difference in the mean thickness of the upper epithelium between the DED group and the control group of about 2 µm. Thus, they suggested that superior corneal epithelial thickness is the first location to be stricken in DED. The measurement of epithelial thickness also included the precorneal tear film. The average tear film thickness was measured using OCT at 4.79 µm by Werkmeister *et al*.[25] It raises the question of the impact of precorneal tear film thickness. However, the lack of significant difference in the measurements of epithelial thickness just after a blink and 10 s after (in both DED and the control groups) would suggest that precorneal tear film may not have a crucial influence in such a measurement.

Liang *et al*[10] featured the relationship between corneal and conjunctival epithelium thickness and ocular surface tests in DED by measuring the central corneal, limbal (LET) and bulbar conjunctival epithelium thickness using spectral-domain OCT. They observed a significant decrease in LET in the DED group (54 patients, mean age 44 years) compared with the control group (32 patients, mean age 43 years), emphasising the involvement of the turnover of epithelial cells in the pathogenesis of DED[26]: (1) decrease in the pool of limbal stem cells during DED,[27–29] (2) increase in the turnover of corneal epithelial cells against depletion,[9] and (3) microenvironment inflammation[30] at the limbus resulting in a decrease in LET. Thus, the peripheral corneal rim could be a clinical indicator for DED. Conversely, in a study comparing a group of 35 women suffering from DED with 35 healthy women, Kanellopoulos and Asimellis reported an overall thickening of the corneal epithelium as measured by Fourier-domain OCT.[12] This intriguing finding may be explained by the young age of the studied population and also in the OCT mapping that differed from that used in our study. The data recorded by the author included maps of corneal epithelial thickness up to the 6 mm diameter cornea, whereas in our study, the region of interest was extended to 9 mm. Thus, by moving away from the centre of the cornea towards the peripheral zone, the corneal epithelium is thinner.

In addition, OCT corneal epithelial mapping may objectively reflect the severity of the disease. Indeed, our results suggest that the severity of DED could correlate with the difference between the upper and lower epithelia. OCT would make it possible to provide a reliable classification of the disease. In stage 1 (mild dryness), we observed a relative thickening of the superior epithelium compared with the inferior one, contrary to what was observed in healthy subjects. This reported thickening of the superior epithelium may be discussed regardless of what was previously reported as epithelial hyperplasia.[31] Using immunofluorescent labelling in a mouse model of induced ocular dryness, Fabiani *et al* observed epithelial hyperplasia (overexpression of Ki67) in the week following the induction of dryness. The mechanisms involved are not yet fully elucidated, but the most likely hypothesis is that DED could cause inflammation and a subsequent increase in proinflammatory cytokines leading to cell proliferation and differentiation. In more severe cases (ie, clinical stages 2 and 3), a progressive and global decrease in epithelial thickness was observed in our study along with a relative thinning of the superior epithelium compared with the inferior one. Accordingly, previous studies reported a decrease in epithelial thickness in patients with severe ocular dryness. Erdélyi *et al* found that epithelial thickness was thinner in 26 patients with severe DED than in 10 healthy people (mean age 62 years), further hypothesising that the progressive destruction of limbal stem cells should lead to such a DED-related thinning.[32] Interestingly, and as stated above, Cui *et al* showed an average decrease in superior epithelial thickness in patients with DED, which correlates with the severity of the disease.[11]

We analysed the putative relationships between OCT parameters and other epidemiological and clinical criteria of DED. Notably, we reported a correlation between the non-invasive BUT and the mean overall epithelial thickness. The worse the tear film was, the thinner the peripheral epithelium, further enlightening the centripetal damage to the corneal epithelium in DED. In a previous study, Reinstein *et al* pioneered the analysis of the corneal epithelium by mapping the corneal epithelium over the entire corneal surface in 56 normal patients coming for refractive surgery using a very high-frequency ultrasound device. They found that epithelial thickness was not uniformly distributed over the cornea with a thinner superior corneal epithelium, further suggesting that this non-uniform morphology of the epithelium was caused by the friction resulting from mechanical dynamics in blinking friction.[33] The connection that was found between the BUT and the mean overall epithelial thickness can be explained by this mechanical hypothesis. The friction resulting from a DED-related increase in blinking could have a mechanical stress effect on the superficial layers and induce superior epithelial thinning.

In addition, we also noticed that patients with blepharitis had a thinner inferior epithelium than patients without an eyelid disease. The question arises as to whether the lower eyelid would have a specific effect on the ocular surface that it is in contact with. In their study, Liang *et al* observed a correlation between the inferior limbal epithelial thickness and the OSDI, the Schirmer test, the BUT, the Oxford score and mean corneal sensitivity.[10] They suggested that these changes in inferior limbal epithelial thickness could be explained by the prolonged contact between epithelial cells and proinflammatory tears within the inferior lacrimal bed. In the same way, we may hypothesise that a close relationship between the inferior cornea and inflammatory eyelids may have led to changes in the epithelial morphology. In a study designed to determine whether MGDs could increase the osmolarity of the tear film and produce ocular surface changes similar to DED, Gilbard *et al* revealed that a closure of the meibomian gland ducts in rabbits resulted in higher tear film osmolarity but also a decrease in corneal epithelial glycogen level.[34] Even if our results suggest a direct influence of eyelid inflammation on epithelial thickness, additional studies are required to better understand this point.

Lastly, multivariate analyses provided a better understanding of the relationship between the recorded features of the patients, from gender to corneal epithelial thickness, in order to present a new scale including OCT measurements that could be used to diagnose DED. Artificial intelligence approach (random forest) and standard logistic regression allowed to identified other parameters which improve the diagnosis performance. As expected, including the criteria of gender, age and the presence of blepharitis increased the power of our diagnosis method. Interestingly enough, our multivariate analysis also identified two other accurate parameters: (1) the difference between superior and inferior

epithelial thicknesses in the peripheral zone and (2) the daily use of antiglaucoma drugs (for at least 3 months, as defined in our inclusion criteria). As a result, integrating OCT epithelial mapping and specific patient features in an integrated scoring scale proved to be highly effective in the diagnosis of DED (sensitivity >86% and specificity >91%), even without an ophthalmological examination of the patient. Such an innovative approach could help better screen DED via paramedical approach in order to detect the disease and then refer the patient to a specialist.

Finally, we have to discuss some limitations of our study. First of all, the relatively small sample size of the patient groups, as well as the recruitment of subjects in a university hospital centre dedicated to ocular surface diseases, may constitute recruitment bias. It also would have been interesting to add a third group including patients with other ocular surface diseases in order to study the way it could impact the diagnostic performances of our model. About OCT, one can notice that the average difference in epithelial thickness between patients with DED and controls was around 5 µm, which has to be assessed against the 5 µm axial resolution of OCT given by the manufacturer. In fact, Kanellopoulos and Asimellis previously analysed the resolution of the same device and reported a repeatability of the measurement of 1 µm and a variability of 0.25.[12] As a result, and given the power of our statistical analysis, one may conclude that the reported differences in OCT measurements are relevant. A further limitation is the inability of OCT to discriminate the precorneal tear film from the corneal epithelium, as stated previously. This raises the uncertainty of the extent that the precorneal tear film could have influenced the measurements. However, it may not impair our model since its diagnostic abilities have been validated, whether or not OCT epithelial mapping is influenced but the tear film. Moreover, we performed three OCT measurements at two different times (ie, immediately after blinking and 10 s after a blink), and no significant differences were found between them. Another way to check the lack of influence of the precorneal tear film on our results would have been to also evaluate the location of the first tear film rupture in relation to the OCT epithelial mapping. We could have also measured the epithelial thickness in the temporal and nasal areas. Even if the previous studies did not report changes in the temporal and nasal ones, it would have been interesting to confirm, or not, these results. The criteria used to define dry eye syndrome in the patient group could also have been optimised by the measurement of tear film osmolarity, which was not routinely done in the present study. Measuring this parameter would have strengthened our inclusion criteria. In addition, the assessment of our patients' meibomian dysfunction was only qualitatively analysed by meiboscopy. A quantitative assessment by eyelid imaging would have made it possible to study other influences of MGD on the corneal epithelium and would have expanded our results about putative direct interactions between ocular surface and lower lids. Finally, it would be interesting to conduct a post-treatment follow-up study to determine the impact of treatments on epithelial thickness. Despite our primary goal being to evaluate the diagnostic abilities of OCT epithelial mapping in a population visiting our specialised consultation for the first time, we found a better epithelial thickness in treated patients as compared with untreated ones. Hence, a post-treatment follow-up may state whether topical treatments are beneficial for epithelial trophicity.

Studies with larger patient groups are needed to validate this model, but it is likely that OCT corneal epithelial mapping will expand the quality of dry eye examinations. On the one hand, precise topographic analysis of epithelial thickness for research purposes will provide a better understanding of the causes and consequences of epithelial changes related to DED and the putative mechanisms and contributing factors involved in the pathogenesis of ocular surface damage. On the other hand, OCT mapping, which could be integrated into an innovative diagnosis score, could become a simple, non-invasive and objective tool to optimise the screening of DED and evaluate the severity and evolution of the disease.

Twitter Alexandre Denoyer @Alexandre DENOYER

Contributors Design of the study: AD, ZD, AEM. Conduct of the study: NAE, AD, CA. Collection and management of data: NAE, AD, ZD. Analysis and interpretation of data: ZD, AD, NAE, AEM. Preparation of the manuscript: NAE, AD, ZD. Review and approval of the manuscript: AD, CA.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. All data are available upon request to the corresponding author: alexandre.denoyer@gmail.com.

ORCID iD
Alexandre Denoyer http://orcid.org/0000-0003-4730-0425

REFERENCES

1 Craig JP, Nichols KK, Akpek EK, *et al*. TFOS DEWS II definition and classification report. *Ocul Surf* 2017;15:276–83.
2 Sullivan BD, Whitmer D, Nichols KK, *et al*. An objective approach to dry eye disease severity. *Invest Ophthalmol Vis Sci* 2010;51:6125–30.
3 Pflugfelder SC, Tseng SC, Sanabria O, *et al*. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea* 1998;17:38–56.
4 Lin P-Y, Tsai S-Y, Cheng C-Y, *et al*. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai eye study. *Ophthalmology* 2003;110:1096–101.
5 Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. *Cornea* 2004;23:762–70.
6 Calonge M, Diebold Y, Sáez V, *et al*. Impression cytology of the ocular surface: a review. *Exp Eye Res* 2004;78:457–72.
7 Møller-Pedersen T, Vogel M, Li HF, *et al*. Quantification of stromal thinning, epithelial thickness, and corneal haze after photorefractive keratectomy using in vivo confocal microscopy. *Ophthalmology* 1997;104:360–8.
8 Zhivov A, Stachs O, Kraak R, *et al*. In vivo confocal microscopy of the ocular surface. *Ocul Surf* 2006;4:81–93.
9 Francoz M, Karamoko I, Baudouin C, *et al*. Ocular surface epithelial thickness evaluation with spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci* 2011;52:9116–23.
10 Liang Q, Liang H, Liu H, *et al*. Ocular surface epithelial thickness evaluation in dry eye patients: clinical correlations. *J Ophthalmol* 2016;2016:1–8.
11 Cui X, Hong J, Wang F, *et al*. Assessment of corneal epithelial thickness in dry eye patients. *Optom Vis Sci* 2014;91:1446–54.
12 Kanellopoulos AJ, Asimellis G. In vivo 3-dimensional corneal epithelial thickness mapping as an indicator of dry eye: preliminary clinical assessment. *Am J Ophthalmol* 2014;157:63–8.
13 Schiffman RM, Christianson MD, Jacobsen G, *et al*. Reliability and validity of the ocular surface disease index. *Arch Ophthalmol* 2000;118:615–21.

14 Behrens A, Doyle JJ, Stern L, *et al*. Dysfunctional tear syndrome: a Delphi approach to treatment recommendations. *Cornea* 2006;25:900–7.
15 Steyerberg EW, Eijkemans MJ, Harrell FE, *et al*. Prognostic modelling with logistic regression analysis: a comparison of selection and estimation methods in small data sets. *Stat Med* 2000;19:1059–79.
16 The epidemiology of dry eye disease: report of the epidemiology Subcommittee of the International dry eye workshop (2007). *Ocul Surf* 2007;5:93–107.
17 Afonso AA, Monroy D, Stern ME, *et al*. Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms. *Ophthalmology* 1999;106:803–10.
18 Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003;22:640–50.
19 Lemp MA, Bron AJ, Baudouin C, *et al*. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol* 2011;151:792–8.
20 Rolando M, Barabino S, Mingari C, *et al*. Distribution of conjunctival HLA-DR expression and the pathogenesis of damage in early dry eyes. *Cornea* 2005;24:951–4.
21 Tsubota K, Fujihara T, Saito K, *et al*. Conjunctival epithelium expression of HLA-DR in dry eye patients. *Ophthalmologica* 1999;213:16–19.
22 C–H C, Furue M, Tamaki K. Selective regulation of ICAM-1 and major histocompatibility complex class I and II molecule expression on epidermal Langerhans cells by some of the cytokines released by keratinocytes and T cells. *European Journal of Immunology* 1994;2411:2889–95.
23 Versura P, Profazio V, Schiavi C, *et al*. Hyperosmolar stress upregulates HLA-DR expression in human conjunctival epithelium in dry eye patients and in vitro models. *Invest Ophthalmol Vis Sci* 2011;52:5488–96.
24 Gulati A, Sacchetti M, Bonini S, *et al*. Chemokine receptor CCR5 expression in conjunctival epithelium of patients with dry eye syndrome. *Arch Ophthalmol* 2006;124:710–6.
25 Werkmeister RM, Alex A, Kaya S, *et al*. Measurement of tear film thickness using ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci* 2013;54:5578–83.
26 Dogru M, Tsubota K. New insights into the diagnosis and treatment of dry eye. *Ocul Surf* 2004;2:59–75.
27 Vera LS, Gueudry J, Delcampe A, *et al*. In vivo confocal microscopic evaluation of corneal changes in chronic Stevens-Johnson syndrome and toxic epidermal necrolysis. *Cornea* 2009;28:401–7.
28 Steger B, Speicher L, Philipp W, *et al*. In vivo confocal microscopic characterisation of the cornea in chronic graft-versus-host disease related severe dry eye disease. *Br J Ophthalmol* 2015;99:160–5.
29 Lee MJ, Ko AY, Ko JH, *et al*. Mesenchymal stem/stromal cells protect the ocular surface by suppressing inflammation in an experimental dry eye. *Mol Ther* 2015;23:139–46.
30 Baudouin C, Messmer EM, Aragona P, *et al*. Revisiting the vicious circle of dry eye disease: a focus on the pathophysiology of meibomian gland dysfunction. *Br J Ophthalmol* 2016;100:300–6.
31 Fabiani C, Barabino S, Rashid S, *et al*. Corneal epithelial proliferation and thickness in a mouse model of dry eye. *Exp Eye Res* 2009;89:166–71.
32 Erdélyi B, Kraak R, Zhivov A, *et al*. In vivo confocal laser scanning microscopy of the cornea in dry eye. *Graefes Arch Clin Exp Ophthalmol* 2006;245:39–44.
33 Reinstein DZ, Gobbe M, Archer TJ, *et al*. Epithelial thickness in the normal cornea: three-dimensional display with ARTEMIS very high-frequency digital ultrasound. *J Refract Surg* 2008;24:571–81.
34 Gilbard JP, Rossi SR, Heyda KG. Tear film and ocular surface changes after closure of the meibomian gland orifices in the rabbit. *Ophthalmology* 1989;96:1180–6.