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REVIEW ARTICLE

# The impact of dry eye disease on corneal nerve parameters: A systematic review and meta-analysis

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## Abstract

**Purpose:** Dry eye disease (DED) is a growing global health problem with a significant impact on the quality of life of patients. While neurosensory abnormalities have been recognised as a contributor to DED pathophysiology, the potential role of in vivo corneal confocal microscopy in detecting nerve loss or damage remains unclear. This systematic review with meta-analysis (PROSPERO registered CRD42022381861) investigated whether DED has an impact on sub-basal corneal nerve parameters.

**Methods:** PubMed, Embase and Web of Science Core Collection databases were searched from inception to 9 December 2022. Studies using laser scanning confocal microscopy to compare corneal nerve parameters of DED with healthy eyes were included. Study selection process and data extraction were performed by two independent members of the review team.

**Results:** Twenty-two studies with 916 participants with DED and 491 healthy controls were included, with 21 of these studies included in subsequent meta-analyses. There was a decrease in total corneal nerve length (−3.85 mm/mm$^2$; 95% CI −5.16, −2.55), corneal main nerve trunk density (−4.81 number/mm$^2$; 95% CI −7.94, −1.68) and corneal nerve branch density (−15.52 number/mm$^2$; 95% CI −27.20, −3.84) in DED eyes compared with healthy eyes, with subgroup analysis demonstrating that these differences were more evident in studies using NeuronJ software, a semi-automated procedure. While this review found evidence of loss of corneal nerve parameters in eyes with DED compared with healthy controls, particularly with the use of a semi-automated image analysis method, it is evident that there is substantial heterogeneity between studies in terms of corneal nerve imaging methodology.

**Conclusions:** Standardisation is required in terms of terminology and analysis, with more research needed to potentially improve the clinical applicability and practicality of corneal nerve imaging. Further investigation is also required to confirm the diagnostic accuracy of this imaging modality and its potential for monitoring DED treatment efficacy.

**KEYWORDS**
corneal nerves, dry eye disease, in vivo corneal confocal microscopy, keratoconjunctivitis sicca

## INTRODUCTION

Dry eye disease (DED) is a growing unmet problem affecting up to one in 10 of the global population.[1] It costs millions in healthcare economic burden worldwide,[2] with substantial adverse impacts on affected individuals' quality of life.[3] Notably, the impact of severe DED has been shown to be similar to that of moderate to severe angina.[4,5] Efforts to seek the optimal DED treatment have risen over the years; however, the multifactorial pathophysiological mechanisms underlying DED contribute to the difficulties in managing this chronic condition. In addition to tear film instability, hyperosmolarity and inflammation, the Tear Film and Ocular Surface Society Dry Eye Workshop (TFOS DEWS) II has identified neurosensory abnormalities as contributors to the development or progression of DED.[6] However, assessment methods of neuronal damage or dysfunction for the diagnosis and management of DED remain limited in clinical settings.

In vivo confocal microscopy is capable of imaging corneal microstructures en face across different layers in a non-invasive and rapid manner. Notably, the cornea is known to be one of the most densely innervated regions of the body.[7] Small nerve fibres in this region are responsible for signalling pain, temperature and mechanical sensations.[8] The sub-basal nerve plexus, which is situated between the basal epithelial layer and Bowman's layer, is often the region of interest in terms of imaging as the nerves in this layer are organised in a relatively homogenous manner compared with other nerve plexi in the cornea.[7] While there have been several iterations of in vivo confocal microscopy over the past few decades, laser scanning confocal microscopy has been the most widely adopted by researchers and clinicians due to its high resolution and magnification.[9]

Dry eye disease has been shown to be associated with neuronal damage or abnormalities, with neurobiological changes associated with neurogenic inflammation and disturbances to the activity of peripheral ocular sensory nerve fibres.[8] Given the impact of DED on these nerves, characterising morphological changes in the sub-basal nerve plexus in a quantitative manner may aid in dry eye diagnosis and potentially guide treatment decisions. This may also impact the identification of more sensitive endpoint measures in evaluating the neuroregenerative or neuroprotective capabilities of current and future therapies. Hence, there has been increasing interest in the investigations of whether structural loss of corneal nerves could be observed in DED. Given the rise in interest and use of the instrument in both clinical and research settings, there is need for deeper discussions of limitations and future directions for in vivo corneal confocal microscopy in addition to providing a timely synthesis of more recent literature.[10] Hence, a systematic review and quantitative analysis was conducted to explore the following question: Do patients with DED have corneal nerve parameter changes as observed with in vivo corneal confocal microscopy?

**Key points**

- Neurosensory abnormalities have been recognised as a contributor to the pathophysiology of dry eye disease; however, the role of corneal nerve imaging in detecting nerve damage or loss remains unclear.
- This systematic review and meta-analysis demonstrated a reduction in several corneal nerve parameters, particularly with the use of a semi-automated image analysis method, although there was substantial heterogeneity between studies.
- Further investigation is required to examine the diagnostic and prognostic capabilities of corneal nerve imaging and to improve its applicability and practicality in real-world clinical settings.

## METHODS

This systematic review and meta-analysis was prospectively registered on PROSPERO (CRD42022381861) and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement[11] and guidelines provided by Rudnicka and Owen.[12]

### Study eligibility criteria

The inclusion criteria were studies, including observational studies (cross-sectional or case–control studies), which compared patients diagnosed with DED of any severity or type (primarily aqueous deficient or evaporative) with individuals without DED (healthy controls) with a sample size $n \geq 10$ participants in each group. Only studies published in the English language were included. Studies must also have explicitly mentioned that DED was diagnosed with a combination of symptoms and signs or made reference to published criteria, which involved these combined diagnostic methods. Only studies using laser scanning confocal microscopy (Heidelberg Engineering GmbH, heidelbergengineering.com) with outcome measures including quantitative corneal nerve parameters of the sub-basal nerve plexus (including length, density, area, width, fractal dimension and tortuosity) produced by manual, semi-automated or automated procedures were included.

Exclusion criteria were review articles, conference abstracts as data are limited from these sources, studies that did not compare patients with DED with healthy controls and studies with sample size <10 participants in either group. Studies involving animals or investigating patients with only other ocular surface issues or conditions such as contact lens discomfort from either soft or rigid contact

lenses, ocular surgeries or injuries, ocular infections, ocular tumours, other ocular inflammatory conditions such as uveitis or neurodegenerative conditions such as glaucoma requiring active treatment, corneal dystrophies or corneal oedema were also excluded from this review. Studies involving patients only affected by systemic comorbidities which may impact corneal nerve parameters were also excluded, including diabetes and Sjögren syndrome. Studies investigating corneal nerve changes qualitatively without quantitative parameters and those that only assessed other corneal layers, microneuromas or non-neuronal features of the sub-basal nerve plexus such as dendritic cells were excluded.

## Literature search strategy

Comprehensive literature searches were conducted using three electronic databases: PubMed, Embase (Ovid) and Web of Science Core Collection. Databases were searched from inception to the date of the search (9 December 2022). Search strategies were developed with assistance from an information specialist with expertise in systematic reviews and are provided in Table S1. The reference lists of included studies were also examined.

## Study selection process

Citation results from each database were first imported into EndNote 20 software (endnote.com), and duplicate entries between database search results were identified by the software and subsequently removed. The Covidence systematic review software (Veritas Health Innovation, covidence.org) was used for study screening. Two members from the review team (JCBC and VT) worked independently to screen studies by referring to the title and abstract of each study. The same members then independently assessed screened studies for eligibility by assessing the full-text article of each study.

## Data extraction

Two members of the review team (JCBC and VT) extracted data from relevant studies while adhering to standardised data extraction forms developed for this systematic review.

For each study, the following information was extracted:

1. Manuscript details including primary author and year of publication.
2. Study details including study type or design, country where the study was performed, funding and conflict of interest details, methods (inclusion and/or exclusion criteria, dry eye diagnostic methods, other potential associated comorbidities investigated, number and laterality of eyes analysed, masking of personnel conducting imaging [imagers] and/or of the outcome assessor, imaging instrument used, number of images selected for analysis and analytical procedure used).
3. Participant demographics (number, age and sex of participants in each group).
4. Results including quantitative corneal nerve parameters. Where studies divided DED groups according to type or severity (potentially multiple levels), data were pooled from these groups as findings for an overall DED group, if this was not already provided in the relevant study. The primary or corresponding authors of investigations that did not explicitly provide exact numerical findings of corneal nerve parameters in their article or supporting information were contacted to provide these data.

## Outcomes

The primary outcome included quantitative sub-basal corneal nerve plexus parameters (including density, length, width, area, fractal dimension and tortuosity) as measured with manual, semi-automated or automated procedures.

## Risk of bias assessment

Two members from the review team (JCBC and VT) worked independently to assess the risk of bias of the included studies. Any discrepancies at any stage of the review were resolved through discussion and consensus. Risk of bias assessment (Table S2) was adapted from tools including the QUADAS-2[13] and Newcastle–Ottawa scale.[14] To provide a more informative assessment of the risk of bias, each element was assessed in terms of the potential for bias (high, low or unclear), instead of one overall determination for the domain or overall study.

## Qualitative synthesis

Prior to conducting a quantitative synthesis, included studies were evaluated in terms of their relevance, potential discordance between studies and appropriateness to be synthesised in subsequent meta-analyses.

## Quantitative synthesis and statistical analysis

To facilitate subsequent quantitative synthesis of results in this review, terminology and measures with common definitions were classified under an overarching, harmonising term and results converted to a uniform unit of measurement where possible. This also included converting standard errors of the mean or median and interquartile range to mean and standard deviation.[15] To ensure consistency between study findings, terminologies used in studies with insufficient detail in their provided definitions to

determine comparability with parameters in other studies were noted and not grouped under the harmonising term (Table 1). Meta-analyses were performed using the Review Manager (RevMan) version 5.4.1 (training.cochrane.org/online-learning/core-software/revman) if a specific corneal nerve parameter had been investigated by a sufficient number of studies using similar methods (≥2). A random-effects meta-analysis with inverse variance method was used for each corneal nerve parameter to assess mean differences (or standardised mean differences if results could not be converted to a uniform unit of measurement) between the dry eye and healthy control groups. This method provides more conservative estimates particularly with the potential presence of significant study heterogeneity. Meta-analyses were grouped by the corneal nerve parameter investigated. Subgroup analyses were also conducted, grouping studies according to the analytical procedure used for measuring corneal nerve parameters (manual, semi-automated or automated) and presented as subtotals in the forest plots because magnitudes of outcome measures may differ substantially across these methods.[16–18] Statistical heterogeneity was assessed using $\tau^2$, $\chi^2$ and $I^2$. In cases where meta-analysis was not possible, the individual study findings were investigated. Statistical significance was considered as $p < 0.05$.

# RESULTS

## Study selection

The electronic searches yielded 509 unique reports following duplicate removal. Full-text articles were obtained and assessed for eligibility for 69 studies following the screening stage by title or abstract. A PRISMA flow diagram of the study selection process is provided in Figure 1, which culminated in 22 included studies for this systematic review (Table S3). There were two unique reports identified from the reference lists of the included studies; however, none were eligible to be included in this systematic review (Figure 1).

## Characteristics of included studies

Of the 22 included studies, a majority were observational studies including 17 cross-sectional controlled studies[19–35] and four retrospective case–control studies.[36–39] Comparisons between DED groups and healthy controls at baseline were undertaken in one randomised controlled trial.[40] A total of 916 participants with DED and 471 healthy controls were included across these studies. Details of funding, conflict of interests and country where the study was conducted are summarised in Table S4.

All included studies mentioned the diagnosis of DED using a combination of symptoms and signs. Ten studies outlined diagnostic methods without reference to published criteria,[20,22,24,27,31,34,36,37,39,40] while seven referred to the TFOS DEWS II,[19,23,25,26,29,30,33] three referred to TFOS DEWS I,[28,32,38] one referred to the 2005 Japanese Dry Eye diagnostic criteria[21] and one referred to the 2013 Chinese Clinical Diagnosis and Treatment Experts Consensus of Dry Eye.[35] Further details on the diagnostic methods are outlined in Table S5.

Only two studies specified the number of personnel involved in imaging the cornea using in vivo corneal confocal microscopy: one study involved one imager[36] and one study involved two imagers.[22] Twenty studies specified

**TABLE 1** Harmonising terminology used for corneal nerve parameters with referencing of the included studies in the meta-analyses.

| Terms, unit | Definition |
|---|---|
| Total corneal nerve length, mm/mm$^2$ | The total length of all corneal nerves per unit area |
| Corneal main nerve trunk length, mm/mm$^2$ | The length of all main nerve trunks per unit area |
| Corneal nerve branch length, mm/mm$^2$ | The length of all nerve branches per unit area |
| Maximum length of corneal nerves, µm | The length of the longest nerve or nerve fragment observed within a frame |
| Minimum length of corneal nerves, µm | The length of the shortest nerve or nerve fragment observed within a frame |
| Mean length of corneal nerves, µm | The mean length of nerves and nerve fragments within a frame |
| Total corneal nerve density, number/mm$^2$ | The total number of all corneal nerves per unit area |
| Corneal main nerve trunk density, number/mm$^2$ | The number of all main nerve trunks per unit area |
| Corneal nerve branch density, number/mm$^2$ | The number of all branches per unit area |
| Corneal nerve branch point density, number/mm$^2$ | The number of branch points per unit area |
| Corneal nerve fibre width, µm or mm/mm$^2$ | The average of a specific number of measures of thickness of long nerve fibres or average width of corneal nerve fibres per unit area |
| Corneal nerve fibre area, mm$^2$/mm$^2$ | The average area occupied by corneal nerve fibres |
| Beading, total number or number/100 µm of nerves | Number of bead-like formations which are alternating broadening areas of corneal nerves |
| Corneal nerve tortuosity, unitless | The degree of twistedness of nerves which could be classified according to a subjective grading system or custom aggregate measure |

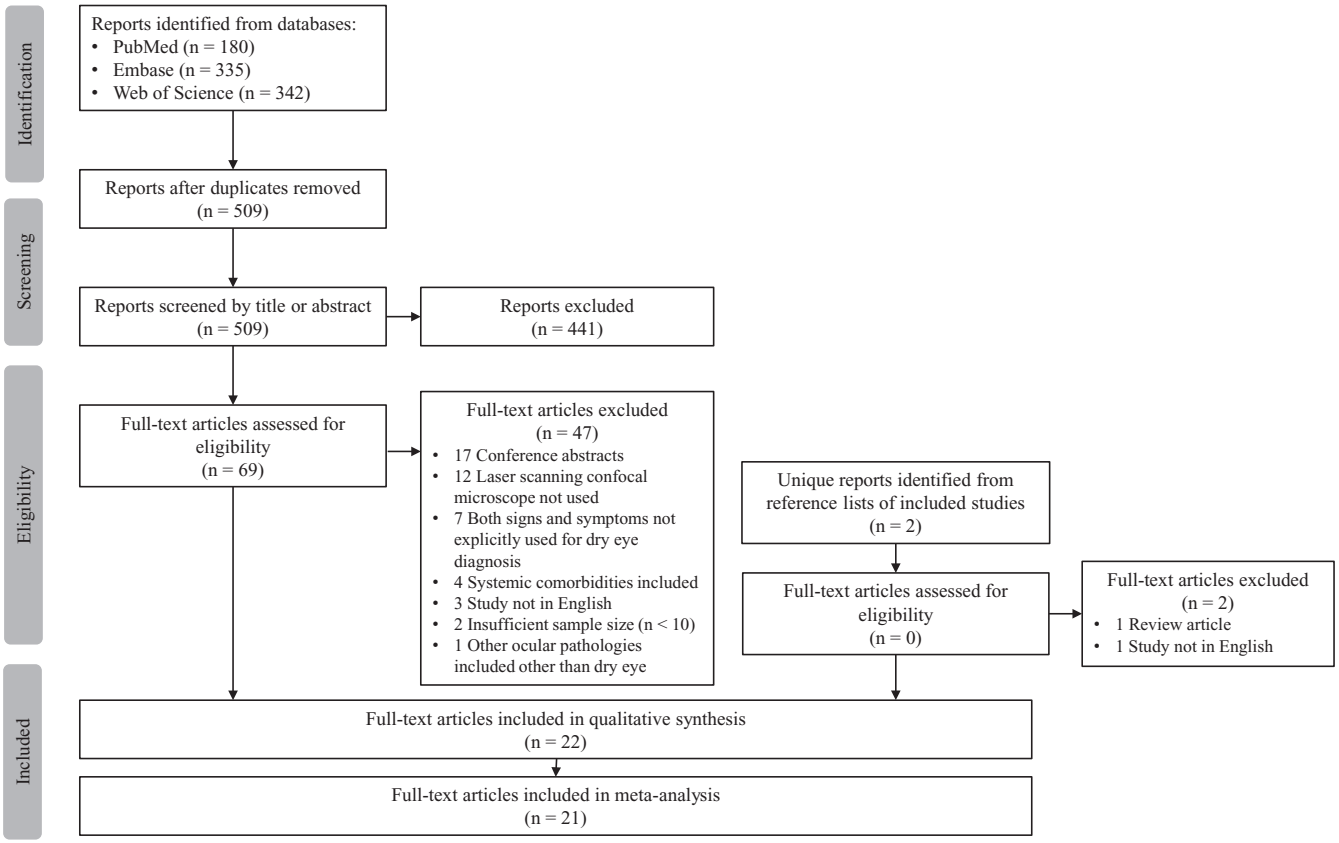


**FIGURE 1** The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flow diagram detailing the search and selection of studies included in the review.

the location of the cornea imaged, all of them included the central cornea,[19–21,23,25–40] with three studies also imaging additional locations: one included the lower third of the cornea,[21] one included mid-peripheral locations (approximately 5 mm in diameter including the central cornea)[19] and one imaged the upper, lower, nasal and temporal cornea.[34] The number of outcome assessors involved in analysing the images obtained were specified in 17 studies: seven studies involved one outcome assessor,[23,26,28,30–32,38] 10 studies involved two outcome assessors,[19,21,22,27,33,34,36,37,39,40] while the remaining five studies did not specify the number of outcome assessors involved.[20,24,25,29,35]

In terms of image analysis, 10 studies primarily used NeuronJ which is a plug-in program of ImageJ with semi-automated capabilities (ImageJ.org),[19,22,27,28,30,36–40] four used the ACCMetrics software with automated capabilities (University of Manchester, sites.manchester.ac.uk/ccm-image-analysis/),[23,26,31,33] two used manual analysis using the ImageJ software,[21,35] two used unspecified manual analysis,[20,32] one used CNS-Net[25] and one used unspecified methods.[24] One study used a combination of NeuronJ and ACCMetrics.[34] Five studies[20–22,28,32] that also graded nerve tortuosity used a manual grading scale,[41] while two studies used a custom aggregate termed Tagg.[29,33] Eighteen studies specified the number of eyes analysed from each participant: nine studies analysed one eye only,[19,21,22,28,29,32,33,38,39] five studies analysed two eyes[25,27,30,34,40] and four studies analysed two eyes for DED participants but one eye for healthy controls.[26,31,36,37] The number of images analysed per eye were provided in 20 studies: eight analysed three images,[20,21,27,32,36,37,39,40] nine analysed five images,[22,23,26,28–31,34,38] one analysed 10 images,[19] one analysed ≥10 images[25] and one analysed ≥5 images.[33]

## Risk of bias in studies

The risk of bias assessment is summarised in Table S6. To provide a more detailed assessment in relation to study characteristics and in vivo corneal confocal microscopy methodology, elements under each domain were judged individually. There were no studies that had low risk of bias across all elements assessed. The element with the most studies judged as having high risk of bias (12 of 22 studies)[19–21,23–25,30,33–35,37,40] was in the domain of comparability between groups, specifically assessing whether there was controlling or matching, or adjusting for both age and sex between groups in statistical analysis. Four of these 12 studies reported matching of groups for age but not sex,[24,30,37,40] while seven of these 12 studies reported no statistical significance between groups in terms of age and/or sex.[19,21,25,33,35,37,40] The elements with the most studies judged as having an uncertain risk of bias

was in the domain of outcome measurement, specifically the masking of personnel conducting confocal microscopy on study participants (20 of 22 studies),[19–21,23–32,34–40] followed by consecutive or random sampling of participants enrolled (17 of 22 studies) in the patient selection domain.[20–27,29–31,33–35,37,39,40]

## Primary outcomes and qualitative synthesis

All studies analysed quantitative corneal nerve parameters, albeit with a variety of terminology; however, one did not provide exact numerical findings for these outcomes and hence was excluded from subsequent meta-analyses.[24] One study did not specify the definition for the term 'number of sub-basal nerves'[20] while another study did not explicitly define corneal nerve maximum length[25]; hence, these findings were not included in the subsequent meta-analyses. One study reported a dichotomous result for beading presence; hence, this was not included in the meta-analysis on the number of beadings.[39] Only one study investigated corneal nerve reflectivity, showing no significant difference between DED and healthy control groups.[28] For the study that used both NeuronJ and ACCMetrics to measure the same corneal nerve parameters from participants,[34] NeuronJ was chosen as the primary data included in the relevant meta-analyses as more corneal nerves are known to be detected through this method. The meta-analyses were then repeated with the NeuronJ results replaced with the ACCMetrics data to evaluate any potential changes in the outcome of the analyses. Table 1 summarises the harmonising terms and units, with a total of 14 being used for the purposes of this review to facilitate comparability of findings between studies included in subsequent meta-analyses.

The most variability in regards to terminology pertains to the total length of corneal nerves measured per unit area, with a majority of studies referring to this parameter as density (10/18 studies),[21,22,25,27,28,30,36–39] while the other studies referred to this parameter as length.[19,23,26,31,33–35,40] It is also common for studies to classify nerves as either main nerve trunks or branches, with the specific terminology, definitions and units used for corneal nerve parameters for each study provided in Table S7. For this review, density refers to the count of nerves, while length refers to the distance of the nerve path in an image.

## Meta-analyses of corneal nerve parameters

This section outlines the meta-analyses conducted on each of the corneal nerve parameters reported. Forest plots of analysis of corneal nerve parameters investigated by a total of five or more studies are presented in this main article; otherwise, the plots are included as Supporting information.

### Length

*Total corneal nerve length*

Eighteen studies,[19,21–23,25–28,30,31,33–40] including a total of 1305 eyes with DED compared with 522 healthy eyes, investigated total corneal nerve length. The total mean difference between DED and healthy eyes was −3.85 mm/mm$^2$ (95% CI −5.16, −2.55; $p < 0.001$; $I^2 = 95\%$; Figure 2). The substantial heterogeneity could partly be due to the different image analytical methods used. A subgroup analysis of studies using semi-automated methods, namely NeuronJ,[19,22,27,28,30,34,36–40] showed significant reduction in total corneal nerve length in DED eyes compared with healthy eyes (−5.70 mm/mm$^2$; 95% CI −6.69, −4.71; $p < 0.001$; $I^2 = 80\%$). Pooled data from the two studies which used manual[21,35] and automated methods revealed no evidence of a significant difference in this parameter between DED and healthy eyes. One study that used a more recently devised technique (CNS-Net)[25] reported a statistically significant and larger decrease in total corneal nerve length (−3.61 mm/mm$^2$; 95% CI −5.04, −2.18) compared with the more commonly used ACCMetrics.[23,26,31,33] Different inclusion criteria, varied DED diagnostic criteria, differences in sample sizes (range of 16–229 DED eyes and 10–58 healthy eyes) and matching of age and sex also potentially contributed to the heterogeneity observed. As Zhang et al.[34] also applied ACCMetrics on the same cohort of participants, repeated meta-analysis with these data showed a similar significant total mean difference of −3.79 mm/mm$^2$ (95% CI −5.09, −2.49; $p < 0.001$; $I^2 = 95\%$), with minimal changes on the subtotals of the NeuronJ and ACCMetrics subgroup analyses (Figure S1).

*Corneal main nerve trunk length and corneal nerve branch length*

Two studies[36,39] (169 DED and 58 healthy eyes) investigated corneal main nerve trunk length. Both used NeuronJ for image analysis and showed a significant reduction in this parameter in DED eyes (−3.34 mm/mm$^2$; 95% CI −6.38, −0.31; $p = 0.03$; $I^2 = 91\%$; Figure S2). Corneal nerve branch length was also investigated in these two studies.[36,39] Conversely, there was no significant decrease in this parameter in DED eyes (−2.81 mm/mm$^2$; 95% CI −7.56, 1.95; $p = 0.25$; $I^2 = 92\%$; Figure S3). Substantial heterogeneity observed may have been due to the difference in sample sizes (Cox et al.[36] with 139 DED and 42 healthy eyes; Moein et al.[39] with 30 DED eyes and 16 healthy eyes) and more extensive DED diagnostic criteria being used in the study by Moein et al.[39]

*Corneal nerve average, minimum and maximum length*

Two studies[28,38] (68 DED and 39 healthy eyes) investigated corneal nerve average length. Both studies used NeuronJ for image analysis. There was no significant reduction in this parameter in DED eyes (−6.31 µm; 95% CI −18.25, 5.63; $p = 0.51$; $I^2 = 0\%$; Figure S4). The same investigations also investigated corneal nerve minimum length. There was

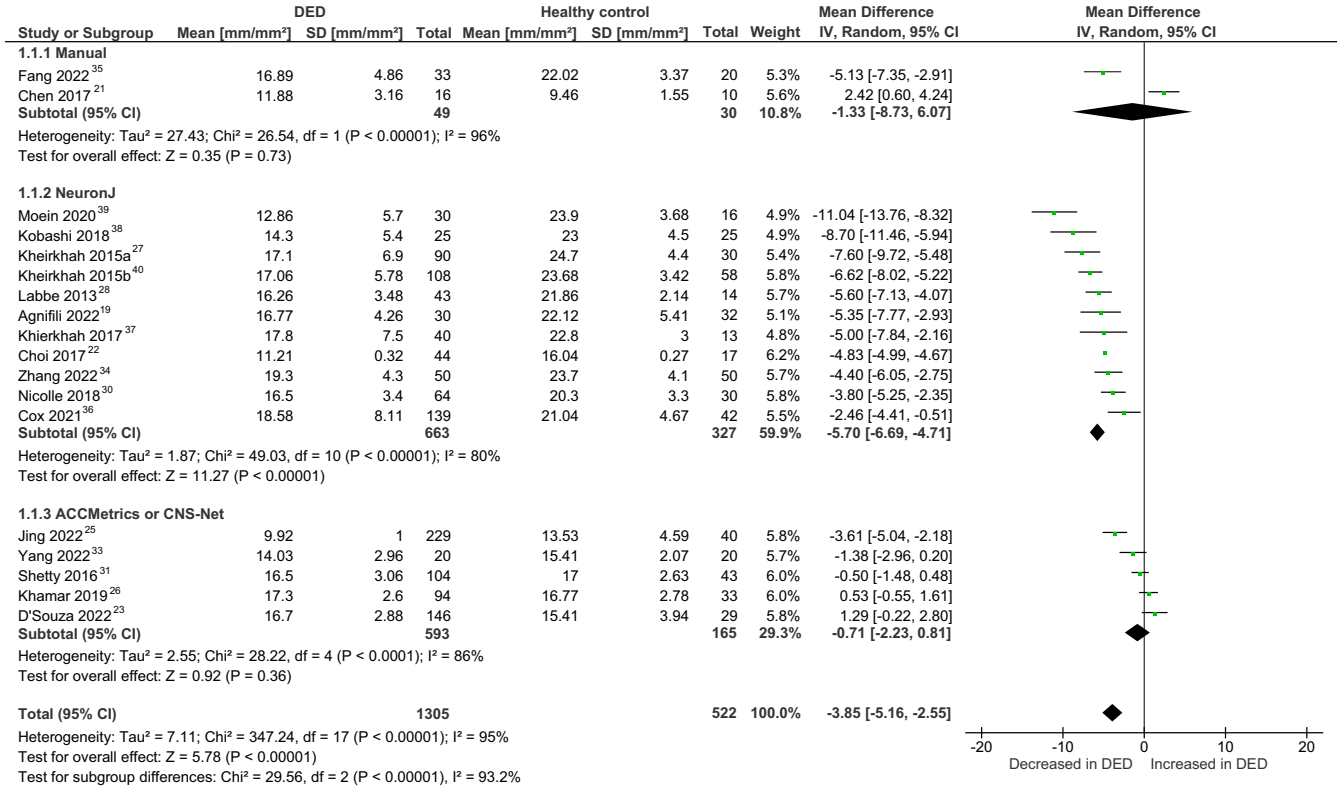


**FIGURE 2** Forest plot of total corneal nerve length measured in mm/mm$^2$ comparing dry eye disease (DED) and healthy control eyes.

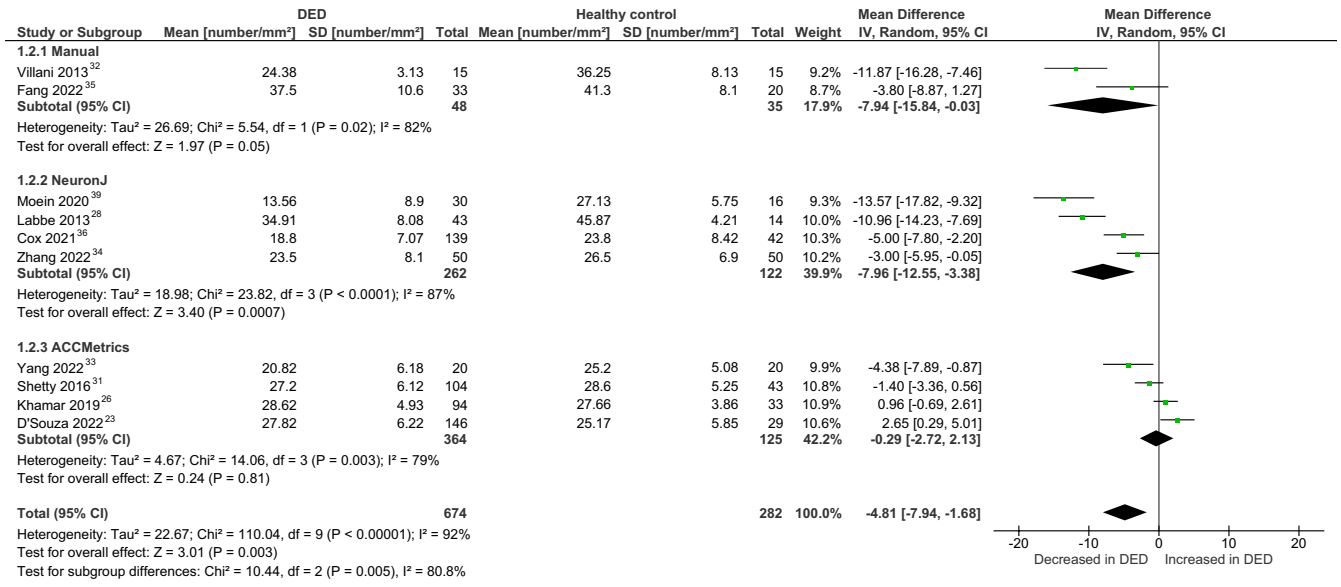


**FIGURE 3** Forest plot of corneal main nerve trunk density measured in number/mm$^2$ comparing dry eye disease (DED) and healthy control eyes.

no significant reduction in this parameter in DED eyes (3.35 μm; 95% CI −1.30, 8.00; $p = 0.16$; $I^2 = 0\%$; Figure S5). In addition to these two mentioned studies, an additional study using CNS-Net by Jing et al. also investigated corneal nerve maximum length.[25,28,38] There was a significant decrease in this parameter in DED eyes, with a total mean difference of −214.45 μm (95% CI −423.37, −7.54; $p = 0.04$; $I^2 = 94\%$; Figure S6). While subgroup analysis of the two studies using NeuronJ showed no significant difference (−86.46 μm; 95% CI −217.32, 44.40; $p = 0.20$; $I^2 = 86\%$), the study using CNS-Net showed a significant large difference between DED and healthy eyes (−533.33 μm; 95% CI −733.69, −332.98; $p < 0.001$). The variability in algorithm and detection methods of sub-basal nerves in these

programs potentially contributed to the heterogeneity observed. Furthermore, while the two studies[28,38] which used NeuronJ included specifically five corneal nerve images for analysis, the study by Jing et al.[25] selected at least 10 without specification of the number chosen for each participant.

## Density

### *Total corneal nerve density*

Three studies[19,36,39] (199 DED and 90 healthy eyes) investigated total corneal nerve density. All these studies used NeuronJ for image analysis and showed evidence of a significant reduction in total corneal nerve density in DED eyes with a total mean difference of −70.86 number/mm$^2$ (95% CI −134.15, −7.58; $p = 0.03$; $I^2 = 95\%$; Figure S7). The substantial heterogeneity may have been due to the varying sample sizes (range of 30–139 DED eyes and 16–42 healthy eyes), differences in the corneal location imaged and number of corneal nerve images analysed.

### *Corneal main nerve trunk density*

Ten studies[23,26,28,31–36,39] (674 DED and 282 healthy eyes) investigated corneal main nerve trunk density. There was a significant total mean difference of −4.81 number/mm$^2$ (95% CI −7.94, −1.68; $p = 0.003$; $I^2 = 92\%$; Figure 3). Subgroup analysis showed significant reduction in DED eyes with manual quantification (two studies; −7.94 number/mm$^2$; 95% CI −15.84, −0.03; $p = 0.05$; $I^2 = 82\%$)[32,35] and NeuronJ (four studies; −7.96 number/mm$^2$; 95% CI −12.55, −3.38; $p = 0.0007$; $I^2 = 87\%$).[28,34,36,39] However, subgroup analysis of the four studies[23,26,31,33] using ACCMetrics showed no significant reduction. Repeated meta-analysis with the ACCMetrics data of the same participants in the study by Zhang et al.[34] showed a similar significant total mean difference of −5.37 number/mm$^2$ (95% CI −8.85, −1.88; $p = 0.003$; $I^2 = 93\%$), with minimal changes on the subtotals of the NeuronJ and ACCMetrics subgroup analyses (Figure S8).

### *Corneal nerve branch density*

Seven studies[19,23,26,28,31,36,39] (590 DED eyes and 209 healthy eyes) investigated corneal nerve branch density. A significant total mean difference of −15.52 number/mm$^2$ was found between DED and healthy eyes (95% CI −27.20, −3.84; $p = 0.009$; $I^2 = 95\%$; Figure 4). Subgroup analysis showed a significant mean difference of −29.91 number/mm$^2$ only in the four studies[19,28,36,39] that used NeuronJ (95% CI −47.75, −12.08; $p = 0.001$; $I^2 = 97\%$).

### *Corneal nerve branch point density*

Six studies[23,26,31,33–35] (447 DED and 195 healthy eyes) investigated corneal nerve branch point density and showed no significant change in this parameter in DED eyes (−5.75 number/mm$^2$; 95% CI −12.21, 0.70; $p = 0.08$; $I^2 = 73\%$; Figure 5). Only one study[34] which used NeuronJ (−21.00 number/mm$^2$; 95% CI −29.17, −12.83; $p < 0.001$) showed significant reduction in DED eyes. As Zhang et al.[34] also applied ACCMetrics on their cohort of participants, repeated meta-analysis with this data replaced showed similar findings (−3.13 number/mm$^2$; 95% CI −6.38, 0.12; $p = 0.06$; $I^2 = 0\%$; Figure S9).

## Corneal nerve fibre width and area

### *Corneal nerve fibre width*

Five studies[19,23,26,28,31] (including 421 DED and 151 healthy eyes) investigated corneal nerve fibre width. There was no significant difference between DED and healthy eyes in this parameter (standardised mean difference: −0.03; 95% CI −0.39, 0.33; $p = 0.88$; $I^2 = 70\%$; Figure 6). The subgroup analysis of either the two studies[19,28] which used NeuronJ or the three studies[23,26,31] with ACCMetrics also showed no significant difference. Some of the overall heterogeneity may be due to methodology differences, with studies using NeuronJ calculating a mean of three[19] or five[28] measurements of long nerve fibre thickness, while ACCMetrics[23,26,31] automatically measured the average thickness of all corneal nerve fibres per unit area.


**FIGURE 4** Forest plot of corneal nerve branch density measured in number/mm$^2$ comparing dry eye disease (DED) and healthy control eyes.

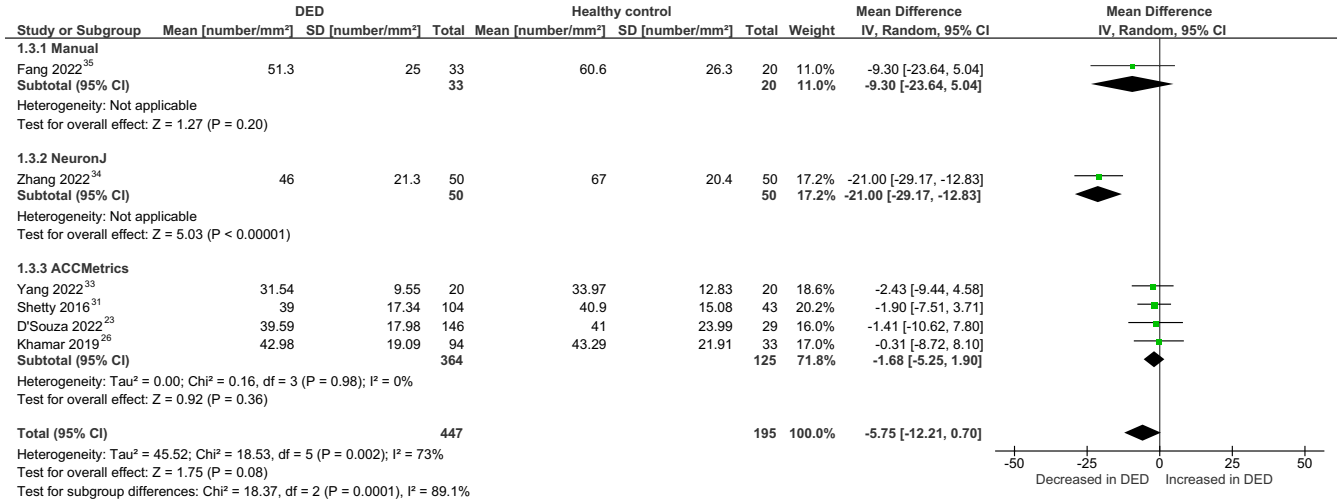


**FIGURE 5** Forest plot of corneal nerve branch point density measured in number/mm$^2$ comparing dry eye disease (DED) and healthy control eyes.

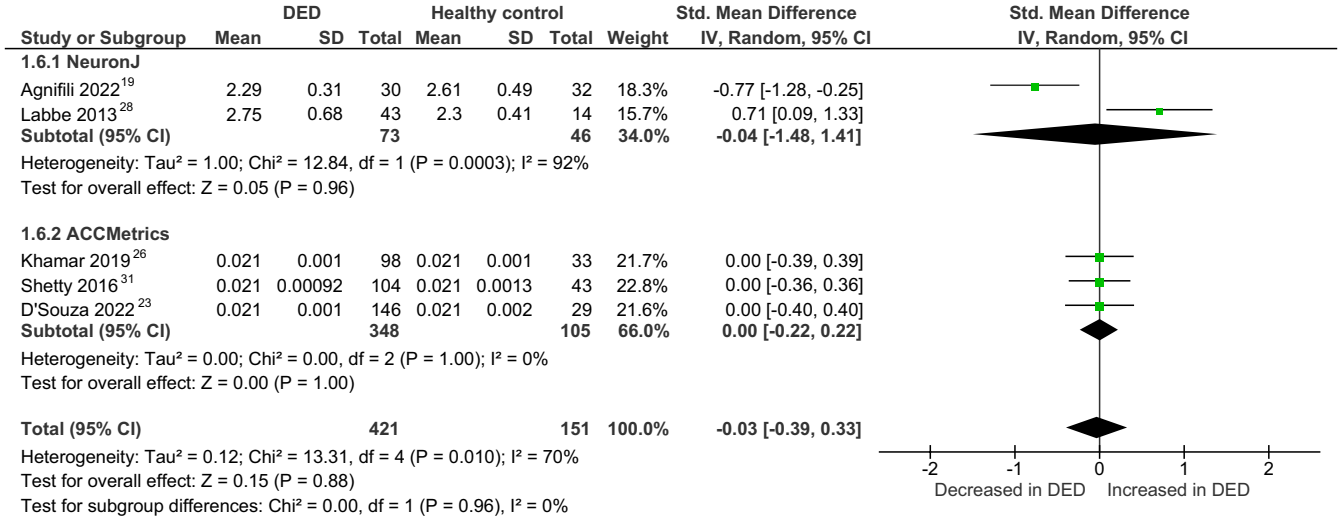


**FIGURE 6** Forest plot of corneal nerve fibre width comparing dry eye disease (DED) and healthy control eyes.

### *Corneal nerve fibre area*

Three studies[23,26,31] (348 DED and 105 healthy eyes) investigated corneal nerve fibre area with ACCMetrics. There were no significant differences between DED and healthy eyes in terms of this parameter (0.17 mm$^2$/mm$^2$; 95% CI −0.17, 0.52; $p = 0.33$; $I^2 = 0\%$; Figure S10).

## Beading and corneal nerve tortuosity

### *Beading*

Three studies[22,28,32] (102 DED and 46 healthy eyes) counted the beading of nerves. There was a significant increase in beading in DED compared with healthy eyes (standardised mean difference: 2.34; 95% CI 1.07, 3.61; $p = 0.0003$; $I^2 = 86\%$; Figure S11). Subgroup analysis of the two studies[22,28] involving the use of NeuronJ showed a significant increase in beading between the groups (2.41; 95% CI 0.34, 4.47; $p = 0.02$; $I^2 = 93\%$), while the one study[32] which used a manual method showed a significant rise in beading number in DED eyes (2.24; 95% CI 1.30, 3.18; $p < 0.001$).

### *Corneal nerve tortuosity*

Seven studies[20–22,28,29,32,33] (190 DED and 125 healthy eyes) investigated corneal nerve tortuosity. Corneal nerves were significantly more tortuous in DED eyes compared with healthy eyes (standardised mean difference: 1.47; 95% CI 0.65, 2.28; $p = 0.0004$; $I^2 = 89\%$; Figure 7). Subgroup analysis of the studies using manual grading showed significantly increased corneal nerve tortuosity in DED eyes (1.83; 95% CI 0.82, 2.84; $p = 0.0004$; $I^2 = 89\%$). Two studies used a custom aggregate (Tagg) which also showed increased tortuosity in DED eyes (0.58; 95% CI 0.15, 1.01; $p = 0.008$; $I^2 = 0\%$).

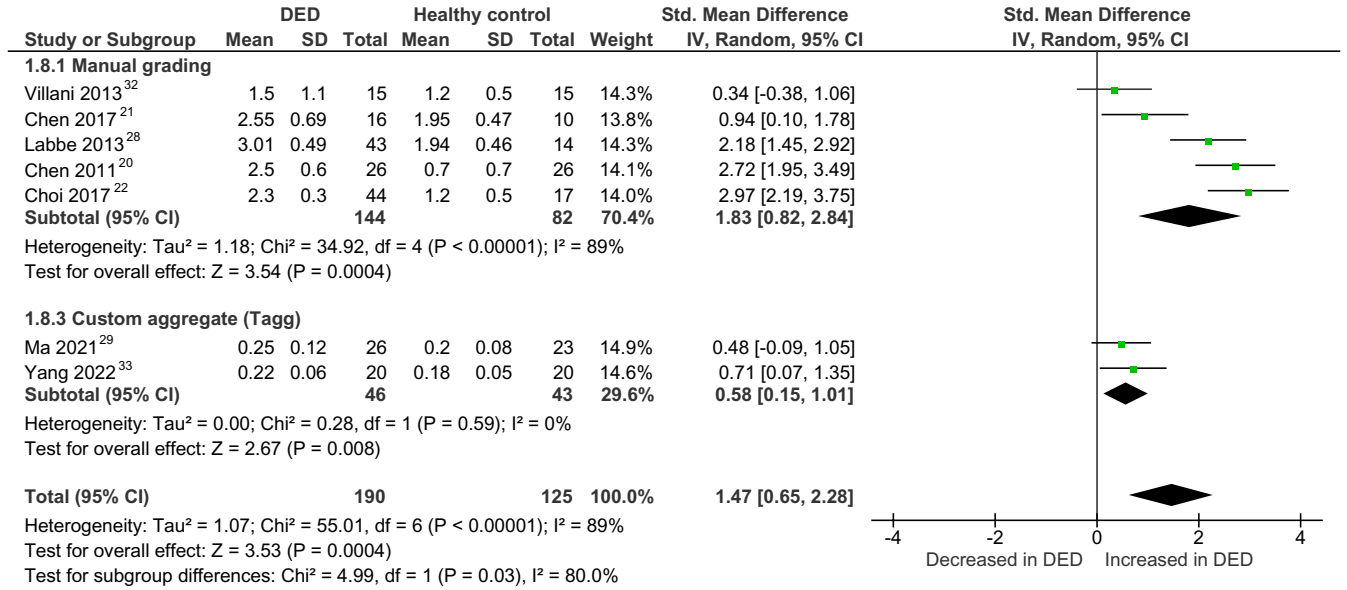


**FIGURE 7** Forest plot of corneal nerve tortuosity comparing dry eye disease (DED) and healthy control eyes.

## DISCUSSION

### Corneal nerve parameters change in DED

This systematic review and meta-analyses showed evidence of corneal nerve morphological changes in DED. This was consistently demonstrated with NeuronJ analysis in certain corneal nerve parameters including reduction in total corneal nerve length, corneal main nerve trunk density and corneal nerve branch density. The usage of this semi-automated method is known to identify and trace more nerves with the guide of an experienced assessor compared with automated methods, particularly the widely used ACCMetrics.[34,42] However, emerging deep learning techniques such as CNS-Net used by a study included in this review[25] and programs investigated by other studies may improve the capability of automated procedures in nerve detection and quantification in a more time-efficient and reliable manner compared with semi-automated procedures.[43,44]

### Other features of the corneal nerve plexus require further work

More complex features of the sub-basal nerve plexus are also commonly investigated by studies, including beading formation and corneal nerve tortuosity. Increased beading formation on sub-basal nerves is thought to be indicative of heightened metabolic processing, which may be a stressor on the health of the corneal nerves.[45] More tortuous corneal nerves are also putative markers of aberrant nerve growth or regeneration following an insult. While most studies that have shown increased corneal nerve tortuosity in DED eyes were based on a crude manual grading scale,[41] emerging automated procedures are also showing similar findings with DED.[29] Corneal nerve reflectivity, fibre width and fibre area are highly dependent on image quality, which may impact analysis reliability. Hence, evidence for the diagnostic or prognostic potential of these parameters is currently limited.

### The need for standardisation of methodology, terminology and images analysed

While evidence for corneal nerve loss seems to be evident in eyes with DED, most studies in this systematic review have uncertain to high risk of bias with substantial heterogeneity between studies. The number of images analysed across studies were not standardised. While greater numbers of minimally or non-overlapping images have been demonstrated to be more likely to represent the 'true mean',[46] this may also depend on the extent of the sub-basal nerve plexus imaged beforehand. The experience of the imaging personnel and tolerability of the patient to the procedure may impact on the total area imaged. While most studies have reported masking of image assessors from the condition of the patients, masking of the imaging personnel may be more difficult in clinical research settings. More standardised widefield imaging with precise localisation capabilities may be required to facilitate repeatable monitoring,[47] akin to the technologies employed in optical coherence tomography. Investigators should clearly define and describe the terminology used to enhance the comparability between studies. While the harmonising terms included in this review were not meant to be prescriptive, the various

terminology used by different studies highlight the heterogeneity in corneal nerve parameters analysed.

## Limitations of the review

While DED diagnosis is becoming more standardised,[48] subtle differences still exist across studies which may impact on generalisability of the findings. As both age and sex are recognised risk factors of DED,[2] these factors should be matched or accounted for in studies comparing differences between groups or cohorts. The current meta-analyses included studies that did not explicitly report this, which may introduce some bias, although most studies did report similar age and sex between groups. Corneal dendritic cells, thought to be increased in DED and indicative of the inflammatory status of the cornea,[49] were also not investigated in this systematic review as the aim was to focus on corneal nerve morphology.

## Future directions

Emerging automated deep learning methods may be crucial to improve the clinical applicability of corneal nerve imaging. Future studies on how in vivo corneal confocal microscopy could guide DED management in real-world clinical settings may also be beneficial. Longitudinal studies which track the development and progression of DED along with corneal nerve imaging may provide insight into the potential intraindividual sub-basal corneal nerve changes. Structure–function concordance is of particular clinical interest, with sparse evidence demonstrating that corneal nerve loss in DED could reduce corneal sensitivity or increase symptoms of ocular surface discomfort,[50,51] although a mouse model of chronic DED has also shown hypersensitivity with loss of intraepithelial corneal nerve terminals.[52]

While evidence for the diagnostic accuracy of in vivo corneal confocal microscopy for DED is limited, there have been several studies that investigated the potential for corneal nerve imaging in monitoring the improvement in nerve morphology in both human clinical studies and animal models of DED. Recent randomised controlled trials of interventions including oral vitamin B1 and mecobalamin,[53] homologous serum eyedrops[54] and oral omega-3 essential fatty acid supplements[55] have demonstrated improvements in corneal nerve parameters as assessed with laser scanning confocal microscopy. However, each study involved different follow-up times, varying image selection and analysis and different sample sizes. The mouse model also showed that instillation of rebamipide 2% ophthalmic solution, a mucin secretagogue, protected nerve density (measured in pixels/frame) against environmental dry eye stress, while artificial tears and hyaluronic acid 0.1% ophthalmic solution did not have such protective effects.[56] An earlier randomised controlled trial further investigated the prognostic capability of in vivo corneal confocal microscopy, demonstrating that DED participants with higher total corneal nerve length at baseline (≥ 16.84 mm/mm$^2$; classified as near-normal) experienced improvements in clinical symptoms and corneal fluorescein staining following 4 weeks of either artificial tear eyedrops or loteprednol etabonate 0.5% eyedrops, compared with those having low total corneal nerve length (<16.84 mm/mm$^2$).[40]

The central cornea has been the primary area of interest in imaging studies; however, other areas of the cornea may provide further insight into potential corneal nerve changes. These include corneal nerves in the far periphery, and the inferior whorl is an inferocentral anatomical landmark where most corneal nerves traverse and converge towards.[7] However, it should be noted that the translatability of terminology and definitions of branches or main nerve trunks as used in conventional central corneal nerve imaging may be difficult given the more complex nerve distributions in these pericentral or peripheral locations.

# CONCLUSION

While the current systematic review and meta-analyses indicate evidence of corneal nerve loss in DED eyes particularly with the semi-automated procedure NeuronJ, more research is required to investigate whether the clinical applicability and practicality of corneal nerve imaging could be improved. Some evidence shows that in vivo corneal confocal microscopy may be useful in monitoring DED treatment effectiveness; however, whether it could be used to diagnose DED accurately and predict the optimal treatment approach for a particular patient requires further investigation.

## AUTHOR CONTRIBUTIONS

**Jeremy Chung Bo Chiang:** Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Vincent Tran:** Methodology (supporting); software (lead); validation (supporting); writing – original draft (supporting); writing – review and editing (supporting). **James S Wolffsohn:** Conceptualization (equal); project administration (lead); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting).

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## CONFLICT OF INTEREST STATEMENT
JSW is on the Executive of the Tear Film and Ocular Surface (TFOS) Society.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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