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Original Article

Comparison of Automated Versus Manual Analysis Programs for Quantification of Corneal Nerve Morphology in Patients With or Without Type 2 Diabetes Mellitus

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Abstract

Objectives: To assess the agreement between the automated analysis program and a manual program for quantification of corneal nerve morphology.

Methods: Twenty-seven non-diabetic controls (mean age: 48.6±5.9 years) and 60 subjects with diabetes (mean age: 52.1±6.5 years) were enrolled. Corneal nerve fiber density (CNFD), branch density (CNBD), and fiber length (CNFL) were quantified by the manual (CCMetrics software, University of Manchester, UK) and automated program (ACCMetrics software, University of Manchester, UK). Bland–Altman plots were generated to assess agreement between the two methods.

Results: There were no differences in gender, age, total cholesterol, and LDL between the two groups, whereas BMI, HbA1c, and triglyceride were significantly higher and HDL was significantly lower in the T2DM group. CNFL was overestimated in the diabetic group and CNFD was underestimated in both groups with ACCMetrics ($p=0.001$, $p<0.001$, respectively). The Bland–Altman plots for both groups demonstrated good agreement for CNFL, with a wide limit of agreement (LoA) of 95% for CNFD and CNBD.

Conclusion: Manual and fully automated protocols for sub-basal nerve evaluation had lower agreement in the measurement of CNFD and CNBD than CNFL in healthy controls and subjects with diabetes.

Keywords: Agreement, Corneal confocal microscopy, diabetes, nerve analysis

Introduction

Corneal confocal microscopy (CCM) has emerged as a significant tool in recent years for detecting small fiber neuropathy. This non-invasive imaging technique is particularly effective in assessing the condition of diabetic neuropathy and other diseases affecting small nerve fibers (1-5).

Diabetic neuropathy is a common and serious complication of diabetes, characterized by damage to nerve fibers, often resulting in pain, numbness, and loss of sensation. CCM has gained prominence in recent years for its ability to visualize and analyze sub-basal nerve plexus (SNP) in the cornea. CCM is used in the early diagnosis of diabetic neu-

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ropathy and can image changes in the density, thickness, and morphology of corneal nerve fibers (5,6). These changes are critical for monitoring disease progression and response to treatment. Studies have shown that a decrease in corneal nerve fiber density, as detected by CCM, is an early indicator of diabetic neuropathy (6).

The advent of automated, semi-automated, and manual programs for SNP analysis has significantly enhanced the efficiency and accuracy of this process. These programs are crucial in clinical and research settings, providing detailed and quantifiable data on nerve morphology and density (7,8). CCMetrics for manual analysis and Neuron J for semi-automated analysis are widely used SNP quantification programs (9). Fully automated analysis techniques such as ACCMetrics have been developed to overcome the time-consuming, laborious, and subjective aspects of manual techniques and to increase diagnostic utility. Manual, semi-automated, and automated programs each have their own distinct advantages and disadvantages.

One of the most significant differences between the manual and automated methods lies in time efficiency and variability depending on the individual conducting the analysis. Automated programs excel in time efficiency, providing rapid results and reducing the time burden on clinicians and researchers. They offer consistency and minimize human error, leading to more reproducible results. However, these programs may struggle with atypical images or poor image quality, sometimes leading to inaccuracies (7,8,10,11). On the other hand, manual programs, while more time-consuming, allow for human intervention and correction, ensuring higher accuracy in challenging cases. This flexibility can be particularly beneficial when dealing with complex or unclear images that automated systems may be unable to analyze. The variability introduced by individual adjustments; however, can lead to less consistency compared to fully automated systems (12,13).

Proving the compatibility and reliability of these two methods is crucial. Demonstrating that manual and automated programs can be used interchangeably, or identifying scenarios where one method is preferable over the other, would significantly enhance the robustness of SNP analysis. Establishing this agreement between the methods is important for ensuring reliable diagnostics and advancing research in neuropathy.

In this study, we aimed to establish an agreement with the automated analysis program compared to a manual program for quantifying corneal nerve morphology.

Methods

Study Participants

Twenty-seven healthy controls and 60 patients with type 2 diabetes mellitus (T2DM) were enrolled. The study was approved by the Ethics Committee of Marmara University and adhered to the tenets of the Declaration of Helsinki and Good Clinical Practice (Protocol No: 09.2024.904). Informed written consent was obtained from all subjects in the study. Inclusion criteria were as follows: to meet the 1999 World Health Organization diagnostic criteria (fasting plasma glucose 7.0 mmol/L and/or 2-h plasma glucose 11.1 mmol/L), a subject must be between the ages of 18 and 65, and have an established diagnosis of T2DM. The exclusion criteria were: contact lens wear; ocular surgery; ocular trauma; acute infection, history of cerebral infarction, connective tissue disease, cervical and lumbar lesions and other causes of peripheral neuropathy, and ocular surface diseases.

Demographic, Medical, and Laboratory Data

Age, gender, height, body weight, and duration of T2DM were all recorded for the subjects. Body mass index (BMI), glycated hemoglobin (HbA1c), total cholesterol (TCh), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were recorded for each participant.

Evaluation of SNP with CCM

As previously reported, all participants underwent CCM imaging (Heidelberg Retinal Tomograph III Rostock Cornea Module [HRT III RCM]; Heidelberg Engineering GmbH, Heidelberg, Germany) by the same investigator (8). Four non-overlapping images were selected for analysis by depth, contrast, and focus position of the SNP. Corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), and corneal nerve fiber length (CNFL) were quantified by a manual (CCMetrics, University of Manchester, UK), and a fully automated program (ACCMetrics, University of Manchester, UK) (Fig. 1). All clearly visible nerves are manually traced with the CCMetrics software and the traces are quantified by the program into CNFL, CNBD, and CNFD measurements (14). The ACCMetrics program distinguishes nerve tracings from the background and quantifies them automatically (15).

Statistical Analysis

The statistical analysis of the data was conducted using SPSS for Windows (Statistical Package for the Social Sciences, version 25.0, IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as mean±standard deviation (SD). Categorical data were assessed using Pearson's Chi-Square and Fisher's Exact tests. The normality of data distribution was determined through the Kolmogorov–Smirnov test and histogram graphs. Mann–Whitney U test was used to compare the values obtained with the two different methods. Bland–Altman plots were used to assess the agreement between the CCM and ACC methods (16). The Pearson correlation was performed to assess the strength of the relationship between automated and manual programs. A P-value below 0.05 was considered statistically significant.

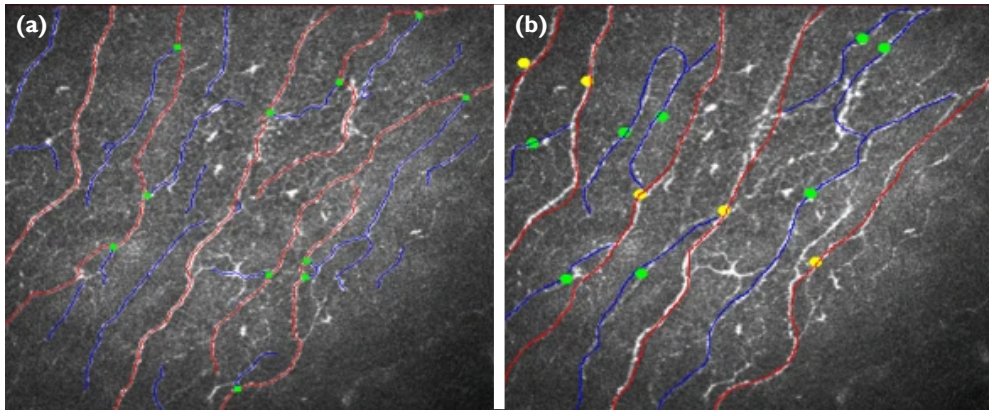

Figure 1. (a) A representative image analysis with CCMetrics. **(b)** A representative image analysis with ACCMetrics.
Red line: CNFD; Green dot: CNBD; Yellow dot: CNFD; Blue line: CNFL.

Results

Patient Characteristics

The demographic and clinical characteristics of patients with T2DM and control subjects are summarized in Table 1. There were no differences in gender, age, TCh, and LDL between the two groups, whereas BMI, HbA1c, and TG were significantly higher and HDL was significantly lower in the T2DM group (Table 1).

Agreements between Manual and Fully Automated Methods

Mean CNFD values obtained with ACCMetrics were lower than those measured with CCMetrics in both groups (Table 2). CNFL was overestimated with ACCMetrics in the T2DM group, whereas no difference in CNFL was observed between the two methods in the control group (Table 2). There was no difference in CNBD measurements between the two methods in both groups (Table 2). Correlation coefficients were 0.44 for CNFD, 0.51 for CNBD, and 0.81 for CNFL ($p<0.001$). These results indicate a moderate correlation for CNBD and a strong correlation for CNFL between the two methods.

The mean of the difference, standard deviation (SD), and 95% limits of agreement (LoA = mean±2 SD) were determined for each group. In the control group, the mean differences between the two methods were; −19.9±15.66 n/mm^2 for the CNFD, 1.6±2.5 mm/mm^2 for the CNFL, and 17.5±26.02 n/mm^2 for the CNDB. In the DM group, the mean differences between the two methods were; −14.9±8.06 n/mm^2 for the CNFD, 1.6±1.83 mm/mm^2 for the CNFL, 6.1±15.51 n/mm^2 for the CNDB. Analysis of agreement between the two measurements using Bland–Altman plots of CNFD and CNBD had a wide 95% LoA for both groups (Fig. 2). Bland–Altman plots

Table 1. The demographic and clinical characteristics of subjects

	Diabetic Group (n=60) (Mean±SD)	Control Group (n=27) (Mean±SD)	p
Age (years)	52.1±6.5	48.6±5.9	0.058
Male/Female	16/44	7/20	0.382
Diabetes duration (years)	9.3±5.2	-	-
BMI (kg/m^2)	32.2±5.89	25.5±1.51	**<0.001**
HbA1c (%)	7.5±1.5	5.3±0.3	**<0.001**
TCh (mmol/L)	204.7±40	226.7±50	0.326
TG (mmol/L)	186.2±103.7	118.3±81.4	**0.005**
HDL (mmol/L)	50.2±8	60.1±13.6	**0.022**
LDL (mmol/L)	123±50.2	141.1±40.3	0.243

BMI: Body mass index; HbA1c: Glycosylated hemoglobin; HDL: High- density lipoprotein; LDL: Low- density lipoprotein; TC: Total cholesterol; TG: Triglyceride; p values in bold are statistically significant.

Table 2. Subbasal nerve plexus analysis in controls and T2DM with manual and fully automatic methods

	Diabetic Group (n=60) (Mean±SD)		p	Control Group (n=27) (Mean±SD)		p
	Manuel	**Full Auto**		**Manuel**	**Full Auto**	
CNFL (mm/mm^2)	12.19±2.04	13.82±2.75	**p=0.001**	17.33±2.48	18.97±4.19	p=0.088
CNBD (no/mm^2)	22.97±8.41	29.06±16.38	p=0.079	30.67±10.91	48.14±31.76	p=0.078
CNFD (no/mm^2)	38.84±8.27	23.95±5.88	**p<0.001**	54.17±10.99	34.25±10.87	**p<0.001**

CNBD; Corneal nerve branch density; CNFD; Corneal nerve fiber density; CNFL; Corneal nerve fiber length; p values in bold are statistically significant.

indicated good agreement between the manual, and fully automated analyses of CNFL for both groups (Fig. 2).

Discussion

In this study, we analyzed the corneal nerve parameters using the CCMetrics and ACCMetrics programs in diabetic patients and healthy controls. We observed a good agreement in CNFL between the two methods in both groups.

Dehghani et al. evaluated the reliability of the CNFL using a manual, a semi-automatic, and an automatic program in diabetic patients (8). In this study, ACCMetrics measured CNFL lower than the other two programs (8). In the Bland–Altman analysis, it was found that the semi-automatic and manual programs had excellent agreement and ACCMetrics had a comparable ability to detect neuropathy as the other two programs (8). Li et al. examined the nerve fibers in DM patients with and without diabetic peripheral neuropathy using ACCMetrics and CCMetrics (17). In their study, Bland–Altman plots showed high agreement in CNFL and CNBD values and weak agreement in CNFD values between manual and fully automated measurements (17). Fully automatic and manual analyses show significant correlations among CNFL, CNBD, and CNFD detection (17). They found that SNP analyzes with ACCmetrics were slightly lower than corresponding manual measurements consistent with the other studies in the literature (8,18,19). In contrast, in our study, ACCMetrics overestimated CNFL in T2DM group and underestimated CNFD in both groups. This inconsistency may be attributed to the small sample size and variations in the severity of peripheral neuropathy.

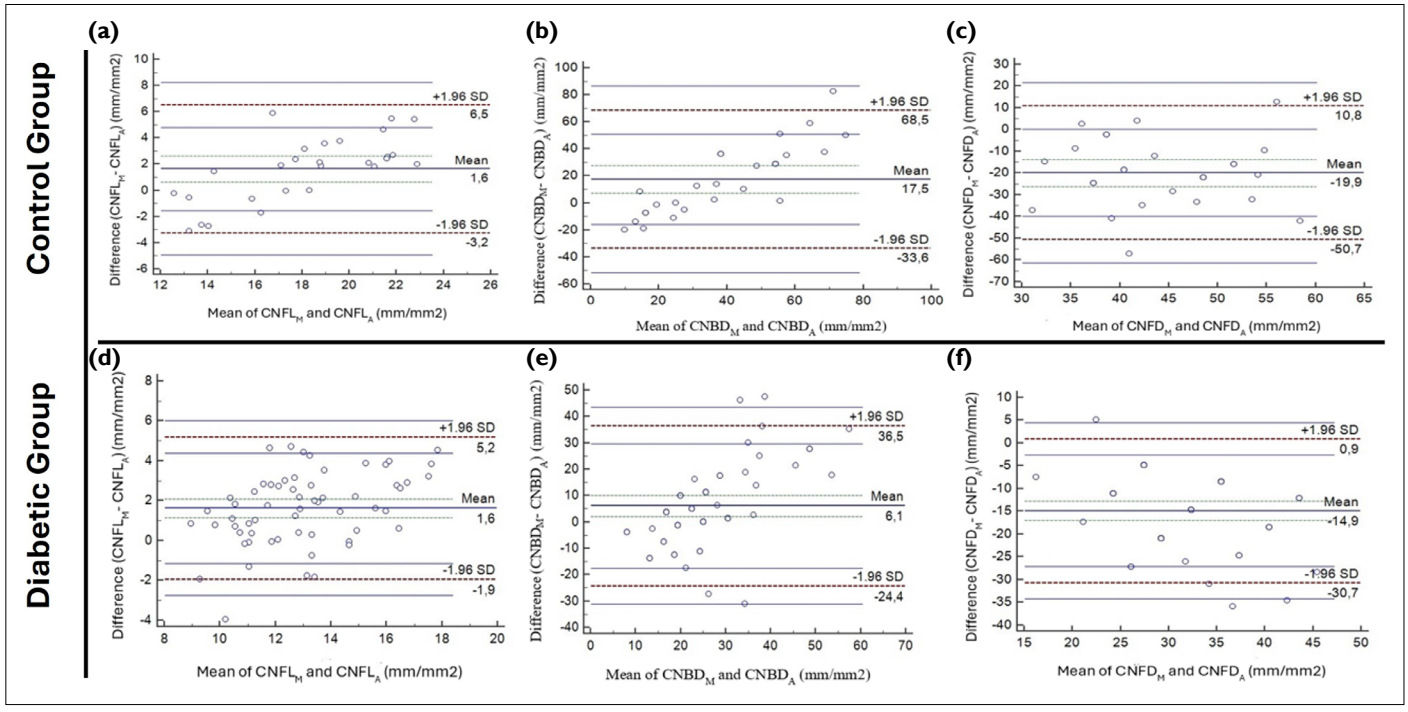

Figure 2. Bland–Altman plots between CCMetrics and ACCMetrics for; CNFL **(a)**, CNBD **(b)**, and CNFD **(c)** in control group, for; CNFL **(d)**, CNBD **(e)**, and CNFD **(f)** in diabetic group. The continuous lines indicate the mean difference. The dashed lines indicate the 95% limits of agreement.

In diabetic patients, the reproducibility of SNP analysis from CCM images was evaluated by Hertz et al. comparing the manual and fully automated methods (20). This study found that both methods showed good reproducibility of CNFL, contrary to CNBD and CNFD (20). In addition manual and fully automated programs demonstrated excellent correlation for CNFL. Zhang et al. examined CCM images of dry eye patients and compared manual and automated programs (21). They demonstrated a significant correlation between the two methods, especially in CNFL (21). Petropolus et al. investigated the interobserver repeatability and agreement of SNP analysis with CCMetrics (13). They observed a high correlation for both CNFL and CNBD values (13). However, this study included only healthy subjects. Based on these studies, CNFL appears to have the highest agreement and reproducibility with the manual and fully automated methods. The CNFL may provide more consistent results as it involves the total length of nerve fibers and branches and does not need to distinguish between fibers and branches. However, in polyneuropathic conditions such as DM, assessment of nerve degeneration by fiber length alone may not be sufficient. Other parameters such as nerve density, tortuosity and branch density are also important in the evaluation of neuropathy (22). The analysis of images using manual programs is a time-consuming process and requires a high level of expertise. It is therefore important to develop fully automated programs with high repeatability that are correlated with manual programs. Machine learning programs have recently been used in medical image analysis and artificial intelligence-based methods have also been developed for SNP analysis (23,24). In the future, advances in artificial intelligence-based automated methods may improve the consistency of various parameters as well as CNFL, providing more comprehensive and reliable analyses in peripheral neuropathy assessment.

The limitations of our study are that we did not perform inter- and intra-observer analysis and also the sample size was small. In addition, categorizing the diabetic group into those with and without peripheral neuropathy and examining the agreement of both methods in different conditions could also be important.

Conclusion

CNFL emerges as a reliable parameter for evaluating corneal nerve morphology in diabetic patients. Identifying reliable parameters and consistent methods in SNP analysis is crucial for improving clinical practice.

Disclosures

Ethics Committee Approval: The study was approved by the Ethics Committee of Marmara University and adhered to the tenets of the Declaration of Helsinki and Good Clinical Practice (Protocol No: 09.2024.904). Informed written consent was obtained from all subjects in the study.

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References

1. Gulkas S, Aydin FO, Turhan SA, Toker AE. In vivo corneal confocal microscopy as a non-invasive test to assess obesity induced small fibre nerve damage and inflammation. Eye (Lond) 2023;37:2226–32.[CrossRef]
2. Dericioğlu V, Turhan SA, Erdem HE, Sevik MO, Erdil E, Sünter G, et al. In vivo corneal confocal microscopy in multiple sclerosis: Can it differentiate disease relapse in multiple sclerosis? Am J Ophthalmol 2023;250:138–48. [CrossRef]
3. Turhan SA, Karlsson P, Ozun Y, Gunes H, Surucu S, Toker E, et al. Identification of corneal and intra-epidermal axonal swellings in amyotrophic lateral sclerosis. Muscle Nerve 2024;69:78–86. [CrossRef]
4. Keskiner-Ozturk E, Akkaya-Turhan S, Toker E, Uluc K, Alibas H, Tanridag T, et al. Corneal nerve fiber involvement in chronic inflammatory demyelinating polyneuropathy. Neurol Sci 2023;44:2509–16. [CrossRef]
5. Celiker H, Erekul G, Turhan SA, Kokar S, Yavuz DG, Gunduz OH, et al. Early detection of neuropathy in patients with type 2 diabetes with or without microalbuminuria in the absence of peripheral neuropathy and retinopathy. J Fr Ophtalmol 2021;44:485–93. [CrossRef]
6. Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, Finnigan J, et al. Corneal confocal microscopy: A novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes care 2010;33:1792–7. [CrossRef]
7. Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, et al. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. Invest Ophthalmol Vis Sci 2014;55:2071–8. [CrossRef]
8. Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Fully automated, semiautomated, and manual morphometric analysis of corneal subbasal nerve plexus in individuals with and without diabetes. Cornea 2014;33:696–702. [CrossRef]
9. Liu YC, Lin MT, Mehta JS. Analysis of corneal nerve plexus in corneal confocal microscopy images. Neural Regen Res 2021;16:690–1. [CrossRef]
10. Dabbah MA, Graham J, Petropoulos IN, Tavakoli M, Malik RA. Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibres in corneal confocal microscopy imaging. Med Image Anal 2011;15:738–47. [CrossRef]

11. Ferreira A, Morgado AM, Silva JS. A method for corneal nerves automatic segmentation and morphometric analysis. Comput Methods Programs Biomed 2012;107:53–60. [CrossRef]
12. Efron N, Edwards K, Roper N, Pritchard N, Sampson GP, Shahidi AM, et al. Repeatability of measuring corneal subbasal nerve fiber length in individuals with type 2 diabetes. Eye Contact Lens 2010;36:245–8. [CrossRef]
13. Petropoulos IN, Manzoor T, Morgan P, Fadavi H, Asghar O, Alam U, et al. Repeatability of in vivo corneal confocal microscopy to quantify corneal nerve morphology. Cornea 2013;32:e83–9. [CrossRef]
14. Dabbah MG, Graham J, Tavakoli M, Petropoulos I, Malik R. Nerve fibre extraction in corneal confocal microscopy images for human diabetic neuropathy detection using Gabor filters. In Medical Image Understanding and Analysis. Berlin: Springer Nature; 2009. p. 254–8.
15. University of Manchester. ACCMetrics User Instructions. Available from: https://research.bmh.manchester.ac.uk/ena/ACCMetricsuserinstructions. Accessed Mar 5, 2020.
16. Giavarina D. Understanding bland altman analysis. Biochem Med (Zagreb) 2015;25:141–51. [CrossRef]
17. Li Q, Zhong Y, Zhang T, Zhang R, Zhang Q, Zheng H, et al. Quantitative analysis of corneal nerve fibers in type 2 diabetics with and without diabetic peripheral neuropathy: Comparison of manual and automated assessments. Diabetes Res Clin Pract 2019;151:33–8. [CrossRef]
18. Ostrovski I, Lovblom LE, Farooqi MA, Scarr D, Boulet G, Hertz P, et al. Reproducibility of in vivo corneal confocal microscopy using an automated analysis program for detection of diabetic sensorimotor polyneuropathy. PLoS One 2015;10:e0142309. [CrossRef]
19. Pacaud D, Romanchuk KG, Tavakoli M, Gougeon C, Virtanen H, Ferdousi M, et al. The reliability and reproducibility of corneal confocal microscopy in children. Invest Ophthalmol Vis Sci 2015;56:5636–40. [CrossRef]
20. Hertz P, Bril V, Orszag A, Ahmed A, Ng E, Nwe P, et al. Reproducibility of in vivo corneal confocal microscopy as a novel screening test for early diabetic sensorimotor polyneuropathy. Diabet Med 2011;28:1253–60. [CrossRef]
21. Zhang Y, Wu Y, Li W, Huang X. Semiautomated and automated quantitative analysis of corneal sub-basal nerves in patients with DED with ocular pain using IVCM. Front Med (Lausanne) 2022;9:831307. [CrossRef]
22. Gad H, Petropoulos IN, Khan A, Ponirakis G, MacDonald R, Alam U, et al. Corneal confocal microscopy for the diagnosis of diabetic peripheral neuropathy: A systematic review and meta-analysis. J Diabetes Investig 2022;13:134–47. [CrossRef]
23. Salahouddin T, Petropoulos IN, Ferdousi M, Ponirakis G, Asghar O, Alam U, et al. Artificial intelligence-based classification of diabetic peripheral neuropathy from corneal confocal microscopy images. Diabetes Care 2021;44:e151–3. [CrossRef]
24. Williams BM, Borroni D, Liu R, Zhao Y, Zhang J, Lim J, et al. An artificial intelligence-based deep learning algorithm for the diagnosis of diabetic neuropathy using corneal confocal microscopy: A development and validation study. Diabetologia 2020;63:419–30.