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# Application and progress of corneal confocal microscopy in the evaluation of diabetes-related peripheral neuropathy

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**Abstract**

Diabetic peripheral neuropathy (DPN) is one of the most common chronic complications of diabetes. As a new detection method for DPN, corneal confocal microscopy (CCM) is characterised by rapid, non-invasive, sensitive, and quantitative characteristics, as well as good repeatability. By detecting changes in the corneal nerves, DPN can be diagnosed early, and the severity of neuropathy evaluated. It is currently an ideal DPN evaluation method and has good clinical application prospects. This paper reviews the application and progress of CCM in the evaluation of DPN and summarises the evaluation methods of CCM, corneal nerve, and DPN to provide new ideas for the clinical diagnosis and treatment of DPN. **(Endokrynol Pol 2024; 75 (4): 395–402)**

**Key words:** *corneal confocal microscopy; diabetic peripheral neuropathy; corneal nerve diagnosis*

## Introduction

According to the latest Diabetes Atlas published by the International Diabetes Federation in 2021, approximately 10.5% of adults worldwide have diabetes, amounting to 536.6 million people. China has the highest number of adults with diabetes, estimated at 140.9 million. The main harm of diabetes is that it can lead to multi-system disease involvement and a variety of chronic complications such as diabetic macroangiopathy, diabetic nephropathy, diabetic retinopathy, and diabetic peripheral neuropathy (DPN), which can lead to disability and death. Diabetic peripheral neuropathy is a common chronic complication of diabetes, including distal symmetric polyneuropathy (DSPN), which is the most common type [1–2], affecting about 30% of patients [3]. Distal symmetric polyneuropathy has considerable morbidity, including reduced quality of life, and an increased risk of lower limb amputations and is associated with declining mortality. Significant progress has been made in understanding the pathogenesis of DSPN, and new techniques for its early diagnosis have emerged in the past decade. Despite advances in uncovering the pathogenesis of pain and its transmission, the management of painful DSPN remains a challenge [4]. It is estimated that 3–25% of patients with DM may experience neuropathic pain [5]. Additionally, DPN was also an independent predictor of all-cause death in diabetic patients [hazard ratio (HR) = 4.4] and diabetes-related death (HR = 11.8) [6]. The treatment of DPN is also difficult; strict blood glucose control can only delay the progression of DPN in type 1 diabetes, but not in type 2 diabetes, and, to date, no effective drugs are available to prevent or reverse DPN. One of the reasons for the slow development of drug therapy for DPN is the lack of effective clinical evaluation indicators.

Based on the lack of unified diagnostic methods and standards for DPN, the condition's prevalence rate has been reported to be between 10% and 90%, and these results indicate large differences [7]. In the course of clinical diagnosis and treatment, it has been found that the disease commonly causes accompanying paraesthesia, sensory loss, and hyperalgesia. Without early intervention, diabetic foot ulcers, gangrene, and subsequent amputation may occur in diabetic patients [8, 9]. Studies have found that more than half of diabetic patients have peripheral neuropathy [10, 11], some at the time of diagnosis or the early onset of diabetes. However, a 2010 survey of 1993 outpatients with type 2 diabetes showed that more than two-thirds of patients in urban areas in China had not

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been screened for DPN [12]. The 2017 American Diabetic Association (ADA) Statement on Diabetic Peripheral Neuropathy asserts that patients with newly diagnosed type 2 diabetes and those with type 1 diabetes for more than 5 years since diagnosis should be evaluated for both large and small fibre neuropathy.

In recent years, several emerging detection methods have been conducted and applied to the diagnosis of DPN, including intraepidermal nerve fibre density (IENFD) [13]. Damage to small nerve fibres can be detected early by measuring the number of nerve fibres at the dermal–epidermal junction, but a skin biopsy is required. This invasive method requires professional laboratory evaluation and is not conducive to clinical promotion and application, and the results still have a degree of variability, even in healthy control groups [14, 15]. The quantitative examination of sweat gland secretion reflex can give information about small nerve fibre function and has the advantage of being non-invasive, but its repeatability has been questioned [16]. Therefore, a rapid, non-invasive, sensitive, and reproducible DPN detection method is urgently needed. By detecting changes in corneal nerves, corneal confocal microscopy (CCM) can diagnose DPN early and evaluate the severity of neuropathy. Studies have shown that this method has the advantages of being rapid, non-invasive, and quantitative, with good repeatability and high sensitivity. It is an ideal DPN evaluation method at present and has good clinical application prospects. This article reviews the application and progress of CCM in the evaluation of DPN and provides ideas for the clinical diagnosis and treatment of DPN.



## Literature retrieval strategy

The English databases PubMed, Cochrane Library, Embase, Ovid, and Web of Science, and the Chinese databases CNKI, VIP, Wanfang, and China Biomedical Literature Service System were systematically searched. Additionally, the reference lists of the retrieved literature were manually checked and screened for any potentially relevant studies that may have been missed in the initial database searches. A combination of the following keywords was used: 'complications', 'CCM', 'diabetes', 'diabetic peripheral neuropathy', and 'diagnosis'. The search strategy was determined by combining subject terms and free terms following several pre-searches.

## Corneal nerves

The cornea is the most densely innervated area of human tissue. The corneal nerve fibres mainly come from the ocular branch of the trigeminal nerve, including the A$\delta$ nerve fibre and the unmyelinated nerve fibre C. Innervation plays an important role in maintaining corneal integrity. In various ocular diseases and systemic disease states, dynamic changes in the distribution and morphology of corneal nerve fibres can be observed, which is of great value for disease diagnosis and therapeutic effect monitoring [17]. The transparent nature of the cornea makes it possible to observe the morphology of nerve fibres directly and non-invasively on living tissue. The corneal plexus in CCM showed a beaded linear homogenous hyperreflective structure. The following definitions are used: (1) corneal nerve fibre density (CNFD) — the number of nerve trunk fibres per square millimetre; (2) corneal nerve fibre length (CNFL) — all nerve fibre lengths per square millimetre; (3) corneal nerve branch density (CNBD) — the number of branching nerves emitted by the main nerve per square millimetre; and (4) corneal nerve fibre tortuosity (CNFT) — the curvature of the total nerve fibres [18, 19].

## Diabetic peripheral neuropathy

Diabetic peripheral neuropathy is one of the most common complications of diabetes and is defined as a symmetric sensorimotor multiple neuropathy (Toronto Consensus Conference, 2009). Its pathogenesis is based on a long-term abnormal rise in blood sugar, metabolic disorders, blood vessels, and other risk factors, which may involve motor nerve, sensory nerve, and autonomic nerve injuries [20]. Diabetic peripheral neuropathy is also the second most common cause of nerve damage after trauma, with lesions affecting all peripheral nerves, including pain fibres, motor neurons, and the autonomic nervous system. The most serious complication of DPN is diabetic peripheral neuralgia, with a reported incidence of 13–26% [21, 22]. With the increase in the prevalence of diabetes worldwide, the pervasiveness of DPN is also increasing [23, 24]. Studies have shown that DSPN and diabetic cardiac autonomic neuropathy are the most common types of DPN, and DSPN accounts for 75% of DPN cases [25]. Peripheral neuropathy is more common in people with early-stage diabetes, and its severity increases with age and disease course. Therefore, early detection and active intervention are very important to improve nerve function and quality of life for patients with DPN. Various notions about the pathogenesis of DPN have been put forward, but its exact mechanism has not been elucidated. Presently, the possible mechanisms are as follows:

1. Schwann cells (neurotrophic cells) participate in the polyol pathway, and sustained hyperglycaemia damages Schwann cell glia, leading to nerve fibre damage.

2. The nodule area between Schwann cells and axons is damaged, slowing down nerve conduction.
3. Endoplasmic reticulum stress occurs, where high blood glucose levels cause the degradation of functional proteins of the endoplasmic reticulum, leading to conduction disorders [26].

Diabetic peripheral neuropathy has a variety of aetiological hypotheses, including impaired neurovascular function, nerve growth factor deficiency, abnormal nerve fibre metabolism and autoimmune factors. Concurrently, microvascular disease, abnormal lipid metabolism and insulin resistance are also believed to play an important role in the pathogenesis of DPN [27]. Among these, abnormal lipid metabolism and insulin resistance are considered to be the main pathogenic factors of type 2 diabetes [28]. The main clinical manifestation of DPN is limb-symmetry pain, especially at the distal end. The most typical symptoms include a feeling of numbness, tingling, or loss of sensation, often described as a "glove and stocking" feel. In severe cases, it may be accompanied by peripheral vascular diseases and foot ulcers, or gangrene caused by infection, and in severe cases, it may lead to amputation or disability. Due to these atypical clinical manifestations, the disease progresses rapidly, which reduces the quality of life of patients, causes physical pain, and increases the economic burden on families and society. Early diagnosis and treatment of DPN are essential to delay the onset and development of diabetic complications and reduce mortality. Because DPN is a key factor in the development and worsening of diabetic foot disease, multiple surgical procedures are required for re-vasoplasty if conservative treatment fails, but in the event of unbearable pain, local gangrene, or even systemic bacteraemia, amputation is the only option to prolong life. The literature has shown that appropriate intervention to control the development of DPN in patients with high-risk factors can reduce the incidence of foot ulcers by 60% and amputation by 85% [29]. Early reduction of the risk factors of DPN, a timely diagnosis, effective aetiological intervention, and early treatment are essential to improve the prognosis of DPN and reduce its social burden. At present, there are no effective drugs for the treatment of DPN on the market, nor any preventive drugs or methods that have been approved by the US Food and Drug Administration. The active control of blood sugar, nutritional supplementation, antioxidant stress, and other means are currently typically used to treat DPN in the early stage, which has some efficacy; however, if the disease has progressed to cause peripheral nerve damage in the later stages, the treatment options for DPN become less effective. Once irreversible nerve damage has occurred, the treatment effect will be poor. Therefore, the early diagnosis of DPN is very important to improve the long-term prognosis of patients.

## Evaluation of diabetic peripheral neuropathy by corneal confocal microscopy

### *Corneal confocal microscopy*

Corneal confocal microscopy is a rapid, non-invasive technique for imaging the cornea *in vivo*. Historically, it has been used in the diagnosis and clinical management of corneal epithelial and stromal diseases. However, over the past 20 years, CCM has increasingly been used to image small sub-basal nerve fibres in various peripheral neuropathies and central neurodegenerative diseases. The method has also been used to identify subclinical nerve damage and predict the development of DPN [30]. At present, the confocal microscope used for the observation of corneal neuromorphology uses a laser as a light source (thus also known as laser-scanning CCM). A laser confocal microscope adopts auto-focusing technology, which does not reduce the resolution when the focus is moved, and its imaging clarity is much higher compared with an ordinary optical microscope. It can quickly scan each layer of corneal tissue in its natural, unaltered state and clearly display the cell morphology and nerve fibre bundle distribution of each layer of the cornea [31]. The imaging principle forms a point light source at a specific depth of the specimen through an illuminating pinhole to realise scanning imaging of a specified point of the focal plane of the specimen. Currently, there are 3 types of confocal microscopes: tandem scanning CCM, with a halogen lamp as a light source, slit-scanning CCM, and laser scanning CCM (LSCM), the latter using a laser as a light source. Since CCM is a non-invasive test, it is an ideal tool for observing changes in the cornea and ocular surface and is widely used in the diagnosis and efficacy monitoring of eye and systemic diseases [32]. Among these, LSCM has good resolution and contrast, and better axial resolution, making it the most commonly used in recent years.

### *Application and progress of corneal confocal microscopy to evaluate diabetic peripheral neuropathy*

#### Advantages of corneal confocal microscopy in early diagnosis of diabetic peripheral neuropathy

Diabetic peripheral neuropathy has complex aetiological mechanisms and diverse clinical manifestations. It is characterised by progressive nerve damage and can involve the somatic and autonomic nervous systems, mainly including distal symmetric polyneuropathy,

DSPN, and diabetic autonomic neuropathy. At present, the detection methods for DPN include nerve conduction studies (NCS), a 10-g nylon wire examination, a 128-Hz tuning fork examination, and quantitative sensory testing (QST), and a skin biopsy to measure IENFD and CCM. Among these, the 10-g nylon wire, tuning fork, and quantitative sensory examination can detect whether the sensory nerve function is damaged; however, the detection results are too dependent on the subjective feelings of patients, with large variability and low repeatability. Although nerve electrophysiological examination has strong objectivity, high specificity, and high repeatability, it cannot be used to diagnose early small nerve injury because it detects large nerve conduction function [33]. Nerve conduction studies are considered a reliable method for the diagnosis of DPN, but they can only evaluate large, myelinated nerve fibres, while the repair evaluation ability of small nerve fibres is limited for injuries, especially after treatment intervention [34]. Quantitative sensory testing is non-invasive, easy to carry out, and has good repeatability, but its disadvantage is that it is greatly affected by patients' subjective cooperation [35]. A skin biopsy to measure IENFD is considered the most objective index for the diagnosis and quantification of small fibre neuropathy [36], but it is invasive and limited in clinical application. Therefore, there is an urgent need for a non-invasive, stable, and sensitive detection method for the diagnosis of small neurofibropathy. Because nerve fibres repair extremely slowly and there are few specific drugs for DPN treatment, early detection and timely intervention measures are important for the treatment of DPN. Small unmyelinated nerve fibres are the first damaged site in the early stage of DPN. A skin biopsy is a clinical examination method used to observe the morphological structure and distribution of small nerve fibres; morphological changes in small nerve fibres can be detected in patients with abnormal glucose tolerance and early diabetes. However, skin biopsies are invasive and difficult for most patients to undergo. Therefore, the non-invasive CCM test has become a more popular means of detecting DPN.

**Diagnosis of diabetic peripheral neuropathy by corneal confocal microscopy and evaluation of diabetic peripheral neuropathy severity**

Recent studies [37] have observed abnormal corneal morphology in diabetic patients via CCM, including decreased corneal thickness, epithelial thinning, irregular changes in the epithelium and endothelium, and a decreased corneal nerve bundle. The corneal nerve bundle decrease preceded the decrease in corneal thickness. Subsequently, a large number of studies found that, compared with healthy people, the CNFD, CNFL, and CNBD in diabetic patients were significantly reduced [38–42]. Recently, Chen et al. [43] studied 89 subjects with and without DSPN (including 63 patients with type 1 diabetes and 26 healthy controls) to compare the diagnostic efficacy of CCM and IENFD. Significantly reduced CNFD, CNFL, CNBD, and IENFD were found in diabetic patients with DSPN compared with the control group ($p < 0.001$); there was no significant difference between the 2 methods for the diagnosis of DSPN based on the ROC curve ($p = 0.14$), which verified that CCM can accurately diagnose small neuropathy and is an ideal alternative method for the diagnosis of DSPN. A recent large meta-analysis on CCM assessment of DPN involving 1680 participants from 13 studies [44] showed that CNFD, CNFL, and CNBD in patients with DPN were significantly reduced compared to healthy controls and diabetic patients who did not have DPN ($p < 0.00001$). Ahmed et al. [41] also determined that $\leq 14.0$ mm/mm$^2$ was the best cut-off value for CNFL, and the sensitivity and specificity reached 85% and 84%, respectively. These studies suggest that CCM is an ideal alternative method for the non-invasive assessment and quantification of nerve damage in patients with diabetes.

Shi et al. [42] used CCM to observe 54 normal corneas, and the results showed that corneal epithelial cells were closely arranged and relatively consistent in size, and their volume gradually decreased with depth, while thin nerve fibres could be seen in basal cells. Bowman's membrane showed an uneven distribution of nerve fibres. The nerve fibres in the stromal layer were thicker, and corneal cells were observed in various states such as rod and moon form. The inner cortex cells were hexagonal and closely arranged. Due to the role that morphological changes play in small nerve fibre injury in the diagnosis of DPN, scholars at home and abroad have begun paying closer attention to CCM; this method can quantitatively analyse morphological changes in the corneal subepithelial nerve fibre plexus. Therefore, in the past decade, some studies have applied CCM to the classification of DPN, early diagnosis, and the evaluation of the effectiveness of treatment measures. Quattrini et al. [45] conducted CCM examination, skin vital tissue examination and neuro-electrophysiological examination, respectively, on 54 patients with diabetes, including 10 patients without DPN, 18 patients with mild DPN, 15 patients with moderate DPN, and 11 patients with severe DPN. The results of 15 non-diabetic healthy volunteers were taken as the control. The results showed that, with an increase in DPN grade, the density of nerve fibres in the epidermis, nerve branch density, and nerve fibre length gradually decreased, and the density of epidermal nerve fibres in patients with diabetes without DPN also decreased significantly compared with healthy

individuals. Corneal subepithelial nerve fibre density, nerve branch density, and nerve fibre length also decreased gradually with DPN grade. Compared with the normal control group, corneal subepithelial nerve fibre density and nerve branch density were significantly decreased in patients who were diabetic but without DPN. Compared with patients who had DPN without obvious pain symptoms, the epidermal nerve fibres and corneal subepithelial nerves were significantly reduced in patients with DPN with pain symptoms. The results of CCM and a skin biopsy were in good agreement with those of the sensory quantitative test. It can be concluded that both CCM and a skin biopsy can accurately reflect the severity of DPN, but CCM may be more sensitive in the diagnosis of early small nerve injury. Wang et al. [46] showed that, compared with patients with non-painful DPN, corneal subepithelial nerve fibre density and nerve fibre length were significantly reduced in patients with painful DPN. The results of the above 2 studies suggest that corneal nerve damage is more severe in patients with painful DPN, and CCM may also be used for early detection of high-risk groups with painful DPN. Furthermore, a significant decline in the use of CCM was also found in patients with prediabetes and newly diagnosed diabetes. Asghar et al. [47] observed 37 patients with impaired glucose tolerance (IGT) and 20 age-matched controls, and found that CNFD, CNFL, and CNBD were significantly reduced in patients with IGT. The results were consistent with those of IENFD and other indicators mainly representing small neurofibropathy, such as thermal and cold sensory thresholds. This suggests that microfibropathy can occur in pre-diabetes and early diabetes, and CCM can detect this change early.

**Corneal confocal microscopy is used to evaluate the therapeutic effect of DPN**

Corneal confocal microscopy can be used to observe early improvements in nerve fibres. In an observational study [48] that included 25 patients with mild/moderate diabetes, it was observed that improvements in cholesterol levels were significantly associated with improvements in CNFD, CNBD, and CNFTO after 24 months of treatment, and a decrease in HbA1c was significantly associated with an increase in CNFD. In other studies, it was found that CNFD and CNFL were significantly improved at 6 months and CNFL, CNFD, and CNBD were all improved at 12 months [49, 50] in patients with type 1 diabetes receiving combined pancreatic–kidney transplantation; furthermore, among all diagnostic methods for DPN, only CCM showed improvement at 12 months [51]. The dynamic observation of corneal nerve changes before and after treatment via CCM is a major advantage, and such changes cannot be detected by skin biopsy or QST. A recent study compared the effects of continuous subcutaneous insulin infusion and multiple insulin injections on DSPN in patients with type 1 diabetes [52]. After 24 months, although the 2 treatment groups received similar glycaemic control, significant increases in CNFD ($p = 0.03$) and CNBD ($p = 0.060$) were observed only in the continuous subcutaneous insulin infusion (CSII) group. This confirms that the stable glycaemic control brought about by CS is beneficial for the recovery of small neurofibropathy, and this improvement can be accurately detected by CCM.

**Progress in corneal confocal microscopy and new technologies for image analysis**

Recent advancements in CCM have led to improved image quality and analysis techniques. High-resolution confocal microscopes with enhanced contrast and axial resolution have enabled better visualisation of corneal nerve fibres [53]. Furthermore, the development of automated image analysis software has facilitated faster and more objective quantification of corneal nerve parameters, reducing the reliance on manual analysis [54]. These technological advancements have contributed to the increasing application of CCM in the evaluation of DPN.

### *Limitations of corneal confocal microscopy*

While CCM offers several advantages in the early diagnosis and assessment of DPN, it is important to acknowledge its limitations. Although CCM is often referred to as a non-invasive procedure, it is a contact examination that requires the application of local anaesthesia, which can be side effect to the ocular surface. In diabetic patients with corneal epithelial defects, CCM may cause corneal decompensation and refractory ulceration, potentially leading to blindness [55]. Therefore, CCM is not suitable for all patients and requires good patient cooperation. Additionally, a CCM is a piece of expensive equipment that requires well-trained personnel to operate it, limiting its availability to specialised university hospitals with corneal departments. Furthermore, the examination is not as rapid as it may seem; it can take up to a couple of minutes to perform [56].

## Prospects and shortcomings

Corneal confocal microscopy is a novel non-invasive detection technique that can accurately and quickly evaluate small nerve fibre damage and repair. It has outstanding advantages in the early diagnosis, quantification of severity, prediction, and efficacy evaluation of DPN. However, there is no consistent standard for

CCM image capture, selection, and analysis, leaving the current research with no standard results. Manual image analysis is time-consuming and subjective, and although automatic image analysis software is available, it is only beginning to be applied in a few research institutions. Corneal confocal microscopy, as a method of treatment, has accumulated more than 20 years of experience in evaluating DPN, and its advantages are that it is non-invasive and highly reproducible, and it is easy to implement patient follow-up. Corneal confocal microscopy has developed as a new method for the detection of DPN, which can be used to study the natural course of DPN, evaluate the severity of neuropathy, and observe nerve fibre regeneration after intervention treatment. Of particular significance is that CCM can detect peripheral neuropathy and high-risk diabetic foot disease early, with moderate to high sensitivity and specificity. Currently, CCM is limited by the expensive nature of this method and is only used in a few medical centres with specialised equipment. The impact of non-neurological and ocular diseases on CCM data remains unclear. As the evidence for the use of CCM in the diagnosis of DPN continues to grow, we look forward to conducting tests and research in more medical centres and accumulating more data. We are looking forward to using CCM to study the effects of various drugs for the treatment of DPN because CCM allows researchers to look directly at small nerve fibre changes and even nerve fibre regeneration. The automatic analysis software of CCM for nerve fibre patterns must be further developed and improved to enable better accuracy and convenience for the quantitative evaluation of neurofibropathy and promote the application of CCM in clinical settings.

In addition, based on the literature review, the research data on CCM for the treatment of DPN are mostly limited to the normal population, while research on Mongolian DPN is still in its early stage, with few relevant studies. Mongolians represent a large minority population in China, and there are large differences between Mongolians and other ethnic groups in terms of geographical location, diet pattern, and genetic characteristics. In recent years, the incidence of diabetes has been increasing annually, and the importance of diabetes and its complications is worrying. Neglecting diabetes management leads to the development of multiple complications. Therefore, CCM is also of great guiding significance in the evaluation of DPN in the Mongolian population.

## Conclusion

Diabetic peripheral neuropathy is one of the most common and costly long-term complication of diabetes. To reduce the severity and progression of DPN, early diagnosis, early and effective intervention, and stable control of blood glucose and blood pressure are extremely important. Some studies have also shown that diet and exercise can improve the symptoms of DPN and even regenerate nerve fibres in the epidermis[54]. Research has to date shown that although there is no clear treatment to cure or reverse DPN, early aggressive and effective treatment can significantly reduce the incidence of DPN and improve patients' quality of life. We should pay attention to the occurrence of DPN and its risk factors and conduct early intervention and treatment for patients with diabetes. Corneal confocal microscopy can be used to detect and diagnose DPN as early as possible. For Mongolian patients with pre-diabetes, especially in cases where education levels are low, knowledge and education should be made available that advocate for the regular detection of relevant indicators; health guidance should be delivered that promotes a healthy diet, smoking cessation, and alcohol restriction, and that highlights the importance of exercise, weight control, and obesity reduction. Lifestyle intervention can clarify the progress and remission of peripheral neuropathy to reduce the incidence and development of diabetes and its complications. Concurrently, the relevant characteristics of neuropathy in type 2 diabetes among the Mongolian population can be analysed to provide a theoretical basis for early clinical diagnosis and prevention and to develop new research ideas for exploring ethnic differentiation.

### *Conflict of interest*

The authors declare no conflict of interests.

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