Corneal Nerve Morphometry for Diabetic Peripheral Neuropathy Assessment

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Abstract— **Diabetic peripheral neuropathy (DPN) is one of the more common complications of diabetes, being associated to 50-75% of non-traumatic amputations. Early diagnosis of DPN often fails or occurs only when patients became symptomatic due to the non-availability of a simple, reliable, non-invasive method.**

We present the results of a pilot study where we used peripheral nerves morphometric information, retrieved from images of the corneal sub-basal nerve plexus, obtained *in vivo* by corneal confocal microscopy, to identify and stage patients with DPN. Nerve segmentation was done manually and using an automatic algorithm. Both standard statistical techniques and classification techniques were used for DPN identification.

With manual segmentation, differences were found between controls and patients with mild and moderate DPN, for the nerve fiber length and density parameters. A simple comparison between individuals with and without DPN results in significant differences for those parameters, as well as for branching pattern and density. No differences were found with automatic segmentation. Simple classification techniques based on manually extracted parameters were tested for identification of DPN patients. The best results were obtained with a binary tree classifier, using PCA transformed data, for a pruning level 9. The classifier accuracy was 87%.

The results confirm that morphometric analysis of corneal nerves images may be used as a complementing technique for DPN diagnosis. A fully automatic tool for identifying and staging DPN patients requires additional work on the nerve segmentation algorithm and the use of more robust classifiers with additional reliance on image features other than the tested morphometric parameters.

Keywords— **diabetic neuropathy, corneal confocal microscopy, morphometric analysis, nerve segmentation, classification.**

I. INTRODUCTION

Diabetic neuropathy is one of the more common complications of diabetes, being the main cause of chronic disability in diabetic patients [1]. Diabetic peripheral neuropathy (DPN) is present in about 8% of newly diagnosed patients [2]. It affects up to 50% of the patients after 25 years of disease and is associated to 50-75% of non-traumatic amputations [2]. Early diagnosis and accurate assessment are very important to define higher risk patients. However, early diagnosis often fails or occurs only when patients became symptomatic due to the non-availability of a simple, reliable, non-invasive method.

The eye cornea is one of the most innervated tissues in the human body [3] and is accessible to optical imaging. In the last 15 years, several researchers proposed to use nerve morphometric parameters, extracted from images of the corneal sub-basal nerve plexus, to assess DPN. These images are obtained *in vivo*, non-invasively, by corneal confocal microscopy (CCM) [4]. It was shown that diabetic patients have lower nerve density [5], even for short diabetes duration [6;7], that CCM can accurately report the extent of corneal nerve damage and repair, using fiber density and branching measurements [8], and that nerve tortuosity correlates with neuropathy severity [9].

Recent works focus on the reproducibility of CCM in the evaluation of corneal nerves, using semi-automated [10] and manual [11] methods, and on accurate methods for automatic segmentation and analysis [12-15]. A review on this research area was recently published [16].

We present results of a pilot study designed to verify if corneal nerves morphometric parameters can diagnose DPN. Nerve segmentation was done manually and using an automatic algorithm [15]. For DPN identification, we used standard statistics and classification techniques.

II. RESEARCH DESIGN AND METHODOLOGY

The study included 12 diabetic patients (type 2, insulin-treated, with mean age of 58±10 years), followed at the Department of Endocrinology, Diabetes and Metabolism of Coimbra Hospital University Center (CHUC), and 8 age-matched non-diabetic control individuals (mean age: 54±7 years). Diabetics were divided in three groups: absent (4 patients, 53±11 years), mild (5, 58±9 years) and moderate DPN (3, 60±9 years). No patient presented severe DPN.

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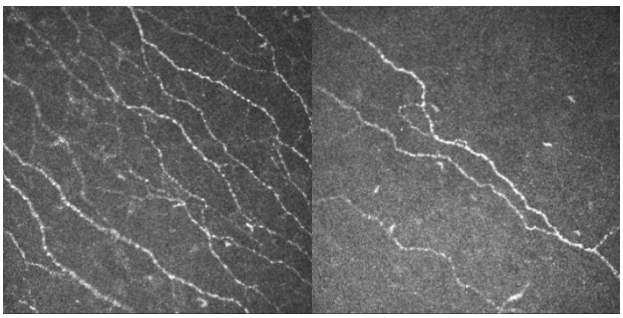
Physical assessment was conducted according to guidelines of the Declaration of Helsinki. Clinical examinations followed the international consensus guidelines for diagnosis and management of DPN [17] and the Michigan Neuropathy Screening Instrument (MNSI) [18], comprising two separate assessments: a 15-item questionnaire and a lower extremity examination, that includes feet appearance evaluation, ankle reflex testing and sensory deficit evaluation (superficial pain, touch perception and vibrating sensation). Physical examination and questionnaire evaluation was done by medical doctors, in a random order, without information of other test results. The patient's clinical history was verified to ensure that peripheral neuropathy is a consequence only of diabetes.

All individuals underwent electromyography (EMG), at the Department of Neurology of CHUC, using a Nicolet Biomedical EA4 (Nicolet Biomedical, Madison, WI, USA) recording device. EMG measurements comprised nerve conduction evaluation, motor (peroneal) and sensory (sural) nerve conduction velocity (NCV) and amplitudes, as well as cutaneous sympathetic response.

CCM images were obtained at the Department of Ophthalmology of CHUC, using a Heidelberg Retinal Tomograph equipped with a Cornea Rostock Module (Heidelberg Engineering, Heidelberg, Germany). The 384x384 pixels images correspond to a 400µm x 400µm area and were saved in JPEG format. All individuals underwent bilateral examination by an ophthalmologist. We recorded 183 nerve images from healthy individuals and 306 images from patients. A reduced set of 200 best images (20 subsets of 10 bilateral images, with one subset per individual) was selected for analysis. All images contained only nerves structures, with clear differentiation between the main corneal nerve trunks and the secondary branches. Image samples are shown in Fig. 1.



(a) (b)

Fig. 1: Images of corneal sub-basal nerve plexus obtained by corneal confocal microscopy: (a) healthy cornea; (b) diabetic cornea.

Nerves were segmented manually and automatically using an algorithm previously described [15]. Briefly, the algorithm starts by normalizing contrast and reducing noise, using contrast equalization, phase symmetry based method and a histogram procedure. A fast search locates candidate regions that are expanded to identify the nerves.

We used parameters reported in similar studies: nerve fiber density (NFD), length (NFL) and tortuosity (TC), as well as nerve branching density (NBD), pattern (NBP) and angle (NBA). NFD is the total number of nerves per image area (nº fibers/mm^2); NFL is the length of all nerve fibers and branches per image area (mm/mm^2); TC quantifies the frequency and magnitude of nerve curvature changes, using the definition proposed by Kallinikos [15]:

$$TC = \sqrt{\sum_{i=1}^{N-1}\left[\left(f'(x_i,y_i)\right)^2 + \left(f''(x_i,y_i)\right)^2\right]} \quad (1)$$

with N the number of pixels of the nerve skeleton, and $f'(x_i, y_i)$ and $f''(x_i, y_i)$ the first and second derivatives at the point (x_i, y_i), respectively.

NBD gives the number of branches emanating from main nerves per image area (nº branches/mm^2); NBP is the percentage of branches per total number of nerve fibers. Finally, NBA is the mean value of the angle formed by the branches and the main nerve. All parameters were calculated, from the segmented images, using a Matlab image processing program developed for this purpose.

III. DATA ANALYSIS AND RESULTS

Table 1 summarizes the data of the study participants.

EMG shown large-fiber abnormalities in all patients with moderate DPN, and in one patient with mild DPN, Progressive axonal losses, according to DPN severity, were found. Normal NCV values were observed for all patients without DPN and for the majority of patients with mild DPN. Nevertheless, the NCV and amplitude values were always lower for patients with mild and moderate DPN, for both motor and sensory nerves.

Table 1: Summary of the four participants groups (baseline evaluation).

Group (DPN Severity)	Mean Age ±Std.Dev. (years)	CCM images	MNSI Quest. (0-15)	MNSI Scoring (0-10)	EMG diagnosis
Control	54±7	80	0	0	Normal
Absent	53± 11	40	0-3	0-1	Normal
Mild	58±9	50	4-6	2-3.5	DPN/Normal
Moderate	60±9	30	6-8	2-3	DPN

The automatic algorithm was evaluated by comparing, on each image, with manually traced nerves. Results are shown in Table 2. The performance was insufficient and lower than that achieved for a different set of corneal nerves images [15].

Table 2: Performance of automatic segmentation algorithm.

	Minimum	Maximum	Average	Std. Dev.
Nerve length correctly detected (%)	19.9	91.0	56.7	20.3
Nerve length falsely detected (%)	0	2.1	0.5	0.8

Nerve morphometric parameters were extracted from manually and automatically segmented images and compared between groups (control, absent, mild, and moderate DPN). ANOVA, with post-hoc Tukey test, was used for normally distributed data. Otherwise, we used the Kruskal-Wallis test, with between group comparisons by the Nemenyi test. The significance level was 95%.

With manual segmentation, differences were found between controls and patients with mild and moderate DPN, for the NFL and NFD parameters (Fig. 2). With automatic segmentation, no differences were found. A simpler comparison (with and without DPN) gave statistically significant differences for the NFL, NFD, NBP and NBD parameters, with manually segmented images.

Classification techniques based on manually extrac-ted parameters were tested for identification of DPN patients. Two classifiers were evaluated: a simple Naive-Bayes classifier and a binary tree classifier. The clas-sifiers used the full set of 489 images, both with raw data provided by the image processing program and with data transformed by Principal Component Analysis (PCA), performed in the feature space. 10% of the data was randomly extracted to build the testing set while the remaining composed the training set.

For the Naive-Bayes classifier, the number of features was reduced according to their importance, as ranked by the chi-square test. The most relevant features were TC, NBD and NBA. With the binary tree classifier, and since all features are continuous, it was necessary to find the position that provides a better data splitting between groups, in the training set. The impurity measure used was the Entropy, given by

$$\text{Entropy}(t) = -\sum_{i=0}^{c-1} p\left(\frac{i}{t}\right)\log_2 p\left(\frac{i}{t}\right) \quad (2)$$

where p(i/t) is the fraction of records belonging to class i, at a given node, and c is the number of classes (here c = 2: without or with DPN). Binary decision tree classification was tested for different levels of pruning.

The best results were obtained with the binary tree classifier, using PCA transformed data, for a pruning level 9, achieving an accuracy of 87%.

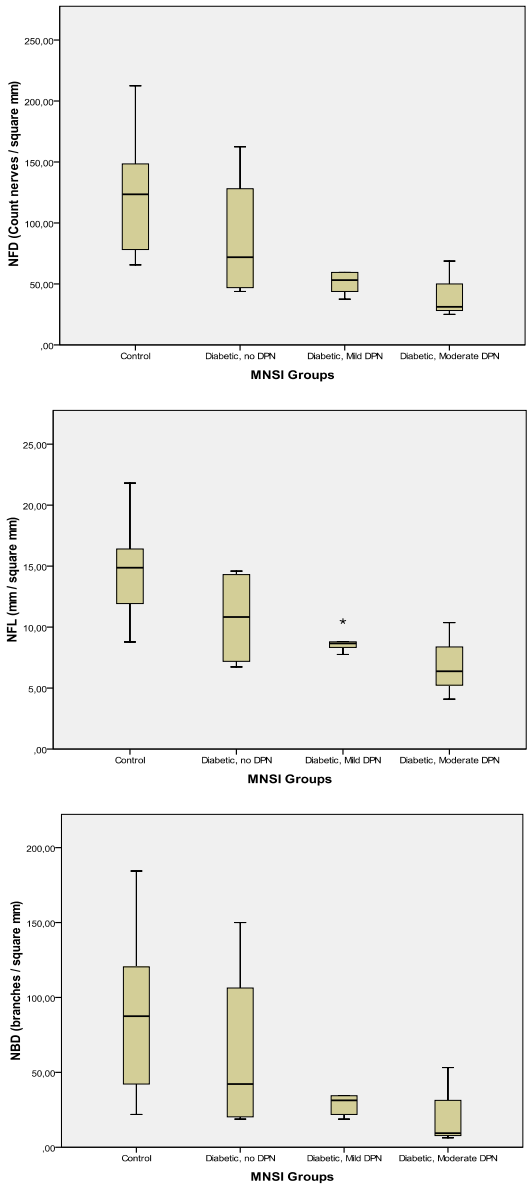


Fig. 2: Representative box-plots (with median, interquartile range, outliers, and extreme cases) for the NFD, NFL and NBD parameters.

IV. DISCUSSION AND CONCLUSIONS

Our results agree with published research, confirming that morphometric analysis of corneal nerves CCM images may be used as a complementing technique for clinical and electrophysiological diagnosis of DPN. We could distinguish healthy individuals from diabetics and between individuals with and without DPN. More important, the technique has the potential to grade the patients by their neuropathy severity. The accurate quantification of NFL,

NFD and NBD let us conclude that increasing corneal nerve degeneration is directly related with DPN severity. Those parameters can identify patients at initial DPN stage (mild) that are at risk of developing clinically significant DPN.

A fully automatic tool for identifying and classifying DPN patients requires additional work. The results obtained with automatically segmented nerves highlighted the algorithm inadequate performance. In fact, it performed worse than previously reported, for a different image set [15]. This may result from differences in the corneal confocal microscopes used for acquiring the images (a scanning slit microscope was used to record the image set tested in [15], while here CCM was performed with a laser scanning microscope), that lead to different image features, namely noise and lateral resolution. The algorithm for automatic segmentation still requires substantial improvements, mainly in post-processing steps, in order to obtain higher accuracy.

The use of classifiers for identifying DPN showed promising results. The work done so far was clearly preliminary and based on simple approaches. The classifiers used the nerve morphometric parameters usually reported in literature for DPN evaluation and did not rely on image features. It is reasonable to expect higher accuracies with more robust classifiers.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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