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ORIGINAL ARTICLE

Comparison of manual & automated analysis methods for corneal endothelial cell density measurements by specular microscopy

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KEYWORDS
Corneal endothelial cell density;
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Abstract
Purpose: To determine the reliability of corneal endothelial cell density (ECD) obtained by automated specular microscopy versus that of validated manual methods and factors that predict such reliability.
Methods: Sharp central images from 94 control and 106 glaucomatous eyes were captured with Konan specular microscope NSP-9900. All images were analyzed by trained graders using Konan CellChek Software, employing the fully- and semi-automated methods as well as Center Method. Images with low cell count (input cells number <100) and/or guttata were compared with the Center and Flex-Center Methods. ECDs were compared and absolute error was used to assess variation. The effect on ECD of age, cell count, cell size, and cell size variation was evaluated.
Results: No significant difference was observed between the Center and Flex-Center Methods in corneas with guttata ($p = 0.48$) or low ECD ($p = 0.11$). No difference ($p = 0.32$) was observed in ECD of normal controls <40 yrs old between the fully-automated method and manual Center Method. However, in older controls and glaucomatous eyes, ECD was overestimated by the fully-automated method ($p = 0.034$) and semi-automated method ($p = 0.025$) as compared to manual method.
Conclusion: Our findings show that automated analysis significantly overestimates ECD in the eyes with high polymegathism and/or large cell size, compared to the manual method. Therefore, we discourage reliance upon the fully-automated method alone to perform specular microscopy analysis, particularly if an accurate ECD value is imperative.

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PALABRAS CLAVE
Densidad celular endotelial corneal;
Método automatizado;
Método de centrado;
Método de centrado flexible;
Microscopio especular

Comparación de los métodos de análisis manual y automatizado para medir la densidad celular endotelial corneal mediante microscopio especular

Resumen
Objetivo: Determinar la fiabilidad de la densidad celular endotelial corneal (ECD) obtenida mediante microscopio especular automático frente a métodos manuales validados y factores predictivos de la fiabilidad.
Métodos: Se capturaron imágenes nítidas de 94 controles y 106 ojos glaucomatosos con un microscopio especular Konan NSP-9900. Todas las imágenes fueron analizadas por examinadores expertos mediante el software Konan CellChek, utilizando los métodos automatizado total, semiautomático y de centrado. Se compararon las imágenes con bajo recuento celular (número de células <100) y/o córnea guttata con el método de centrado y centrado flexible. Se compararon las ECD, utilizándose el error absoluto para valorar la variación. Se evaluó el efecto de la ECD sobre la edad, el recuento celular, el tamaño celular y la variación del tamaño celular.
Resultados: No se observó diferencia significativa entre los métodos de centrado y centrado flexible en las córneas con guttata ($p = 0{,}48$) o baja ECD ($p = 0{,}11$). No se observó diferencia ($p = 0{,}32$) en cuanto a ECD en los controles normales < 40 años entre el método totalmente automatizado y el método de centrado manual. Sin embargo, en los controles mayores y en los ojos glaucomatosos, la ECD fue sobreestimada por el método totalmente automatizado ($p = 0{,}034$) y el método semiautomático ($p = 0{,}025$), en comparación al método manual.
Conclusión: Nuestros hallazgos muestran que los análisis automatizados sobreestiman considerablemente la ECD en los ojos con alto polimegatismo y/o gran tamaño celular, en comparación al método manual. Por tanto, no recomendamos confiar en el método totalmente automatizado por sí solo para realizar estudios mediante microscopio especular, particularmente en casos en que la precisión del valor de ECD sea imperativo.

Introduction

Endothelial cell density (ECD) values are routinely used in clinical practice to evaluate the status of the corneal endothelium to make treatment and surgical decisions and to gauge the safety of new drugs, devices and surgical processes during clinical trials.

Non-contact specular microscopes such as the Konan NSP-9900 capture sharp images with sufficient magnification for reliable ECD determination or morphometric analysis. The fixed-frame method for determine ECD allows quantitative analysis of cell structure, including ECD, coefficient of variation (CV), and percentage of hexagonal cells (HEX).[1–3]

The Konan CellChek software uses several different approaches, varying in speed and complexity, to obtain ECDs, from fully- and semi-automated to manual.[4–8] The automated analysis method, or Auto-Trace, automatically outlines endothelial cells and calculates cell density, cell size and hexagonality. In the fully-automated method, the default cell size S pattern is used, while in the semi-automated method, the cell size is manually selected from S to XL. The most commonly manual analysis methods are the Center Method and the Flex-Center Method. In the Center Method, the user marks the center of each cell in a contiguous group, and the software then counts the number of cells by determining cell area from a polygon digitization by locating cell border intersections.[9] In the Flex-Center Method, based on the Center Method, the outer boundary of all visible cells is outlined by clicking the intersection of three cells. The use of Flex-Center Method is suggested by the manufacturer when fewer contiguous cells are visible.

We compared automated and manual methods of ECD measurements to determine the optimal methods in terms of accuracy under different conditions and the factors that predict reliability of ECD values in normal and glaucomatous eyes.

Methods

Patients and endothelial photography

Ninety-nine normal control eyes and 112 open angle glaucomatous eyes with or without uveitis spanning a wide range of cell densities were recruited from the Doheny Eye Center between May 2013 and May 2014, and were included in the study. None of the eyes had any history of prior intraocular surgery, ocular trauma, keratitis or contact lens wear. Central images of corneal endothelium were captured for each eye with a Konan NSP-9900 specular microscope (Konan Medical USA Inc., Irvine, CA). This study was approved by the Institutional Review Board of University of Southern California (at that time the affiliation of Doheny Eye Institute). Informed consent was obtained from all participants, and the study followed the tenets of the Declaration of Helsinki.

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