ORIGINAL ARTICLE

Comparison of corneal endothelial cell density estimated with 2 noncontact specular microscopes

Yakov Goldich[1], Arie L. Marcovich[2], Yaniv Barkana[1], Morris Hartstein[1], Yair Morad[1], Isaac Avni[1], David Zadok[1]

[1]Department of Ophthalmology, Assaf Harofeh Medical Center, Zerifin - Israel
[2]Department of Ophthalmology, Kaplan Medical Center, Rehovot - Israel

PURPOSE. *To assess the repeatability of endothelial cell density (ECD) measurements by the EM-935 endothelial microscope and their agreement with those of the Konan-Noncon Robo SP 6000 (Noncon Robo) specular microscope.*

METHODS. *We assessed the agreement between automated and semiautomated methods of analysis of the EM-935 and the Noncon Robo by measuring ECD in 40 eyes of 20 healthy subjects and calculating the 95% limits of agreement (LoA) and plotting Bland-Altman graphs. We then evaluated the repeatability of both the methods of the EM-935 by calculating the intraclass correlation coefficients (ICC) and coefficient of variation (CV).*

RESULTS. *The mean ECD of the 40 eyes was 2531±244 cells/mm^2 for Noncon Robo and 2483±159 cells/mm^2 for EM-935-automated. The difference between these 2 methods was not statistically significant. The mean ECD for EM-935-semiautomated was 2635±190 cells/mm^2 and was statistically significantly higher than the 2 other methods (p<0.001). The 95% LoA were –435 to 339 cells/mm^2 for the Noncon Robo and EM-935-automated, –230 to 438 cells/mm^2 for the Noncon Robo and EM-935-semiautomated, and –347 to 43 cells/mm^2 for the EM-935-automated and EM-935-semiautomated. Repeatability was better for EM-935-semiautomated method compared with automated method as expressed by ICC (95% CI) of 0.80 (0.52–0.95) vs 0.50 (0.09–0.84) and coefficient of variation of 2.43% vs 2.85%, respectively.*

CONCLUSIONS. *The EM-935 specular microscope showed better repeatability for the semiautomated mode compared with the automated mode. Although measurement agreement with the Noncon Robo was somewhat better for the semiautomated mode, agreement was only moderate for both methods. This leads us to recommend that these instruments should not be used interchangeably. (Eur J Ophthalmol 2010; 20: 825-30)*

KEY WORDS. *Corneal endothelium, Endothelial cell density, Noncontact specular microscopy*

INTRODUCTION

Specular microscopy provides an objective assessment of the number and quality of corneal endothelial cells. It is commonly used to augment preoperative decision-making and postoperative assessment of procedures such as implantation of phakic intraocular lenses, cataract extraction, and corneal grafting (1-3). It is also used to assess the effect on the endothelium of new ophthalmic pharmaceuticals and surgical procedures (4, 5).

In specular microscopy, images of the corneal endothelium are obtained through the tangential illumination of the corneal surface. The central surfaces of endothelial cells reflect light brightly and the boundaries of these cells appear

as dark lines. Using these images, endothelial cells can be quantitatively and qualitatively assessed, either manually or automatically.

The clinical employment of endothelial cell count has been hampered by publications that have indicated significant differences between instruments and even with repeated measurements using the same instrument. For example, in one multicenter study it was found that the repeatability of specular microscopy could be improved from 20% when one measurement is obtained to 4% if the average of 3 measurements is used (1).

In another multicenter study, 2 measurements of the same eye 12 weeks apart showed a large range of differences with a 95% limit of agreement of ±8.2% (McCarey BE, Lynn MJ, Edelhauser HF. Noncontact specular microscopy: accuracy and repeatability. Invest Ophthalmol Vis Sci 2002;43:ARVO meeting abstract 3177).

While specular microscopes have been stationary, table-mounted devices, the new endothelial microscope EM 935 (Rhine-Tec, Krefeld, Germany) is a portable device that is installed on the standard slit lamp. It provides automated and semiautomated (manually corrected) endothelial analysis. To our knowledge, no study assessing repeatability of this new device has been conducted. The aim of this study was to assess the repeatability of EM 935 measurements in normal corneas using both automated and semiautomated modes, and to compare them with those of Konan Noncon Robo SP 6000 (Konan Medical Inc., Hyogo, Japan).

METHODS

The study was approved by the Institutional Review Board of Assaf Harofeh Medical Center and a written informed consent was obtained from each subject. The study protocol was consistent with the tenets of the Declaration of Helsinki.

Subjects

Healthy subjects were prospectively recruited from the medical personnel of the Assaf Harofeh Medical Center. Subjects with any abnormality of the anterior segment or previous ocular surgery or contact lens wearers were excluded. Written informed consent was obtained from each subject after the nature and intent of the study had been fully explained. All research procedures followed the tenets

of the Declaration of Helsinki. The Institutional Review Board of Assaf Harofeh Medical Center approved this study. In the first experiment, the central endothelial cell density (ECD) was measured and compared with 2 different instruments operated by 2 trained operators (3 different methods): Noncon Robo, EM 935-automated, and EM 935-semiautomated methods. The order of examination with the 2 devices was alternated with each successive subject. Forty eyes of 20 subjects aged 25–59 years (mean 36.0±9.5 years, 11 male and 9 female) were included in this study.

In the second experiment, the repeatability of the EM 935 was determined. Three successive scans were obtained by the same operator in the right eye of each of 9 subjects aged 19–50 years (mean 33.1±11.2 years, 4 male and 5 female).

Image acquisition

Both specular microscopes were positioned in the same dimly illuminated room so that the ambient lighting conditions were the same. No eyedrops were used. Both instruments capture an image of the endothelium relying on the corneal reflex. In the attempt to obtain photographs of the same endothelial area with both microscopes, the person's head was carefully aligned on the chin rest and the person was instructed to look straight ahead. With the Noncon Robo, the endothelial image was instantly displayed on the monitor, and if outlines of endothelial cells were not sharp and in focus, the process was repeated. Adequate images were stored and used for further analysis.

The EM 935 was mounted on a slit lamp (Haag Streit BQ 900, Switzerland). The operator achieved focus on the corneal endothelium manually and the EM 935 automatically captured the image and transferred the data to the attached computer.

Image analysis

With Noncon Robo, after the adequate image was chosen, the center of each cell in a contiguous group of at least 100 cells was manually marked and further analyzed by computer algorithm. We used the fixed frame method, as previously described by Gundersen for marking and counting endothelial cells while minimizing counting bias caused by border effects (6). After the good quality image was accepted, the operator defined a rectangular frame. All cells

lying completely within the cell area were manually marked with central dot. For cells intersected by frame borders, we marked and counted all cells along 2 adjacent sides of the frame and did not mark these lying along 2 other sides. With the EM 935, a central corneal image was captured automatically as soon as the operator obtained a focused view of the cornea. The counting frame, including a minimum of 100 cells, was placed by the operator within the area of well-seen endothelial cells. After choosing the frame, the analysis started automatically, and the resulting ECD was recorded. EM 935 specular microscope provides an automated and semiautomated method of analysis. The automated analysis mode uses an edge detection algorithm to detect cell borders within the chosen frame. With the semiautomated method, drawing and erasing tools enable the examiner to correct manually for what he or she determines to be cells falsely recognized or falsely unrecognized by the device software.

Statistical analysis

The ECD values of the different methods were analyzed by an Excel spreadsheet (Excel version 2003; Microsoft, Redmond, WA) and SPSS for Windows software (version 14.0: SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to check for a normal distribution of quantitative data. Intermethod difference was evaluated using repeated-measures analysis of variance (ANOVA). Significance was determined as $p < 0.05$. To assess interdevice/intermethod agreement and interchangeability, we used the method suggested by Bland and Altman (7). Intermeasurement differences were plotted against their mean, and the 95% limit of agreement (LoA) was determined as mean difference ± 1.96 standard deviation (SD). To assess intraoperator repeatability of the EM 935, we calculated the coefficient of variation that was defined as the SD of the difference from the mean of the repeat measurements divided by the mean response. We also calculated the intraclass correlation coefficients (ICC) and 95% confidence intervals (CI).

RESULTS

The mean ECD measurements with Noncon Robo and with EM 935-automated and semiautomated method are presented in Table I. The mean interdevice differences in ECD for the Noncon Robo vs EM 935-automated, Noncon Robo vs EM 935-semiautomated, and EM 935-automated vs EM 935-semiautomated mode were 48, 104, and 152 cells/mm², respectively. Measurements of ECD with the EM 935 semiautomated method differed statistically significantly from both Noncon Robo and EM 935-automated ($p < 0.001$), whereas those of Noncon Robo and EM 935-automated did not ($p = 0.13$).

Agreement between the instruments and methods are presented as Bland-Altman plots for the measurement of ECD (Figs. 1–3).

For measurement of ECD, 95% LoA were −435 to 339 cells/mm² for the Noncon Robo and EM 935-automated, −230 to 438 cells/mm² for the Noncon Robo and EM 935-semiautomated, and −347 to 43 cells/mm² for the EM 935-automated and EM 935-semiautomated. In each graph, a trend line was plotted and suggested that intermethod difference decreased as the cell count increased. To test the validity of this impression, we calculated the correlation coefficient between the X and Y values of each graph, and indeed obtained weak negative statistically significant correlations for each of the 3 graphs.

The ICC (95% CI) for the 3 measurements of 9 subjects

TABLE I - ENDOTHELIAL CELL DENSITY VALUES WITH NONCON ROBO SP 600, EM 935 AUTOMATED, AND SEMIAUTOMATED METHOD

Method	Endothelial cell density (cells/mm²)		p value
	Mean ± SD	**Range**	
Noncon Robo SP 600	2531±244	1870–2994	
EM 935 automated	2483±159	2111–2883	0.13*
EM 935 semiautomated	2635±190	2095–2996	<0.001†

*Comparing EM 935 automated to Noncon Robo SP 6000.
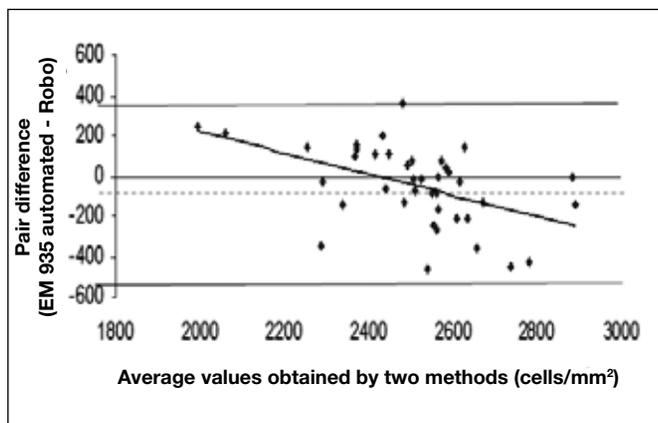†Comparing EM 935 semiautomated to Robo SP 6000 and EM 935 automated.

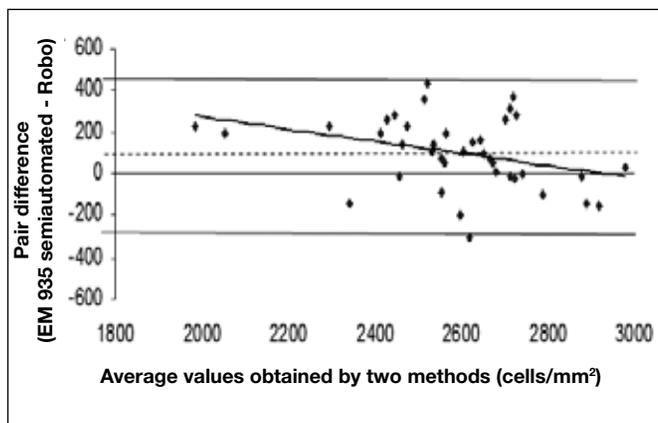Fig. 1 - *Comparison between EM 935-automated and Noncon Robo SP 6000.*

Fig. 2 - *Comparison between EM 935-semiautomated and Noncon Robo SP 6000.*

TABLE II - COEFFICIENTS OF VARIATION OF THE EM 935 AUTOMATED METHOD AND EM 935 SEMIAUTO-MATED METHOD

Patient	EM 935 automated	EM 935 semiautomated
1	0.92	2.47
2	4.41	2.61
3	4.59	0.81
4	3.33	2.38
5	3.29	0.15
6	2.65	3.42
7	1.51	3.11
8	4.03	4.71
9	0.92	2.24
Mean	2.85	2.43

Values are coefficient of variation (%).

Fig. 3 - *Comparison between EM 935-automated and EM 935-semiautomated methods.*

was 0.50 (0.09–0.84) for the EM 935 automated mode of analysis and 0.80 (0.52–0.95) for the EM 935 semiautomated method. The mean coefficient of variation was 2.85% for the EM 935 automated method and 2.43% for the EM 935 semiautomated method (Tab. II).

DISCUSSION

Corneal specular microscopy should provide rapid, objective, and accurate evaluation of endothelial cells. Before acceptance of a new instrument into routine clinical practice, it is important to know whether its measurements are reproducible and in agreement with previously available and familiar instruments. If they are, the new and previous instruments may be used interchangeably and their results
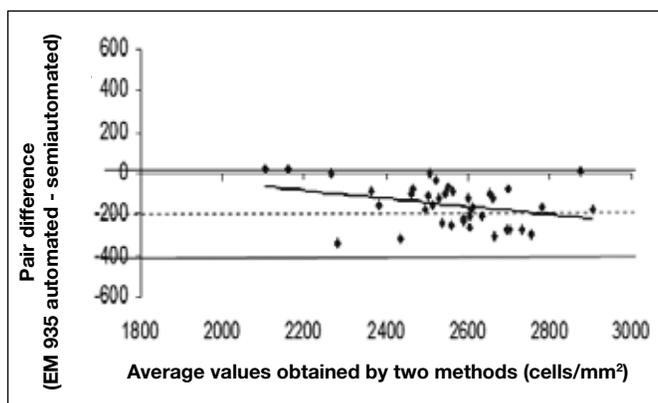
may be compared directly. If measurements are not in agreement, interdevice differences must be taken into account when measurements are obtained during the follow-up of a single patient or compared between study groups.

In the current study, we compared the automated and semiautomated modes of the new EM 935 specular microscope with the well-known Konan Noncon Robo SP 6000 in measuring ECD in healthy subjects. On average, measurements with the EM 935-semiautomated method were significantly higher compared with both EM 935-automated method and Noncon Robo, which were statistically similar. Presumably, in this study, when the human operator corrected the automatic cell-recognition algorithm, he more often added nonrecognized cells than he deleted wrongly recognized cells.

In order to obtain a more clinically meaningful compari-

son, agreement between these methods was evaluated as suggested by Bland and Altman (7). This method helps clinicians to determine for any given use whether the measurements provided by 2 methods are interchangeable. Numerically, the 95% LoA gives an indication of how much the devices may differ in 95% of cases—that is, in most patients. The narrower the 95% LoA, the better the intermethod agreement. This comparison shows that although the Noncon Robo and EM 935 automated mode had the smallest mean difference of 48 cells/mm^2, concluding that the 2 devices provide "similar" measurements would be incorrect. The 95% LoA and Bland-Altman plots (Figs. 1–3) show a relatively large range of interdevice differences for all comparisons, and this may be too broad for some clinical applications, such as evaluation of influence of new procedures on ECD, for example surgery involving phakic intraocular lenses. Interdevice agreement was somewhat better, expressed by the narrower 95% LoA, when the semiautomated method was used.

In each Bland and Altman graph, a trend line was plotted and suggested that intermethod difference decreased as the cell count increased irrespective of whether automated (Fig. 1) or semiautomated mode (Fig. 2) was used. This finding is not new and has been previously reported in fully automated analysis of ECD (8, 9). This implies that the (absolute) percentage error of the ECD from the EM935 decreased with increasing ECD until zero error is reached at an ECD = 2500 cells/mm^2 and then saturates at ECD > 2500 cells/mm^2. There is no obvious explanation for this unexpected effect. This intermethod difference may be attributed to several factors. Software, especially for images with poor quality and lack of sufficient contrast, fails to correctly identify the cell borders, resulting in the recognition of cells of abnormal size (too large or too long) that actually represent poorly separated cells. Semiautomated counting with the analyzer, with the observer selecting the counting zone, choosing the best threshold levels, and retracing the cell contours, overcomes most of the drawbacks observed with the automated and manual techniques.

We measured the repeatability of the EM 935, i.e., the variation in measurements taken by a single operator on the same subject and under the same conditions over a short period of time. Repeatable measurement of ECD is dependent on repeatable identification of cells. Whether done manually by a human operator or automatically by the device software, identification of cell borders is directly related to the image quality. Repeatability for ECD assessment sho-

wed higher ICC value (0.80 vs 0.50) and lower coefficient of variation (2.43% vs 2.85%) with EM 935 semiautomated mode than with EM 935 automated mode. These data are consistent with the study of Landesz et al (10) that reported a 2.2%–3.9% coefficient of variation in ECD repeatability study using noncontact specular microscopes. Additionally, it is in agreement with previous studies that found the semiautomated–manually corrected method was the most accurate of all assessment methods, with the fewest measurement errors (11, 12). We note that our results reflect measurements in healthy corneas. Repeatability may vary in diseased corneas, especially when ECD are low.

Some previous studies (13, 14) reported that the precision of ECD measurements depends on the number of analyzed cells and recommended to include at least 75 cells in each measurement, so expecting accuracy of ECD measurement to be within 2%. Even though it appears more time-consuming, we chose a minimal number of 100 cells for both endothelial microscopes following recommendations of the American Academy of Ophthalmology to choose a sample of at least 75 to 100 endothelial cells (15, 16).

Although operator-dependent focusing of EM 935 seems to result in longer image acquisition than automatic image acquisition of Noncon Robo (not measured in this study), portability of this novel instrument and possibility of installation on a slit lamp makes it a unique alternative to stationary endothelial microscopes.

CONCLUSIONS

The EM 935 specular microscope showed better repeatability for the semiautomated mode compared with the automated mode. Although measurement agreement with the Konan Noncon Robo SP 6000 microscope was somewhat better for the semiautomated mode, agreement was only moderate for both methods. This leads us to recommend that these instruments should not be used interchangeably.

Address for correspondence:
Yakov Goldich, MD
Department of Ophthalmology
Assaf Harofeh Medical Center
Beer Yaakov
Zerifin 70300, Israel
doctor.goldich@gmail.com

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