Quantitative Endothelial Biomicroscopy

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SUMMARY

Twenty patients were evaluated by quantitative endothelial biomicroscopy and the resulting endothelial cell densities compared with those from a specular microscope. Estimated cell densities by this technique, when compared with the specular microscope, demonstrated a Pearson correlation coefficient of +0.977, an average error of −7% and an absolute error of 12%. There were no errors greater than 26% except for one extremely low cell density of 318 cells/mm² for which the estimate was 476 cells/mm².

This rapid, inexpensive technique requires counting the number of endothelial cells seen across the horizontal diameter of the 0.2-mm projected spot beam of a standard biomicroscope. From this count and the known spot size the endothelial cell density may be accurately calculated. A simplified four-step technique for performing specular microscopy using only the standard biomicroscope and a method for accurately measuring the size of the projected slit lamp spot beam are explained.

The importance of the corneal endothelium in maintaining the deturgescence and optical clarity of the cornea has been well documented in recent years. New developments with the specular microscope have resulted in high quality photographs or video displays which allow accurate determination of the cell density.

Clinically, the endothelial cell density has been used preoperatively to identify those patients with low cell densities who are at higher risk for corneal decompensation with anterior segment surgery. Endothelial cell loss is known to occur with cataract extraction, glaucoma procedures, secondary intraocular lens implantation and secondary discussions of the posterior capsule. Measuring endothelial cell densities has also been helpful in determining progressive endothelial cell loss with various styles of intraocular lenses so that they can be removed before corneal decompensation occurs. The major impediment to routine, widespread use of the specular microscope has been its prohibitive cost. We have developed an inexpensive, rapid, and convenient method of estimating the endothelial cell density which uses the standard slit lamp and is accurate within a 7% absolute error of that obtained with a specular microscope.

SUBJECTS AND METHODS

Twenty-one subjects, nine males and 12 females, were selected for inclusion in the study. Age range from 21 to 97 years. Of the eight left eyes studied, none had a history of prior trauma. Nine of the 21 eyes were normal, one was a Fuch’s endothelial dystrophy with pterygium, one had chronic open angle glaucoma, and had a cataract and open angle glaucoma.

Two observers (JTH and JEB) independently counted the number of endothelial cells seen inside the 0.2-mm diameter of the 0.2-mm circle projected from the Haag-Streit slit lamp. The same standard was used to evaluate all patients. Care was taken to position the margin of the projected circle was in clear view of the eyepiece was critically focused monocularly on the endothelial cells.

The exact projected spot size of the beam measured photographically to be 0.24 mm in diameter. This dimension was then divided to find the millimeter conversion factor of the slit lamp. This factor represents the number of mm per 1 mm. The number of endothelial cells counted on the horizontal diameter of the spot was then multiplied by this factor to obtain the number of cells/mm².
to find the number of endothelial cells along 1 mm. This number was then squared to find the cell density in cells/mm².

The amount of cellular pleomorphism and guttata were also graded on a clinical scale from 0 to 4+. In those patients with guttata, the calculated cell density was reduced by 10% times the clinical scale grading (i.e., 1+ = 10% reduction, 2+ = 20% reduction, ..., 4+ = 40% reduction).

Photographs were taken with the Pocklington specular microscope, and the endothelial cells were counted using the standard technique as specified by the manufacturer. Specular photographs of sufficient clarity to permit accurate determination of cell density could not be obtained in one eye. On the remaining 20 eyes a Pearson correlation coefficient was calculated and used to compare the estimated and specular microscopic cell densities.

RESULTS

Table 1 lists the endothelial cell densities for all 20 subjects as estimated by each observer and that obtained with the specular microscope. The absolute and percentage algebraic difference between the mean cell density estimates and that obtained with the specular microscope are listed for each subject. The average algebraic difference between the two methods of assessing cell density was −188 cells/mm². The mean absolute error of the estimates was 12.1%, and the mean algebraic error was −6.7%.

These data are further illustrated in Figure 1. For each
subject the average percentage difference from the specular microscopic cell density is plotted as well as the absolute cell density difference, in parentheses. The 99% confidence interval of the mean and the percent deviation for each observer are also depicted for each of the 20 subjects. In Figure 2 a best fit linear regression line was determined between the mean estimated cell density and the cell density determined with the specular microscope. A Pearson product-moment correlation of $R = +0.977$ was shown to be highly significant ($p < .0005$).

**DISCUSSION**

Several studies have shown the value of determining the endothelial cell density as a predictor of the cornea's ability to withstand anterior segment surgery. There is no established cell density below which the cornea will decompensate with surgery since there are many other factors that contribute to the optical clarity of the cornea. Some of these other factors include the functional capability of each cell, the total number of cells distributed over the posterior cornea, cellular pleomorphism, the intraocular pressure, and the corneal thickness. Although these other factors are important, the endothelial cell density is a major factor and is also one of the easiest to measure.

Using endothelial photographs, early researchers were able to determine the importance of the endothelial cell density in vivo. Newer instruments are still quite expensive and primarily reserved for research purposes. These newer
generations of endothelial cell cameras allow instant feedback by using a video monitor display. Cell densities can be obtained easily and rapidly by having a standardized grid on the monitor screen. These instruments are superb technical devices, but, unfortunately their cost is prohibitive to many ophthalmologists.

Since anterior segment surgery, such as cataract extraction, primary and secondary intraocular lens implantation, and glaucoma procedures is commonly performed by most ophthalmologists, there is a need for a simple, convenient, rapid, and inexpensive technique to determine the endothelial cell density. In an effort to satisfy these requirements for the clinician, two estimation techniques have been developed previously.

The first method, a 25X eyepiece with reticle, allows the observer to view the endothelium and compare it with an adjacent reticle that is graduated in four steps from 500 to 4000 cells/mm². This technique has been reported to have a mean algebraic error of 10% to 21% with a range from 25% to 105%. The cost is very reasonable, and the only disadvantage is having to remove the special 25X eyepiece with reticle from the slit lamp for routine biomicroscopy.

The second technique has been called “mosaic matching.” This method requires the observer to memorize the pattern of endothelial cells within the small spot on the slit beam and then match this pattern with that shown on a printed comparative card (Personal communication, John R. Karickhoff, M.D., May, 1982). No published data is available on the accuracy of this subjective technique. It also may be difficult for most observers to remember the image of the endothelial cells as seen through the

FIGURE 2: (Holladay, Bishop and Prager). The correlation of endothelial cell densities estimated by quantitative endothelial biomicroscopy and photographic specular microscopy is shown. A linear regression line is also plotted.
biomicroscope until they look at the comparative card.

Because of the disadvantages mentioned above with each of the estimation techniques and the prohibitive cost of the endothelial cell cameras, we decided to develop a technique using only the standard biomicroscope. This technique requires counting the number of endothelial cells along the horizontal diameter of the 0.2-mm spot beam as seen on the posterior cornea during specular microscopy. Since the diameter of the spot is known, the number of cells counted along the horizontal diameter can be multiplied by the number of spots necessary to fill one millimeter. This would then give the number of endothelial cells along one millimeter. If this number is then squared, the density of endothelial cells per square millimeter is thus determined. For the standard Haag-Streit slit lamp in which the spot beam is nominally 0.2 mm, it would require multiplying the horizontal cell count by 5 (the millimeter conversion factor) and then squaring this number. For example, if 10 cells were counted along the horizontal diameter of the spot, there would be 50 cells in one millimeter or 2500 cells/mm².

The data in Figures 1 and 2 show the average accuracy of this technique to be approximately 12%. For spot sizes greater than 0.25 mm, such as found on the Zeiss slit lamp (0.30 mm), estimates are more difficult due to the greater number of cells which must be counted.

In teaching this technique to our residents and other ophthalmologists, it has become apparent that the most difficult part of the entire procedure is the technique of specular microscopy. We have, therefore, described in detail four simple steps to easily visualize the corneal endothelial mosaic.

**Step 1 (Figure 3):** In preparation, the slit lamp should be placed so that the angle between the slit beam and the oculars is between 60° and 80°. The slit lamp should be adjusted to the 0.2-mm spot beam and set at the highest available magnification (25X with the Haag-Streit or 40X with the Zeiss).

The patient is now positioned at the slit lamp for normal viewing of the cornea. When the slit beam is directed from the observer's left, three images will be seen on the cornea as shown in Figure 3. The lower two corneal reflexes will be the anterior and the posterior spot beam circles on the epithelial and endothelial cell surfaces. The bright upper corneal reflex will be the mirror image of the slit lamp filament, which will be slightly above and to the left of the previous two images. If the filament image cannot be located easily, enlarge the size of the slit beam momentarily, and the image will become brighter. Once the mirror image of the filament is located, return to the 0.2-mm spot size. It is very important to note that the only point at which the specular microscopic reflex can be seen is where the mirror reflex of the filament appears on the cornea.

To see the specular microscopic reflex, it is necessary to take the posterior endothelial circle and move it upward and to the left so that it is superimposed on the mirror image of the filament. When this occurs, a brilliant light reflex will be projected back into one of the observer's eyes, confirming that the proper endpoint has been achieved. By carefully keeping the epithelial reflex out of this mirror image, some of the glare from the unwanted epithelial specular reflex can be avoided.

**Step 2 (Figure 4):** Once the endothelial spot is superimposed on the mirror image of the filament, as shown in Figure 4, it is then necessary to assure that the margin of the projected spot is in clear focus on the corneal