Volumetric Analysis of Macular Edema by Scanning Laser Tomography in Immune Recovery Uveitis

Dirk-Uwe G. Bartsch, PhD; Ajay Aurora, MD; Nuttawut Rodanant, MD; Lingyun Cheng, MD; William R. Freeman, MD

Objectives: To evaluate the macular volume in eyes with immune recovery uveitis (IRU) and to describe a new method to quantify macular edema with the use of confocal scanning laser tomography (cSLT).

Methods: A prospective study was performed to assess the macular volume with cSLT in patients with and without IRU. None of the patients enrolled had cytomegalovirus retinitis within 3000 µm of the fovea. Eight eyes had healed cytomegalovirus retinitis with IRU and cystoid macular edema (group A); 4 eyes had healed cytomegalovirus retinitis with IRU and clinically normal maculas (group B); 18 eyes had no IRU (group C); and 3 eyes underwent pars plana vitrectomy and epiretinal membrane peeling for epiretinal membranes associated with cystoid macular edema and IRU (group D). Patients with IRU underwent standard clinical examinations and cSLT. On cSLT, volume above the reference plane was calculated within a fovea-centered circle of 3 mm in diameter. We devised a novel system for defining the reference plane. Measurements were performed 3 times in masked fashion and the mean was used for analyses.

Results: The mean macular volume was highest in group A (1.97 mm³). This was significantly higher (P<.001) than that in groups B (1.15 mm³), C (1.02 mm³), and D (0.86 mm³).

Conclusions: Macular edema in patients with IRU can be consistently and objectively quantitated by cSLT. The method of defining the reference plane used in our study is novel and can be used in other disorders causing macular edema.

Arch Ophthalmol. 2003;121:1246-1251

Combination antiretroviral therapy leads to decreased plasma levels of human immunodeficiency virus RNA and increased CD4 T-lymphocyte counts, with impaired immune function in patients with human immunodeficiency virus infection. The ocular inflammation associated with immune recovery in patients taking potent antiretroviral combination therapy is known as immune recovery vitritis or immune recovery uveitis (IRU). Our group first described this entity in 5 patients with symptomatic vitritis, papillitis, and cystoid macular edema (CME) or epiretinal membrane formation. Presence of macular edema is traditionally assessed clinically by slitlamp biomicroscopy (with contact or noncontact lens), stereoscopic fundus photography, and fluorescein angiography. Fluorescein angiography has been the gold standard in diagnosing and monitoring macular edema. None of these techniques, however, can objectively quantify macular edema. This makes grading between different observers difficult, and subtle changes cannot be assessed. Nussenblatt et al used 4 standard photographs to estimate the degree of center thickening, but their technique did not provide objective measures.

Confocal scanning laser tomography (cSLT) was first described in 1987 and found its clinical use in the analysis of optic nerve head topography. We later adapted it for the assessment of the macula. In brief, this ophthalmoscope uses a red laser diode (670 nm) to illuminate the eye with a maximal corneal irradiance of 0.160 mW, which is well below the limits established by the American National Standards Institute and other international standards. Two galvanometric mirrors provide the horizontal and vertical scanning. The optics of the instrument allow spherical aberration compensation between −12 and +12 diopters. The system uses a 3-mm illumination beam diameter, and the full aperture of eye is used to collect light from the posterior pole. The instrument allows scanning with a field of view.
10°, 15°, or 20°. A confocal pinhole located in front of the photodetector ensures that each image represents a thin optical section with a full-width and half-maximum thickness of approximately 300 µm. During an axial scan series, 32 consecutive, equidistant, overlapping optical slices are captured over a total depth of between 0.5 and 4 mm. The software calculates the location of the retinal surface with high reproducibility of better than 25 µm. Furthermore, it has been shown that the cSLT used in this study is telecentric and thus allows determination of true retinal sizes after correcting for refractive power and axial length. These findings have been confirmed in a study where optic nerve measurements with a cSLT were compared with direct measurements obtained during vitreoretinal surgery.

The Heidelberg Retinal Tomograph (HRT; Heidelberg Engineering, Carlsbad, Calif) has shown good reproducibility for point height and mean depth measurement at the macula and recently has been used to describe retinal reflectivity as a function of scan depth (z-profile) and hence to derive a topographic macular edema map. Zambarakji et al measured macular volume above an arbitrary reference plane drawn at the lowest point of the contour line with the use of the HRT in normal eyes and in eyes of diabetic patients with early macular edema. To be able to measure the macular volume above a reference plane, the reference plane needs to be stable and consistently identifiable.

Simplistically, one would consider that the retinal pigment epithelium (RPE) would form a good candidate for such a reference plane. The RPE, however, is not visible on cSLT. The “outer band” of the optical coherence tomography (OCT) image is believed to represent the RPE. The exact correlation of the colored bands and anatomic equivalents is still being studied.

However, OCT can yield high-resolution information on apparent optical thickness of retina at a specific point rather than the topographic edema distribution in the macula, which would be clinically desirable. Also, it may be difficult to measure the retinal thickness at a particular point in the retina with OCT consistently on follow-up examination over the same point.

Neubauer et al conducted a study of 21 normal eyes with 2 different instruments: the OCT and the retinal thickness analyzer (RTA). They measured a mean foveal thickness of 153 µm (OCT) and 181 µm (RTA). The coefficient of variation was 10% and 9% for the OCT and the RTA, respectively.

The aim of this study is to describe a new method of quantification of macular edema in patients with IRU with the use of cSLT. To our knowledge, this technique has not been applied to this disease previously.

**METHODS**

**STUDY SUBJECTS**

Twelve eyes of 11 patients with healed cytomegalovirus (CMV) retinitis with IRU that did not have CMV retinitis within 3000 µm of the fovea formed the study group. These patients had been referred to the University of California, San Diego, AIDS Ophthalmic Research Unit in La Jolla, Calif. All patients underwent complete vision assessment with Early Treatment Diabetic Retinopathy Study charts, anterior segment evaluation with slitlamp biomicroscopy, and fundus evaluation after pupillary dilation with slitlamp biomicroscopy with the use of 78-diopter noncontact lens and indirect ophthalmoscopy. All patients also underwent fundus photography and fluorescein angiography in which stereo images recorded in the early phase. Midphase images and late-phase images (around 10 minutes postinjection) were also recorded. Fluorescein angiograms of each patient were read and graded by 2 of us (A.A. and W.R.F.). This group of patients with CMV retinitis with IRU was subdivided into 2 groups: group A, 8 eyes with healed CMV retinitis with IRU and CME; and group B, 4 eyes with healed CMV retinitis with IRU and clinically and angiographically normal macula.

**CONTROL SUBJECTS**

Eighteen age-matched subjects formed the control group (group C). None of them had IRU. All had corrected visual acuity of 20/20 or better. This group consisted of 15 eyes of patients who were human immunodeficiency virus positive but had no CMV retinitis and 3 eyes of patients who were negative for both human immunodeficiency virus and CMV retinitis. All patients underwent detailed anterior segment and fundus evaluation similar to the study group.

**SURGICALLY TREATED GROUP**

This group (group D) comprised 3 eyes that had undergone pars plana vitrectomy with removal of epiretinal membrane during the past 12 months. These subjects had undergone surgery for epiretinal membranes associated with macular edema and IRU. This group represents an intermediate group of subjects.

**HRT IMAGE ACQUISITION TECHNIQUE**

The HRT scans were done on the HRT with the use of software version 2.01. All HRT examinations were done with a dilated pupil (1% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride eyedrops). Dilation was not necessary for HRT examination; however, the patients were examined during the course of their complete routine examination. The patients were comfortably positioned with the chin placed in the chin rest and asked to fixate on a target fixed on the wall (1 m away) with their other eye. We opted for a scan size of 20° × 20° and the scan depth was adjusted between 0.50 and 4.00 mm. The standard software of the HRT performs a check of illumination, axial scan range, and defocus compensation. If the examination variables do not adhere to the appropriate settings, the user is prompted to adjust the values accordingly. In addition, the stack of 32 images is aligned by the software with the use of vascular landmarks to cancel out eye movements. A scan depth of 1.5 to 2.0 mm was routinely used. For patient convenience, patients were scanned after 5 to 10 minutes on the same day, and this was considered to simulate a repeat examination. For each eye on a particular day, 3 fovea-centered scans were finally stored. Of these, the best-quality scan (ie, a scan that is clear, well illuminated, and centered on the fovea and does not show eye movements in the image series) was selected for analysis.

On the best scan, a circle of 0.25-mm radius was drawn by means of the circle draw feature of the standard software in the peripapillary retina within the arcade. It was ensured that the circle was drawn in a well and uniformly illuminated area that did not overlap any obvious retinal vessels and was well away from the clinically edematous area of retina. Fluorescein angiography was performed in all patients, and the fluores-
tical comparison between the 4 groups (From the 3 readings of the volume above the reference plane, a
the 3000-µm circle.
ness, we were thus able to calculate the retinal volume within
all 3 readings. On the basis of our assumption of retinal thick-
After each measurement, the circle was erased and a new circle
with the study reference plane. This was performed 3 times.
The circle draw feature and the macular volume was calculated
in the RPE plane. A fovea-centered circle was drawn with
ence plane. We assume that this reference plane is thus lo-
calculated reference plane reading to arrive at our study refer-
surface was 160 µm. Thus, we added 110 µm to the HRT-
sumed that the retinal thickness from the RPE to the retinal
was performed 3 times for each patient image. The av-
centration for detection with the instrument, the central fo-
tended into the imaging area were excluded, as this would
We ensured that all patients whose CMV retinitis ex-
biomicroscopy and stereofundus photography.20 In cases
of IRU with angiographic macular edema, clinical ex-
amination missed the diagnosis of macular edema in more
than half of the cases.21 In the present study, all eyes with
angiographic macular edema showed swollen maculas
when they were analyzed by our technique of macular
volume measurement with cSLT. The mean macular vol-
In these eyes was 1.97 mm³. Our study documents
that this did occur in some cases. This, however,
We ensured that all patients whose CMV retinitis ex-
extended into the imaging area were excluded, as this would
falsey reduce macular thickness from retinal atrophy sec-
ary to CMV retinitis.
There are potential artifacts in evaluating macular edema by means of scanning laser tomography. In cases
where the foveal tissue is extremely thinned because of
CME, if the reflectivity of the tissue is below the thresh-
old set for detection with the instrument, the central fo-
veal thickness may be underestimated.9,22 Indeed, we
found that this did occur in some cases. This, however,
would tend to lead to an underestimation of macular
volume in the cases with the most severe cystoid macular edema and would not affect the results of our study. In-
deed, one could interpolate the inner retinal surface po-
sition from the perifoveal position; however, we chose
not to manipulate the raw data. Such adjustments would
only increase the statistical significance of our findings.
Another potential problem in using this technology is de-
termining the so-called baseline level of the retina. This

<table>
<thead>
<tr>
<th>Characteristics of Patient Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>

*Group A consisted of eyes with healed cytomegalovirus retinitis, immune recovery uveitis, and cystoid macular edema; group B, eyes with healed cytomegalovirus retinitis and immune recovery uveitis without angiographic cystoid macular edema; group C, control eyes; and group D, surgically treated eyes.
†P<.001. The macular volumes between groups B and C (P = .22) as well as between groups D and C (P = .28) were not statistically significant.

There were 8 eyes in group A (healed CMV retinitis with IRU and angiographic CME), 4 eyes in group B (healed CMV retinitis, IRU without CME), 18 eyes in group C (control eyes without IRU and CME), and 3 eyes in group D (eyes with healed CMV retinitis, IRU, and CME that had undergone pars plana vitrectomy with peeling of the epiretinal membrane in the past year). As outlined in the ‘Methods’ section, each macular volume measurement was performed 3 times for each patient image. The average reproducibility for the repeat measurement was 0.008 mm³, ranging from 0.001 mm³ to 0.04 mm³.

The mean macular volume in eyes with healed CMV retinitis, IRU, and CME (group A) was significantly greater than that in the control eyes (group C), the eyes with CMV retinitis and IRU, but without angiographic CME (group B), and the surgically treated eyes (group D) (all P<.001) (Table). The macular volumes in eyes in groups B and C
were not statistically significantly different (P = .22); similarly, the macular volumes in groups D and C were not statistically significantly different (P = .28). Images from representative patients in groups A and B are shown in Figure 1 and Figure 2, respectively.

COMMENT

Although patients with CMV retinitis with immune recovery associated with combination antiretroviral therapy achieve effective control of CMV retinitis, they develop complications like cataract, macular edema, and epireti-
nal membrane because of their reconstituted immune sys-
tem. Macular edema is an important cause of vision loss and can be sensitively detected by fluorescein angiogra-
phy. The aim of the present study was to establish a reli-
able method of quantifying macular edema by using optical
scanning laser technology with the HRT.

Small changes in retinal thickness are difficult to es-
timate with the traditional methods, including slitlamp
biomicroscopy and stereofundus photography.20 In cases
of IRU with angiographic macular edema, clinical ex-
amination missed the diagnosis of macular edema in more
than half of the cases.21 In the present study, all eyes with
angiographic macular edema showed swollen maculas
when they were analyzed by our technique of macular
volume measurement with cSLT. The mean macular vol-
ume in these eyes was 1.97 mm³. Our study documents
that the macular volume in eyes with CME secondary to
IRU is higher than in eyes without IRU, control eyes, or
eyes with surgically treated IRU. The reliability of 3 in-
dependent macular volume readings is apparent from the
observed miniscule variation of SD for individual sets
of 3 readings. This means that, once the reference plane
has been set, the volumes above that reference plane
obtained by drawing fovea-centered circles are consist-
tently reproducible. In conducting this study, we ob-
served that defining the reference plane is of critical
importance. An important factor that contributes to this
is the quality of image and its illumination. The image
should be neither too bright (ie, in photographic termi-
ology, overexposed) nor too dark (ie, underexposed).
We ensured that all patients whose CMV retinitis ex-
tended into the imaging area were excluded, as this would
falsely reduce macular thickness from retinal atrophy sec-
ondary to CMV retinitis.

There are potential artifacts in evaluating macular edema by means of scanning laser tomography. In cases
where the foveal tissue is extremely thinned because of
CME, if the reflectivity of the tissue is below the thresh-
old set for detection with the instrument, the central fo-
veal thickness may be underestimated.9,22 Indeed, we
found that this did occur in some cases. This, however,
would tend to lead to an underestimation of macular
volume in the cases with the most severe cystoid macular edema and would not affect the results of our study. In-
deed, one could interpolate the inner retinal surface po-
sition from the perifoveal position; however, we chose
not to manipulate the raw data. Such adjustments would
only increase the statistical significance of our findings.
Another potential problem in using this technology is de-
termining the so-called baseline level of the retina. This
problem is inherent to any topographic measurement that uses reflecting optical instrumentation. Some researchers have chosen a method similar to ours, while others have chosen to ignore this consideration. Jaakkola and associates determined the reference area by selecting a region of the retina that was considered to remain flat during the follow-up. However, they did not correct for the difference in retinal thickness between the default value used in glaucoma analysis and our retinal thickness value. We chose to correct for this potential problem. To do this, we chose an area near the major vascular arcades that was clearly not involved by thickening or edema on fluorescein angiography. This was used as a positional baseline in the z-dimension (depth dimension).

The use of OCT has given good images of edematous macula. However, OCT as currently used performs only limited 3-dimensional mapping reconstruction. In addition, the OCT software does not always locate the anterior and posterior retina interface. This may lead to erroneous volumetric measurements. Furthermore, the instrument takes only 6 radial sections through the fovea and, from this, interpolates the entire macular area. The number of points of data in the retinal plane with OCT technology is 600 vs 65,356 with HRT in the retinal plane. For this reason, we chose to use the cSLT technology for this preliminary investigation. Future studies with enhanced OCT hardware and software may allow higher-resolution depth images of eyes with immune recovery macular edema to be obtained and analyzed. We also believe that simultaneous use of OCT and cSLT on the same patient needs to be considered. Two groups of researchers have been developing prototype instruments that allow an OCT image to be captured while a scanning laser ophthalmic image is recorded. However, neither instrument currently supports cSLT imaging. If such a device were available, OCT could help find the exact retinal thickness in an area of normal retina, define the RPE, and give a point thickness of the macula. These data may then be used for analysis with the cSLT.

Zeimer et al showed promising results with a new method for mapping retinal thickness at the posterior pole with the use of the RTA. The technique allows the analysis of the central 20° of the macula by way of 9 scans,
the macular region requires capturing 5 overlapping fields to cover a $20^\circ \times 20^\circ$ area in approximately 3 to 4 minutes (http://www.talia.com). The RTA records about 200 points per scan line and 16 scan lines in each 3 by 3-mm region, for a total of 3200 points. The HRT records 65 536 pixels per image plane in about the same area ($10^\circ \times 10^\circ$ field of view). The HRT takes 1.6 seconds for a scan, which consists of 32 independent scans obtained axially along the optical axis and aligned electronically by means of the vascular landmarks.

Light levels used in the scanning laser ophthalmoscope are safe and comfortable to the patient and are much lower (70 µW/cm²) than those used during indirect ophthalmoscopy (100 000 µW/cm²) or fluorescein angiography (4 000 000 µW/cm²). Hence, the cSLT, with its high light efficiency and the ability to reconstruct a 3-dimensional image from the 32 sections it records along the optical axis, is an ideal system for detecting and quantifying macular edema.

In the present study, we have not tried to correlate the visual status with the macular volume. This would require careful observation of macular volume over time, and we are collecting such data at present.

In summary, this study demonstrates a novel technique of quantifying macular edema in eyes with CMV retinitis with IRU and CME by means of the HRT. We believe that this technique will be applied to other conditions with macular edema and may help to monitor macular edema and the results of treatment in these eyes.

Submitted for publication October 23, 2001; final revision received April 11, 2003; accepted May 14, 2003.

This study was supported in part by grant EY13304 from the National Eye Institute, National Institutes of Health, Bethesda, Md (Dr Bartsch), grant EY07366 from the National Institutes of Health (Dr Freeman), and Research to Prevent Blindness Inc, New York, NY (to University of California, San Diego).

Corresponding author: Dirk-Uwe G. Bartsch, PhD, University of California, San Diego, Shiley Eye Center, 9415 Campus Point Dr, La Jolla, CA 92039-0946 (e-mail: dbartsch@ucsd.edu).

REFERENCES

8. Bartsch DU, Intaglietta M, Bille JF, Dreher AW, Gharib M, Freeman WR. Confo
cal laser tomographic analysis of the retina in eyes with macular hole formation
9. Bartsch DU, Freeman WR. Axial intensity distribution analysis of the human
retina with a confocal scanning laser tomograph. Exp Eye Res. 1994;58:161-
173.
11. Weinreb RN, Lusky M, Bartsch DU, Morsman D. Effect of repetitive imaging on
topographic measurements of the optic nerve head. Arch Ophthalmol. 1993;111:
636-638.
12. Rudnicka AR, Burkh RO, Edgar DF, Fitzke FW. Magnification characteristics of fun-
13. Bartz-Schmidt KU, Weber J, Heimann K. Validity of two-dimensional data ob-
tained with the Heidelberg Retina Tomograph as verified by direct measure-
profile signal width as an objective index of macular retinal thinning. Br J Oph-
thalmol. 1998;82:121-130.
15. Zambarakji HJ, Evans JE, Amoaku WM, Vernon SA. Reproducibility of volumet-
16. Zambarakji HJ, Vernon SA, Spencer AF, Amoaku WM. Reproducibility of vol-
metric macular measurements in diabetic patients with the Heidelberg Retina To-
17. Zambarakji HJ, Amoaku WM, Vernon SA. Volumetric analysis of early macular
edema with the Heidelberg Retina Tomograph in diabetic retinopathy. Ophthal-
18. Chauhan DS, Marshall J. The interpretation of optical coherence tomography im-
19. Neubauer AS, Priglinger S, Ullrich S, et al. Comparison of foveal thickness mea-
sured with the retinal thickness analyzer and optical coherence tomography. Retina.
109:1115-1119.
21. Robinson MR, Reed G, Csaky KG, Polis MA, Whitcup SM. Immune-recovery uve-
itis in patients with cytomegalovirus retinitis taking highly active antiretroviral
22. Bartsch DU, Freeman WR. Laser-tissue interaction and artifacts in confocal scan-
ing laser ophthalmoscopy and tomography. Neurosci Biobehav Rev. 1995;17:
459-467.
in the evaluation of retinal elevation in age-related macular degeneration. Ophthal-
24. Hudson C, Charles SJ, Flanagan JG, Brahma AK, Turner GS, McLeod D. Objec-
tive morphological assessment of macular hole surgery by scanning laser to-
25. Kooszkanani O, Boyer K, Roberts D. Retinal thickness measurements from op-
tical coherence tomography using a Markov boundary model. IEEE Trans Med
26. Podoleanu AG, Rogers JA, Jackson DA, Dunne S. Three dimensional OCT im-
27. Hitzengerer OK, Zhou Q, Trost P, Schmode S, Baumgartner A. High-speed three-
dimensional optical coherence tomography for retinal imaging [ARVO ab-
of the retinal thickness at the posterior pole. Invest Ophthalmol Vis Sci. 1996;
thickness in diabetic retinopathy with the scanning retinal thickness analyzer.