Effect of pupil dilation on retinal nerve fibre layer thickness measurements and their repeatability with Cirrus HD-OCT

Abstract

Purpose To assess whether pupil dilation influences retinal nerve fibre layer (RNFL) thickness measurements provided by spectral-domain optical coherence tomography (SD-OCT) in healthy individuals.

Patients and Methods In this observational case series, carried out in a private clinical practice, 32 eyes of 32 participants were investigated. Using Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA) three individual 200 × 200 cube optic disc scans were obtained before and after pupil dilation. The RNFL thickness was the outcome measure. Coefficient of variation (COV) and test–retest variability were calculated.

Results Pupil size did not influence RNFL thickness measurements: mean values did not change in any sector (except the 9 o’clock hour) after dilation. Excellent repeatability was achieved both before and after mydriasis. In the former condition, COV ranged between 1.37% (for average RNFL) and 4.46% (for clock hour 2 RNFL) and test–retest variability between 2.17 (for temporal quadrant RNFL) and 9.18 μm (for clock hour 6 RNFL). In the latter condition, COV ranged between 1.36% (for average RNFL) and 4.48% (for clock hour 2 RNFL) and test–retest variability between 2.41 (for average RNFL) and 9.29 μm (for clock hour 6 RNFL). The repeatability was higher than that previously reported for time-domain OCT.

Conclusion In eyes with clear media highly repeatable measurements of the RNFL thickness can be obtained by SD-OCT both before and after mydriasis.

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shown to correlate positively with ex vivo histological measurements of the retina. Spectral-domain OCT (SD-OCT) has now replaced TD-OCT and a large number of instruments by different manufacturers are commercially available. This technology provides increased axial resolution and scanning speed as it records the interferometric information using a spectrometric method instead of adjusting the position of a reference mirror. Hence, it does not need to measure reflectivity changes between retinal layers in each A-scan one at a time. Instead, the spectrometer detects the relative amplitudes of many optical frequencies simultaneously within the backscattered light. Many points are thus sampled at the same time, with all of the retinal layer depths of each A-scan calculated using Fourier transformation. Resolution in Fourier transformation is up to five times higher and imaging speed is up to 50 times faster than in conventional TD-OCT. Showing the accuracy of RNFL thickness measurements is an important first step towards validating latest-generation SD-OCTs for the diagnosis of glaucoma and glaucoma progression, as well as any other disease of the optic nerve.

Recently, several authors showed that the Cirrus HD-OCT has very good intra-observer repeatability in both healthy and glaucomatous eyes. Similar results were obtained by González-García et al. with another instrument (RTVue; Optovue Inc., Fremont, CA, USA).

Materials and methods

Between February and March 2009, 35 eyes of 35 subjects were enrolled in a private practice for this prospective observational case series. The study population consisted of consecutive patients with minor refractive disorders. All individuals underwent a complete ophthalmological examination, including visual acuity measurement, intraocular pressure measurement, slit-lamp biomicroscopy, and indirect ophthalmoscopy, to determine eligibility. Inclusion criteria were: best corrected visual acuity above 20/25, refractive error between −3 and +3 dioptres of sphere or between −2 and +2 dioptres of cylinder, normal intraocular pressure <21 mm Hg, normal appearance of the optic disc, no significant ocular disease found by routine ophthalmological examination, no history of glaucoma in the family, and/or systemic diseases with possible ocular involvement, such as diabetes mellitus.

Before pupil dilation, three individual 200 × 200 cube optic disc scans were obtained with Cirrus OCT (software version 3.0) as previously described by Knight et al. Once the subject was seated and properly aligned, the iris was brought into view. The scanning laser ophthalmoscopic image was focused by adjusting for refractive error. As the optic nerve head was centred on the live scanning laser ophthalmoscopic image using the internal fixation cross, centring (Z-offset) and enhancement (polarization) were optimized. A 6 × 6 mm square of data was acquired. If the subject had an involuntary saccade during the scan, this was discarded and retaken (Figure 1). Ultimately, the signal strength had to be 6 or higher. Each optic disc scan captured a 6 × 6 × 2 mm ‘cube’ of data consisting of 200 A-scans from 200 linear B-scans (40 000 points) in 1.5 s (27 000 A-scans per second).

Because the data collected by Cirrus are a ‘cube,’ rather than a single circle as in TD-OCT, a comparable circle (radius 1.73 mm) must be extracted from the data. In the Glaucoma Analysis, Cirrus HD-OCT algorithms find the optic disc, even if it is not well centred in the scan image.
and automatically place a calculation circle of 3.46 mm diameter evenly around it. Layer-seeking algorithms find the RNFL inner (anterior) boundary and RNFL outer (posterior) boundary for the entire cube, except the optic disc. The system extracts from the data cube 256 A-scan samples along the path of the calculation circle. The resulting temporal, superior, nasal, inferior, temporal profile map is equivalent to the Stratus peripapillary RNFL scan.

The same procedure was carried out after pupil dilation induced by 1% tropicamide.

All participants gave their informed consent. The study was conducted according to the Declaration of Helsinki Principles and was approved by the internal review board of the Department of Neurological Sciences at the University of Bologna.

Statistics

For the purposes of this study, the term repeatability was used according to the definition of the British Standards Institute, which considers it as a part of accuracy.\textsuperscript{30} Accuracy, as formally defined by BS ISO 5725-1, includes trueness and precision. Trueness is the inverse of bias and is obtained by comparing the measurement result with the accepted reference (conventional true) value. Precision is the inverse of statistical uncertainty, and is normally expressed in terms of standard deviation (SD). The factors involved include (1) the operator, (2) the equipment used, (3) the equipment calibration, (4) the environment, and (5) the elapsed time between measurements. Precision has two conditions: repeatability and reproducibility. Under repeatability conditions, factors such as 1–5 are considered to be constant and do not contribute to the variability of the measurement result. Under reproducibility conditions, the factors can vary. Repeatability and reproducibility are the two extremes of precision.

We assessed repeatability by the following methods:

- Repeated-measures analysis of variance (ANOVA): this was automatically calculated using InStat software 3.0 (GraphPad, San Diego, CA, USA, 2003) for each of the RNFL parameters (average, four quadrants and 12 sectors) both before and after pupil dilation.
- Coefficient of variation (COV): this was calculated as the within-subject SD of the three repeated measurements divided by the within-subject mean, and was expressed as a percentage.\textsuperscript{8,31}
- Intra-session test–retest variability in RNFL thickness: this was measured in micrometres and was calculated as twice the SD of the three repeated measurements for each measure of RNFL thickness.\textsuperscript{8}

Differences between mean measurements before and after pupil dilation were assessed by a paired t-test. We postulated that a mean difference of 3.5 µm in the average RNFL thickness measurement could have been regarded as clinically significant, as this value corresponds to the intra-session test–retest variability reported by Budenz et al\textsuperscript{25} for Stratus OCT (data for Cirrus HD-OCT have not yet been published). Using PS version 3.0.12 (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize, checked on 4 February 2010), we estimated a sample size of 29 eyes to detect such a difference with an SD of ±4.4 µm (corresponding to the SD of the difference between Stratus OCT average RNFL thickness measurements before and after mydriasis),\textsuperscript{26} and a power of 95% at a significant level of 5%. A smaller sample would have been required to detect differences higher than 3.5 µm.

In all cases, differences were considered statistically significant when P-value was <0.05.

Results

Two patients were excluded, as the pupil diameter was too small to allow visualization of the optic nerve before pharmacological dilation. Another subject was excluded because of motion artefacts. Hence the statistical analysis was carried out on a final sample of 32 eyes (10 men and 22 women, mean age 59 ± 19 years) out of 35.

Repeated-measures ANOVA did not show statistically significant differences among the three series of measurements either before or after mydriasis, in any of the quadrants and sectors, including the 360° average measurement (P > 0.05 in all the cases). For each measurement, the mean of the three values did not show statistical differences before and after dilation, the only exception being the nasal sector at 9 o'clock, where the mean value was higher after mydriasis (Table 1).

On the basis of the results shown in Table 1, therefore, pupil dilation did not significantly affect RNFL thickness measurements by Cirrus HD-OCT. Nor did it affect the mean signal strength (7.77 ± 1.09 before and 8.00 ± 1.18 after pupil dilation, P > 0.05).

As far as repeatability is concerned, excellent results were obtained for the COV and test–retest variability (Table 2).

Discussion

The present investigation shows that pupil dilation does not affect the RNFL thickness measurements provided by Cirrus HD-OCT: signal strength, mean values, and repeatability did not change before and mydriasis. Moreover, only in a small minority of subjects (2 out of 35, 5.7%) could the scan not be acquired because of an insufficient pupil diameter. These findings reveal noteworthy differences compared with the Stratus OCT.
Table 1 Mean values of three measurements of the RNFL thickness before and after pupil dilation

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Mean thickness (± SD) before pupil dilation (µm)</th>
<th>Mean thickness (± SD) after pupil dilation (µm)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>88.53 ± 11.10</td>
<td>88.87 ± 10.81</td>
<td>NS</td>
</tr>
<tr>
<td>Temporal</td>
<td>59.19 ± 7.8</td>
<td>59.54 ± 7.8</td>
<td>NS</td>
</tr>
<tr>
<td>Superior</td>
<td>114.42 ± 17.13</td>
<td>111.68 ± 16.65</td>
<td>NS</td>
</tr>
<tr>
<td>Nasal</td>
<td>67.53 ± 11.20</td>
<td>68.10 ± 10.65</td>
<td>NS</td>
</tr>
<tr>
<td>Superior</td>
<td>111.42 ± 17.13</td>
<td>111.68 ± 16.65</td>
<td>NS</td>
</tr>
<tr>
<td>Nasal</td>
<td>67.53 ± 11.20</td>
<td>68.10 ± 10.65</td>
<td>NS</td>
</tr>
<tr>
<td>8 o’clock</td>
<td>115.97 ± 18.82</td>
<td>115.73 ± 19.35</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2 COV and test–retest variability of intra-session RNFL thickness measurements with Cirrus HD-OCT compared to intra-session values obtained by Budenz et al. with TD-OCT and a standard 3.4 mm scan

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>COV</th>
<th>Test–retest variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mydriasis No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>1.37</td>
<td>2.41</td>
</tr>
<tr>
<td>Temporal</td>
<td>1.84</td>
<td>2.55</td>
</tr>
<tr>
<td>Superior</td>
<td>2.47</td>
<td>5.17</td>
</tr>
<tr>
<td>Nasal</td>
<td>2.97</td>
<td>3.60</td>
</tr>
<tr>
<td>Inferior</td>
<td>2.27</td>
<td>3.96</td>
</tr>
<tr>
<td>8 o’clock</td>
<td>3.16</td>
<td>3.49</td>
</tr>
<tr>
<td>9 o’clock</td>
<td>2.49</td>
<td>2.73</td>
</tr>
<tr>
<td>10 o’clock</td>
<td>2.07</td>
<td>3.92</td>
</tr>
<tr>
<td>11 o’clock</td>
<td>2.85</td>
<td>8.14</td>
</tr>
<tr>
<td>12 o’clock</td>
<td>3.69</td>
<td>8.28</td>
</tr>
<tr>
<td>1 o’clock</td>
<td>3.54</td>
<td>7.30</td>
</tr>
<tr>
<td>2 o’clock</td>
<td>4.46</td>
<td>7.32</td>
</tr>
<tr>
<td>3 o’clock</td>
<td>3.65</td>
<td>4.15</td>
</tr>
<tr>
<td>4 o’clock</td>
<td>4.10</td>
<td>4.60</td>
</tr>
<tr>
<td>5 o’clock</td>
<td>4.33</td>
<td>7.85</td>
</tr>
<tr>
<td>6 o’clock</td>
<td>3.70</td>
<td>9.29</td>
</tr>
<tr>
<td>7 o’clock</td>
<td>3.00</td>
<td>7.40</td>
</tr>
</tbody>
</table>

In a study involving 38 glaucoma patients with a mean age similar to that in our own sample, Smith et al. reported that in almost a quarter of cases (23.7%) it was not possible to acquire high-quality OCT images without pupil dilation and that dilated scans were more reproducible and gave different RNFL thickness mean values. A significant effect of pupil dilation was found also by Paunescu et al. In a previous study on a group of patients undergoing cataract surgery, our group reported that pupil dilation slightly affected RNFL thickness measurements by Stratus OCT, as RNFL thickness values were statistically higher in the 360° average measurement and in the nasal quadrant and some differences were found in the other quadrants as well, although statistical difference was not reached.

Just one study found that pupil dilation did not induce any statistical difference in RNFL thickness measurements, but the sample of this investigation was probably too small (10 eyes) to detect such a difference. Overall, therefore, we can conclude that SD-OCT is less influenced than TD-OCT by pupil size and this represents a further step forward for this technology.

Our study results also confirm the excellent repeatability of Cirrus HD-OCT RNFL thickness measurements and suggest that the measurements provided by Cirrus HD-OCT and RTVue have similar repeatability.

Intrinsically, we also confirmed that RNFL thickness measurements are slightly less repeatable in the nasal quadrant. This finding has already been reported with both TD-OCT and SD-OCT, and, as previously suggested, may be related to the fact that the angle of incidence of the illuminating beam is such that the RNFL is dimmer nasally, so that the edges of the RNFL are less reliably identified.
This study did not address two important issues: repeatability in glaucomatous eyes and both inter-observer and inter-visit reproducibility. Although the former evaluation has been recently carried out (with excellent results), the latter will be the subject of further investigations.

In conclusion, our study data confirm that Cirrus HD-OCT measurements of the RNFL thickness show excellent repeatability, on condition that no artefacts are produced during the scan acquisition, and are not influenced by pupil size.

Summary

What was known before

- Spectral-domain OCT provides faster scanning of the retina and optic nerve than time-domain OCT.

What this study adds

- Spectral-domain OCT measurements of the retinal nerve fibre layer thickness provide also the highest repeatability, with and without pupil dilation.

Conflict of interest

The authors declare no conflict of interest.

References


