EXTENDED REPORT

Effect of pupillary dilatation on glaucoma assessments using optical coherence tomography

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Aims: To examine the effect of pupillary dilatation on the reliability of retinal nerve fibre layer (RNFL) and optic nerve head (ONH) assessments using Stratus OCT in a glaucoma clinic.
Methods: Observational study of 38 patients attending a glaucoma clinic. The ''fast optic disc'' and ''fast RNFL thickness'' programs on Stratus OCT were used to measure the RNFL thickness and ONH cup to disc ratio (CDR). Two scans were done before dilatation and two after dilatation with tropicamide 1% drops. The mean values and reproducibility before and after dilatation were compared, along with the quality of scans as indicated by the ''signal strength'' score.
Results: In nine patients (23.7%) no images were obtained undilated but after dilatation examination was possible in all patients. Inability to obtain an undilated scan was associated with smaller pupil size and increasing cataract. The scan quality, as judged by the signal strength score, was higher dilated than undilated for both RNFL thickness ($p=0.011$) and ONH CDR ($p=0.007$). Reproducibility was higher with dilated scans for RNFL thickness but not for ONH CDR. There were significant differences between the dilated and undilated examinations for three of the five RNFL thickness variables and two of the three ONH CDR categories.
Conclusions: Acquisition of high quality OCT images was not possible without pupillary dilatation in about 25% of the patients. The dilated scans were more reproducible and of higher quality than the undilated scans. The two methods of examination do not appear to be interchangeable, suggesting that in follow up examinations the pupil should be in the same condition as at baseline. Pupillary dilatation is recommended before glaucoma assessments using Stratus OCT.

Optical coherence tomography (OCT) is a non-invasive method of imaging the optic nerve head and retina. It has been shown to provide a reproducible measure of the optic nerve head (ONH) and retinal nerve fibre layer (RNFL), and studies have suggested it has a role to play in both the diagnosis and monitoring of glaucoma.[1–8] In addition it compares well with other optical imaging techniques designed for the same purpose.[9]

Glaucoma assessment using the OCT is largely automated and from the patients' perspective only requires them to keep their eye still for a few seconds. It is unclear, however, whether pupillary dilatation is necessary for an adequate examination. This is important as pupillary dilatation is time consuming and results in visual blurring for several hours afterwards. On a practical level this means that patients are unable to drive to and from their appointments, which many find very inconvenient. The aim of this study was to determine whether the reliability of OCT is influenced by pupillary dilatation.

METHODS

After approval from the South Devon research ethics committee, 45 consecutive patients attending the glaucoma clinic were invited to take part in this prospective study. The inclusion criterion was a confirmed or suspected diagnosis of glaucoma or ocular hypertension. There were no exclusion criteria. Thirty eight patients agreed to take part and underwent an assessment of the optic disc and peripapillary retinal nerve fibre layer using Stratus OCT (Model 3000, software version 3.0, Carl Zeiss Meditech, Dublin, California, USA). ONH and RNFL analyses were both done using the ''fast optic disc'' and ''fast RNFL thickness'' programs. Only right eyes were included in the study. Two test–retest examinations with each program were carried out undilated (examinations 1 and 2) and then a further two test–retest examinations were done after pupillary dilatation with tropicamide 1% drops (examinations 3 and 4). Between scans the patient was asked to sit back from the machine for at least one minute and the machine was realigned. In addition to OCT analysis, horizontal pupil diameters were measured before dilatation. This was done using a clear rule by a single observer in standardised dim light conditions (the same conditions as used for the scans). The lighting conditions were kept constant during all examinations. After pupillary dilatation the degree of lens opacification was also recorded using the Lens Opacities Classification System (LOCS) III.[10] All examinations were done by a single examiner (MS).

The Stratus OCT produces a wealth of data. We examined the following:

- Retinal nerve fibre layer (RNFL) thickness, average
- RNFL thickness, superior quadrant
- RNFL thickness, inferior quadrant
- RNFL thickness, nasal quadrant
- RNFL thickness, temporal quadrant
- Optic nerve head (ONH), cup/disc area ratio
- ONH, cup/disc horizontal ratio
- ONH, cup/disc vertical ratio

For each variable the reproducibility dilated and undilated was analysed, and the dilated and undilated data were compared.

Abbreviations: CDR, cup to disc ratio; LOCS, lens opacities classification system; OCT, optical coherence tomography; ONH, optic nerve head; RNFL, retinal nerve fibre layer

The Stratus OCT software gives each scan a quality indicator score known as ''signal strength'' from 1 to 10. This score is based on a combination of signal to noise ratio and the uniformity of the signal strength within a scan, but for commercial reasons the manufacturer does not provide further details.[11] The higher the signal strength the higher the quality of scan, and for low scores the results of the analysis may be unreliable. We recorded the signal strength measurements for all the scans performed.

Statistical analysis

Using the SPSS package, univariate and multivariate regression analyses were employed to analyse the effect of sex, age, cataract, and undilated pupil size on the ability to carry out undilated examinations. Probability (p) values were calculated using *t* tests; $p<0.05$ was considered statistically significant.

To analyse the reproducibility of the OCT examinations with pupils undilated we calculated the differences in results between the undilated test–retest examinations (examination 2 minus examination 1). To analyse the reproducibility of the OCT examinations with pupils dilated we calculated the differences in results between the dilated test–retest examinations (examination 4 minus examination 3). For each comparison the distribution of differences between ''test'' and ''retest'' was analysed by calculating the maximum, minimum, mean, and standard deviation. In addition we calculated the 95% tolerance limits for change (95% TLC). This figure was calculated as 1.96 times the standard deviation and provides a quantity by which individual paired measurements must differ in order to show evidence of a true change. If the difference between serial tests is less than the 95% TLC then the difference is more likely to reflect measurement ''noise'' than a genuine change in clinical condition. We calculated the 95% TLC as a decimal figure and also as a percentage of the absolute scale of measurement and as a percentage of the working scale. The absolute scale of measurement is based on the difference between the maximum and minimum RNFL thickness measurements that we identified from published reports, and on a theoretical scale of 1.0 for ONH cup to disc ratio (CDR). For the working scale, both RNFL thickness and ONH CDR values were based on the maximum and minimum figures measured in all patients in this study. In expressing the 95% TLC as a proportion of the overall scale of measurements we have attempted to allow a more meaningful comparison between different measurement variables.

RESULTS

Of the 45 consecutive patients invited to take part in the study, 38 agreed to participate. There were 20 women and 18 men and their average age was 64.3 years (range 39 to 88). Three patients were pseudophakic, with the remaining 35 phakic. Four patients had established glaucoma, while 12 were being monitored for ocular hypertension or suspected glaucoma. The remaining 22 patients were attending the eye clinic for the first time after referral with suspected glaucoma. No patients were using pilocarpine drops.

In nine patients (23.7%) no images were obtained undilated but examination was possible in all patients after dilatation. The group in whom undilated scans were not possible had higher scores on the LOCS, and this reached significant levels ($p<0.05$) for nuclear opalescence and cortical and posterior subcapsular opacity (table 1).

In addition, a smaller undilated pupil was associated with inability to carry out the test undilated. There was no association with sex or age.

The patient group where undilated examination was not possible was excluded from the analyses below. For the 29 patients in whom examination was possible with both dilated and undilated pupils the RNFL thickness and ONH CDR measurements are summarised in table 2. Tables 3 and 4 show the reproducibility of the measurements, both dilated and undilated.

The related values of standard deviation of the differences between ''test'' and ''retest'', ''95% TLC as % of scale'', and the ''95% TLC as % of working range of scale'' were lower dilated than undilated for all of the RNFL thickness measurements except temporal. This suggests that the reproducibility for these measurements was better when the pupils were dilated. For ONH analysis the standard deviation was similar dilated and undilated and there appeared to be no difference in reproducibility.

Table 5 compares the measurements made before and after dilatation. In the dilated examinations the RNFL was apparently thicker than in the undilated examinations, whereas the ONH CDR was higher undilated than dilated. These differences reached statistical significance for three of the five RNFL thickness categories and for two of the three ONH categories.

The quality of scans carried out before and after dilatation is summarised in table 6. The ''signal strength'' scores for the two scans undilated and the two scans dilated for all 29 patients are included in the analysis. For RNFL thickness the mean score undilated was 5.24 (range 1 to 10, median 5) whereas the mean dilated score was 6.09 (range 3 to 10, median 6). For ONH CDR examination the mean undilated score was 5.82 (range 2 to 10, median 6) but this improved to 6.86 (range 3 to 10, median 7) on pupillary dilatation. Therefore for both RNFL thickness and ONH analysis the signal strength score was significantly higher for dilated scans, suggesting that pupillary dilatation produced better quality scans.

Table 1 Comparison of patients in whom scans were possible and not possible with the pupils undilated

Variable	Undilated scans possible (n = 29)	Undilated scans not possible (n = 9)	p Value
Sex	44.8% male	55.6% male	>0.1
Age	62 (39 to 80)	72 (44 to 88)	>0.1
Pupil*	4.0 (2.5 to 7.0)	3.0 (2.5 to 5.0)	0.024
NO	1.5 (0.5 to 3.5)	2.5 (1.0 to 3.5)	0.030
NC	2.0 (0.5 to 3.5)	2.65 (0.5 to 3.1)	0.08
C	1.0 (0 to 2.5)	2.0 (0 to 2.5)	0.024
P	0.5 (0 to 1.5)	1.0 (0 to 2.0)	0.037

For pupil size, NO, NC, C and P, the median (range) is given.
*Pupil size undilated (mm).
C, cortical grade; NC, nuclear colour grade; NO, nuclear opalescence grade; P, posterior subcapsular grade.

DISCUSSION

Most studies looking at the reproducibility of OCT in glaucoma have been undertaken following pupillary dilatation, and the manufacturer recommends dilated examination. Zafar *et al* examined the need for pupillary dilatation and concluded that it is not necessary for adequate RNFL measurements.[12] Their study, however, involved only 10 volunteers and all were young (mean age 32 years) with no eye disease. Another study found that the reproducibility of RNFL and ONH measurements was better when the pupils were dilated.[2] Again this study involved only 10 healthy volunteers. The volunteers in those studies were not representative of the glaucoma population, which tends to be older and often has coexistent eye disease such as cataract. Although the patients in a study by Savini *et al*—examining the influence of cataract and pupil size on RNFL thickness measurements by Stratus OCT—had cataracts and an average

Table 2 Summary of results: each of the 29 patients had two examinations with pupils dilated and two with pupils undilated

	n	Minimum of all readings	Maximum of all readings	Mean of all readings	SD
RNFL thickness					
Average thickness	29	40.63	120.54	87.6	16.0
Superior quadrant	29	29	152	102.3	23.3
Inferior quadrant	29	53	160	113.3	24.5
Nasal quadrant	29	13	129	67.9	22.0
Temporal quadrant	29	37	104	66.6	13.4
Cup to disc ratio					
Area	29	0.135	0.959	0.512	0.213
Horizontal	29	0.341	0.994	0.726	0.170
Vertical	29	0.393	0.966	0.670	0.144

RNFL, retinal nerve fibre layer.

age of 73 years, patients with glaucoma were excluded, as were patients with pupil diameters less than 3 mm.[13]

The aim of the present study was to examine whether the reliability of OCT is influenced by pupillary dilatation. This study was conducted in an unselected glaucoma clinic population. We believe this is more representative of the population in which OCT is being used in clinical practice.

In this study we were unable to obtain images in almost a quarter of the patients with undilated pupils. These patients had smaller pupils and a higher incidence of lens opacity than the patients in whom undilated scans were possible. A previous study looking at image quality during confocal scanning laser ophthalmoscopy came to similar conclusions.[14] In addition, the study by Savini cited above excluded 12 patients (32%), three in whom the pupil diameter was less than 3 mm and nine with cataract of a degree which "precluded adequate RNFL imaging".[13] This suggests that in clinical practice a significant proportion of patients may require pupillary dilatation for glaucoma assessment using OCT.

In comparing the precision of the measurements made before and after pupillary dilatation (tables 3 and 4), we calculated the 95% TLC as both a percentage of the absolute range of scale and as a percentage of the range of values in this study. This aids comparison between different variables such as RNFL thickness and ONH CDR. For both dilated and undilated examinations in this study, the 95% TLC as a percentage of both the absolute and working range of scale is generally higher for RNFL thickness measurements than for ONH CDR measurements. However, as there is also significant overlap between the ONH and the RNFL thickness 95% TLC values these data provide no firm conclusions on the relative merits of each as a marker for significant change.

The other advantage of using 95% TLC values is that it allows comparison between different methods of examination. Although to our knowledge there are no previous reports of this form of data in relation to OCT glaucoma assessments, we compared our results with previous publications which examined other variables measured in glaucoma. The precision of OCT appears to compare favourably with other methods of optic disc examination, such as a stereophotography and binocular indirect ophthalmoscopy.[15 16]

In this study, four of the five RNFL thickness parameters examined showed poorer reproducibility when the pupil was undilated. This suggests that RNFL thickness measurements are more reliable when the pupils are dilated. For ONH CDR measurements we found no significant difference. Pupillary dilatation may be less important for the precision of ONH CDR measurements. This may be explained by the differences in technique used to undertake these examinations. The RNFL analysis involves circular scans in the peripapillary area, whereas the ONH analysis is based on line scans across the ONH. Therefore a smaller "window" is necessary for ONH analysis and this may explain why it appears to be less dependent on pupillary dilatation.

Although pupillary dilatation appears to have little effect on the precision of ONH CDR measurements (measured by examining the differences between test–retest data), the study results suggest that there is a systematic difference (bias)

Table 3 Reproducibility of retinal nerve fibre layer thickness and optic nerve head measurements undertaken within individuals before pupillary dilatation (examination 2 minus examination 1)

	n	Maximum difference	Minimum difference	Mean difference	SD*	Scale range†	Working range of scale	95% TLC as % of scale	95% TLC as % of working range of scale
RNFL thickness									
Average thickness	29	10.97	−21.54	−2.08	6.48	136	79.91	9.34	15.89
Superior quadrant	29	22	−48	0.28	13.33	134	123	19.21	21.24
Inferior quadrant	29	14	−41	−4.76	13.07	147	107	18.84	23.94
Nasal quadrant	29	34	−41	−2.38	14.38	120	116	20.72	24.30
Temporal quadrant	29	9	−16	−1.59	5.71	130	67	8.23	16.70
Cup to disc ratio									
Area	29	0.09	−0.12	0.00	0.05	1	0.82	10.00	12.19
Horizontal	29	0.14	−0.13	0.01	0.05	1	0.65	10.58	16.28
Vertical	29	0.08	−0.14	−0.01	0.05	1	0.57	10.19	17.88

The average thickness of the total peripapillary area was examined, as were the quadrant subdivisions. For ONH analysis the total area and the horizontal and vertical cup to disc ratios were analysed.
*Standard deviation of the differences between "test" and "retest".
†From published reports.
ONH, optic nerve head; RNFL, retinal nerve fibre layer; TLC, tolerance limits for change.

Table 4 Reproducibility of retinal nerve fibre layer thickness and optic nerve head measurements undertaken in individuals after pupillary dilatation (examination 4 minus examination 3)

	n	Maximum difference	Minimum difference	Mean difference	SD*	Scale range†	Working range of scale	95% TLC as % of scale	95% TLC as % of working range of scale
RNFL thickness									
Average thickness	29	16	−10.25	0.98	5.38	136	79.91	7.75	13.20
Superior quadrant	29	42	−17	3.83	11.95	134	123	17.48	19.04
Inferior quadrant	29	14	−18	−1.59	7.76	147	107	10.35	14.21
Nasal quadrant	29	25	−16	2.21	9.72	120	116	15.88	16.42
Temporal quadrant	29	15	−14	−0.34	6.31	130	67	9.51	18.46
Cup to disc ratio									
Area	29	0.12	−0.12	0.00	0.05	1	0.82	10.39	12.67
Horizontal	29	0.08	−0.13	−0.01	0.05	1	0.65	9.02	13.87
Vertical	29	0.12	−0.08	0.01	0.05	1	0.57	10.19	17.88

The average thickness of the total peripapillary area was examined, as were the quadrant subdivisions. For ONH analysis the total area and the horizontal and vertical cup to disc ratios were analysed.
*Standard deviation of the differences between ''test'' and ''retest''.
†From published reports.
ONH, optic nerve head; RNFL, retinal nerve fibre layer; TLC, tolerance limits for change.

between dilated and undilated measurements. The undilated examinations tended to measure the RNFL as thinner and the ONH CDR as higher compared with the dilated examinations (table 5). The reasons for this are unclear but the differences reached statistical significance for three of the five RNFL thickness parameters and two of the three ONH categories. This suggests that the two methods of examination are not interchangeable when using serial OCT examinations to monitor glaucomatous progression, and follow up examinations should be done with the pupil in the same condition as at the baseline examination.

Finally, we looked at the quality of the scans by analysing the signal strength score on the RNFL thickness printout. This serves as a quality indicator for the scan, producing a score from 0 to 10, 10 being the strongest signal strength. For signal strength scores less than 5 the manufacturers warn that the result may be unreliable. We found that for both the RNFL and ONH CDR the dilated examinations had a higher mean signal strength score. This suggests that pupillary dilatation produces better quality scans.

Our findings appear to be in conflict with previous studies examining the issue of pupil size in glaucoma assessments using OCT. However, as previously discussed the studies by Panescu and Zafar are not directly comparable to this study as they involved healthy volunteers while our study was conducted in an unselected glaucoma population.[2 12] The study by Savini *et al* involved patients with an average age of 73 years undergoing cataract surgery[13] but glaucoma patients were excluded. In addition, although the investigators concluded that cataract but not pupil size had an effect on RNFL thickness measurements, their statement must be viewed with caution as they excluded patients with undilated pupil diameters smaller than 3 mm and those with cataract of a degree which ''precluded adequate RNFL imaging.'' These exclusions for small pupils and cataracts comprised 32% of the original sample and question the relevance of the study for the general glaucoma clinic population. We believe these exclusions explain the differing findings in our study.

There are some limitations to our study. In our analysis we made multiple statistical comparisons in a relatively small number of patients, increasing the likelihood that some of the differences may have arisen by chance. In addition, it would have been interesting to compare pseudophakic and phakic patients, but this was not possible owing to the small number of pseudophakic patients in the study.

Conclusions

Our study compared glaucoma assessments using Stratus OCT in a glaucoma clinic sample before and after pupillary dilatation. In almost a quarter of the patients glaucoma assessments were not possible without pupillary dilatation. The dilated scans were more reproducible and of higher quality than the undilated scans. In addition, the two methods of examination do not appear to be interchangeable, suggesting that follow up examinations should be undertaken with the pupil in the same condition as at baseline. In view of these

Table 5 Comparison of mean retinal nerve fibre layer thickness and optic nerve head measurements before and after pupillary dilatation

	Mean of examinations 1+2 (undilated) (a)	Mean of examinations 3+4 (dilated) (b)	Difference (a−b) (SD)	p Value for difference
RNFL thickness				
Average thickness	85.3	89.8	−4.49 (11.05)	0.037
Superior quadrant	100.3	104.3	−3.98 (18.1)	0.25
Inferior quadrant	110.6	116.0	−5.38 (13.2)	0.037
Nasal quadrant	64.2	71.7	−7.54 (16.6)	0.021
Temporal quadrant	66.2	67.1	−0.90 (9.3)	0.61
Cup to disc ratio				
Area	0.527	0.496	0.032 (0.078)	0.037
Horizontal	0.739	0.713	0.026 (0.058)	0.023
Vertical	0.676	0.663	0.013 (0.050)	0.19

RNFL, retinal nerve fibre layer.

Table 6 Comparison of scan quality with dilated and undilated pupils

	n	Mean score of undilated scans	Mean score of dilated scans	p Value
RNFL thickness	29	5.24	6.09	0.011
ONH analysis	29	5.82	6.86	0.007

Each of the 29 patients had two examinations with pupils dilated and two with pupils undilated.
ONH, optic nerve head; RNFL, retinal nerve fibre layer.

findings we recommend pupillary dilatation before glaucoma assessments using Stratus OCT.

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