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ORIGINAL ARTICLE

Comparing Optic Nerve Head Analysis Between Confocal Scanning Laser Ophthalmoscopy and Spectral Domain Optical Coherence Tomography

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ABSTRACT

Purpose/aim of the study: Confocal scanning laser ophthalmoscopy, HRT3, and spectral domain optical coherence tomography (OCT), RTVue-100, are able to give 3-dimensional (3D) topography images of optic nerve head (ONH) and to derive stereometric parameters and sectorial analysis. The purpose of the study is to evaluate the agreement of these two devices and their diagnostic accuracy to discriminate eyes with glaucoma from those without.

Materials and methods: Glaucoma patients and healthy control subjects were included. All of them underwent a complete ophthalmological examination, including slit lamp evaluation and visual field (VF) test. After pupil dilatation, HRT3 and RTVue-100 were performed. The following stereometric parameters were recorded: disc area, rim area, rim volume, cup volume, cup area, cup/disk ratio, and the following sectors, superotemporal, superonasal, inferotemporal, inferonasal.

Results: Forty-six eyes of 46 glaucoma patients and 58 eyes of 58 healthy subjects were included in the study. In both groups, HRT3 rim area and rim volume were statistically higher than RTVue-100 (glaucomas: 0.95 ± 0.38 versus 0.44 ± 0.33 and 0.19 ± 0.13 versus 0.02 ± 0.03, $p < 0.01$. controls: 1.41 ± 0.30 versus 1.08 ± 0.37 and 0.37 ± 0.13 versus 0.14 ± 0.11, $p < 0.01$), while cup area was statistically higher by RTVue-100 (glaucomas: 1.42 ± 0.57 versus 1.14 ± 0.58, $p < 0.01$. controls: 1.05 ± 1.35 versus 0.65 ± 0.48). Bland and Altman plots confirmed the presence of a fixed bias. The parameters with largest AUROC were rim volume, rim area and cup/disk ratio for both instruments. HRT3 inferotemporal sector had the highest sensitivity (80.43%, at 75.9% specificity), while for RTVue-100, the superotemporal sector had the highest sensitivity (76.1%, at 81% specificity). The agreement was moderate for inferotemporal sector and fair for the others.

Conclusions: HRT3 and RTVue-100 are not interchangeable for ONH analysis. They both have good diagnostic accuracy, but RTVue-100 shows slightly better performance, at least with regard to rim area volume.

Keywords: Glaucoma, HRT3, imaging, optic nerve head, Rtvue-100

INTRODUCTION

Glaucoma is a chronic disease characterized by progressive loss of retinal ganglion cells that leads to morphological changes in the optic nerve head (ONH) that can be appreciated by ophthalmoscopy.[1] Nevertheless ophthalmoscopy is a subjective qualitative method of evaluation of the ONH and, despite it has been reported that when performed by glaucoma experts, it represents still the most accurate way of detecting morphological ONH glaucomatous changes its accuracy suffers by a wide inter-operator variability and subjectivity and doesn't allow quantitative measures and accurate follow-up.[2–5]

Heidelberg Retina Tomograph (HRT; Heidelberg Engineering GmbH, Heidelberg, Germany) is a confocal scanning laser ophthalmoscope that creates 3-dimensional (3D) topography images and then

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derives several structural measurement parameters representing the ONH globally and in predefined segments. The last HRT version (HRT3) incorporates a larger normative database and thus allows an analysis that is more specific to ethnic background, age, and disc size.[6]

Optical coherence tomography (OCT) is a non-invasive technology that has been extensively used to evaluate many retinal and optic nerve diseases. It obtains high-resolution cross-sectional images of the retina and ONH. Spectral domain OCT (SD-OCT) has higher axial resolution and scanning speed than time-domain OCT (TD-OCT) and can provide a significant reduction in motion artifacts and an increased signal to noise ratio.[7,8] The RTVue-100 (Optovue Inc, Fremont, CA) is one of the currently available ultra high-speed, high-resolution OCT based on SD-OCT technology. Because of its high frame transfer rate and fast Fourier transform algorithm, RTVue-100 can perform 26,000 A scans per second with a depth resolution of 5 μm: the depth scan can be immediately calculated by a Fourier-transform from the acquired spectra, without movement of the reference arm, improving imaging speed. There is limited information in the literature on the agreement of ONH parameters generated by the CSLO HRT3 and the SD-OCT RTVue-100 and on the comparison of their accuracy in detecting ONH glaucomatous changes. The aim of this study was to compare the ONH analysis performed by the CSLO HRT3 and by the SD-OCT RTVue-100, to assess their agreement to evaluate their interchangeability and their diagnostic accuracy to discriminate eyes with glaucoma from those without.

MATERIALS AND METHODS

This prospective evaluation of clinical diagnostic tests was conducted at the IRCCS-Fondazione G. B. Bietti, Rome, Italy, in accordance with the Declaration of Helsinki and the ethics committee of the institution that approved the protocol. Written informed consent was obtained from all enrolled subjects.

Two groups of people matched by age were involved, glaucoma patients and healthy subjects as the control group. Glaucoma was defined as the presence of a repeatable visual field (VF) defect, commensurate with optic nerve damage. A glaucomatous VF change was defined as the consistent presence of a cluster of three or more non-edge points on the pattern deviation plot with a probability of occurring in <5% of the normal population with one of these points having the probability of occurring in <1% of the normal population, a pattern standard deviation with $p<5\%$, or a glaucoma hemifield test result outside normal limits. VF defects had to be reliable (false positive <15%; fixation losses and false-negative responses <25%) and confirmed in at least two tests no more recent than 1 month.

Healthy subjects had to have intraocular pressure (IOP) of less than 22 mmHg, normal-appearing optic disc, and normal VF test result.

Participants in both groups were excluded if they had spherical refractive error greater than ±6 diopters, astigmatism greater ±3 diopters, any active or past retinal pathologies (including diabetic retinopathy or age-related macular degeneration), opacities of optic media that could bias functional and structural testing, history of ocular surgery (except for uncomplicated cataract or glaucoma surgery).

All eligible subjects underwent a complete ophthalmological examination, including slit lamp evaluation, gonioscopy, Humphrey field analyzer Swedish interactive threshold algorithm standard 24-2 test (Carl Zeiss Meditec, Dublin, CA), IOP measurement using Goldmann applanation tonometry and indirect dilated ophthalmoscopy with a 90 diopters lens. After pupil dilatation, HRT3 and RTVue-100 were performed in random order within the same session.

Scanning Laser Ophthalmoscope

HRT3 (software version 3.0; Heidelberg, Germany) uses a diode laser (λ 670 nm) to scan the retinal surface at multiple consecutive parallel focal planes. The instrument and its image acquisition are described in detail elsewhere. The corneal curvature radius was entered in the HRT software for all patients and cylindrical lenses were adapted for those who had astigmatism greater than or equal to 1.0 D. Only high-quality images with acquisition sensitivity of more than 90% and a standard deviation of less than 40 were considered acceptable and used for the study. The optic disc margin, defined as the inner edge of Elschnig's scleral ring, was manually drawn by a single trained operator (M. M.). The HRT3-derived stereometric ONH parameters are automatically calculated using a standard reference plane automatically set at 50 μm posterior to the mean retinal height between 350 and 356° along the contour line.

The following stereometric parameters were recorded for the study purposes: disc area, rim area, rim volume, cup volume, cup area, and cup/disk ratio.

From the same scan, six sectors were obtained and considered: temporal quadrant (with angle extend from −45° to 45°), superotemporal octant (45° to 90°), inferotemporal octant (−90° to −45°), nasal quadrant (135° to 225°), superonasal octant (90° to 135°), and inferonasal octant (−135° to −90°).[9]

Moorfields Regression Analysis classification outcomes were considered for each ONH sector: considering the rim area of the relative segment compared with values predicted for a healthy subject with the same disc size and age, each sector is classified as abnormal if the observed rim area is smaller than 99.9% prediction limits (derived by linear regression of log rim area and disc area) and as borderline if it is smaller than the 95% prediction limits.

For the analysis of this study, borderline results were considered as abnormal results.

Spectral-Domain OCT

The RTVue-100 software version 5.1.0.90 (Carl Zeiss Meditec Systems GmbH, Pirmasens, Germany) uses a scanning laser diode to emit a scan beam with a wavelength of 840 ± 10 nm to provide images of ocular microstructures. The 3D disc and nerve head map 4-mm diameter (NHM4) RTVue protocols were used to obtain optic disc imaging and parameters. The 3D protocol consists of a 4×4-mm raster scan fixed on the optic disc and is composed of 101 B scans each containing 512 A scans. This results in a 3D image of the optic disc and surrounding area. This version of the software automatically draws the contour line of the disc margin by estimating the retinal pigment epithelium edges, and then generates optic disc parameters in the NHM4 protocol. The NHM4 protocol consists of 12 radial scans 3.4 mm in length (452 A scans each) and six concentric ring scans ranging from 2.5 to 4.0 mm (587–775 A scans each), all centered around the optic disc contour line automated by the 3D protocol. The areas between the A scans are interpolated and various parameters are generated to describe the optic disc. The RTVue-100 puts the cup plane at 150 μm above the defined disc plane.[10]

The integrated signal strength index (SSI) was used to control for image quality. The SSI represents the ratio between the measuring beam and the reference beam. SSI measurements of 50 and above were accepted.

As for HRT3, stereometric parameters recorded were disc area, rim area, rim volume, cup area, cup volume and cup/disk ratio.

From the same scan, eight sectors (octants) were obtained, 45° each and only sectors corresponding to HRT3 sectors were considered in the analysis for comparison purposes (superotemporal, superonasal, inferotemporal, and inferonasal).

RTVue-100 normative classification, similarly to HRT3, considers measurements with $p > 5\%$ within normal limits, $p < 5\%$ but >1% borderline, and $p < 1\%$ outside normal limits.

We considered borderline measurements as outside normal limits.

Statistical Analysis

Descriptive analysis was expressed as mean ± standard deviation (SD).

Comparisons of continuous variables between groups were performed by paired *t*-test or Wilcoxon sign rank test as appropriate after normality distribution of the data was checked by the Shapiro–Wilk test and *p* values <5% were considered statistically significant.

The mean difference of corresponding global ONH parameters obtained by the two imaging devices were plotted against their means to evaluate their agreement (Bland–Altman plots).[11] The mean difference is the estimated bias, and the SD of the differences measures the random fluctuations around this mean. If the mean value of the difference differs significantly from 0 on the basis of a 1-sample *t*-test, this indicates the presence of fixed bias. We also calculated 95% limits of agreement for each comparison (mean difference $\pm 1.96 \times$ SD).

ONH sectorial analysis was considered to determine the sector with highest sensitivity considering normative classifications. The Kappa statistic was used to quantify and evaluate the agreement between sectorial analyses.

To describe the diagnostic accuracy of each ONH global parameters (disc area, rim area, rim volume, cup volume, cup area, and cup/disk ratio) receiver operating characteristic (ROC) curves were constructed, by plotting sensitivity against 1-specificity. The area under each ROC curve (AUROC) was then calculated for each parameter and compared using the method described by DeLong et al.[12]

RESULTS

Forty-six eyes of 46 glaucoma patients and 58 eyes of 58 healthy subjects matched by age (61 ± 12.9 years versus 58.5 ± 11.3 years, p=0.28) were included in the study.

As expected, patients had worse VF defect compared with healthy subjects (MD -7 ± 5.9 dB versus -0.8 ± 0.8 dB, $p < 0.01$ and PSD 6.9 ± 4.6 dB versus 1.4 ± 0.2 dB, $p < 0.01$).

Mean ONH results from RTVue-100 and HRT3 are presented in Table 1. All parameters except cup volume among glaucoma patients and disc area between both groups were statistically different between the two instruments. In both groups, HRT3 rim measurements (rim area and rim volume) were statistically higher than RTVue-100 rim measurements, while the cup area was statistically higher by RTVue-100.

Except for cup volume among glaucoma patients and disc area among healthy subjects, Bland and Altman plots showed the presence of fixed bias as indicated by the significant deviation from zero of the mean difference between RTVue-100 and HRT3 measurements (Table 2), that is HRT rim area and rim volume tended to be higher than rim area and rim

TABLE 1 Mean ONH results from HRT3 and RTVue-100 for both groups. Data are presented as mean ± standard deviation and compared by paired *t*-test.

	HRT3	RTVue-100	*p*
Glaucoma patients			
Disc area (mm²)	2.1 ± 0.6	2.1 ± 0.52	0.73
Cup area (mm²)	1.14 ± 0.58	1.42 ± 0.57	<0.01
Rim area (mm²)	0.95 ± 0.38	0.44 ± 0.33	<0.01
Cup/disk ratio	0.52 ± 0.17	0.76 ± 0.17	<0.01
Rim volume (mm³)	0.19 ± 0.13	0.02 ± 0.03	<0.01
Cup volume (mm³)	0.38 ± 0.34	0.44 ± 0.26	0.14
Healthy subjects			
Disc area (mm²)	2.07 ± 0.58	1.98 ± 0.51	0.09
Cup area (mm²)	0.65 ± 0.48	1.05 ± 1.35	0.01
Rim area (mm²)	1.41 ± 0.30	1.08 ± 0.37	<0.01
Cup/disk ratio	0.29 ± 0.15	0.42 ± 0.22	<0.01
Rim volume (mm³)	0.37 ± 0.13	0.14 ± 0.11	<0.01
Cup volume (mm³)	0.17 ± 0.19	0 ± 0.27	0.04

TABLE 2 Mean difference (Rtvue100-HRT3) of all global parameters in both groups, and limits of agreement.

Parameter	Mean difference (Rtvue100-HRT3)	*p** Value	Fixed bias	Limits of agreement
Glaucoma patients				
Disc area (mm²)	−0.23	0.02	Yes	1.10, −1.56
Cup area (mm²)	0.25	<0.01	Yes	1.22, −0.66
Rim area (mm²)	−0.51	<0.01	Yes	0.27, −1.29
Cup/disk ratio	0.23	<0.01	Yes	0.48, −0.02
Rim volume (mm³)	−0.16	<0.01	Yes	0.07, −0.39
Cup volume (mm³)	0.05	0.14	No	0.52, −0.42
Healthy subjects				
Disc area (mm²)	−0.09	0.09	No	0.69, −0.87
Cup area (mm²)	0.39	0.01	Yes	2.70, −1.92
Rim area (mm²)	−0.33	<0.01	Yes	0.55, −1.21
Cup/disk ratio	0.13	<0.01	Yes	0.36, −0.10
Rim volume (mm³)	−0.22	<0.01	Yes	0.09, −0.53
Cup volume (mm³)	0.05	0.04	Yes	0.40, −0.30

*One-sample *t* test.

TABLE 3 Sensitivity and specificity of ONH sectors and agreement between tests.

	Sensitivity (%)	Specificity (%)	Agreement (k)
HRT3 infero-temporal	80.43	75.86	0.41
RTVue-100 infero-temporal	65.22	91.38	
HRT3 infero-nasal	76.09	68.97	0.21
RTVue-100 infero-nasal	50	93.10	
HRT3 supero-temporal	71.74	68.97	0.34
RTVue-100 supero-temporal	76.09	81.03	
HRT3 supero-nasal	76.05	67.24	0.23
RTVue-100 supero-nasal	60.87	82.76	

TABLE 4 Area under receiver operator characteristic curves (AUROCs), cut-off values, and sensitivity at fixed specificity (95%) for all ONH global parameters.

	AUROC	Cut-off	Sensitivity at fixed specificity (95%)
HRT3			
Disc area (mm²)	0.50	0.01	6%
Cup area (mm²)	0.76	0.05	10%
Rim area (mm²)	0.83	0.42	45%
Cup/disk ratio	0.83	0.44	50%
Rim volume (mm³)	0.84	0.47	52%
Cup volume (mm³)	0.72	0.14	19%
RTVue-100			
Disc area (mm²)	0.56	0.07	13%
Cup area (mm²)	0.74	−0.03	2%
Rim area (mm²)	0.89	0.68	73%
Cup/disk ratio	0.88	0.40	45%
Rim volume (mm³)	0.92	0.60	65%
Cup volume (mm³)	0.75	0.05	10%

volume by RTVue-100 while cup area measurements tended to be smaller by HRT3 than by RTVue-100 in both groups.

HRT3 inferotemporal sector had the highest sensitivity (80.43%, at 75.9% specificity), while for RTVue-100, the superotemporal sector had the highest sensitivity (76.1%, at 81% specificity). The agreement was moderate for the inferotemporal sector and fair for the others. Results are shown in Table 3.

The parameters with the highest diagnostic accuracy as expressed by AUROCs were rim volume, rim area, and cup/disk ratio for both instruments, while cup area and cup volume had fair diagnostic accuracy (Table 4).

Only RTVue-100 rim volume showed statistically significant difference in glaucoma discrimination capability compared with HRT3 rim volume ($p = 0.04$) (Figure 1A–C).

JMP 10 statistical discovery software from SAS (SAS Institute Inc., Cary, NC) was used for statistical analysis.

DISCUSSION

As far as we know this is the first study comparing ONH analysis by HRT3 and RTVue-100. We included 46 eyes of 46 glaucoma patients and 58 eyes of 58 healthy subjects matched by age.

There was no significant difference in the average disc area measured by the two instruments in both groups (glaucoma, HRT3 $2.1 \pm 0.6\,mm^2$, RTVue-100 $2.1 \pm 0.52\,mm^2$, $p = 0.73$; healthy subjects, HRT3 $2.07 \pm 0.58\,mm^2$, RTVue-100 $1.98 \pm 0.51\,mm^2$, $p = 0.09$).

However, the mean measurements of all other ONH global parameters were different between the two devices, suggesting they are not interchangeable: in both groups rim area and rim volume were statistically higher by HRT3 than RTVue-100, while the cup area was statistically higher by RTVue-100 than HRT3.

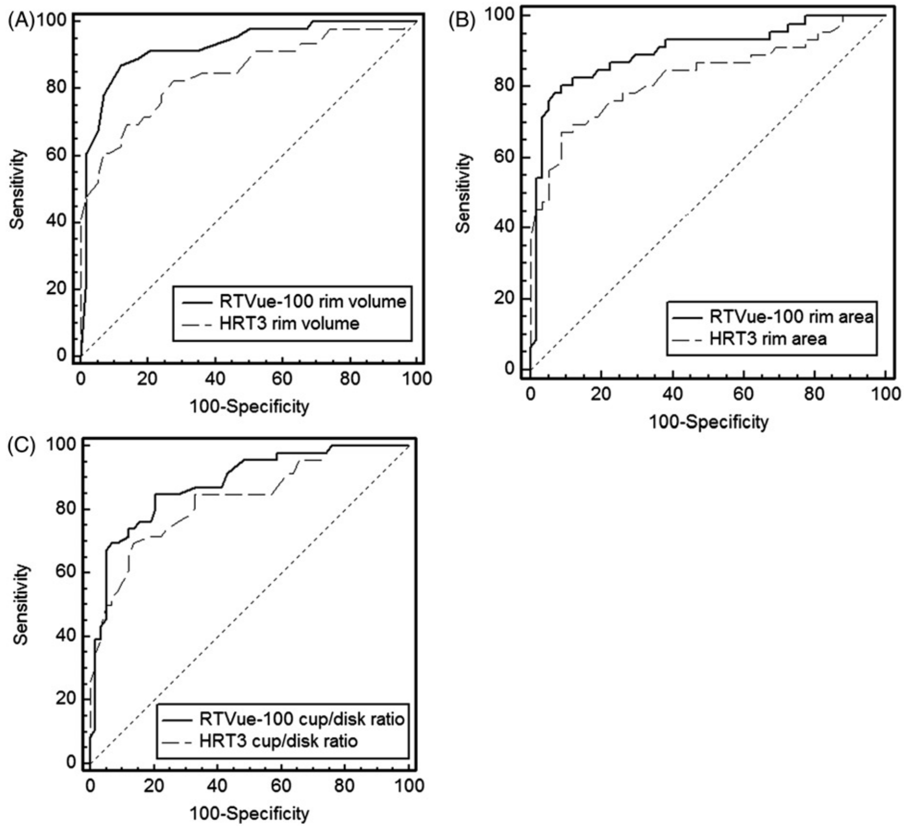

FIGURE 1 (A) Comparison between HRT3 rim volume AUROC and Rtvue-100 rim volume AUROC. (B) Comparison between HRT3 rim area AUROC and RTVue-100 rim area AUROC. (C) Comparison between HRT3 cup/disk ratio AUROC and RTVue-100 cup/disk ratio AUROC.

Bland–Altman plots confirmed discrepancies between the measurements and fixed bias: HRT3 rim area and rim volume tended to be higher than rim area and rim volume by RTVue-100 while cup area measurements tended to be smaller by HRT3 than by RTVue-100 in both groups.

Although some lack of agreement between different methods is inevitable, what matters is the amount by which the methods disagree. This will indicate whether or not the instruments can be used interchangeably. For example, for the parameter cup volume (glaucoma patients), although no fixed bias was detected, the 95% limits of agreement were wide, ranging from 0.52 mm^3 to −0.42 mm^3. This means that RTVue-100 cup volume could be as much as 0.52 mm^3 higher or as much as 0.42 mm^3 lower than HRT3 cup volume.

Basically, the main difference between the two devices is the position of the reference plane in relation to the optic disc margin[1].

The RTVue-100 automatically determines the edge of the ONH as the end of the retinal pigment epithelium (RPE)/choriocapillaris layer. A straight line connects the edges of the RPE/choriocapillaris, and a parallel line is constructed 150 µm anteriorly. Structures below this line are defined as the disc cup and above this line as the neuroretinal rim. In contrast, the HRT machine automatically calculates a reference plane that is located 50 µm posterior to the retinal surface. Structures underneath the reference plane and within the contour line are defined as the disc cup. Structures above the reference plane and within the contour line are defined as the neuroretinal rim.[13] Hence, cup measurements are higher by RTVue-100 than HRT3, and rim measurements are higher by HRT3 than RTVue-100.[14]

Tan et al.[15] already demonstrated that HRT rim area has the highest variability due to reference plane position. In fact, rim area is measured where the reference plane intersects the ONH on the *z*-axis, so any *z*-axis shifts between the ONH and the reference plane due to variation in ONH and peripapillary topography, reference plane or both, could affect rim area measurements.

Similar results are reported in studies comparing HRT3 and another available spectral-domain OCT.[16–19] So, when measuring optic disc topography, examiners should be aware that results are likely to be influenced by the method of measurement used and that each of the instruments may provide different results when tested on the same individual and follow-up examinations should be performed always with the same device.

The sectorial analysis made by these two devices is different because HRT3 divides ONH into six sectors (two quadrants and four octants), while RTVue-100 divides ONH into eight sectors (eight octants). Only the supero-temporal, infero-temporal, supero-nasal and infero-nasal sectors were comparable, showing fair agreement while the infero-temporal sector had moderate agreement. The latter had the highest sensitivity for HRT3 (80.43%) and our results are in agreement with previous studies,[20–23] while the supero-temporal sector was the sector with highest sensitivity by RTVue-100 (76.09%) and this might be interesting to consider for structure–function relationship as infero-nasal defects are very common at early to moderate stages of glaucoma. Furthermore, RTVue-100 sectorial analysis has a greater level of specificity, rather than sensitivity while the opposite happens for HRT3 (Table 3). So, to get the sector with the best diagnostic accuracy, it would be useful to consider the HRT3 sensitivity and the RTVue-100 specificity for the same sector, but as they are not interchangeable, this is not possible.

Although rim volume, rim area, and cup/disk ratio had the highest diagnostic accuracy for both instruments (HRT3 AUROCs 0.84, 0.83, 0.83; RTVue-100 AUROCs 0.92, 0.89, 0.88) RTVue-100 showed slightly better performance that was statistically significant only for rim volume ($p = 0.04$).

Previous studies found different AUROC values for these global ONH parameters, due to different study groups included. Schultze et al.[24] reported that the RTVue-100 parameter with the best discriminating ability was cup/disk ratio (AUROC = 0.85), closely followed by rim volume (AUROC = 0.83) and rim area (AUROC = 0.83), but they included three groups of patients (glaucoma, ocular hypertension, and controls) with different ages and they adjusted their results for it. Iester et al.[25] showed that cup shape measure, rim area, cup/disk ratio, and rim volume (AUROCs = 0.81, 0.80, 0.80, and 0.76) were the most sensitive HRT variables, using one of the first version of this device. Ferreras et al. reported cup/disk ratio, rim volume, and rim area AUROCs similar to our results using the last version of HRT, as we did in this study (0.86, 0.84, and 0.84).[26]

Although our results confirm the good diagnostic accuracy of these two devices, it is worth considering the recent developments in the ONH rim measurements.

Chauan et al. have in fact recently shown that the termination of Bruch's membrane is a logical anatomic outer border of the rim and they have derived a new parameter using a spectral-domain OCT that accounts for the orientation of the rim tissue relative to the point of measurement, while both HRT3 and RTVue-100 derive rim width measurements from the plane of the perceived disc margin without reference to the orientation of the rim tissue. They found that this new parameter has even better diagnostic performance compared with current rim measurements by HRT3 and RTVue-100.[27]

We can then conclude that our results demonstrate that HRT3 and RTVue-100 are not interchangeable in ONH assessment due to the poor agreement in global parameters measurements and sectorial analysis. Both instruments have good diagnostic accuracy and the improvements in OCT technology have led to an available alternative for quantitative ONH analysis which has been the domain of HRT since the 90s.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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