Observer interpretation variability of peripapillary flow using the Heidelberg Retina Flowmeter

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Abstract

Purpose To evaluate the intraobserver and interobserver reproducibility of the automatic full field perfusion image analysis (AFFPIA) program on Heidelberg Retina Flowmeter (HRF) derived perfusion images in a multicentre study group.

Methods A total of 10 subjects were consecutively recruited in the study. One eye was randomly selected for each patient. Blood flow was assessed by HRF and flow measurements were analyzed by using the AFFPIA program. AFFPIA calculates the Doppler frequency shift and the haemodynamic variables: flow for each pixel. Intraobserver and interobserver reproducibility was calculated for AFFPIA program. The retinal blood flow was calculated in the superior and inferior section, furthermore, each section was divided into three parts: the temporal area, the nasal, and the rim area, as for software, but only the temporal and nasal areas were considered in this study. The blood flow and the area considered were evaluated for each part.

Results When the intraobserver and intrainage reproducibility was studied, the coefficient of variation ranged from 0.4 to 1.9%. When the interobserver and intrainage reproducibility was studied, the retinal blood flow coefficient of variation ranged from 0.52 to 3.30% for the supero-temporal area, from 0.13 to 2.67% for the inferotemporal area, from 0.15 to 2.75% for the supero-nasal area, and from 0.04 to 5.65% for the infero-nasal area.

Conclusion Our results with AFFPIA showed an interobserver coefficient of variation of retinal blood flow measurements always less

than 6% in both temporal and nasal areas. No significant difference was found among the four observers for the flow measurements.

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Keywords: optic disc; blood flow; Doppler; confocal scanning laser; reproducibility; glaucoma

Introduction

Recent technical developments have made possible a quantification of retinal blood flow. Some of the first users of these new tools were Michelson and Schmauss, who found high reliability coefficients of flow, volume, and velocity suggesting that there was only 15–16% of variability by using the Heidelberg Retina Flowmeter (HRF), Heidelberg Engineering, Heidelberg, Germany).¹ Furthermore, they found that a significant linear relationship was present between HRF and the ocular perfusion pressure.¹ After some years, Michelson *et al*^{2,3} introduced automatic full field perfusion image analysis (AFFPIA) program able to analyse HRF measurements in a quantitative way. Indeed, they found that for the evaluation of retinal blood flow the interobserver reproducibility was significantly better when the analysis was performed with a larger frame.^{2–6} A coefficient of reliability of 0.74 for the intraobserver reproducibility and 0.95 for the interobserver reproducibility were found.^{1,2} Iester *et al*⁷ showed an intraobserver and intrainage reproducibility (coefficient of variation (COV) range: 0.1–5.3%) when temporal and nasal areas

were considered, while a higher COV was found when the flow of rim area was analyzed (range: 0.5–28%).

The purpose of this study was to evaluate the intraobserver and interobserver reproducibility of the AFFPIA program on HRF-derived perfusion images in a multicentre study group.

Methods

This is a cohort study. In total, 10 subjects were included in this study and only one eye for each subject was randomly included in the study. The research followed the tenets of the Declaration of Helsinki and informed consent was obtained from all enrolled patients. The study was approved by the Institutional Review Board.

Patients were excluded if they had glaucoma, diabetes, high blood pressure, vasospasm symptoms, or other ocular diseases that can affect the optic nerve head (ONH) structure and circulation. Intraocular pressure (IOP) had to be less than 21 mmHg when the retinal blood flow analysis was performed. Patients with an ametropia of more than ± 5 diopters or a lens opacity were not included in the study.

Heidelberg retina flowmeter

The HRF combines the principles of a confocal scanning laser and a laser Doppler flowmeter (670 nm; 100 μ W). The details of this technique have been described elsewhere.^{1–6} After 128 scans of each examined point, the HRF calculates a bi-dimensional map of the laser Doppler shift within a 400 μ m slice of tissue, over a rectangular area ($5^\circ \times 20^\circ$) of the posterior pole of the eye. The calculation is made using a fast Fourier transformation. The laser Doppler shift values recorded at different locations are displayed on the monitor in a colour code image. For each pixel a frequency shift is calculated.^{1–6}

Automatic full field perfusion image analyzer

HRF perfusion images were analyzed using a program called automatic full field perfusion image analyzer (AFFPIA). The details of this program have been published elsewhere.^{2,3} In brief, AFFPIA calculated the Doppler frequency shift, and the haemodynamic factor or flow of each pixel according to the theory of Bonner and Nossal. For a valid estimation of retinal blood flow some assumptions must be made: adequate brightness, no ocular movement, Doppler shift considerably lower than 2000 Hz. To meet these requirements, the resulting perfusion image is processed to account for underexposed and overexposed pixels, saccades, and the

retinal vessel tree. With AFFPIA the operator marks saccades and the location of the outer and inner edge of the rim area; in a further step the capillaries and large vessels of the retina are identified automatically by a vessel detection algorithm based on the intensity and the perfusion image.^{2,3} Underexposed and overexposed pixels and the saccades previously detected by the user, are excluded. The local inhomogeneities of the perfusion map are softened by a moving average procedure, performed with a size of 5×5 pixel.² For capturing each image, the time needed is 2 s. This allows averaging the cardiac cycle for areas larger than a pixel.

The blood flow was automatically analyzed in the temporal, nasal, and rim area. The heart beat associated pulsation of capillary blood flow was taken into account for plotting the mean capillary flow of each horizontal line against time.

Acquisition

All the subjects were examined at the same time (between 1200 and 1400 hours). Blood pressure, heart rate, and IOP were assessed just before each HRF examination.

All the optic nerve heads were theoretically divided into three horizontal sections (superior, central, and inferior sections). In this study, the superior and inferior ONH sections were considered and 10 images for each

Figure 1 Each ONH was divided into two different sections for the analysis: one section was superior and the other was inferior. The user positioned the HRF frame on the optic disc as in the figure, and then the system acquired the perfusion maps (the images). For each perfusion map, the AFFPIA calculated three different flow measurements: the rim flow, the adjacent temporal, and nasal retina flow, after drawing the inner and outer rim contourline. In this study only temporal and nasal retina areas were considered.

section were acquired and analyzed (Figure 1). Patients were instructed to look straight ahead at a fixation target located at 2 m with the contralateral eye, while the user focused on the peripapillary retina. The camera distant from the eye was around 1.5 cm. In each HRF image, the superior or inferior rim area and the adjacent temporal and nasal peripapillary areas were analyzed by AFFPIA program (Figure 2). For each analysis, the users (MC, MI, TR, and OV) from four different centres (and computers) had to individuate the eventual presence of micro-saccades in the image and to delete those points, and then to position the inner and outer optic rim

Figure 2 AFFPIA results. The highest frame is the DC image, which is used by the observer to draw the contourline. Then the program automatically eliminates extracellular matrix and larger vessels, and calculates the flow and area measurements in the temporal, rim, and nasal area, as for software. In this study only temporal and nasal area values were included in the analysis.

contourline. Then the program automatically calculated the flow in the following three areas: rim, temporal, and nasal areas. In this study only nasal and temporal peripapillary areas were considered.

Statistical analysis

Primary objectives were as follows:

- To determine the intraobserver and intrainage reproducibility. All the observers analyzed 20 HRF perfusion maps. The same perfusion map was analyzed three times by each of the four observers and the COV was calculated.
- To determine the interobserver and intrainage reproducibility. The four observers analyzed the HRF maps and the COV was calculated.

In both reproducibility studies, two parameters were obtained for each analysis and for each sector: the 'flow', measured in arbitrary units, and the 'area' used to calculate the flow, measured as a percentage of the total area.

For each analysis the temporal and nasal retinal 'flow' were considered together with the 'area' analyzed, while the rim 'flow' and 'area' were not considered in this study.³

In both the 'intraobserver and intrainage' and 'interobserver and intrainage' variability study, the mean values of the two parameters (eg flow and area) were compared by using F-test.

Furthermore, the intraobserver and interobserver repeatability of the flow values was also calculated to evaluate by using a Bland–Altman plot.

Results

The mean age was 47.3 ± 7.7 years (mean $\pm$ standard deviation) and the mean refractive error was 0.3 ± 0.7 diopters. Mean blood pressure was 126.3/81.3 mmHg (systolic/diastolic blood pressure) and mean heart rate was 67.4 ± 3.4 .

HRF images were considered adequate for analysis when the focus was on the superficial vessels, capillaris in flow image had to be visible and HRF DC values ranged from 70 to 200.

Intraobserver and intrainage reproducibility

When the intraobserver and intrainage reproducibility was studied, for the retinal blood flow the mean COV was 0.7% in all four considered areas for observer 1. The mean COV was 0.4% in the supero-temporal area, 0.5% in the supero-nasal and infero-temporal areas, and 1.9% in the infero-nasal area for observer 2. The mean COV for

the flow was 0.7% in the supero-temporal area, 0.8% in the supero-nasal area, 0.5% in the infero-temporal area, and 0.7% in the infero-nasal area for observer 3. The mean COV for the flow was 0.5% in the supero-temporal area, 0.8% in the supero-nasal area, 0.5% in the infero-temporal area, and 0.6% in the infero-nasal area for observer 4 (Table 1) (Figure 3).

In the intraobserver and intrainage study, the 'area' of measurements showed a slightly higher COV. In particular, the mean COV for the area was 2.3% in the supero-temporal area, and 2.7, 2.6, and 2.1% in the supero-nasal, infero-temporal, and inferonasal areas, respectively, for observer 1. The mean COV was 2.3% in the supero-temporal area, 1.8% in the supero-nasal area,

Table 1 Intraobserver coefficient of variation for 'flow' analysis

Patient	Obs 1	Obs 2	Obs 3	Obs 4	Patient	Obs 1	Obs 2	Obs 3	Obs 4
<i>Supero-temporal</i>					<i>Supero-nasal</i>				
1	0.12	0.78	0.10	0.14	1	0.06	0.41	0.31	0.08
2	0.54	0.50	1.21	0.33	2	0.70	0.13	1.38	0.58
3	2.19	0.56	2.19	1.40	3	1.94	0.60	1.94	3.89
4	0.55	0.35	0.31	0.21	4	0.45	0.25	1.88	0.24
5	1.06	0.18	0.55	0.52	5	0.14	0.16	0.58	0.11
6	0.56	0.66	0.84	0.28	6	1.37	0.25	0.78	0.22
7	0.90	0.73	0.67	0.90	7	1.27	2.65	0.38	1.58
8	0.00	0.07	0.00	0.32	8	0.06	0.03	0.09	0.14
9	0.81	0.16	0.59	0.23	9	0.60	0.42	0.34	0.46
10	0.27	0.04	0.20	0.35	10	0.41	0.31	0.41	0.60
<i>Infero-temporal</i>					<i>Infero-nasal</i>				
1	0.29	0.69	0.41	0.36	1	0.15	0.68	0.17	0.51
2	0.18	0.04	0.15	0.21	2	0.39	0.40	0.06	0.08
3	0.09	0.76	1.41	0.76	3	1.04	0.88	0.76	0.29
4	2.17	1.01	0.95	2.17	4	1.79	8.21	2.24	1.79
5	2.22	0.82	0.27	0.46	5	0.45	5.55	0.38	0.08
6	0.91	0.56	0.31	1.10	6	1.93	1.63	0.73	1.04
7	0.52	0.36	0.10	0.53	7	0.04	0.05	0.11	0.14
8	0.18	0.05	0.40	0.22	8	0.72	0.87	0.39	0.23
9	0.44	0.44	0.79	0.48	9	0.55	0.71	1.85	1.67
10	0.36	0.19	0.36	0.09	10	0.13	0.03	0.05	0.01

Obs: observer.

Figure 3 Interobserver and intrainage for mean flow measurements (arbitrary unit), on the X-axis the subjects, on the Y-axis the flow. Each symbol represents one of the four users.

1.9% in the infero-temporal area, and 3.0% in the infero-nasal area for observer 2. The mean COV was 2.1% in the supero-temporal area, 3.1% in the supero-nasal area, 2.3% in the infero-temporal area, and 2.2% in the infero-nasal area for observer 3. The mean COV was 2.2% in the supero-temporal area, 2.3% in the supero-nasal area, 1.9% in the infero-temporal area, and 1.6% in the infero-nasal area for observer 4 (Table 2) (Figure 4).

Interobserver and intrainage reproducibility

When the interobserver and intrainage reproducibility was studied, the retinal blood flow COV ranged from 0.52 to 3.30% for the supero-temporal area, from 0.13 to 2.67% for the inferotemporal area, from 0.15 to 2.75% for supero-nasal area, and from 0.04 to 5.65% for the infero-nasal area (Table 3). Furthermore,

Table 2 Intraobserver coefficient of variation for 'area' analysis

Patient	Obs 1	Obs 2	Obs 3	Obs 4	Patient	Obs 1	Obs 2	Obs 3	Obs 4
Supero-temporal					Supero-nasal				
1	2.03	4.75	0.44	1.60	1	3.86	2.82	3.09	0.62
2	3.65	1.85	3.94	3.44	2	5.27	2.61	5.65	3.84
3	2.89	0.79	1.61	1.51	3	2.32	2.08	5.33	3.71
4	1.34	1.34	0.64	0.64	4	2.18	2.18	4.92	1.27
5	3.14	4.13	3.14	2.42	5	4.57	3.43	3.83	1.95
6	1.91	3.08	4.25	4.61	6	1.84	1.27	1.59	5.26
7	1.41	2.05	1.35	0.77	7	1.27	1.01	0.68	2.01
8	2.44	1.00	1.67	1.46	8	2.44	0.84	2.11	1.08
9	1.95	1.15	1.66	0.90	9	1.89	0.61	2.29	0.28
10	2.24	3.53	1.84	2.43	10	1.23	1.12	1.09	3.00
Infero-temporal					Infero-nasal				
1	4.06	3.61	4.38	2.27	1	0.79	2.21	0.15	2.28
2	0.71	0.12	0.97	0.88	2	2.05	1.04	2.58	2.58
3	2.56	2.23	5.76	1.44	3	2.67	3.41	3.81	0.99
4	2.90	3.35	2.30	1.56	4	1.29	7.41	2.57	3.87
5	2.94	1.87	0.57	1.81	5	1.13	5.38	0.91	0.20
6	3.49	1.46	2.33	2.62	6	3.77	3.12	2.24	2.02
7	2.36	1.51	2.37	1.94	7	0.38	0.39	2.36	1.05
8	1.45	1.26	2.57	1.29	8	1.70	1.75	1.23	0.84
9	1.89	2.08	1.20	2.29	9	2.79	2.25	3.13	0.94
10	4.04	1.64	0.15	2.86	10	4.81	3.47	3.29	1.09

Obs: observer.

Figure 4 Interobserver and intrainage for the area obtained for the analyzes (%), on the X-axis the subjects, on the Y-axis the area in %. Each symbol represents one of the four users.

Table 3 (a) Interobserver coefficient of variation for 'flow' analysis and (b) Interobserver coefficient of variation for 'area' analysis

Patient	TS	TI	NS	NI
<i>(a) Flow analysis</i>				
1	0.52	0.39	0.73	0.73
2	0.65	0.40	0.71	0.72
3	0.73	0.74	2.75	1.08
4	0.53	1.68	1.16	5.65
5	0.59	2.67	0.15	1.26
6	0.75	0.13	1.25	0.19
7	0.67	0.46	1.97	0.06
8	0.21	0.24	0.20	1.48
9	3.30	1.00	1.80	2.03
10	0.81	0.19	0.65	0.04
<i>(b) Area analysis</i>				
1	1.76	5.64	9.12	4.68
2	1.99	0.78	11.99	4.65
3	1.83	1.66	5.24	8.49
4	0.03	2.61	6.50	6.56
5	3.30	8.52	1.19	3.52
6	5.11	2.77	4.50	1.51
7	1.33	3.04	2.13	0.98
8	1.27	3.12	4.80	3.25
9	4.72	2.08	7.03	3.91
10	2.09	2.22	4.92	1.37

TS = supero-temporal area; TI = infero-temporal area; NS = supero-nasal area; NI = infero-nasal area.

the 'area' COV ranged from 0.03 to 5.11% in the supero-temporal area, from 0.78 to 8.52% the for infero-temporal area, from 1.19 to 9.12% for supero-nasal area and from 0.98 to 8.49% for the infero-nasal area (Table 3).

In the intraobserver retinal blood-flow analysis, no significant difference was found within the same observer for the flow measurements (Table 4), while for the area values few significant differences were found (Table 5).

The interobserver retinal blood flow and area were obtained by the four observers, and no significant difference was found among them (Tables 4 and 5).

No difference was found in the reproducibility between superior and inferior areas in both the intraobserver and interobserver study.

No significant difference for flow measurements was found among the four different sectors in the intraobserver analysis (observer 1, $P = 0.085$; observer 2, $P = 0.094$; observer 3, $P = 0.109$; observer 4, $P = 0.086$) and in the interobserver analysis ($P = 0.092$).

When the Bland-Altman plot was applied in the intraobserver analysis the mean difference was 0.09 and the coefficient of repeatability was 4.56 (Figure 5a), while in the interobserver analysis the mean difference was 0.07 and the coefficient of repeatability was 11.54 (Figure 5b).

Discussion

Several technologies are competing to allow clinicians to evaluate ocular blood flow.⁸⁻¹³ In this study we used

Table 4 (a) Intraobserver retinal blood 'flow' mean values obtained by each observer and (b) Interobserver retinal blood 'flow' values

	Observer 1 ^a		Observer 2 ^a		Observer 3 ^a		Observer 4 ^a		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TS	251.92	0.64	250.06	0.36	249.49	0.66	250.73	0.45	
TI	216.86	1.09	218.13	0.26	216.99	0.20	217.16	0.49	
NS	204.77	0.49	204.21	0.41	204.65	0.35	204.07	0.88	
NI	223.62	0.04	224.51	1.50	222.41	0.75	224.25	0.25	
(b) Interobserver retinal blood ‘flow’ values									
	Observer 1		Observer 2		Observer 3		Observer 4		F-test
	First analysis		First analysis		First analysis		First analysis		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TS	252.56	99.59	249.87	98.01	248.93	98.12	250.51	98.33	NS
TI	215.43	79.70	217.82	80.10	216.76	79.70	216.48	80.50	NS
NS	204.16	76.98	204.40	76.91	204.73	77.64	203.43	77.39	NS
NI	223.58	83.55	226.52	82.95	222.31	84.29	224.47	83.12	NS

^aNo significant difference was found among the three-intraobserver measurements by F-test; NS = no significant difference was found comparing the first image analysed by the four observers; SD: standard deviation.

Table 5 (a) Intraobserver mean 'area' values obtained by each observer and (b) Interobserver mean 'area' values

(a) Intraobserver mean 'area' values

	Observer 1			Observer 2			Observer 3			Observer 4		
	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
TS	46.65	0.76	0.004	46.85	0.58	0.107	46.92	0.10	0.938	47.53	0.76	0.006
TI	42.58	0.43	0.266	41.57	0.20	0.645	43.11	0.36	0.464	43.36	0.21	0.541
NS	42.57	0.37	0.452	40.93	0.14	0.759	39.34	0.20	0.800	41.45	0.34	0.398
NI	43.23	0.42	0.267	41.94	0.22	0.798	41.96	0.73	0.003	42.88	0.04	0.978

(b) Interobserver mean 'area' values

	Observer 1 First analysis		Observer 2 First analysis		Observer 3 First analysis		Observer 4 First analysis		F-test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P-value
TS	45.97	4.39	47.32	2.75	46.88	2.90	48.07	3.77	0.077
TI	43.07	5.70	41.80	5.82	43.52	6.16	43.17	6.06	0.223
NS	42.99	3.20	40.79	3.48	39.54	5.21	41.47	3.32	0.075
NI	42.81	6.82	41.81	7.09	41.87	5.75	42.89	6.30	0.544

SD = standard deviation; TS = supero-temporal area; NI = infero-nasal area; NS = supero-nasal area; TI = infero-temporal area.

Figure 5 Bland-Altman plot: (a) Intraobserver analysis, the mean difference was 0.09 and the 95% confidence interval was -4.65 and 4.57, (b) Interobserver analysis, the mean difference was 0.07 and the 95% confidence interval was -11.47 and 11.61.

HRF, a noninvasive technique based on Doppler effect, which is able to evaluate the retinal and ONH capillary flow. The HRF uses a complex technique where the two main variables are the acquisition of the images and the software to analyze them.

Our results with AFFPIA showed an intraobserver COV of retinal blood flow measurements always less than 1%, and the interobserver COV was always less

than 6% in all the considered areas, while using the Bland-Altman plot we found a coefficient of repeatability of 4.56 and 11.54, respectively. Furthermore, no significant difference was found among the four observers for the flow measurements.

Using AFFPIA program to analyze HRF measurements, Michelson *et al.*² found a coefficient of reliability of 0.74 for the intraobserver reproducibility and 0.95 for the interobserver reproducibility. These data were significantly better than the results obtained with a 10 × 10 pixel box. Indeed, they found the reliability coefficients of flow, volume, and velocity to be 0.84, 0.85, and 0.84, respectively, suggesting that there was only a 15–16% of variability due to intrinsic errors component.³ Also Nicolela *et al.*⁴ found an interobserver reliability coefficient ranging from 0.90 to 0.98 and a COV ranging from a median of 17 to 34%.

Since in most centres this type of examination is carried out by the same person using the same instrument, it is important to assess the intraobserver variability; however, the possibility of reading the same perfusion map in the same way is an important parameter. In this study four observers working in different centres were included in the study to analyze the images of 10 normal superior and inferior ONHs.

The main result of this study is that the bidimensional HRF maps can be read easily and with a low COV both by the same observer and by different observers confirmed also by Bland-Altman plots (Tables 1, 3, Figure 5). The flow measurements obtained by the four different users did not show any significant difference

(Table 4). In the intraobserver study they found that the supero-temporal area had the highest flow followed by infero-nasal, infero-temporal, and supero-nasal areas, even if no significant difference was found.

The flow of the rim area was not considered, due to some technical problems such as the low reflectivity of the rim making focusing on it not repeatable, the height where flow measurements were made in the optic disc, and the higher COV ranging from 22 to 40%.^{2,4,6}

The different 'areas' obtained in both the intraobserver and interobserver study suggested that the global flow values are independent from the size of the considered area. This does not mean that there is some uniformity of blood flow in the retina. But although there are different blood flows in different retinal areas, when a global analysis is performed a mean values is obtained. Indeed, when the HRF map is analyzed by using a single pixel is possible to find a lot of variation from one area to another. However, if we analyze a large amount of pixels this measurement could be probably independent of area because a large area includes many differences inside itself.^{14,15}

Using AFFPIA, the peripapillary retinal flow is measured globally, independently from retinal locations and the cardiac cycle during the measurement too. Indeed when a 10 × 10 pixels box is used to measure retinal blood flow, it is important to know in which cardiac phase the measurement is obtained, since the flow can accordingly increase or decrease. However, if the flow is measured globally, systolic and diastolic phases are averaged, and the mean retinal flow will include the entire cardiac circle.

Using pixel by pixel analysis of the entire image, Kagemann *et al*⁵ showed that HRF measurements were independent from the angle between the laser beam and the fundus angle and were influenced by the distance of the camera from the eye. Since this study is limited to the intraobserver and interobserver reproducibility, other variables like the brightness of the image, the distance between the eye and the instrument have not been considered.

The position where to draw the rim contourline is always important; however, there was some variation among the observers in the parameter 'area', which probably suggests that there was some variability in the identification of the outer rim edge (the rim flow was not considered in this study). However, these variations did not affect the final flow measurements as the average of the measurements from thousands of pixels and despite a small change in disc margin the vast majority of the pixels included in the different analysis were the same.

Different observers can analyze the same HRF perfusion maps obtaining similar results when AFFPIA program is used.

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