Title: A 30 second test for quantitative assessment of a Relative Afferent Pupillary Defect: the Infrared Pupillary Asymmetry

Authors: Nathalie Stéphanie Meneguette[1,2], J. Emanuel Ramos de Carvalho[2,3], A. Petzold[2,4,5]

1. Department of Neurology and Ophthalmology, Federal University of the State of Rio de Janeiro, Brazil
2. Moorfields Eye Hospital, London, United Kingdom
3. Department of Ophthalmology, Amsterdam UMC – AMC Amsterdam, The Netherlands
4. The National Hospital for Neurology and Neurosurgery, London United Kingdom
5. Department of Ophthalmology, Neuro-ophthalmology Expertise Centre, Amsterdam UMC, The Netherlands

Address correspondence to:
Dr. Axel Petzold
Moorfields Eye Hospital, Department of Neuro-ophthalmology
162 City Road, EC1V 2PD,
London, UK
Tel: 020 7253 3411
Email: a.petzold@ucl.ac.uk

Abstract

Background: Detection of a relative afferent pupillary defect (RAPD) by the swinging-light test can be challenging in clinical practice (dark eyes, anisocoria). We developed a new method of RAPD quantification based on the recording of the Infrared Pupillary Asymmetry (IPA) with a standard optical coherence tomography (OCT) device.

Methods: The diagnostic value of the IPA for detection of the RAPD was determined by Receiver Operating Characteristic (ROC) curves, Area Under the Curve (AUC).

Results: Twenty-nine subjects were included in this study (17 controls, 12 unilateral optic neuropathies). The IPA was significantly greater in unilateral optic neuropathies (0.39) compared to controls (0.18, $p = 0.001$). The diagnostic value was good with a ROC-AUC of 0.843. Importantly, the IPA correlated significantly with the inter-eye difference of the macular ganglion cell-inner plexiform layer (mGCIPL) thickness ($R = 0.53$, $p = 0.01$). Assessment of the IPA took less than 30 seconds.

Conclusion: Present data show that the IPA is a practical and rapid test that can be applied in a clinical setting. The IPA may be a valuable functional outcome measure for clinical trials, complementing structural retinal OCT data in a biological meaningful way. The IPA should be further investigated for suitability for optic neuritis treatment trials.

Keywords

Optical coherence tomography, Infrared Pupillary Asymmetry, IPA, Relative Afferent Pupillary Defect, RAPD.

Objective

Pupillary reaction testing is a crucial step in the clinical examination of patients with optic neuropathies. Presence of a relative afferent pupillary defect (RAPD) is one of the most informative clinical signs, indicating unilateral dysfunction at the level of the *afferent* visual pathways [1-3]. The RAPD is not helpful in situation of bilateral pathology [1]. In patients with optic neuritis, a RAPD can be detected prior to development of inner retinal layer atrophy [2,4-6]. Whilst clinically useful, the RAPD has not yet been included as a functional outcome measure in clinical trials and can be challenging to detect clinically in patients with dark eyes, or in the presence of anisocoria [7-9]. This is further accentuated by the need to assess the pupillary light reflex in the dark[1]. Even with an optimal setup there remain difficulties interpreting the results because the physiological moment-to-moment variation of the pupillomotor output results in dissimilar pupillary amplitudes and velocity profiles to the same light stimulus [8]. Finally, the test is dependent on the examiners experience and is subjective. Many commercially available, hand held pupillography devices are monocular. Binocular pupillography devices are, because of their wrap around both eyes design, prone to inadvertent accommodation which can be difficult to detect to the non experienced examiner. Both issues are severe limitations to the test and source of misinterpretation of the data.

The accurate, quantifiable assessment of the RAPD requires binocular assessment of the pupil responses in absence of accommodation. The last decade has seen an ever increasing use of retinal optical coherence tomography (OCT) devices not only in routine clinical practice, but also with high street opticians. Access to these devices has become relatively easy. In this study we make use of the infrared (IR) camera of such an optical coherence tomography (OCT) device to quantify the RAPD.

Methods

This retrospective study was approved by the IRB of the Moorfields Eye Hospital, London, UK (Protocol number ROAD 17/011) in accordance with the tenets of the 1964 Declaration of Helsinki.

Study design and patient population

Retrospective data from 29 patients seen at Moorfields Eye Hospital were retrieved and assessed following consensus guidelines which had been incorporated into the trust Standard Operating Procedures (SOP)[1]. All subjects underwent a full neuro-ophthalmic examination and OCT examination on the same day and at the same site. Diagnosis of optic neuropathy was based on clinical assessment and preceded the OCT recording.

IPA and OCT assessment

All subjects were examined with the SD-OCT (Heidelberg Engineering, Inc., Heidelberg, Germany). For assessment of the pupil response, subjects were asked to sit back from the infrared (IR) camera and look at s fixation target at 3 meter distance to avoid near accomodation (figure 1). Room lighting was dimmed. The pupil light response was elicited with a solar powered ophthalmoscope (Arclight®). The pupil diameters and inter-canthus distances (figure 2) were quantified from IR images in (1) dim light, (2) light shone into the right eye and (3) light shone into the left eye. For each photo the frames of the IR images were averaged for about 1 second in order to account for the physiological moment-to-moment variation of the pupillomotor output [8]. We also corrected for two important variables which can interfere with the reproducibility of IR pupil diameter measurements, eye to camera distance and convergence.

The IPA assessment was followed by an OCT examination, as previously described [10]. No pharmacological pupil dilation was used. Data on the pRNFL was obtained using a 12° ring scan centered around the optic nerve head. Data on the macular area for the mGCIPL were acquired using a macular volume scan (20×20° field, 49 B-scans, vertical alignment) centred on the fovea. Automated segmentation of the pRNFL and mGCIPL was performed (Heidelberg Engineering, software version 1.10.2.0). For pRNFL thickness the global mean of the entire pRNFL was used. For mGCIPL thickness the average thickness of the inner four quadrants of the 1 mm, 2.22 mm, 3.45 mm grid was calculated. Scans were excluded from the analyses if manual correction for algorithm segmentation failures was not possible or if the validated quality control criteria were not met (OSCAR-IB)[11].

IPA calculation

In order to calculate the IPA, we corrected for potential variability in distance between the eye and the camera by revising the differences between inner intercanthal distance for each measurement. A larger intercanthal distance difference indicates the head was closer to the camera during scan acquisition. To adjust for this variation, we calculated a correction factor by dividing the baseline intercanthal distance value by the value for each measurement and each eye. The formulae presented here are for the right eye (RE). Calculations are identical for the left eye (LE):

$$(1)\quad correctionfactorR = \frac{intercanthusRE_{dark}}{intercanthusRE_{light}}$$

↓

Next, we considered the possibility of anisocoria (physiological or pathological). Therefore, we calculated the change of pupil diameter from dark (defined in formula as ‘dark_r’) to light (defined as ‘light_r’) taking the potential change of measurement distance into account. Larger values indicate a

↓

more dilated pupil. The formula applies to light shone into the RE (defined as 'light_r'). Calculations are identical for light shone into the LE.

$$\text{(2a) } RE_{light_r} = \frac{light_r \times correctionfactor\Re}{(dark_r)}$$

$$\downarrow$$

$$\text{(2b) } LE_{light_r} = light_r \times correctionfactor \leq \frac{}{dark_r}$$

$$\downarrow$$

$$\text{(3)} = mean\left(RE_{light_r}, LE_{light_l}\right)$$

Finally we were able to calculate the IPA as follows:

$$\downarrow$$

$$\text{(4) } IPA = -$$

For dichotomised (RAPD yes/no) statistical analyses we used the positive values of the IPA as: $|\text{IPA}|$.

To simplify the reproducibility of above approach all formulas have been incorporated in a simple excel sheet provided as supplementary material.

Statistical analyses

Data were assessed for normality using the Shapiro-Wilk test and by graphical inspection in SAS (version 9.4). The inter-eye percentage difference (IEPD) was calculated as before [12,13]. Because of non-Gaussian data, we used the non-parametric Kruskal-Wallis Test Receiver Operator Characteristics (ROC) curves to calculate the Area Under the Curve (AUC) to describe the level of diagnostic accuracy. The diagnostic value was rated as 'no or low discriminatory power' for an AUC 0.5-0.7, as 'moderate discriminatory power' for an AUC of 0.7-0.9 and as 'high discriminatory power' for an AUC >0.9. The ROC were plotted to determine graphically optimised IPA cutoff values as the shortest distance from the top left corner to the ROC curve. The dichotomised IPA was then used to compare groups on a categorical level using the two-tailed Fisher's Exact Test. P values of < 0.05 were accepted as significant.

Results

Twenty-nine subjects were included in this retrospective study. The baseline characteristics are shown in Table 1. Seventeen were healthy controls and twelve were afflicted with an unilateral optic neuropathy secondary to multiple sclerosis, sarcoidosis or non-arteritic anterior ischaemic optic neuropathy. As examples for anisocoria we had also included two patients with Holmes-Adie pupils

and one with a Horner syndrome (figure 3).There was a significant increase in pRNFL atrophy in the affected eye (p=0.01, figure 4a).

The IPA was significantly larger in subjects with an optic neuropathy (0.39) when compared to healthy control subjects (0.18, figure 4b, p=0.001). The ROC-AUC (0.843) indicated a 'moderate discriminatory power' of the IPA to separate both groups. The optimised cutoff for the IPA was 0.11. Based on this cutoff a significant higher proportion of patients with an optic neuropathy had a pathological IPA (58%) when compared to controls (6%, p = 0.003) There was a linear relationship between the IPA and the mGCIPL-IEPD (R = 0.53, figure 4c, p = 0.0). For the pRNFL-IEPD there was no such relationship (R = 0.16, p = 0.52).

Discussion

This retrospective study suggests that the IPA provides a rapid and quantifiable metric for the RAPD in cases with unilateral pathology. There are two novel observations. First there is a physiological IPA. Second the IPA correlates with the macular GCIPL. Therefore the IPA is biological meaningful. Finally the IPA can be reliably assessed and documented in clinically challenging situations. The calculation of the IPA is straightforward and we provide an excel worksheet practical equation for the readers to test and validate the IPA in their clinical practices.

Consistent with previous work, the intercanthal distances were taken as reference point so to overcome the variability induced by differences in positional acquisition of the scans. Previous studies have shown a correlation between the presence of a RAPD and pRNFL and mGCIPL[4], the latter deemed more sensitive as opposed to the pRNFL[2]. A positive RAPD, as assessed by an experience clinician, is expected to become subjectively visible only once at least 25% of the pRNFL has been atrophied[5]. In our study, we used a highly sensitive measure, the IEPD [12,13]. The finding of a significant linear relationship between the mGCIPL-IEPD and IPA is important because the IPA could be a valuable functional outcome measure to complement structural OCT data in clinical trials and research. This will require independent confirmation as there will be a likely need for validation of the inter-laboratory IPA cutoff points with differences in device settings, lighting conditions and distance to IR camera. Finally. The IPA allows diagnosis of anisocoria, such as from a Horner syndrome [10] or a Holmes-Adie pupil which is clinically relevant.

The novel, but logical findings from this study are first a "physiologically RAPD". Second, the strong correlation between the IPA and the inter-eye difference of the GCIPL thicknesses is biologically meaningful. The eye with a higher macular GCIPL density elicits compared to the contralateral eye a stronger light response which will result in a relative stronger pupil constriction. A physiological RAPD has not been described previously, but can be implied indirectly from results based on use of neutral density filters for calibration of a commercial pupillography device [14]. In their method paper

the authors showed that a RAPD could be induces by placing 0.0, 0.3, 0.6, and 0.9 log units neutral density filters in front of one healthy eye. Neutral density filters have also been used to accentuate the RAPD [14]. In clinical practice however assessment of the RAPD by placing a dark object in front of an already dark eye does make assessment of the pupil response even more difficult. For this reason we have not used this approach in our routine clinical practice, other than for bedside teaching purposes in a subject with light blue irises. Future prospective studies aiming to validate the IPA for a multi-center setting will need to consider calibration of the method with neutral density filters in subjects to travel around sides and be re-assessed across the variable setups. This clearly was beyond the scope of this retrospective study.

One important conclusion from the observation of a physiological RAPD is that there will be a need to determine and validate a normal range for the IPA in a multi-center setting. This is a crucial paradigm shift from the classical clinical teaching which was based on a subjective yes/no answer to whether or not a RAPD was present in the diagnostic work up of a patient with a suspected optic neuropathy. The new question is when does the IPA become pathological. This will require validation of the here proposed cutoff of 0.11 for the IPA in a multi-center setting. Similar to lessons learned from other clinical tests we anticipate that during this validation process a list of other relevant variables will emerge which we have not yet identified. Factors which likely will require to be investigated prospectively relate to opacities in the visual pathway, visual field defects of variable size and location and outer retinal layer pathology not reflected in pRNFL and mGCIPL atrophy. The IPA is unlikely to be valid in cases of bilateral optic nerve pathology, just as the RAPD can be absent in these cases [1].

Our findings are also consistent with another new approach for detection of the RAPD, ultrasound, has been published whilst our paper was under review [15]. The study by Schmidt *et al.* was of comparable patient numbers and took only 5 minutes per patient. The authors found a reduced constriction amplitude of the pupil in the affected eye and calculated a constriction ratio. They concluded that a cutoff of this constriction ratio of >1.3 was useful in discriminating eyes with a RAPD from healthy controls. The constriction ratio correlated with visual acuities, but OCT data have not been available.

In conclusion the IPA is a practical, easily applied and quick test for quantitative assessment of the RAPD in patients with a suspected optic neuropathy, even in clinically challenging situations. The novel observation of a physiological RAPD and the biological meaningful correlation of the IPA with macular GCIPL structure could introduce a paradigm shift from the RAPD as a subjective bedside expert opinion to become a valuable quantitative measure worthwhile to undergo multi-center validation as a promising secondary outcome marker for clinical trials.

Table legends

Table 1: Patient characteristics. The median (IQR), mean (SD) are shown. The pRNFL and macular GCIPL are given for the better and worse (atrophy) eye. Not significant = NS. Abbreviations - GCIPL: ganglion cell-inner plexiform layer; IEPD: inter-eye percentage difference; IPA: infrared pupillary asymmetry; pRNFL: peripapillary retinal nerve fibre layer.

Characteristics	**Control**	**Optic neuropathy**	**P value**
Age (yrs)	34 (28-48)	44 (42-57)	0.046
Gender (F:M)	10:7	7:5	
IPA	0.06 ± 0.05	0.17 ± 0.11	0.0019
pRNFL (worse eye) [μm]	99 ± 18	71 ± 20	0.011
pRNFL (better eye) [μm]	97 ± 17	92 ± 12	NS
IEPD-pRNFL	7.36 ± 11.02	12.85 ± 11.02	0.037
GCIPL (worse eye) [mm^3]	0.40 ± 0.08	0.29 ± 0.08	0.018
GCIPL (better eye)[mm^3]	0.41 ± 0.09	0.40 ± 0.05	NS
IEPD-GCIPL	8.2 ± 12	13 ± 12	NS

Figure legends

Figure 1: A photograph of the setup. The subject (NM) looks at the distant target to avoid accommodation. Note that the dark pupils are difficult to assess clinically. The IR images on the screen highlight the pupils. The assessment takes less than 30 seconds and can be performed even in an only moderately dimly-lit room. Abbreviations – IR: infrared; OCT: optical coherence tomography.

Figure 2: Measurements recorded for the IPA assessment. (A) Measurements from a healthy control subject with dark irides with a final IPA of 0.04 (B) Measurements from a patient with a left RAPD and dark irides with a final IPA of 0.18 An IPA of larger than 0.11 indicated an RAPD in our cohort. Abbreviations - IPA: infrared pupillary asymmetry; RAPD: relative afferent pupillary defect.

Figure 3: Pupillary reactions demonstrated by photos of the IR camera of the Spectralis SD-OCT device. (A) The IPA for the right RAPD is 0.20 (B) A left Horner Syndrome with greater anisocoria in the dark (IPA=0.01). (C) A left Holmes-Adie pupil with greater anisocoria in light (IPA=0.05). An IPA greater than 0.11 indicated an RAPD in our cohort. Abbreviations - IPA: infrared pupillary asymmetry; IR: infrared; RAPD: relative afferent pupillary defect; SD-OCT: spectral-domain optical coherence tomography.

Figure 4a: The pRNFL of the better (red bar) and worse (blue bar) eyes are shown for controls and patients. There was a statistically significant amount of atrophy in the affected (blue bar) eye ($p = 0.01$). Abbreviations – pRNFL: peripapillary retinal nerve fibre layer.

Figure 4b: The IPA was significantly higher in patients with an optic neuropathy if compared to when compared to controls subjects ($p = 0.001$). Abbreviation – IPA: infrared pupillary asymmetry.

Figure 4c: There is a linear relationship ($R = 0.53$, $p = 0.01$) between the IPA and the IEPD for the ganglion cell inner plexiform layer density (GCIPL-IEPD). Abbreviations – GCIPL: ganglion cell-inner plexiform layer; IEPD: inter-eye percentage difference; IPA: infrared pupillary asymmetry.

Disclosures

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