Mitochondrial Disease Ophthalmology Test Guidance

1. GENERAL INSTUCTIONS

A consistent protocol and uniform approach to measurement and analysis is needed for studies with eye and vision outcomes. Devices/machines used should be consistent for the length of a study. This uniform approach should apply to the test conducted, e.g., the plates used in color vision testing should be consistent, as should the method of visual field measurement. Units of measure and the study outcomes should be collected.

2. OPTICAL COHERENCE TOMOGRAPHY

A. Introduction

Optical coherence tomography (OCT) studies in Leber's hereditary optic neuropathy (LHON) re-defined quantitatively the changes that characterize the preclinical (Savini et al., 2005), acute and chronic stages (Barboni et al., 2005) of the disease. These OCT studies also described the dynamic events characterizing the natural history of disease conversion from unaffected mutation carrier to affected (Barboni et al., 2010). Furthermore, optic nerve head (ONH) analysis suggested that a larger optic disc is a protective factor in carrier and in participants who recover vision (Ramos et al., 2009). Similarly, OCT studies have led to define the concept that participants with Dominant Optic Atrophy (DOA) have a congenital lower amount of axons and smaller optic discs (Milea et al., 2010, Barboni et al., 2010), that the rate of progression (loss of fibers) parallels that of age-dependent thinning of retinal nerve fiber layer (RNFL) (Barboni et al., 2011) and finally that the earliest event is loss of macular retinal ganglion cells (RGCs), followed by thinning of the retinal nerve fiber layer (RNFL), progressing from the papillomacular bundle to the remaining quadrants (Ronnback et al., 2013, Barboni et al., 2014). All these studies support the usefulness of a series of standardized exams based on OCT to monitor mitochondrial optic neuropathies.

Refractive Error and Axial Length measures may be needed for magnification adjustment.

There are newer imaging options and emerging technologies available; however, additional research is needed in optic neuropathies in mitochondrial disease, as not much data is available. For radial scans of the optic nerve, along with the Heidelberg Spectralis, there is the Bruchs-Membrane-Opening Minimal Retinal Rim Width measure of the nerve (BMO-MRRW). Enhanced Depth Imaging (EDI-OCT) has been used for Optic Disc Drusen assessment of the optic disc, as well as choroidal thickness measurement.

OCT Angiography (OCT-A) (macular and peripapillary) is also being widely implemented in research. Previously this has focused on the macular angiography, but there has been increasing literature on peripapillary changes which is of interest in mitochondrial optic neuropathy. Nominative data is still being collected in this area. OCT-A equipment and the training to interpret collected data is not available to all researchers. OCT-A has variability in data across different manufacturers' devices. Measurements are currently considered proprietary. OCT-A has a custom based approach to analysis, it is not provided by the manufacturer, which makes standardization difficult. Ideally, the same machine should be used for the length of the study with the machine type and software version recorded.

B. Assessment of macular and optic nerve thickness

Available platforms: Cirrus, Optoview and Spectralis

These machines do not produce comparable data and the same platform should be used to standardize data collection.

I. Macular cube (512x128) – centered on the fovea

Available platforms: Cirrus and Optoview

Cirrus provides a measurement of the macular retinal ganglion cell and inner plexiform layer (GC-IPL) thickness, whereas Optoview provides measurement of the ganglion cell complex (GCC+RNFL). The thickness measurements (μm) are provided as an average and as 6 individual sectors.

II. Optic disc cube (200x200) – centered on the optic disc

Available platforms: Cirrus, Optoview and Spectralis

For the measurement of peripapillary retinal nerve fiber layer thickness and optic nerve parameters, including (i) average RNFL thickness (μm), (ii) temporal, superior, nasal, and inferior quadrants RNFL thickness, (iii) individual clock hours RNFL thickness, (Cirrus, Optoview and Spectralis), (iv) optic disc area (mm^2), (v) optic cup volume (mm^3), (vi) rim area (mm^2), and (vii) average cup to disc ratio (Cirrus and Optoview).

C. Assessment of retinal structure at the macula

Available platforms: Cirrus, Optoview, and Spectralis

Vertical and horizontal 6mm HD Raster lines centered on the fovea are recommended to identify inner nuclear layer (INL) cysts.

Platform: Spectralis
The Spectralis Fast Macula scans are volume scans, and the following parameters are frequently used: (i) 20 degree x 20 degree, (ii) 512 A-scan, and (iii) Automatic Real Time (ART) between 9 and 16. Higher ART (≥ 50) provides higher resolution images but take longer to acquire.

Platform: Cirrus and Optoview
HD Raster collapsed to a single line. Depending on the extent of the lesions the researcher is trying to image, the length (3mm, 6mm and 9mm are available) and orientation of the line can be altered.

D. Methods
RNFL thickness and GC-IPL thickness are measured by SD-OCT.
Scans are acquired using the Optic Disc Cube 200x200 and the Macular Cube 512x128 protocols in eyes with pupil dilation. After the participant has been properly seated and aligned, the iris is brought into view using the mouse-driven alignment system and the fundus image is focused.

In order to acquire the Optic Disc Cube, the ONH is centered on the live image before the centering and enhancement are optimized. The ONH parameters reported result from a fully automatic algorithm that defines both the optic disc and cup margins within the three-dimensional data cube. The disc margin is defined as the termination of Bruch's membrane (also referred to as "neural canal opening" or "Bruch's membrane opening").

In order to acquire the Macular Cube, the participant is asked to fixate on the central target. The Ganglion Cell Analysis (GCA) algorithm of the Cirrus platform detects and measures the thickness of the macular GC-IPL within a 14.13-mm^2 elliptical annulus area centered on the fovea. The GCA algorithm processes data from 3-dimensional volume scans and measures the thickness of the macular ganglion cell-inner plexiform layer (GC-IPL). The average, minimum, and six sectoral (superotemporal (ST), superior (S), superonasal (SN), inferonasal (IN), inferior (I), inferotemporal (IT)) GC-IPL thicknesses were measured from the elliptical annulus centered on the fovea.

E. Data Collection
   I. The study proformas need to be designed carefully, preferably in consultation with an experienced reading center, to ensure a uniform protocol for data collection across multiple centers.
   II. Adequate training for the technicians performing the OCT measurements and ongoing quality control are also essential.
   III. The OCT signal strength should be ≥ 7, without RNFL discontinuity or misalignment, involuntary saccade or blinking artifacts, and absence of algorithm segmentation failure on careful visual inspection.

3. REFRACTION
   A. Refraction right eye (OD):
   B. Refraction left eye (OS):

4. BEST CORRECTED VISUAL ACUITY
   A. ADULTS
      I. Corrected Distance Visual Acuity: ETDRS (logMAR) R EYE:
      II. Corrected Distance VA: ETDRS (logMAR) L EYE:
      III. Corrected Near VA: N-notation R EYE:
      IV. Corrected Near VA: N-notation L EYE:
      V. Corrected Near VA: BINOCULAR:
      Near add right eye (OD):
      Near add left eye (OS):

   B. CHILDREN

Older children who are able to read should be tested in the same way as adults using logMar chart for distance and N notation for near. Younger children should be tested at distance only using letter matching charts (crowded where possible) or if that is not possible, picture matching tests. Visual acuity should be recorded in logMar notation and both the type of test and test distance recorded. Younger children should have a baseline cycloplegic refraction.

   I. Corrected Distance VA: R EYE:
   II. Corrected Distance VA: L EYE:
   Acuity test used:
   Refraction right eye (OD):
   Refraction left eye (OS):
   III. Corrected Near VA: N-notation R EYE:
   IV. Corrected Near VA: N-notation L EYE:
   V. Corrected Near VA: BINOCULAR:
   Near add right eye (OD):
   Near add left eye (OS):
   Cycloplegic refraction: right eye (OD):
   Cycloplegic refraction: left eye (OS):

Instructions:

- Refraction should be measured using cycloplegic autorefraction at initial visit and when there are changes in vision. Subsequent visits should use non-cycloplegic autorefraction.
- Testing distance: Distance VA at 4 m and Near VA at 40 cm.
- If the largest print cannot be read at 40 cm, then bring the participant closer and measure the distance at which the largest print N48 is read. Although it is assumed that “off chart” logMar adjustments can be made, this may not be accurate. Many LHON patients are off the chart, and this may be a problem.
- Room illumination: Well lit (60–120 candela/m^2).
- The participant should be encouraged to read/guess until more than half the letters on a line are missed. There is variability in how this influences the VA – this is not a problem in completely masked trials but may be subject to bias if not masked and participants are encouraged more.

- For “off chart” very poor levels of visual acuity, there are some additional methods that can be used such as the Freiburg Acuity and Contrast Test (FrACT) and the Berkely Rudimentary Vision Test (BRVT).

5. CONTRAST SENSITIVITY
   A. R EYE:
   B. L EYE:
   Instructions:
   - Chart Type: Pelli-Robson chart
   - Testing distance: 1 meter
   - Room Illumination: Well lit

6. COLOR VISION
   A. R EYE:
   B. L EYE:
   Instructions:
   - The Hardy Rand and Rittler (HRR) plates are preferable to the Ishihara plates
   - Other approaches to color vision testing are available
   - Test with correction
   - Testing distance: N/A
   - Illumination: Well lit
   - Complete the HRR testing document

7. TONOMETRY
   A. ADULTS
      I. Applanation tonometry R EYE:
      II. Applanation tonometry L EYE:
      If pressure above 21 mmHg:
      Pachymetry - R EYE:
      Pachymetry - L EYE:
      Specify type: ☐ Optical ☐ Ultrasound ☐ Other, specify:
   Instructions:
   - Optical is preferable to ultrasound if equipment is available
   - Pachymetry may only be measured at the initial visit, but if repeated the same type of equipment should be used for all measurements

   B. CHILDREN
      I. Tonopen or Icare R EYE:
      II. Tonopen or Icare L EYE:
      Specify which test was used:

8. VISUAL FIELDS
   A. Central
      I. Humphrey perimetry (Zeiss): SITA Standard 24-2, 30-2 (Size III stimulus) or Fastpac 30-2 (Size V stimulus)
      II. Octopus perimetry (Haag Streit): G1 protocol
      III. Microperimetry
   Instructions:
   - The Humphrey visual field analyzer provides target sizes ranging from 0.25 mm^2 to 64.00 mm^2 that are represented by the Roman numerals I through V. A size III stimulus (4 mm^2) is conventionally used for participants with good visual acuities of 20/200 or better. A size V (64 mm^2) stimulus can be used to collect more reliable visual field information with poorer levels of vision.
   - We recommend the use of the Fastpac 30-2 (Size V stimulus) testing strategy for participants with significant visual impairment and dense central visual field loss (e.g., participants with LHON).
   - The G1 protocol measures the central 30° of the visual field at 59 positions. The spatial resolution increases towards the center, being maximal in the 2° macular region.

B. Peripheral
   I. Humphrey perimetry (Zeiss): FASTPAC 60-4 (Size V stimulus)
   II. Octopus perimetry (Haag-Streit): Kinetic protocol
   III. Microperimetry

Instructions:

- There are several advantages to using the size V stimulus instead of the size III stimulus in assessing the peripheral visual field. Based on previously published validated studies, the size V stimulus provides higher sensitivity values, a larger dynamic range and a more reliable measurement of the far periphery. The larger size V stimulus also has a better test-retest and within test variability than the smaller size III stimulus. Furthermore, there does not appear to be any disadvantage in detecting subtle or small visual field defects with a size V stimulus compared with a size III stimulus.
- When using the kinetic protocol of the Octopus to assess the visual field beyond 30° eccentricity, we recommend the use of 2 to 3 isopters and spot checks with the V4e, II4e and I3e stimulus targets to provide sufficient coverage of the far peripheral visual field.
- We do not recommend the use of the Goldmann kinetic perimetry due to operator variability and inherent difficulties in standardizing the test protocols across multiple study centers.

C. Reliability Indices
   I. Humphrey perimetry: Fixation Losses and False Negative Errors < 33% and False Positive Errors < 15%
   II. Octopus perimetry: Fixation Losses, False Negative Errors and False Positive Errors < 33%

Instructions:

- The reliability indices above are benchmark figures based on previously published studies. They are not absolute and they should be analyzed in the context of both the severity of the visual loss for the particular disease being studied and the trend observed with repeat measurements at multiple time points.

D. Data Collection
   I. The study proformas need to be designed carefully, preferably in consultation with an experienced reading center, to ensure a uniform protocol for data collection across multiple centers.
   II. Adequate training for the technicians performing the visual field tests and ongoing quality control are also essential.

9. RETINAL IMAGING

A. OPTIC DISC
   R EYE – normal / abnormal
   If abnormal – provide description:
   L EYE – normal / abnormal
   If abnormal – provide description:

B. MACULA
   R EYE – normal / abnormal
   If abnormal – provide description:
   L EYE – normal / abnormal
   If abnormal – provide description:

C. PERIPHERAL RETINA
   R EYE – normal / abnormal
   If abnormal – provide description:
   L EYE – normal / abnormal
   If abnormal – provide description:

D. BLOOD VESSELS
   R EYE – normal / abnormal
   If abnormal – provide description:
   L EYE – normal / abnormal
   If abnormal – provide description:

   Retinal images should be taken to allow for reliable serial analysis, especially if specific abnormalities are noted as part of the examination.

10. AXIAL LENGTH
    - A new readily available measure is Axial Length which is done optically in any clinic that does cataract surgery.
    - Different retinal dystrophies are associated with increasing axial length and myopia.
    - Additionally, for much of the Retinal Imaging there is a need to make magnification adjustments based on axial length of the eye involved.

11. STRABISMUS
    Parameters to be collected:
    A. Prism Cover Test
       Distance and Near
    B. Eye Movements

12. PTOSIS
    A. Margin Reflex Distance (MRD)
    B. Lid crease height in upper lid
    C. Levator excursion

13. VISUAL ELECTROPHYSIOLOGY
    Instructions: Research should be conducted with the same machines, laboratory settings, and lighting levels. Additionally, calibration should be performed on a regular basis. The aforementioned information should be documented.

    For participants with optic nerve disease, it is more practical and valuable to collect electrophysiology parameters related to optic atrophy (e.g., OCT and visual acuity) than parameters related to retinal dystrophy (e.g., electroretinography [ERG]).

    Electrophysiology Parameters (to be collected for each eye):

    Measurements to be collected in accordance with International Society for Clinical Electrophysiology of Vision

    A. PATTERN VEP
       P100 amplitude (mean of onset and offset amplitudes; μV)
       P100 peak time (ms)

    B. FLASH VEP
       P2 amplitude (mean of onset and offset amplitudes; μV)
       P2 peak time (ms)

    C. PERG
       P50 amplitude (μV)
       P50 peak time (ms)
       N95 amplitude (μV)
       Steady-state amplitude (μV)
       Steady-state amplitude (π rad)

    D. FULL-FIELD ERG
       DA 0.01 (cd·s·m-2)
       b wave amplitude (μV)
       b wave peak time (ms)

       DA 10.0 (cd·s·m-2)
       a wave amplitude (μV)
       a wave peak time (ms)

b wave amplitude (μV)
b wave peak time (ms)

LA 30Hz flicker
Peak amplitude (μV)
Peak time (ms)

LA 3.0 (cd·s·m-2)
a wave amplitude (μV)
a wave peak time (ms)
b wave amplitude (μV)
b wave peak time (ms)

14. NIH CDE REPOSITORY

CDEs related to eye and vision outcomes can also be found in the National Eye Institute (NEI) section of the NIH CDE Repository: https://cde.nlm.nih.gov/home

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