Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

Pediatric Optic Neuritis Prospective Outcomes Study (PON1)

Protocol

Version 2.0
April 11, 2016

PEDIATRIC OPTIC NEURITIS PROSPECTIVE OUTCOMES STUDY (PON1)

PROTOCOL AMENDMENT I (4-11-16)

Proposed Change # 1

Current Protocol
A cycloplegic refraction is required at the time of enrollment for all subjects.

Proposed Change
If a cycloplegic refraction has been done by a pediatric ophthalmologist or pediatric optometrist prior to referral for optic neuritis within 1 month of enrollment, then that refraction can be used for testing visual acuity and does not need to be repeated.

Rationale for Change
Allowing a cycloplegic refraction within 1 month of enrollment better matches clinical practice and referral patterns for subjects who might be enrolled into the study.

Proposed Change # 2

Current Protocol
At the 2-year visit, if monocular distance best-corrected visual acuity is below age norms in either eye as defined in Table 1, a cycloplegic refraction should be completed.

Proposed Change
To only require a cycloplegic refraction at the 2-year visit if one was not already done by a referring pediatric ophthalmologist or pediatric optometrist within 1 month.

Rationale for Change
Allowing a cycloplegic refraction within 1 month of the 2-year visit if monocular distance best-corrected visual acuity is below age norms in either eye is acceptable to ensure an accurate visual acuity and better matches clinical practice and referral patterns for subjects who complete the 2-year visit.

Proposed Change # 3

Current Protocol
The current protocol is not clear with respect to whether collection of blood or serum for serological analysis is being done for the study and reads as follows:

- Serological analysis –NMO antibody measurement by ELISA is collected in most centers. If done, the results will be entered on the case report form (CRF). In addition the study will collect serum for analysis by a lab which will be used as the definitive NMO marker by the study, as well as for other antibodies.

Proposed Change

The protocol has been revised to make it clear that blood or serum will only be collected if the parent/s (and child, if old enough to provide assent) agrees to participate and is already having blood or serum collected as part of standard care. The proposed change to the protocol is as follows:

- Serological analysis – NMO antibody measurement by ELISA is collected in most centers. If done, the results will be collected.
    - If blood or serum has already been (or will be) collected as part of standard care, the parent/s (and child, if old enough to provide assent) will be given the option to participate in a study to measure NMO antibodies. Participants in this optional study will have blood or serum collected for analyses as described in a separate procedures manual.

Proposed Change # 4

Current Protocol

The protocol was not clear with respect to whether or not the MRI testing cited at the 2-year visit was done for research purposes as part of the study.

Proposed Change

At two years, children with optic neuritis typically will undergo another MRI of the brain and orbits with intravenous gadolinium contrast as part of standard care to evaluate if there are new white matter lesions. The protocol has been revised to make it clear that MRI data will only be collected at the 2-year visit if an MRI was already done as part of standard care. The protocol now reads as follows:

- Data from the last MRI done since enrollment as part of standard care (or from an MRI if ordered as part of standard care at the time of the 2 year visit), will be collected and sent to the JAEB Center for analyses.

Additional edits to the protocol have been made to be consistent with the above proposals.

Miscellaneous Changes:

The analysis chapter has been updated to be specific to primary and secondary objectives. Exploratory analyses will be described in the analysis plan.

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CHAPTER 1: BACKGROUND AND SUMMARY

This study is being conducted by the Pediatric Eye Disease Investigator Group (PEDIG) with the cooperation of the Neuro-Ophthalmology Research Disease Investigator Consortium (NORDIC) and is funded through a cooperative agreement between the National Eye Institute and PEDIG.

1.1 Background

A literature review for the background was conducted via a Medline search using the PubMed database (National Library of Medicine) through 2015. A combination of key words including: optic neuritis, pediatric optic neuritis, multiple sclerosis, neuroretinitis, clinically isolated syndrome, neuromyelitis optica, and acute disseminated encephalomyelitis were used and cross-referenced with various key words to retrieve specific articles. The search was confined to articles written in English and other-language publications with an English abstract. Cross references cited in the reference list of the major retrieved articles, as well as from the PubMed link "related articles," were also reviewed.

Optic neuritis (ON) is an acute inflammatory disease of the optic nerve.[1] It can be the initial manifestation of multiple sclerosis (MS) in children and adults. Patients develop vision loss, pain with eye movements, dyschromatopsia, and visual field defects.[2]

The pathogenesis of pediatric optic neuritis (PON) is not well understood. Our understanding about causation, natural history of PON, response to therapy, and prognosis is primarily based on case reports and case series, in addition to extrapolation from adult data.[3-7] In the 1950s, corticosteroids were used first to treat demyelinating diseases including optic neuritis and MS. Corticosteroids have become the mainstay of treatment for acute optic neuritis despite a lack of proven long-term efficacy and well-documented side effects. In adults, the Optic Neuritis Treatment Trial (ONTT) has informed the evaluation, treatment, and outcome of demyelinating diseases in adults.[8] The ONTT was designed to evaluate whether corticosteroids were effective as a treatment for optic neuritis in adults (ages 18-46 years).[9] The primary outcome measures were visual fields and contrast sensitivity at 6 months; secondary outcomes included visual acuity and color vision.[10] The study showed that the administration of intravenous corticosteroids hastens visual recovery in adults but did not affect the long-term visual or neurologic prognosis.[11-13] Furthermore, in the ONTT initial treatment with oral corticosteroids was associated with an increased risk of relapse; however, the relation between treatment and risk of relapse remains unclear.[14] Moreover, intravenous corticosteroids did not prevent optic nerve atrophy[15] and may be harmful.[16] Importantly, the ONTT did not include any pediatric patients and therefore is not generalizable to the pediatric population.

There are differences between adult and pediatric optic neuritis that may impact visual recovery and development of MS. First, children often present with a poorer visual acuity than adults. Children are more likely to present with bilateral involvement than adults. In addition, a greater percentage of children present with visible optic disc swelling than adults. This finding was found to be protective in adults with ON. A recent meta-analysis of cases involving children with ON revealed that the risk of MS was higher in children who were older and in those who presented with white matter lesions on magnetic resonance imaging (MRI).[17] However, this meta-analysis could not draw definitive conclusions regarding the natural history of the disease or provide evidence that treatment was beneficial since it was a retrospective review of multiple studies with varying treatment protocols and patient populations.

Despite the lack of evidence of long-term efficacy and the potential for adverse reactions even with short-term therapy, most pediatric neuro-ophthalmologists and neurologists prescribe a short course of intravenous corticosteroids as a treatment for acute PON. However, no prospective study or randomized controlled trial has been performed to guide the management of children with ON. In a recent survey, 86% of physicians felt that not all acute attacks of central nervous system (CNS) demyelinating disease require treatment.[18] Furthermore a survey involving a hypothetical treatment trial of PON revealed that 98% of the 49 eye professionals queried would enroll their patients into such a trial,[19] thereby demonstrating the need for an evidence basis for care of PON.

In order to resolve the controversy concerning the benefit of corticosteroids for PON and to establish appropriate treatment guidelines, a randomized, placebo-controlled trial of the effects of corticosteroids in pediatric participants with acute optic neuritis – a Pediatric Optic Neuritis Treatment Trial (PONTT) - is needed. Clinical trials often have difficulty with enrollment and the incidence of PON is low. Therefore, we propose a pilot study with two primary aims: to determine the feasibility of a PONTT and to test study procedures. A secondary aim is to prospectively study a large group of children with optic neuritis which will provide data to guide patients and physicians with regard to treatment trends, prognosis, and associated diseases. These data will be far more robust than the few retrospective studies reported to date.

This pilot study includes two years of enrollment in a prospective data collection including PEDIG and NORDIC investigators to assess the ability to enroll sufficient patients for a future RCT and to evaluate clinical outcomes in this population. Outcome measures will be visual acuity (low and high contrast) and optical coherence tomography (OCT) of the retinal nerve fiber layer (RNFL). Treatment decisions will be at investigator discretion. The primary outcome is visual acuity at 6 months. Secondary aims will be to characterize optic neuritis in a large multicenter cohort of children and to assess the risk of development of MS at two years.

1.2 Study Objective and Specific Aims

Primary Aims:

1. To determine our investigators' ability to enroll children with PON into a research protocol.
2. To develop estimates of visual acuity outcomes in children with PON 6 months following enrollment.

Secondary Aims:

1. To describe visual acuity outcomes at 1 month, 1 year, and 2 years after enrollment.
2. To estimate the risk of an acute optic neuritis recurrence in the same eye at two years after enrollment.
3. To estimate the risk of developing acute PON in the fellow eye at two years after enrollment for children with unilateral PON at presentation.
4. To estimate the risk of diagnosis of MS 2 years after enrollment.
5. To characterize PON including prevalence of white matter lesions on MRI and neuromyelitis optica (NMO) antibodies at enrollment.
6. To determine retinal nerve fiber thickness and ganglion cell layer thickness - using spectral domain OCT at 1 month, 6 months, 1 year and 2 years after enrollment.
7. To collect MRIs for future analyses.
8. To assess quality of life (QOL) outcomes using current pediatric instruments at 6 months.
9. To describe low contrast visual acuity outcomes at 6 months, 1 year and 2 years after enrollment.

The study will enroll children and teenagers 3 to <16 years of age who present with unilateral or bilateral optic neuritis over a 2-year enrollment period, or until 100 children are enrolled, whichever comes first.

1.3 Synopsis of Study Protocol

Children and teenagers (3 to <16 years of age at the time of enrollment) who present within two weeks of onset of unilateral or bilateral optic neuritis will be invited to participate.

Written informed consent will be obtained from the parent or guardian and a child assent form where required. After consent, study-specified visual function measurements are made including low contrast visual acuity and visual acuity measurements using the amblyopia treatment study protocol specific for age (ATS-HOTV for <7 years of age, E-ETDRS for ≥7 years) using cycloplegic refraction in trial frames if current refractive correction is not optimal or not available.[20, 21] If these tests are standard care for the clinic and thus collected as part of the patient's normal evaluation, they do not need to be repeated after the consent. If a cycloplegic refraction has been done by a pediatric ophthalmologist or pediatric optometrist prior to referral for optic neuritis within the past 1 month, then that refraction can be used for testing visual acuity and does not need to be repeated.

Quality of Life (QoL) surveys (EyeQ, PedsQL)[22, 23] will be administered at the 6-month follow-up visit. Spectral domain optical coherence tomography (OCT) data will be collected when performed as part of usual care.

Participants will be enrolled if there is a clinical diagnosis of optic neuritis based on diminished visual acuity, abnormal visual field, abnormal color vision, a relative afferent pupillary defect, or optic disk edema. Children with optic neuritis typically undergo MRI of the brain and orbits with intravenous gadolinium contrast as part of standard care. The cohort of primary interest will have optic nerve enhancement on fat suppression or abnormal signal on short tau inversion recovery (STIR) imaging. Children without evidence of optic nerve enhancement ipsilateral to the visual loss on MRI may have been enrolled prior to MRI completion, and will be followed but analyzed separately.

The treatment of participants is not determined by the protocol, but rather will be determined by the managing physicians. Participants will be asked to return after enrollment for follow-up visits at 1-month (+/-1 week), 6-months (+/- 2 weeks), 1-year (+/- 1 month) and 2-years (+/- 2 months) following enrollment, which is consistent with standard care. Testing at follow-up visits will include Electronic Visual Acuity (EVA) visual acuity, low contrast visual acuity (2.5%) on the EVA test platform, and quality of life questionnaires (6 months only). OCT testing is not required for enrollment but may be performed as standard care at follow up visits when indicated and available.

CHAPTER 2: SUBJECT ENROLLMENT

2.1 Eligibility Assessment and Informed Consent/Assent

A child is considered for the study after undergoing a routine eye examination (by a study investigator as part of standard care) that identifies optic neuritis in at least one eye appearing to meet the eligibility criteria. The study will be discussed with the child's parent(s) or guardian(s) (referred to subsequently as parent(s)). Parent(s) who express an interest in the study will be given a copy of the informed consent form to read. An assent will be obtained from children according to IRB requirements. Written informed consent / assent must be obtained from a parent and child prior to performing any study-specific procedures that are not part of the participant's routine care.

2.2 Eligibility and Exclusion Criteria

2.2.1 Eligibility

Children who present with unilateral or bilateral optic neuritis will be eligible for the study if they meet each of the following criteria:

1. 3 to < 16 years of age
2. Optic neuritis in at least one eye based upon clinical diagnosis
3. Visual loss and/or pain on eye movements for 2 weeks or less
4. Ipsilateral relative afferent pupillary defect if unilateral PON
5. At least one of the following in the affected eye:
   - Visual acuity deficit at least 0.2 logMAR below age-based norms from EVA ATS-HOTV or e-ETDRS testing (see Table 1); OR
   - Diminished color vision; OR
   - Abnormal visual field; OR
   - Optic disk swelling
6. Requirements for refractive error correction (based on a cycloplegic refraction performed at enrollment or as part of the examination by a referring pediatric ophthalmologist within the past 1 month): Myopia of • -0.75D, hyperopia • 4.00D, and cylinder • 1.50D must be corrected using habitual glasses or trial frames if there is a change in the best correction.
   a. Refractive error correction prescribing instructions:
      - Hyperopic spherical equivalent (SE) must not be under corrected by more than 2.00D SE
      - Cylinder power in both eyes must be within 0.75D of fully correcting the astigmatism.
      - Axis must be within +/- 10 degrees if cylinder power is ” 1.00D, and within +/- 5 degrees if cylinder power is >1.00D.
      - Myopia SE must not be under-corrected by more than 0.25D or over corrected by more than 0.75
7. MRI of the orbits has been done with evidence of enhancement of the optic nerve (s) or will be done in the next two weeks as standard care.
8. Idiopathic optic neuritis, optic neuritis associated with MS, and optic neuritis associated with other causes not known at presentation.

Table 1: Normal Visual Acuity Values Based on Age

Age range	Normal Visual Acuity	Visual Acuity Deficit
36-47 months	20/50 or better	20/63 or worse
48-71 months	20/40 or better	20/50 or worse
72-83 months	20/32 or better	20/40 or worse
≥84 months	77 letters	<77 letters

Normal values for ATS-HOTV for children <84 months of age based on a study by Yamada et al[24] and Pan et al.[25] Normal values for children ≥84 months of age based on a study by Drover et al.[26] The lower limit for the 95% tolerance interval was used to establish normal range of E-ETDRS visual acuity.

2.2.2 Exclusion

1. Study participants who do not expect to remain in area for the next two years and follow up at another study center is not possible
2. Participants whose parents/guardians in the opinion of the Investigator, may be non-compliant with study schedules or procedures.
3. Neuro-retinitis (if the diagnosis is made after enrollment they will be followed, but not included in the primary cohort)
4. Previous episode of optic neuritis in the same (currently affected) eye (established or presumed due to optic atrophy). Prior episode in fellow eye does not exclude enrollment.
5. CSF or laboratory values suggesting a condition other than optic neuritis.
6. Ocular or systemic findings suggestive of non-demyelinating cause for optic neuritis in the affected eye(s) such as an associated history of sarcoidosis, systemic lupus erythematosus, collagen vascular disease, or Lyme disease with overt symptoms beyond serum markers such as rash, peripheral neuropathy, or liver disease.
7. Preexisting ocular abnormalities that might affect assessment of visual function in the affected eye(s). (Preexisting ocular abnormalities in the fellow eye are not an exclusion criterion.)

2.3 Procedures at Enrollment Visit

Demographic and clinical information will be collected from medical records and during the baseline examination, including presenting signs/symptoms. Informed consent and assent (if applicable) will include permission for the Coordinating Center to receive personal health information from participants or from medical records extracted by the clinic staff. This information will include birth history, demographic and socioeconomic information, medical history, and family history. The Contact Information Form will be completed and transmitted via a secure link to the Jaeb Center.

1. Distance Visual Acuity Testing: Distance visual acuity testing will be performed in current refractive correction (if prescribed) monocularly in each eye (right, then left) by a certified examiner using the electronic ATS-HOTV visual acuity protocol for children <7 years and the E-ETDRS visual acuity protocol for children 7 years on a study-certified acuity tester displaying single surrounded optotypes as described in the ATS Testing Procedures Manual.

   - A cycloplegic refraction is generally performed at enrollment for all participants. If a cycloplegic refraction has been done by a pediatric ophthalmologist or pediatric optometrist prior to referral for optic neuritis within the past 1 month, then that refraction can be used for testing visual acuity and does not need to be repeated.

- If a participant is found to have refractive error that does not meet the conditions for uncorrected or corrected visual acuity testing listed below, then visual acuity testing will be repeated with appropriate refractive correction in a trial frame determined from cycloplegic refraction. The best visual acuity (prior to cycloplegia, or after cycloplegia in trial frames if done) will be used to assess eligibility with respect to visual acuity age norms in section 2.2.1.
    - Myopia of • -0.75D, hyperopia • 4.00D, and cylinder • 1.50D must be corrected using habitual glasses or trial frames if there is a change in the best correction.
    - Refractive error correction for visual acuity and low-contrast testing:
        - Hyperopic spherical equivalent (SE) must not be under corrected by more than 2.00D SE
        - Cylinder power in both eyes must be within 0.75D of fully correcting the astigmatism.
        - Axis must be within +/- 10 degrees if cylinder power is ” 1.00D, and within +/- 5 degrees if cylinder power is >1.00D.
        - Myopia SE must not be under-corrected by more than 0.25D or over corrected by more than 0.75D.

2. Monocular low contrast visual acuity using 2.5% low contrast charts presented on the EVA Tester using the ATS-HOTV visual acuity protocol for children <7 years and the E-ETDRS visual acuity protocol for children 7 years. Refractive error correction procedures as for visual acuity testing in section 2.3.1.

3. MRI of the brain and orbits with and without gadolinium using a standard care technique for optic neuritis. The techniques in standard use include fat-saturation for orbital images and STIR. (A participant may be enrolled and undergo visual acuity testing before the MRI is performed.) MRI data will be collected and sent to the Jaeb Center for analyses.

4. OCT data will be collected if performed (but not required for study enrollment) and will include mean peripapillary retinal nerve fiber layer thickness (RNFL). Optic disc centered and foveal-centered images will be obtained.

5. Serological analysis – NMO antibody measurement by ELISA is collected in most centers. If done, the results will be collected.
    - If blood or serum has already been (or will be) collected as part of standard care, the parent/s (and child, if old enough for assent) will be given the option to participate in a study to measure NMO antibodies. Participants in this optional study will have blood or serum collected for analyses as described in a separate procedures manual.

CHAPTER 3: TREATMENT AND FOLLOW-UP

Participants are expected to have standard care visits at 1 month (+/-1 week), 6 months (+/- 2 weeks), 1 year (+/- 1 month) and 2 years (+/- 2 months) after enrollment.

Sites will follow their local IRB requirements with respect to the assent of participants who reach the age of assent during the study.

At each study visit, testing will include the following:

- Monocular distance visual acuity in each eye using the same testing protocol (ATS-HOTV or e-ETDRS) as used at the time of enrollment
- Monocular low contrast acuity in each eye using the same testing protocol (ATS-HOTV or e-ETDRS) as used at the time of enrollment
- OCT if performed (but not required) to include optic disc centered and foveal centered imaging using the same type of instrument as used at the time of enrollment
- Assessment by investigator if there has been a new diagnosis of acute PON in a previously unaffected eye based on criterion 5 as defined in section 2.2.1 or a recurrence of acute PON in either eye, defined as a reduction of visual acuity of at least 0.2 logMAR lines from the prior exam.
    - Note: A recurrence will not be diagnosed if there is loss of vision within two weeks of discontinuation of steroids.
    - A Protocol Chair should be contacted after any visit to confer regarding the diagnosis of a previous or current episode of optic neuritis if there is evidence of the following:
        - *f* A non-demyelinating cause for optic neuritis based on ocular or systemic findings
        - *f* The condition may be due to another cause on the basis of clinical findings, CSF, imaging or laboratory values (if collected)
- Recording any treatment prescribed (medication and dosage)

<u>Additional testing and data collection at one month will include the following:</u>

- A questionnaire will be administered to each participant to determine whether the child is pre-pubertal, pubertal, or post-pubertal.
- The presence or absence of white matter lesions of the brain on the T2 or FLAIR images, not including the optic nerves, in the enrollment imaging, will be documented by the investigator.
- The PON Study neurologic symptom questionnaire will be administered and the PON neurological summary diagnosis form will be completed.
    - The investigator should use the results of the neurologic symptom questionnaire and a neurological exam to complete the summary diagnosis form concerning whether the patient may have ON associated with MS, acute disseminated encephalomyelitis (ADEM), or NMO.
        - *f* A neurological exam by a pediatric neurologist or MS specialist may be obtained at investigator discretion to aid in making the neurological diagnosis.
        - *f* If no pediatric neurologist or MS specialist has been consulted, a neurological examination will be performed by the investigator.
    - The investigator will report neurological results as isolated optic neuritis, ADEM, Clinically Isolated Syndrome, or NMO spectrum (antibody positive or antibody negative)

- The completed form will be reviewed along with imaging by study MS expert for verification of classification.

Additional testing and data collection at 6 months will include the following:

- Binocular visual acuity and binocular contrast visual acuity.
- QOL surveys (Eye Q and PedsQL).

Additional testing and data collection at 1 year will include the following:

- The PON Study neurologic symptom questionnaire will be administered and the PON neurological summary diagnosis form will be completed as previously outlined (refer to additional testing and data collection at one month).

Additional testing and data collection at 2 years will include the following:

- Binocular visual acuity and binocular contrast visual acuity.
- The PON Study neurologic symptom questionnaire will be administered and the PON neurological summary diagnosis form will be completed as previously outlined (refer to additional testing and data collection at one month).
- At two years, children with optic neuritis typically will undergo another MRI of the brain and orbits with intravenous gadolinium contrast as part of standard care to evaluate if there are new white matter lesions.
    - Data from the last MRI done since enrollment as part of standard care (or from an MRI if ordered as part of standard care at the time of the 2 year visit), will be collected and sent to the Jaeb Center for analyses.
- If monocular distance best-corrected visual acuity is below age norms in either eye as defined in Table 1, a cycloplegic refraction should be completed (if not done by the referring pediatric ophthalmologist or pediatric optometrist within the past 1 month).
    - If the cycloplegic refraction is different (as defined below) than the optical correction used for initial testing, monocular and binocular distance and low-contrast visual acuities should be retested in trial frames or with a phoropter. A significant difference is defined as:
        - > 0.25 D decrease in hyperopia
        - > 0.25 D increase in myopia
        - > 0.50 D change in cylinder power
        - > 10 degrees change in axis

Summary of Study Visits and Associated Testing to be Performed:

Test	Baseline	1 Month	6 Months	1 Year	2 Years
Visual Acuity	X (M only)	X (M only)	X (M, B)	X (M only)	X (M, B)
2.5% Low Contrast Acuity	X (M only)	X (M only)	X (M, B)	X (M only)	X (M, B)
Cycloplegic Refraction	X				X if vision below age norms
OCT (optional)	X	X	X	X	X
Serology NMO, Lyme	X*				
EyeQ			X		
PedsQL			X		
Puberty Questionnaire		X			
MRI	X**				X**
MS Questionnaire & Neurological Examination		X		X	X

505 M: monocular, B: binocular, MS Questionnaire: Neurologic Symptom Questionnaire for development of MS

*If blood or serum has already been (or will be) collected as part of standard care, and parent/s and participant agree to participate
** MRI will be collected if done as part of standard care

Additional non-study visits can be performed at the discretion of the investigator.

After completion of the two-year follow-up visit, if participants and their parents have given permission for future contact in their site-specific IRB-approved consent, they may be contacted again in the future.

CHAPTER 4: MISCELLANEOUS CONSIDERATIONS

4.1 Contacts by the Jaeb Center for Health Research

The Jaeb Center serves as the PEDIG Coordinating Center. The Jaeb Center will be provided with the parent's contact information. The Jaeb Center may contact the parents of the participants. Permission for such contacts will be included in the Informed Consent Form. The principal purpose of the contacts will be to develop and maintain rapport with the participant and/or family and to help coordinate scheduling of the outcome examinations.

4.2 Participant Withdrawals

Parents may withdraw their child from the study at any time. A participant who has reached 18 years of age before the two-year outcome may withdraw at any time. Consent of the participant to continue would be needed. These are expected to be very infrequent occurrences in view of the study design's similarity to routine clinical practice and recruitment only up to the 16th birthday.

4.3 Adverse Events/Risks

Adverse events will not be collected per se, as the study is a prospective data collection without a study protocol to be followed and includes no specific intervention or use of a device or drug as part of the protocol. The risk to the participant for participation in the registry is related to the unlikely chance that an unauthorized person views sensitive personal health information. Efforts to ensure this does not occur are described in section 4.7.

4.4 Benefits for Participants

There are no direct benefits for the participants. Results of this study may provide important knowledge that will be generalizable to the care of children with pediatric optic neuritis.

4.5 Travel Reimbursement

Parents of each participant will be compensated $50 per visit for completion of each visit: enrollment, 1-month, 6-month, 1-year and 2-year protocol-specified visits, for a maximum of $250. If there are extenuating circumstances, and the participant is unable to complete study visits without additional funds for travel costs, additional funds may be provided.

4.6 Study Costs

The participant or his/her insurance provider will be responsible for the costs at all visits as they are considered standard care. Any clinical and laboratory costs (including the cost for any OCT or MRI testing, and any blood or serum draw) will be the participant's/parent's responsibility just as they would be if they were not taking part in the study.

4.7 Confidentiality

Each participant will be assigned a study unique identification number. All data and other information sent to the coordinating center for the project will be identified with this number.

In addition, to help identify the study participant, the informed consent form and the assent form (if applicable) will include permission for the Coordinating Center to receive the child's initials (first, middle, and last name initial).

4.8 General Considerations

The study is being conducted in compliance with the policies described in the study policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice.

Data will be directly collected in electronic CRFs, which will be considered the source data.

There is no restriction on the number of participants to be enrolled by each site towards the overall recruitment goal.

A risk-based monitoring approach will be followed, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013).

It is the investigators' opinion that the protocol's level of risk falls under DHHS 46.404, which is research not involving greater than minimal risk.

CHAPTER 5: SAMPLE SIZE ESTIMATIONS AND STATISTICAL ANALYSIS

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to any tabulation or analysis of any study outcome data, excepting recruitment data. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan, which will supersede these sections when it is finalized.

The primary and secondary objectives are to characterize PON and clinical outcomes in a cohort of children with presentation of acute optic neuritis; therefore, the analysis is largely descriptive.

5.1 Sample Size

A convenience sample size of 100 patients was chosen. The goal is to recruit 100 patients with PON over a two-year enrollment period. If enrolled, participants without evidence on MRI of optic nerve enhancement ipsilateral to visual acuity loss will be included as part of the total sample size.

5.2 Primary Analyses

The primary objectives of the study are as follows:

1. To determine investigators' ability to enroll children with PON into a research protocol.
2. To develop estimates of visual acuity outcomes in children with PON 6 months after enrollment, regardless of treatment.

5.2.1 Recruitment Feasibility & Laterality

The average number of participants recruited per site per month will be calculated (overall and according to laterality at presentation as defined below) and used to evaluate recruitment feasibility for any future randomized trial.

Participants may contribute one or both eyes in the study (referred to as study eye(s)) as defined in section 5.3. Laterality at PON presentation will be characterized as follows:

1. Unilateral - if an acute PON attack (as defined in section 2.2), has occurred in only one eye with no previous episode of acute PON in the other eye (referred to as fellow eye)
2. Bilateral simultaneous - if onset of acute PON occurs in both eyes within 1 month of each other
3. Bilateral sequential - if onset of acute PON occurs in both eyes more than 1 month apart

If the fellow eye develops acute PON after enrollment, participants classified as unilateral PON will be reclassified as bilateral simultaneous if onset of acute PON for both eyes occurs within 1 month of each other; otherwise, laterality at PON at presentation will remain classified as unilateral.

Depending on the sample sizes within each laterality subgroup, the subgroup with the smallest sample size may be combined with one of the two remaining subgroups that more closely resembles the clinical profile of the disease.

5.2.2 Visual Acuity Outcomes at 6 Months

The distribution of monocular visual acuity at 6 months after enrollment will be tabulated with computation of descriptive statistics for study eyes. For those with 2 study eyes, monocular visual acuity at 6 months after enrollment will be cross tabulated between the 2 eyes.

The primary outcome will be visual acuity deficit in the study eye(s) at 6 months after enrollment, defined as the difference between the observed visual acuity and the normal visual acuity for age at the time of the 6-month visit. The mean visual acuity deficit in the study eye(s) at 6 months after enrollment and the corresponding 95% confidence interval (CI) will be calculated by age group using a linear mixed model when necessary to adjust for the inter-eye correlation due to some participants contributing 2 study eyes.

Whether the visual acuity deficit in the study eye(s) differs by laterality of PON at presentation will also be evaluated in the linear mixed model. If there is a difference according to laterality at PON presentation, the analysis will be stratified by laterality.

5.3 Principles to be followed for Analyses

1. Analyses for eye-level outcomes will only include study eyes, consisting of eligible eyes diagnosed with a first episode of acute PON at enrollment (defined in section 2.2), and fellow eyes unaffected at enrollment, but with onset of acute PON within 1 month of the initially enrolled eye. Participants classified as having bilateral simultaneous PON at presentation may contribute 2 study eyes for the analyses if either (1) both eyes meet eligibility criteria at enrollment (section 2.2) or (2) the fellow eye develops a first episode of acute PON after enrollment (chapter 3) and PON onset between both eyes occurs within 1 month of each other. To be considered a study eye, the following criteria will be applied with respect to ipsilateral optic nerve enhancement in eligible eyes:
    - If only one eye is eligible, there must be evidence of ipsilateral optic nerve enhancement on MRI for that eye to be counted as a study eye.
    - For participants with bilateral simultaneous PON at presentation with 2 eligible eyes, both eyes will be counted as study eyes if there is evidence of optic nerve enhancement for at least one of the eyes. The number of eyes without ipsilateral optic nerve enhancement will be reported.
2. Participant- and eye-level outcomes from participants without evidence of optic nerve enhancement for any eligible eyes at PON presentation will be analyzed separately from those with optic nerve enhancement on MRI. Descriptive statistics will be computed to characterize visual acuity outcomes in this cohort.
3. Analyses of eye-level outcomes or covariates at the 1-month visit will only include study eyes with a diagnosis of acute PON at enrollment. All study eyes will be included for eye-level analyses occurring after the 1-month visit.
4. Distance visual acuity and low contrast visual acuity scores will be converted to the logMAR scale so that E-ETDRS letter scores and HOTV scores can be pooled for analyses.
5. Visual acuity deficit is defined as the difference between the observed visual acuity and the normal visual acuity for age based on the participant's age at the visit.
6. Unless otherwise specified, data will be included only from participants who complete the visit within the predefined analysis window. There will be no imputation of data for participants who miss the visit or who are lost to follow-up or withdraw from the study prior to the visit.

5.4 Secondary Analyses

<u>Factors Associated with Visual Acuity Deficit at 6 Months</u>

In addition to laterality at PON presentation, other person-level and/or eye-level factors will be tested for association with deficit in visual acuity in the study eye(s) at 6 months using linear mixed models with adjustment for inter-eye correlation.

Binocular Summation and Inhibition at 6 Months

Binocular visual acuity will be compared to the better monocular visual acuity of the 2 eyes at 6 months after enrollment. For each participant, a measure of summation/inhibition will be computed as the difference between the binocular and monocular (Binocular – Monocular) visual acuity score (logMAR) at 6 months after enrollment. The proportion of participants will be tabulated according to the following categories:

1. Binocular summation (binocular visual acuity is better than visual acuity in the better eye alone)
2. Binocular inhibition (binocular visual acuity is worse than visual acuity in the better eye alone)
3. No difference

Analyses will be stratified by laterality at PON presentation if there is an association between laterality and binocular summation.

Additional secondary objectives include the following:

1. Describe visual acuity outcomes at 1 month, 1 year and 2 years after enrollment in children with PON

   Descriptive analyses of monocular visual acuity at 1 month, 1 year and 2 years after enrollment will be performed as defined in section 5.2.2. The primary analyses of visual acuity deficit by age group will be repeated at 1 month, 1 year and 2 years after enrollment as defined for the 6-month visit (section 5.2.2). Binocular summation and inhibition at the 2-year visit will be evaluated as previously described for the 6-month visit. The principles outlined in section 5.3 will also be applied to secondary visual acuity outcomes, and analyses will exclude data from visits completed outside of the predefined analysis windows.

   An analysis of variance will be used to compare unilateral versus bilateral disease at PON presentation with respect to visual acuity deficit at 2 years after enrollment. The visual acuity in the study eye will be used for participants with unilateral or bilateral disease at PON presentation who have only one study eye. For those with bilateral disease with 2 study eyes, the analysis will be performed using (1) the better visual acuity of the 2 study eyes, (2) the worse visual acuity of the 2 study eyes, and (3) the average visual acuity of the 2 study eyes.

2. Estimate the risk of acute optic neuritis recurrence in the same eye at two years after enrollment

   A binomial regression analysis that adjusts for clustered data will be performed to estimate the crude recurrence rate of acute PON in the study eye(s) at the 2-year visit. Study eyes without a 2-year outcome will be included in the 2-year analysis if there was a diagnosis of acute PON recurrence at a previous visit. Multiple imputation will be used to impute a status for missing study eye outcomes at the 2-year visit only for study eyes where acute PON recurrence has not been diagnosed at a previous visit.

   If the crude recurrence rate of acute PON differs according to laterality at PON presentation, the analysis will be stratified by laterality (defined in section 5.2.1).

3. Estimate the risk of developing acute PON in the fellow eye two years after enrollment for participants with unilateral PON at presentation

For participants with unilateral PON at presentation, the crude rate of acute PON in the fellow eye at 2 years after enrollment will be calculated and an exact 95% confidence interval will be computed on the rate. Fellow eyes without a 2-year outcome will be included in the 2-year analysis if there was a diagnosis of acute PON at a previous visit. Multiple imputation will be used to impute a status for missing fellow eye outcomes at the 2-year visit only for fellow eyes with no history of acute PON during follow-up.

4. Estimate the risk of diagnosis of multiple sclerosis at 2 years after enrollment
   A binomial regression analysis will be performed to estimate the crude diagnosis rate of multiple sclerosis (MS) at 2 years after enrollment and an exact 95% confidence interval will be computed on the rate. Participants without a 2-year visit will be included in the 2-year analysis if there was a diagnosis of MS at a previous visit. Multiple imputation will be used to impute a status for missing outcomes at the 2-year visit only for participants where MS has not been diagnosed at a previous visit.

   If the crude diagnosis rate of MS differs according to laterality at PON presentation, then the analysis will be stratified by laterality.

5. Characterize PON including prevalence of white matter lesions on MRI and NMO antibodies at enrollment
   The proportion of participants with white matter lesions on MRI and NMO antibodies at enrollment will be tabulated separately and combined.

6. Determine the change in retinal nerve fiber layer thickness and ganglion cell layer thickness at 1 month, 6 months, 1 year and 2 years after enrollment
   Descriptive analyses of change in RNFL thickness and GCL thickness for the study eye(s) at 1 month, 6 months, 1 year and 2 years after enrollment will be performed as defined in section 5.2.2.

   Linear mixed models that adjust for inter-eye correlation and for correlation between repeated measures of the study eye(s) over time will be used to describe eye-level changes in RNFL and GCL thickness for the study eye(s) across the follow-up visits. If the change in OCT measures differ according to laterality at PON presentation, then the analysis will be stratified by laterality.

7. Collect MRIs for future analyses
   Images from MRI scans submitted at the time of enrollment and at 2 years after enrollment will be collected and reviewed by a MS specialist.

8. Assess quality of life (QOL) outcomes using current pediatric instruments
   Responses to the pediatric quality of life (QOL) survey instruments (Eye Q and PedsQL) will be tabulated separately at 6 months after enrollment, and descriptive statistics computed. Exploratory analyses may be performed to assess the relationship between the QOL survey scores and visual acuity outcomes at 6 months after enrollment.

9. Describe low contrast visual acuity outcomes at 6 months, 1 year and 2 years after enrollment
   Descriptive statistics and cross-tabulations of the distribution of low contrast monocular visual acuity will be computed at the 6-month, 1-year and 2-year visits as described in section 5.2.2. Low contrast visual acuity outcomes in the study eye(s) will be computed at

each of the aforementioned study visits. If these outcomes differ according to laterality at PON presentation, the analyses will be stratified by laterality as described for the visual acuity primary outcome analysis.

CHAPTER 6:
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Pediatric Optic Neuritis Prospective Outcomes Study (PON1)
Baseline Characteristics and 6 Month Visual Acuity Outcomes

Statistical Analysis Plan
Version 1.1

Statistical Analysis Plan Version: 1.1, 8Apr20
Protocol Version: 2.0

VERSION HISTORY

The following table outlines changes for the analysis plan:

VERSION NUMBER	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION*
1.0	E. Lazar	M. Melia	12-19-17	Initial Version
1.1	R. Henderson	M. Melia	4-8-20	Version revised upon work of and completion of the manuscript.

Robert Henderson
I have reviewed this document
2020-04-08 16:10-04:00

Michele Melia
I am approving this document
2020-04-08 16:01-04:00

Pediatric Optic Neuritis Prospective Outcomes Study (PON1)
Baseline Characteristics and 6 Month Visual Acuity Outcomes

Statistical Analysis Plan Version 1.1 (Protocol Version 2.0)
April 7, 2020

1.0 Background and Objectives

The Pediatric Optic Neuritis protocol prospectively studies children and teenagers (3 to <16 years of age at time of consent) who present within two weeks of onset of optic neuritis (unilateral or bilateral). The primary and secondary objectives are to characterize pediatric optic neuritis (PON) and clinical outcomes in this cohort of children with presentation of acute optic neuritis; therefore, the analysis is largely descriptive.

Primary Objectives

The primary objectives of this study are as follows:

1. To determine investigators' ability to enroll children with PON into a research protocol
2. To develop estimates of visual acuity (VA) outcomes in children with PON 6 months after enrollment, regardless of treatment

This statistical analysis plan provides a general overview of the methods and statistical approaches for the primary and secondary objectives outlined in the protocol. Given the broad spectrum and number of proposed study objectives, analysis results of grouped outcomes will be reported across a series of manuscripts. The protocol statistical analysis plan may not be sufficient to address the statistical methods/approach of planned (and post-hoc) analyses for each manuscript. In this case, a manuscript-specific analysis plan will be drafted to provide further details and will supersede relevant sections from this plan.

1.1 Sample Size

A convenience sample size of 100 participants was chosen. The goal is to recruit 100 participants with PON over a 2-year enrollment period. If enrolled, participants without evidence on magnetic resonance imaging (MRI) of optic nerve enhancement ipsilateral to visual acuity loss will be counted towards the total sample size, but only eyes with evidence of optic nerve enhancement ipsilateral to visual acuity loss based on the site's assessment will be counted as study eyes.

The total number of participants with at least one study eye and rate of recruitment of such participants during the 2-year enrollment period will be used to evaluate recruitment feasibility for any future randomized trial.

1.2 Laterality at PON Presentation

Participants may contribute one or both eyes if eligible for the study, subsequently referred to as study eye(s) (defined in section 2.3 of protocol).

At enrollment, laterality at PON presentation will be characterized as follows:

1. Unilateral – if acute PON attack occurred in only one eye with no previous episode of acute PON in the other eye (referred to as the fellow eye)
2. Bilateral simultaneous - if onset of acute PON occurred in both eyes within 1 month of each other
3. Bilateral sequential - if onset of acute PON occurred in both eyes more than 1 month apart

If the fellow eye develops acute PON after enrollment, participants initially classified as unilateral PON will be re-classified as bilateral simultaneous at enrollment and subsequent visits if onset of acute PON for both eyes occurs within 1 month of each other. Otherwise, laterality at PON presentation for all visits will be defined based on the enrollment classification.

For the analyses, laterality at PON presentation may be collapsed into two subgroups depending on the sample sizes within each laterality classification. In this case, the subgroup with the smallest sample size will be combined with one of the two remaining subgroups that more closely resembles the clinical profile of the disease. Given that we expect few (if any) participants to be classified as bilateral sequential, both bilateral subgroups will be combined unless clinical evidence suggests otherwise.

1.3 Defining Study Eyes

According to the protocol (section 5.3), analyses for eye-level outcomes will only include study eyes, defined as follows:

- Eligible eyes diagnosed with a first episode of acute PON at enrollment and history of visual loss and/or pain on eye movements for ≤ 2 weeks prior to enrollment (protocol, section 2.2)
- Fellow eyes unaffected at enrollment, but with onset of acute PON within 1 month after the initially enrolled eye (protocol, chapter 3).

After preliminary review of the data, there was only one fellow eye that developed a first acute episode of PON after enrollment, which was reported at the 1-month visit. A decision was made to exclude this eye from analyses of study eyes given that the enrollment data could not be used to assess eye-level, clinical characteristics at PON presentation and no ocular assessment was conducted at the 1-month visit. Therefore, there would be limited eye-level data collected at PON presentation for this study eye. Laterality at PON presentation will still be reclassified as bilateral simultaneous (defined in section 1.2) at enrollment and follow-up visits for this participant.

Participants classified as having bilateral simultaneous PON at presentation (defined in section 1.2) may contribute 2 study eyes if both eyes meet eligibility criteria at enrollment (see Protocol, section 2.2).

Ipsilateral Optic Nerve Enhancement

To be considered a study eye, there must be evidence of ipsilateral optic nerve enhancement on MRI (based on site assessment) for at least one eligible eye. In other words, if only one eye is eligible, then there must be evidence of optic nerve enhancement for that eye, whereas if 2 eyes are eligible, both eyes will be counted as study eyes if there is evidence of optic nerve enhancement for at least one eye. Because the protocol did not require completion of the baseline MRI by enrollment (protocol, section 2.2), it is possible that children without evidence of optic nerve enhancement on MRI ipsilateral to the visual loss could be enrolled prior to MRI completion. Therefore, the proportion of participants with and without evidence of optic nerve enhancement (if applicable) on MRI at PON presentation will be computed.

In the event there are enrolled participants without evidence of ipsilateral optic nerve enhancement on MRI, data from these participants will be analyzed separately from those with optic nerve enhancement at PON presentation. Note that the sample size may limit (or preclude) the possibility of conducting descriptive analyses for the subset of participants without evidence of optic nerve enhancement.

1.4 General Principles for Analysis

The following principles will be applied to all analyses (unless otherwise noted):

1. Analyses for participant-level outcomes will include all participants with at least one study eye as stipulated in section 1.3. Laterality at PON presentation will be classified as defined in section 1.2.
2. Analyses for eye-level outcomes will only include eyes that are classified as study eyes (defined in section 1.3) *at enrollment*.
3. For each study eye, the visit of PON presentation will be defined as the study visit where the initial episode of acute PON was *first* reported for that eye. Given that analyses of study eyes will only include data from eligible eyes with an initial diagnosis of acute PON at enrollment (section 1.3), the visit of PON presentation for eye-level measures will be defined as enrollment.

4. Unless otherwise specified, analyses of mean estimates or proportions for eye-level outcomes will be adjusted for inter-eye correlation due to some participants contributing 2 study eyes.
5. For each visit, data will be included only from participants who complete the visit within the predefined analysis window (specified below). There will be no imputation of data for participants who miss the visit or who are lost to follow-up or withdraw from the study prior to the visit.

The analysis window for each study visit will be as follows:

- 1 month: 1 week to 2.5 months (7 days to 75 days) after enrollment
- 6 months: >2.5 to 9 months (>75 to 272 days) after enrollment

A visit will be considered missed if it is completed out of window or not completed, but a later visit is completed. Unless otherwise specified, analyses will be limited to visits completed within window for both the primary and secondary outcome measures.

1.4.1 Presentation of Acute PON in Study Eyes

Analyses of eye-level visual acuity measures coinciding with the first acute episode of PON will be based on data collected at enrollment (visit of PON presentation). This definition will also apply to eye-level measures collected from the optic coherence tomography (OCT) scan except for retinal nerve fiber layer (RNFL) thickness. Optic nerve swelling during an acute PON attack precludes accurate measurement of RNFL thickness, so it would not be appropriate to use the RNFL data at enrollment. Therefore, RNFL data collected from study eyes at the 1-month visit (if completed) will be used for the analyses.

1.4.2 Interpretation of MRI Scans

Magnetic resonance imaging (MRI) findings specific to study eye eligibility (evidence of optic nerve enhancement) and clinical outcomes/diagnoses will be based on site interpretation of the MRI scans. These scans will also be read by an independent, masked examiner for confirmatory purposes. Scans with discrepant MRI findings between the site and masked examiner assessment will be flagged for review and adjudicated appropriately. In the event discrepant or indeterminate findings cannot be resolved, the site assessment will be used given that the masked examiner's interpretation is based on observed MRI patterns alone without consideration of other clinical characteristics and medical treatment that may account for these discrepancies.

1.4.3 Visual Acuity

High-contrast monocular and binocular distance VA was tested with refractive correction (if worn) using one of two testing methods, the ATS single-surround HOTV for participants aged 3 to <7 years at enrollment, or the ETDRS for participants aged 7 to <16 years at enrollment. The Electronic Visual Acuity (EVA) Tester was used for both methods. The former method yields Snellen equivalent values, whereas the latter yields letter scores.

Low contrast monocular and binocular VA was measured with refractive correction (if worn) using 2.5% low contrast charts presented on the EVA Tester using the same test method (defined above) performed for distance VA.

Distance VA and low contrast VA (LCVA) scores will be converted to the logMAR scale so that E-ETDRS letter scores and HOTV scores can be pooled for analyses. If a retest was required for distance VA and LCVA at enrollment and/or 2-year visit (refer to chapters 2.3 and 3 of the protocol), distance VA and LCVA will be calculated as the better of the initial test and retest (if done) acuities for each eye (monocular) and for binocular acuity (if required at the visit). Otherwise, distance VA and LCVA scores will be based on the initial test.

2.0 Visual Acuity

2.1 Primary Outcome

The primary outcome is deficit in best-corrected distance VA for study eye(s) at 6 months after enrollment, defined as the difference between the observed monocular VA at distance and the lower limit of the normal distance VA range for age (defined in Table 1) at the time of the 6-month visit. In the event the ATS HOTV method is used for a participant aged ≥ 7 years at enrollment, the normal VA range for age will be defined as 20/25 or better.

Table 1: Normal Distance Visual Acuity Values Based on Age[a]

Age range	Normal Visual Acuity	Visual Acuity Deficit
36-47 months (3 to <4 years)	20/50 or better	20/63 or worse
48-71 months (4 to <6 years)	20/40 or better	20/50 or worse
72-83 months (6 to <7 years)	20/32 or better	20/40 or worse
≥84 months (≥ 7 years)[b]	≥ 77 letters (if E-ETDRS)	<77 letters (if E-ETDRS)

[a] Normal values for ATS-HOTV for children <84 months of age based on a study by Yamada et al[24] and Pan et al.[25] Normal values for children ≥84 months of age based on a study by Drover et al.[26] The lower limit for the 95% tolerance interval was used to establish normal range of E-ETDRS visual acuity.

[b] If monocular distance VA is tested with the ATS HOTV method, the normal VA for age will be defined as 20/25 or better.

The mean VA deficit in the study eye(s) at 6 months after enrollment and the corresponding 95% confidence interval (CI) will be calculated by age group (defined in Table 1) using a linear mixed model when necessary to adjust for the inter-eye correlation due to some participants contributing 2 study eyes. Least squares means will be computed to estimate the mean VA deficit and 95% CIs for the age groups (3 to <4 years, 4 to <6 years, 6 to <7 years and ≥ 7 years), adjusted for inter-eye correlation. Study eye(s) of participants without evidence of ipsilateral optic nerve enhancement on MRI will be analyzed separately from those with optic nerve enhancement at PON presentation (section 1.3).

Whether the distance VA deficit in study eye(s) differs by laterality of PON at presentation (unilateral vs bilateral) will be evaluated by repeating the analysis after stratifying by laterality. For those classified as having bilateral PON at presentation, mean VA deficit and a corresponding 95% CI will be calculated using a linear mixed model that adjusts for inter-eye correlation for participants with 2 study eyes. Given that we are unlikely to have adequate power for performing formal group comparisons according to laterality of PON at presentation, a clinically meaningful difference will be based on the group estimates and results will be considered exploratory. In the event there is evidence to suggest that distance VA deficit may differ by laterality at PON presentation, laterality subgroups (defined in section 1.2) will be analyzed and reported separately. Otherwise, data will be pooled across laterality for the primary analysis.

2.1.1 Sensitivity Analyses

Sensitivity analyses will be conducted by repeating the primary analysis in the following ways:

- Limit the analysis to study eye(s) of participants who completed the 6-month visit within the pre-specified protocol window (169 to 197 days after enrollment).

If the primary analysis and sensitivity analyses produce similar results, the primary analysis will be considered the definitive analysis and the sensitivity analyses will be used to provide supportive evidence of the estimated mean VA deficit for each age group. However, if results differ, exploratory analyses may be performed to evaluate potential factors that may have contributed to these differences.

2.1.1 Subgroup Factors

Exploratory analyses may be performed to examine whether deficit in distance VA for study eyes at 6 months may be associated with other factors (listed below) in addition to PON laterality at presentation. Descriptive statistics of distance VA deficit will be computed within subgroups of interest having at least 5 eyes, but no formal subgroup analyses will be conducted. If distance VA deficit is analyzed according to laterality at PON presentation (section 2.1), subgroup analyses will be performed separately for each laterality group.

The following person-level subgroups will be explored (in addition to laterality at PON presentation):

- Gender
- Puberty status at 1-month visit (pre-pubertal, pubertal)
  - Pre-pubertal is defined as having a Tanner stage score of I for questionnaire items 2-3 (both genders) and a response of 'No' or 'Unknown' for onset of menstrual periods (females only)
- Race/ethnicity (White/non-Hispanic, Non-White/Hispanic)
- Steroid treatment (yes/no) at PON presentation based on current, prescribed or prior treatment (if steroid IV) reported at enrollment
- Presence of white matter lesions on brain MRI scan (yes, no/unknown) based on site assessment*
- Diagnostic category (clinically isolated vs neurological) based on site assessment*
- NMO blood test results (positive, negative) based on site assessment (if done)†
- Myelin oligodendrocyte glycoprotein (MOG) blood test results (if done)†

* Data collected at enrollment or shortly after enrollment (reported at 1-month visit). 'Unknown' responses will be reviewed and adjudicated based on additional site feedback with guidance from independent review of the MRI scans.
† Data collected at enrollment but reported at the 1-month visit.

2.2 Secondary Visual Acuity Outcomes

Secondary VA outcomes will be performed in accordance with the general principles for analysis (section 1.4) and those outlined for the primary analysis (section 2.1). In accordance with the primary outcome, secondary analyses will be stratified (or conducted separately) by laterality of PON presentation if there is a clinical rationale or evidence suggesting that VA outcomes may differ according to laterality.

2.2.1 Descriptive Analyses

The distribution of monocular distance VA and LCVA will be tabulated with computation of descriptive statistics for study eyes across each follow-up visit. For those with 2 study eyes, monocular distance VA will be cross tabulated between the 2 eyes according to eye laterality (right vs left eye) with computation of descriptive statistics.

Ranked acuity scores will be tabulated for monocular distance VA and LCVA to evaluate the distribution of acuity scores. For eyes with very poor visual acuity, defined as distance VA or LCVA scores of <20/800 (if ATS HOTV) or 0 letters (if E-ETDRS), an attempt will be made to further subdivide this category based on site comments regarding the depth of visual acuity. Ranked acuity variables will be created such that logMAR values for scores <1.7 logMAR are preserved while scores of 1.7 logMAR will be subdivided into 4 ordered categories with assigned logMAR value as follows: counting fingers (1.7 logMAR), hand motion (1.8 logMAR), light perception only (1.9 logMAR) and no light perception (2.0 logMAR). A value of 1.7 will be assigned for 1.7 logMAR scores where there is no additional information regarding the depth of acuity.

2.2.2 Normal Distance VA for Age

Each study eye will be classified according to whether the monocular distance VA falls within the normal VA range for age. The normal VA range for age will be determined based on participants' age *at the time of the visit* as shown in Table 1. A cross-tabulation will be performed to evaluate the proportion of eyes within normal VA range for age at each follow-up visit by age normal VA status at enrollment.

The proportion of study eyes within normal VA range for age and the corresponding 95% CI will be calculated at each follow-up visit using a binomial regression analysis that adjusts for clustered data due to some participants contributing 2 study eyes. If the binomial regression model does not converge, outcomes will be

analyzed using Poisson regression with robust variance estimation or an exact method (without adjustment for inter-eye correlation).

2.2.3 Binocular Summation/Inhibition at 6 Months (unilateral PON at presentation)

For participants with unilateral PON at presentation, binocular distance VA will be compared to monocular distance VA (better of the 2 eyes, including non-study eye) at 6 months and at 2 years after enrollment. For each participant, a measure of summation/inhabitation will be computed as the difference between the binocular VA and monocular VA (binocular VA – monocular VA) as logMAR values. Based on these results, participants will be classified into one of the 3 subgroups defined below and the proportion of participants within each group will be calculated.

- Binocular summation (binocular VA is better than monocular VA in the better eye)
- Binocular inhibition (binocular VA is worse than monocular VA in the better eye)
- No difference

This analysis will be repeated for LCVA (binocular LCVA – monocular LCVA in the better eye) at 6 months and at 2 years after enrollment for participants with unilateral PON at presentation.

3.0 Additional Secondary Outcomes

Additional secondary analyses will be performed in accordance with the general principles outlined in chapter 1.

3.1 Baseline Characteristics

The number and proportion of enrolled participants will be reported according to whether there was evidence of optic nerve enhancement (if applicable) on MRI (defined in sections 1.3 and 1.4.2) at PON presentation.

The distribution of the following person-level characteristics at PON presentation will be tabulated separately for those with and without optic nerve enhancement at enrollment:

- Gender
- Race/ethnicity (refer to demographic information form)
- Age (3 to <4 years, 4 to <6 years, 6 to <7 years, 7 to <10 years, 10 to <13 years, ≥ 13 years)
- Puberty status at 1-month visit (pre-pubertal, pubertal)
- Family history of PON (yes/no)
- Family history of autoimmune conditions (yes/no)
- Family history of cardiovascular conditions (yes/no)
- Systemic symptoms within 2 weeks prior to enrollment (yes/no) and type of symptoms (if reported):
  - headaches
  - focal weakness
  - loss of bowl or bladder control
  - focal numbness
  - difficulty with coordination
  - tinnitus
  - nausea
  - vomiting
  - altered mental status
  - diplopia
  - neck stiffness/pain
- Current medications at enrollment (yes/no) and type of medications (if reported):
  - Ethambutol
  - Isoniazid
  - Amiodarone
  - Accutane
  - Linezolid
  - Sildenafil
  - ADHD meds
  - Seizure meds
  - Analgesics
  - Anti-TNF agents (infliximab/remicade, etanercept/enbrel, adalimumab/humira, golimumab/simponi
  - Other
- Laterality at PON presentation (unilateral, bilateral simultaneous, bilateral sequential)
- Steroid treatment (yes, no) (current, prescribed or prior treatment (if steroid IV) = yes)

- Presence of white matter lesions on brain MRI scan (yes, no/unknown) based on site assessment*
- History (prior to enrolling episode) of demyelination (yes/no)†
- NMO blood test results (positive, negative) based on site assessment (if done)‡**
- Diagnostic category (based on investigator assessment)‡
    - Clinically isolated syndrome/optic neuritis alone
        - Unilateral [¥]
        - Bilateral (bilateral simultaneous, bilateral sequential) [¥]
    - Neurological
        - Acute disseminated encephalomyelitis (ADEM)
        - ADEM followed by optic neuritis
        - Chronic relapsing inflammatory optic neuropathy
        - Myelin oligodendrocyte glycoprotein (MOG) positive demyelinating disorder**
        - Multiple sclerosis (MS)
        - Neuromyelitis optica spectrum disorder

* Data collected at enrollment or shortly after enrollment (reported at 1-month visit).
**Results of NMO will be reported as aquaporin 4 test (the actual test performed) in the manuscript. Both NMO and MOG results are based on blood samples collected from participants whose parents consented to optional additional testing with assays performed by the Mayo Clinic.
† Based on 1-month assessment
‡ Data collected at enrollment but reported at the 1-month visit. "Unknown" responses will be adjudicated based on additional site feedback with guidance from independent assessments/tests (if done).
[¥] Defined according to laterality at PON presentation (section 1.2)

The following baseline characteristics will also be described according to laterality (unilateral, bilateral) at PON presentation:

- Age at enrollment (years), continuous
- Diagnostic category (clinically isolated versus neurological) based on site assessment‡

The number of enrolled study eyes will be reported and the distribution of the following eye-level characteristics at PON presentation (enrollment) will be tabulated for study eyes:

- Anterior chamber reaction (yes, no/unknown)
- Vitreous cellular reaction (yes, no/unknown)
- Optic disk edema (yes, no/unknown)
- Subretinal fluid (yes, no/unknown)
- Retinal exudates (yes, no/unknown)
- Retinal hemorrhage (yes, no/unknown)
- Monocular distance visual acuity (quartiles, continuous)
- Monocular low contrast visual acuity (quartiles, continuous)

Descriptive statistics will be calculated for age, monocular distance VA and monocular low contrast VA. Baseline characteristics at PON onset for person-level and eye-level factors will be evaluated separately for participants without evidence of optic nerve enhancement on MRI.

The baseline characteristics will also be tabulated by laterality at PON presentation if there is evidence to suggest that VA outcomes differ between laterality groups.

3.2 Risk of Acute Optic Neuritis in the Fellow Eye at 6 Month after Enrollment

For participants with unilateral PON at presentation, number developing acute PON in the fellow eye at 6 months after enrollment will be reported.

3.3 Characterize PON based on MRI and Antibody Markers

The proportion of participants with white matter lesions on MRI and NMO antibodies at PON presentation will be tabulated separately (section 3.1) and combined.

3.4 Collect MRIs for Future Analyses

Images from MRI scans performed at or shortly after enrollment were collected and reviewed by a MS specialist who served at a masked examiner. The proportion of discrepant MRI findings between the site and masked examiner assessments will be computed for the following:

- Evidence of optic nerve enhancement on MRI (yes, no/unknown)
- Presence of white matter lesions (yes, no/unknown)
- Diagnosis (defined in section 3.1)

3.5 Assess Quality of Life (QOL) Outcomes using Current Pediatric Instruments

Responses to the pediatric quality of life (QOL) survey instruments (Eye Q and PedsQL) will be tabulated separately at 6 months after enrollment with computation of descriptive statistics. For each QOL instrument, descriptive statistics of survey scores will be computed within subgroups of (1) laterality at PON presentation (unilateral vs bilateral) and (2) diagnosis type (neurological vs isolated optic neuritis) provided there are ≥ 5 observations per subgroup. No formal subgroup analyses will be conducted to test for associations between QOL survey scores and each of the aforementioned factors.

References

1. Pan Y, Tarczy-Hornoch K, Cotter SA, et al. Visual acuity norms in pre-school children: the Multi-Ethnic Pediatric Eye Disease Study. Optometry and Vision Science 2009;86:607-12.
2. Drover JR, Felius J, Cheng CS, Morale SE, Wyatt L, Birch EE. Normative pediatric visual acuity using single surrounded HOTV optotypes on the Electronic Visual Acuity Tester following the Amblyopia Treatment Study protocol. Journal of AAPOS: American Association for Pediatric Ophthalmology & Strabismus 2008;12:145-9.

eTable 1. Study Inclusion and Exclusion Criteria

The following criteria must be met for a participant to be enrolled in the study:

Eligibility Criteria:

- 3 to < 16 years of age
- Optic neuritis (ON) in at least one eye based upon clinical diagnosis
- Visual loss and/or pain on eye movements for 2 weeks or less
- Ipsilateral relative afferent pupillary defect if unilateral PON
- At least one of the following in the affected eye:
    - Visual acuity deficit at least 0.2 logMAR below age-based norms from EVA ATS-HOTV or e-ETDRS testing (20/50 or better if 3 to <4; 20/40 or better if 4 to <6; 20/32 or better if 6 to <7; and ≥77 letters if 7 or older); OR
    - Diminished color vision; OR
    - Abnormal visual field; OR
    - Optic disk swelling
- Requirements for refractive error correction (based on a cycloplegic refraction performed at enrollment or as part of the examination by a referring pediatric ophthalmologist or pediatric optometrist within the past 1 month):
    - Myopia of ≥-0.75D, hyperopia ≥4.00D, and cylinder ≥1.50D must be corrected using habitual glasses or trial frames if there is a change in the best correction.
        - Refractive error correction prescribing instructions:
            - Hyperopic spherical equivalent (SE) must not be under corrected by > 2.00D SE
            - Cylinder power in both eyes must be within 0.75D of fully correcting the astigmatism.
            - Axis must be within ±10° if cylinder power is ≤1.00D, and within ±5° if cylinder power is >1.00D.
            - Myopia SE must not be under-corrected by > 0.25D or over corrected by > 0.75
- MRI of the orbits has been done with evidence of enhancement of the optic nerve(s) or will be done in the next 2 weeks as standard care.
- Idiopathic optic neuritis, optic neuritis associated with MS, and optic neuritis associated with other causes not known at presentation.

Exclusion Criteria:

- Study participants who do not expect to remain in area for the next two years and follow up at another study center is not possible
- Participants whose parents/guardians in the opinion of the Investigator, may be non-compliant with study schedules or procedures.
- Neuro-retinitis (if the diagnosis is made after enrollment they will be followed, but not included in the primary cohort)
- Previous episode of optic neuritis in the same (currently affected) eye (established or presumed due to optic atrophy). Prior episode in fellow eye does not exclude enrollment.
- Co-existent meningitis or laboratory abnormalities suggesting CNS infection or malignancy at the time of enrollment
- Ocular or systemic findings suggestive of non-demyelinating cause for optic neuritis in the affected eye(s) such as an associated history of sarcoidosis, systemic lupus erythematosus, collagen vascular disease, or Lyme disease with overt symptoms beyond serum markers such as rash, peripheral neuropathy, or liver disease.
- Preexisting ocular abnormalities that might affect assessment of visual function in the affected eye(s). (Preexisting ocular abnormalities in the fellow eye are not an exclusion criterion.)

eTable 2. Neurological Summary Diagnosis Worksheet

PON1: Pediatric Optic Neuritis Outcomes Study Study ID:
Neurological Summary Diagnosis Worksheet (to be completed by the treating physician) Date:
Visit: ____ 1-month ____ 1-year ____2-year

For the 1 month visit only:
Does the patient have a history (prior to this attack) of demyelination? Yes No
If yes, specify: MS NMO ADEM ON
Other demyelinating disease? Specify_____

For the 1 and 2-year visit only:
Has the patient had any further attacks of demyelination? Yes No
If yes, specify: optic nerves supratentorial white matter brainstem cerebellum spinal cord
Other? Specify _____

At 1-month, 1 and 2-year visits only:
Based upon the published criteria for diagnosis of MS[1] what is the patient's diagnostic category?

Select	Category	Notes (formal criteria attached)
	Unilateral optic neuritis only	(1) Unilateral ON (2) No signs, symptoms, or exam abnormalities outside the affected eye (3) MRI of the brain is normal (outside the affected optic nerve)
	Bilateral optic neuritis only	(1) Bilateral ON (may be asymmetric) (2) No signs, symptoms, or exam abnormalities outside the affected eye (3) MRI of the brain is normal (outside the affected optic nerves)
	Multiple sclerosis	(1) ON in one or both eyes (2a) History of MS attack in the past OR (2b) Signs, symptoms, exam or MRI abnormalities typical of MS outside the affected eye(s)
	Clinically isolated syndrome	(1) ON in one or both eyes (2) No history of demyelination in the brain or spinal cord (3) Signs, symptoms, exam or MRI abnormalities outside the affected eye(s), but the patient does not meet criteria for MS
	Neuromyelitis optica spectrum disorder	(1) ON in one or both eyes, longitudinally–extensive myelitis, area postrema (brainstem syndrome), (2) NMO-IgG seropositive or seronegative (new criteria stratify by whether the patient is positive or negative, see attached)
	Acute disseminated encephalomyelitis (ADEM)	(1) No history of demyelination in the brain or spinal cord (2) Encephalopathy (3) Multifocal neurologic signs – signs, symptoms, exam or MRI abnormalities in multiple parts of the brain or spinal cord
	ADEM followed by optic neuritis	(1) Initial attack fulfills criteria for ADEM (2) ON diagnosed at least 4 weeks after ADEM with objective evidence of loss of visual function (3) Initial MRI lesions consistent with ADEM have resolved or nearly resolved
	Chronic relapsing inflammatory optic neuropathy	(1) Recurrent optic neuritis (2) No signs, symptoms, or exam abnormalities outside the affected eye (3) MRI of the brain normal outside the affected nerve(s)
	Unknown	Comments:

1. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol.* 2011;69(2):292-302.

eTable 3. Diagnosis at Optic Neuritis Presentation

	Overall (N=44)	
	n	**%**
Diagnosis based on initial site impression[a]		
Isolated optic neuritis	22	50%
Bilateral isolated optic neuritis	9	20%
Unilateral isolated optic neuritis only	13	30%
Acute disseminated encephalomyelitis (ADEM)	8	18%
Multiple sclerosis	5	11%
Neuromyelitis optica spectrum disorder (NMO)	5	11%
Myelin oligodendrocyte glycoprotein positive demyelinating disorder (MOG)	1	2%
MS vs seronegative NMO	2	5%
Unknown/Not Reported[b]	1	2%
Six-month diagnosis based on a combination of site impression[a] , MRI review, and additional serological testing		
Isolated optic neuritis	21	48%
Bilateral isolated optic neuritis	9	20%
Unilateral isolated optic neuritis only	12	27%
Acute disseminated encephalomyelitis (ADEM)	7	16%
Multiple sclerosis	5	11%
Neuromyelitis optica spectrum disorder (NMO)	3	7%
Myelin oligodendrocyte glycoprotein positive demyelinating disorder (MOG)	8	18%

[a] Site-reported diagnosis was recorded at the 1-month visit. Cases where the investigator indicated that the diagnosis was clinically isolated syndrome were reclassified as either unilateral optic neuritis only or bilateral optic neuritis only according to laterality at optic neuritis presentation.

[b] Site did not report a diagnosis because the participant missed the 1-month visit.