AMBLYOPIA TREATMENT STUDY

ATS9

A Randomized Trial Comparing Patching Versus Atropine for Amblyopia in 7 to <13 Year Olds

Protocol Chairs: Richard W. Hertle, M.D., Mitchell M. Scheiman, O.D.

PROTOCOL

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Contact Information

Coordinating Center

Roy W. Beck, M.D., Ph.D. (Director)
Jaeb Center for Health Research
15310 Amberly Drive, Suite 350
Tampa, FL 33647
Phone (888) 79PEDIG or (813) 975-8690
Fax (888) 69PEDIG or (813) 975-8761

Protocol Chairs

Richard W. Hertle, M.D.
Business: (412) 692-8940
Home: (412) 364-1792
Pager: (412) 456-8673
Cell: (412) 861-0129
richard.hertle@chp.edu

Mitchell M. Scheiman, O.D.
Business: (215) 276-6057
Home: (610) 664-5120
Cell: (484) 716-0815
mscheiman@pco.edu

CHAPTER 1: BACKGROUND AND SUMMARY

1.1 Study Objectives

To compare the effectiveness of weekend atropine plus near activities and daily patching plus near activities for moderate amblyopia (20/40 to 20/100) and severe amblyopia (20/125 to 20/400) in 7 to <13 years olds.

To determine the maximum improvement with each treatment.

1.2 Rationale for the Study

Although there is consensus that amblyopia can be treated effectively in young children, many eye care practitioners believe that treatment beyond a certain age is ineffective. Some clinicians have believed that a treatment response is unlikely after the age of 6 or 7 years, while others have considered age 9 or 10 years to be the upper age limit for successful treatment. The American Academy of Ophthalmology Preferred Practice Pattern for amblyopia recommends treatment up to age 10 years. The opinion that amblyopia treatment is ineffective in older children may have arisen because the age of 6 to 7 years is thought to be the end of the "critical period" for visual development in humans. This belief, however, was not based on adequate prospectively-collected data.

To address this issue of the response of amblyopia to treatment in children 7 years and older, the Pediatric Eye Disease Investigator Group (PEDIG) conducted a randomized trial of 507 patients (aged 7 to <18 years) with amblyopic eye visual acuity ranging from 20/40 to 20/400. [1] Patients were provided with optimal optical correction and then randomized to a Treatment Group (2 to 6 hours per day of prescribed patching of the sound eye combined with near visual activities for all patients plus atropine one drop per day in the sound eye for 7 to <13 year olds) or an Optical Correction Group (optical correction alone). Patients whose amblyopic eye acuity improved 10 or more letters (2 lines) by 24 weeks were considered *responders*. In the 7 to <13 year olds (N=404), 53% of the Treatment Group were responders compared with 25% of the Optical Correction Group (P<0.001). In the 13 to <18 year olds (N=103), the responder rates were 25% and 23% respectively overall (adjusted P=0.22), but 47% and 20% respectively among patients not previously treated with patching and/or atropine for amblyopia (adjusted P=0.03). Most patients, including responders, were left with a residual visual acuity deficit.

The use of multiple modalities (patching, atropine, near visual activities) in the treatment regimen for the 7 to <13 year olds in this trial (ATS3) was an effort to maximize the therapeutic response. Patients age 13 years and older were prescribed patching but not atropine because of concern that the continual optical blur from the atropine could have a deleterious effect on their ability to drive and perform other activities. Prescribed patching was 2 to 6 hours a day to limit patch wear to nonschool hours and because our prior studies of 3 to <7 year olds demonstrated that as little as two hours of patching a day (when combined with near visual activities) is as effective as a greater number of hours. Instructing patients to perform at least one hour of near activities while wearing the patch was based on the unproven clinical opinion that near activities can augment the effect of the occlusion therapy. A PEDIG pilot study suggested that near activities are beneficial and this question of benefit of near activities is currently being studied in another randomized clinical trial. Atropine placed in the sound eye once a day [2] and two days a week [3] has been demonstrated in younger children to be beneficial to the acuity of the amblyopic eye, presumably due to its cycloplegic effect of blurring vision in the sound eye especially at near fixation. In a study comparing daily and weekend atropine, daily atropine was not found to be superior. [3]

The unanswered question from this completed clinical trial is whether prescribing patching or atropine alone could have produced a response similar to the combination therapy, or whether in this age group, one treatment is better than the other. A poll of PEDIG investigators at an investigator meeting on January 15, 2005 indicated that very few are following the treatment regimen used in the prior study (ATS3); rather, most are prescribing monotherapy—either patching or atropine—as the initial treatment for amblyopia in the 7 to <13 year age range. Thus, a trial comparing atropine and patching as amblyopia treatments in 7 to <13 year olds is needed.

There are data available from prior PEDIG trials on the effect of patching in moderate and severe amblyopia. However, for atropine, at present, prospective data are only available for moderate amblyopia. Although a randomized treatment trial of atropine for severe amblyopia has not been conducted, there are several published case series on the outcome of pharmacological penalization that specifically discuss the outcomes for patients with severe amblyopia. In nearly all of the following studies atropine was prescribed daily. Ron and Nawratzki reported on 16 patients with initial acuities of 20/200 and 13 patients with initial acuities of 20/100. [4] In all but two children the vision improved and in 18 improved to 20/30 or better. North and Kelly included only one such case in their series of 20 patients with longitudinal follow-up. [5] Repka and Ray reported on 79 patients treated with atropine.[6] They stratified patients on the basis of initial amblyopic eye acuity, 20/100 or worse, 20/80 to 20/50, and 20/40 or better. They found that the 20/100 or worse group improved the most. Of those children who improved an octave (3 logMAR lines) or more, the initial mean acuity was 20/113. Foley-Nolan and colleagues reported a prospective randomized study of occlusion versus atropine. [7] The treatments appeared equally effective. Ten of the 18 patients randomized to atropine had acuity worse than 20/100. After treatment all 10 children were better than 20/80 and 7 were 20/40 or better. Simons and coworkers specifically mentioned that they observed visual acuity improvement with atropine treatment for initial amblyopic eye acuities of <20/100. [8] Eight patients with 20/200 to 20/600 best corrected initial visual acuity (geometric mean = 20/287) were managed recently at the Wilmer Institute with atropine penalization and best glasses correction (unpublished data – Michael X. Repka). Visual acuity testing was not masked. The acuity of 6 of 8 patients improved, with the mean geometric acuity of 20/80 at outcome for all 8 patients.

1.3 Synopsis of Study Design

Major Eligibility Criteria *(see section 2.2 for a complete listing)*

- Age 7 to <13 years
- Amblyopia associated with strabismus, anisometropia, or both
- Visual acuity in the amblyopic eye 19 to 71 letters on E-ETDRS (20/40 to 20/400 inclusive)
- Visual acuity in the sound eye 79 letters or better on E-ETDRS (20/25 or better)
- Interocular difference >=15 letters (3 lines)
- No amblyopia treatment (other than spectacles) in the last 6 months
- No myopia (more than -0.25D spherical equivalent) in either eye
- Spectacles, if needed, worn for at least 16 weeks or visual acuity documented to be stable

Treatment Groups

Each patient is randomized to either:

- Atropine 1% once each weekend day in the sound eye plus near activities for at least one hour every day (with increase to daily atropine at 5 weeks if acuity not improved by at least 5 letters)

- Patching 2 hours per day plus near activities for one hour while patching (with increase to 4 hours per day for moderate amblyopes and ≥ 4 hours per day for severe amblyopes at 5 weeks if acuity not improved by at least 5 letters).

Sample Size

Moderate Amblyopia (20/40 to 20/100): a minimum of 180 patients
Severe Amblyopia (20/125 to 20/400): patients to be enrolled until enrollment ends in the moderate amblyopia trial

Visit Schedule

- Visits at 5 weeks and 17 weeks. Patients who are using atropine at the time of the 17-week visit will return for an additional off-atropine visit in two weeks.
- Partial responders at 17 weeks (see section 4.3 for definition) will continue to be followed at 8-week intervals after the 17-week visit until there is no further improvement.

At baseline and at each visit, distance visual acuity will be assessed in each eye using the E-ETDRS protocol. At the 17-week visit, the visual acuity testing will be done by a masked examiner.

Primary Analysis

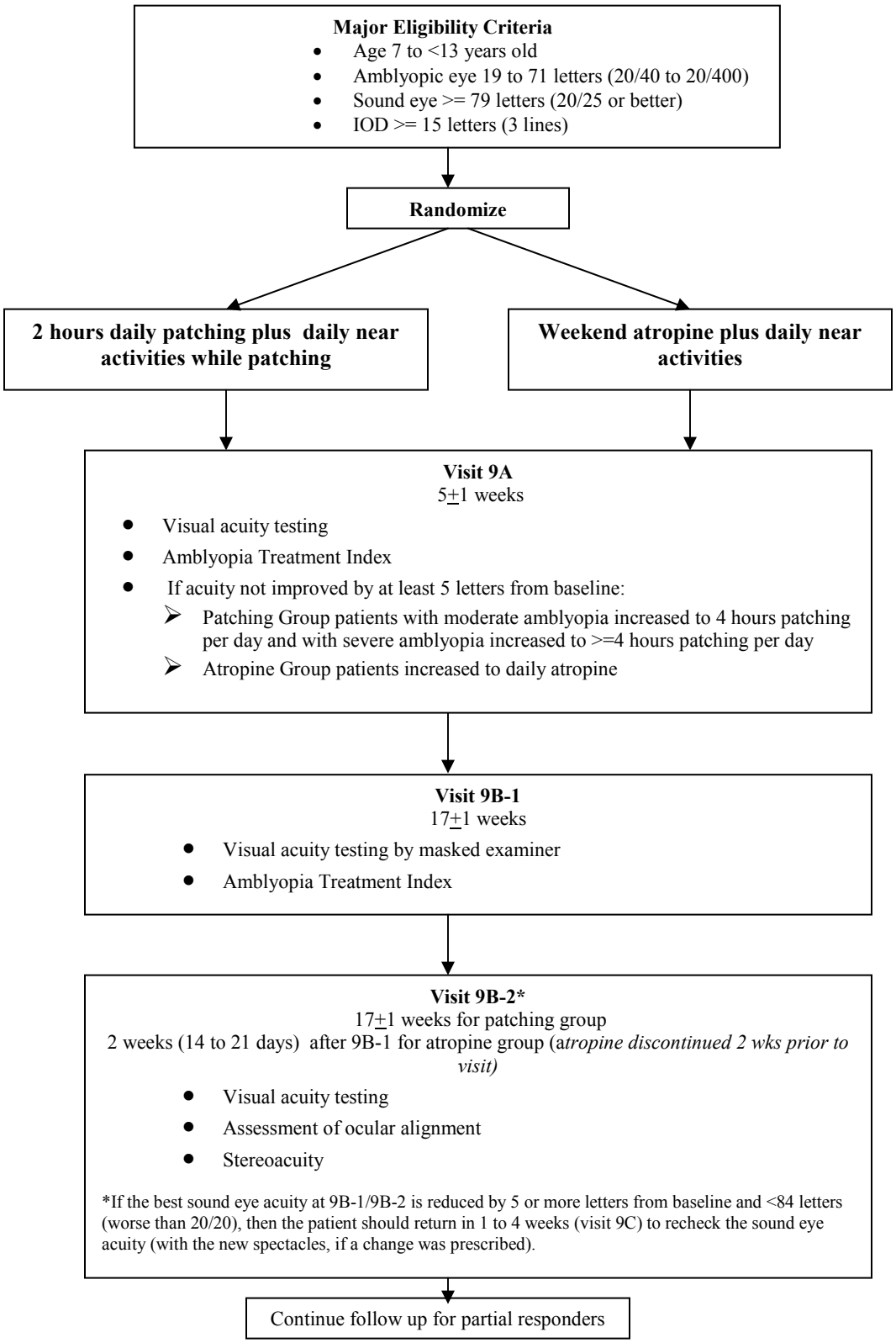
All primary analyses will include only the patients with moderate amblyopia (20/40 to 20/100). Analyses of data from patients with severe amblyopia (20/125-20/400) will be exploratory.

The primary outcome assessment is visual acuity at 17 weeks for the amblyopic eye, and 17 weeks or 19 weeks for the sound eye.

The primary analytic approach for the amblyopic eye acuity will involve constructing two one-sided confidence intervals on the difference in means obtained in an analysis of covariance model. Treatment equivalence will be declared if the two 1-sided 95% confidence intervals constructed on the difference between adjusted mean visual acuity scores for the two groups are completely contained within the designated equivalence interval of ± 0.1 logMAR.

Sound eye acuity data will be reported for each treatment regimen at the 17-week visit for patching group patients and 2 weeks after the 17 week visit for atropine group patients as mean change from baseline and as the distribution of the numbers of lines of change from baseline.

1.4 Study Summary Flow Chart



CHAPTER 2: SCREENING AND ENROLLMENT VISIT

2.1 Eligibility Assessment and Informed Consent

A patient is considered for the study after undergoing a routine eye examination by an investigator (as part of standard care) that identifies amblyopia meeting the eligibility criteria. As noted in subsequent sections, refractive error if present must be corrected with glasses (as is the case in standard patient care) before a patient can be enrolled into the trial.

For patients who appear eligible for the study following a "standard-care" or preliminary examination, the study will be discussed with the child and the child's parent(s) or guardian. Parents or guardians who express an interest in the study will be given a parent information sheet and a copy of the informed consent form to read. Written informed consent must be obtained from the parent or guardian and written assent from the child (unless waived by the IRB) prior to performing any study-specific procedures that are not part of the patient's routine care.

2.2 Eligibility Criteria for Enrollment

Below are the eligibility criteria for enrollment.
The following criteria must be met for the patient to be enrolled in the study:

1. Age 7 to < 13 years
2. Amblyopia associated with strabismus (comitant or incomitant), anisometropia, or both
   a. Criteria for strabismus: At least one of the following criteria must be met:
      - Heterotropia at distance and/or near fixation on examination (with or without spectacles)
      - History of strabismus surgery (or botulinum)
      - Documented history of strabismus which is no longer present (which in the judgment of the investigator could have caused amblyopia)
   b. Criteria for anisometropia: At least one of the following criteria must be met:
      - ≥0.50 D difference between eyes in spherical equivalent
      - ≥1.50 D difference between eyes in astigmatism in any meridian
   c. Criteria for combined mechanism amblyopia: Both of the following criteria must be met:
      - Criteria for strabismus are met (see above)
      - ≥1.00 D difference between eyes in spherical equivalent **OR** ≥1.50 D difference between eyes in astigmatism in any meridian
        - *Note: the spherical equivalent requirement differs from that in the definition for refractive/anisometropic amblyopia*
3. Visual acuity, measured in each eye without cycloplegia within 7 days prior to randomization using the E-ETDRS protocol on Electronic Visual Acuity Tester, as follows:
   1. Visual acuity in the amblyopic eye between 19 to 71 letters inclusive (20/40 to 20/400 inclusive)
   2. Visual acuity in the sound eye ≥79 letters (20/25 or better)
   3. Inter-eye acuity difference ≥15 letters (i.e., amblyopic eye acuity at least 3 lines worse than sound eye acuity)
4. No amblyopia treatment (other than spectacles) in the past 6 months
   - *any treatment more than 6 months prior to enrollment is acceptable*
5. No current vision therapy or orthoptics

6. Ocular examination within 6 months prior to enrollment showing no ocular cause for reduced visual acuity
    - *nystagmus per se does not exclude the patient if the above visual acuity criteria are met*
7. Cycloplegic refraction within 6 months prior to enrollment
8. No myopia (no more than -0.25 D spherical equivalent) in either eye.
9. Spectacle correction for measurement of enrollment visual acuity must meet the following criteria and be based on a cycloplegic refraction that is no more than 6 months old (refractive error must be corrected with spectacles and not contact lenses):

    a. Requirements for spectacle correction:

    1) For patients meeting criteria for strabismus (see #2a above)
        - Hypermetropia if corrected must not be undercorrected by more than +1.50 D spherical equivalent, and the reduction in plus sphere must be symmetric in the two eyes. Otherwise, spectacle correction is at investigator discretion.

    2) For patients meeting criteria for anisometropia or combined-mechanism (see #2b,c above)
        - Spherical equivalent must be within 0.50 D of fully correcting the anisometropia
        - Hypermetropia must not be undercorrected by more than +1.50 D spherical equivalent, and reduction in plus must be symmetric in the two eyes
        - Cylinder power in both eyes must be within 0.50 D of fully correcting the astigmatism
        - Cylinder axis in the spectacle lenses in both eyes must be within 6 degrees of the axis of the cycloplegic refraction when cylinder power is $\geq$ 1.00 D

    b. Spectacles meeting above criteria must be worn either:

    1) for at least 16 weeks immediately prior to enrollment, or

    2) until visual acuity in amblyopic eye is stable (defined as two consecutive visual acuity measurements by the same testing method at least 4 weeks apart with no improvement of one logMAR line or more)
        - An acuity measurement done any of the following ways may be considered the first of two consecutive measurements: 1) in current glasses , 2) in trial frames with full correction of hypermetropia with cycloplegia, or 3) by having the patient return in new glasses for first measurement. The second acuity measure does not have to be made through the same prescription as the first, if the second measure is made through a more accurate prescription. *Note: since this determination is a pre-study procedure, the method of measuring visual acuity is not mandated although E-ETDRS testing is preferred if done as part of usual care.*
10. No prior intraocular or refractive surgery
11. No known reaction to or development of systemic side effects (e.g., confused mental state, somnolence, skin flushing, exacerbation of asthma) with prior use of atropine or other cycloplegics and no known skin allergy to patch or bandage adhesives
12. Down Syndrome not present
13. Parent willing to accept randomized treatment, available for at least 4 months of follow-up, has home phone (or access to phone), and willing to be contacted by Jaeb Center staff

2.3 Examination Procedures

2.3.1 Historical Information

Historical information to be elicited will include: date of birth, gender, race, ethnicity, prior amblyopia therapy (e.g., glasses, patching, pharmacologic, filters), history of strabismus surgery, and history of allergy/intolerance to bandage adhesive or atropine. Results of eccentric fixation assessment of the amblyopic eye, if done as part of usual care within 1 month prior to randomization, will be recorded on the enrollment form.

2.3.2 Screening/Enrollment Examination Procedures

The examination procedures are performed after the investigator has diagnosed amblyopia and has determined that the patient is likely eligible for the study. The examination procedures are listed below and detailed in the ATS Procedures Manual.

Examination procedures include:

1. Measurement of visual acuity in each eye (right eye first) by the E-ETDRS testing protocol on the Electronic Visual Acuity Tester. The protocol for conducting the visual acuity testing is described in the ATS Testing Procedures Manual. Aspects of the testing protocol that are specific to this study are indicated below:
   - Testing must be done without cycloplegia (with spectacles, if worn) no more than 7 days prior to randomization.
   - Since the patient needs to be wearing spectacles that provide best visual acuity to be enrolled, trial frames/phoropter with a different correction cannot be used to measure acuity at enrollment.
2. Ocular motility examination
   - Measurement of predominant alignment by Simultaneous Prism and Cover Test (SPCT) in primary position at distance and near; and recording of the presence of primary position nystagmus (with and without monocular occlusion).
   - Testing must be done without cycloplegia (with spectacles, if worn) no more than 7 days prior to randomization.
3. Ocular examination as per investigator's clinical routine to rule out a cause for reduced visual acuity other than amblyopia, within 6 months prior to randomization.
4. Cycloplegic refraction using cyclopentolate 1% as per investigator's usual routine within 6 months prior to randomization
5. Binocularity testing (prior to cycloplegia): Titmus fly, Randot Preschool test

2.4 Randomization of Eligible Patients

1. The Jaeb Center will construct a separate Master Randomization List for moderate amblyopia and severe amblyopia using a permuted block design stratified by site and by visual acuity (20/40 to 20/100 and 20/125 to 20/400), which will specify the order of treatment group assignments. A patient is officially enrolled when the website randomization process is completed.
2. Once a patient is randomized that patient will be included regardless of whether the assigned treatment is received or not. Thus, the investigator must not randomize a patient until he/she is convinced that the parent/guardian will accept either of the treatment regimens.
3. Treatment must commence within 48 hours following randomization (patching group) or the most immediate weekend day following randomization (atropine group); therefore, a patient should not be randomized until both the investigator and parent are ready to start treatment.

4. Visual acuity and ocular alignment testing must be performed within 7 days prior to randomization. If patient randomization is delayed beyond 7 days, the visual acuity and ocular alignment testing must be repeated to confirm eligibility and establish the baseline acuity and amblyopia classification for the study.

2.5 Additional Testing in Patients Assigned to the Atropine Group

2.5.1 Assessment of Reading Ability

Reading ability after cycloplegia of the sound eye will be assessed binocularly in patients randomized to the Atropine Group.

Patients who (1) are unable to read the grade-appropriate print with the sound eye cyclopleged and (2) will be attending school while on treatment will be prescribed single vision reading glasses to use for desk (near) work in school and home (paid for by the study).

- The power of the reading glasses can either be prescribed as +2.50D greater than the distance optical correction for each eye or at investigator discretion.
- If reading glasses are not prescribed at randomization but the patient later has difficulty in school due to the reduced acuity in the sound eye, reading glasses will be prescribed at that time.
- *Note: single vision reading glasses are being prescribed rather than bifocals to avoid the problem of the bifocals being worn all of the time, thus potentially compromising the effect of the atropine.*

If both eyes were cyclopleged during the randomization visit, near correction will be added for the amblyopic eye.

2.5.2 Fixation Preference

Fixation preference will be assessed in patients randomized to the Atropine Group. Patients in whom both eyes were cyclopleged during the randomization visit will skip this assessment. Fixation preference testing will be done after cycloplegia of the sound eye. The procedures for strabismic and non-strabismic patients are described in the ATS procedures manual.

CHAPTER 3: RANDOMIZATION AND TREATMENT GROUP ASSIGNMENT

3.1 Randomization Groups

Each patient will be randomly assigned to one of two treatment groups:

1. 2 hours of daily patching of the sound eye combined with one hour of near visual activities while patching (increased at 5 weeks if acuity has not improved by at least 5 letters: increased to 4 hours patching per day for patients who were enrolled with amblyopic eye acuity 48-71 letters (20/40-20/100) and increased to 4 or more hours patching per day for patients who were enrolled with amblyopic eye acuity 19-47 letters (20/125-20/400)).
2. Atropine 1% once each weekend day in the sound eye combined with one hour of near visual activities every day (increased to daily atropine at 5 weeks if acuity has not improved by at least 5 letters).

The study will provide the patches and atropine.

Spectacle wear, if prescribed, will be continued.

3.2 Patching Group

The Patching Group is initially prescribed two hours of patching per day plus near visual tasks to be done while wearing the patch for at least one hour per day.

At the 5-week visit, if the amblyopic eye acuity has not improved at least 5 letters from baseline, patching will be increased. Patients who were enrolled with moderate amblyopia (amblyopic eye acuity 48-71 letters (20/40-20/100)) will be increased to 4 hours daily. Patients who were enrolled with severe amblyopia (amblyopic eye acuity 19-47 letters (20/125-20/400)) will be increased to 4 or more hours daily at investigator discretion. If amblyopic eye acuity has reached 20/25 or better by the 5-week visit, the patching regimen may be continued or tapered but must be at least one hour per day (patching should not be stopped prior to the 17-week outcome exam unless an adverse effect of treatment occurs).

3.3 Atropine Group

The Atropine Group is prescribed atropine 1% to be placed in the sound eye on Saturday and Sunday of each week plus near visual tasks to be done at least one hour per day. If reading glasses have been prescribed, near activities must be done without the use of reading glasses for at least an hour a day.

If the amblyopic eye acuity has not improved at least 5 letters from baseline to the 5-week visit, atropine will be increased to 1 drop in the sound eye daily. Atropine should not be stopped prior to the 17-week outcome exam unless an adverse effect of treatment occurs.

Patients using atropine will be advised to wear spectacles or sunglasses with UV protection and a brimmed hat when outdoors.

As noted in section 2.5, patients in the Atropine Group who (1) are unable to read the grade-appropriate print with the sound eye cyclopleged and (2) will be attending school while on treatment will be prescribed single vision reading glasses to use during school and for necessary homework (paid for by the study).

3.4 Near Activities

The investigator may use his/her discretion with regard to which specific visual activities to prescribe based on what he/she believes are suitable for the child's age and may be beneficial. These tasks may include:

- Crafts, coloring, tracing, cutting out objects, dot-to-dot connecting, 'fill in the symbols,' 'symbol sequence,' or other activities requiring eye-hand coordination
- Hidden pictures and word finds
- Video games (e.g., Game Boy/Nintendo)
- Computer/internet
- Written homework
- Reading
- Building models, knitting, stringing beads
- Other near accommodative tasks

3.5 Home Calendar Logs

A calendar will be provided on which the child/parent will record the treatment received each day. These logs will be turned in at each of the protocol visits. At each visit, the logs will be reviewed and an assessment of compliance will be recorded on the Follow-up Examination Form.

CHAPTER 4: FOLLOW-UP EXAMINATIONS

4.1 Visit Schedule

Protocol-specified follow-up visits will occur at the following times from randomization:

- Visit 9A: 5 ± 1 week
- Visit 9B-1: 17 ± 1 week
- Vist 9B-2: 17 ± 1 week for the Patching Group and 2 weeks (14 to 21 days) after 9B-1 for the Atropine Group (2 weeks after atropine is discontinued)

For the Patching Group and for patients in the Atropine Group who have discontinued use of atropine, visits 9B-1 and 9B-2 can be performed on the same day.

Additional visits can be performed at the discretion of the investigator. A Follow-up Examination Form should be completed on the study website for every exam (not just the minimum required exams).

4.2 Testing Procedures

The table below summarizes the testing procedures at each follow-up visit.

Test	9A *5±1 wk*	9B-1 *17 ± 1 wk* *Masked exam*	9B-2 *17 ± 1 wk for patching group and 2 weeks (14-21 days) after 9B-1 for atropine group*
Distance acuity amblyopic eye*	X	X	X
Distance acuity sound eye*	X	X	X
Amblyopia Treatment Index	X	X	
Ocular alignment**			X
Stereoacuity***			X

*Using E-ETDRS acuity testing protocol on the EVA. The testing at visit 9B-1 will be done by a masked examiner

**Assessed at each visit but only quantified at visit 9B-2 after atropine discontinued (at other visits, the development of a new or increased deviation will be reported)

***Titmus fly, Randot Preschool Test

4.2.1 Visual Acuity Testing

At each visit, visual acuity is measured by a certified examiner using the E-ETDRS acuity protocol on the Electronic Visual Acuity Tester.

- While atropine is being used, the sound eye will be tested with the current full cycloplegic correction (if the glasses do not correct the full amount of hyperopia, clip-ons or trial frames will be used).
- The visual acuity measurement of the amblyopic eye at visit 9B-1 (17 weeks) will be done by a masked examiner. This is accomplished by having the sound eye patched before the examiner sees the patient.

4.2.2 Amblyopia Treatment Index

The Amblyopia Treatment Index (questionnaire) is completed by the patient and by a parent or guardian at visit 9A and 9B-1.

- The questionnaire consists of 18 questions concerning the effect of the patching and atropine on the child and parent. For the parent, the questionnaire will be self-administered and for the patient, it will be administered by site staff
- The questionnaire should be completed prior to the investigator's examination of the patient.
- The questionnaire is meant for the child's parent or guardian who is responsible for administering the patching or atropine drop. If the child is brought to the visit by an

individual who is not involved in the treatment, this is indicated on the questionnaire and it is not completed.

4.2.3 Testing at Visit 9B-2

For patients in the Patching Group and for patients in the Atropine Group who have discontinued use of atropine, visit 9B-2 can occur on the same day as the masked examination (visit 9B-1). For patients in the Atropine Group who are still using atropine at the time of the 9B-1 visit, the atropine will be discontinued and the patient will return in two weeks for the 9B-2 visit.

At this visit, testing will include the following:

- Visual acuity testing of both the amblyopic eye and sound eye using the E-ETDRS protocol
  - If the patient completes visit 9B-1 on the same day as 9B-2, visual acuity will be tested again even though it was already tested once this day. The testing may be done by the same or a different examiner.
  - See section 4.2.3.1 for protocol regarding retesting of visual acuity in the sound eye.
- Titmus fly and Randot Preschool test
- Ocular alignment assessed with the SPCT
- Cycloplegic refraction and retesting of acuity in sound eye if it is decreased from baseline (see below)

4.2.3.1 Retesting of Visual Acuity in the Sound Eye at Visit 9B-2

If the better sound eye acuity at visits 9B-1 and 9B-2 is 5 or more letters (1 line) worse than the baseline sound eye acuity and is <84 letters (worse than 20/20), a cycloplegic refraction should be performed on the sound eye, lenses adjusted in trial frames/phoropter, and then the acuity should be tested again, modifying the refractive correction in trial frames if indicated.

If acuity is still reduced by 5 or more letters from baseline and <84 letters (worse than 20/20), then a change in spectacle lens should be prescribed if indicated from the results of the cycloplegic refraction (*the study will pay for this change*). The new prescription is at investigator discretion, but should provide maximum visual acuity. If the best sound eye acuity at visits 9B-1 and 9B-2 is decreased 5 or more letters from baseline, the patient should return in 1 to 4 weeks (visit 9C) to recheck the sound eye acuity (with the new spectacles, if a change was prescribed). The patient should remain off amblyopia therapy for either eye.

4.3 Additional Follow-up for Partial Responders

After visit 9B (or visit 9C if needed), follow up will end for patients who meet one of the following criteria:

- Amblyopic eye acuity (better of 9B and 9C acuities) is $\geq$84 letters (20/20 or better) OR if worse than 20/20 is no more than 3 letters worse than sound eye acuity (better of 9B and 9C acuities)
- Amblyopic eye acuity (better of 9B and 9C acuities) has improved by less than 5 letters from baseline OR less than 3 letters from visit 9A (if not missed)
- Sound eye acuity has worsened from baseline such that investigator believes reverse amblyopia has occurred and does not believe that the randomized treatment should be continued
- Patient in the Atropine Group received patching for one or more weeks or patient in the Patching Group received atropine for one or more weeks

Follow up will continue for patients who are considered to be *partial responders*. To be considered a partial responder, both of the following criteria must be met:

- amblyopic eye acuity (better of 9B and 9C acuities) has improved 5 or more letters from baseline and improved at least 3 letters from visit 9A (if not missed)
- amblyopic eye acuity (better of acuities at 9B and 9C) is <84 letters (worse than 20/20) and 4 or more letters worse than sound eye acuity (better of acuities at 9B and 9C)

Patients continuing in follow up will continue using the randomized treatment or with an increased dosage of the randomized treatment (at investigator discretion) and will have a follow-up visit every 8± 1 weeks until at least one of the following criteria are met:

(1) amblyopic eye acuity is ≥84 letters (20/20 or better) OR if worse than 20/20 is no more than 3 letters worse than sound eye acuity
(2) treatment is discontinued because the sound eye worsened
(3) treatment other than the randomized treatment is used for one or more weeks
(4) there is no further improvement in the amblyopic eye acuity.
    - 'No further improvement' is defined as visual acuity in the amblyopic eye that is no more than 2 letters better than the measured acuity at the prior visit on two tests of acuity at the same visit. At the first 8-week visit, the better amblyopic eye acuity at visit 9B or 9C is used as the 'baseline' for evaluating improvement. At subsequent visits, the acuity from the prior visit is used (or better acuity if acuity was tested twice at that visit).

At each visit, distance visual acuity will be measured in each eye using the E-ETDRS acuity protocol on the Electronic Visual Acuity Tester. If the amblyopic eye acuity is not improved by 3 or more letters from the prior visit, the visual acuity test will be repeated.

For patients remaining in the study, a cycloplegic refraction is encouraged if not performed within the prior 6 months.

CHAPTER 5: MISCELLANEOUS CONSIDERATIONS

5.1 Management of Optical Correction

A refraction should be performed at any time the investigator suspects that refractive error may not be optimally corrected.

5.2 Management of Strabismus

Strabismus surgery is not expected to be needed during the 17 weeks of the study prior to the masked outcome exam. However, for patients who remain in the study as a *partial responder*, surgery may be indicated before the patient completes the study. It is preferred that strabismus surgery not be performed in the first 17 weeks, but such surgery is allowed at the discretion of the clinician. This will be recorded in the comment section of the Follow-up Examination Form.

5.3 Intercurrent Events

If visual acuity should worsen in the amblyopic eye (or in the sound eye and does not recover with cessation or reversal of treatment), the investigator should evaluate this condition using best clinical judgment and perform whatever work up is clinically indicated to assess for an alternate cause (i.e., other than amblyopia) for the visual loss. Patients found to have a cause other than amblyopia that fully explains the visual loss (i.e., amblyopia was never present) will be dropped from the study. This will be reported on the Patient Final Status Form.

Eye injuries or the development of an eye problem that might affect vision will be reported on the Follow-up Examination Form. Likewise, the development of a serious medical problem that might affect the patient's study participation will be recorded.

5.4 Patient Withdrawals

A patient (and in this case the parents or guardian) may withdraw from the trial at any time. This is expected to be a very infrequent occurrence in view of the short duration of follow up and the limited number of follow up visits. If the parents or guardian indicate that they want to withdraw the child from the study, the investigator personally should attempt to speak with them to determine the reason.

Patients who receive alternate treatment (e.g., patching for at least one week for patients assigned to atropine and vice versa) will be dropped from the study after completion of the main outcome exam (9B).

5.5 Adverse Events/Risks

The risks involved in the study are identical to those that would be present for a patient treated with the study treatment regimens who is not participating in the study.

5.5.1 Side Effects of Atropine

Local side effects of minimal severity include allergic lid reactions, local irritation, conjunctival hyperemia, and follicular conjunctivitis. Potential systemic side effects include dry skin and mouth, tachycardia, fever, flushing irritability, mental confusion, constipation, aggravation of asthma, and seizures. Systemic effects occur very uncommonly in the dosage schedule (one drop a day) suggested in this protocol as noted in the previous studies of atropine treatment. Simons and coworkers [8] found the risk of allergic reactions to be less than 1%. Additional safety data can be derived from the literature describing the chronic topical atropine treatment for the attempted prevention of myopia. In the study by Brodstein and colleagues,[9] 253 patients were treated for an

average of 33 months with daily atropine 1% drops. Neither local nor systemic side effects of any significance were noted. In ATS1,[2] among 204 patients <7 years old, an ocular side effect was reported at least once for 26% of patients, most commonly light sensitivity (18%), lid or conjunctival irritation (4%), and eye pain or headache (2%). Parents were queried about the occurrence of a systemic side effect at each follow-up visit during atropine treatment. Facial flushing was reported for two patients, one of whom remained on atropine with no further problems and one of whom was switched to homatropine. Atropine was not discontinued because of side effects in any other patients. No other systemic side effects of atropine were reported. In ATS3, among 201 patients 7 to <13 years old, atropine was generally well tolerated. Two patients were switched from atropine to homatropine because of possible side effects although the relationship to atropine was uncertain (vomiting in 1 patient, tachycardia in 1 patient) and atropine was discontinued prior to the end of the randomized trial phase in 9 (4%) patients due to symptoms related to cycloplegia (difficulty with near vision that was not satisfactorily treated with reading glasses).

Atropine produces dilation of the pupil, which can increase the light that enters the eye. Although it has not been demonstrated that atropine used for several months duration will have harmful ocular effects, excessive exposure to light theoretically could be toxic to the retina. Atropine has been used long-term to prevent the progression of myopia without an apparent adverse effect on acuity.[9-12]

To minimize risks, the following steps will be taken:

- Atropine will be dispensed in child-proof containers and parents will be instructed to keep the atropine away from children.
- To minimize risk from pupil dilation, non-prescription sunglasses with UV protection (or flip-ups, for patients who require glasses) will be provided, and the wearing of a brimmed hat will be encouraged.

When a patient develops adverse effects serious enough to discontinue atropine, the investigator should call a Protocol Chair to discuss the protocol to follow. If atropine is discontinued, then the patient can be switched to homatropine 5%. Such a change in the treatment regimen will be recorded on a follow-up examination form. Homatropine, if needed, will be sent directly to the patient from the Jaeb Center.

5.5.2 Side Effects of Patching

Patching could cause mild skin irritation. However, in view of the small number of hours of daily patching prescribed in the study, substantial skin irritation is highly unlikely.

5.5.3 Reverse Amblyopia

The study treatment for amblyopia could decrease the visual acuity in the sound eye, although this is almost always reversible and is rare in the age group included in this study.

The diagnosis and management of reverse amblyopia is left to the investigator's judgment.

5.5.4 Development of Strabismus or Diplopia

The study treatment could precipitate the development of a manifest ocular deviation. If treatment precipitates the development of an ocular deviation (e.g., esotropia), the parent will be advised to have the patient see the investigator as soon as possible. If the deviation is confirmed on examination, the decision as to whether to continue or discontinue therapy will be left to the investigator's and parent's decision. If amblyopia treatment is to be discontinued during the

treatment period of the study, a Protocol Chair should be called to discuss the case. The development of a new heterotropia is an accepted risk of amblyopia therapy as part of standard care. Such an event occurred with both patching and atropine therapy with atropine in about 18% of cases in ATS1. About 13% of patients had resolution of their preexisting strabismus >8Δ with treatment. In ATS4, 2 of 168 patients developed a new esotropia >8Δ, while 5 patients had resolution of a small angle esotropia. This risk in this study is no greater than it would be with standard care of amblyopia.

Diplopia has been considered to be a possible adverse effect of treating amblyopia in older children. However, in ATS3, no patients developed constant diplopia during the randomized trial phase. In the 7 to <13 year olds among patients not reporting diplopia at baseline, intermittent binocular diplopia occurring more than once a day was reported by 4 patients in the Treatment Group and by 1 patient in the Optical Correction Group. For 3 of the 4 patients in the Treatment Group, diplopia was not reported at the last study visit; 1 patient at the last visit reported diplopia once a day, while the parent reported the diplopia once a week. While still on treatment after the end of the randomized trial phase, an 8-year-old in the Treatment Group, who had a history of a prior sixth nerve palsy and an esotropia at near at baseline, developed intermittent daily diplopia; at the last visit the patient indicated diplopia was occurring several times a day but the parent indicated once a week. In the 13 to <18 year olds, no patients reported binocular diplopia occurring more than once a day.

5.5.5 Risks of Examination Procedures

The procedures in this study are part of daily ophthalmologic and optometric practice in the United States and pose no additional known risks. Dilating/cycloplegic eye drops may normally be used as part of an exam.

5.6 Reporting of Adverse Events

Each investigator is responsible for informing his/her IRB of serious treatment-related adverse events and for abiding by any other reporting requirements specific to his or her IRB.

Data on the complications of the study treatments will be tabulated regularly by the Coordinating Center for review by the Steering Committee. Serious complications will be reported expeditiously to the Data and Safety Monitoring Committee, which will receive a full adverse event report semi-annually. Following each DSMC data review, a summary will be provided to IRBs.

5.7 Patient Payments

The parent/guardian of each patient will be compensated $25 per visit for completion of 9A and 9B exams. (Completion of 9B-1 and 9B-2 exams will result in one visit payment for patients who complete both on the same visit (i.e., patching group). Completion of 9B-1 and 9B-2 exams will result in 2 visit payments for patients who complete 9B-2 two weeks after 9B-1 (i.e., atropine group).) For patients remaining in follow up after the 9B visit, $25 will be paid for each study visit, up to a maximum of $75 (maximum of $150 for the entire study). If there are extenuating circumstances, additional funds may be provided for travel if expenses exceed $25 and the patient will be unable to complete the visit without the reimbursement of the travel expenses.

5.8 Discontinuation of Study

The study may be discontinued by the Steering Committee (with approval of the Data and Safety Monitoring Committee) prior to the preplanned completion of enrollment and follow-up for all patients.

5.9 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/guardian's contact information. The Jaeb Center will maintain direct contact with the parents or guardian of each patient. 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 family and to help coordinate scheduling of the outcome examination. One phone contact is planned for each patient in the first month after enrollment. Additional phone and mail contacts will be made if necessary to facilitate the scheduling of the patient for follow-up visits. A patient newsletter, study updates, and a study logo item may be sent. Patients will be provided with a summary of the study results in a newsletter format after completion of the study by all patients.

CHAPTER 6: SAMPLE SIZE ESTIMATION AND STATISTICAL ANALYSIS

The estimation of sample size and statistical analysis plan are summarized below and detailed in separate documents. A detailed statistical analysis plan will be written and finalized prior to the completion of the study. The analysis plan synopsis in section 6.2 contains the framework of the anticipated final analysis plan, which will supersede section 6.2 when it is finalized.

6.1 Sample Size Estimation

The sample size estimate has been computed for the primary study objective, to determine whether the visual improvement at 17 weeks obtained with pharmacologic therapy and occlusion therapy are equivalent, in concert with the analytic approach for this objective (analysis of covariance) as described in section 6.2.

The primary analysis will include only patients with moderate amblyopia (visual acuity of 20/40 to 20/100) because of the lack of available data on the outcome of atropine treatment when acuity is worse than 20/100; therefore the sample size was estimated in order to have the needed power for analysis of these patients. Based on data from ATS3, we assumed a standard deviation for the 17-week outcome score of 0.20 logMAR and a correlation between baseline and 17-week outcome score of 0.30.

To select a sample size for the trial, it is necessary to set the equivalence limit, which represents the end of the 95% confidence interval for the difference in mean acuity between groups. A limit of 1 logMAR line (5 letters) was considered by the Steering Committee to provide sufficient evidence for equivalence. Because the study question is equally interested in whether patching is equivalent to atropine and atropine is equivalent to patching, it will be necessary to construct two one-sided confidence intervals in analysis.

For a 1 logMAR line equivalence limit, a 95% confidence interval (in actuality, two 1-sided 95% confidence intervals), 90% power, a standard deviation of 0.20 and correlation between the baseline and outcome acuities of 0.30, the sample size required for the study was estimated to be 162, equally divided between the two groups. This was increased to 180 to account for up to 10% loss to follow up.

6.2 Primary Analysis for Efficacy

All primary analyses will include the cohort of patients with moderate amblyopia (20/40 to 20/100).

Because ATS9 is designed as an equivalence trial, treatment equivalence will be declared if the two 1-sided 95% confidence intervals constructed on the difference between adjusted mean visual acuity scores for the two groups are completely contained within the designated equivalence interval of ±1 logMAR unit.

The adjusted means mentioned above will be obtained through application of an Analysis of Covariance (ANCOVA) model using baseline visual acuity scores as the covariate and six-month visual acuity scores as the dependent variable. Thus, a confidence interval will be formed around the difference between 17-week visual acuity mean scores after these means have been adjusted for the baseline acuity scores.

The primary analysis will follow the “intent-to-treat” principle.

As a secondary analysis, the proportions of patients in each group with acuity at 17 weeks of ≥20/25 and the proportions improving 3 or more lines from baseline will be compared by constructing 2 one-sided 95% confidence intervals on the difference in proportions.

To explore whether there is a difference in the initial effect of treatment, the visual acuity data from the 5-week visit will be used to compare the treatment groups in an analysis of covariance similar to that described for the primary analysis.

The treatment effect in subgroups based on baseline factors will be assessed in preplanned secondary analyses. The subgroups of most interest will be those based on baseline amblyopic eye visual acuity (≥20/50, <20/50), cause of amblyopia, age, and prior treatment.

6.3 Primary Safety Analysis

The primary safety analysis will be a treatment group comparison of logMAR sound eye visual acuity score obtained at visit 9B-2, adjusted for baseline acuity scores in an analysis of covariance (ANCOVA) model.

A second analysis will compare the proportion of subjects with a 3 or more line decrease from baseline to visit 9B-2 in sound eye acuity with a Fisher's exact test.

6.4 Severe Amblyopia

In view of the small sample size, analyses will be exploratory. An equivalence analysis will not be performed. Power will be limited for a direct treatment group comparison. However, point estimates can be obtained to provide an indication as to (1) whether severe amblyopia can improve with atropine and (2) the degree of expected improvement to help in the planning of a larger trial if indicated.

6.5 Interim Analyses

No formal interim efficacy analyses of the outcome data are planned in view of the timing of its collection as it seems unlikely that there would be sufficient data and reason to terminate the trial early. However, an efficacy and safety report will be provided to the DSMC twice a year. DSMC reports also will include tabulations of local and systemic adverse effects of atropine. Nevertheless, a minimal amount of alpha spending (0.001) will be allocated for each DSMC review of the data. There are projected to be two DSMC data reviews prior to the end of the trial. The final alpha level at the end of the trial will be accordingly adjusted to 0.048 for the overall statistical comparisons of the two treatment groups for declaring superiority of one treatment over the other.

CHAPTER 7: A STUDY OF RETINAL NERVE FIBER LAYER THICKNESS IN AMBLYOPIA

7.1 Introduction

This ancillary study is part of the ATS9 protocol. Participation is optional for sites and, at participating sites, participation is optional for each subject.

7.1.1 Study Objective

To determine whether amblyopia is associated with structural abnormalities of optic nerve fiber layer.

7.1.2 Background

Amblyopia is generally attributed to abnormal development of the visual cortex due to strabismus, image blur from refractive error, monocular form deprivation, or a combination of these factors. A change in size of cell bodies in the lateral geniculate body of the thalamus have been seen with amblyopia and amblyopia therapy in non-human primate models of amblyopia.[13] However, some have suggested that amblyopic eyes may also have abnormalities of the retinal ganglion cell, retinal nerve fiber layer (RNFL), and optic nerve. It is not known if changes to the pregeniculate visual system are congenital or acquired from abnormal postnatal sensory input. If the lack of development of normal structures or loss of normal structures occurs prior to treatment, normal structures may not be present and treatment might not be as likely to succeed.

It is possible that these changes in the optic nerve may be due to abnormal apoptosis. It has been argued that correctly controlled apoptosis of the optic nerve needs a focused image. Abnormal focused input due to amblyopia could lead to either too few or too many fibers remaining in the optic nerve. Loss of optic nerve axons may also be due to transsynaptic degeneration following a cortical lesion. These anatomic changes in the optic nerve fiber layer could play a role in the visual acuity outcome with treatment.

Using computerized analysis of magnification-corrected optic disc photos from 205 amblyopic subjects Lempert and Porter identified an abnormal optic disc appearance, they have termed optic disc dysversion in 45.4% of eyes.[14] A second study of 275 patients found such an appearance in 48% of the amblyopic eyes (mean age = 39.8 years).[14] (The patients from the first study were possibly included in this study.) The disc area was significantly smaller in the amblyopic eyes compared to the sound fellow eyes, 1.58 mm^2 compared to 1.77 mm^2. However, these authors also found that 74% of amblyopic eyes and 57% of fellow eyes had disc areas less than 2.05 mm^2, suggesting that the condition may be bilateral.[15] They also noted microphthalmos and optic nerve hypoplasia in the amblyopic eye in a more recent report.[14] Lempert also reported that the optic disc area of anisometropic amblyopic eyes was smaller than their non-amblyopic eyes, even when adjusted for axial length.[16] These authors presumed that the small size of the optic disc implied a subnormal number of optic nerve fibers. However, this analysis is complicated by the fact that optic disc size and nerve fiber counts are not well-correlated.[17]

The RNFL arises from the unmyelinated axons of the retinal ganglion cells. The thickness is greatest in the inferior and superior poles of the optic disc. The thickness of retinal nerve fiber layer around the optic disc is a surrogate measure of the total number of optic nerve axons. A positive correlation of RNFL thickness and optic nerve head size has been shown with optical coherence tomography (OCT) (Carl Zeiss-Humphrey-Meditec, Dublin, CA).[18] This complements earlier histological studies in non-human primates and humans that showed that optic nerve fiber count increases as optic disc size increases. [19,17]

The overall RNFL thickness is reduced in eyes of adults and children with optic nerve atrophy from glaucoma. [20] [21] However, Hoh and colleagues found significant overlap between normal eyes and eyes with optic nerve pathology, primarily glaucoma in their study.

7.1.3 RNFL and Amblyopia

The RNFL thickness in strabismic amblyopia has been measured with scanning laser polarimetry. [22, 23, 24] The amblyopic eyes were mainly strabismic, and the RNFL thickness did not differ significantly from the sound eyes in overall average, superior or inferior thickness values. [22] Colen and coworkers (N=20) and Baddini-Caramelli et al (N=21) showed no difference in RNFL with strabismic amblyopia. [23, 24] In a more recent study Yen and colleagues found the RNFL with OCT to be thicker in the amblyopic eyes compared to the fellow eyes of children with anisometropic amblyopia, but no difference between eyes of children with strabismic amblyopia. [25] Another abstract from Rabbione and colleagues found no difference in the RNFL of the amblyopic eyes compared to fellow sound eyes.[26] Atilla and colleagues measured the RNFL of hypermetropic and anisometropic amblyopia adults (mean age about 17 years) and found no difference between amblyopic eyes and eyes from a control group of non-amblyopic eyes.[27]

Each of the published studies included small numbers. To date studies of the RNFL have not resolved the question whether there is a difference in the number of optic nerve axons in the optic nerve of amblyopic eyes compared to sound fellow eyes.

7.1.4 Optical Coherence Tomography

Optical coherence tomography has evolved to be a widely used, non-invasive technique that measures nerve fiber layer thickness and other retinal features. The currently available instrument is the OCT3. The OCT obtains cross-sectional, high-resolution images of the RNFL. The OCT measurement is not substantially affected by axial length or refractive error between -5.00D and +5.00D. Multiple techniques of sampling the retina and the retinal nerve fiber layer are included in the instrument software. OCT testing requires dilated pupils. However, it is a non-contact device, so no anesthetic drops are required. The testing requires steady fixation which may be difficult for younger children and those with poorer central acuity. Testing of both eyes can be completed in less than 15 minutes.

The simplest imaging software for the peripapillary optic nerve is the rapid RNFL analysis. In this technique a circular sample is taken around the optic disc with a diameter of 3.44 mm. The circle is manually centered by the technician while the patient fixates a target. The software takes three images around each optic nerve which are averaged in the output. Each scan is performed in just a few seconds. The thickness is determined by image processing software which identifies the first bright reflection at the vitreoretinal interface and the posterior border of the bright RNFL image as it changes to a less bright image. Average thickness values for each quadrant (superior, nasal, inferior, temporal), each clock hour, and the RNFL as a whole are provided. Signal strength is rated on a 10 point scale, with 5 or more considered acceptable.

No comparisons of amblyopic children with normal age-matched patients have been reported, possibly because there are few normative data reported for OCT3 in children and young adults. The commercially-distributed software does not include any children in the normal database. Hess and colleagues reported RNFL data for 104 normal patients age 3 to 17 years using the OCT3 sampling two concentric circles. [21] They found fixation difficulties and movement artifact to be a problem in a few patients. They reported data from superior and inferior quadrants for two different peripapillary diameters, 2.9 and 6.8 mm. A difference between glaucomatous and normal eyes was

an average of 26%. These authors, focusing on glaucoma, published superior and inferior thickness values, but did not include temporal thickness values. Temporal RNFL might be important for children with amblyopia as a better marker of central visual function. Thus there are no normative data available, either by sector or as a whole for a nerve, to be used as a comparison group with young amblyopic patients.

RNFL measurements in adults with the OCT are reproducible. [28] No such test-retest data are available for children. In addition, interpatient variability in adults is substantial which if also true in children may make it difficult to detect relatively small differences in amblyopic eyes. Given the likely small difference present in amblyopic eyes, the variability of thickness in the population, and no available large database of normal adolescents, it is not possible to compare the RNFL of amblyopic children to a normal population at this time. We propose comparing sound to amblyopic eyes as our primary aim of this ancillary study. We have considered but do not believe it is feasible in our network to develop a database of optic nerves from normal patients for analysis and comparison to amblyopic eyes.

No prospective data are available correlating RNFL measurements or optic disc size with visual acuity at baseline for both eyes, visual acuity at outcome, and the improvement of visual acuity of the amblyopic eye. Thus the secondary study aim is to explore these relationships.

In a pilot study using rapid RNFL (4.0.3.1) software at the Wilmer Eye Institute, 18 amblyopic patients 5 to 20 years were studied. Only one amblyopic eye could not be imaged (eETDRS letter count on the EVA Tester = 17, 20/80 equivalent) (based on shape of NFL thickness plot and placement of scan path around optic nerve image). The other amblyopic eyes, many with worse acuity were successfully imaged. For the 17 patients in whom both eyes were imaged, the mean thickness of the sound eye was 109.2 microns, the amblyopic eye was 104.2 microns, and the average difference (sound eye – amblyopic eye) was 5.0 microns. The sound eye was 10 or more microns thicker than the amblyopic eye in 4 patients; the amblyopic eye was 10 or more microns thicker than the sound eye in 1 patient; and the difference was within 10 microns in 12 patients.

7.2 Study Protocol

7.2.1 Informed Consent

At participating sites, written informed consent for participation in the ancillary study will be obtained from the parent (guardian) and assent from the subject. Participation will be optional.

7.2.2 Eligibility criteria

- Enrolled in ATS9
- Refractive error in both eyes between -0.25 and +5.00D, inclusive (to avoid the need to adjust for refractive error)
- Birth weight $\geq$1500 grams
- No history of CNS disease (e.g. IVH, PVL, meningitis, developmental abnormalities of the brain, hydrocephalus, cerebral palsy, hypoxic ischemic encephalopathy)

7.2.3 Testing Procedures

The OCT RNFL imaging will be performed either at the baseline examination or at one of the study follow up visits.

The subject's pupils will need to be dilated, if not already dilated as part of the exam. The RNFL scan will be performed on the right eye, followed by the left eye. The scan will then be repeated on both the right eye and the left eye. The procedures to be followed by the OCT operator will be

detailed in a separate procedures manual. The results of the scans will be sent to the coordinating center.

7.2.4 Risks

The OCT may cause discomfort from the flashing lights. This lasts just during the procedure. However there is no known risk. The drops to dilate the pupils may cause sensitivity to light for several hours.

7.2.5 Payments

Subjects will be compensated $25 for participation in this ancillary study.

7.3 Statistical Considerations

Twenty-five subjects initially will be scanned. From those data, an assessment will be made as to the variability and the sample size will be re-estimated. Up to 100 subjects may be included in the study.

The analysis plan is detailed in a separate document. The primary analysis will be a binomial sign test for two dependent samples, testing for association between RNFL thickness and amblyopia. This will be based on the proportion of patients with greater thickness in the sound eye, out of all patients with a difference in thickness between the sound and amblyopic eye. Test-retest data for the first 25 patients will be reviewed to determine the level of difference to be considered within the variability of testing, and thus the range of size difference to be considered 'no difference'.

Secondary analyses will include exploratory analyses of the difference in thickness based on cause of amblyopia, baseline acuity and best acuity of the sound and amblyopic eyes.

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