Fixation instability in amblyopia: Oculomotor disease biomarkers predictive of treatment effectiveness

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Introduction

Fusion Maldevelopment Nystagmus (FMN) is one of the most common subtypes of pathologic nystagmus seen in children. The National Institutes of Health Committee on Eye Movement and Strabismus classification has recommended utilizing a new etiologic description from 2001, replacing the term latent nystagmus. This type of nystagmus has initially been called latent because its severity increases, or became evident when an eye is covered. However, it is now known that true latent nystagmus is rare, with the majority of patients have manifest latent nystagmus seen with both eyes uncovered as identified on eye movement recordings. (Abadi and Scallan 2000) Amblyopia is a neurodevelopmental disorder that occurs due to de-correlated binocular input to the visual cortex. Investigations in non-human primate models have revealed that loss of horizontal binocular connections within area V1 in infancy is the necessary and sufficient cause of FMN. (Tychsen et al. 2010) The new terminology describes the strong correlation with a binocular fusion maldevelopment that occurs during the infancy, like strabismus, amblyopia or any monocular vision deprivation. (Tychsen 1992)

Studies by the pediatric eye investigator group have compared part-time occlusion to full-time occlusion and found similar levels of improvement in visual acuity. Thus the current standard of treatment is part-time occlusion ranging from 2-6 hours/eye depending on the severity of amblyopia. (Holmes et al. 2003) The slow phase velocity (SPV) of FMN increases under monocular viewing conditions and therefore in patients with FMN occlusion was initially considered contraindicated because it could enhance the nystagmus intensity or amplitude. (Duke-Elder and Wybar 1973) Successively evidence was provided in a small cohort of patients that a significant improvement of visual acuity was obtained with full-time patching during all waking hours. (von Noorden al. 1987) Similarly, Simonsz demonstrated a compensatory drift changes in nystagmus direction and lower magnitude over days of full-day occlusion in 5 patients with latent nystagmus.(Simonsz 1989) Despite good compliance, up to 40% of children treated by occlusion therapy have residual amblyopia. Some baseline risk factors that predict the presence of residual amblyopia include severe amblyopia at time of diagnosis and older age at treatment initiation. (PEDIG Group 2011)

Amblyopes are known to have increased fixation instability.(Niechwiej-Szwedo et al. 2012; Subramanian et al. 2013). This instability could be due to the presence of FMN. Amblyopic patients without nystagmus have an increase in the amplitude of fixational saccades with increase inter-saccadic drift, which contributes to the instability in both the fellow and amblyopic eye. (Shaikh et al 2016; Shi et al 2012; Chen et al 2018) In the current manuscript, we characterized the fixational eye movement waveforms of amblyopic patients. We hypothesize that the presence of FMN and increase slow phase velocity in patients with nystagmus would predict treatment responsiveness. In addition, we hypothesize that in patients without nystagmus, the presence of increased fixational saccade amplitude and inter-saccadic drift would be correlated with poor treatment response.

Methods:

The records of 80 amblyopic patients from the practice of FG who had eye movement recordings performed between 2013 to 2019 were reviewed. The experimental protocol was approved by the Cleveland Clinic Institutional review board and written informed consent was obtained from each participant or parent/legal guardian in accordance with the Declaration of Helsinki. After review, 53 patients, who had at least 12 months of follow up after diagnosis of amblyopia and were prescribed patching treatment were included in the study (Table 1).

The clinical categorization of amblyopia subtype and severity at the time of diagnosis were based on PEDIG studies (Manh, Holmes et al. 2018). Type of amblyopia: Amblyopia associated with strabismus, anisometropia, or both meeting the following criteria: *1) Strabismic amblyopia*: At least one of the following criteria must be met and criteria are not met for combined-mechanism amblyopia: a) Heterotropia at distance and/or near fixation on examination (with or without spectacles) b) History of strabismus surgery c) documented history of strabismus which is no longer present (and which, in the judgment of the investigator, is the cause of amblyopia) *2) Anisometropic amblyopia*: At least one of the following criteria must be met: a) ≥0.50 D difference between eyes in spherical equivalent ≥1.50 D difference between eyes in astigmatism in any meridian 3) *Combined mechanism amblyopia*: Both of the following criteria must be met: a) criteria for strabismus are met (see above) b) ≥1.00 D difference between eyes in spherical equivalent or ≥1.50 D difference between eyes in astigmatism in any meridian

Severity of amblyopia: Mild amblyopia: if worse eye visual acuity (VA) was <0.30 LogMAR, moderate if ≥ 0.30 and <0.70, severe if ≥0.70; VA of amblyopic eye at baseline. Visual acuity was measured in each eye using the participant's optimal spectacle correction with Snellen linear optotype. For patients younger than 7 years of age, crowding bars HOTV or Allen pictures were used as per the child's ability to perform the test if they were unable to do the Snellen linear optotype. There were only four patients that were diagnosed before their ability to perform optotype testing- they all had manifest strabismus with strong fixation preference. They were all assigned as having severe amblyopia at the time of diagnosis.

Treatment considered was part-time occlusion (2-6 hours/day), prescribed depending on the severity of amblyopia. Patients with manifest strabismus were treated according to the American Academy of Ophthalmology Preferred Practice Pattern. Investigators judged compliance with patching treatment to be excellent (>75%), good (51%–75%), fair (26%–50%), or poor (≤25%), based on discussions with the parents.

Of the recruited patients, 21 had no nystagmus, 21 had nystagmus without FMN, and 11 had FMN. The subjects were also grouped based on the type of amblyopia (anisometropic = 19, mixed = 28, strabismic = 7). Due to an inadequate number of subjects, we were not able to do subgroup analysis per eye movement waveform within each clinical type of amblyopia. There was no difference in the follow up time (None: 56 ± 34, Nyst No FMN: 71 ± 37, FMN: 75 ± 43, p =0.32), The follow up duration for amblyopic patients of anisometropic patients was lower than the other two groups (Aniso = 46 ± 31, Strab = 109 ± 32, Mixed= 71.5 ± 32, p= 0.0001).

Clinical data and Outcome Measures:

The clinical parameters were extracted from a retrospective chart review for all the enrolled subjects (Table 1). The ages at follow up visits, visual acuity of fellow and amblyopic eye, strabismus measurements in the primary position, stereopsis and compliance to treatment were noted. Stereoacuity was measured with the Titmus Stereoacuity Test. Stereoacuity scores in seconds of arc were: 40'', 60'', 100'', 200'', 400'', 800''; 3500" was the value of patients able to see only the fly; subjects with no detectable (nil) stereoacuity were assigned a value of 7000". For analyses, stereoacuity scores in seconds of arc were converted to log values as follows: 40" (1.60), 60" (1.78), 100" (2.00), 200" (2.30), 400" (2.60), 800" (2.90), 3500" (3.55) and 7000" (3.85). The total duration in months of patching treatment till visual acuity was stabilized with no further improvement or deterioration ≥ 2 consecutive visits ≥ 6 weeks apart was computed for all the patients with at least 50% compliance. The improvement in visual acuity as expressed in arc minutes were calculated as the difference of acuity at the final visit from that of the acuity at the start of treatment. Patients were stratified based on the degree of vision improvement in response to treatment as < 3 arc min, 3-6 arc min and > 6 arc min. In addition residual amblyopia at end of treatment was defined as mild<0.30 LogMAR, moderate if ≥ 0.30 and <0.70 and severe if ≥0.70 log MAR scale. Final stereopsis was assessed and patients were classified to have good stereopsis (better than 100 sec arc), some stereopsis (100-400 sec arc) and gross/absent stereopsis (3500 or absent stereo).

Eye movement recording and analysis: A high-resolution video-based eye tracker (EyeLink 1000®, SR Research, Ontario, Canada) was used to measure binocular horizontal and vertical eye positions at a temporal resolution of 500 Hz during a fixation task as described previously. Briefly, eye position data was analyzed after removal of blinks and partial blinks. To measure eye velocity, we differentiated the eye position signal using MatlabTM (Mathworks, Natick, MA) diff function. Differential value (velocity signal) was further smoothened with Savitzkey-Golay filter, a function that can be applied to a set of digital data points for the smoothing purpose. (Shaikh et al. 2016)

Fixational saccades and quick phases of nystagmus were identified using an unsupervised clustering method(Otero-Millan, Castro et al. 2014). Drifts were defined as epochs between fixational saccades and blinks. We removed 20 msec data at the beginning and end of each of the drifts to exclude periods of acceleration and deceleration of the eye during fixational saccades and blinks. The composite eye velocity of the ocular drifts and the composite variability of eye position were computed using the following equation: composite=$(horizontal^2+vertical^2)^{1/2}$. All analyses were performed in Matlab (Mathworks, Natick, MA, USA) and GraphPad Prism 7 (La Jolla, CA, USA). A Kruskal-Wallis analysis of variance test was used to compare the demographics and clinical outcomes across amblyopia subtype. We used one-way ANOVA to compare the clinical and oculomotor parameters across fixation eye movement characteristics. An unpaired t-test was used to analyze clinical/oculomotor parameters between the two groups.

Results:

Fixational stability in amblyopes is determined by the frequency and amplitude of fixational saccades and inter-saccadic drifts in patients without nystagmus and by quick and slow phases in patients with nystagmus. We characterized fixational eye movements in amblyopic patients based on their waveform characteristics as those without nystagmus, those with nystagmus but without the classic reversal in quick phase of nystagmus seen in fusion maldevelopment nystagmus patients and those with fusion maldevelopment nystagmus. In the current study, we wanted to discern the effects of fixation waveforms and amblyopia type on treatment effectiveness. We hypothesize that eye movement waveforms were better predictors of treatment effectiveness than clinical type. Besides the clinical type and waveform, there are several variables such as age at diagnosis, visual acuity at the time of diagnosis and compliance to treatment that could be related to final visual acuity at the end of treatment. There was no difference in the age at months of start of patching across eye movement waveforms (No nystagmus = 63 ± 24,Nystagmus no FMN = 56 ± 25, FMN = 57.9 ± 40.5, p = 0.71) and type of amblyopia (Aniso = 76 ± 14, Strab = 59 ± 38, Mixed = 65 ± 29, p= 0.19). Similarly, there was no difference in compliance (None: 58 ± 21, Nyst no FMN: 63 ± 21, FMN: 59 ± 16, p = 0.73) and visual acuity expressed in arc min at time of diagnosis across eye movement waveforms (None: 9.3 ± 15.75, Nyst no FMN: 7.78 ± 16.5 and FMN: 4.9 ± 3.3, p = 0.32). The compliance to patching (Aniso: 67 ± 11, Strabismic: 51 ± 17 and Mixed: 59 ± 18, p = 0.09) and visual acuity at the time of start of treatment (Aniso: 8.1 ± 17.4,Strabismic: 9.0 ± 8.5, Mixed: 6.9 ± 12.9, p = 0.9) was comparable across clinical types.

Treatment outcome measures as a function of clinical subtype of amblyopia:

Anisometropic patients either did not have nystagmus or had nystagmus no FMN. There was only 1 subject with anisometropia and FMN. This subject was noted to have intermittent esotropia on subsequent clinical visits. Thus we categorized this patient as having mixed mechanism amblyopia for statistical analysis. All the patients with strabismic and mixed amblyopia had strabismus surgery and were either orthotropic with glasses or had microstrabismus with glasses. Figure 2 A plots the visual acuity improvement in arc min which was comparable across all three clinical subtypes (Aniso: 6.9 ± 16.9; Strabismic: 6.4 ± 8.4 and Mixed: 4.3 ± 12.3, p = 0.16). The total duration of treatment (Fig 2b) was similar across the three subtypes (Aniso: 19.2 ± 22; Strabismic: 27 ± 14 and Mixed: 19.75 ± 20, p = 0.16). Anisometropic amblyopes were more likely to have better stereopsis at the end of treatment compared to the other two groups (Fig 2C- Aniso: 1.9 ± 0.51; Strabismic: 3.5 ± 0.69, Mixed: 3.0 ± 0.89, p <0.0001).

Treatment outcome measures as a function of fixation eye movement waveforms:

The presence of FMN serves as a signature oculomotor disease biomarker indicative of a disruption in binocularity in the first six months of life. Because we wanted to discern the correlation between characteristics of fixation eye movements and response to amblyopia treatment, we categorized the amblyopic subjects without nystagmus from subjects with nystagmus no FMN and those with FMN that would be indicative of early-onset amblyopia.

Patients with FMN had less improvement in visual acuity (Fig 3A) compared to the other groups however it did not reach statistical significance (None: 7.7 ± 14.5, Nystagmus no FMN: 5.0 ± 16, FMN: 3.02 ± 3.4, p =0.2). Patients with FMN had longer duration of treatment (Fig 3B) compared to the other two groups (No nystagmus: 9.5 ± 6.3,

Nystagmus no FMN: 22 ± 22, FMN: 38 ± 19, p = 0.01). The stereopsis was worse in patients with FMN (Fig 3C) compared to the other two groups (No Nystagmus: 2.4 ± 0.9, Nystagmus no FMN: 2.6 ± 0.9, FMN: 3.3 ± 0.8, p =0.04).

Eye movement parameters and clinical outcomes:

Our previous studies have shown that patients without nystagmus have increased fixational saccade amplitude and inter-saccadic drift compared to controls. (Shaikh et al 2016) We have also found increased slow phase velocity in patients with FMN compared to the inter-saccadic drift velocity in amblyopic patients without nystagmus and controls. (Kang et al submitted under review) During occlusion therapy, the amblyopic eye is the viewing eye. Thus we wanted to investigate whether the fast and slow eye movement properties of the amblyopic eye correlate with the presence of residual amblyopia, the treatment duration, and stereopsis at the end of treatment.

Fig 4 plots the composite drift velocity (4 A) and eye position variance (4D) measured during inter-saccadic drifts in patients without nystagmus as a function of the degree of residual amblyopia defined as mild (20/30 or better), moderate (20/40 and better than 20/100) and severe (20/100 or worse). The composite drift velocity was higher in patients with severe residual amblyopia compared to the other two groups (Mild: 0.8 ± 0.95°/s; Moderate: 0.64 ± 0.54°/s, Severe: 0.94 ± 0.94°/s, p= 0.03). Similarly, the variance in eye position during the inter-saccadic drift was greater in patients with severe residual amblyopia (Mild: 0.03 ± 0.06°; Moderate: 0.028 ± 0.04°, Severe: 0.04 ± 0.08°, p= 0.04). Fig 4 B and E plots the composite drift velocity and drift variance respectively as a function of the duration of treatment (≤ 6 months versus 7-24 months). Unlike the other two groups, none of the patients without nystagmus required > 2 years of treatment. Patients with prolonged duration of treatment before their vision stabilized tend to have greater composite drift velocity (Fig 4 B)(≤ 6 months: 0.52 ± 0.41°/s > 6 months: 0.91 ± 1.07°/s; p = 0.0007). The variance of eye position was similar across the two groups (Fig 4 E) (≤ 6 months: 0.02 ± 0.04° and 7-24 months: 0.03 ± 0.07°, p=0.3). Fig 4 C and F plot the composite drift velocity and drift variance respectively as a function of stereopsis at the end of treatment. Patients with poor stereopsis tend to have greater composite drift velocity (good stereo: 0.59 ± 0.51°/s; some stereo: 0.53 ± 0.53°/s; poor stereo: 1.03 ± 1.1°/s; p < 0.0001). Similarly, the variance of eye position was greater in patients with poor stereopsis (good stereo: 0.01 ± 0.03°; some stereo: 0.03 ± 0.06°; poor stereo: 0.05 ± 0.12°; p < 0.0001) compared to the other two groups.

Fig 5 plots the cumulative sum histogram of composite fixational saccade amplitude of the amblyopic eye as a function of residual amblyopia (Fig 5 A), treatment duration (Fig 5 B) and stereopsis at the end of treatment (Fig 5C). The fixation saccade amplitude of the amblyopic eye was greater in patients with residual amblyopia (Mild: 0.93 ± 0.95; Moderate: 1.3 ± 1.04; Severe: 2.6 ± 1.7; p < 0.001). This is consistent with the rightward shift of the distribution of fixational saccades in patients with moderate and severe amblyopia compared to mild amblyopia (Fig 5A). Patients requiring longer than 6 months of treatment had greater amplitude of the fixational saccade of the amblyopic eye (Fig 5 B) (≤ 6 months: 0.68 ± 0.55 ° and 7-24 months: 1.2 ± 1.2°, p < 0.0001). Patients with poor stereopsis had greater amplitude of the fixational saccade of the amblyopic eye (Fig 5 C) (good stereo: 0.86 ± 0.60°; some stereo: 1.2 ± 1.3°; poor stereo: 2.0 ± 1.4°, p < 0.0001).

In patients with nystagmus with and without FMN, we have found increased slow phase velocity compared to the inter-saccadic drift in patients without nystagmus. We investigated whether there is a correlation between the amblyopic eye velocity and eye position variance elicited during the pathologic slow phase of the nystagmus and treatment outcomes.

Fig 6 plots the composite slow phase velocity (6A) and eye position variance (6 D) during the slow phase of the nystagmus of the amblyopic eye and the degree of residual amblyopia in patients with nystagmus no FMN. The composite drift velocity was comparable across the three groups (Mild: 0.90 ± 0.82°/s; Moderate: 0.97 ± 0.98°/s, Severe: 1.08 ± 1.3°/s, p= 0.14). However, the variance in eye position during the slow phase of nystagmus was greater in patients with severe residual amblyopia (Mild: 0.02 ± 0.12°; Moderate: 0.03 ± 0.08°, Severe: 0.22 ± 0.91°, p <0.0001). Fig 6 B and E plots the same two parameters elicited during the slow phase of nystagmus as a function of the duration of treatment. Patients with shorter duration of treatment (6 months or less) before their vision stabilized tend to have lower composite drift velocity (Fig 6B) (≤ 6 months: 0.65 ± 0.51°/s; > 7-24 months: 1.21 ± 1.1°/s and > 24 months: 1.12 ± 1.16°/s; p < 0.0001) with reduced variance of eye position (Fig 6E) (≤ 6 months: 0.01 ± 0.02°; > 7-24 months: 0.04 ± 0.08° and > 24 months: 0.04 ± 0.13°; p <0.001) compared to the other two groups. Fig 6 C and F plots the composite drift velocity and drift variance respectively as a function of stereopsis at the end of treatment. Patients with good stereopsis tend to have lower composite drift velocity (good stereo: 0.44 ± 0.31°/s; some stereo: 0.94 ± 0.98°/s; poor stereo: 1.02 ± 1.1°/s; p =0.02). The variance of eye position was lower in patients with good stereopsis (good stereo: 0.01 ± 0.02°; some stereo: 0.03 ± 0.08°; poor stereo: 0.04 ± 0.05°; p <0.001).

Fig 7 plots the composite slow phase velocity (7 A) and eye position variance during the slow phase (7 D) of the amblyopic eye and the degree of residual amblyopia in patients with FMN. None of the patients had severe amblyopia at the end of treatment. The composite drift velocity was greater in patients with moderate residual amblyopia compared to mild residual amblyopia. (Mild: 1.29 ± 1.15°/s; Moderate: 9.9 ± 10°/s, p < 0.0001). Similarly, the variance in eye position during the slow phase of nystagmus was greater in patients with moderate residual amblyopia (Mild: 0.02 ± 0.05°; Moderate: 0.74 ± 1.04°, p <0.0001). Fig 7 B and 7E plots the same two parameters elicited during the slow phase of nystagmus as a function of duration of treatment. All the patients with FMN required > 6 months of treatment before their vision stabilized. Patients with prolonged duration of treatment before their vision stabilized tend to have greater composite drift velocity ((7-24 months: 1.5 ± 1.2°/s, and > 24 months: 7.1 ± 9.6°/s, p < 0.0001) with increased variance of eye position (7-24 months: 0.02 ± 0.07 °, and > 24 months: 0.52 ± 1.06°/s, p < 0.0001). Fig 7 C and F plots the composite drift velocity and drift variance respectively as a function of stereopsis at the end of treatment. None of the patients with FMN had good stereopsis. Patients with poor stereopsis tend to have greater composite drift velocity (some stereo: 1.33 ± 0.95°/s; poor stereo: 5.68 ± 8.6°/s; p <0.0001). The variance of eye

position was greater in patients with poor stereopsis (some stereo: 0.02 ± 0.08°; poor stereo: 0.38 ± 0.98°; p <0.001) compared to those with some stereopsis.

Discussion:

The current standard of treatment for amblyopia patients comprises of refractive correction followed by part-time occlusion or atropine penalization of the non- amblyopic eye. (PEDIG Group 2005) Previous studies have shown no additional benefit of full-time occlusion over part-time occlusion.(Holmes et al 2003). In addition, atropine optical penalization has similar efficacy to part-time occlusion in the treatment of moderate and severe amblyopia.(PEDIG Group 2002) In younger children, aged 3 to 7 years, although current treatments with part-time occlusion and atropine drops are effective, residual amblyopia (20/32 or worse) is present in 54% of children at age 10 years(PEDIG Group 2008) and 40% at age 15 years.(Repka et al 2014) Previously the PEDIG group *(*PEDIG Group 2011) in a multicenter randomized clinical trial analyzed children with residual amblyopia who had stopped improving with daily patching or daily atropine and they found that an intensive combined treatment of patching and atropine did not result in better visual acuity outcomes after 10 weeks compared with a control group in which treatment was gradually discontinued. The purpose of this study was to identify oculomotor biomarkers that can be used to predict treatment effectiveness of part-time occlusion therapy. In the current manuscript, we characterized fixational eye movements in amblyopia patients. The subjects enrolled in the study had comparable visual acuity at the time of diagnosis and age at initiation of patching across the groups categorized per their eye movement waveforms. We found that rather than clinical subtype (anisometropia, strabismic or mixed), eye movement characteristics were better in predicting treatment outcomes. This is in agreement with previous studies that have shown that baseline visual acuity and younger age at enrollment were associated with the best improvement, but not the cause of amblyopia. (Wallace et al 2015) We found that children with FMN required longer duration of treatment compared to those without nystagmus. Despite the improvement in visual acuity the recovery of stereopsis was poor in patients with FMN.

Very few studies to date have examined occlusion therapy effectiveness in amblyopic patients with FMN. Von Noorden et al. (von Noorden et al 1987) was the first to show in 12 patients with FMN noted on the clinical exam that patching during all waking hours was useful in improving VA, while it was previously considered contraindicated. The study had examined the effects of full time patching with no eye movement recordings. This is the first study to our knowledge examining the impact of fixation instability on the effectiveness of part-time patching in amblyopia patients. In addition to FMN patients, amblyopic patients can have nystagmus without the classic reversal of the direction of the quick phase. These patients also had improvement in visual acuity albeit requiring a longer duration of treatment compared to those without nystagmus.

Besides the waveforms, the slow phase velocity of patients with nystagmus and inter-saccadic drift velocity in patients without nystagmus were increased in patients with less visual acuity improvement and requiring longer durations of treatment. Also, patients without nystagmus, the amplitude of fixation saccades was greater in patients with residual amblyopia. This is in agreement with previous studies that have shown that amblyopic patients have increased amplitude of fixational saccades with fewer microsaccades (< 1 deg). The increase in amplitude correlated with the severity of amblyopia. (Shaikh et al 2016; Shi et al 2012; Chen et al 2018) The treatment duration was longer in patients with greater fixational saccade amplitude. Microsaccades have shown to play an important role in visual perception by counteracting visual fading.(Martinez-Conde et al 2009) Reduced microsaccade frequency could be an end result of amblyopia versus could be a contributing factor to amblyopia. It is possible that the pathologic increase in the amplitude of fixational saccade in amblyopic patients could hamper the treatment effectiveness.

The analyses were performed independently for different eye movement waveforms and the type of amblyopia. Strabismic patients have an increase in the drift velocity with greater velocities in patients with nystagmus. Our previous study has shown that the drift velocity and variance increase with an increase in the strabismus angle. (Ghasia et al 2018). All of our patients with strabismic and mixed amblyopia had microstrabismus (defined as < 10 prism diopters) at the time of eye movement recordings. In the future, a larger cohort of patients will allow us to independently analyze the effects of eye movement waveforms within each clinical subtype of amblyopia as well as delineate the effects of degree of strabismus.

An important limitation of the current study is the eye movement recordings were obtained at the end of treatment and the treatment effectiveness was determined based on a retrospective chart review. In addition, only, a small cohort of patients with residual amblyopia was treated with atropine and majority of them had no further improvement in visual acuity but the decision to treat was based on discussions with family and the children with greater deficits of visual acuity were more likely to try an alternate treatment. The mean duration of follow up was greater in our study compared to most amblyopia treatment studies. Thus, we were able to identify regression soon after the treatment was stopped or while it was being tapered for patients who initially had severe amblyopia at diagnosis. The analysis from the current study suggests that eye movement characterization and quantification can play an important role in providing information about prognosis and amblyopia treatment effectiveness. A prospective clinical trial of obtaining eye movement recordings at the time of diagnosis and following the patients longitudinally to determine treatment effectiveness of part-time occlusion will be necessary to confirm the findings of the current observational study. In addition, the study suggests that the timing of amblyopia development seems to play an important role in determining part-time patching treatment effectiveness. Additional prospective studies evaluating alternative treatments such as optical penalization and newer binocular amblyopia treatments in a cohort of amblyopic patients with FMN would help further tailor the treatment.

Figure Legends:

Figure 1. Representative eye position traces obtained during fixation in amblyopia patients without nystagmus, nystagmus no FMN and FMN. In patients without nystagmus there is an increase in the amplitude of the fixational saccade with an increase in the inter-saccadic drift. In patients with nystagmus no FMN there is no reversal of the quick phase of nystagmus as seen in patients with FMN. In patients with FMN, there is an increase in slow phase velocity of the amblyopic eye during amblyopic eye viewing condition. Of note, in all three patients abnormalities are seen during binocular viewing condition particularly of the amblyopic eye.
Figure 2. Clinical outcomes sub grouped by the type of amblyopia. Visual acuity improvement and patching duration are not significantly different between types. Final stereopsis is significantly better in anisometropic patients.
Figure 3. Clinical outcomes sub grouped by the eye movement waveforms. Visual acuity improvement is not significantly different between the waveform groups. However, in FMN patients the duration of patching is significantly longer, and the final stereopsis is significantly worst compared to the other two groups.
Figure 4. Composite drift velocity and variance of eye position during inter-saccadic drift of the amblyopic eye of patients without nystagmus obtained under amblyopic eye viewing condition, as a function of severity of residual amblyopia (**A** and **D**), duration of treatment (**B** and **E)** and stereopsis at end of treatment **(C** and **F)**.
Figure 5. Cumulative sum histogram of fixational saccades of amblyopic eye of patients without nystagmus as a function of: **A** severity of residual amblyopia (green = mild, blue = moderate and red = severe), **B** duration of treatment (green = duration of 6 months or less, blue = duration of treatment 6-24 months), **C** stereopsis at end of treatment (green = good stereo, blue = some stereo and red = poor stereo).
Figure 6. Composite drift velocity and variance of eye position elicited during slow phase of the nystagmus in amblyopic eye of patients with nystagmus, but no FMN, obtained under amblyopic eye viewing condition, as a function of severity of residual amblyopia (**A** and **D**), duration of treatment (**B** and **E)** and stereopsis at end of treatment **(C** and **F)**.
Figure 7. Composite drift velocity and variance of eye position elicited during slow phase of the nystagmus in amblyopic eye of patients with FMN, obtained under amblyopic eye viewing condition, as a function of severity of residual amblyopia (**A** and **D**), duration of treatment (**B** and **E)** and stereopsis at end of treatment **(C** and **F)**.

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