

USE OF NFL ATROPHY IN DIAGNOSING GLAUCOMA SUSPECTS

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INTRODUCTION

There has been some recent controversy among eye care professionals as to whether or not subtle changes in the retinal nerve fiber layer (NFL) can provide an early indication of the onset of primary open-angle glaucoma. The prevalent view seems to be that just visualizing the NFL, let alone being able to make meaningful and consistent evaluations, is prohibitively difficult. This paper presents the findings of a related literature search.

An early indicator is obviously needed since practitioners face the problem of when to begin treatment of a glaucoma suspect. Should they begin treatment early, and perhaps needlessly, using expensive and potentially harmful drugs, or wait, and risk added damage? The preponderance of the literature deals with the use of the conventional glaucoma indicators (physiological cupping of the optic nerve head, increased intraocular pressure, visual field defects, etc.). Some of the literature^{1,2,3,4}, however, outlines procedures to be used in visualizing the NFL, describes, classifies, and assigns levels of significance to subtle changes, and states flatly that discernible changes in the NFL occur four to six years in advance of any visual field defects! If this is so, then evaluation of NFL changes represents an extremely valuable, but grossly underused, adjunct to the normal diagnostic procedures. Treatment initiated on the basis of this early indicator could significantly retard the progress of the disease and delay the onset of visual field defects.

NERVE FIBER LAYER STUDIES

Several extensive studies have been conducted to correlate changes in NFL appearance with the quantity of nerve fibers lost and the related vision loss. In general, these studies set out to determine:

- 1) The typical number of axons present in the retinal nerve fiber layer to establish a "normal" standard.
- 2) The absolute thicknesses of the NFL throughout the retina, and the relationship between NFL thickness and the brightness of the ophthalmoscopic reflex. This was done to provide an objective basis for estimating the extent of atrophy.
- 3) The reduction in thickness of the NFL required to cause just discernible changes in appearance.
- 4) The amount and type of NFL change associated with a visual field loss.
- 5) The typical time span between the occurrence of just discernible NFL changes and visual field loss.

Obviously, these tasks entailed a combination of histological and clinical studies.

HISTOLOGICAL & CLINICAL STUDIES

It has long been recognized that there is a gradual diminution of visual function with advancing age in normal eyes. Quantifying this age related loss with respect to axonal loss could provide a basis for determining when glaucoma related nerve fiber loss becomes significant. This diminution could be determined by nerve fiber counts of the optic nerve at various ages. Such counts in the past have resulted in estimates ranging from 40,000 to 1,435,543.^{6,7}

In an attempt to obtain more precise information, Balazi, Rootman, et al⁶ conducted a study which set out to determine the effects of age on the number of axons in the optic nerve and to determine the consistency of count among individuals of the same age. They examined normal human optic nerves from sixteen subjects ranging in age from three and one half years to eighty two years. Specimens were obtained from autopsies, surgical enucleations, and corneal graft donor material. A nerve specimen was taken from a point 1 mm behind the globe. This section was dehydrated, embedded in plastic, and photographically recorded in order to make a nerve fiber count. The globe was sectioned at the equator to allow the optic nerve head and retina to be studied. The number of nerve fibers, established to a 99% confidence level, was between 1,192,400 and 1,295,610.⁶

Some data collected during this study suggests that there was either a fairly large difference in optic nerve fibers between individuals or a large difference in the magnitude of the loss of fibers with age. The study found that a typical rate of decay of fibers with age was 5,637 axons per year.⁶ The age effect may therefore account for loss of approximately 400,000 fibers during a seventy year life span. This suggests that approximately 30% of the optic nerve fibers may be lost on a diffuse basis due to age alone. Although the results of the study were less specific than would be desired for quantifying glaucoma related loss, the study yielded significant new information.

In an attempt to correlate NFL atrophy with changes in NFL appearance, Quigley and Addicks² conducted a study where lesions were experimentally produced in the optic nerves of monkeys and the change in appearance of the NFL was photographically recorded. As soon as a detectable change in the photo occurred, the monkey was sacrificed to allow examination of the minute changes in tissue structure.

They found that a loss of 50% of the NFL thickness in any area of the retina produced a just detectable change in appearance, and that NFL thicknesses equal to or less than 25 microns could not be recorded photographically.

The study also yielded a quantitative basis for NFL examination. They found that the thickness of the normal NFL in monkeys is nearly 200 microns at the twelve and six o'clock positions of the rim and only 60 microns at the foveal and nasal positions. By two disc diameters from the nerve head the NFL thins to less than 40 microns in every meridian. This thickness variation of the NFL was found to be directly proportional to the brightness of the reflexes as seen in green light. Hence, the brightness is a measure of the thickness (number of axons in the bundles) and can be used as a semi-quantitative standard of atrophy. Green light was used to cut down the reflection from the fundus and thereby provide a darker background for the silvery striations of the NFL.

The preceding monkey study was based on nerve fiber layer atrophy caused by lesions to the optic nerve, and the results were compared to a pooled set of data. A follow-up study by Quigley & Addicks² caused glaucoma-like damage to one of a subjects otherwise healthy eyes by using laser treatment of the trabecular meshwork to induce elevated intraocular pressures. The companion eye was used as the control. The subjects of the follow-up study were observed clinically for extended periods of time. As the study progressed they were divided into groups according to the measured increase in IOP and clinical observations of the retina. Finally, the animals were sacrificed and histological studies were conducted. This procedure made it possible to both study the normal progression of glaucoma related NFL atrophy and to conduct histological studies related to various levels of progression.

Results of Follow-Up Monkey Study

Group one included those subjects that had no clinically detectable NFL changes. Their mean IOP was from 25 to 30 mm Hg. Their cup to disc ratios remained unchanged. Serial observations lasted up to 20 months. At the termination of the study it was found that the cross sectional area of the optic nerve in the glaucomatous eyes was 90% or more of that in the companion eyes. In other words, there was a loss of up to 10% of the optic nerve fibers with no clinically detectable signs of atrophy.

Group two developed mild localized NFL defects. Their mean IOP was 26 to 34 mm Hg. Their cup to disc ratios had minimal enlargement. Serial observations lasted up to 16 months. Although some reports state that initial NFL atrophy appears in the form of slit-like defects that are approximately one arteriole in width,^{1,2,3,15} no such defects were observed. This group developed mild localized NFL defects that were manifested by a change in the normal pattern of striations covering approximately one hour of the clock face. The brightness of the striations decreased in this area, and the visibility of the retinal blood vessels increased. In the areas where striations were deficient, the NFL was only 45% as thick as in the normal eye. Based on an evaluation of the remaining neural area, there was a loss of approximately 25% of the nerve fibers.

The eyes in group three developed moderate diffuse NFL atrophy. Group three included subjects whose mean IOP was 24 to 39 mm Hg. Their cup to disc ratios were .8 or .9. Serial observations lasted up to several months. Clinical findings in this group were more extreme than in the previous groups. The cups were greatly enlarged, and the eyes would have been recognized as having glaucoma-type damage even if their original appearance was unknown. The remaining neural area of the optic nerve was from 50% to 69% of normal. Nerve fiber layer atrophy extended over 2 to 3 clock hours at both the superior and inferior

disc poles. There was a decrease in brightness in these areas accompanied by increased visibility of retinal blood vessels. There was no evidence that the atrophy started as, or passed through, a phase which included localized slit-like defects. A nerve fiber count was not made, but based on a combination of reduced neural area and reduced fiber density, it was estimated that between one third and one half of the usual number of fibers remained. Two of the eyes in this group were measured histologically, and their NFL thickness had been reduced by 47% to 75%. At this stage of loss, NFL atrophy no longer serves as a primary indicator.

The eyes in group four ultimately developed severe loss of NFL striations. These subjects had mean IOP's in the range of 24 to 39 mm Hg. Their cup to disc ratios were .9 or 1.0. With this group it was possible to observe all levels of damage as the atrophy ran its course. Even though a diffuse loss of fibers was involved, some areas were affected more than others resulting in local wedge-shaped areas of greater atrophy. Blood vessels became more visible as the atrophy progressed, with the larger vessels becoming bared first followed by their first and second order branches. Finally, the actual wall of the blood vessel was seen as a white outline of the red blood column. In the eyes that had detail fiber counts made, fewer than 10% of the axons remained.

A strong feature of the monkey studies was that glaucoma-like conditions could be induced, and the histological and pathological results could be studied at various stages by sacrificing the subject. The problem was that these results were on monkeys, and it was not known if they applied to humans. Therefore, it was necessary to conduct at least limited human eye studies.

Human Eye Study

The eyes for the human eye study were obtained from eye banks, at autopsy, or from surgical enucleation. Only eyes for which

prior clinical photographs were available were used. One normal eye was obtained as a result of an exenteration for sinus cancer involving the orbit. The eyes were prepared in a manner similar to that used in the monkey studies. NFL thicknesses were measured in various retinal areas with constant reference to the clinical photographs. The human eye study lacks the orderly progression of atrophy that was available in the monkey study, and in general, the mild or moderate phases are missing. The lack of mild or moderate damage was overcome in part by attempting to compare variably damaged areas to undamaged, or normal, areas within the same eye.

Normal Eye Findings (one eye)

The one normal eye served as the primary control for the human eye study. The thickness of the rim of neural fiber at the superior and inferior poles of this eye was 400 microns. On the foveal side of the disc the thickness was 275 microns. The thickness 1 mm above or below the nerve head was 150 microns to 200 microns. While these thicknesses are greater than those found in the monkey studies, the thickness distribution is similar.

Neurologic Disease Findings (2 eyes)

One eye was affected by a meningioma of the sella, and the other was presumed to have optic neuritis. The clinical photographs were in the form of standard color slides. It was found that in the polar areas of the eye affected by the meningioma the NFL thickness was reduced to 115 to 150 microns (vs. 400 microns for the normal), but the striations were still easily detected. A nerve fiber count showed a loss of about one third of the axons. The eye with the presumed optic neuritis had a history of temporal pallor of the disc, decreased visual acuity, and a central scotoma. Striations were clearly visible in the arcuate zones of the NFL, and the thickness was found to be normal at 100 to 150 microns. The foveal side of the disc rim was 50 microns versus

275 microns for the normal eye, and the slides suggested a loss of the NFL pattern in this area. We might draw the broad general conclusion that NFL thicknesses of 100 to 150 microns are clearly visible while thicknesses of 50 microns are not.

Ocular Hypertension (2 eyes)

One of these eyes had been the subject of a three year serial study of NFL appearance with accompanying Goldmann perimetry and Peritest automated field testing. No field defects were found using the Goldmann instrument, but some single points of abnormality were found in the superior field with the Peritest device. The NFL was rated normal in appearance during repeated clinical examinations. Based on the remaining neural area of the optic nerve there appeared to be a mild loss of fibers. No fiber count or neural area measurements were made, so no quantitative conclusions can be drawn.

Asymmetric Glaucoma Damage (5 eyes)

The color photograph of the first eye in this group showed a lack of NFL striations and a lack of vessel blurring at 11 o'clock in the superior arcuate zone. The NFL at the disc was 100 microns thinner superiorly than inferiorly, and the inferior thickness was somewhat less than that for the normal eye. In the arcuate zones the NFL thickness was 50 microns superiorly compared to 100 microns inferiorly with an anticipated thickness of 150 microns based on the normal eye. The second eye in this group had a history of long-standing open-angle glaucoma with complete loss of inferior disc rim, dense upper field loss, and major loss of neural area of the disc, especially the inferior nerve area. Vessels were blurred superiorly, but not inferiorly. Histologically it was found that 125 microns of NFL remained superiorly, but only 65 microns inferiorly. Histologic examination of the remaining three eyes found that a NFL thickness of 140 microns produced some pattern and vessel masking, but thicknesses of 50 microns or less were poorly seen. Again, we see that a remaining NFL thickness of approximately

50 microns is not readily visible, and fails to cause vessel blurring, while thicknesses of 125 to 140 microns are clearly seen.

Severe Glaucoma Damage (5 eyes)

The eyes in this group typically had cup to disc ratios of .9, loss of central fixation and a 90% loss of nerve fibers. The clinical photographs show no evidence of NFL pattern, and only in one area was there a retinal NFL thickness of greater than 100 microns. In general, the remaining areas of the NFL were less than 75 microns thick.

A summary of the findings will provide a basis for developing clinical guidelines.

SUMMARY

- 1) Thirty percent of the nerve fibers may be lost on a diffuse basis due to aging alone by age seventy without detectable changes in NFL appearance and without gross changes in vision.
- 2) A local thickness reduction of approximately 50% is required to produce clinically detectable changes in the NFL pattern. Pattern changes are due more to percent reduction than to absolute reduction in thickness.
- 3) NFL thicknesses of 50 microns or less are difficult to see.
- 4) As the NFL thickness decreases, retinal blood vessel visibility increases.
- 5) The brightness of the NFL reflex serves as a quantitative indicator of thickness. The thicker the NFL, the brighter the reflex.
- 6) At least 25% of the total nerve fibers can be lost on a diffuse basis due to factors other than aging with no apparent change in pattern.
- 7) There was no evidence of an early phase of NFL loss involving slit-like defects.

- 8) The typical NFL defect appears first as a localized diffuse loss covering approximately one hour of the clock face and converging on the disc in the 6 or 12 o'clock areas.
- 9) This diffuse loss subsequently widens to include two to three hours of the clock face and may include localized areas of greater loss in the form of wedges. At this stage, NFL atrophy no longer serves as a primary indicator.

CLINICAL GUIDELINES

The nerve fiber layer can be readily examined in the clinic using the following systematic approach:

- 1) Begin with a maximally dilated pupil.
- 2) Use a bright direct ophthalmoscope with a green filter.
- 3) Precise focusing is required.
- 4) Have the patient change fixation of the opposite eye until you obtain the best reflex from the NFL.
- 5) Examine the area at 12 o'clock just outside the disc margin.
- 6) Look for a striated pattern of alternate bright and dark streaks.
- 7) If you do not see the NFL clearly, keep in mind that there may be media opacities, thinned or absent NFL, or a blonde fundus which reduces contrast.

Once the basic technique for viewing the NFL has been established, more thorough exams, including photographic records, can be used to monitor glaucoma suspects.

INTERPRETATION

Proper interpretation requires a knowledge of the normal appearance of the retinal NFL.

Normal Appearance of the NFL

The NFL appears as fine silver linear striations in the anterior retinal layer.² These striations form a repeated bright, dark, bright pattern.¹ The bright striations represent a reflected NFL bundle, and the dark separating stripes are made up of the end feet of Muller cell glia.^{1,2,9} The brightness of the NFL reflex is related to its thickness with the reflex becoming brighter as you get closer to the optic nerve head. It is most prominent in the superior and inferior arcades. The pattern gradually fades out as you travel further from the optic nerve. The NFL casts a white haze on the underlying retinal layers.¹

Appearance of Retinal NFL Defects

Nerve fiber layer defects can be of three general types: slit, wedge, and diffuse defects.^{1,2,4,10} In all of these defects, the bright, dark, bright pattern is disrupted, and the underlying retina appears redder and darker. The blood vessels become more prominent, and the clinician can judge damage by the degree of vessel visibility. When NFL atrophy occurs, the decreasing bulk of NFL on top of the vessel leads to a readily distinguishable sequence of changes in vessel appearance.² First, the red column of blood is seen crisply. Second, as further loss of NFL occurs, the vessels actually stand up in relief due to the recession of the NFL. Next, the vessel walls come into view and appear white. The underlying retina will appear increasingly granular as the NFL exposes RPE.

Classification of NFL Defects in Glaucoma

Dark striations in the peripapillary area are classified as slits. The slit defects are larger than an arteriole in width and travel back to the nerve head. The slits become broader as you go away from the nerve head. Slit defects represent a focal defect where a 50 micron loss of thickness has occurred.¹ Controlled studies in monkeys have failed to identify slit

defects as a primary stage of glaucoma related NFL damage, and other studies of human subjects have found slit like defects in approximately 10% of normal eyes,^{2,5,16} so this indicator must be used with caution.

Wedge defects also represent a localized loss and are easiest to identify. Again, the defect becomes narrower as you approach the disc. Wedge defects are usually associated with notching of the nerve head and with a visual field defect.¹

Diffuse defects are most common in glaucoma and most difficult to detect.¹ These defects represent a generalized loss and there is an associated loss in brightness of the striated pattern. Up to 60% of the axons can be lost diffusely without a localized visual field defect because of the overlapping receptor fields in the retina.^{11,12}

NFL ATROPHY AS A DIAGNOSTIC TOOL

A study of optic fundus changes in glaucoma have demonstrated that visible NFL atrophy precedes visual field loss by as much as five years.^{3,4} However, the presence of NFL atrophy alone is not sufficient to identify a glaucoma suspect. This indicator must be used in conjunction with other indicators and risk factors to improve the accuracy of the diagnosis. The risk factors, as we know them at the moment, include high intraocular pressure, family history of glaucoma, advancing age, cardiovascular and microvascular disease, and the presence of pseudoexfoliation.¹⁴ Diabetes, myopia, and the presence of intraocular pigment may also represent risk factors over which the optometrist has no control. These risk factors are used in conjunction with eye-to-eye differences in NFL appearance to diagnose a glaucoma suspect in the same manner that the ubiquitous cup to disc ratio is used. NFL atrophy can be thought of as a precursor of cup to disc ratio change.

EXAMINATION & RECORDING TECHNIQUES

Examinations can be performed using various instrument and lens combinations. Where pupil size and media clarity permit its use, photography represents the most effective way of maintaining progressive records of NFL change. Anthony B. Litwak of the Veterans Administration Medical Center in Baltimore, Maryland has developed the following set of guidelines:

Evaluation of the Retinal NFL

Bright light source and maximally dilated pupil

- Direct Ophthalmoscope
- Biomicroscope
- Fundus Camera

Filters

- Red Free

Green light absorbed by RPE and creates a dark background to highlight white reflected NFL

Slit Lamp Lenses

- 90, 78, or 60 Volk Lens (clear)
- Fundus or Hruby Lens
- Center of the Zeiss four-mirror lens

Photography of the Retinal NFL

Cameras

- Any fundus camera (30 degree angle)
- Don't use stereo
- Focus ocular with filter in place (focus is critical)
- Move camera around to get the right reflection off NFL
- Need to focus on NFL not the optic nerve head
- Flash setting 36 (Plus X), 150 (Tech Pan) (Topcon 50 V)

Black and White Film

- Kodak Technical Pan 2415 (Developed at ASA 25)
- Kodak Plus X (ASA 125)

Photography of the Retinal NFL (cont'd)

Filters

- Red Free. Green light absorbed by RPE.
- Fluorescein Exciter Filter
- Short Pass 560 nm cut off filter (Ditric Optical)

Developing Process

- D11 1:1 12 Min @ 68 F (Plus X)
- HC 110 Dilution B 8 Min @ 68 F (Tech Pan)
- Produces a negative image
- Use contact printing to make a positive image

Comments

- Sometimes clinical exam is better (it is difficult to photograph NFL with media opacity), but photographs allow better comparison of the NFL between the two eyes and allows you to compare the NFL in future examinations.

The examination and recording procedures presented by Litwak represent an eminently practical approach which uses readily available equipment, but the use of more sensitive techniques could add to the "early indicator" value of NFL damage.

IN THE SEARCH OF MORE SENSITIVE TECHNIQUES

During a study conducted by Sommer, Kues et al¹³ cross-polarized photography was used to enhance the visibility of the arcuate bundles of the NFL. A fundus camera was modified to include a linear polarizer in front of the flash and another in front of the film plane. The axes of the two polarizing filters were kept at 90 degrees to one another while the orientation with respect to the eye being photographed was changed from zero to 180 degrees in 22.5 degree increments. A "short pass" 560 nm filter was used. It was found that cross-polarization accentuated the apparent defects in the NFL when compared with standard red free illumination. Maximum enhancement occurred when the

polarizer axes were aligned within ± 22.5 degrees of the vertical and horizontal meridians of the eye.

A study by Katsuhico & Schwartz,³ also aimed at allowing earlier detection of NFL atrophy, was based on the assumption that axon loss in the NFL would logically be associated with vascular damage to the optic disc. Fluorescein angiography of the optic disc had shown that localized areas of fluorescein filling defects occur in glaucomatous, ocular hypertensive, and normal eyes, but the number and size of filling defects are greater in patients with glaucoma and ocular hypertension. The study set out to evaluate the correlation between NFL defects and absolute fluorescein filling defects of the optic disc.

In general, a close correspondence was found between fluorescein filling defects and both wedge-shaped and diffuse NFL defects. The results pertaining to slit defects were inconclusive. One of the difficulties involved in establishing absolute correspondence for wedge-shaped and diffuse defects may be that more sensitive techniques are required. The inconclusive results with regard to slit defects may simply add credence to the view of investigators who believe that most slits are pseudo-defects. Techniques employing computerized image enhancement are now available, and studies of sensitivity and specificity are beginning.¹⁴ These techniques could provide greater sensitivity and enhance the early indicator value of NFL damage.

DISCUSSION

It seems patently obvious that glaucoma related NFL atrophy begins long before visual field defects or obvious changes in rim tissue or cup to disc ratio occur. The assumption that vascular damage in the disc area will accompany axon loss also seems obvious, but again, the evaluation techniques are lacking in sensitivity. Until the various evaluation techniques are improved, and their application systematized, the use of NFL damage as an early indicator to aid in identifying glaucoma suspects will be dependent upon the experience-based skill

and judgement of the clinician. In order to build this experience-based skill it is necessary to take the time to evaluate the NFL in normals as well as in those with defects. When examining glaucoma patients, the pattern of NFL atrophy should be correlated with their visual field loss.

Contrary to the "prohibitively difficult" task mentioned in the introduction, while working at the Optometric Institute and Clinic of Detroit, I found that the NFL was readily visible in healthy young black patients. For initial experience in visualizing the NFL, such patients seem like a good place to start. The only tools required are a direct ophthalmoscope with a red free filter and a bit of care in focusing.

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