Published in final edited form as:

Ophthalmology. 2017 December ; 124(12 Suppl): S76–S82. doi:10.1016/j.ophtha.2017.10.011.

The Future of Imaging in Detecting Glaucoma Progression

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Abstract

Ocular imaging has been heavily incorporated into glaucoma management and provides important information that aids in the detection of disease progression. Longitudinal studies have shown that the circumpapillary retinal nerve fiber layer is an important parameter for glaucoma progression detection, while other studies demonstrated that macular parameters, such as the ganglion cell inner plexiform layer and optic nerve head parameters are also useful for progression detection. The introduction of novel technologies with faster scan speeds, wider scanning fields, higher resolution, and improved tissue penetration has enabled the precise quantification of additional key ocular structures, such as the individual retinal layers, optic nerve head, choroid, and lamina cribrosa. Furthermore, extracting functional information from scans such as blood flow rate and oxygen consumption provides new perspectives on the disease and its progression. These novel methods promise improved detection of glaucoma progression and better insight into the mechanisms of progression that will lead to better targeted treatment options to prevent visual damage and blindness.

Glaucoma is a multi-factorial optic neuropathy characterized by structural damage of retinal ganglion cells (RGC) and their axons that is associated with vision loss and may lead to irreversible blindness (1). Because glaucomatous damage is irreversible and effective treatment is available to halt further damage, glaucoma management should be optimized with precise micron scale quantifications of ocular structures that improve detection of the disease and its progression (2–4). The introduction of optical coherence tomography (OCT) technology over 20 years ago provided in vivo detailed visualization of the optic nerve head (ONH) and retina and enabled the quantitative evaluation of these structures (5) (6). Circumpapillary retinal nerve fiber layer (RNFL) thickness is a common OCT measurement that provides comprehensive evaluation of all RGCs in an eye as they converge into the ONH (2). When measured with spectral-domain (SD-) OCT, the RNFL has been shown to differentiate between healthy and glaucomatous eyes (7). The steady evolution of OCT technology has led to imaging with better resolution, higher scanning speeds, and advanced

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Conflict of Interest: Dr. Schuman receives royalties for intellectual property licensed by Massachusetts Institute of Technology to Zeiss.

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imaging patterns that has improved the reliability of OCT measurements and allowed for detection of minute changes that can improve the sensitivity of progression detection.

Assessment of glaucoma progression is usually based on event or trend analysis. Event-based progression determines when a measurement exceeds a pre-established threshold for change from baseline. Trend-based analysis quantifies the rate of a parameter's progression over time (2) (8). Conventionally, structural progression has been assessed using RNFL measurements; however, longitudinal studies have shown that other parameters including the macular ganglion cell complex (GCC), ganglion cell/inner plexiform layer (GCIPL) and ONH parameters (rim area, cup area and cup-to-disc ratio) are also useful for evaluating glaucoma progression (9–11).

Despite the utility of OCT, there are also challenges in glaucoma detection and its progression with this technology. These challenges are the result of structural variability in healthy eyes, overlap in structural measurements between healthy and early glaucomatous eyes and abnormal appearing eyes that do not show any evidence of disease progression over time (e.g., physiologic cupping). Additionally, normal age-related structural loss can confound the interpretation of longitudinal glaucoma assessment (12–15).

OCT comparison with functional visual testing, such as standard automated perimetry (SAP), also introduces a substantial challenge when assessing disease progression. The measurement variability of SAP is high and can be influenced by many confounding factors (16) (17). Additionally, the association between structural and functional abnormalities in glaucoma varies with disease severity, which should also be considered when assessing progression with these methods. In early stages of the disease, the high variability in SAP measurements often delay the possibility of detecting functional progression at a time when changes might be noticed with structural assessment. A “tipping point” was reported, illustrating the point in disease severity before which structural and functional changes often disagree and after which there is a strong association between the two (18). Other studies have demonstrated that the SAP 24-2 testing strategy misses more central points compared with the 10-2 strategy in early glaucomatous losses (19). Given the improved ability to detect macular thinning of RNFL and GCL in recent iterations of the technology, an improved agreement between macular structure and visual function might be present in earlier stages than previously described.

In advanced disease the opposite situation occurs in which the RNFL, as measured with OCT, is less sensitive than SAP in detecting progression after a minimum measurable thickness is reached; this is referred as the “floor effect” (20) (21). It should be noted, however, that there are also limitations in visual field diagnosis of progression, with mean sensitivities between 19 to 15dB due to a reduction in the asymptotic maximum response (22) (23). Furthermore, longitudinal analysis of OCT macular and ONH parameters demonstrated their ability to detect structural progression even at stages where the peripapillary RNFL reached the “floor effect” thickness (24). In a cohort of advanced glaucoma, defined by visual field mean deviation (MD) ≥ 21 dB, another study showed a significant rate of GCIPL thinning in 31% of the eyes (25). It has also been demonstrated in an advanced glaucoma group that GCIPL had a rate of change of $-0.21 \mu\text{m}/\text{year}$ and that the

dynamic range above the measurable floor was larger than peripapillary RNFL and minimum rim width (MRW) (26). Therefore, macular OCT measurements provide an alternative for objective and quantitative monitoring of subjects with advanced glaucoma.

In order to address these challenges and explore opportunities in glaucoma progression detection, several new technologies, modalities and image processing tools are being studied. The assessment of the structure-function association with newer software and the introduction of advanced statistical methods to analyze and predict progression from large longitudinal data sets are promising tools to be incorporated into clinical practice. Additionally, novel modalities and technologies are enabling the introduction of new biomarkers that may further enhance the assessment of glaucoma progression and improve the understanding of the disease pathophysiology.

Novel OCT Technologies and Applications

Swept-Source (SS-) OCT is a newer generation of OCT that uses a short cavity swept laser with a tunable wavelength of operation. It has a longer central wavelength (1050nm) compared to conventional SD-OCT (840nm), providing deeper penetration and eliminating the depth-dependent signal drop-off observed with earlier generations of OCT. These capabilities enable imaging of deeper ocular structures, such as the choroid and lamina cribrosa (LC) (27–29). Additionally, improved visualization of the RCG layer allowed for the introduction of a model to estimate RGC quantity, which may be useful for understanding the longitudinal structure-function association (30).

OCT angiography (OCTA) employs decorrelation motion contrast between rapidly repeated OCT cross-section scans to document retinal vessels. This is achieved by detecting variations in the intensity and/or phase properties of the OCT signal resulting from the movement of red blood cells (31). OCTA allows the peripapillary, ONH and macular vasculature to be evaluated at various depths, exposing several vascular networks (32). The peripapillary and ONH capillaries have been demonstrated to be associated with glaucoma (33–37). The flow index (mean decorrelation value on the en-face retinogram) and vessel density (area occupied by large vessels and microvasculature), have been shown to be associated with disease severity as reflected by SAP global indices (38). Additional studies demonstrated a reduction in peripapillary vessel density in the hemispheres corresponding to hemifield defects in visual fields (39) (40). In eyes with glaucoma and a single-hemifield defect, diminished vascular density of the macular and peripapillary regions was also demonstrated in unaffected hemifields (41). OCTA of eyes with glaucoma and LC defects portrayed diminished vascular density corresponding to the locations of these defects (42).

A recent longitudinal study with a short follow-up period of less than 14 months showed a significantly faster rate of change of the superficial capillary plexus density in glaucomatous eyes compared to glaucoma suspects and healthy eyes (43). This initial promising result should be corroborated by additional studies evaluating changes in other regions.

Color Doppler imaging (CDI) showed significantly lower blood flow velocities and higher resistive indexes in the central retinal artery, short posterior ciliary arteries, and ophthalmic

artery in patients with clinically worsening primary open angle glaucoma (POAG) (44) (45). **Doppler OCT** was previously reported to measure total flow around the ONH, but small vessel flow could not be detected (46). Recently, high-speed en face Doppler OCT showed reduced total retinal blood flow in diabetic eyes compared with healthy eyes (47). The value of vascular flow quantification in assessing glaucoma progression is yet to be determined.

The incorporation of **adaptive optics** (AO) systems into ophthalmic imaging has further improved achievable image quality (48). AO corrects for the monochromatic optical aberrations of the eye, thus improving the resolution of ocular imaging technologies (49). For example, AO-OCT improves transverse resolution from the typical 20 μ m to approximately 5 μ m (50). Pruning of RGC dendrites has been shown to be an early indicator of glaucomatous damage (51); using an AO system conjugated with scanning laser ophthalmoscope (SLO) investigators showed that imaging of individual RGC bodies is possible in humans (52). Additional studies with AO systems have demonstrated changes in the LC microstructure in glaucomatous eyes (53) (54). Others have allowed detection of damage down to individual retinal fiber layer bundles and the inner and outer retinal layers in glaucoma (55) (56). This technology, however, still presents some barriers to its widespread adoption, namely high cost, limited field of view, narrow depth of focus, and extended acquisition time (48).

Other experimental OCT systems that are potentially useful for glaucoma follow-up are **polarization-sensitive (PS-) OCT** and **visible light (Vis-) OCT**. PS-OCT generates images with tissue-specific contrast based on properties that alter the polarization state, such as birefringence (sclera, RNFL), polarization-preservation (photoreceptors) and depolarization (RPE) (57). Vis-OCT can provide both structural and functional information from the same scan. The device uses a broad wavelength within the visible light spectrum that can be parsed to determine oxygenated and de-oxygenated blood and thus determine the oxygen consumption of the tissue as a functional surrogate. The utility of these novel biomarkers for longitudinal assessment is yet to be determined.

Conventional RNFL assessment is based on pre-defined sectoral analysis; however, in some instances, this approach may overlook localized defects and their progression. Focusing on the affected area, also known as the region of interest (ROI), was tested as an alternative approach in eyes with disc hemorrhage, a high-risk population for progression (58). The ROI approach was superior to global RNFL thickness in detection of localized loss. This approach could also be applied for measuring and monitoring disease progression in other structures.

The improvements to OCT scan speed and resolution have resulted in better imaging of the ONH. Parameters such as rim area, vertical rim thickness and vertical cup-to-disc ratio have been shown to allow detection of glaucoma progression (59). In cross-sectional studies, MRW showed a high association with glaucomatous functional changes on SAP (60) (61).

The LC has been suggested as an important target for axonal injury in glaucoma, as certain structural and biomechanical features within this structure may lead to neuronal damage. Advanced imaging offered by current technologies allows for in vivo evaluation of LC

structures, including prelaminar tissue, LC thickness, localized LC defects, LC insertion depth, and posterior displacement (62) (63) (53) (54). SS-OCT was used for a three-dimensional evaluation of the LC in normal tension glaucoma, which demonstrated a significant correlation between LC thickness and both RNFL thickness and the MD of the visual field (64). Statistically significant differences in LC microstructure have been reported between healthy and glaucomatous eyes (65).

The biomechanics of the LC determine tissue deformation in response to its pressure environment. Locations with marked deformations might strangle the passing axons, leading to impaired functionality (66) (67). Given the LC's pivotal role in glaucoma pathophysiology, its parameters are potential biomarkers for glaucoma progression management.

Other Ophthalmic Imaging Modalities

Multispectral imaging (MSI) is a noninvasive tool for examining layers of the retina and choroid by using light wavelengths from 550 to 850nm. By combining images along a range of wavelengths, maps can be generated to reflect the oxygenated and de-oxygenated blood content within the vasculature (68). Retinal oxygen saturation was reported to be positively correlated with visual field and RNFL thickness; a negative correlation was seen between oxygen saturation in the choroid plexus and RNFL thickness (69). MSI has also been used to measure increased venular retinal oxygen saturation in POAG compared to healthy subjects (70). Measurements using MSI have illustrated differential light absorption in neuroretinal rim tissue that is associated with visual field sensitivity in POAG (71).

As glaucoma is an optic neuropathy often suggested to have a central nervous system component (72), **magnetic resonance imaging (MRI)** technology has been introduced as a potential tool for glaucoma progression detection. Diffusion tensor imaging (DTI) MRI studies have demonstrated abnormalities in the optic radiation and optic nerve that correlate with glaucoma severity (73) (74). In vivo MRI on glaucomatous animal models has been successfully used to demonstrate increased retinal and choroidal blood flow with topical dorzolamide therapy (75). Each of these findings supports the role of MRI as a potential modality in detecting glaucoma progression.

Magnetization transfer imaging (MTI) is an MRI technique that detects macromolecules and their associated water molecules. In POAG patients, this ability to detect axonal and myelin loss has been used to reveal optic nerve atrophy and degeneration of the optic pathway (76). Researchers have used this tool in humans to identify increased demyelination in the optic chiasm, calcarine fissure, geniculocalcarine, and striate area in POAG compared to control eyes (76) (77). While this is a step towards understanding the pathogenesis of optic nerve changes in glaucoma, MTI applicability towards detecting POAG progression has not been delineated (77).

Functional MRI (fMRI) is capable of producing cortical mapping of visual function (78). Human eye studies have revealed the ability of fMRI to detect inner retinal layer thinning, optic nerve cupping and reduced visual cortex activity prior to any evidence of visual field

impairment on SAP testing (79). Pursuing additional functional imaging techniques, researchers have also used **proton magnetic resonance spectroscopy** (MR spectroscopy) to assess cross sectional glaucoma diagnosis. These methods have revealed a correlation between choline metabolism disruptions within the visual field and disease severity on diffusion tensor MRI and visual field assessment (79). Longitudinal studies with larger sample sizes are still needed before conclusions can be drawn about the clinical utility of these methods.

Combining Structure and Function

As discussed above, the association between structural and functional changes varies along the spectrum of glaucoma severity. Novel technologies now offer automated and precise co-localization of structural and predicted functional information as a first step toward better determination of disease progression (80) (81).

Another approach is using structural and functional information to estimate the number of RGC axons in the eye. This model estimates the structural RGC axon quantity from OCT RNFL thickness. Functional estimates of RGCs are based on conversion models developed from histological studies of monkeys (82–84). Because SAP is less sensitive in detecting early glaucomatous progression, the combined technique utilizes a weighted scale that takes into account the differences in OCT and SAP performances in different stages of disease. This method was shown to predict glaucomatous progression better than functional and structural evaluations independently (82) (85) (86).

Artificial Intelligence and Telemedicine

Artificial intelligence is a computerized method that can be trained to detect relationships between information from multiple inputs and an output of interest. This method has been applied to visual function testing and optical imaging and has indicated an ability to accurately diagnose and detect disease change over time (87). One approach of artificial intelligence, namely deep learning (DL), has gained recent popularity as a promising tool for sensitive detection of disease progression. This method was developed to imitate multilevel processing performed by the human brain using linear and non-linear models. DL has been shown to be valuable in improving image quality (88) (89), enhancing reliability of automated segmentation (90) and diagnosing glaucoma (91) (92).

The application of **telemedicine** expands throughout medicine; accordingly, in glaucoma care, most uses are in disease screening with optic nerve photographs transmitted to glaucoma specialists (93). The ease of acquiring multiple images opens new opportunities for detection of disease progression.

Novel Biomarkers for Glaucoma Progression

Direct RCG imaging offers exciting prospects for diagnostic imaging in humans. Detection of apoptosing retinal cells (DARC) is one such method, which has offered encouraging results thus far. Annexin-5 is an endogenous extracellular calcium-dependent membrane-binding protein, ubiquitously expressed in human cells. In an early step in apoptosis,

phosphatidylserine is translocated to the outer plasma membrane, where it is vulnerable to high-affinity binding with a fluorescently labeled annexin-5 molecule, which can subsequently be visualized and counted using confocal scanning laser ophthalmoscopy (CSLO) (94) (95). In a proof-of-concept study it was demonstrated that this method could be effectively used in human eyes in vivo. Interestingly, in their post-hoc analysis, authors found high DARC counts could predict increased glaucoma progression rates. Phase 2 studies are being undertaken to continue to assess the potential role of DARC in evaluating disease progression.

Pre-Clinical Biomarkers

Fluorescently tagged cholera toxin subunit B, injected intravitreally, was used in mice to successfully label RGCs (96). Although this technique has been deemed safe in humans (97), its use has been limited thus far by its low specificity. In mouse models as many displaced amacrine cells have wound up labeled as RGCs (98).

Genetically encoded calcium indicators like GCaMPs are being explored as possible functional imaging tools to measure RGC damage (98). Fluorescently labeled GCaMP3 retinal cells can be detected in vivo with CSLO when directly exposed to UV light. While originally explored in transgenic mice, this labeling has now been performed using viral vectors, paving the way for its development into a tool for imaging human eyes in vivo (99).

RGC labeling with retrograde tracer molecules, such as Fluorogold, is highly effective in mouse models (98). Transgenic mouse models have also been used with a number of RGC-specific markers, such as THY monocyte differentiation antigen 1 (Thy1), ckit and Brn3b (95). Transgenic Thy1 mice that have been modified to express yellow fluorescent protein (Thy1-YFP) permit detailed visualization of neurons within the RGC. Once labeled, these retinas can be visualized in vivo with CSLO. Unfortunately, however, specificity can be lost as labels are phagocytosed by microglia and macrophages, and in many cases the tracer's use is limited by its transient presence (98). Electroporation, an in vivo application of voltage to a membrane for transfection of genes or contrast agents into cells, is another labeling technique employed in animal models, which though very sensitive, is limited by its reduced specificity (100). Thus far none of these methods can be applied to humans (98).

Conclusion

Ophthalmic imaging innovations have revolutionized glaucoma diagnosis and management, and the tools, algorithms and biomarkers that are relevant to clinical practice are constantly evolving. As these parameters undergo constant research and development, they may contribute to further improvements in glaucoma progression detection. These novel biomarkers and approaches might aid in the understanding of specific etiologies and pathological mechanisms of glaucoma, such as those involving the biomechanics of the LC, thus contributing to the development of new future therapies.

Acknowledgments

Financial Support: Supported in part by National Institutes of Health R01-EY013178.

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