The Nine-Step Minnesota Grading System for Eyebank Eyes With Age Related Macular Degeneration: A Systematic Approach to Study Disease Stages

Timothy W. Olsen,1,2 Albert Liao,2 Hershonna S. Robinson,2 Neal V. Palejwala,3 and Nicholas Sprehe4

1Mayo Clinic, Department of Ophthalmology, Rochester, Minnesota, United States
2Emory Eye Center, Emory University Department of Ophthalmology, Atlanta, Georgia, United States
3Retinal Consultants of Arizona, Phoenix, Arizona, United States
4The Lions Eye Institute for Transplant and Research (LEITR), Tampa Bay, Florida, United States

Purpose. To refine the Minnesota Grading System (MGS) using definitions from the Age-Related Eye Disease Studies (AREDS) into a nine-step grading scale (MGS-9).

Methods. A nine-step grading scale descriptive analysis using three key phenotypic features (total drusen area, increased, and decreased pigmentation) of human eyebank eyes that were graded according to definitions from the AREDS criteria in order to harmonize results among researchers when grading human tissue using MGS criteria. Most importantly, the MGS-9 links definition of Age Related Macular Degeneration: A Systematic Approach to Study Disease Stages

Age related macular degeneration (AMD) is a leading cause of irreversible blindness globally.1–14 According to the United Nations, there will be a dramatic global increase in the number of those over age 60, especially in the developing world.8 This demographic is expected to double over the next 35 years with the highest populations of individuals over age 60 in developed nations, such as North America, Europe, areas in China, Japan, Australia, South Korea, and Iran.

Both Age Related Eye Disease Study (AREDS) 1 and 2 are multicenter, randomized, controlled clinical trials that were designed to evaluate the efficacy and safety of a dietary supplement on AMD progression.9,10 In the course of these studies, prospectively acquired data was obtained on 4203 individuals (50–85 years of age) for 5 years in AREDS 1 with 3549 of this cohort that were followed through 10 years. In AREDS 2, the study examined 4757 higher-risk individuals who were followed for 5 years. Stereoscopic, color fundus photographs were obtained for each subject at baseline and annually. In addition, a standardized early treatment of diabetic retinopathy study (ETDRS) visual acuity and a comprehensive eye exam was also performed. Thus, the rates of progression to advanced disease for those with early or intermediate AMD have been well characterized in the AREDS database.

In 2004, we developed a four-step, Minnesota Grading System (MGS or MGS-4) for evaluating eyebank eyes using color fundus photographs.11 The grading system was based on key clinical and epidemiologic grading systems. In 1991, the Wisconsin Age-Related Maculopathy Grading System (WARMGS) defined and then quantified specific AMD macular features.12 The system quantified fundus features by including stereoscopic color fundus photographs with an overlying ETDRS grading template from a large, population-based epidemiologic study of AMD.13 In 1995, a modified international classification and grading system was developed to harmonize various epidemiologic studies.14 In 2001, the AREDS study modified the WARMGS grading system for use in the longitudinal, multicenter AREDS studies.15 AREDS used a four-level, progressive classification with end-stage AMD, either center involving geographic atrophy (GA) or subfoveal choroidal neovascularization (CNV), defined as level 4.15

Conclusions. The MGS nine-step grading scale enables researchers using human tissue to refine the risk assessment of donor tissue. This analysis will harmonize results among researchers when grading human tissue using MGS criteria. Most importantly, the MGS-9 links directly to the known risk for progression from the AREDS.

Keywords: eyebank, grading system, human tissue

Correspondence: Timothy W. Olsen, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA; olsen.timothy@mayo.edu.

Submitted: May 2, 2017
Accepted: September 26, 2017
Citation: Olsen TW, Liao A, Robinson HS, Palejwala NV, Sprehe N. The nine-step Minnesota grading system for eyebank eyes with age related macular degeneration: a systematic approach to study disease stages. Invest Ophthalmol Vis Sci. 2017;58:5497–5506. DOI:10.1167/iovs.17-22161

PURPOSE. To refine the Minnesota Grading System (MGS) using definitions from the Age-Related Eye Disease Studies (AREDS) into a nine-step grading scale (MGS-9).

METHODS. A nine-step grading scale descriptive analysis using three key phenotypic features (total drusen area, increased, and decreased pigmentation) of human eyebank eyes that were graded according to definitions from the AREDS criteria in order to harmonize studies of disease progression for research involving human tissue. From 2005 through February 2017, we have analyzed 1159 human eyes, procured from two eyebanks. Each macula was imaged using high-resolution, stereoscopic color fundus photography with both direct- and transillumination. Fundus images were digitally overlaid with a grading template and triangulated for foveal centration.

RESULTS. We documented and stratified risk for each globe by applying the AREDS nine-step grading scale to the key clinical features from the MGS-9. We found a good distribution within the MGS categories (1–9) with few level eight globes. Eyes were processed within 12.1 ± 6.3, hours from the time of death through imaging, dissection, and freezing or fixation. Applying the MGS-9 to 331 pairs (662 eyes were simultaneously graded), 84% were within one-grading step and 93% within two steps of the fellow eye. We also document reticular pseudodrusen, basal laminar drusen, and pattern dystrophy.

CONCLUSIONS. The MGS nine-step grading scale enables researchers using human tissue to refine the risk assessment of donor tissue. This analysis will harmonize results among researchers when grading human tissue using MGS criteria. Most importantly, the MGS-9 links directly to the known risk for progression from the AREDS.

Keywords: eyebank, grading system, human tissue
The WARMGS and AREDS grading system served as the basis for the original MGS-4. The unique aspects of the MGS-4 was the ability to create stereoscopic, color fundus photographs of the macula in postmortem eyebank eyes that correspond to the AREDS grading. The MGS demonstrated that high-resolution images of the macula could be reliably obtained and key features quantified with the ETDRS template. Such features include drusen type and size, drusen area, pigmentary changes, and GA. In 2008, the MGS was also adapted for quantitative fundus autofluorescence (FAF) or MGS-FAF. Finally, in 2015, we confirmed detailed histopathology and mapping of key clinical findings from the MGS that are consistent with our current understanding of AMD pathogenesis and progression.

In 2005, Davis et al. defined a nine-step severity scale that was capable of predicting the 5-year risk of progression. From this study, the risk of progressing to advanced AMD was based on both a six-step drusen area and a five-step pigmentary abnormality scale that stratifies phenotype into nine categories. Risk ranged from less than 1% to 50%.

In this study, we incorporated the nine-step AREDS severity scale with the MGS to create the MGS-9, a refined classification of eyebank eye tissue that corresponds to the AREDS severity scale. We propose that researchers using human eyebank eyes to study AMD apply these criteria to standardize results and accurately risk assess the tissue.

METHODS

The principle investigator obtained institutional review board exemption from both the University of Minnesota and Emory University for the conduct of this study. Eyebank tissue was de-identified by using a unique identifier(s) from either the Minnesota Lions Eyebank (MLE) or the Lions Eye Institute for Transplant and Research (LEITR) in Tampa Bay, Florida. The study was conducted in full compliance with Health Insurance Portability and Accountability Act of 1996 regulations and adhered to the tenets of the Declaration of Helsinki.

Eyes were procured by a highly trained and skilled team at either the MLE or the LEITR from either the Minnesota region or the Tampa Bay region of Florida. The larger Tampa Bay region in the United States has a high prevalence of retiree population (age >50 years). After death, trained procurement personnel promptly enucleate globes in the field, and a limited medical history was obtained. The eyes were immediately transported to the eyebank headquarters and processed for use in either transplantation or research. Demographic information was obtained and included age, sex, race, cause and time of death, time of emucleation, an ocular history, and any major, medical conditions, such as diabetes, hypertension, infectious disease, or ocular conditions, such as prior ocular surgery, glaucoma, or AMD. Additional postmortem medical history from the next of kin was obtained when possible, especially when researcher required a more detailed history (i.e., smoking history estimates). The time of globe dissection was documented and each globe was assigned a unique identifier.

Once the tissue is designated for research, the globes are processed according to a flexible protocol, depending upon specific research needs and requests. First, a standard, predissection imaging was performed for both eyes of the pair along with high-resolution postdissection imaging of either one or both eyes (depending upon the protocol). These photos are referred to as a scout images and can be sent via text message to the principle investigator for immediate screening. Based on the scout, and the existing research protocols, the eyes are assigned to a specific protocol. Sometimes, one eye is not dissected and remains intact. Thus, one eye is graded postdissection (more accurate) while the second eye is not dissected (no detailed grading). Therefore, some study protocols require an undissected globe that is preserved (e.g., for histology) using a specific, predetermined methodology. Because we have previously found that by using the MGS-4 there is a high level of correlation (95% concordance), the fellow eye, especially with the supplemental scout images, are highly likely to be at the same or very similar to the graded eye of the pair. Thus, when one eye only is dissected in this stage, the second eye is either fixed, frozen, or shipped on ice, according to the investigators needs. Therefore, in some pairs, both eyes have detailed grading while in other pairs, only one eye has detailed grading, and the fellow eye is only graded with the neurosensory retina intact (a less accurate methodology that relies upon fellow eye for step-level assessment).

Globe Processing and Predissection Images

Anterior segments are removed with a coronal incision, just anterior to the equator using a razor blade. The vitreous is removed in order to obtain an unobstructed view of the posterior segment with the neurosensory retina intact. Next, a 1000 ± 2.5-μm ruby colored sphere (Meller Optics, Inc., Providence, RI, USA) is placed on or near the optic nerve to serve as a size reference. The globes are photographed using digital color camera (DXC-S500; Sony, Tokyo, Japan) mounted on a dissecting microscope (SMZ 1500; Nikon, Tokyo, Japan). High-resolution, predissection, stereoscopic color images are obtained at each posterior segment by taking the initial photograph and tilting the stage slightly to generate stereo-pair images as previously described.

High-Resolution Macular Imaging

One globe of the pair is dissected further for detailed macular imaging. The fellow eye was either dissected in a similar fashion, fixed, flash frozen, or shipped fresh on ice to the researcher following initial photography. For high-resolution, stereoscopic macular images, the neurosensory retina was carefully dissected from the underlying retinal pigment epithelial (RPE) layer as described. The dissected neurosensory retina is carefully placed en face in cell culture media and was available either whole or the macular region can be made selectively removed using a trephine punch of any size (typically ranging from 1.5 to 5 mm in diameter), depending upon the extent of tissue required. Next, high-resolution, color, stereoscopic photographic pairs were taken of the RPE cell layer in the macular region along with the ruby sphere. Two gooseneck lights (Schiott-Fostec, Auburn, NY, USA) illuminate the tissue tangentially to highlight macular features, such as drusen. The globes were then transilluminated using an illuminated stage, thus projecting light from the posterior side of the globe, and the stereoscopic photographic image of the stereoscopic pair was repeated.

Composite Images

Stereoscopic mages were uploaded to a digital server as a high-resolution, composite image (70-MB files; Fig. 1) into a digital folder. The folder also contained a separate AREDS grading template with de-identified patient information (age, race, sex, etc.). In Figure 1, the top two images (row A) are the initial globe processing scout images that illustrate the status of the entire posterior pole with a description of each subsequent row in the figure legend. These images are important to detect tumors, panretinal photocoagulation, or other retinal pathologies. Next, a modified ETDRS grading template is placed on one of the images along with a proportional triangle that
localizes the foveal center relative to two independent choroidal vascular branching landmarks from predissection images. Note that the ETDRS grading template has all prior demarcations yet there are more subcircles, increasing from five to seven circles, as was done in the AREDS study. Importantly, the details of the drusen and pigment are less visible (due to the opaque retina) in the predissection images. The actual drusen and RPE changes were much more clearly imaged postdissection, after removal of the semiopaque neurosensory retina.

**Grading**

Images are analyzed by using the criteria from AREDS nine-step grading to generate a final, corresponding MGS-9 level for the 1159 globes (MGS steps 1-9; Table 1). While each eye was graded independently, represented in Table 1, the pair was given an MGS score for the higher-level eye in the pair. For example, if one eye has a disciform scar, and the fellow eye does not, the highest-level score is given to the pair, MGS level 9. If one eye was an MGS-3, and the fellow eye was an MGS-4, each receives an independent score, yet the pair was given the score of MGS-4. When only one eye was graded using high-resolution images postdissection, and the fellow eye was graded using the initial scout image (i.e., with the neurosensory retina intact, thus less accurate assessment), both eyes were assigned the score of the eye with the postdissection, high-resolution image. Some research protocols require that the neurosensory retina remain intact. In order to ensure accuracy of high-resolution grading and classification. All globes were graded based upon high-resolution imaging, either directly or of the fellow eye of the pair and are listed in Table 1. In some circumstances, the eye graded with the high-resolution image is not at end stage, yet the scout film of the fellow eye clearly demonstrates either central GA or CNV, then the appropriate, more advanced MGS level was assigned to the pair.

**Grading Parameters**

The key phenotypic features used to assign an MGS one through nine grade for each globe include the criteria listed in Table 1. The 5-year relative risk for that particular MGS grade as derived from the AREDS is also listed (0.3%-53%). Because other factors are important in AMD risk, yet were not specifically graded, comments are added with important the key criteria listed in Table 2 (e.g., fibrovascular RPE detachments).

Drusen are characterized and quantified in terms of type, size, and total drusen area. The drusen area were assessed within the entire grid, and all subfields within the outer ring of the grading template. Drusen area were highlighted on the images using a 10% green enhancement filter in the digital edit colors feature on the image software (Adobe Illustrator CS6, v 16.0.0; San Jose, CA, USA). The area of all or any drusen choroidal vascular landmarks. Note that the retina is semiopaque. Row C: right eye, transilluminated stereo pair. Note that the predissection image on the left still has the neurosensory retina intact with foveal triangulation while in the fellow image on the right, the retina is removed, thus the pigmentary changes are more visible. Row D: right eye, direct illumination, high-resolution color stereo-pair with the grading template centered using triangulation. Note that drusen are much easier to grade, postdissection of the retina. Row E: left eye, corresponds to row B for the right eye; row F left eye, corresponds to row C, and row G, left eye, corresponds to row D (drusen much easier to grade).
present was best estimated by mentally pushing the drusen together to approximate the template size reference, as originally described in WARMGS.\textsuperscript{12}

Pigmentation was graded only within the inner zone of the ETDRS grading template. This region included the central subfield (1000-\textmu m diameter) and the inner subfields (total area is 3000 \textmu m in diameter). Pigmentary changes were quantified as either increased pigment, decreased RPE pigmentation or GA. Changes noted outside of the inner subfield or pigmentary changes found in the outer subfields may be associated with disorders other than AMD.\textsuperscript{10} Also, GA was defined as an area from 2005 through February 2017, we evaluated and graded 1159 human eyes using the MGS-9 as defined by the nine-step severity scale from AREDS.\textsuperscript{18} Of these eyes, 331 pairs were graded using detailed macular analysis of each globe. In other words, some globes were not graded bilaterally with the neurosensory retina removed. In these cases, the protocol was to assign the classification to the undissected eye of the pair to the step of the dissected eye, unless it was clear that the undissected eye was at end-stage (either central geographic atrophy or disciform scar). Table 1 lists the total number of eyes (% of the total 1159) that fit the criteria outlined for each step. There was a good distribution of globes from one to nine. Approximately 50% were either step one or two (low risk for progression).

Table 2. Additional Grading Features Gathered From MGS Images

<table>
<thead>
<tr>
<th>Feature</th>
<th>Number of Eyes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular pseudodrusen (definite diagnosis; not questionable)</td>
<td>49 (4)</td>
</tr>
<tr>
<td>Calcified drusen (definite diagnosis; not questionable)</td>
<td>39 (3)</td>
</tr>
<tr>
<td>Cuticular drusen*</td>
<td>66 (6)</td>
</tr>
<tr>
<td>Pattern dystrophy</td>
<td>12 (1)</td>
</tr>
<tr>
<td>Soft indistinct drusen present</td>
<td>172 (15)</td>
</tr>
<tr>
<td>Drusenoid RPED</td>
<td>110 (9)</td>
</tr>
<tr>
<td>Fibrovascular RPED</td>
<td>74 (6)</td>
</tr>
<tr>
<td>Serous RPED</td>
<td>72 (6)</td>
</tr>
<tr>
<td>Hard exudates</td>
<td>15 (1)</td>
</tr>
<tr>
<td>Subretinal fibrosis</td>
<td>46 (4)</td>
</tr>
<tr>
<td>Laser photoagulation scars</td>
<td>23 (2)</td>
</tr>
<tr>
<td>Largest drusen diameter</td>
<td>845 (73)</td>
</tr>
<tr>
<td>&lt;63</td>
<td>144 (12)</td>
</tr>
<tr>
<td>63–125</td>
<td>125–250</td>
</tr>
<tr>
<td>125–250</td>
<td>83 (7)</td>
</tr>
<tr>
<td>&gt;250</td>
<td>66 (6)</td>
</tr>
<tr>
<td>Unable to grade drusen</td>
<td>21 (2)</td>
</tr>
</tbody>
</table>

* Basal laminar drusen or dominant drusen.

RESULTS

From 2005 through February 2017, we evaluated and graded 1159 human eyes using the MGS-9 as defined by the nine-step severity scale from AREDS.\textsuperscript{18} Of these eyes, 331 pairs were graded using detailed macular analysis of each globe. In other words, some globes were not graded bilaterally with the neurosensory retina removed. In these cases, the protocol was to assign the classification to the undissected eye of the pair to the step of the dissected eye, unless it was clear that the undissected eye was at end-stage (either central geographic atrophy or disciform scar). Table 1 lists the total number of eyes (% of the total 1159) that fit the criteria outlined for each step. There was a good distribution of globes from one to nine. Approximately 50% were either step one or two (low risk for progression).

Specific AMD-associated features (Table 2) and the distribution of these features were quantified. Interestingly, there were 49 cases of reticular pseudodrusen (4%), 6 cases of pattern dystrophy (1%), 172 cases of high-risk, soft, indistinct drusen (15%), 110 cases of drusenoid RPED (9%), and 46 eyes (4%) with subretinal fibrosis. Drusen size distribution results...
showed mostly small hard (<63 μm) drusen (845 or 73%), intermediate or medium drusen (144 or 12%), fewer large drusen (83 or 6%), and occasional very-large or giant drusen (66 or 6%).

The MGS-4 and MGS-9 system differ in criteria and Table 3 outlines the distribution of globes and correlates the distribution breakdown. The MGS-4 criteria prioritize drusen type (small hard, intermediate, etc.) while the MGS-9 emphasizes total drusen area without regard to type of drusen. Also, in the MGS-4 system, pigment quantification is less important than in the MGS-9. Thus, we broke down the category of MGS-4 grades (denominator) into MGS-9 subcategories, to determine the percentage of globes that fell into each of the four levels of MGS-4. For example, most of the subdivision of MGS-4 (level 3) was broken down into MGS-9 (levels 4–8). The MGS-9 level four, represents 50 of 236 or 21% of MGS-4 level three eyes (5% risk of 5-year progression to advanced disease; Table 1). At the other end of the risk for MGS-4 level three are the 36 MGS-9 level seven eyes (15%; Table 3) that have a 28% 5-year risk of progression (Table 1). Finally, there are rare globes (only one in this series) that fit the criteria for step eight (0.09%). We suspect that many of these very high-risk eyes may have been classified as end stage (or step 9). Thus, such extremely high-risk eyes are difficult to find, yet their fellow eye, if not at end-stage, would be at high risk (50% 5 year, or 80% 10 year) for progression to advanced disease. Also, it’s important to note that the total MGS-4 eyes in Table 3 included all globes (1159) while the MGS-9 eyes in Table 3 (673) were only those graded directly with the neurosensory retina removed. It’s not possible to see the details of MGS-9 with the neurosensory retina intact.

There was good correlation between globe pairs (Table 4). Of 331 pairs, 225 (68%) had identical grades using the MGS-9. Also, 278 or 84% were within 1 grade level of the fellow eye, and 309 or 93% within 2 grade levels. In the MGS-4, 86% of the pairs had identical scores and 98% were within one grade level of the fellow eye.

Representative and Illustrative Images

The MGS allows for high-resolution color images that correlate complex clinical findings and diagnosis for more accurate classification of eyebank tissue.

A composite panel is available for each standard pair of eyebank eyes (Fig. 1). Each individual image, for at least one eye of the pair) was examined at very high resolution.

Table 3. Comparison of Distribution of Eyes Between MGS-4 and MGS-9

<table>
<thead>
<tr>
<th>MGS-4 Step Grade (%) of Total Eyes</th>
<th>MGS-9 Step Grade (%) of Corresponding MGS-4 Step Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 424 (37)</td>
<td>1 319 (75)</td>
</tr>
<tr>
<td>2 88 (21)</td>
<td>4 50 (21)</td>
</tr>
<tr>
<td>3 236 (20)</td>
<td>4 36 (15)</td>
</tr>
<tr>
<td>4 75 (6)</td>
<td>5 38 (16)</td>
</tr>
<tr>
<td>5 36 (16)</td>
<td>6 36 (15)</td>
</tr>
<tr>
<td>6 38 (16)</td>
<td>7 36 (15)</td>
</tr>
<tr>
<td>7 36 (15)</td>
<td>8 1 (0.4)</td>
</tr>
<tr>
<td>8 1 (0.4)</td>
<td>9 60 (80)</td>
</tr>
</tbody>
</table>

Comparison of the same eyes with either the four-step or nine-step grading.

* Percentages listed are derived from a numerator of total MGS-9 globes to the denominator from the corresponding MGS-4 globes.

Table 4. Right and Left Eye Grade Comparison

<table>
<thead>
<tr>
<th>MGS-9 Grading Level Difference</th>
<th>Pairs, N = 331 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same grade</td>
<td>225 (68)</td>
</tr>
<tr>
<td>1 step</td>
<td>53 (16)</td>
</tr>
<tr>
<td>2 steps</td>
<td>31 (9)</td>
</tr>
<tr>
<td>3 steps</td>
<td>7 (2)</td>
</tr>
<tr>
<td>MGS-4 grading Level difference</td>
<td></td>
</tr>
<tr>
<td>Same grade</td>
<td>284 (86)</td>
</tr>
<tr>
<td>1 step</td>
<td>39 (12)</td>
</tr>
<tr>
<td>2 steps</td>
<td>7 (2)</td>
</tr>
<tr>
<td>3 steps</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

Figure 2. Right macular image of a 90-year-old Caucasian female who died of a cerebral vascular accident and had a history of cataract surgery in both eyes, had been using antioxidant vitamins with zinc for 15 years. End-stage AMD features are present and include: (1) AMD-related hyperpigmentation within the inner zone; (2) depigmentation graded as the most advanced stage (≥500 μm), (3) drusen in the superior temporal outer subfield, with some appearing calcified, (4) thin subretinal hemorrhage in the center subfield as well as the inner and outer temporal subfields, and (5) a whitish area of subretinal fibrosis located in the inner inferior subfield.

Figure 3. Right macular image of an 89-year-old Caucasian female who died of a myocardial infarction. There is central GA involving the foveal center and nearly the entire central subfield. There are areas of hyperpigmentation within the central and inner subfield, also referred to as the central zone. There are few small hard drusen.
An example of an eye with advanced AMD is shown in Figure 2 and illustrates the high-resolution macular images for the right eye of a 90-year-old Caucasian female who died of a cerebral vascular accident, had cataract surgery in both eyes, and had been using antioxidant vitamins with zinc for 15 years. Classic, end-stage AMD clinical features are demonstrated that include pronounced AMD-related hyperpigmentation within the inner zone of the ETDRS grading template, depigmentation graded as the most advanced stage ($\geq 3000 \text{ mm}^2$), and drusen, including calcified drusen as noted in the superior temporal outer subfield and even outside of the outer ring, suggestive of reticular pseudodrusen. While subretinal hemorrhage was sometimes difficult to differentiate from postmortem changes, this eye appears to have thin subretinal blood in the center subfield as well as the inner and outer temporal subfields. The inner inferior subfield appears to have a whitish area of subretinal fibrosis. Other postmortem changes in the RPE or choroid were not appreciated during this brief time from procurement to imaging, unlike critical molecular events that are highly time dependent.

Figure 3 represents an example of central GA. The donor is an 89-year-old Caucasian female who died of a myocardial infarction. As an example of expedient tissue collection (LEITR), the donor died at 11:12 AM, eyes were enucleated at 2:34 PM (3 hours 22 minutes later), globes were processed at 3:30 PM (4 hours 18 minutes-postmortem), and frozen at 4 PM and 4:55 PM for the right and left eyes, respectively (4 hours 43 minutes for the right and left eyes, respectively). The GA clearly involves the foveal center and nearly the entire central subfield. There are areas of hyperpigmentation within the central and inner subfield (the central zone).

Figure 4 represents an example of high-risk drusen. The donor is an 86-year-old Caucasian female who died of a pulmonary embolus and had a history of AMD and had cataract surgery in each eye. The image of the left macula demonstrates the presence of large, soft, distinct drusen, a drusenoid RPED, hyperpigmentation within the central zone, and areas of depigmentation overlying the drusen.

Figure 5 illustrates the advantage of color digital enhancement to recognize the presence of drusen, in particular, indistinct drusen (similar to filters used in the AREDS). This case was from an 82-year-old Caucasian male. The two images differ only by the application of a 10% green enhancement (image right) of the original photograph (image left) taken of the right macula. Note that the extent of drusen is difficult to determine and quantifying the drusen was challenging (image left). However, in the image with the green enhancement (image right), both the drusen and the extent are much easier to determine, particularly in the central and all inner subfields.

Figure 6 illustrates an eye with mostly intermediate drusen. This 90-year-old Caucasian male has numerous intermediate drusen (estimated 354- to 650-mm$^2$ total drusen area) with questionable hyperpigmentation and less than 354 mm$^2$ of depigmentation. Thus, using the MGS-4 step grading system, this would be classified as MGS-5 while in the MGS-9 step grading system, this would be classified as MGS-5 with a 6%, 5-year risk of progression to advanced disease.

Figure 7 illustrates an eye with basal laminar or cuticular drusen. This 76-year-old Caucasian male has numerous small hard drusen that are highlighted best by using transillumination (right). The homogenous, small drusen are symmetric and bilateral.

Figure 8 illustrates a pattern dystrophy. In this 76-year-old Caucasian female, there is an elevated yellow luteal lesion at the level of the pigment epithelium (left), best seen in stereo (not shown). Prior to dissection of the neurosensory retina, the donor died of a myocardial infarction. As an example of expedient tissue collection (LEITR), the donor died at 11:12 AM, eyes were enucleated at 2:34 PM (3 hours 22 minutes later), globes were processed at 3:30 PM (4 hours 18 minutes-postmortem), and frozen at 4 PM and 4:55 PM for the right and left eyes, respectively (4 hours 43 minutes for the right and left eyes, respectively). The GA clearly involves the foveal center and nearly the entire central subfield. There are areas of hyperpigmentation within the central and inner subfield (the central zone).

Figure 4. Left macular of an 86-year-old Caucasian female who died of a pulmonary embolus and had a history of AMD and had cataract surgery in each eye. There are large, soft, distinct drusen, a drusenoid RPED, hyperpigmentation within the central zone, and areas of depigmentation overlying the drusen.

Figure 5. Two right macular images with the application of a 10% green digital enhancement (image right) of the original photograph (image left). Drusen are difficult to detect (left) while indistinct drusen are more readily noted in the central and all inner subfields.
The globe was imaged using transillumination (right) and this clearly demonstrates a dark shadow, a characteristic clinical finding in support of the diagnosis of a pattern dystrophy. Such imaging is performed clinically during biomicroscopy with the same phenotypic result.

Figure 9 illustrates reticular pseudodrusen. This 95-year-old Caucasian male has a lace-like pattern of reticular pseudodrusen that are located in the superior-nasal and superior-temporal quadrants of each eye (only left eye is pictured). The pattern was readily visible, even on the pre-dissection image.

Table 3 demonstrates the distinction between the original MGS 4-step scale as applied to our data set when compared with the MGS 9-step scale. The main category that deserves attention is the subdivision of risk in the MGS-3 in the original scale that is separated into more distinct risk categories in the MGS 9-step grading scale. See explanation above for the discrepancy in globe numbers between the four-step and nine-step analysis.

Table 4 examines the similarity between donor pairs. Specifically, by using the nine-step grading, we would anticipate a greater separation of left to right correlation. Indeed, we found that most all pairs (84%) were either the same or within one step of the fellow eye using the nine-step system compared with 98% in the four-step grading.

**DISCUSSION**

At present, an animal model is not available to study human AMD. While some animal models may replicate certain features of AMD, such as deposit that resemble drusen or surgically induced CNV, the effects of aging are unique and are difficult to replicate in animals, especially in animals without a true macula, such as mice or other rodents. Maximum lab animal life-span may be 2 to 5 years, thus aging mechanisms, as in humans, is lacking. Furthermore, AMD is a complex disorder with complex genetics, environmental influences, inflammatory, and immunologic factors that contribute to AMD progression. Thus, the use of eyebank eyes with AMD represents a noninterventional system to study AMD in human eyes that are affected and risk assessed.

We propose a system that leverages the same definitions as those applied by the AREDS and translate these criteria directly to human eyebank eyes. Thus, the strong, prospectively collected data derived from the AREDS applies directly to the tissue graded using the MGS. By modifying and improving upon our original classification, we are now able to stratify the risk of a particular tissue from human eyes with AMD, procured by qualified eyebank personnel, and apply the AREDS nine-step grading system. The use of both the MGS-4 and MGS-9 will help to harmonize research data.

In earlier work, tissue graded using the original MGS 4-step system led to biochemical, proteomic, and molecular changes that were particularly relevant, especially at the transition between MGS-2 and -3. Importantly, this represents a critical transition from AREDS level two to three that is the key step to identify patients who benefit from the use of high-dose antioxidant vitamins with zinc. As an example, during the transition from MGS-2 to -3, we found a quantitative upregulation of the copper-zinc superoxide dismutase (SOD), manganese SOD, and other heat-shock proteins that protect against oxidative damage. This finding may help to at least partially explain and provide biochemical evidence to support the beneficial and therapeutic effect of antioxidant systems in the pathogenesis of AMD. Thus, the MGS has enabled a quantitative proteomic approach to study AMD. Other studies using the MGS have led to both proteomic and genomic data in support of biologic pathways involving mitochondrial function that are likely involved in the pathogenesis of AMD.

Since the original description of the MGS, other imaging analysis has supplemented the grading of eyebank tissue...
including both FAF and confirmatory histopathology. In the FAF study, variables were standardized and 262 human eyes were examined. No correlation between total FAF and age (range, 42–100 years) was found, yet there was a statistically significant inverse correlation with MGS level (1–4). Next, the clinical features imaged in eyebank eyes were found to be consistent with those found in human eyes with AMD. The MGS images, followed by histopathologic analysis of the fellow eye using mapping techniques, confirmed the presence and extent of AMD features along with ultrastructural findings to add further validation to this methodology.

Herein, applying the MGS-9 step grading system to a large number of donor eyes (1159) leverages the risk-stratification data from the AREDS study by correlating the known risk of progression (thus, severity of AMD) with clinical features. The MGS-9 allows for an accurate phenotypic documentation of the clinical features found in AMD, along with several other related conditions that are illustrated (see Table 2). Common examples of associated macular pathologies that are readily identified using MGS imaging techniques, and commonly confused clinically, may help to clarify the role that each entity may play in AMD. For example, the presences of basal laminar drusen, reticular pseudodrusen, pattern dystrophies, and others (see Table 2) are likely to be important variables in studying the pathogenesis of AMD. By demonstrating key clinical and phenotypic features of AMD, we demonstrate the power and utility of this methodology and also help to link a translational research bridge that harmonizes the clinical assessment of AMD in eyebank eyes with the AREDS database. Thus, by using the MGS-4 and -9, researchers will be better able to understand the cellular, biochemical, proteomic, genomic, metabolomic, and other molecular changes that occur in each stage of AMD.

In Table 3, the distribution of the globes are reassigned from the MGS-4 into the MGS-9 step grading. The key clinical phenotype transition in the AREDS grade level occurs mostly at level three. Using the MGS-4 grading at level three, the 5-year risk of progression to advanced disease as defined by the MGS-9 levels four to eight ranges from 5% to 47% (Table 1). Thus, further subdividing this category and incorporating the MGS-9 grading adds greater risk stratification to the phenotype assessment. Furthermore, by using the MGS-9, researchers can take advantage of this risk stratification in order to better...
apply individual molecular studies that are performed on human tissue that examine AMD progression.

The paired globe correlation of the MGS warrants a more detailed explanation. First, one eye of each pair may be photographed, dissected, and rephotographed to accurately examine the detailed features seen by not only directly examining the RPE sheet. The preliminary (predissection) or scout photographs are also taken of the fellow eye and obtained in order to obtain the best clinical information without disturbing the tissues. Scout images can be transmitted immediately (text messaging or e-mail) for review by the investigator for acceptability of a particular tissue or pair of eyes into a study. A detailed macular grade cannot be obtained by taking photographs with the neurosensory retina intact because the retinal tissue opacifies postmortem and has been demonstrated to underestimate the level of AMD in the vast majority of eyes. However, given the relatively high rate of correspondence between right and left eyes of a donor pair, the fellow, the undissected eye is assumed to have a similar grade. Using the MGS-9 grading, we found that the relatively high rate of correspondence between right and left eyes of a donor pair, the fellow, the undissected eye is assumed to have a similar grade. Using the MGS-9 grading, we found that the second eye is at the same level (±1 step) approximately 84% of the time and within two steps in 93% of cases. Updating our MGS-4 correspondence between globes, we found that 80% had the same grade level and 98% were within one-grade level of the fellow eye, similar to prior results.

Should a research protocol require that the neurosensory retina remain intact, without dissection, the second eye of a pair may be used with a reasonably high level of MGS-9 correlation with the grade level of the first eye (Table 4). For example, a researcher may require a specific fixation protocol, the tissue immediately dissected, flash frozen, or shipped immediately on ice. Such conditions largely depend upon the investigator’s specific needs or requirements. Other research protocols may require processing both globes along with a detailed macular analysis that is performed on each globe of the pair, followed by local tissue dissection and either freezing or fixing the tissue. For example, the macular RPE may be required for detailed study and can be obtained using a 5-mm punch biopsy. Technicians are trained to gently brush the RPE from the central button and separate the cells from the underlying Bruch’s membrane for flash freezing and immediate shipment of the fresh tissue.

As illustrated in our example images, the transillumination technique is very helpful to detect specific macular features. Transillumination of the macula highlights subtle RPE alterations and can help determine the presence of drusen, type of drusen, and help to quantify both the increased and decreased pigment scores. Transillumination is helpful to distinguish basal laminar drusen and also detect classic features of pattern dystrophies. Likewise, failure to identify these macular findings compromises the value of the research tissue and confuses the final data in studies designated for AMD and may add unnecessary variables to the researcher’s final analysis and conclusions.

In summary, we have evaluated over 1159 human eyebank eyes using the MGS-4 and -9 grading systems. Such analysis adds critical clinical information to the original MGS-4 by applying the grading criteria derived from the AREDS nine-step system. The corresponding MGS-9 may be used to generate and supply consistent, risk-stratified tissue for researchers globally. Consistency in high-quality tissue grading has the potential to better harmonize studies and data from AMD researchers. Most importantly, because the definitions are based on the AREDS 1 and 2, WARMGS, and the international system, MGS-9 links human tissue analysis to the powerful data from prospectively studied populations. Importantly, AREDS 1 documents the natural history of untreated AMD progression in a large number of subjects with AMD obtained in a well-designed, prospective, multicenter clinical trial that is not likely to be repeated. Thus, human tissue graded using modified MGS-4 grading criteria, referred to as the MGS-9, allows researchers to have access to a more detailed, risk-adjusted human eyebank tissue for independent study. We encourage investigators who use the eyebank tissue model to study AMD to use both MGS-4 and -9 criteria in order to harmonize research data. Development of a tissue repository in combination with a robust bioinformatics infrastructure would enable query for specific disease-causing mechanisms and lead to new therapeutic options.

Acknowledgments

Supported by an unrestricted departmental grant from Research to Prevent Blindness (RPB; New York, NY, USA).

Disclosure: T.W. Olsen, iMacular Regeneration, LLC (I); Lions Eye Institute for Transplant and Research (LEITR) (S); A. Liao, None; H.S. Robinson, None; N.V. Palejwala, None; N. Sprehe, None

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