Quantifying Drusen in AMD

An interview with Duncan Friedman, MD, MPH; and Christine A. Curcio, PhD

A paper in *Retina*[1] last year described the use of a novel method of comparing fluorescein angiography (FA) and fundus photography images to quantify the number of drusen present in eyes with age-related macular degeneration (AMD). The authors found that roughly half of all drusen per macula stained on fluorescein. This approach was built upon a smaller study of an inherited disorder by Russell and colleagues.[2] *Retina Today* spoke with the first author and senior author of the paper to learn the background behind the study and its possible implications for clinicians and other researchers.

Retina Today: *For background, please explain what is known about the significance of drusen in the pathophysiology of AMD.*

Duncan Friedman, MD, MPH: From a clinical standpoint, drusen are the first lesions seen—they define macular degeneration. A greater number of drusen, especially a subtype of drusen—the larger, softer drusen—indicate a greater predisposition to the formation of wet or neovascular macular degeneration. These diffuse drusen that we find in the macula tend to be more clinically significant than small, hard drusen—a clinical predictor of progression.

Christine A. Curcio, PhD: What you ask is a question that has been around for a long time. That is, whether these lesions affect disease progression directly or whether they are some kind of epiphenomenon—whether the disease is really something else and the drusen are just tombstones or signposts. The answer I would put forward in that debate is that, first of all, drusen are only 1 part of the story. The soft drusen that Dr. Friedman mentioned as being more clinically significant are part of what we call the "oil spill" in Bruch membrane, which is basal linear deposits. There are 2 forms of the same lesion: the lumps, which are the clinically visible drusen, and this oil slick on the inner surface of Bruch membrane. We don't see that oil slick because it is too thin to be detected on optical coherence tomography (OCT).

There are multiple ways that the oil spill can get in the way of healthy functioning. It can form a barrier, causing dysfunction of Bruch membrane. Everything traveling between the retinal pigment epithelium and the photoreceptors, both to and from, has to pass through that membrane. When this lipid-rich material collects in Bruch membrane and on the surface as basal linear deposits and drusen, transport is impaired in both directions.

RT: *And this oil slick is not seen clinically?*

Dr. Curcio: That's correct; it is just below the radar of OCT. In a few years, I think we will be able to see the thickest examples of it, but its median thickness is under 2 µm.

RT: *Is that what is showing up on FA in your study?*

Dr. Friedman: Is the fluorescein highlighting the oil spill? That is not likely, actually, because the oil is hydrophobic and therefore less likely to interact with fluorescein, which is hydrophilic. The drusen are just a marker that there is a barrier.

Dr. Curcio: That's true. They are blocking transport to and fro, and soft drusen and basal linear deposits are 2 aspects of the same barrier. When you see soft drusen—and they only appear in the macula—it's like seeing tar balls in the oil spill. They are very obvious, but that thin layer underneath is also there. The fluorescein is hydrophilic and will tend to diffuse into lesions that have less lipid in them. So 1 possibility is that on FA we see drusen that are perhaps less dangerous.

There are other things that drusen do that are impor-

tant in the progression of the disease. Because they are lipid-rich, the lipids can become peroxidized—they react with unstable molecules called reactive oxygen species. Peroxidized lipids are very toxic. They partition into membranes and kill cells.

Basal linear deposits and soft drusen both are biomechanically fragile, by which I mean that they fall apart. We determined that in microdissection studies, and I see it every day in histology. This is important because it creates a cleavage plane through which choriocapillaries dissect, so that process abets the formation of choroidal neovascularization.

Dr. Friedman: All of these deposits form an environment that allows choroidal neovascularization to progress because of inflammation, as Dr. Curcio said: setting up pathways for blood vessels to grow.

Dr. Curcio: Because the oil spill is there, things like high-density lipoprotein (HDL), the "good" cholesterol in plasma, factors that would normally have capacity for clearance, for healing, have trouble getting in. These lipoproteins, and other agents that could help resolve the situation, have reduced access because the lesions block the way.

Finally, because drusen are tall—they are visible clinically at 30 µm, but they can be greater than 200 µm in height—they increase the path link between the cells and the blood supply, which carries oxygen. So that can contribute to ischemia and promote blood vessel growth, helping to keep the disease process going. So, to that question I mentioned earlier, in many ways drusen are not bystanders or signposts; they are the disease.

RT: *Please tell us what you hoped to show in your study and describe your methods.*

Dr. Friedman: We started with images that are commonly obtained in the clinic, both standard color fundus photos and FA images—late FA images in which drusen are most likely to fluoresce. We obtained both types of images in each study eye, and then by using software provided by our coauthor Francois C. Delori, PhD, we were able to directly overlay those 2 images so that they lined up with each other. By putting them on the computer screen and rapidly alternating the color photo and the FA image, we could determine whether a particular druse was lighting up on FA or not. We could see it on the color photo, and then if we immediately flipped over to the FA image there would either be an obvious appropriate signal or a lack of signal, and that way we could quantify how many of those drusen actually lit up in each FA image.

RT: *And only about half of the drusen seen in the color photo showed up on FA, correct?*

Dr. Friedman: Yes. Previous studies have reported about 50%, and in our study just a bit under 50% of those drusen (49.57%) had a corresponding signal on FA.

Dr. Curcio: It was very good to get these numbers with a reproducible method because I've heard from clinicians over the years that about half of drusen stain with fluorescein. It's 1 of those numbers that gets passed down and you ask, where does that come from? The data that were gathered many years ago were done with film methods that would be hard to reproduce. So digital photography has now made it possible to address this in a more quantitative and rigorous fashion.

RT: *Do you have an idea of why only half of the drusen appear on FA? Does it have to do with their hydrophobicity/hydrophilicity?*

Dr. Friedman: That is probably the underlying reason. Drusen are made up of different subcomponents, and some of those subcomponents are more hydrophilic and some more hydrophobic. Therefore you would expect that drusen with more hydrophilic components will take up the dye more readily. Does that mean that those drusen without a fluorescein signal do not contain hydrophilic substances? No; they might be sequestered and unable to interact with the dye. But nonetheless, some are more apt to take up more dye than others.

Dr. Curcio: These ideas came from work done by Alan Bird, Daniel Pauleikhoff, and John Marshall[3,4] years ago at Moorfields Eye Hospital in London. They speculated that FA could reveal the composition of drusen, and they had ideas about the relative hydrophobicity of drusen. The data our group has collected are the first quantitative tests of that hypothesis.

RT: *What does an understanding of this phenomenon do for our understanding of AMD?*

Dr. Friedman: That's a good question. Ultimately it helps us to quantify how many drusen there are in the eye, and of what types. Does this finding serve as a clinical predictor? Not necessarily. It could have some significance in that, as Dr. Curcio hinted, maybe certain subclasses of drusen that are more apt to take up the dye are less likely to promote progression. This needs to be studied. Perhaps in the future we will be able to say that these drusen that are more hydrophilic are less likely to

be associated with progression of the disease. We cannot say that right now and cannot make that assumption. But it may be worth further investigation as to whether more lipid-rich drusen are predecessors to neovascular AMD. Each person with AMD is different, and the environment and milieu that leads to progression to wet AMD will be different for each person.

Dr. Curcio: If further work confirms that this technique of examining drusen in color photographs and angiography can reliably identify drusen that are particularly lipid-rich or not lipid-rich, it would be interesting to see how these characteristics relate to AMD's associations with genes historically part of plasma HDL metabolism. These kinds of analyses could be used along with detailed genotyping studies to see if the expression of different alleles of those genes affects the composition of drusen.

Dr. Friedman: Another lesson that can be taken from the study is that we found a good correlation between the size of the drusen on fundus photograph and the angiographic signal. This was true across the range of drusen sizes. This suggests that different druse subregions do not exhibit differences in relative fluorescein-binding capacity.

RT: *Can you see applications for this overlay technique, whether manual or automated, in future studies?*

Dr. Friedman: Absolutely. The method we used in the study was a time-consuming process, and it would be better if it were automated so that a computer could recognize the major vessels, the nerves, and other landmarks and automatically overlay and then rapidly alternate the fundus and FA images. But the ability to directly overlay the color and the angiographic images and highlight signs of choroidal neovascularization and other vascular pathology could have clinical significance in many other diseases, not just AMD. This is just thinking off the cuff, but it could help identify hot spots lighting up in diabetic eye disease. So when we do, for instance, focal laser photocoagulation in diabetic retinopathy, the ability to overlay the clinical and the angiographic image could help guide our laser application and pinpoint the accuracy of our treatment.

As I suggested, with the right type of technology, the process that we used could be automated. Someone better with computers than I could design the right algorithm or software to take the color and angiographic images, overlay them, and look for corresponding signals between the two images. I did this by hand in the study, and our coauthor John S. Parker used a different method to validate the findings, also by hand. But a computer-based model would not be too hard to construct. The computer could recognize the appearance of drusen on the fundus photo and quantify how many of them appear on the corresponding angiogram.

RT: *What are the take-away points that you would want readers to understand from your study?*

Dr. Curcio: We are still learning about drusen. Drusen still have things to teach us about AMD. Use of digital fundus photography, converting traditional methods to digital, offers tremendous potential benefit in terms of understanding this disease, selecting patients who might be suitable for clinical trials, and so on.

Dr. Friedman: These studies point out that we should not discount the usefulness of FA. With newer methods of retinal imaging available, clinicians may tend to let FA fall by the wayside, but it can still be a powerful diagnostic tool. It still has important clinical applications in AMD.

Dr. Curcio: When we started this study, 1 of the rationales was to explore other lesions in the subretinal space that do not take up fluorescein, subretinal drusenoid debris called reticular pseudodrusen.[5] We thought that some of the nonstaining drusen might be these reticular pseudodrusen. We didn't actually see them, so that is a subject for a future study. ■

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