Kinetics of Retinal Lipoprotein Precipitation and Elimination after Closure of Subretinal New Vessels

Nina Charlotte Bille Brabe Taarnhøj,1 Ole Kjeka,2 and Michael Larsen1

PURPOSE. To study the kinetics of retinal precipitation and elimination of lipoprotein in relation to photodynamic therapy of subretinal new vessels.

METHODS. This was a retrospective observational study of subretinal precipitate before and after photodynamic therapy for subfoveal new vessels secondary to age-related macular degeneration in 14 eyes demonstrating precipitate at one or more visits, using digital red-free gray-scale fundus photography, intravenous fluorescein angiography, optical coherence tomography, and morphometric mapping.

RESULTS. In 14 eyes of 14 patients, the area covered by lipoprotein precipitate increased from a mean of 0.54 optic disc areas (range, 0.2–1.61) before treatment to a mean of 0.65 optic disc areas (range, 0.01–3.04) at 45 d after treatment (P = 0.18). Eyes with a serous detachment at baseline, before the initial treatment (n = 10) demonstrated more treatment-related precipitation than eyes without a serous detachment at baseline (n = 4; P = 0.034). Two eyes with a large amount of precipitate and a long follow-up demonstrated net elimination of precipitate from approximately 100 days after the initial treatment, with unexponential precipitate half-lives of 43 and 52 days.

CONCLUSIONS. Photodynamic therapy for subfoveal new vessels may be associated with retinal precipitation of lipoprotein, presumably because early extraction of water and salts from the subretinal fluid increases the concentration of leaked plasma proteins. Despite reperfusion of the subretinal vessels, macromolecular leakage appears to cease within 100 days, indicating that functional maturation of the new vessels has occurred, with an associated decrease in pore size. (Invest Ophtalmol Vis Sci. 2003;44:1680–1685) DOI:10.1167/iovs.01-1132

Retinal precipitation of plasma lipoprotein is a common feature of retinopathies characterized by exudation from the blood stream. Although the amount of precipitate varies with the severity of the disease, the dynamics of exudation, precipitation, and elimination appear to be complex and highly variable. The commonly applied term “hard exudate” is a misnomer, because the primary exudate from leaking retinal or choroidal vessels is clear and invisible. Only the fraction of the exudate that cannot be resorbed by adjacent retinal vessels or by the retinal pigment epithelium becomes visible after having been concentrated and deposited within the retina as a white, amorphous precipitate. Histochemical analysis of the diabetic retina has shown that the precipitate is composed of polysaccharides, glycoproteins, glycolipids, unsaturated lipids, and phospholipids of plasma origin.

Having previously documented a therapeutically induced precipitation phenomenon in two case reports on branch retinal vein occlusion,2,3 we proceeded to study lipoprotein precipitation in the retina in patients with age-related macular degeneration (AMD) and subfoveal new vessels of choroidal origin (CNV) who had undergone photodynamic therapy (PDT) for this condition. Theoretically, these eyes are ideally suited for studying the kinetics of lipoprotein precipitation and elimination, because the source of pathologic leakage is solitary, is well circumscribed, and can be effectively closed in a single treatment session.

METHODS

Subjects

This study was a retrospective observational study of 41 eyes with AMD (age range, 48–90 years) and subfoveal CNV in patients who underwent PDT. Inclusion criteria were subfoveal neovascularization secondary to AMD treated by PDT, retrievable fundus photographs from baseline (<2 weeks before the first application of PDT), and from at least one follow-up examination within approximately 1 to 2 months after the initial treatment. A review of the records concerning 41 eyes in 40 consecutive patients who underwent PDT for subfoveal CNV secondary to AMD at the Herlev Hospital identified 13 eyes in 13 patients (nine women and four men) in which lipoprotein precipitate (hard exudate) was identified at one or more visits. An additional case (patient 14; Table 1) from the Haukeland Hospital was included in the study because it presented a highly informative and prominent precipitation phenomenon.

Of the 14 patients included in the study, 6 had 100% classic CNV, 4 had 50% to 90% classic CNV, 3 had less than 50% classic CNV, and one had 100% occult CNV (Table 1). We did not deliberately treat eyes with minimally (<50%) classic CNV, but three eyes in three patients were classified as such after review of their angiograms. In 11 patients, the fellow eye had no CNV, whereas 3 patients had subfoveal CNV in the fellow eye. The mean of the greatest linear lesion dimension was 2449 μm (range, 1200–3428). Transfoveal optical coherence tomograms (Zeiss Humphrey Systems, Dublin, CA) before and after PDT were available in 10 of the 14 eyes.

PDT was applied after intravenous infusion of 30 ml verteporfin (6 mg/m2 body surface area) over 10 minutes. Fifteen minutes after the start of the infusion, a laser beam at 689 nm delivered 50 J/cm2 at an intensity of 600 mW/cm2 over 83 seconds and a spot size with a diameter 1000 μm larger than the greatest linear dimension of the CNV lesion. Retreatment was applied 1 month after the initial treatment if fluorescein angiographic reperfusion with leakage was present and a serious foveal detachment could be documented by optical coherence tomography. At subsequent follow-up examinations, retreatment with the same regimen was applied if angiography showed fluorescein leakage, if there was evidence of new vessel growth by comparison with the preceding fluorescein angiogram, or if a newly developed
subretinal hemorrhage was found. The study adhered to the tenets of the Declaration of Helsinki.

**Fundus Photography and Morphometry**

Clinical examination and fundus photography were repeated every 2 months after the initial treatment, with a follow-up of up to 9 months (range, 1–9). Fundus photography was performed in red-free illumination and with optical filters for fluorescein angiography, using a 50° fundus camera with an 8-bit single-chip 1024 × 1024-pixel digital focal plane detector. The patients were examined no more than 1 week before and at approximately 45 days (range, 33–68) after the initial PDT session and from then on approximately every 3 months. The best 50° fovea-centered photograph from each session was selected for further analysis.

Optical coherence tomograms were visually graded in the following categories: serous foveal detachment, cystoid intraretinal edema, diffuse intraretinal edema (thickening), and absence of edema, with the latter category comprising subnormal retinal thickness.

Retinal lipoprotein precipitate was identified visually, with specific attention on the differential diagnosis between precipitate, drusen, atrophic choriretinal scars, and subretinal fibrosis. The morphometric analysis was conducted with a computerized fundus morphometry system (RetinalLyze; Retinalyze A/S, Horsholm, Denmark). Each precipitate element or confluent cluster of precipitates was identified visually on the computer screen and outlined by manual cursor control (Fig. 1). The precipitate mapping was followed by automated calculation of the total fundus area (in pixels) covered by precipitate. The optic disc area (DA) was used for internal reference and for interindividual comparison, the optic nerve head area being normalized to a mean area of 15,000 pixels (range, 11,500–19,000) on a 50° × 1024 × 1024-pixel digital fundus photograph. The reproducibility of the procedure was assessed by a second analysis of all photographs from all 14 patients at two different sessions. First, all baseline and follow-up photographs (n = 28) were mapped by one of the authors, and approximately 3 months later the mapping was repeated by the same author. The reproducibility, determined as the numerical difference between the consecutive mappings divided by the mean, was 27%.

**Statistical Analysis**

Median shifts within and between groups with respect to precipitate-covered fundus area were analyzed by Wilcoxon’s nonparametric signed rank test and the Mann-Whitney test. The primary end point was the relative change in lipoprotein precipitate coverage between the baseline examination (performed no more than 1 week before the first PDT) and the first follow-up examination before renewed treatment (1 to 2 months after the initial treatment).

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**TABLE 1. Characteristics of Patients with Age-Related Macular Degeneration and Subretinal Neovascularization before and after PDT**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex (M/F)</th>
<th>Lesion Type (% Classic)</th>
<th>Greatest Linear Dimension of Subretinal Neovascularization (µm)</th>
<th>Serous Detachment (Y/N)</th>
<th>Precipitate Area before Treatment (DA)</th>
<th>Precipitate Area after Treatment (DA)</th>
<th>Visual Acuity before Treatment</th>
<th>Visual Acuity after Treatment</th>
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<td>1</td>
<td>67</td>
<td>F</td>
<td>10</td>
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<td>0.08</td>
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<td>2</td>
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<td>1875</td>
<td>Y</td>
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<td>1.14</td>
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<tr>
<td>3</td>
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<tr>
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<td>0.10</td>
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<tr>
<td>6</td>
<td>58</td>
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<td>5</td>
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<td>0.04</td>
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<td>7</td>
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<tr>
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<td>100</td>
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<td>Y</td>
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<td>0.08</td>
<td>0.45</td>
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<tr>
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<td>3.04</td>
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<tr>
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<td>Y</td>
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1 DA, 1 optic disk area. Greatest linear dimension of subretinal neovascular membranes was calculated assuming a standard vertical optic disk diameter of 1500 µm. The mean interval between treatment and follow-up was 45 days.

* Log-weighted mean.

† P = 0.18.
RESULTS

In the 14 patients in whom lipoprotein precipitate was found at one or more examinations the total fundus area covered by precipitate was found to have increased from baseline to the first postoperative examination by 20%, from a mean of 8,392 (range, 0–40,506) to 9,638 (range, 135–47,044) pixels ($P = 0.18$; Table 1). The mean interval between treatment and follow-up was 45 days. An increase in precipitate was found in 9 of the 14 eyes, whereas a reduction was found in the remaining 5 eyes (Fig. 2). When normalized to an optic DA of 15,000 pixels (range, 11,500–19,000), the fundus area covered by precipitate was 0.54 DA before and 0.65 DA after treatment. Fluorescein angiography at follow-up demonstrated a reduced extent of the subfoveal new vessels and reduced leakage in all eyes, and a well-demarcated round area of early-phase hypofluorescence corresponding to the treated area.

Biomicroscopically, 10 of the 14 eyes had a serous retinal detachment over or around the CNV before the initial treatment, whereas four eyes had no conclusive signs of serous detachment (Table 1). Eyes with a serous detachment before treatment demonstrated an increase in precipitate area after treatment, from a mean of 9,042 (range, 30–40,506) pixels before treatment to a mean of 11,091 (range, 473–47,044) pixels after treatment ($P = 0.093$). Eyes without serous detachment at baseline demonstrated a decrease in precipitate from a mean of 6,768 (range, 0–18,095) pixels before treatment to a mean of 6,005 (range, 135–17,420) pixels after treatment ($P = 0.144$). The difference between the two groups (Fig. 3) is statistically significant ($P = 0.034$).

Two patients (Table 1, patients 1 and 14) with extensive lipoprotein precipitation after PDT were examined regularly until the precipitate had completely resolved at 8 and 9 months, respectively, after the initial treatment (Fig. 4, showing fundus photographs of patient 14). Eyes in both patients demonstrated a large serous detachment before treatment and at the first follow-up but only a moderate amount of precipitate at baseline (i.e., before the initial treatment). The first and in patient 14 also the second treatment were followed by marked precipitation (Fig. 5, patient 14; Fig. 6, patient 1). No serous detachment was noted at subsequent follow-up examinations, and the area covered by precipitate then decreased exponentially with half-lives of 32 d (patient 1) and 43 d (patient 14) (Fig. 7). The subretinal neovascular membrane remained angiographically perfused without definite leakage of fluorescein. A third treatment was administered in one of the two patients, because fresh hemorrhage from the rim of the neovascular membrane was suspected.

All 14 patients were under observation for at least 2 months. Mean visual acuity at baseline was 0.12 (Table 1) and remained unchanged at 2 months after the initial PDT. The statistical results were not qualitatively influenced by the inclusion of patient 14 in the study.

DISCUSSION

We have demonstrated evidence of lipoprotein precipitation in the retina secondary to closure of subretinal new vessels by PDT. Significant increases in the precipitate-covered fundus area occurred only in eyes that had a serous retinal detachment before treatment. Although the natural history of subfoveal new vessels includes maturation of the vessels, reduction of leakage, and disappearance of precipitate, we have never observed such drastic spontaneous increases in the amount of precipitate as in this study. Although an untreated control group would be desirable, we believe that there is substantial evidence that the phenomenon was induced by therapeutic closure of the leaking subretinal vessels by PDT.

Precipitation of lipoprotein after treatment of exudative retinopathy was first described by Bernard et al.1 in patients with diabetic retinopathy and macular edema. The investigators found an increased amount of precipitate after photocoagulation, whereas fluorescein angiography showed reduced

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932920/)

FIGURE 2. Fundus area covered by lipoprotein precipitate before and after PDT for subfoveal neovascularization secondary to age-related macular degeneration. The area increased in nine eyes and decreased in five eyes ($P = 0.18$) during a mean follow-up period of 45 days.

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932920/)

FIGURE 3. Change in fundus area covered by lipoprotein precipitate from the pretreatment examination to the first follow-up examination after PDT for subfoveal neovascularization in patients with age-related macular degeneration. Follow-up was conducted at a mean interval of 45 days after treatment (range, 33–68). Eyes with a serous detachment of the retina before treatment had a significant increase in area of precipitate when compared with eyes without a serous detachment before treatment ($P = 0.034$).
edema and leakage. The resorption of precipitate lasted from 18 months to 3 years, which is considerably longer than in our patients, probably because a prompt and total cessation of leakage cannot be obtained by photocoagulation treatment of diabetic macular edema. In a case of branch retinal vein occlusion Christoffersen and Larsen recorded a resorption time of 18 months.

Subretinal new vessels of choroidal origin represent a solitary source of pathologic leakage that can be abolished in a single treatment session. We chose to study patients with age-related macular degeneration and subfoveal neovascular lesions who underwent PDT, because this treatment is generally effective in relieving leakage after a single treatment session while imparting minimal effects on adjacent structures.

Quantitative digital analysis of lipoprotein precipitation as seen on fundus photographs may be of value in the study of other retinopathies, but the pattern of precipitation and elimination is likely to be much more complex with multiple ill-defined sources of leakage, as seen in diabetic retinopathy. It is of fundamental importance, however, to understand that an increased amount of hard exudate may be evidence of a beneficial therapeutic effect.

The precipitation of lipoprotein is a dynamic process governed by factors that include the balance between rates of production and resorption of extracellular fluid from the neovascular membrane, the pore size of the leaking vessels, and the phagocytic activity of cells in the retina. The unexponential elimination of lipoprotein observed after two treatments in the present study indicates that, at this point, the subretinal

![FIGURE 4. Gray-scale fundus photographs in red-free illumination from the left eye of a 56-year-old woman (patient 14, Table 1, Fig. 5) with age-related macular degeneration and entire classic subfoveal neovascularization (greatest linear dimension of lesion, 3265 μm) with a surrounding serous detachment of the retina. At the initial examination (A), the precipitate covered a total area of 1495 pixels (image format, 904 × 904 pixels). Seventy days after photodynamic treatment the precipitate area had increased to 12,296 pixels (B). At this time, a second treatment was administered, and, at 100 days after the initial examination, the area covered by precipitate had increased to 25,308 pixels (C). Subsequently, a monotonous decrease in precipitate area with a halflife of 43 days was noted, resulting in near-complete resolution at 240 days after presentation, when the precipitate covered only 2840 pixels (D).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932920/)

![FIGURE 5. Fundus area covered by lipoprotein precipitate (■) in relation to time after initial photodynamic treatment (treatment indicated by □) for subfoveal neovascularization secondary to age-related macular degeneration in a 56-year-old woman (patient 14, Table 1; Fig. 4). Serous detachment and angiographic leakage were noted before both treatments. The area of the optic disc was 10,800 pixels. When performed on the same day, fundus photography preceded PDT.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932920/)
vessels had matured functionally in the sense that their pore size had decreased to a level at which no further exudation of plasma protein occurred.

A methodological limitation is the use of two-dimensional projection (the fundus photographic image) to study three-dimensional objects. Speculation on the relevant geometric transformation was avoided in favor of an empiric approach to the analysis of retinal lipoprotein kinetics.

An additional potential methodological problem is presented by the optical shower curtain phenomenon, which may arise when the retina is detached from the pigment epithelium. Precipitate located on the surface of the retinal pigment epithelium is then seen through the detached retina, and scattering of light in the retina may reduce the contrast and edge definition of the precipitate. The phenomenon increases with increasing distance between the retina and the precipitate. When reattached, however, the retina transmits a clearer image of the subretinal precipitate. This phenomenon alone may result in an apparent increase in precipitate, even when no real change has occurred. Our visual impression is that the postoperative increase in precipitate cover is so impressive in several of our cases (Fig. 4) that the shower curtain effect is unlikely to be responsible for more than a minor fraction of the changes measured in this study.

Although our clinical guideline required that patients treated by PDT have predominantly classic CNV, a review of the pretreatment angiograms for the purpose of the present study demonstrated that four patients had minimally classic CNV and one patient had 100% occult CNV. We chose to...
accept all types of subfoveal CNV, because hard exudate in the subretinal space can be seen in any of these conditions.

Our current understanding of the fluid dynamics of pathologic extracellular and extravascular fluid in the retina can be summarized as follows: Leakage from classic subretinal new vessels of choroidal origin occurs in a pathologic subretinal compartment between the photoreceptor layer and the retinal pigment epithelium (Fig. 8). In steady state, the compartment is maintained by the constant production of a clear exudate from the leaking vessels, which is balanced by resorption of water and salts across the pigment epithelium and the endothelium of the intraretinal capillaries. Fluid will flow from the neovascularization toward the sites of resorption of fluid and salt, with the concentration of plasma proteins increasing with the distance from the new vessels. The absence of specific resorption mechanisms for plasma macromolecules leads to an increase in concentration until the fluid can penetrate. This is often at the rim of the retinal detachment or possibly inside the retina, which may be open to the penetration of extracellular fluid from the subretinal compartment. This is suggested by the frequent presence of cystoid macular edema in eyes with subfoveal new vessels. Alternatively, the cystoid macular edema may be a consequence of impaired drainage of intraretinal fluid that would normally be eliminated by way of the retinal pigment epithelium.

Intraretinal lipid deposition also occurs in other exudative retinopathies, such as diabetic retinopathy and branch retinal vein occlusion. In the present study of subfoveal new vessels, the lipid precipitate appears, when examined visually by stereoscopic biomicroscopy or fundus photography, to be deposited exclusively or predominantly in the subretinal space. In addition, the lipid is confined to the area where a serous detachment of the neurosensory retina existed before treatment and the pattern of lipid deposition does not conform with the anatomy of any of the intraretinal extracellular compartments.

The same fundamental mechanisms are likely to be responsible for precipitation of lipoprotein both in the subretinal compartment and within the neurosensory retina, but differences in the dynamics of precipitation and resorption and in spatial distribution are likely to exist because of the variation in anatomic relation between sources of leakage and sites of resorption and in the configuration of the retinal extracellular compartment.

The serous detachment of the retina is maintained by a dynamic balance between leakage and resorption. When leakage exceeds the local resorption capacity the subretinal compartment expands circumferentially away from the CNV, resulting in more subretinal fluid being exposed to direct resorption by the pigment epithelium and in the subsequent establishment of a new equilibrium. Cessation of leakage is followed by early resorption of water and salts with a secondary increase in precipitation, especially if long-standing leakage under a highly elevated detachment has allowed the accumulation of large amounts of protein-saturated fluid. This is followed by a prolonged phase of resorption of precipitate by phagocytosis. Potential sources of variation in the dynamics of precipitation and resorption of subretinal lipid include the surface area of the source of leakage and its effective pore size, the surface area of the site(s) of resorption, the active fluid and salt resorption capacity of the retinal pigment epithelium, the phagocytic activity of the retinal pigment epithelium and infiltrating macrophages, and the degree of infiltration of phagocytic cells in the subretinal space.

Acknowledgments

The authors thank Jannik Godt for technical assistance.

References