Oxidized Low-Density Lipoprotein and the Incidence of Proliferative Diabetic Retinopathy and Clinically Significant Macular Edema Determined From Fundus Photographs

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**IMPORTANCE** Studies have shown oxidized low-density lipoprotein to be associated with the incidence of proliferative retinopathy and other complications of type 1 diabetes mellitus. Because low-risk interventions are available to modify oxidized low-density lipoprotein, it is important to examine the relationships between this factor and the incidence of proliferative retinopathy and of macular edema, 2 important causes of visual impairment in people with type 1 diabetes.

**OBJECTIVE** To determine the association of oxidized low-density lipoprotein with the worsening of diabetic retinopathy and the incidence of proliferative retinopathy and of macular edema.

**DESIGN, SETTING, AND PARTICIPANTS** Of 996 participants with type 1 diabetes in the Wisconsin Epidemiologic Study of Diabetic Retinopathy, 730 were examined up to 4 times (1990-1992, 1994-1996, 2005-2007, and 2012-2014) over 24 years and had assays of oxidized low-density lipoprotein and fundus photographs gradable for diabetic retinopathy and macular edema. Analyses started July 2014 and ended February 2015.

**MAIN OUTCOMES AND MEASURES** Worsening of diabetic retinopathy, incidence of proliferative diabetic retinopathy, and incidence of macular edema as assessed via grading of color stereo film fundus photographs. The levels of oxidized low-density lipoprotein collected from serum samples at the time of each examination were measured in 2013 and 2014 from frozen serum.

**RESULTS** The cohort at baseline had a mean (SD) level of oxidized low-density lipoprotein of 30.0 (8.5) U/L. While adjusting for duration of diabetes, glycated hemoglobin A1c level, and other factors, we found that neither the level of oxidized low-density lipoprotein at the beginning of a period nor the change in it over a certain period was associated with the incidence of proliferative diabetic retinopathy (hazard ratio [HR], 1.11 [95% CI, 0.91-1.35], \( P = .30 \); odds ratio [OR], 1.77 [95% CI, 0.99-3.17], \( P = .06 \)), the incidence of macular edema (HR, 1.04 [95% CI, 0.83-1.29], \( P = .74 \); OR, 1.08 [95% CI, 0.44-2.61], \( P = .87 \)), or the worsening of diabetic retinopathy (HR, 0.94 [95% CI, 0.83-1.07], \( P = .34 \); OR, 1.32 [95% CI, 0.83-2.09], \( P = .24 \)).

**CONCLUSIONS AND RELEVANCE** Our findings do not provide evidence for a relationship between increasing levels of serum oxidized low-density lipoprotein and the incidence of macular edema or the worsening of diabetic retinopathy in persons with type 1 diabetes. The potential increase in the HR for incident proliferative retinopathy, with an increase in oxidized low-density lipoprotein level over the preceding period, warrants further investigation of this relationship.
The generation of oxidizing compounds is physiologically normal and is an important component of the body’s inflammation and tissue-repair processes.\textsuperscript{1,2} Oxidizing compounds represent part of the normal mechanism of defense against invading microorganisms and malignant cells, and they form during tissue healing and remodeling. Oxidative stress may occur when oxidizing compounds accumulate owing to the tissue’s inability to metabolize them. Oxidative stress in persons with type 1 diabetes mellitus has been attributed to hyperglycemia, nonenzymatic protein glycation, and the decreased presence of antioxidants, resulting in less removal of reactive oxygen species.\textsuperscript{3} This may lead to the peroxidation of low-density lipoprotein (LDL), which has been hypothesized to cause the death of retinal vessel pericytes, endothelial cells, and retinal cells of Müller, resulting in exacerbation of diabetic retinopathy (DR).\textsuperscript{4}

Few long-term cohort studies have examined the association of serum oxidized LDL (ox-LDL) with the incidence and worsening of DR in persons with diabetes.\textsuperscript{4} In our report, we examine relationships of serum ox-LDL level with the incidence of proliferative DR (PDR), the incidence of diabetic macular edema (DME), and the worsening of DR by 2 or more steps in participants with type 1 diabetes in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR).

Methods

Study Population
Detailed descriptions of the WESDR cohort, participation statistics, and reasons for nonparticipation have appeared elsewhere.\textsuperscript{5-11} In brief, the WESDR identified 1210 persons with type 1 diabetes living and receiving care in an 11-county area of south central Wisconsin in 1979-1980. A group of 996 persons 3 to 79 years of age with 1 to 59 years of type 1 diabetes was first examined in 1980-1982. These persons participated in up to 6 follow-up examinations in 1984-1986 (n = 903), 1990-1992 (n = 816), 1994-1996 (n = 667), 2000-2001 (n = 567), 2005-2007 (n = 520), and 2012-2014 (n = 414).\textsuperscript{5,11} No stereoscopic fundus photographs were taken at the 2000-2001 examination, and data from that examination were not used in the analyses. No individuals with a history of kidney or pancreas transplant or renal dialysis were included in the analyses. The tenets of the Declaration of Helsinki were followed, and institutional review board approval was obtained from the University of Wisconsin in Madison. Written informed consent was obtained from each participant.

Examination
Pertinent parts of the examination included the measurements of blood pressure, height, and weight and the recording of answers to questions regarding the use of tobacco products and lipid-lowering medications.

Laboratory Procedures
Nonfasting blood samples were collected, processed within 1 hour of collection, and frozen at –80°C beginning at the third examination in 1990-1992 and through 4 follow-up examinations between 1990-1992 and 2012-2014. During the 1990-1992, 1994-1996, 2005-2007, and 2012-2014 examinations, serum samples obtained from 638, 501, 425, and 289 participants, respectively, were frozen. At the time of the 2012-2014 examination, all of these frozen serum samples were sent to the University of Minnesota Advanced Research and Diagnostics Laboratory in Minneapolis for analysis. These serum samples were measured for ox-LDL levels in 2 batches, one in 2013 and the other in 2014. The ox-LDL level was measured by use of an enzyme-linked immunosorbent assay (Merckodia, Uppsala, Sweden). The intensity of the color was measured on a SpectraMax spectrophotometer (Molecular Device). The interassay coefficient of variation reported in the kit insert is from 4.0% to 6.2%. A set of quality-control samples accompanied each batch to ensure comparability of the ox-LDL levels between the batches. The methods used to measure the levels of other lipids, glycated hemoglobin A\textsubscript{1c}, serum creatinine, microalbuminuria, and gross proteinuria can be found in Klein et al.\textsuperscript{12}

Assessment of Presence and Severity of DR
After the participants’ pupils were dilated, 30° stereoscopic color film photographs of the Early Treatment Diabetic Retinopathy Study 7 standard fields of the retina were obtained for each eye at each examination, except the 2000-2001 examination. The Airlie House classification system was used for grading retinopathy, and a modified concatenated 15-step Early Treatment Diabetic Retinopathy Study severity scale was used to define the presence and severity of DR in which the eye with more severe retinopathy was given more weight.\textsuperscript{13,14} An increase in DR severity by 2 or more steps for a person defined worsening. Incidence of PDR was defined as developing severity level 60 or greater in 1 or both eyes in a person who did not have PDR in either eye at the beginning of a study interval. Diabetic macular edema was defined as the presence of retinal thickening involving the macula.

Definitions
Age was defined as the age at the time of each examination. Age at diagnosis of diabetes was defined as the age at the time...
the diagnosis was first recorded by a physician in the participant’s medical record or in a hospital record. The duration of diabetes was defined as the period between the age at diagnosis and the age at the specific examination. Systolic and diastolic blood pressures were defined as the average of the 2 measurements taken according to the Hypertension Detection and Follow-up Program protocol.15

A participant was classified as a never smoker if he or she had smoked fewer than 100 cigarettes in his or her lifetime, an ex-smoker if he or she smoked 100 or more cigarettes in his or her lifetime but had stopped smoking before the examination, and a current smoker if he or she had not stopped smoking. Body mass index (BMI) was defined as the participant’s weight in kilograms divided by the height in meters squared. Proteinuria was defined as a urine protein concentration of 30 mg/dL or more as measured by Labstix (Ames).

Statistical Methods
While adjusting for duration of diabetes, HbA1c level, statin use, sex, proteinuria, smoking status, BMI, systolic blood pressure, WESDR visit, and the DR severity level at the time the serum sample of ox-LDL was obtained, we examined the association of the ox-LDL levels at the beginning of 3 periods (1990-1992, 1994-1996, and 2005-2007) with the incidence of PDR, the incidence of DME, and 2 or more steps of worsening of DR at the end of each period (1994-1996, 2005-2007, and 2012-2014). Data from the 3 periods were analyzed together using models that accounted for the varying lengths of time between examinations (4 years between the 1990-1992 and 1994-1996 examinations, 11 years between the 1994-1996 and 2005-2007 examinations, and 7 years between the 2005-2007 and 2012-2014 examinations). Duration of diabetes was used as the time scale, and the baseline hazard ratio (HR) was assumed to be piecewise constant with duration of diabetes categorized as less than 20 years, 20 to 29 years, and more than 30 years.

The association of an increase in ox-LDL level during the 4-year period between the 1990-1992 and 1994-1996 examinations with the subsequent 11-year incidence of PDR and of DME and the worsening of DR between the 1994-1996 and 2005-2007 examinations was modeled using logistic regression in SAS version 9.1 (SAS Institute). Models were adjusted for sex and duration of diabetes, as well as changes in HbA1c level, statin use, proteinuria, smoking status, BMI, systolic blood pressure, and DR severity from the beginning to the end of the 4-year period.

Deming regression16 was used to evaluate the comparability of the ox-LDL levels obtained from the quality-control samples between the first and second batches sent to the laboratory. We found that the ox-LDL levels in the quality-control samples in the first batch were not comparable to those in the second batch (P < .05). Based on these results, we transformed the levels for all the samples in the second batch to be comparable to those in the first batch. The distribution of baseline ox-LDL levels, after the transformation of the samples obtained from participants in the second batch, is presented in eFigure 1 in the Supplement.

Results
Participant Characteristics
There were 813 persons (1886 person-intervals) who participated in the WESDR examinations in 1990-1992, 1994-1996, and/or 2005-2007. Of the 1886 person-intervals, 322 were excluded because frozen serum samples were unavailable to measure the ox-LDL level; 709 were excluded because the participant was not at risk for any of the outcomes of interest or because there was missing data on DR severity, DME, or PDR status; and 16 were excluded because of missing data on covariates. After these person-intervals were excluded, 428 participants remained eligible, and they contributed 832 person-intervals to the analyses (728 person-intervals for worsening of DR and incidence of PDR and 720 person-intervals for incidence of DME). At the baseline WESDR examination, the participants included in the analyses were more likely to be younger and, after adjustment for age and sex, were more likely to have a shorter duration of diabetes, a lower HbA1c level, a lower ox-LDL level, a lower serum total cholesterol level, and lower systolic blood pressure, and were less likely to have proteinuria, prevalent PDR, or DME compared with those who were excluded (Table 1).

Distribution of Oxidized LDL Levels and Associated Risk Factors
The mean (SD) ox-LDL level was 30.0 (8.5) U/L. We found significant inverse trends of age, duration of diabetes, and history of using statins. We found positive associations of HbA1c level, systolic blood pressure, BMI, presence of proteinuria, and presence of PDR with increasing quartile ranges of ox-LDL level (Table 2). The results were similar when the ox-LDL level was treated continuously (data not shown). We did not find any associations of sex, systolic blood pressure, and history of current smoking with ox-LDL levels.

Incidence of PDR and DME and Worsening of DR
Among the 428 participants studied, DR worsened in 227 of 356 participants at risk (63.8%) by 2 or more steps, 90 of 356 participants at risk (25.3%) developed PDR, and 64 of 382 participants at risk (16.8%) developed DME over the 22-year period. While adjusting only for duration of diabetes, ox-LDL level was associated with a 53% increase in the hazard of incident PDR (HR per 10 U/L = 1.53 [95% CI, 1.30-1.79]; P < .001) (Table 3). Oxidized LDL level was also associated with the incidence of DME (HR per 10 U/L = 1.27 [95% CI, 1.05-1.54]; P = .01) and worsening of DR (HR per 10 U/L = 1.13 [95% CI, 1.02-1.26]; P = .02). Oxidized LDL level remained significantly related to the incidence of PDR but not the incidence of DME or the worsening of DR after inclusion of HbA1c level in the model (Table 3). After further adjustment for sex, statin use, examination phase, proteinuria, smoking status, BMI, and baseline DR severity, ox-LDL level was no longer associated with the incidence of PDR (HR, 1.11 [95% CI, 0.91-1.35]; P = .30), the incidence of DME (HR, 1.04 [95% CI, 0.83-1.29]; P = .74), or the worsening of DR (HR, 0.94 [95% CI, 0.83-1.07]; P = .34). Adjusting for other factors, we did not find significant relation-
ships between ox-LDL levels modeled as quadratic terms or in quartiles and the incidence of PDR or DME or the worsening of DR (data not shown).

Change in ox-LDL Level and Incidence of PDR and DME and Worsening of DR
There were 318 persons who participated in examinations in 1990-1992, 1994-1996, and 2005-2007. Of these 318 participants, 78 were excluded because they did not have serum samples for analysis or were missing laboratory data on ox-LDL levels at either the 1990-1992 or 1994-1996 examination, 74 were excluded because their retinal photographs taken at the 1994-1996 and 2005-2007 examinations were not gradable or because they were not at risk for any of the outcomes of interest, and 15 were excluded because they were missing data on covariates. After these 167 participants were excluded, 151 participants remained eligible, with 144 contributing to analyses of the 11-year worsening and incidence of PDR.

Table 1. Characteristics of Participants at the Baseline Examination Included and Excluded From Analyses of Incidence, Wisconsin Epidemiologic Study of Diabetic Retinopathy, 1990-1992

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Participants</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Included</td>
<td>Excluded</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>35.7 (11.1)</td>
<td>38.5 (12.1)</td>
</tr>
<tr>
<td>Duration of diabetes, mean (SD), y</td>
<td>21.1 (8.3)</td>
<td>25.0 (10.0)</td>
</tr>
<tr>
<td>HbA1c, mean (SD), %</td>
<td>9.1 (1.5)</td>
<td>9.5 (1.8)</td>
</tr>
<tr>
<td>Oxidized LDL, mean (SD), U/L</td>
<td>32.1 (10.3)</td>
<td>35.9 (12.1)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>25.8 (3.8)</td>
<td>25.8 (4.4)</td>
</tr>
<tr>
<td>Serum total cholesterol, mean (SD), mg/dL</td>
<td>190.1 (42.4)</td>
<td>205.5 (47.0)</td>
</tr>
<tr>
<td>SBP, mean (SD), mm Hg</td>
<td>123.5 (16.3)</td>
<td>129.1 (21.3)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>50.6</td>
<td>48.0</td>
</tr>
<tr>
<td>Currently smoking, %</td>
<td>40.1</td>
<td>44.0</td>
</tr>
<tr>
<td>Proteinuria present, %</td>
<td>20.3</td>
<td>44.0</td>
</tr>
<tr>
<td>Currently using statins, %</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Prevalent PDR, %</td>
<td>17.0</td>
<td>64.5</td>
</tr>
<tr>
<td>Prevalent macular edema, %</td>
<td>6.3</td>
<td>43.3</td>
</tr>
</tbody>
</table>

Table 2. Unadjusted Associations of Covariates With Oxidized Low-Density Lipoprotein Levels by Quartiles

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Quartile 1 (9.3-24.5 U/L)</th>
<th>Quartile 2 (24.6-28.2 U/L)</th>
<th>P Valuea</th>
<th>Quartile 3 (28.3-34.2 U/L)</th>
<th>P Valuea</th>
<th>Quartile 4 (34.3-90.4 U/L)</th>
<th>P Valuea</th>
<th>Overall P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>40.3 (12.1)</td>
<td>40.4 (11.5)</td>
<td>.15</td>
<td>37.4 (10.9)</td>
<td>.10</td>
<td>39.9 (11.3)</td>
<td>.002</td>
<td>.006</td>
</tr>
<tr>
<td>Duration of diabetes, mean (SD), y</td>
<td>26.2 (10.0)</td>
<td>24.8 (9.3)</td>
<td>.22</td>
<td>24.0 (9.0)</td>
<td>.11</td>
<td>24.4 (8.1)</td>
<td>.002</td>
<td>.006</td>
</tr>
<tr>
<td>HbA1c, mean (SD), %</td>
<td>8.2 (1.4)</td>
<td>8.4 (1.5)</td>
<td>.35</td>
<td>8.6 (1.5)</td>
<td>&lt;.001</td>
<td>9.2 (1.6)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SBP, mean (SD), mm Hg</td>
<td>123.3 (17.6)</td>
<td>122.9 (15.0)</td>
<td>.64</td>
<td>123.3 (15.7)</td>
<td>.66</td>
<td>128.1 (17.9)</td>
<td>.02</td>
<td>.05</td>
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<tr>
<td>BMI, mean (SD)</td>
<td>25.8 (4.1)</td>
<td>26.7 (4.0)</td>
<td>.20</td>
<td>26.4 (4.2)</td>
<td>.23</td>
<td>27.5 (4.7)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>44.6</td>
<td>52.4</td>
<td>.15</td>
<td>52.7</td>
<td>.21</td>
<td>52.4</td>
<td>.11</td>
<td>.12</td>
</tr>
<tr>
<td>Proteinuria present, %</td>
<td>8.5</td>
<td>11.5</td>
<td>.64</td>
<td>13.0</td>
<td>.29</td>
<td>30.6</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Currently using statins, %</td>
<td>14.2</td>
<td>10.1</td>
<td>.33</td>
<td>6.8</td>
<td>.05</td>
<td>7.8</td>
<td>.41</td>
<td>.17</td>
</tr>
<tr>
<td>Prevalent PDR, %</td>
<td>36.0</td>
<td>41.4</td>
<td>.11</td>
<td>39.1</td>
<td>.57</td>
<td>41.8</td>
<td>.50</td>
<td>.61</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HbA1c, glycated hemoglobin A1c; LDL, low-density lipoprotein; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure.

*Conversion factors: To convert HbA1c to proportion of total hemoglobin, multiply by 0.01; and to convert total cholesterol to millimoles per liter, multiply by 0.0259.

* Adjusted for age and sex except where noted.

* Adjusted for sex.

* Adjusted for age.

Change in ox-LDL Level and Incidence of PDR and DME and Worsening of DR
There were 318 persons who participated in examinations in 1990-1992, 1994-1996, and 2005-2007. Of these 318 participants, 78 were excluded because they did not have serum samples for analysis or were missing laboratory data on ox-LDL levels at either the 1990-1992 or 1994-1996 examination, 74 were excluded because their retinal photographs taken at the 1994-1996 and 2005-2007 examinations were not gradable or because they were not at risk for any of the outcomes of interest, and 15 were excluded because they were missing data on covariates. After these 167 participants were excluded, 151 participants remained eligible, with 144 contributing to analyses of the 11-year worsening and incidence of PDR.
and 123 contributing to the analyses of the 11-year incidence of DME. At the baseline examination, the participants included in the analyses were more likely to have a shorter duration of diabetes, a lower \( \text{HbA}_{1c} \), a lower serum total cholesterol level, a lower ox-LDL level, lower systolic blood pressure, and less prevalent proteinuria, PDR, or DME compared with those excluded after adjusting for age and sex (Table 4).

The distribution of change in ox-LDL level between the baseline and 4-year follow-up examinations is shown in eFigure 2 in the Supplement. Change in ox-LDL level was slightly skewed to the right with a mean (SD) of 1 (7.0) U/L.

Among the 151 participants remaining in our study, DR worsened in 49 of 144 participants at risk (34.0%) by 2 or more steps, 21 of 144 participants at risk (14.6%) developed PDR, and 4 of 123 participants at risk (3.3%) developed DME over the subsequent 11-year period. Adjusting for age and sex, we found that the change in ox-LDL level was marginally associated with the incidence of PDR (odds ratio [OR], 1.63 [95% CI, 0.96-2.76]; \( P = .07 \)) but not with the incidence of DME (OR, 1.20 [95% CI, 0.47-3.05]; \( P = .70 \)) or the worsening of DR (OR, 1.40 [95% CI, 0.91-2.14]; \( P = .12 \)). After further adjustment for change in \( \text{HbA}_{1c} \) level, history of statin use, proteinuria, smoking status, BMI, and DR level over the same period, there remained a marginal association of a 77% higher subsequent 11-year incidence of PDR with a 10-U/L increase in the ox-LDL level between the 1990-1992 and 1994-1996 examinations (OR, 1.77 [95% CI, 0.99-3.17]; \( P = .06 \)) (Table 5). Change in ox-LDL level was not associated with the worsening of DR (OR, 1.32 [95% CI, 0.83-2.09]; \( P = .24 \)) or the incidence of DME (OR, 1.08 [95% CI, 0.44-2.61]; \( P = .87 \)).

### Discussion

In the WESDR, while adjusting for duration of diabetes and other covariates, we found no significant relationships between serum ox-LDL level and the incidence of PDR, incidence of DME, or worsening of DR. To our knowledge, there are no cohort studies that have measured the relationships between change in serum ox-LDL level and the subsequent incidence of PDR and DME and the worsening of DR to which WESDR findings can be compared. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study used a different measure of ox-LDL that is found in immune complexes, which is a result of the formation of autoantibodies due to the activation of the T cells by ox-LDL. In this study, while adjusting for \( \text{HbA}_{1c} \) level,
Table 5. Associations of Change in ox-LDL Level Between Baseline and 4-Year Follow-up Examination With 11-Year Incidence of PDR and DME and Worsening of DR

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Model 1a OR (95% CI)</th>
<th>P Value</th>
<th>Model 2b OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDR</td>
<td>1.63 (0.96-2.76)</td>
<td>.07</td>
<td>1.77 (0.99-3.17)</td>
<td>.06</td>
</tr>
<tr>
<td>DME</td>
<td>1.20 (0.47-3.05)</td>
<td>.70</td>
<td>1.08 (0.44-2.61)</td>
<td>.87</td>
</tr>
<tr>
<td>Worsening of DR</td>
<td>1.40 (0.91-2.14)</td>
<td>.12</td>
<td>1.32 (0.83-2.09)</td>
<td>.24</td>
</tr>
</tbody>
</table>

Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; OR, odds ratio; ox-LDL, oxidized low-density lipoprotein; PDR, proliferative diabetic retinopathy.

a Adjusted for duration of diabetes.

b Adjusted for duration of diabetes, sex, and change in glycated hemoglobin A1c.

d Level, body mass index, systolic blood pressure, smoking habits, presence of proteinuria, statin use, and DR severity level (categorized as none, mild/moderate, and severe nonproliferative DR).

"Per 10-U/L change in ox-LDL level."
Conclusions
In summary, we did not find evidence to support our hypothesis that serum levels of ox-LDL are associated with the incidence of DME and the worsening of DR independent of other risk factors. The increase in the HR for incident PDR, with an increase in the ox-LDL level over the preceding period, warrants further investigation. The limited power in our analysis and the selection biases (with regard to those at higher risk of worsening disease being less likely to be included in the analyses) must be considered.

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Author Contributions: Ms Myers had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: R. Klein.

Critical revision of the manuscript for important intellectual content: All authors.

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REFERENCES


