Diabetes mellitus and rhegmatogenous retinal detachment
Fokkens, Bernardina Teunisje

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Pilot study: Skin autofluorescence is increased in patients with a severe retinal detachment

B.T. Fokkens, D.J. Mulder, C.G. Schalkwijk, R.A. Bank, M. van Deemter, A.J. Smit, L.I. Los
Abstract

Purpose
Proliferative vitreoretinopathy (PVR) is the main cause of a poor surgical outcome in patients with a rhegmatogenous retinal detachment (RRD). In this pilot study, we assessed the concentration of advanced glycation endproducts (AGEs) in various tissues, in addition to clinical characteristics, to identify RRD patients at high risk of PVR.

Methods
In 33 RRD patients (19 male, aged 19 – 89 years), vitreous biopsy was performed and severity of retinal detachment, i.e. intraoperative PVR grade and surface area of retinal detachment, was established. Vitreous pentosidine values and later obtained plasma AGE values were analyzed using high or ultra performance liquid chromatography. Skin autofluorescence (SAF), as a marker of tissue AGE accumulation, was measured at the forearm using an AGE reader.

Results
Patients with severe retinal detachments were older and had higher vitreous pentosidine, SAF and HbA1c values. Vitreous pentosidine and SAF remained significantly associated with the severity of detachment after correction for age.

Conclusion
Our results show that both SAF and vitreous pentosidine, but not plasma AGE levels, were elevated in patients with a severe retinal detachment. These data suggest that SAF holds promise to identify RRD patients with higher risk of developing PVR and increased surgical failure.
Introduction
Rhegmatogenous retinal detachment (RRD) is a sight-threatening disease with an annual incidence of 18.2 per 100,000 in the Netherlands. Surgical treatment results in primary reattachment of the retina in 64% to 94% of cases. Persistent detachments and redetachments are associated with poor functional results and are mainly due to traction to the retina by proliferative vitreoretinopathy (PVR). The prevalence of PVR is reported to range from 5% to 10% in older studies and as 16.4% or even higher in recent reports.

Attempts to improve the outcome of surgical treatment in RRD patients have been made by the intravitreal use of drugs in order to prevent PVR. However, due to mixed results and/or retinal toxicity, none of these has led to a safe or sufficiently effective drug for routine use. Therefore, several studies have emphasized the need to identify high-risk patients who may gain benefit from a specific treatment. At present, because of the complexity of risk factors associated with the development of PVR, it is difficult to select patients at high risk.

One factor that may play a role in PVR is increased tissue glycation, caused by the accumulation of advanced glycation endproducts (AGEs), which are formed by glycation and oxidation of free amino groups of proteins, lipids and nucleic acids. These AGEs promote tissue dysfunction in vitro and in vivo through cross-linking of long-lived molecules and through binding to the receptor for advanced glycation endproducts (RAGE). AGEs play a role in several age-related conditions, such as atherosclerosis and diabetes mellitus, and AGEs have been found at sites of known ocular pathology.

Considering the potential role of AGEs in PVR, AGEs might represent a biomarker for increased risk of PVR. AGE content of the vitreous body is not quite suitable for large-scale studies, since this can only be assessed invasively. Plasma AGE assays may be used, but because of the high turnover rate of plasma proteins, these may not be representative for the accumulation of AGEs in tissues with a slow turnover rate, such as the vitreous body. Moreover, both methods would cause a delay in treatment, since vitreous and plasma AGE values are not directly available. A possible solution would be the use of skin autofluorescence (SAF), which is a non-invasive technique to assess dermal tissue deposition of AGEs using its fluorescent properties.

In this pilot study, we explored the concentration of AGEs in various tissues in relation to the severity of retinal detachment, i.e. intraoperative PVR grade and surface area of retinal detachment, which are commonly seen as predictors of the development of postoperative PVR and surgical failure. We evaluated whether SAF, vitreous pentosidine, and plasma AGE levels differ between RRD patients with a regular and a severe retinal detachment in order to identify patients at high risk of PVR.
Methods
Patients were selected from a previous study, performed at the Department of Ophthalmology of the University Medical Center Groningen, in which vitreous samples were obtained from 120 adult (≥18 years of age) patients undergoing trans pars plana vitrectomy surgery because of RRD. Exclusion criteria for the present study were based on the presence of the following conditions, which may affect AGE levels: diabetes mellitus, vascular diseases, renal dysfunction and pseudophakia. The study was approved by the Medical Ethics Committee of the University Medical Center Groningen and adhered to the Declaration of Helsinki. All patients gave written informed consent.

Questionnaire
Patients were asked about their general characteristics, including age, gender, height, weight, medical history, as well as current and previous smoking habits. Patients were classified as smoking if they were current smokers or if they had stopped smoking in the last 15 years. Patients were classified as non-smoking if they never had smoked or if they had stopped smoking more than 15 years before the start of the study.

Vitreous body AGE levels
Vitreous body pentosidine levels were analyzed in the previous study using high performance liquid chromatography (HPLC). The concentration of pentosidine is expressed as moles per mol of collagen assuming 300 hydroxyproline residues per collagen molecule.

Skin autofluorescence
Skin autofluorescence (SAF) was measured on the left forearm using the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands), a non-invasive desk-top device using the characteristic fluorescent properties of certain AGEs to estimate the level of AGE accumulation in the skin. Technical details concerning the optical technique have been extensively described elsewhere. The AGE reader has been validated against AGEs measured in dermal skin biopsy, and the device considers measurements not reliable when the UV light reflectance level is ≤ 12%, occurring in persons with dark skin colour. In the current study, the mean value of three SAF measurements (expressed in Arbitrary Units (AU)) was used for analysis. Values obtained in our study were compared to standard values obtained in healthy controls.

Since previously obtained values of vitreous pentosidine were used for analysis, 3.0 to 4.2 years had elapsed between vitreous sampling and skin measurement with the AGE-reader. Because SAF values increase linearly over time according to a formula established by Koetsier et al., we corrected the measured SAF values to create an estimated corrected value for the time of vitreous sampling by using the formula: estimated SAF = SAF – 0.024 * elapsed time (in years).
Plasma levels
Pentosidine residues in plasma protein were measured using HPLC with a fluorescence detector, as described in detail elsewhere. Intra- and inter-assay coefficients of variation for pentosidine were 6.5% and 3.1%, respectively. Plasma levels of protein-bound N\(^\epsilon\)-(carboxymethyl) lysine (CML) and N\(^\epsilon\)-(carboxyethyl) lysine (CEL) were measured using ultra performance liquid chromatography - tandem mass spectrometry. Intra- and inter-assay coefficients of variation were 4.8% and 7.0% for CML, 5.0% and 9.7% for CEL. Plasma glucose, HbA1c, and serum creatinine were measured using standard procedures to ensure that no diabetes mellitus or renal dysfunction was present.

Severity of detachment
Retrospectively, by using preoperative and intraoperative patient data from patient files and surgical reports, the severity of retinal detachment was scored by: 1. surface area of detached retina, more or less than 50%, in relation to total retinal surface; 2. degrees A to C of PVR according to the Retina Society PVR classification. When PVR grade A was present, patients were classified as ‘non-PVR’; when PVR grades B or C were present, patients were classified as ‘PVR’.

Statistics
Normally distributed variables were analyzed using Pearson correlation. When needed (Shapiro-Wilk test: p <0.05), data were normalized using log-transformation. Other variables were analyzed using Spearman correlation. Correction for age was performed on statistically significant (p < 0.05) correlations by using binary logistic regression analysis. The Mann-Whitney test was used to determine any deviation of SAF from the healthy population. Statistical analyses were performed using SPSS version 20.

Results
Based on the following conditions, 87/120 patients were excluded from participation in this study: 1. previously known or newly discovered diabetes mellitus (n = 11); 2. previously known vascular diseases (n = 4); 3. newly discovered renal dysfunction (n = 1); 4. preoperatively present pseudophakia (n = 45); 5. inability to reach the patient or unwillingness to participate (n = 26). Therefore, 33/120 patients remained for analysis.
Patient characteristics are summarized in Table 1. Missing data: 1. one patient did not consent to donate blood; 2. vitreous pentosidine was not measurable in two samples; 3. smoking habits were unknown in three patients. Figure 1 shows the regression line of SAF on age, established in the healthy population by Koetsier et al.\textsuperscript{12} In the current study, SAF values did not differ significantly from this regression line (mean: 1.97 vs. 2.05; p=0.14).

### Severity of retinal detachment

Intraoperatively, fourteen patients had a severe retinal detachment: 7/14 patients had both a surface area of retinal detachment ≥50% and PVR; 4/14 patients had only PVR; 3/14 patients had only a surface area of retinal detachment ≥50%.

Table 1 compares general characteristics and measurements in patients with a regular retinal detachment and a severe retinal detachment. Patients with a severe retinal detachment were significantly older and they had significantly higher vitreous pentosidine values, higher SAF values, and higher HbA1c values. Plasma AGEs did not correlate with the severity of detachment. Vitreous pentosidine and SAF values remained significantly associated with the severity of detachment after correction for age, while HbA1c was not. Figure 1 shows SAF values in relation to age for patients with a severe and a regular retinal detachment.

### Table 1. Summary of patient characteristics, measurements and univariate comparison.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients</th>
<th>RegularRD</th>
<th>Severe RD</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51 ± 14.8</td>
<td>45 ± 13.0</td>
<td>60 ± 13.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.77 ± 0.090</td>
<td>1.75 ± 0.079</td>
<td>1.80 ± 0.010</td>
<td>0.173</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77 ± 12.0</td>
<td>76 ± 11.0</td>
<td>77 ± 13.7</td>
<td>0.757</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>19 / 14</td>
<td>9 / 10</td>
<td>10 / 4</td>
<td>0.177</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>7 / 23</td>
<td>4 / 13</td>
<td>3 / 10</td>
<td>0.978</td>
</tr>
<tr>
<td>Medication use, yes/no</td>
<td>10 / 23</td>
<td>4 / 15</td>
<td>6 / 8</td>
<td>0.189</td>
</tr>
<tr>
<td>ARB/ACEi, yes/no</td>
<td>5 / 28</td>
<td>2 / 17</td>
<td>3 / 11</td>
<td>0.404</td>
</tr>
<tr>
<td>Hypertension, yes/no</td>
<td>6 / 27</td>
<td>3 / 16</td>
<td>3 / 11</td>
<td>0.689</td>
</tr>
<tr>
<td>Vitreous pentosidine, mmol/mol collagen</td>
<td>4.3 (0.3 – 68.0)</td>
<td>3.7 (0.3 – 6.7)</td>
<td>5.0 (1.8 – 68.0)</td>
<td>0.015</td>
</tr>
<tr>
<td>Skin autofluorescence, arbitrary units</td>
<td>1.89 ± 0.474</td>
<td>1.65 ± 0.335</td>
<td>2.22 ± 0.443</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma pentosidine, nmol/mmol lysine</td>
<td>0.47 (0.23 – 2.09)</td>
<td>0.42 (0.27 – 0.89)</td>
<td>0.53 (0.23 – 2.09)</td>
<td>0.215</td>
</tr>
<tr>
<td>Plasma CML, nmol/mmol lysine</td>
<td>75.3 ± 15.19</td>
<td>71.1 ± 12.33</td>
<td>80.7 ± 17.18</td>
<td>0.075</td>
</tr>
<tr>
<td>Plasma CEL, nmol/mmol lysine</td>
<td>42.5 ± 8.94</td>
<td>44.0 ± 9.51</td>
<td>40.7 ± 8.11</td>
<td>0.308</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>37 ± 3.4</td>
<td>36 ± 3.3</td>
<td>38 ± 3.1</td>
<td>0.041</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.4 ± 0.89</td>
<td>5.4 ± 0.89</td>
<td>5.3 ± 0.93</td>
<td>0.776</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>75 ± 14.2</td>
<td>73 ± 14.2</td>
<td>77 ± 14.2</td>
<td>0.370</td>
</tr>
</tbody>
</table>

Data are displayed in mean ± standard deviation, median (range) or number. Vitreous pentosidine values of two patients were not measurable, blood values of one patient were missing and smoking habits of three patients were unknown. ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; CEL, carboxyethyllysine; CML, carboxymethyllysine; RD, retinal detachment. *Probability value for Pearson or Spearman correlation coefficient.
Figure 1. The linear regression line (−−) for skin autofluorescence (SAF) and age is shown, as well as the reference line (−) for SAF and age (SAF = 0.83 + 0.024 * age), established by Koetsier et al. (Diabetes Technol Ther 2010).

Tissue and plasma AGEs
Vitreous pentosidine concentrations ranged widely from 0.3 to 68.0 mmol/mol collagen, with a median value of 4.3 mmol/mol collagen. SAF values varied between 1.15 and 2.68 AU, with a mean value of 1.89 AU. Plasma pentosidine varied between 0.23 and 2.09 nmol/L (median 0.47), plasma CML between 48.5 and 108.6 nmol/L (mean 75.3) and plasma CEL between 27.5 and 61.5 nmol/L (mean 42.5).

Table 2 shows univariate correlations between AGEs from various tissues and other plasma parameters. Plasma CML was related to plasma pentosidine and plasma CEL. None of the measured plasma AGEs were related to vitreous pentosidine or SAF.
Discussion

In this pilot study, we investigated whether SAF, vitreous pentosidine, and plasma AGE levels, as markers of AGE accumulation in various tissue compartments, differ between RRD patients with a regular and a severe retinal detachment. Our results show that both SAF and vitreous pentosidine, but not plasma AGE levels, were elevated in patients with a severe retinal detachment.

In literature, little is known about the role of AGEs in patients with RRD, but some evidence exists for a potential role of AGEs in PVR. First, vitreous AGEs and RAGE were elevated in patients with PVR in a previous study.\(^{16}\) Furthermore, AGEs can induce the expression of several cytokines that are elevated in PVR, e.g. monocyte chemo attractant protein-1 (MCP-1) and vascular endothelial growth factor (VEGF).\(^{17-19}\)

SAF appears to represent AGE accumulation in several tissues with slow turnover, such as skin and vessel wall. SAF has been shown to be strongly related to several AGEs in skin biopsies of diabetic patients, hemodialysis patients and healthy controls.\(^{10,11}\) SAF has also been found to have a strong relation with the collagenase digestible collagen fraction, as a measure of AGE content, in graft material from patients with coronary heart disease and to have a strong association with arterial pulse wave velocity, an accepted procedure for evaluation of central arterial changes.\(^{20}\)

Furthermore, it has been shown that SAF is related to autofluorescence of the lens, and that SAF is elevated in patients with neovascular age-related macular degeneration and diabetic retinopathy.\(^{55}\) Based on these former studies, and since the half-life of vitreous collagen is assumed to be comparable to that of skin collagen,\(^{21}\) we expected to find a strong correlation between SAF and vitreous pentosidine. However, our results did not show a significant relation between SAF and vitreous pentosidine, even though both were related to the severity of retinal detachment.

### Table 2. Univariate correlation coefficients (Spearman or Pearson) between AGEs from various tissues and other plasma parameters.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitreous pentosidine</th>
<th>Skin autofluorescence</th>
<th>Plasma pentosidine</th>
<th>Plasma CML</th>
<th>Plasma CEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitreous pentosidine</td>
<td>-</td>
<td>0.291</td>
<td>0.124</td>
<td>0.030</td>
<td>0.026</td>
</tr>
<tr>
<td>Skin autofluorescence</td>
<td>0.291</td>
<td>-</td>
<td>0.289</td>
<td>0.112</td>
<td>-0.237</td>
</tr>
<tr>
<td>Plasma pentosidine</td>
<td>0.124</td>
<td>0.289</td>
<td>-</td>
<td>0.720**</td>
<td>0.179</td>
</tr>
<tr>
<td>Plasma CML</td>
<td>0.030</td>
<td>0.112</td>
<td>0.720**</td>
<td>-</td>
<td>0.417*</td>
</tr>
<tr>
<td>Plasma CEL</td>
<td>0.026</td>
<td>-0.237</td>
<td>0.179</td>
<td>0.417*</td>
<td>-</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.320</td>
<td>0.350*</td>
<td>0.040</td>
<td>0.049</td>
<td>0.158</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.155</td>
<td>0.212</td>
<td>0.137</td>
<td>-0.130</td>
<td>-0.049</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.034</td>
<td>0.148</td>
<td>0.359*</td>
<td>0.379*</td>
<td>0.011</td>
</tr>
</tbody>
</table>

\(\text{CEL, carboxyethyllysine; CML, carboxymethyllysine }^* p < 0.05; ** p < 0.001.\)
This discrepancy could be due to the fact that vitreous pentosidine is expressed as pentosidine per triple helix, whereas SAF is related to skin surface area. Alternatively, it is possible that vitreous pentosidine and SAF are indeed not interrelated, and that they are independent predictors of PVR. However, a possible shared mechanism of accumulation of AGEs could be through their induction by inflammation and oxidative stress. These effects do not necessarily occur at the same rate at various body sites. Vitreous pentosidine would then be a reflection of the severity and duration of local inflammation and oxidative stress in the eye, and SAF of the overall systemic inflammatory response and other risk factors that do not necessarily influence AGE accumulation in the eye.

The hypothesis that inflammation has an important role in PVR is recently supported by a finding of Schröder et al.\textsuperscript{22} They have shown that breakdown of the blood-ocular barrier as determined by aqueous flare, is a major risk factor for PVR redetachment. Breakdown of the blood-ocular barrier is responsible for protein leakage and diffusion of inflammatory cells into the eye, which could promote the development of PVR.

The role of plasma AGEs in disease is complex. Plasma AGEs could partially influence AGE accumulation in various tissues, e.g. in the skin and also in the vitreous after breakdown of the blood-ocular barrier. However, it is difficult to establish the actual role of plasma AGEs in disease, because plasma AGEs are quite variable and because AGEs are also formed within cells and tissues.

Previously, mixed results in the relation of plasma AGEs with SAF and disease state have been shown in various diseases, for example: plasma CML and CEL, but not pentosidine, were related to SAF in 88 COPD patients\textsuperscript{23}; plasma pentosidine (CML and CEL were not measured) was weakly associated with SAF in a group of 128 dialysis patients\textsuperscript{24}; neither plasma CML, CEL, nor pentosidine were related to SAF in another group of 43 dialysis patients.\textsuperscript{25} In these studies, an association of plasma AGE with disease state was less frequently seen than an association of SAF with disease state. This finding is consistent with our study, in which SAF was better associated with the severity of retinal detachment than was plasma AGE.

Several limitations to this pilot study need to be acknowledged. The small sample size did not allow correction for all possible confounders, such as glucose levels, kidney function and smoking. To limit the influence of these factors, patients with impaired glucose levels, elevated HbA1c values and increased creatinine values were excluded from the study. Another limitation is the interval between vitrectomy and assessments of SAF and plasma AGEs. To overcome differences in time interval, SAF values were adjusted for the time interval using a correction formula. However, this does not correct for inter-individual differences in AGE accumulation over time, depending on a number of factors, such as inflammation, oxidative and metabolic stress. Furthermore, it was not possible to correct for plasma AGEs regarding time interval. Therefore, no strong conclusions can be made based on our plasma AGE results.
Measuring SAF has also some limitations, because SAF is an indirect marker for AGEs in the skin. Not all AGEs exhibit fluorescent properties and SAF is influenced by other skin fluorophores. Nonetheless, previous studies have shown that SAF is representative of the AGE pool, based on strong correlations with both fluorescent and non-fluorescent AGE values in skin biopsies.\textsuperscript{10,11} In the context of these limitations, future studies should be performed to further explore the role of AGEs in patients with RRD.

To conclude, SAF was shown to be increased in patients with a severe retinal detachment and, therefore, SAF is promising as a non-invasive and practically applicable detector of RRD patients who have higher risk of developing PVR and increased surgical failure.
References


Pilot study: SAF is increased in severe RRD


