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Skin autofluorescence is elevated in neovascular age-related macular degeneration

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ABSTRACT

Background/aims Skin autofluorescence (AF) is a non-invasive marker for advanced glycation endproducts (AGE) in tissues, making use of their characteristic AF pattern. The aim of this study was to investigate whether skin AF is increased in patients with neovascular age-related macular degeneration (AMD) compared with healthy controls.

Methods Skin AF was assessed in 73 consecutive patients with active and documented neovascular AMD without evidence for diabetic or hypertensive retinopathy and in 31 healthy age-matched controls. Exclusion criteria were: known renal disease, current inflammatory or malignant disease, or skin type V or VI. Skin AF was measured on the forearm and was calculated as a ratio of mean intensities detected from the skin between 420–600 and 300–420 nm. Student t test and χ^2 test were used to compare differences between groups.

Results Skin AF was increased in neovascular AMD compared with controls (2.57 ± 0.68 vs 2.23 ± 0.63 arbitrary units $\times 10^{-2}$; $p=0.018$). In patients without vascular risk factors or cardiovascular disease, skin AF was not significantly higher than that of the controls. Skin AF correlated with age in both patients and controls.

Conclusion Skin AF is increased in patients with neovascular AMD, suggesting that AMD is accompanied by enhanced systemic AGE accumulation, which may indicate a role in the pathophysiology of AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is a multifactorial disease, characterised by degeneration of the macular retina and choroid due to atrophy or detachment, and to scarring caused by choroidal neovascularisation.¹ These events are driven by inflammation- and oxidative stress-related pathways: this is supported by detection of modified proteins and lipids in the affected retinal tissue that are derived from oxidative stress.^{2–3} Since these pathophysiological events strikingly resemble those observed in atherosclerotic disease,⁴ it has been postulated that AMD may actually be an expression of atherosclerotic disease in the eye.²

AMD might be a manifestation of a systemic disease such as atherosclerosis, as it is associated with the same risk factors, and patients with AMD are more likely to develop cardiovascular disease (CVD).^{5–6} Furthermore, risk factors for atherosclerosis are associated with progression of intermediate to advanced AMD.⁷ Atherosclerosis is associated with increased accumulation of advanced glycation endproducts (AGEs), which are the final products of non-enzymatic glycation of proteins that form cross-links with long-lived

proteins such as collagen, but may also accumulate as a result of oxidative stress.⁸ In dry AMD, AGEs accumulate and then promote neovascular AMD by inducing inflammation and vascular endothelial growth factor (VEGF) expression, suggesting that AGEs are also involved in the pathophysiology of AMD.⁹

We have developed a technique to estimate the amount of AGE accumulation in the skin by measuring skin autofluorescence (AF), which makes use of the typical fluorescent properties some AGEs encompass when illuminated with ultraviolet (UV) light.¹⁰ The technique has been validated with AGEs measured directly from skin biopsies taken from the same body site.^{11–12} Skin AF is elevated in several diseases associated with atherosclerosis, such as diabetes,¹¹ end-stage kidney disease¹² and coronary artery disease,¹³ and skin AF is a strong predictor of future cardiovascular morbidity and mortality.^{12–14}

The aim of the current study was to investigate whether skin AF is elevated in patients with neovascular AMD compared with healthy age-matched controls.

MATERIALS AND METHODS

This cross-sectional study was performed between January 2005 and July 2005, and included 79 consecutive patients with neovascular AMD visiting the outpatient clinic of the ophthalmology department of our university hospital for fluorescein angiography or photodynamic therapy, and 31 age-matched healthy controls from an historical control group. The inclusion criteria were active neovascular AMD in which the lesion contained subretinal haemorrhage associated with choroidal neovascularisation, as documented on fluorescein angiograms without evidence for diabetic or hypertensive retinopathy. The following exclusion criteria applied for all subjects: known renal disease, current inflammatory or malignant disease, skin photo type V or VI (ie, skin of colour). Because of the high incidence of these conditions at advanced age, known CVD and hypertension were accepted as co-morbidity for this study. Clinical data were obtained by chart review and questionnaires and all measurements were performed prior to therapeutic or investigational interventions, including the intravenous administration of sodium fluorescein.

Assessment of skin AF

Skin AF was assessed on the ventral site of the lower arm with a prototype of the current AGE-Reader (DiagnOptics BV, Groningen, The Netherlands). This method has been extensively described elsewhere.¹⁰ In short, the prototype

of the AGE-Reader consists of a 29×13×9 cm (length×width×height) box, containing an excitation light source (4 W black-light) emitting light with wavelengths of 300–420 nm (peak ~360 nm). Light is transmitted through a 4 cm² window on the upper side of the box, directly illuminating the skin. Only light reflected and emitted from the skin is measured with an integrated spectrometer (Avantes Inc., Eerbeek, The Netherlands) in the range 300–600 nm, using a 50 µm UV-glass fibre (Avantes Inc.). In addition, dark and white reference measurements are performed before every measurement to correct for dark current background light and to calculate reflectance, respectively. All actions are performed automatically. Each AF measurement is composed of the average of 50 (individual) scans, each of approximately 200 ms, depending on skin reflectance. The entire AF measurement takes approximately 30 s to be performed. To correct for differences in light absorption, skin AF is calculated by dividing the mean value of the emitted light intensity per nm between 420 and 600 nm by the mean value of the excitation light intensity per nm between 300 and 420 nm, expressed as arbitrary units.¹⁰ The intra-individual Altman error is 5.0% on a single day and 5.9% for seasonal changes.¹¹

Statistical analysis and power calculation

We determined that with an expected SD of 0.4, a sample size of 62 patients and 31 matched controls would have 80% power to detect an absolute difference between groups of at least 0.25 at $\alpha=0.05$. However, to allow reliable insight in potential confounders (including vascular risk factors (VRF) and CVD) a larger patient group was recruited. The patient group was divided in subgroups consisting of subjects with and without VRF or CVD. Patients and controls were matched for age, which may be an important confounder. Descriptive statistics are presented as mean values±SD, as number of subjects, or as percentages. For comparison between groups, continuous variables were analysed by Student t test. In case of categorical variables the χ^2 test was used. For correlations Pearson correlation coefficient (r) was calculated. A two-sided p value <0.05 was considered statistically significant. All statistical analyses were carried out with the Statistical Package for Social Science (SPSS, version 12.0.2, 24 March 2005; SPSS Inc., Chicago, Illinois, USA).

RESULTS

As shown in table 1, patients and controls were matched for age. All subjects were Caucasian. Patients with neovascular AMD were more likely to be female, whereas controls were more likely to be male. Smokers and subjects with a history of CVD were significantly overrepresented in the patient group, which was characterised by a more frequent use of cardioprotective medication accordingly.

Table 1 Subject characteristics

	Controls	AMD	p Value
Age (years) (mean±SD)	77±5	78±7	0.77
Male/female (n/n)	20/11	29/44	0.031
Smoking (n (%))	1 (3)	37 (51)	<0.001
Hypertension (n (%))	9 (29)	23 (32)	1.00
Smoking and hypertension (n (%))	0	12 (16)	0.016
Cardiovascular disease (n (%))	0	13 (18)	0.009
Medication (n (%))			
Statins	1 (3)	8 (13)	0.26
ASA	1 (3)	14 (22)	0.018
Antihypertensives	8 (26)	23 (37)	0.36

AMD, neovascular age-related macular degeneration; ASA, acetylsalicylic acid. Between group differences were tested with the Student t test or the χ^2 test.

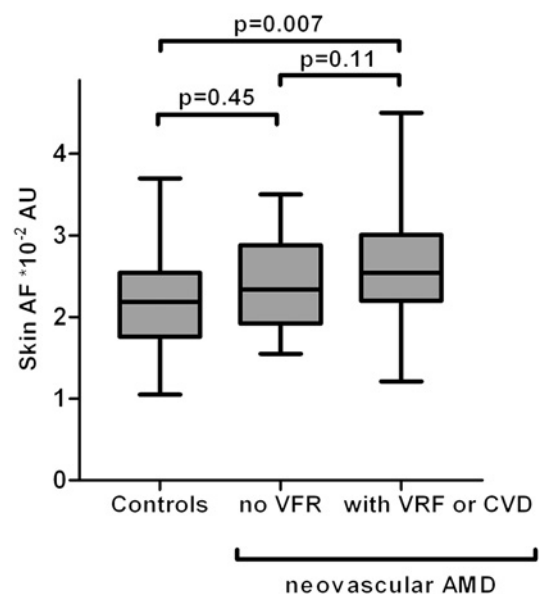


Figure 1 Box plot of skin autofluorescence (AF) expressed as arbitrary units (AU) in patients with neovascular (age-related macular degeneration (AMD)) and in healthy age-matched controls. The AMD group is divided in one consisting of subjects with vascular risk factors (VRF) or cardiovascular disease (CVD) and one consisting of subjects without the conditions ('no VRF'). p Values were calculated using Student t test.

Skin AF was significantly higher in patients than controls (2.57 ± 0.68 vs 2.23 ± 0.63 arbitrary units (AU) $\times 10^{-2}$; $p=0.018$). Figure 1 shows that, when dividing the patient group into subgroups containing subjects without VRF (referred to as 'no VRF' in the figure) and another consisting of those who were current smokers or had a history of hypertension or CVD ('with VRF or CVD'), skin AF was not significantly higher in the first group than in the controls. However, the no-VRF subgroup was small ($n=20$). After exclusion of smokers in the patient and control group ($n=1$), skin AF remained significantly higher in the AMD group (2.57 ± 0.69 ($n=35$) vs 2.18 ± 0.59 ($n=30$) AU $\times 10^{-2}$; $p=0.020$). Excluding subjects with CVD alone preserved the significant difference between patients and controls (2.54 ± 0.69 ($n=59$) vs 2.23 ± 0.63 ($n=31$) AU $\times 10^{-2}$; $p=0.037$).

Figure 2 shows that skin AF increased with age in patients ($r=0.24$; $p=0.044$) as well as in controls ($r=0.56$; $p=0.001$). In the patient group no difference in skin AF was observed between those

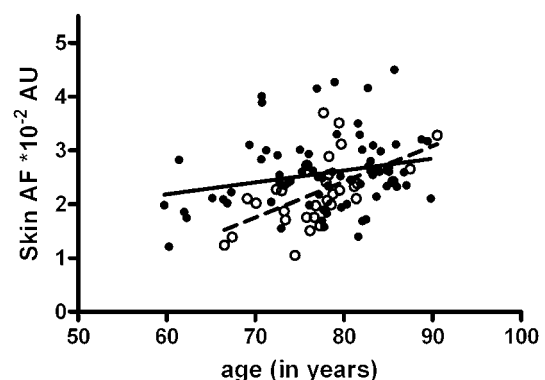


Figure 2 Correlation plot of the association between skin autofluorescence (AF) expressed as arbitrary units (AU) and age in patients with neovascular age-related macular degeneration (AMD) (black circles, continuous line; $r=0.24$; $p=0.044$) and in healthy age-matched controls (white circles, dashed line; $r=0.56$; $p=0.001$).

Table 2 Co-morbidity and vascular risk factors in patients with neovascular AMD

	Absent		Present		p Value
	Mean \pm SD	n	Mean \pm SD	n	
Smoking	2.57 \pm 0.70	35	2.56 \pm 0.68	37	0.96
Hypertension	2.51 \pm 0.67	50	2.70 \pm 0.71	23	0.26
Smoking and hypertension	2.56 \pm 0.71	61	2.61 \pm 0.54	12	0.80
Cardiovascular disease	2.54 \pm 0.69	59	2.69 \pm 0.69	13	0.49

Between group differences were tested with the Student t test.
AMD, neovascular age-related macular degeneration.

who were current smokers, or had a history of hypertension or CVD, compared with those in which these conditions were absent (table 2). Between these groups, no difference in age was observed.

DISCUSSION

The results presented in this study suggest that skin AF—a non-invasive marker for systemic accumulation of AGEs—is elevated in patients with neovascular AMD compared with healthy age-matched controls. In the current study, it cannot be excluded that this elevation is associated with the coexistence of smoking, hypertension and CVD. As skin AF increases with age, it may be that AMD is a progressive chronic disease that is accompanied by enhanced systemic age-related AGE accumulation.

Studies in animal models have shown that oxidative stress plays a pivotal role in the development and progression of AMD.³ In human studies this is supported by the fact that smoking, which is a recognised source of oxidative stress, significantly increases the risk of AMD.¹⁵ Moreover, antioxidant therapy may retard the progression of AMD.¹⁶ In addition, AGEs, which are the endproduct of glycation and oxidative stress, seem to be involved in the development of AMD.⁹ An oxidative stress agent derived exclusively from AGE (carboxy methyl-lysine (CML)) has been found in drusen,¹⁷ and a variety of other AGEs accumulate in drusen and in Bruch's membrane with age and occur at a higher level in patients with AMD.³ The characteristic basal membrane thickening as a consequence of AGE cross-linking found in diabetes resembles the progressive thickening of the Bruch's membrane, as it occurs in older patients.¹⁸ Moreover, AGEs have the capacity to interact with several receptors that have been found to be enhanced in the subretinal membranes of patients with AMD and co-localise with CML.¹⁷ Ligation of the AGE receptor (RAGE) in AMD triggers activation of nuclear factor kappa B and promotes the expression of abnormal levels of vascular endothelial growth factor (VEGF) in retinal pigment epithelium (RPE) cells.¹⁷

In the current study we have shown that the accumulation of AGEs and hence oxidative stress in the eye may be accompanied by accumulation of AGEs in the skin. In previous reports we have shown that skin AF is strongly related to skin accumulation of AGEs, as evidenced by a strong correlation with specific AGEs measured from skin biopsy homogenates.¹¹ Since these measured AGEs included both carbohydrate-derived AGEs (pentosidine) and lipid-derived AGEs (CML and carboxyethyl-lysine), we concluded that skin AF is a non-invasive marker for both glycation and oxidative stress. This was also supported by the observation that skin AF was inversely related to plasma vitamin C levels, a strong antioxidant, in patients with renal failure.¹⁹ In addition, skin AF strongly correlates with serum levels of soluble RAGE and inflammation.¹³

AGEs may also be derived from exogenous sources, such as tobacco smoke²⁰ and industrially processed foods,²¹ and accu-

mulate in tissues. Since smoking is a major risk factor for AMD,²² exogenously derived AGEs might be of importance in the development of AMD. Although in current study 50% of the patients were current smokers, there was no significant difference in skin AF levels between smokers and non-smokers. A possible explanation may be that most patients may have been former smokers and hence have enhanced accumulation of AGEs. Indeed, in previous studies we have shown a clear relation between smoking and skin AF.¹³

Recent development in AMD research suggests common antecedents in AMD and CVD, since they share several risk factors for their development.⁶ Although partly explained by selection bias, it is not surprising that in the current study common atherosclerotic risk factors were more prevalent in the patients with neovascular AMD than in healthy controls. However, after correction for these factors, there was little difference between both groups. This can probably be explained by the small sample size after multiple corrections, since the significant difference was preserved after correction for CVD only. This endorses the hypothesis that both AMD and CVD have a common aetiology, which entails a risk of statistical overcorrection in the regression model. The small sample size may also explain why patients in the AMD group who were smokers or had a history of hypertension, diabetes or CVD did not have higher levels of skin AF. These limitations stress the need for a prospective study to evaluate the role of skin AF and VRF in the progression of AMD.

Intraocular AF measurement, using a fundus spectrophotometer or scanning laser ophthalmoscope has been extensively studied in patients with AMD.²³ However, these methods are largely directed at measuring lipofuscin, using different excitation/emission spectra from the ones that we used in the current study. Although lipofuscin is considered to be a downstream endproduct of oxidative stress and ageing, it is not an AGE. Therefore, data collected in the current study cannot be directly compared with the above-mentioned methods. It would be of great interest to compare skin AF measurement with these eye AF methods in a future study.

From previous investigations we have learnt that skin AF cannot be reliably measured in subjects with skin photo type V or VI¹⁰ with the prototype of the AGE reader in the configuration used in our study. Therefore, for this study we excluded subjects with these skin types. Variations in skin pigmentation were accounted for by the use of a ratio between excitation and emission light intensity. Further development of the reader for improving the measurement in dark skin types is ongoing, and a newer version of the instrument will be capable of reliably measuring skin AF in darker skin types. Since not all AGEs encompass fluorescent properties, skin AF is representative of only part of the total AGE burden. However, in an earlier validation study we found that skin AF also correlated strongly with non-fluorescent AGEs, such as CML.¹¹ Photo-ageing, which is a direct effect of sun exposure to the skin, has been suggested as a factor in skin AF elevation in the AMD group. This cannot be directly assessed or corrected for. In subjects with dark skin types, skin AF seems to increase with age to a similar extent as observed in those with lighter skin types.¹³ Since skin pigmentation protects against photo-ageing by absorbing UV light, the effect of sun exposure is probably negligible in this study.

Although skin AF is strongly elevated in patients with renal failure,¹² it does not seem to be related to renal clearance in subject with (near) normal kidney function.¹³ Although kidney function was not assessed in this study, those with known renal disease were excluded and therefore it can be assumed that the

difference in skin AF between patients and controls is not explained by a difference in kidney function.

In this study, only patients with neovascular AMD were included. However, AGEs have been shown to accumulate in different stages of dry AMD as well, including normal retinal ageing, drusen formation and geographical atrophy. Geographical atrophy may be associated with a large burden of AGE formation. Since this is the first study to investigate skin AF in AMD, we chose to include only those with wet AMD, since these were logistically more easy to include and represent a group of subjects with advanced AMD only. To determine a prognostic/causal relation between skin AF and AMD it would be of interest to prospectively follow patients with dry AMD and to determine whether higher levels of skin AF predict the progression to neovascular AMD.

It has recently been shown that AMD is associated with a polymorphism in the complement factor H gene, suggesting that AMD is actually a genetic disease. However, risk factors such as hypertension and smoking, which are known to be associated with AGE accumulation, are environmental factors needed for the phenotypic expression of the disease.

Since local therapy of AMD with current treatment strategies is difficult, more systemically orientated treatments in early stages of AMD might be useful. Established cardioprotective drugs such as statins and aspirin might reduce the risk of AMD progression. However, this has not been tested in a prospective and/or randomised trial.²⁴ Antioxidant therapies are only clinically effective in specific patients groups.¹⁶ Our data support a search for agents and dietary measures directed to reduce AGE formation as a new therapeutic strategy in preventing or treating AMD. Although several drugs that reduce AGE formation or break AGE cross-links are promising, none of these have been shown to be consistently effective and/or safe in human studies⁸ and there remains a need for innovative drug development.

CONCLUSION

This study demonstrates that with a non-invasive technique designed to measure AGEs in the human skin, increased AGE accumulation could be detected in Caucasian subjects with neovascular AMD. This finding provides evidence that AMD may not only be accompanied by enhanced AGE formation in the eyes, but is also accompanied by increased AGE deposition in other tissues such as the vascular wall. Because confounding of these findings by other cardiovascular risk factors cannot be excluded, these data should be interpreted with caution and should be considered as preliminary, as they need further confirmation in a larger prospective study. Nonetheless, we believe that these data strengthen the hypothesis of atherosclerosis and AMD having a common aetiology.

Competing interests R. Graaff and A. J. Smit are founders of DiagnOptics, The Netherlands, which manufactures the AGE Reader, based on the prototype used in the study reported here. The remaining authors have no competing interests to declare.

Ethics approval This study was conducted with the approval of the Medical Ethics Committee of University Medical Center Groningen, The Netherlands.

Contributors We state that the manuscript has been approved by all the authors and that all requirements for authorship have been met by each author.

Provenance and peer review Not commissioned; externally peer reviewed.

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