Skin Autofluorescence is Elevated in Acute Myocardial Infarction and Predicts the One Year Incidence of Major Adverse Cardiac Events

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Submitted
Abstract

Background: ST-elevation myocardial infarction (STEMI) is associated with increased inflammation and oxidative stress, enhancing the formation of Advanced Glycation Endproducts (AGEs). Some AGEs encompass a characteristic fluorescence pattern, which can be non-invasively measured as skin autoflourescence (AF). In this study we investigate whether skin AF is elevated in STEMI, its association with inflammatory and glycemic stress and its predictive value for clinical outcomes.

Methods: Skin AF was measured in 88 STEMI patients (age: 64±13 years; male: 76%; diabetes: 28%) within 72 hours following symptom onset and >150 days after discharge, in 81 stable coronary artery disease (sCAD) patients (age: 64±10 years; male: 74%; diabetes: 15%), and in 32 healthy controls (age: 63±11 years; male: 72%). The cumulative one year incidence of all cause mortality and hospitalization for myocardial infarction or heart failure was documented.

Results: Skin AF was significantly higher in STEMI compared with sCAD and controls, irrespective of confounders, and was independently associated with HbA1c and C-reactive protein. Skin AF decreased significantly in STEMI patients, when measured >200 days after discharge. In STEMI patients, skin AF above the median was predictive of future events (hazard ratio, 11.6; 95% CI, 1.5-90.8; P=0.019), even after partial correction for known confounders.

Conclusion: Skin AF is elevated in STEMI, is associated with inflammation and glycemic stress, and predicts future major adverse cardiac events in STEMI.
Introduction

Oxidative stress plays a pivotal role in the inflammatory process leading to atherosclerotic plaque formation. In clinical studies, several novel markers of oxidative stress have been found to be associated with acute coronary syndromes (ACS) and also to predict future cardiovascular events, independent of traditional cardiovascular risk factors. However, since the measurement of most of the biomarkers requires sophisticated techniques, clinical applicability is limited.

Oxidative modification of carbohydrates and lipids enhances the formation of Advanced Glycation Endproducts and Advanced Lipoxidation Endproducts, generally referred to as AGEs. Although classically associated with diabetes and renal failure, these compounds are also implicated in the pathophysiology of atherosclerosis. We developed a device to non-invasively assess skin accumulation of AGEs by measuring skin autofluorescence (AF). It has been validated with glycaemic and oxidative stress derived AGEs measured in skin biopsies and independently predicts cardiovascular mortality in patients with renal failure and in patients with type 2 diabetes. Furthermore, we have recently demonstrated that skin AF is elevated in patients with stable coronary artery disease (sCAD) and is associated with neopterin, a serum monocyte activation marker and with serum levels of the soluble isoform of the receptor for AGEs (sRAGE), making it a potentially non-invasive marker for the inflammatory process that characterizes atherosclerosis.

In this study we investigate whether skin AF is elevated in acute ST-elevation myocardial infarction (STEMI) compared with sCAD and healthy controls. Furthermore, we investigate whether skin AF is associated with clinically available markers of inflammation and glycaemic stress in these patients, and whether it predicts the one year incidence of major adverse cardiac events (MACE) in STEMI.

Materials and Methods

Subjects

This observational study was performed between May 2004 and August 2005, including 88 patients with acute STEMI admitted to our Coronary Care Unit and 81 patients with sCAD admitted for elective coronary angiography (CAG). Diagnostic criteria for STEMI were: typical chest pain of >30 minutes duration and significant ST segment elevation of >1 mm (0.1 mV) in more than 2 adjacent leads on electrocardiography. sCAD was defined as typical chest pain, or a history of an ACS, or vascular intervention, combined with the presence of at least one coronary artery with mild stenosis (>30% luminal narrowing) on CAG. Additionally, 31 healthy age and sex matched controls without a history of cardiovascular disease (CVD), with <2 vascular risk factors, and with normal carotid and femoral arteries on ultrasound examination, were recruited by advertisement in a local newspaper. Exclusion criteria were: recent ACS <3 months before admission, known renal disease or serum creatinine >1.7 mg/dL at admission, severe inflammatory or current malignant disease, and skin photo type V/VI (i.e. a skin of colour). The study was approved by the local ethics committee and all patients gave written informed consent.
Follow-up measurement and event scoring

After discharge, STEMI patients were routinely prescribed statins, β-blocking agents, aspirin, and clopidogrel. In a subgroup of 29 STEMI patients a second skin AF measurement was performed >150 days after STEMI. Since most patients were transferred back to a regional hospital <3 days after percutaneous coronary intervention (PCI), it was not possible to perform this measurement in all initial STEMI patients. Additionally, for all STEMI patients the one year incidence of all cause mortality and hospitalization for myocardial infarction or heart failure was documented (major adverse cardiac events (MACE)). Event scoring was performed blinded for patient characteristics and skin AF levels, obtained from questionnaires and verified with patient’s hospital records. Non responders were contacted by telephone or their status was verified with the family doctor. Three patients were lost to follow-up. Myocardial infarction was defined as typical clinical presentation, combined with troponine levels above normal or typical ECG changes, and heart failure was defined as hospitalization for New York Heart Association (NYHA) Class III or IV heart failure.

Skin Autofluorescence

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) as described elsewhere\textsuperscript{12,13}. In short, the AGE-Reader consists of a tabletop box, containing a black light excitation light source (peak wavelength ~360 nm). Light emitted from the skin is measured with an integrated spectrometer. Measurement is fully automated and takes approximately 30 seconds to complete, giving an average value over 50 individual scans. Skin AF is calculated by dividing the mean value of the emitted light intensity per nm between 420-600 nm by the mean value of the excitation light intensity per nm between 300-420 nm, expressed as arbitrary units (AU). The intra-individual Altman error percentage is 5.0\% on a single day and 5.9\% for seasonal changes\textsuperscript{12}.

Laboratory assessments

Laboratory parameters were routinely measured at the local clinical laboratory and collected at admission, prior to CAG/PCI in sCAD and prior to or shortly after PCI in STEMI. However, since no admission blood was collected from approximately 20\% of sCAD patients, the most recent (<3 months before study entry) values were used. C-reactive protein (CRP) was measured on a normal sensitivity machine with detection limit of 4 mg/dl (Vitros 250; Ortho Clinical Diagnostics, Rochester, USA). Creatinine clearance was estimated with the Cockcroft-Gault formula.

Statistical analysis

Based on a pilot study of 37 STEMI patients, we determined that a sample size of 85 STEMI and sCAD patients would have 80\% power to detect a difference of at least 10\% at α=0.05 between STEMI and sCAD patients\textsuperscript{14}. Normal distribution of variables was tested with the Kolmogorov-Smirnov test. For comparison between groups, continuous variables were analyzed by analysis of variance with correction for multiple comparisons (Bonferroni). In case of categorical variables the Fisher’s exact test was used. Descriptive statistics are presented as mean values ± SD, as median (inter quartile range) for skewed variables, or
Skin autofluorescence is elevated in acute myocardial infarction as percentages. To test whether between-group differences in skin AF could be explained by differences in potential confounders, one-way analysis of covariance (ANCOVA) was performed and F statistic and estimation of effect size (eta-squared, $\eta^2$) were calculated. Skewed variables (i.e. glucose, HbA1c, triglycerides, and peak cardiac enzymes) were log transformed for a better linear fit. CRP was analysed as a dichotomous variable with separation by values above and below 4 mg/d. The Pearson correlation or Mann-Whitney test were used as appropriate for unadjusted associations. Stepwise, forward selection was used to construct multivariate models and variables with P-values <0.05 which were retained in the model. The one-year survival period after STEMI until the occurrence of the composite endpoint was assessed by the Kaplan–Meier method and comparison was made between STEMI patients with skin AF values above and below the median, using the log-rank test. For assessment of the influence of confounding factors on skin AF as a predictor of the composite endpoint, Cox proportional hazard method was used. Due to the small sample size and thus a low number of events, we could only correct for one potential confounder at a time. The following covariates were corrected for separately: age, sex, smoking, peak troponin levels, anterior infarct location, previous myocardial infarction, and admission blood pressure, glucose, and serum creatinine. Since CRP and HbA1c were measured in only 64 and 74 STEMI patients respectively, these values were not included in this analysis. 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).

Results

Subject characteristics
Subject characteristics are shown in Table 1. In 5% of STEMI patients no primary PCI was performed because the coronary occlusion had resolved spontaneously. Median myocardial ischemia time was 3.2 (2.5-4.3) hours for STEMI.

Differences in skin AF between groups
Skin AF was measured at a median of 21.2 (12.3-28.2) hours after symptom onset (63% <24h; 27% 24h-48h; 11% >48h) and 16.6 (8.8-16.6) hours after PCI in STEMI patients. Figure 1 demonstrates that skin AF was significantly higher in STEMI compared with sCAD and controls. Also in sCAD, skin AF was significantly greater than in controls. These between-group differences in skin AF remained significant after correction for diabetes, smoking, cholesterol, and serum creatinine ($F=5.2$, $\eta^2=0.035$, $P=0.024$ for STEMI compared with sCAD and $F=8.9$, $\eta^2=0.079$, $P=0.004$ for STEMI compared with controls).

Skin AF and markers for glycemic and inflammatory stress within STEMI group
Skin AF correlated positively with age ($r=0.44$; $P<0.001$), HbA1c ($r=0.43$; $P<0.001$), and glucose ($r=0.24$; $P=0.027$), negatively with systolic blood pressure ($r=-0.23$; $P=0.034$), and was higher in subjects with CRP >4 mg/l ($P=0.007$) or with previous CVD ($P<0.001$). Skin AF was not significantly associated with lipid parameters, peak cardiac enzymes, or gender. After multivariate adjustment for glucose, blood pressure, lipid parameters, peak
cardiac enzymes, and gender, age (β, 0.24; 95% CI, 0.02-0.46; P=0.033), CRP (β, 0.26; 95% CI, 0.05-0.48; P=0.018), HbA1c (β, 0.36; 95% CI, 0.14-0.58; P=0.002), and previous CVD (β, 0.24; 95% CI, 0.01-0.44; P=0.042) were independently associated with skin AF (r²=0.45; P<0.001).

Follow-up measurement and one year incidence of composite endpoint in STEMI

Patients measured within 200 days following STEMI (N=15) did not show a significantly decrease in skin AF, however in patients measured >200 days after STEMI (N=14) skin AF did decrease significantly (P=0.018). Figure 2 demonstrates that during one year follow-up, the composite endpoint occurred in 11 patients; 4 patients died, 4 were hospitalized for a new myocardial infarction and 3 for heart failure and that patients with skin AF values above the median (2.5 *10^-2 AU) had a hazard ratio of 11.6 (95% CI, 1.5-90.8; P=0.019) for developing MACE. Partial corrections for potential confounders revealed that skin AF predicted MACE independent of age (hazard ratio 8.7, 95% CI 1.0-74.4; P=0.048), sex (10.3, 1.3-83.7; 0.030), current smoking (10.5, 1.3-84.1; 0.027), peak troponin levels

Table 1. Clinical characteristics of study groups

<table>
<thead>
<tr>
<th></th>
<th>STEMI (n = 88)</th>
<th>sCAD (n = 81)</th>
<th>Controls (n = 32)</th>
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<tr>
<td>Age (yrs)</td>
<td>64 ± 13</td>
<td>64 ± 10</td>
<td>63 ± 11</td>
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<tr>
<td>Men</td>
<td>67 (76%)</td>
<td>60 (74%)</td>
<td>23 (72%)</td>
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<tr>
<td>Current smokers</td>
<td>36 (44%)*†</td>
<td>15 (20%)‡</td>
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<td>Diabetes mellitus</td>
<td>25 (28%)*†</td>
<td>12 (15%)‡</td>
<td>0 (0%)</td>
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<tr>
<td>known</td>
<td>11 (13%)*†</td>
<td>12 (15%)‡</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>new onset</td>
<td>14 (16%)*†</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CrCl (ml/min./1.73 m²)</td>
<td>80 ± 28</td>
<td>74 ± 25</td>
<td>81 ± 21</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>130 (111 - 160)*†</td>
<td>92 (83 - 106)‡</td>
<td>86 (83 - 95)</td>
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<tr>
<td>Cholesterol (mg/dL)</td>
<td>215 ± 39*</td>
<td>183 ± 39‡</td>
<td>222 ± 31</td>
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<td>BMI (kg/m2)</td>
<td>27 ± 3</td>
<td>27 ± 4</td>
<td>25 ± 3</td>
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<tr>
<td>Vascular history</td>
<td></td>
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<tr>
<td>previous ACS</td>
<td>6 (7%)*</td>
<td>27 (34%)</td>
<td>0 (0%)</td>
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<tr>
<td>previous PTCA</td>
<td>3 (3%)*</td>
<td>35 (44%)‡</td>
<td>0 (0%)</td>
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<tr>
<td>previous CABG</td>
<td>2 (2%)*</td>
<td>19 (24%)‡</td>
<td>0 (0%)</td>
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<tr>
<td>previous stroke</td>
<td>3 (3%)</td>
<td>4 (5%)</td>
<td>0 (0%)</td>
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<td>previous PAD</td>
<td>4 (5%)</td>
<td>10 (13%)</td>
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<tr>
<td>Statin use</td>
<td>11 (13%)*</td>
<td>67 (85%)‡</td>
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<td>Antihypertensive agent use</td>
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<td>β-blocking agents</td>
<td>20 (23%)*†</td>
<td>63 (80%)</td>
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<td>diuretics</td>
<td>8 (9%)</td>
<td>9 (11%)</td>
<td>1 (3%)</td>
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<td>ACE-inhibitors</td>
<td>7 (8%)*</td>
<td>28 (35%)‡</td>
<td>2 (6%)</td>
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<tr>
<td>ARBs</td>
<td>5 (6%)</td>
<td>10 (13%)</td>
<td>0 (0%)</td>
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<tr>
<td>Calcium antagonists</td>
<td>5 (6%)*</td>
<td>38 (48%)‡</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>7 (8%)*</td>
<td>62 (78%)‡</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Data are mean ± SD, medium (quartiles), or number of subjects (%). Between group differences were tested with student’s t-test, or Fisher’s exact test when appropriate. * indicates P<0.05 for STEMI vs. sCAD; †, STEMI vs. controls; ‡, sCAD vs. controls. Medication use indicates situation before admission. STEMI = ST-elevation myocardial infarction; sCAD = stable coronary artery disease; CrCl = estimated Creatinine Clearance; BMI = body mass index; ACS = acute coronary syndrome; PTCA = percutaneus transluminal coronary angioplasty; CABG=coronary artery bypass graft; PAD = peripheral artery disease; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker
Skin Autofluorescence is elevated in acute myocardial infarction (12.3, 1.6-97.3; 0.017), anterior infarct location (11.1, 1.4-87.7; 0.022), previous myocardial infarction (12.4, 1.6-97.8; 0.017), admission systolic (11.7, 1.5-92.0; 0.019) or diastolic (11.5, 1.5-90.5; 0.020) blood pressure, glucose (10.3, 1.3-82.7; 0.028), or serum creatinine (11.2, 1.4-88.0; 0.022).

Discussion

In this study we demonstrate that skin AF is elevated in acute ST-elevation myocardial infarction compared with stable coronary artery disease and healthy controls. Even after multivariable adjustment for known confounders, skin AF remained significantly higher in
STEMI, and was age independently related to CRP and HbA1c. The elevation of skin AF within 72 hours following STEMI appeared to be partly of a transient nature, showing a tendency to decrease after 200 days. Furthermore, STEMI patients with an elevated skin AF were substantially more likely to die or to be hospitalized for a new myocardial infarction or heart failure in the following one year, which seemed to be independent of other risk factors such as age or infarction size. Our data suggest that skin AF may serve as a simple non-invasive measure of hyperglycaemia and inflammation derived oxidative stress involved in the development of ACS and may identify those patients with a potentially adverse outcome.

Comparison with previous clinical studies using skin AF
In previous reports we have already demonstrated that skin AF is strongly related to skin accumulation of AGEs, as evidenced by a high correlation with specific AGEs measured from skin biopsy homogenates. Since these measured AGEs included both exclusively carbohydrate derived AGEs (pentosidine), but also mainly lipid derived AGEs (carboxymethyllysine and carboxyethyllysine), we concluded that skin AF may be 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. Furthermore, subjects with diabetes, especially those with evidence of micro- or macrovascular disease, and subjects with renal failure had significantly higher levels of skin AF and skin AF was an independent predictor of CVD related mortality in these patients. The data from the present study are in agreement with our previous observations, and suggest that skin AF may also be of potential use in euglycaemic patients without impaired kidney function.

Oxidative stress and AGEs in CVD
Data on the potential value of oxidative stress markers in endothelial dysfunction and clinically overt CAD is extensive. Since most markers for oxidative stress require specialized laboratories, they are not readily available for clinical practice. AGEs are stable and non-invasively quantifiable using skin AF and may, therefore, serve as a potential new marker in CVD. Data from other clinical studies support this hypothesis. Plasma AGEs have been shown to be associated with the number of stenotic coronary arteries in non-diabetic subjects and to predict the long term incidence of cardiovascular mortality in non-diabetic women.

Basic research has shown that in atherosclerotic plaques AGEs interact with their receptor (RAGE), which results in increased production of inflammatory mediators and proteolytic enzymes, rendering plaques more vulnerable to rupture. Recently, the key role of RAGE in the generation of oxidative stress and subsequent cellular damage was pointed out in an animal model of ischemia/reperfusion injury after myocardial infarction. It was demonstrated that lipoxidation derived AGEs are generated by ischemia/reperfusion and activate RAGE, thereby augmenting vascular and inflammatory cell activation. Animals lacking cellular expression of RAGE were less susceptible to ischemia/reperfusion damage. This supports previous animal intervention studies showing that lowering AGE levels or antagonizing their receptors may attenuate the atherosclerotic process. We recently reported that skin AF is strongly associated with serum levels of sRAGE in patients with sCAD, thus making the link...
between inflammation and oxidative stress and skin AF by means of the AGE-RAGE axis\(^6\). In clinical studies it has been shown that lowering AGE in the circulation by dietary intake low in glycotoxins, results in marked decreases in serum levels of inflammatory mediators such as CRP and vascular adhesion molecule-1\(^3\). Because in the present study skin AF was related to CRP, a marker of inflammation known to be strongly associated with CAD\(^2\), and predicted future events, our data are in agreement with the abovementioned reports.

**Study limitations**

Due to the limited number of events in the one year follow-up period, it was impossible to differentiate between the individual contributions of all cause mortality, myocardial infarction, and heart failure. CRP was measured with a normal sensitivity kit with a detection limit of 4 mg/dl. Because a significant number of patients had CRP levels <4 mg/dl it could only be analysed as a dichotomous variable. CRP and HbA1c could also not be included in the Cox regression analysis, because these were only measured in a subset of STEMI patients. More importantly, the small sample size did not allow for multivariable correction for more than one potentially confounding covariate in the Cox regression analysis. Additionally, because we measured skin AF after PCI, whether the elevation in skin AF was related to atherosclerotic plaque formation, ischaemia, or reperfusion, or a combination of those, cannot be determined from these data. Also, the observation that skin AF decreased after 200 days was based on a small number of patients, and should be considered as preliminary. The objection that skin AF was performed within a wide time range (i.e. some >72 hours after symptom onset) is valid, however we have seen that skin AF does not actually change in the first few days following STEMI (data not shown). In the current study, we did not measure circulating AGEs or other markers for oxidative stress. In order to determine the independent prognostic value of skin AF over these markers, a larger study in STEMI patients is warranted. This would also make multivariable correction possible in the Cox proportional hazard model. For it to be of clinical use in STEMI patients, it should add incremental information to currently available risk scores such as the Thrombolysis In Myocardial Infarction (TIMI), Platelet glycoprotein IIb/IIIa in unstable Agina: Receptor Suppression Using Integrilin (PURSUIT), and Global Registry of Acute Coronary Events (GRACE) risk score. Finally, we are currently studying its value in patients with unstable angina or non ST-elevation myocardial infarction. These patients are more of a challenge, since it is currently very difficult to select those patients that are at the highest risk of an adverse outcome. From previous investigations we have learned that skin AF cannot be reliably measured in subjects with skin photo type V-VI\(^3\). Therefore, for this study we excluded these skin types and further development for improving the measurement in dark skin types is ongoing and a newer version of the instrument is capable of reliably measuring skin AF in darker skin types. Since not all AGEs encompass fluorescent properties, skin AF is only representative of part of the total AGE burden. However, in our validation study we found that skin AF also correlated strongly with non-fluorescent AGEs\(^1\). Additionally, two major lipid peroxidation products, 4-hydroxynonenal and malondialdehyde — after binding to free aminogroups of protein — also encompass characteristic fluorescent properties\(^3\). Since the AGE-Reader covers a wide excitation/emission spectrum, skin AF measured with the AGE-Reader may therefore have a miscellaneous origin.
Conclusion

To the best of our knowledge, this is the first study to demonstrate an association of a non-invasive marker for cumulative hyperglycaemia and inflammation related oxidative stress with acute myocardial infarction. Despite the small sample size of this study, our prospective data do indicate that skin AF may provide an easily applicable tool to identify those patients at risk of developing major adverse cardiac events after myocardial infarction. However, since this was a relatively small study, these observations should be confirmed in a larger follow-up study in patients with ACS to establish the usefulness of skin AF in a clinical setting.
Reference List


