DiagnOptics
The pioneer
in non-invasive diagnosis and risk assessment on diabetes and its complications

OVERVIEW

Established in 2003, DiagnOptics is the pioneer and inventor of cutting edge diagnostic devices that can non-invasively diagnose and assess the risk of diabetes and its complications.

Its technological advance in detecting fluorescence of the AGEs (Advanced Glycation Endproducts) has led to the invention of the AGE Reader™ - a state of the art non-invasive device which can determine the tissue accumulation of AGEs within 30 seconds.

AGEs are essential biomarkers of metabolic and glycemic stress and have been implicated as causative factors in the progression of a host of age-related diseases, such as atherosclerosis, diabetes, renal failure and Alzheimer. The amount of AGE in tissue serves as an important risk predictor of diabetes and its complications.

The introduction of the AGE Reader™ marks a technological breakthrough and has profound impact on the discovery and clinical intervention of diabetes and other ageing related chronic disorders. This innovative product not only enables instantaneous (diagnosis and) risk assessment on diabetes and its complications, but also provides clinicians with much needed information to make a correct treatment plan.

Since the CE certification on the AGE Reader™ in 2006, DiagnOptics has been selling this device to diverse end users in applicable markets. Currently the company is working on obtaining additional regulatory approvals in other regions and is engaged in new product development activities. The head office of DiagnOptics Technologies BV is in Groningen, the Netherlands.

MISSION

DiagnOptics is committed to the development and innovation of skin autofluorescence detection through continuous dedication on non-invasive biomedical technologies.

Our mission is to improve the standard of clinical diagnosis and risk assessment on diabetes and its complications.

MANAGEMENT

Bart A. van den Berg (MSc) - Managing Director

Joined the company in June 2004. Mr. van den Berg is former general manager and co-founder of two pharmaceutical contract manufacturing companies and founder of VenGen BV, a life sciences start-up management company. He was trained in business economics at Tilburg University, the Netherlands.

Dr. Andries J. Smit (MD) - Medical Director

Dr. Smit founded DiagnOptics in 2003 and has been the medical director since 2003. He has been the head of the Vascular Unit of the University Medical Center Groningen for more than 15 years and has authored or co-authored over 200 scientific publications. He was trained in internal medicine and is associate professor in hypertension and atherosclerosis.

Dr. Reindert Graaff (MSc PhD) - Technical Director

Dr. Graaff founded DiagnOptics in 2003 and has been the technical director since 2003. He works within the Department of Biomedical Engineering at the University Medical Center of Groningen He was educated in applied physics and is specialized in biomedical optics. He wrote a thesis on tissue optics applied to reflectance pulse oximetry.

Dr. Jibin Chi (MD, MBA, MBI) - Commercial Director

Dr. Chi joined DiagnOptics in February 2007. He has over 18 years of international sales, marketing and business development experience in medical industry. In the last decade, he has worked for international pharmaceutical, medical device and biotech companies across Europe, Australasia and North America. He received a medical degree from The Medical University of China and MBA and MBI dual degrees from The Rotterdam School of Management.

HISTORY

1995 Dr. Smit and his co-workers made a coincidental finding of increased autofluorescence of the skin in many diabetes patients when they were performing fluorescein capillary leakage studies in diabetes.

1996 Dr. Smit and Dr. Graaff developed dedicated prototypes to assess skin autofluorescence in human subjects.

1998 The first clinical studies in diabetes and renal failure were started with an advanced prototype of the AGE Reader™ in the University Hospital Groningen, the Netherlands.

1999 Filed patent application for the AGE Reader and the first scientific report published on the clinical use of AGE Reader™ prototype in diabetic patients.

2000 Initiation of the first large scale follow-up study in the Zwolle region.

2003 Dr. Smit and Dr. Graaff founded DiagnOptics in Groningen, the Netherlands.

2004 The first clinical study on AGE Reader™ was published in Diabetologia.

2005 AGE Reader™ was first shown to future users (EASD, Athens 2005).

2006 AGE Reader™ received CE approval for commercialization in Europe.

Advanced Glycation Endproducts

Causative factor
of many age related chronic diseases such as
diabetes, atherosclerosis, renal failure and Alzheimer

WHAT ARE AGEs ?

Advanced Glycation Endproducts (AGEs) have originally been described as the result of a non-enzymatic chain of chemical reactions between sugars and proteins. In 1912 Louis-Camille Maillard, a French Chemist, conducted a simple experiment in his lab that turned out to be a shortcut that created meat flavor and aroma through heating sugar and amino acids. This chemical reaction has thus been called Maillard Reaction or Browning Reaction. The intermediate products are known, variously, as Amadori, Schiff base and Maillard products, named after the researchers who first described them.

Following extensive research on the Maillard Reaction across diverse fields from food, nutrition to theraputics, a term of Advanced Glycation Endproducts or AGEs has been introduced to describe the end products of the Maillard Reaction that form under normal physiological conditions in living organisms, but also in vitro.

AGEs can be formed external to the body (exogenously) through heating and cooking sugars with fats or proteins; or inside the body (endogenously) through metabolism or aging and oxidative stress. In particular, those endogenous AGEs are known to contribute to vascular and renal dysfunction[1-2].

Once proteins are exposed to elevated level of sugar, a series of non-enzymatic chemical reactions occur and the proteins thus become glycated. The initial glycated product, Schiff base, can be formed within hours of the reactions. However the Schiff base is very unstable and can easily break down or undergo further rearrangement to become Amadori Adducts within a few days. If no reverse reaction occurs, the Amadori Adducts can eventually evolve into irreversible chemical substances -AGEs and crosslinks. This entire process may take weeks or months to be completed. AGEs can also be formed much more rapidly from so-called reactive carbonyl compounds, often formed during oxidative stress.

THE ROLE OF AGEs

AGEs result from non-enzymatic glycation and oxidation of proteins, are biomarkers of glyco-metabolic and oxidative stress and can cause major damage to tissue and cells. They can initiate a wide range of abnormal responses in cells and tissues such as inappropriate expression of growth factors, alteration in growth dynamics, accumulation of extracellular matrix, promotion of vasoregulatory dysfunction and initiation of death pathways[3].

AGEs crosslink with proteins, lipids and nucleic acids and affect their structure and function. Crosslinking to longlived proteins like collagen results for example in loss of elasticity. Since many cells in the body (for example endothelial cells, smooth muscle or cells of the immune system) bear the Receptor for AGEs (RAGE), AGEs affect nearly every type of cell and organ. The half-life and the internal microenvironment of a protein dictate the number and stages of AGE modifications present, ranging from reactive intermediates to later non-reactive AGEs[4]. Similarly cells with long half lives such as brain cells are more exposed to the damaging effect of AGEs.

AGEs accumulate with ageing in normal persons, but this process occurs more rapidly in patients with conditions such as diabetes and renal failure. They have been implicated as causative factors in the progression of a host of chronic, age-related diseases, such as diabetes, atherosclerosis, renal failure and Alzheimer. A sub-study of the Diabetes Control and Complications Trial (DCCT) first established a causal relationship between chronic hyperglycemia, skin AGE accumulation and long term diabetic complications[5]. Besides accelerated AGE formation, increased formation of mitochondrial reactive oxygen species (ROS) by hyperglycemia may modulate the activity of multiple biochemical pathways such as aldose reductase and protein kinase C(Figure 2)[6].

AGEs AND DIABETES[7]

In diabetes the body does not properly control the amount of sugar in the body, characterised by varying or persistent hyperglycemia (elevated blood sugar levels), especially after eating. Diabetes has become an epidemic health problem with around 300 million people in the world suffering from this disease. Diabetes has major health-economic consequences, and the costs involved have a considerable impact on the economy. There are two major challenges facing clinicians in the case of fighting diabetes. One is that a large percentage of diabetic patients remain unidentified until they develop

one or more serious complications. The other is that dealing with the complications of diabetes costs considerably more than controlling the disease. It is suggested that at least a third of type 2 diabetes patients are undiagnosed and unaware of their condition. Even more alarmingly, there are probably twice as many people that have pre-diabetes and are on the path to becoming diabetes.

The morbidity caused by diabetes has traditionally been classified into macro and microvascular complications. Although macrovascular complications have received greater attention, microvascular complications are more typical for diabetes.

Persons with diabetes have higher levels of AGEs than non-diabetic subjects because hyperglycemia and oxidative stress both contribute to their accumulation. Studies have shown 20-30% higher AGE levels in persons with uncomplicated diabetes and 40-100% higher levels in subjects with type 2 diabetes complicated by coronary artery disease or microalbuminuria. Multivariate analyses in subjects with diabetes have identified renal function, age, urinary albumin-to-creatinine ratio, systolic blood pressure, and anemia as independent predictors of AGE levels. Renal impairment decreases clearance of AGEs in both diabetic and non-diabetic populations. Subjects with end-stage renal disease have significant elevations in circulating and tissue AGEs compared with healthy control subjects (by 5-100 fold). Renal transplantation has been shown to lower serum AGE levels.

Several observations indicate that AGE turnover is more dynamic than previously appreciated and that endogenous AGEs are determined by AGE production (endogeneous glycemia and oxidative stress) as well as renal AGE excretion. Hyperglycemia, renal insufficiency, and aging are prooxidant states that contribute to the endogenous levels of AGEs.

The relationship of AGEs to their receptors reflects both positive and negative roles in the actions and fate of AGEs. In their positive role, some receptors normally aid in clearing AGEs from the circulation and may help to mitigate the prooxidant effects of AGEs. In contrast, RAGE and other receptors appear to activate a stress response leading to inflammation and cellular dysfunction.

AGEs AND DIABETIC COMPLICATIONS[8]

Monnier first described the relation between AGE accumulation in skin collagen and the severity of long term diabetic complications. In a subsequent study of a subset of type I diabetic patients from the Diabetes Control and Complications Trial (DCCT), they concluded that skin AGE levels could explain 19-36% of the variance in incidence of diabetic complications in the intensively and 14-51% in the conventionally treated patients. The association remained significant after adjustment for HbA1c.

Thus tissue AGE accumulation was superior to (single) HbA1c measurements in predicting the progression of diabetic complications.

Macrovascular complications

Diabetic patients have a clearly increased risk of cardiovascular morbidity and mortality. Both the DCCT and the United Kingdom Prospective Diabetes Study (UKPDS) trial have shown that hyperglycemia contributes to the increased cardiovascular risk. Serum levels of AGEs in patients with type 2 diabetes with coronary heart disease (CHD) are increased compared to patients without CHD, and correlate with CHD severity. Even after correction for other cardiovascular risk factors, AGEs remained associated with CHD. AGE deposits have been demonstrated in atherosclerotic plaques and within myocardial fibers. Serum levels of AGEs in type 1 diabetic patients are associated with isovolumetric relaxation time of the left ventricle, as a marker of left ventricular diastolic dysfunction. The EURODIAB prospective complication study showed a strong correlation between pulse pressure and plasma levels of AGEs in type 1 diabetic patients.

AGEs may promote cardiovascular disease in diabetes through endothelial dysfunction, by accelerating inflammation, and by inducing lipid abnormalities. AGE accumulation may be related to endothelial dysfunction, and endothelial RAGE has been proposed as the major key in such an interaction. Binding of AGEs to RAGE activates endothelial cells, resulting in higher levels of endothelial adhesion molecules like VCAM-1, and activation of transcription factor NF-kB. Release of endothelial adhesion molecules by NF-kB further increases monocyte adhesivity and vascular permeability, accelerating atherosclerosis.

Binding of AGEs to RAGE and to other receptors initiates inflammation-mediated proliferative processes and propagates inflammation in established macrovascular disease. AGEs also modify low density lipoproteins, limiting their clearance, promoting uptake by macrophages and inducing inflammation. The peroxidation step of lipid transformation is an essential step in the initiation of atherosclerosis, and calcium antagonists have been shown to reduce oxidative stress and the formation of AGE-modified lipoproteins.

Microvascular complications

- Nephropathy

One major mechanisem by which AGEs may contribute to the development of diabetic nephropathy is through effects on the structure and function of extracellular matrix components. The formation of AGEs on type 4 collagen inhabits lateral association of molecules into a normal network, and decreases binding of anionic heparin sulphate proteoglycans to matrix molecules. This may lead to an increase in capillary leakage. The podocyte slit diaphragma maintains a size-selective barrier in glomerular permselectivity. A correlation between nephin expression and proteinuria has been observed. AGE levels in diabetic nephropathy correlate with down regulation of nephin.

AGEs and AGE specific receptors (e.g. RAGE) may be a second major mechanism by which AGEs contribute to the development of diabetic nephropathy. RAGE is widely distributed on cells throughout the body and has been identified on moncytes, glomerular mesangial cells, and endothelial cells. Interaction with RAGE results in the production of cytokines, which stimulate proliferation of mesangial cells, arterial smooth cells and increase collagen synthesis. So both AGEs themselves and the cellular response to AGEs via AGE-receptors provoke matrix changes which may explain the histopathological changes seen in diabetic nephropathy.

In diabetic patients with end-stage renal failure total serum AGE levels increase up to 4 times compared to healthy subjects. Tissue levels of AGEs increase with increasing albumin excretion rate (AER) and basement membrane thickness. Intensive glycaemic control in patients from the DCCT trial led to a lower risk for diabetic nephropathy compared to conventional treatment. The lower risk was associated with lower AGE levels, even after

adjustment for HbA1c. Similar associations were found for albumin excretion rate (AER) and rate of change of AER, and AGE accumulation.

- Retinopathy

The retina is one of the most highly perfused and metabolically active organs and is susceptible to substrate imbalance or ischemia. Retinal cells are sensitive to glycaemic control, they are freely permeable to formation of AGEs. Skin AGE levels are increased in diabetic patients with retinopathy and correlate with the severity of retinopathy. Diabetic patients with proliferative retinopaty show increased levels of AGEs by immunohistochemistry in the entire retina, both neuroglial and vascular.

Diabetic retinopathy is characterized by an increased retinal neovasuclarization, probably in response to the action of the angiogenic factor, vascular endothelial growth factor (VEGF). The increase of VEGF expression by AGEs, as well as the above described effects of AGEs on tissue proteins establish an association between AGE accumulation and the pathogenesis of diabetic retinopathy.

- Neuropathy, and dementia

Diabetic polyneuropathy is characterized by a decrease in nerve blood flow and structural changes in nerves resulting in alterations in conduction velocity.

The pathogenetic role of AGE accumulation in the development of diabetic neuropathy is as yet more obscure. The lack of clinical data on neuropathy and AGEs is in part the result of limited tissue available for research. Furthermore the development of diabetic neuropathy is a complex interrelationship between nerve and vascular changes. The relationship between AGEs and diabetic sensorimotor neuropathy is correlative. Results with the AGE Reader™ have documented that skin autofluorescence is closely related to nerve conduction velocities. Even in the pre-clinical stage (Meerwaldt 2005), skin autofluorescence was also related to clinical neuropathy scores in these studies.

Epidemiologic and experimental clinical studies have suggested that diabetes significantly increases risk for the development of Alzheimer’s disease and vascular dementia, independent of vascular risk factors. Insulin dysregulation

may contribute to Alzheimer's disease pathology through several mechanisms including decreased cortical glucose utilization particularly in the hippocampus and entorhinal cortex; increased oxidative stress through the formation of advanced glycation endproducts; increased β-amyloid aggregation through inhibition of insulin-degrading enzyme[9]. Some studies reported increased serum AGE levels in Alzheimer's Disease. AGE levels are also high locally in plaques in the brain.

AGEs AND END STAGE RENAL DISEASE (ESRD)[8]

AGE levels are significantly increased in patients with ESRD and are related to age, renal function, presence of diabetes, and dialysis duration. Serum levels of low molecular weight AGEs increase with declining renal function, and both serum and tissue AGE levels are markedly increased in patients with ESRD. Currently available methods of renal replacement therapy are only partially effective in clearing AGEs from the plasma of patients with ESRD.

Classically, AGE formation has been described as a nonenzymatic reaction between proteins and glucose. Because AGEs are increased in plasma and collagens of normoglycaemic, uremic patients, the increase in AGE levels in ESRD cannot be attributed only to increased glycation of proteins. Thus alternative mechanisms that may contribute to the increase in AGE formation in ESRD are:

Increased pro-oxidant activity
Decreased anti-oxidant activity
Decreased detoxification and excretion of AGE precursors
Less efficient renal excretion of AGE peptides (this is probably by far the most important mechanism)
Insulin resistance resulting in increased formation of AGEs
Abnormalities in lipid metabolism

ESRD is characterized by increased (intracellular) oxidative stress as indicated by increased lipid peroxidation, and a decrease in the ratio between oxidized glutathione to reduced glutathione. Renal impairment is associated with a state of oxidative stress, even before the start of renal replacement therapy. Differences in biocompatibility between dialysis membranes may be responsible for acute further aggravation of oxidative stress and related endothelial dysfunction.

Normally AGEs cross linked to proteins are excreted by breakdown of proteins to peptides and amino acids and subsequent renal excretion of AGE-peptides and AGEs linked to amino acids. Impaired glucose tolerance and hypertension are important contributors in the rise of patients with ESRD, and insulin resistance is an universal phenomenon in uremic patients. Insulin resistance may also increase ROS formation and so might increase AGE accumulation.

Carbonyl compounds may form through non-oxidative mechanisms, e.g. 3-deoxyglucosone (3-DG) by hydrolysis and rearrangement of glucose adducts to protein. 3-DG is also produced as a by-product of the polyol pathways. In ESRD, decreased catabolism of 3-DG may be due to the loss of 3-DG reductase activity.3-DG is reactive with other proteins, increasing the formation of AGEs .

AGEs AND COMPLICATIONS OF END STAGE RENAL DISEASE(ESRD)[8]

Cardiovascular disease (CVD) is extremely common in patients with ESRD, and accumulation of AGE is significantly increased in those patients. Accumulation of AGEs is thought to play a pivotal role in this major increase in CVD. Skin autofluorescence, assessed with the AGE Reader™, has indeed been shown to be an strong and independent predictor of CVD in ESRD.

Cardiovascular complications

ESRD is associated with accelerated atherosclerosis, increased arterial stiffness and myocardial dysfunction. AGE accumulation occurs in all tissues prone to these complications in ESRD, such as coronary vessels, ahterosclerotic plaques of the aorta, and in cardiac myocytes. And interestingly tissue inhibition or breaking of AGEs also prevents or reduces myocardial diastolic dysfunction and arterial stiffness. At least two studies with the AGE Reader™ in ESRD have shown that diastolic dysfunction is closely and independently related to diastolic dysfunction.

One major mechanism by which AGEs may contribute to the development of vascular complications in general is through effects on the structure and function of extracellular matrix proteins. The formation of AGEs on type 4 collagen inhibits lateral association of molecules into a normal network and decreased binding of anionic heparin sulphate proteoglycans to matrix molecules. Decreased degradation of basement membrane components and increased binding of plasma proteins may further enhance tissue AGE accumulation. AGE modifications of lipoproteins may increase vascular deposition of LDL, as a consequence of impaired LDL receptor-mediated clearance. This may induce vascular inflammation and the development of atherosclerosis.

Interactions between AGEs and AGE specific receptors (RAGE) are a major mechanism by which AGEs are thought to contribute to vascular pathology. Stimulating of RAGE in endothelial cells by AGEs results in accelerated

release of ICAM, VCAM and other endothelial adhesion molecules and in endothelial dysfunction. Endothelial dysfunction contributes to the increased incidence of cardiovascular disease in ESRD. AGE accumulation is related to endothelial dysfunction and endothelial RAGE has been proposed as the major key in such interaction. Furthermore, AGEs also react with and inactivate nitric oxide (NO), affecting vascular relaxation and reducing the antiproliferative effect of NO on aortic smooth and myocardial musclecells.

Connective tissue complications

Destructive spondylarthropathy, carpal tunnel syndrome, and lytic bone cysts are major clinical features of ESRD. When proteins lose their original configuration and are converted into a predominantly beta-sheet form, this increases the formation of insoluble aggregates; a process known as amyloidosis. Accumulation of amyloid deposits has been linked to ESRD-related connective tissue disease, and β-$_2$microglobulin (β_2M) is a major protein of amyloid deposits. The mechanism of AGE modified β_2 microglobulin (AGE-β_2M) formation is unknown, but AGEs accumulate on long lived products, such as amyloid fibrils.

Peritoneal damage

Vascular proliferation and extracellular structural changes are histopathological characteristics in peritoneal membrane failure. With increasing time on peritoneal dialysis, peritoneal molecule transport increases and so reduces ultrafiltration capacity. AGE–RAGE interaction in human peritoneal mesothelial cells results in overexpression of vascular and intercellular cell adhesion molecules, which may promote local inflammation and injury.

Presence of reactive carbonyl compounds in peritoneal dialysis fluids may further enhance local AGE formation. Levels of those compounds may be limited by shortening heat sterilization time, lowering pH during processing, removing metal ion and trapping of the reactive carbonyls, or use of purer glucose solutions. In clinical studies, dialysis fluids with low levels of reactive carbonyl compounds (precursors of AGEs) result in less mesothelial cell damage.

Clinical relevance of AGEs in ESRD

In the general population, well-established risk factors for cardiovascular disease have been defined, such as hypertension and smoking. However, in patients with ESRD the role of traditional risk factors is less well understood, since some factors are not related to increased vascular morbidity and mortality.

It is probable that AGEs or AGE precursors are an important component of uremic toxins, contributing to accelerated atherosclerosis and connective tissue disease in ESRD. Up till recently results are mainly based on in vitro analysis and animal models of ESRD. The study of Meerwaldt et al with the AGE Reader™ was the first prospective cohort study to evaluate the extent to which AGEs are related to and predictive of ESRD complications. Other studies using AGE inhibition are necessary to establish whether it may be a useful intervention to reduce the deleterious impact of ESRD on tissues.

AGE detection in blood and urine using AGE specific fluorescence or ELISA has been described, but these AGEs are in part derived from diet, are short-lived and do not reflect tissue accumulation of AGEs linked to long-lived proteins. Studies with the AGE Reader™ have clearly demonstrated markedly increased tissue AGE accumulation in ESRD. The rate of AGE accumulation is influenced by the half-life of proteins, which may explain discrepancies between tissue and blood levels of AGEs.

Chemically stable AGEs bound to long-lived proteins are biomarkers of cumulative metabolic/oxidative stress. Because AGEs are associated with and predictive of complications in ESRD, like in diabetes, tissue AGE assays provide important information on metabolic and oxidative control. The simple estimate of AGE accumulation using the AGE Reader™ in skin tissue aids in the assessment of risk for long term complications in ESRD.

AGEs ACCUMULATION MEASUREMENTS[8]

From a clinical point of view, it would be desirable to follow up AGE accumulation in diabetic patients. Measurements of blood glucose or glycated hemoglobin is useful as a measure of glycaemic control, but provides limited information on resultant tissue damage. Hyperglycaemia, dyslipidaemia and oxidative stress have perhaps co-equal roles in the mediation of tissue damage, so that measurement of glycaemic control alone has limited value. Quantitation of AGE accumulation in tissue provides an useful estimate of tissue injury as a consequence of the complex metabolic burden of diabetes and so offers a predictive measure for the development of long term complications. Analysis of AGE accumulation at accessible body sites (skin, lens or cornea) show a strong relationship with the progression of diabetic complications, with the strongest correlations observed when measuring skin AGE levels.

The characteristic florescence spectrum of several AGEs has classically been used to determine AGE accumulation, e.g. from skin biopsies taken under local anaesthesia from the buttock. Analysis of collagen-linked fluorescence or other specific AGE assays always required invasive procedures, requiring tissue biopsies. Consequently, skin AGE analysis never reached the clinic, despite its potential clinical value.

Thus if skin autofluorescence can be measured non-invasively and is related to skin AGE accumulation, it certainly offers a simple and useful clinical tool for assessment of risk for AGE related long term complications.

AGE Reader™

Shine a non-invasive light into life

HISTORY OF THE AGE READER™

Diabetes mellitus is associated with increased leakage of capillaries in various parts of the human body. The transcapillary and interstitial diffusion of intravenously administered sodium fluorescein is used as a marker for capillary permeability and for microvascular endothelial dysfunction.

In 1995, Dr. Smit and his colleagues at the University Medical Center Groningen in the Netherlands performed a fluorescein capillary leakage study in diabetes. In this study, they deployed non-invasive fluorescence videomicroscopy, a technique which has been described in detail in a published paper (Jager et al. Int. J Microcirc 1997; 17:150-158). This led to the unexpected finding that the background fluorescence levels in diabetic and healthy subjects are significantly different. This was the first report on increased skin autofluorescence in diabetes patients to be measured non-invasively.

Following on this coincidental and serendipitous finding, Dr. Smit, Dr. Graaff and their co-workers have developed dedicated prototypes to assess the fluorescence in human non-invasively. After increasingly encouraging results, they started the first clinical studies on diabetes and renal failures with an advanced prototype of the AGE Reader™ in the University Medical Center Groningen in the late 90s.

In 1999, they submitted patent applications on the methodology and subsequently presented their findings at various scientific meetings.

In 2004, the first clinical study on the AGE Reader™ was published in Diabetologia. Soon after that, the commercial model of the AGE Reader™ was developed and eventually was granted with CE certification in the middle of 2006.

INTRODUCTION TO SKIN AUTOFLUORESCENCE[8]

Tissue autofluorescence

The phenomenon of autofluorescence was known by the middle of the nineteenth century. British scientist Sir George G. Stokes first made the observation that the mineral fluorspar exhibited fluorescence when illuminated with ultraviolet light, and he coined the word "fluorescence". He described the theoretical basis of fluorescence in 1852.

Skin autofluorescence may reflect biochemical composition of skin tissue. Light that is transmitted to the skin will only partly be directly reflected at the surface (specular reflection), whereas the largest part will be scattered and/or absorbed by its tissue. A part of the remaining light will be reflected and this is called diffuse reflection. The amount of diffuse reflection is related to skin chromophores, like melanin. Melanin absorbs light and thus will decrease reflection.

Absorption of a photon may excite the absorbing molecule to a higher energy level. However, fluorescent molecules may rapidly return to a more stable energy state by re-emission of a photon with lower energy and a higher wavelength: fluorescent emission. Skin autofluorescence is the fluorescent emission of skin tissue fluorophores upon absorption and excitation of light for certain wavelengths.

Tissue fluorophores

Several fluorophores in the skin, including collagen and elastin, have been identified. These natural tissue fluorophores have their own specific excitation-emission spectrum. Autofluorescence has been used in different ways for the analysis of several diseases. Autofluorescence imaging is used to distinguish normal from malignant tissue in several organs, such as skin and colon. Others have related autofluorescence of e.g. the lens to ageing and diabetes. The non-enzymatic glycation and subsequent Maillard reaction between reducing sugars and proteins, leading to the formation of advanced glycation endproducts, are held, in part, responsible for lens changes upon ageing or in diabetes.

Besides lens fluorescence, also skin autofluorescence is increased in ageing and chronic age-related diseases, such as diabetes. The characteristic

fluorescence spectrum of collagen linked-AGEs at 440nm upon excitation at 370nm has classically been used to determine tissue AGE accumulation, for example in extracts from homogenates of skin biopsies. The most frequently studied fluorescent skin AGE is pentosidine, a crosslink with lysine and arginine residues.

METHODOLOGY OF THE AGE READER™

In contrast to the methods that use autofluorescence to detect local variations in skin autofluorescence for example to detect tumours, the AGE Reader™ detects the changes that occur in the apparently clinically healthy normal skin with aging. Therefore, localized imaging measurements, as performed for instance for the detection of cancer are not required. In contrast, the area of the tissue that is seen by the detecting optical fibre should be large enough to prevent gross variations of the result by particular contributions that occurs on small displacements of the probe. The setup of the AGE Reader™ consists of an UV-tube to emit ultraviolet light (UVA), an optical fibre and a spectrometer. The measurements are made in a semi-dark environment with an excitation light source that mainly emits within the range 350-420 nm. The optical fibre is located at some distance from the skin, leading to an integration area of approximately 0.5 cm^2.

The UV light that is used to illuminate the tissue enters the skin almost perpendicularly over an area that exceeds the detected area in such a way that the measured fluorescence does not depend on the size of the illuminated area. The location of the detection area is thereby chosen in the middle of the illumination area. Furthermore, by measuring with the detection fibre oriented at a certain angle, the influence of specular reflection (light directly reflected by skin) has been restricted.

With a fixed geometry, the amount of fluorescence that is captured by the detector is affected by the intensity of the source and the optical properties of the skin. The measurement of autofluorescence is the average light intensity per nm in the range between 420-600nm divided by the average light intensity per nm in the range between 300-420nm (AF in a.u.). The AF ratio is calculated off-line by automated analysis and is observer-independent.

METHODOLOGICAL VALIDATION OF THE AGE READER™

Methodology

Source: Advanced Biomedical and Clinical Diagnostic Systems III. Edited by Vo-Dinh, Tuan; Grundfest, Warren S.; Benaron, David A.; Cohn, Gerald E. Proceedings of the SPIE, Volume 5692, pp. 111-118 (2005).

Title: Instrumentation for the measurement of autofluorescence in human skin

Authors: Graaff R, Meerwaldt R, Lutgers HL, Baptist R, de Jong ED, Zijp JR, Links TP, Smit AJ and Rakhorst G.

Abstract:

A setup to measure skin autofluorescence was developed to assess accumulation of advanced glycation endproducts (AGE) in patients noninvasively.

The method applies direct blacklight tube illumination of the skin of the lower arm, and spectrometry. The setup displays skin autofluorescence (AF) as a ratio of mean intensities detected from the skin between 420-600 nm and 300-420 nm, respectively. In an early clinical application in 46 patients with diabetes mellitus and 46 control subjects matched for age and gender, AF was significantly increased in the patients ($p = 0.015$), and highly correlated with skin AGE's that were determined from skin biopsies in both groups.

A large follow-up study on type 2 diabetes mellitus, ongoing since 2001 with more than 1000 subjects, aims to assess the value of the instrument in predicting chronic complications of diabetes. At baseline, a relation with age, glycemic status and with complications present was found.

In a study in patients with end stage renal disease on dialysis AF was a strong and independent predictor of total and cardiovascular mortality. A commercial version of this AGE-reader is now under development and becomes available early 2005 (DiagnOptics B.V., Groningen, The Netherlands).

One of the remaining questions, that will be answered by measuring so-called Exciation-Emission Matrices (EEM's) of the skin tissue in vivo, is whether a more selective choice of wavelengths is more strongly related to clinical characteristics. An experimental instrument to measure these EEM's was, therefore, developed as well. Clinical measurements are underway of EEM's in patient groups with diabetes mellitus and in healthy volunteers.

Validation against skin biopsies –1

Source: Diabetologia 2004; 47: 1324-1330

Title: Simple non-invasive assessment of advanced glycation endproduct accumulation

Authors: Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans RO, Smit AJ.

BACKGROUND:
The accumulation of AGE is thought to play a role in the pathogenesis of chronic complications of diabetes mellitus and renal failure. All current measurements of AGE accumulation require invasive sampling. We exploited the fact that several AGE exhibit autofluorescence to develop a non-invasive tool for measuring skin AGE accumulation, the Autofluorescence Reader (AFR). We validated its use by comparing the values obtained using the AFR with the AGE content measured in extracts from skin biopsies of diabetic and control subjects. METHODS: Using the AFR with an excitation light source of 300-420 nm, fluorescence of the skin was measured at the arm and lower leg in 46 patients with diabetes (Type 1 and 2) and in 46 age- and sex-matched control subjects, the majority of whom were Caucasian. Autofluorescence was defined as the average fluorescence per nm over the entire emission spectrum (420-600 nm) as ratio of the average fluorescence per nm over the 300-420-nm range. Skin biopsies were obtained from the same site of the arm, and analysed for collagen-linked fluorescence (CLF) and specific AGE: pentosidine, N(epsilon)-(carboxymethyl)lysine (CML) and N(epsilon)-(carboxyethyl)lysine (CEL).

RESULTS:
Autofluorescence correlated with CLF, pentosidine, CML, and CEL (r=0.47-0.62, p</=0.002). In 32 of 46 diabetic patients (70%), autofluorescence values were above the 95% CI of the mean value in control subjects, and correlated with age, diabetes duration, mean HbA(1)c of the previous year and creatinine levels.

CONCLUSIONS/INTERPRETATION:

The AFR offers a simple alternative to invasive measurement of AGE accumulation and, to date, has been validated in non-pigmented skin. The AFR may prove to be a useful clinical tool for rapid risk assessment of AGE-related long-term complications in diabetes mellitus and in other conditions associated with AGE accumulation.

Validation against skin biopsies –2

Source: Ann N Y Acad Sci. 2005 Jun;1043:290-8.

Title: Simple noninvasive measurement of skin autofluorescence.

Authors: Meerwaldt R, Links T, Graaff R, Thorpe SR, Baynes JW, Hartog J, Gans R, Smit AJ

Abstract:

Accumulation of advanced glycation end products (AGEs) is thought to play a role in the pathogenesis of chronic complications of diabetes mellitus and renal failure. Several studies indicate that AGE accumulation in tissue may reflect the cumulative effect of hyperglycemia and oxidative stress over many years. Simple quantitation of AGE accumulation in tissue could provide a tool for assessing the risk of long-term complications. Because several AGEs exhibit autofluorescence, we developed a noninvasive autofluorescence reader (AFR).

Skin autofluorescence measured with the AFR correlates with collagen-linked fluorescence and specific skin AGE levels from skin biopsy samples. Furthermore, skin autofluorescence correlates with long-term glycemic control and renal function, and preliminary results show correlations with the presence of long-term complications in diabetes.

The AFR may be useful as a clinical tool for rapid assessment of risk for AGE-related long-term complications in diabetes and in other conditions associated with AGE accumulation.

CLINICAL VALIDATION OF THE AGE READER™

Clinical study in type II diabetes on vascular damage

Source: Diabetes Care. 2006 Dec; 29(12):2654-9

Title: Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes

Authors: Lutgers HL, Graaff R, Links TP, Ubink-Veltmaat LJ, Bilo HJ, Gans RO, Smit AJ.

OBJECTIVE:
Advanced glycation end products (AGEs) are thought to have a role in the pathogenesis of diabetes complications. We recently reported the association between skin autofluorescence, as a measure of tissue AGE accumulation, and diabetic neuropathy in a selected diabetic population. In this study, we investigated the relation between skin autofluorescence and clinical variables including micro- and macrovascular complications in a type 2 diabetes primary care population.

RESEARCH DESIGN AND METHODS: Clinical data and skin autofluorescence were obtained in the type 2 diabetes group (n = 973) and in a control group (n = 231). Skin autofluorescence was assessed by illumination of the lower arm with a fluorescent tube (peak intensity approximately 370 nm).

RESULTS: Skin autofluorescence was significantly higher in type 2 diabetic patients compared with control subjects in each age category. Multiple regression analysis showed significant correlation of skin autofluorescence with age, sex, diabetes duration, BMI, smoking, HbA1c, plasma creatinine, HDL cholesterol, and albumin-to-creatinine ratio in the type 2 diabetes group (R2 = 25%) and with age and smoking in the control group (R2 = 46%). Skin autofluorescence was significantly higher in the type 2 diabetes group, with both micro- and macrovascular disease, compared with the group without complications and the group with only microvascular complications.

CONCLUSIONS: This study confirms in a large group of type 2 diabetic patients that skin autofluorescence is higher compared with age-matched control subjects and is associated with the severity of diabetes-related complications.

Skin autofluorescence reflecting vascular damage might be a rapid and helpful tool in the diabetes outpatient clinic for identifying diabetic patients who are at risk for developing complications.

Clinical study in diabetic neuropathy

Source: Diabetologia. 2005 Aug;48(8):1637-44. Epub 2005 Jul 14

Title: Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy

Authors: Meerwaldt R, Links TP, Graaff R, Hoogenberg K, Lefrandt JD, Baynes JW, Gans RO, Smit AJ.

AIMS/HYPOTHESIS:
The accumulation of AGE is related to the progression of the renal, retinal and vascular complications of diabetes. However, the relationship with diabetic neuropathy remains unclear. We recently showed that skin autofluorescence, measured non-invasively with an AutoFluorescence Reader (AFR), could be used to assess skin AGE accumulation. We evaluated the relationship between skin autofluorescence and the severity of diabetic neuropathy.

MATERIALS AND METHODS:
Skin autofluorescence in arbitrary units (AU) was assessed in 24 diabetic patients with a history of neuropathic foot ulceration (NP(+)), 23 diabetic patients without clinical neuropathy (NP(-)) and 21 control subjects, using the AFR. Arterial occlusive disease was excluded in all. The severity of foot ulceration was assessed by the Wagner score. Peripheral nerve function was assessed by neurography, measuring motor and sensory nerve conduction velocity and amplitude of the median, peroneal and sural nerves. Heart rate variability (HRV) and baroreflex sensitivity (BRS) were measured by Finapres to assess autonomic nervous function.

RESULTS:
Autofluorescence was increased in NP(-) compared with control subjects. In NP(+) patients, autofluorescence was further increased and correlated with the Wagner score. Autofluorescence correlated negatively with nerve

conduction velocity and amplitude, HRV and BRS in both NP(+) and NP(-) groups. Autofluorescence correlated with age, diabetes duration, mean HbA(1)c of the previous year, serum creatinine level, presence of microalbuminuria and severity of diabetic retinopathy.

CONCLUSIONS/INTERPRETATION:
Skin autofluorescence correlates with the severity of peripheral and autonomic nerve abnormalities in diabetes, even before being clinically manifest. The AFR may be a convenient and rapid clinical tool for assessing risk of progression of long-term diabetic complications.

Clinical study in diabetes on risk for retinopathy and neuropathy

Source: American Diabetes Association 2007 annual meeting Poster

Title: Skin Autofluorescence: a Tool to Identify Type 2 Diabetic Patients at Risk for Developing Retinopathy or Neuropathy

Authors: Gerrits EG, et al.

Skin autofluorescence (AF) as a measure of tissue AGE-accumulation, might become a helpful non-invasive tool for predicting microvascular and macrovascular disease in type 2 diabetic patients (T2DM). Recent studies have already shown a relationship between skin AF and diabetic complications and its predictive value in total and cardiovascular mortality in T2DM-patients. In this analysis, we studied the predictive value of skin AF for the development of diabetic retinopathy and neuropathy.

Our study group consisted of 973 well-controlled T2DM-patients: 47% men and 19% smokers; mean age 66 years, mean HbA1c 7.0% and mean diabetes duration 6.3 years. After 3.5 years follow up (FU), 881 patients (86 died and 6 were lost to follow up) were included in the analysis. Skin AF was assessed at the lower arm at baseline (BL) and expressed as arbitrary units (AU).

At BL, presence of retinopathy and neuropathy was related to higher levels of skin AF. Retinopathy developed in 61 patients (7%); skin AF in those 7% did not significantly differ from the skin AF of patients who already had retinopathy at BL. Skin AF was significantly higher in these two groups compared to patients who did not develop retinopathy during follow up (p

0.048*, respectively p 0.001**). Comparable results were seen for neuropathy; newly developed neuropathy was diagnosed in 151 patients (17.2%). Skin AF at BL was higher in both patient groups with neuropathy at BL or at FU compared to patients who did not develop neuropathy (p < 0.01#). Seventy subjects (8%) showed signs of neuropathy at BL and not at FU.

Retinopathy	**BL - / FU-**	**BL - / FU +**	**BL + / FU +**
N (%)	647 (73.8)	61 (7)	169 (19.3)
Skin AF (AU)	2.69 ± 0.73	2.88 ± 0.74 *	2.91 ± 0.72**
Neuropathy	**BL - / FU-**	**BL - / FU +**	**BL + / FU +**
N (%)	511 (58.3)	151 (17.2)	145 (16.5)
Skin AF (AU)	2.65 ± 0.72	2.86 ± 0.72#	2.94 ± 0.74#

Our study shows that skin autofluorescence may help to identify T2DM-patients at risk for developing retinopathy or neuropathy.

Clinical study in diabetes on cardiac mortality

Source: Diabetes Care 2007, 30:107-112

Title: Skin autlfluorescence is a strong predictor of cardiac mortality in diabetes

Authors: Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans RO, Smit AJ.

OBJECTIVE:
Advanced glycation end products (AGEs) are biomarkers of metabolic stress and are thought to contribute to the increase of coronary heart disease (CHD) in diabetes. Tissue autofluorescence is related to the accumulation of AGEs. The aim of the present study was to evaluate the relationship between skin autofluorescence and metabolic burden (hyperglycemia and hyperlipidemia) and its relationship with CHD and mortality.

RESEARCH DESIGN AND METHODS:
Skin autofluorescence was measured noninvasively with an autofluorescence reader in 48 type 1 and 69 type 2 diabetic patients and 43 control subjects.

The presence of CHD was observed at baseline and mortality during a follow-up period of 5 years.

RESULTS:
Autofluorescence correlated with mean A1C, triglycerides, and LDL. Autofluorescence values further increased with age, microalbuminuria, dialysis treatment, and diabetes duration. Autofluorescence was strongly related to the presence of CHD (odds ratio 7.9) and predicted mortality (3.0).

Multivariate analysis showed that autofluorescence was more strongly associated with CHD and mortality compared with A1C, triglycerides, and LDL.

CONCLUSIONS:
Skin autofluorescence is strongly related to cumulative metabolic burden. Skin autofluorescence seems strongly associated with cardiac mortality and may provide important clinical information for risk assessment.

Clinical study in hemodialysis

Source: J. Am. Soc. Nephrol 2005; 16:3687-93

Title: Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation endproducts, predicts mortality in hemodialysis patients.

Authors: Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, Thorpe SR, Baynes JW, Navis G, Gans RO, Smit AJ.

Abstract

Tissue advanced glycation end products (AGE) are a measure of cumulative metabolic stress and trigger cytokines driven inflammatory reactions. AGE are thought to contribute to the chronic complications of diabetes and ESRD. Tissue autofluorescence is related to the accumulation of AGE. Therefore, skin autofluorescence (AF) may provide prognostic information on mortality in hemodialysis (HD) patients.

Skin AF was measured noninvasively with an AF reader at baseline in 109 HD patients. Overall and cardiovascular mortality was monitored prospectively during a period of 3 yr. The AF reader was validated against AGE contents in skin biopsies from 29 dialysis patients.

Forty-two of the 109 (38.5%) HD patients died. Cox regression analysis showed that AF was an independent predictor of overall and cardiovascular mortality (for overall mortality odds ratio [OR] 3.9), as were pre-existing cardiovascular disease (CVD; OR 3.1), C-reactive protein (OR 1.1), and serum albumin (OR 0.3).

Multivariate analysis revealed that 65% of the variance in AF could be attributed to the independent effects of age, dialysis and renal failure duration, presence of diabetes, triglycerides levels, and C-reactive protein. AF was also independently linked to the presence of CVD at baseline (OR 8.8; $P < 0.001$). AF correlated with collagen-linked fluorescence ($r = 0.71$, $P < 0.001$), pentosidine ($r = 0.75$, $P < 0.001$), and carboxy(m)ethyllysine (both $r = 0.45$, $P < 0.01$).

Skin AF is a strong and independent predictor of mortality in ESRD. This supports a role for AGE as a contributor to mortality and CVD and warrants interventions specifically aimed at AGE accumulation.

Clinical study in renal transplant

Source: Nephrol Dial Transplant. 2006 Aug;21(8):2263-9

Title: Risk factors for chronic transplant dysfunction and cardiovascular disease are related to accumulation of advanced glycation end-products in renal transplant recipients.

Authors: Hartog JW, de Vries AP, Bakker SJ, Graaff R, van Son WJ, van der Heide JJ, Gans RO, Wolffenbuttel BH, de Jong PE, Smit AJ.

BACKGROUND:
Accumulation of advanced glycation end-products (AGEs) has been implicated in the pathogenesis of chronic transplant dysfunction and cardiovascular disease in renal transplant recipients. We aimed to investigate which factors are associated with tissue AGE accumulation in renal transplant recipients.

METHODS:
The AGE accumulation was assessed using a validated skin-autofluorescence reader (AFR) in 285 consecutive renal transplant recipients (57% male, aged 50+/-12 years) visiting the outpatient clinic at a median (interquartile range) time of 73 (32-143) months after transplantation. Furthermore, various transplant- and recipient-related factors of interest were collected.

RESULTS:
Average skin-autofluorescence of lower arm and leg was 2.7+/-0.8 a.u. Skin-autofluorescence was positively determined by recipient age, systolic blood pressure, smoking, high-sensitivity C-reactive protein, duration of pre-transplant dialysis, and negatively by plasma vitamin C levels, creatinine clearance at baseline, and change in creatinine clearance since one year after transplantation in linear multivariate regression analysis. Together, these factors explained 41% of the variance of skin-autofluorescence.

CONCLUSIONS:
Skin-autofluorescence was associated with several risk factors for cardiovascular disease and chronic renal transplant dysfunction. These results are in line with the hypothesis that AGEs play a role in the pathogenesis of these conditions in renal transplant recipients. Prospective studies are required to investigate whether the AFR can be used as a simple, non-invasive tool to identify and monitor patients at risk for chronic renal transplant dysfunction and cardiovascular disease.

Clinical study in renal transplant dysfunction

Source: Am J Kidney Dis. 2004 Jun;43(6):966-75

Title: Advanced glycation end products in kidney transplant patients: a putative role in the development of chronic renal transplant dysfunction.

Authors: Hartog JW, Smit AJ, van Son WJ, Navis G, Gans RO, Wolffenbuttel BH, de Jong PE.

Abstract

Chronic renal transplant dysfunction is one of the leading causes of graft failure in kidney transplantation. A complex interplay of both alloantigen-

related and alloantigen-unrelated risk factors is believed to underlie its development. We propose that advanced glycation end products (AGEs) are involved in the development of chronic renal transplant dysfunction.

AGE formation is associated with different alloantigen-unrelated risk factors for chronic renal transplant dysfunction, such as recipient age, diabetes, proteinuria, hypertension, and hyperlipidemia. In vitro studies have shown that AGEs induce the expression of various mediators associated with chronic renal transplant dysfunction.

Furthermore, AGE-induced renal damage has been found in multiple experimental studies. This renal damage shows similarity to the damage found in chronic renal transplant dysfunction. Together, several lines of evidence support a role of AGEs in the development of chronic renal transplant dysfunction and suggest that preventive therapy with AGE inhibitors may be helpful in preserving renal function in transplant recipients.

Clinical study in Coronary Artery Disease

Source: Atherosclerosis. 2007 May 11

Title: Skin autofluorescence is elevated in patients with stable coronary artery disease and is associated with serum levels of neopterin and the soluble receptor for advanced glycation end products.

Authors: Mulder DJ, van Haelst PL, Gross S, de Leeuw K, Bijzet J, Graaff R, Gans RO, Zijlstra F, Smit AJ.

AIMS:
To investigate whether skin autofluorescence (AF), a non-invasive marker for advanced glycation end products (AGEs), is elevated in stable coronary artery disease (sCAD) and to investigate its relationship with serum levels of the soluble receptor for AGEs (sRAGE), neopterin and C-reactive protein (CRP).

METHODS AND RESULTS:
Skin AF and serum levels of sRAGE, neopterin and CRP were assessed in 63 sCAD patients (mean age: 64.7+/-10.5 years), comprising 78% males, 19% subjects with diabetes, and 22% current smokers and in 33 (mean age: 63.4+/-10.0 years) healthy non-diabetic and non-smoking age and gender

matched controls. Skin AF was significantly increased in sCAD compared with controls, irrespective of diabetes, current smoking and renal function. Levels of sRAGE (standardized beta: 0.43 (explaining 17% of variance in skin AF); P<0.001), neopterin (beta: 0.36 (11%); P=0.003) and glucose (beta: 0.29 (8%); P=0.0011) as well as current smoking (beta: 0.26 (6%); P=0.024) were independently associated with skin AF (R(2) 0.42), whereas the association of gender, former smoking, body mass index, CRP, lipids, creatinine clearance and pulse pressure with skin AF was not significant in this model.

CONCLUSION:
These data demonstrate that skin AF is elevated in sCAD and is related to sRAGE and neopterin, making it an easily applicable tool to improve our understanding of inflammatory and oxidative stress in cardiovascular disease.

Clinical study in Acute Myocardial Infarction

Source: Circulation, AHA 2005 Abstract

Title: Skin Autofluorescence is an in dependent Marker for Acute Myocardial Infarction

Authors: Mulder DJ, van Haelst PL, Graaff R, Smit AJ, Gans RO, Zijlstra F,

INTRODUCTION:
Acute myocardial infarction is associated with an increased burden of oxidative stress, enhancing the intracellular formation of stable Advanced Glycation Endproducts (AGEs) and their precursors. Some AGEs encompass a characteristic fluorescence pattern, which can be non-invasively measured as skin fluorescence (AF), using the AGE-reader. This is a new device for assessing oxidative stress in tissue in cardiovascular disease.

AIM:
A case control study to investigate whether AF is associated with acute ST-elevation myocardial infarction (STEMI) as compared with patients with stable coronary artery disease (SCAD), matches for age and sex.

METHODS:

AF was defined as the average light intensity in the range of 420-600 nm divided by average light intensity in the range of 300-420 nm (emission/excitation) and was measured on the lower arm, using the AGE-reader. AF was assessed in patients with STEMI within 48 hours following PCI and in SCAD patients scheduled for elective CAG. Patients with impaired renal function, age<30 years, heart failure, infection or a dark skin type were excluded. Additionally, routine admission blood results were evaluated.

RESULTS:
Ninety-two STEMI (mean age:64.3+/-12.8 years; 77.2% male; smoking:42.&%; diabetes:27.2%) and 81 SCAD (mean age: 63.9 +/- 10.3 years; 74.1% male; smoking:23.9%;diabetes:15.0%) patients were included. The mean AF was significantly higher in STEMI patients, after correction for diabetes, smoking cholesterol. WBC and cardiovascular history (OR 2.98; p 0.017). In STEMI patients, 53.3% of the total variance in AF could be explained by age, history of peripheral vascular disease, HbA1c and—remarkably– CRP(p<0.0001).

CONCLUSION:
These data demonstrate that enhance skin autofluorescence is independently associated with STEMI and is strongly related to CRP and HbA1c, making it a promising tool for assessing inflammation and/or (glyc)oxidative stress in high-risk cardiovascular patients.

Skin Autofluorescence is additive to the UKPDS Risk-score

Source: American Diabetes Association 2007 annual meeting Poster

Title: Skin Autofluorescence is Additive to the UKPDS Risk-score in the Prediction of Cardiovascular Complications in Type 2 Diabetes

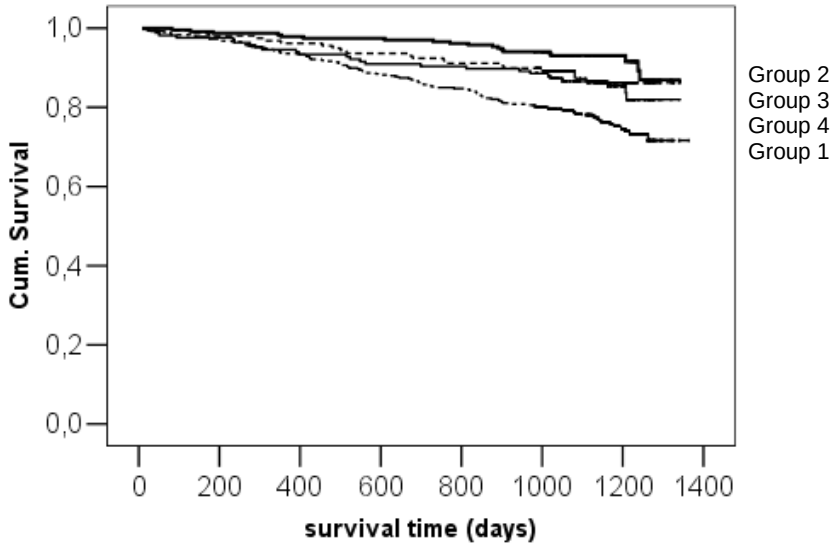
Authors: Helen L. Lutgers, Esther G. Gerrits, Henk J. Bilo, Andries J. Smit.

Increased formation and accumulation of advanced glycation endproducts (AGEs) is one of the pathogenetic mechanisms of accelerated atherosclerosis in T2DM. Some AGEs have characteristic fluorescent properties, which can be non-invasively measured as skin autofluorescence (AF). We have previously demonstrated the relation between skin AF and chronic diabetes complications. This study aims to assess whether skin AF is additive to

classical risk factors combined in the UKPDS risk-engine in predicting cardiovascular disease.

In 2001, 973 primary care T2DM-patients were included (mean age 66 yrs, mean HbA1c 7.0% and mean diabetes duration of 6.3 yrs). Skin AF, using a prototype of the current AGE reader, and UKPDS risk scores were assessed at baseline. The UKPDS risk-engine, downloaded from the UKPDS website, was used. Follow-up ended at January 2005. Primary endpoint was mortality or non-fatal cardiovascular event (CHD, CVA, PVD). Patients were categorized in four groups: both AF and UKPDS risk score respectively above (1) and below (2) the median, and, either AF (3) or UKPDS risk score (4) above the median (Fig 1).
During a follow-up of about 3.5 years, 6 patients were lost to follow-up. In the remaining 967 patients, there were 161 events. Kaplan-Meier survival curves (Fig 1) showed significant differences between the groups, except between group 3 and 4 ($p<0.05$).

Autofluorescence has an additional value to the UKPDS risk-score in the risk assessment of the occurrence of cardiovascular complications.



Overview of current clinical studies

Source: Diabetes Technology & Therapeutics Oct 2006, Vol. 8, No. 5 : 523 -535

Title: Skin Autofluorescence, a Novel Marker for Glycemic and Oxidative Stress-Derived Advanced Glycation Endproducts:

An Overview of Current Clinical Studies, Evidence, and Limitations

Authors: Mulder DJ, van de Water T, Lutgers H, Graaff R, Gans RO, Zijstra F, Smit AJ.

BACKGROUND:
Advanced glycation endproducts (AGEs) predict long-term complications in age related diseases. However, there are no clinically applicable markers for measuring AGEs in vivo.

METHODS:
We have recently introduced the AGE-Reader (DiagnOptics B.V., Groningen, The Netherlands) to noninvasively measure AGE accumulation in the human skin of the forearm, making use of the characteristic autofluorescence (AF) pattern that AGEs encompass. Skin AF is calculated as a ratio of mean intensities detected from the skin between 420–600 nm and 300–420 nm. It correlates with collagen-linked fluorescence and specific skin AGE levels from skin biopsies in diabetes, renal failure, and control subjects. Skin AF levels are increased in patients with diabetes and renal failure and are associated with the presence of vascular complications. Additionally, skin AF is strongly related to the progression of coronary heart disease and mortality, independently of traditional risk factors. Since skin pigmentation might influence skin AF, we have investigated the relation of relative skin reflectance (R%) to skin AF in subjects with varying skin phototypes (SPT).

RESULTS:
The data presented in this article suggest that only in subjects with an SPT of V and VI or R% <12%, no reliable measurement can be performed. Therefore, the current prototype of the AGE-Reader is suitable for subjects with SPT I–IV or R% >12%, and more research is needed for a broader application.

CONCLUSION:
The AGE-Reader is useful as a noninvasive clinical tool for assessment of risk for long-term vascular complications in diabetes and in other conditions associated with AGE accumulation.

PUBLICATION LIST

Skin autofluorescence is a strong predictor of cardiac mortality in diabetes
Meerwaldt R, et al.
Diabetes Care 2007, 30: 107-112

Skin Autofluorescence is Additive to the UKPDS Risk-score in the Prediction of Cardiovascular Complications in Type 2 Diabetes
Lutgers HL, et al.
ADA 2007 Poster

Skin Autofluorescence: a Tool to Identify Type 2 Diabetic Patients at Risk for Developing Retinopathy or Neuropathy
Gerrits EG, et al.
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Advanced glycation end products and the absence of premature atherosclerosis in glycogen storage disease Ia.
den Hollander NC, et al. ,
J Inherit Metab Dis. 2007 Jun 14;

Skin Autofluorescence is a Non-invasive Marker for Inflammatory Stress in Stable Coronary Artery Disease
Mulder DJ. et al.
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Skin autofluorescence is elevated in patients with stable coronary artery disease and is associated with serum levels of neopterin and the soluble receptor for advanced glycation end products.
Mulder DJ, et al
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The Effect of Aggressive Versus Conventional Lipid-lowering Therapy on Markers of Inflammatory and Oxidative Stress.
Mulder DJ, et al.
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Blaauw J, et al.
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Risk factors for chronic transplant dysfunction and cardiovascular disease are related to accumulation of advanced glycation end-products in renal transplant recipients
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Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation endproducts, predicts mortality in hemodialysis patients
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Simple noninvasive measurement of skin autolfuorescence
Meerwaldt R, et al.
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Hartog JW. et al.
Ann N Y Acad Sci. 2005 Jun;1043:299-307.

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Graaff R et al.
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Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy
Meerwaldt R, et al.
Diabetologia. 2005;48:1637-44.

Skin Autofluorescence is an independent marker for Acute Myocardial Infarction
Mulder DJ, et al.
Circulation, AHA 2005 abstract.

Advanced glycation endproducts in kidney transplant patients: a putative role in the development of chronic renal transplant dysfunction
Hartog J. et al.
Am J Kidn Dis 2004; 43:966-975

Simple non-invasive assessment of advanced glycation endproducts accumulation
Meerwaldt R et al.
Diabetologia 2004; 47:1324-1330

The clinical relevance of advanced glycation endproducts (AGE) and recent developments in pharmaceutics to reduce AGE accumulation.
Smit AJ, Lutgers HL.
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AGE Reader™ VALUE IN HEALTHY CONTROLS FOR DIFFERENT AGE CLASSES

The measurement unit of autofluorescence has been based on the average light intensity per nm in the range between 420-600nm divided by the average light intensity per nm in the range between 300-420nm (AF in a.u.), as measured with the prototype system that was used by Lutgers et al.

It should be noted that the AGE Reader has been validated with the prototype AGE Reader used by Lutgers et al.

Results obtained by Meerwaldt et al. have been obtained with an older prototype. The AF number in those results are, therefore, not exactly comparable in absolute terms with the numbers obtained by Lutgers et al, although the relative changes between different groups remain.

Furthermore, it should be pointed out that compared to these publications the current AGE Reader has introduced a multiplication factor of 100 to make the results more user-friendly (the skin AF value is given in arbitrary units, it is in fact a ratio).

The table below and calculation rule were generated from a study which has involved healthy controls from different decades (appr. 25-40 persons per decade, both sexes combined between 20 and 80 years, with check for absence of diabetes and renal function abnormalities, and all within American Society of Anesthesiologists functional class I-II).

Part of the reference values given below have been published by Lutgers (Diabetes Care 2006):

Skin autofluorescence of Control Subjects

age	ASA1+2	s.d.	Fit	s.e.m.
20-30	1.68	0.31	1.53	0.075
30-40	1.84	0.46	1.75	0.07
40-50	1.84	0.39	1.97	0.06
50-60	2.10	0.34	2.20	0.04
60-70	2.51	0.60	2.42	0.10
70-80	2.71	0.56	2.65	0.08

The Column "Fit" shows results obtained with the calculation rule $AF = 1.5 + 0.000215 \times age^2$, is a parabolic fit of AF as a function of calendar age data, which allows to compare measured autofluorescence value to confidence intervals which have been derived from autofluorescence values.

It should be noted that these results include smokers (36%). For the non-smokers the values are somewhat lower, and for the smokers somewhat higher. A more detailed publication of the results in control subjects is in preparation. Soon AF values for younger childhood age ranges will become available.

FREQUENTLY ASKED QUESTIONS

Clinical questions

What are the normal ranges of AGE levels for healthy people ?

The normal ranges of the AGE levels increase as the age group is getting older. In addition, it varies slightly between male and female. For people under the age of 60 years old, the normal AGE value should be below 2.2.

As a rule of thumb:
If the AGE level is higher than 3, there is definitely increased risk
If the AGE level is lower than 2, there is definitely no increased risk
Anything in between should be evaluated against age and gender etc.

Please refer to the table on page 32 for detailed explanation

How soon can we see improvement of the AGE level after treatment or corrective actions have been taken?

In stable patients changes will be slow, thus a measurement each half year is a reasonable time frame to see the improvement.

What additional information does the AGE-Reader give me in my diabetes patients, and does this have any effect on treatment decisions?

Although current guidelines advise more or less similar treatment with a combination of drugs together with antidiabetics in all type II diabetes patients, it is beyond doubt that the cardiovascular risk varies quite considerably within this group of patients. The results from the AGE reader can help you to identify those at highest risk. This additional information can help you to more aggressively treat those patients who are at high risk.

As far as I know no intervention is available to prevent or treat AGE accumulation. Why should I then measure the AGE level ?

Indeed no AGE-breakers have reached the market so far. However, all antidiabetics reduce AGE formation, and some antihypertensive drugs like angiotensin-converting enzyme inhibitors and angiotensin receptor blockers do the same. Moreover, as discussed above in the previous question, the AGE level may also affect treatment decisions when targeting blood pressure or lipid levels.

Is the AGE-reader only useful in patients with diabetes and renal failure ?

No, the AGE reader has also in non-diabetic persons with normal renal function at moderately increased cardiovascular risk independent and additional power to identify those with high IMT. Also in some acute conditions like acute coronary syndromes and severe infections the AGE reader provides relevant information.

Technical questions

Can I use the AGE-Reader safely in all my patients?

The AGE-Reader has been extensively tested in thousands of adults for several years without any adverse effects. The AGE Reader has CE approval to be used as a medical device and thus has passed the rigid safety standard. The amount of ultraviolet light exposure is actually far below the safety limits.

Can I use the AGE Reader to measure other parts of the body other than the forearm?

Yes, it can be used on other parts of the body and we have performed extensive sets of measurements on the lower leg (which give slightly higher values). The point is that no clear reference values are available for these other sites. Measurements at the hand palms have been done and are not equal to those on the lower arm!

Will the AGE level be influenced by the different measurement time of the day or types of food eat before the measurement?

No clear effects of the time of the day are present. When a person eats and drinks a meal very high in AGE content this may result in a small 5-7% increase in skin AF.

How to operate the AGE reader? Do I need trained personnel?

No, the device is almost fully automatic and gives straightforward measurement results. Thus anyone who is able to use a computer can operate the device.

Are there any precautions on the use of the AGE reader?

It is important to know that a good measurement should be conducted at a healthy skin area with no presence of abnormal looking skin such as eczema, scars or tattoo. In addition please be aware that applications of skin care cream, sun block and skin browning agents at the site of measurement in the hours before can make the measurement results unreliable.

The advice is to routinely ask the patients before the measurement if he/she has used such agents at the site of measurement. If this is the case, thorough cleaning of the skin may often help. But it is preferable to perform the measurement at another site, not exposed to such agents, or to do the measurement on another occasion when no skin agents have been used in the preceding hours.

CLINICAL CASE STUDY

An example about how the AGE value affects the prognosis of a diabetes patient, independent of the UKPDS score

Suppose a female patient, 60 years old, diabetes type II since 10 years, not smoking, does have peripheral vascular disease, her systolic blood pressure is 150 mmHg, HbA1c 7.5%; total cholesterol 4.5 mmol/l, HDL-cholesterol 1.2 mmol/l.

If we enter these data in the UKPDS risk engine, she has a 10-year risk of fatal coronary heart disease of 7.6%, and of fatal stroke of 1 %.

If this lady has an AF-value = 3.6, her median life expectancy will be 9.5 year (using a multivariate Cox-regression model).

If in this same lady on the other hand her AF is 2.4, her median life expectancy becomes then 14.4 years.

So, substantial differences exist in life expectancy which just depend on her AF value. These differences may directly affect the clinical treatment policy and target levels of blood pressure and cholesterol.

Additional Reference (see elsewhere for those specific for AGE reader)

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