Advanced glycation end products in patients with peripheral artery disease

de Vos, Lisanne Carlijn

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Chapter 1

General introduction
Cardiovascular disease

Cardiovascular disease is the number one cause of death in the world. An estimated 17.5 million people died from cardiovascular disease in 2012, representing 30% of all global deaths.¹

Unlike the well-known cardiovascular diseases, such as myocardial infarction and stroke, there are also other vascular diseases, which are lesser-known to the general public. Since several guidelines use different definitions, we will use the term peripheral vascular disease for all vascular diseases besides coronary artery disease and cerebrovascular disease in this thesis. From a pathophysiological view, peripheral vascular disease can be divided into occluding and dilating diseases. In this thesis, we will elaborate on peripheral artery disease as an example of occluding vascular disease and abdominal aortic aneurysm as an example of dilating vascular disease.

Peripheral artery disease

Peripheral arterial occlusive disease, in this thesis referred to as peripheral artery disease (PAD) can cause stenosis or occlusion of the arteries of the lower limbs. The most common underlying disease is atherosclerosis. Atherosclerosis is characterized by inflammation of the tunica intima of the arterial wall, followed by increased endothelial permeability, infiltration with macrophages, entry and retention of cholesterol, and recruitment of smooth muscle cells which form a fibrous cap.² Within years, these pathophysiological changes may result in development of symptoms in patients.

The main presenting symptom of these patients is intermittent claudication, which is typical leg pain during walking that disappears during rest. The severity of symptoms may vary from mild intermittent claudication after walking ≥100 meters to rest pain, with or without ulceration or necrosis of the distal part of affected limb(s) and is generally categorized using the so-called Fontaine classification (Table 1). In addition to the local burden of disease, PAD patients often suffer from systemic atherosclerosis with a subsequent increased risk for cardiovascular events such as myocardial infarction, ischemic stroke and death.

<table>
<thead>
<tr>
<th>Stage</th>
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<tbody>
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<tr>
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Several risk factors contribute to the development of PAD. Most of these risk factors are similar to the risk factors of other cardiovascular diseases. Age and smoking are the most important risk factors for PAD. PAD rarely occurs below 40 years of age, while the prevalence increases to 16% in the general population above 80 years. As a result of the improving life expectancy, the prevalence of PAD has increased with almost 25% within ten years (2000-2010). The number of pack-years of cigarettes is directly related to the incidence of PAD. Conversely, the number of years since cessation of smoking is negatively associated with the incidence of PAD. Men are at increased risk for severe PAD as compared to women, however this sex difference between men and women are less clear than in other cardiovascular disease. Other treatable risk factors are diabetes mellitus, hypertension and hypercholesterolemia. The incidence of PAD increases with the number of risk factors present in the patient.

Treatment of patients with PAD consists of improving the impaired blood flow of the affected limb as well as reducing general burden of atherosclerosis and the associated risk of cardiovascular events. Patients with intermittent claudication are advised to perform supervised exercise training to stimulate revascularization through the formation of collaterals. If progression of the symptoms occurs even with exercise training, invasive treatment options may be considered, including percutaneous transluminal angiography or endoluminal recanalization, bypass surgery, or endarterectomy procedures. The choice between the different procedures depends on the severity, the length, and the location of the stenosis or occlusion. Pharmacological treatment of local symptoms in PAD has very limited efficacy, and is usually limited to use of intravenous vasodilators such as prostanoids in Fontaine stage III-IV patients in whom reconstructive treatment is not possible or has failed. Finally, amputation is treatment of last resort in Fontaine stage IV patients, especially in case of concomitant local infections. Treatment of cardiovascular risk factors to prevent cardiovascular events consists of lifestyle improvement and pharmacotherapy similar to that in other forms of cardiovascular disease. Life style advice includes smoking cessation, increase of the level of physical activity, and reduction of overweight.

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Table 1. Fontaine classification

Pharmacological
treatment consists of lipid-lowering drugs, antihypertensive drugs, platelet aggregation inhibitors and optimization of glycemic control of diabetes mellitus if present.  

**Abdominal aortic aneurysm**

The most frequent type of dilating vascular disease is an aneurysm of the abdominal aorta (AAA). The definition of an AAA is a dilation of the aorta of ≥30 mm. AAA is characterized by inflammation of the tunica media with metalloproteinase (MMP) activation, causing proteolysis and smooth muscle cell apoptosis.

An AAA is generally asymptomatic until sudden rupture of the aneurysm occurs. In case of rupture, the clinical presentation usually includes acute abdominal pain and hemodynamic shock. The mortality rate in patients with a ruptured AAA is about 80-90%. The risk of rupture is associated with the maximal diameter of the aneurysm. The 12-month rupture risk increases with diameter, with a 0% rupture risk between a diameter of 30-39 mm, 1-11% risk between 50-59 mm, and 30-33% risk of rupture above a diameter of 70 mm.

Although the pathophysiological changes in dilating disease (media weakening) and occlusive disease (intima inflammation) differ, AAA and PAD share several risk factors such as smoking, age, male sex, and hypertension. Consequently, AAA and PAD often coexist in one patient. Still, while hypercholesterolemia is a strong risk factor for atherosclerosis, it is not associated with the presence of AAA. An even larger contrast between atherosclerosis and AAA concerns the presence of diabetes mellitus, which is a major risk factor for atherosclerosis, but has an inverse association with the prevalence of AAA and growth of the aneurysm.

Treatment options in AAA consist of surveillance or intervention in combination with pharmacological treatment. Surgical treatment of the aneurysm is recommended from a diameter of ≥55 mm for men and ≥50 mm for women. The risk of complications during surgery is larger than the risk of rupture in small aneurysms, and, therefore, surveillance is recommended in these cases. In men with small aneurysms up to 39 mm, imaging of the aneurysm can be performed every three years, every two years in aneurysms with a diameter between 40-44 mm, and yearly in aneurysms with a diameter between 45-54 mm. In case the diameter of the aneurysm reaches the cutoff point, intervention should be scheduled. An intervention can be performed with endovascular aneurysm repair (EVAR) or with open surgery. Besides surveillance and
intervention, pharmacological treatment may be considered. Statins have besides lipid-lowering effects, anti-inflammatory and anti-oxidative stress effects. Inhibition of inflammation and oxidative stress with statin therapy results into decreased AAA progression in several animal studies. A meta-analysis of five clinical cohort studies revealed a decreased growth rate in patients with an AAA which use statins. Angiotensin-converting enzyme (ACE) inhibitors are also associated with decreased AAA growth in two large cohort studies. Although no randomized controlled trials are yet performed to confirm the effect of both medicines, the European Society of Cardiology guideline recommend consideration of statins and ACE-inhibitors in patients with an AAA. Due to the communal risk factors for atherosclerosis, antiplatelet drugs may be considered as well.

Pathophysiological markers in PAD and AAA

Identification of patients with high cardiovascular risk for primary and secondary prevention is a key part of the current cardiovascular research. Over the past two decades, endothelial dysfunction and vulnerable plaques have received most attention as initiating events in cardiovascular disease. Other mechanisms, possibly leading to cardiovascular disease, such as plaque calcification and arterial stiffness, have received less attention, and still more so in PAD, even while they are promising parameters. All mentioned markers for cardiovascular events and the association with PAD or AAA will be elaborated on in the following paragraphs.

The endothelial layer of the artery connects the blood and vascular tissue. Functions of the endothelial layer encompass vasoconstriction and vasodilation, thrombogenesis and smooth muscle cells inhibition or stimulation. Endothelial dysfunction is an early marker for atherosclerosis, causing endothelial permeability, leukocyte recruitment and production of pro-inflammatory cytokines. Several methods to measure endothelial dysfunction are known, including acetylcholine induced or flow-mediated dilatation, and circulating markers such as Factor VIII/von Willebrand factor or endothelial progenitor cells (EPCs). Indeed, flow-mediated dilatation predicts cardiovascular end points in patients with PAD after a follow-up of 1.2 years. The function and number of EPCs are also increased in PAD compared to controls.

Another important pathophysiological parameter in cardiovascular disease is plaque formation. New insights suggest that vulnerability of the plaque may serve as a marker to predict cardiovascular events. Several criteria have been marked to identify
vulnerable plaques, including inflammation, thin cap thickness and large lipid core, endothelial erosion with platelet aggregation.\textsuperscript{21} Classification of a vulnerable plaque can be assessed by plaque morphology as well as with \textit{in vivo} imaging techniques. However, the PROSPECT study showed that vulnerable plaques, detected with intravascular ultrasound (IVUS), had no additional value to predict cardiovascular events in patients with coronary artery disease.\textsuperscript{22} Future studies should indicate the additional effects to detect vulnerable plaques for cardiovascular events with different techniques and in other cardiovascular diseases.

Furthermore, details of plaque development can be assessed noninvasively with intima-media thickness (IMT). Thickness of the tunica intima combined with the tunica media can be measured with B-mode ultrasound, most frequently assessed in the carotid artery. Single measurement of IMT is an independent predictor for cardiovascular events in cardiovascular disease in general.\textsuperscript{23,24} Until data shows the additional relevant value of IMT in favor of traditional risk estimation, IMT will not be used for cardiovascular risk assessment in the general population, and even then, the labour-intensive character of the measurement will limit its widespread use. As for the predictive value of IMT in PAD patients, no supportive evidence exists.

Also, calcification is an important process which occurs in atherosclerosis. Several mechanisms are involved in the formation of calcification, including debris from apoptotic cells, loss of inhibition of mineralization molecules and induction of bone formation.\textsuperscript{25} Several imaging techniques are available to detect calcification of which computer tomography (CT) is the most important. CT-based coronary artery calcification (CAC) score can be used to reclassify patients with intermediate risk for coronary artery disease for primary prevention.\textsuperscript{26} Calcification in PAD is an important factor for the vascular surgeon, since clamping and suturing is more difficult in calcified arteries. Furthermore, medial artery calcification, also known as media sclerosis, causes an elevated ankle-brachial index in patients with diabetes mellitus and renal insufficiency.\textsuperscript{27,28} Low ankle-brachial index, but also high ankle-brachial index predict mortality.\textsuperscript{29} In patients with an AAA, a calcification score on CT is associated with rupture of the aneurysm, independently of the diameter of the aneurysm.\textsuperscript{30}

Finally, arterial stiffness increases with age as a result from a loss of elasticity of the arterial wall. Arterial stiffness is a marker for cardiovascular events and mortality in the general population, but also in patients with cardiovascular risk factors.\textsuperscript{31} Pulse wave velocity is a noninvasive technique and the gold standard to assess arterial stiffness.
Patients with PAD have increased arterial stiffness compared to patients with an AAA. Evidence for the predictive value for arterial stiffness measurements like pulse wave velocity in PAD is still lacking.

Thus, the search for good prognostic parameters or biomarkers remains challenging. One promising biomarker which is known to have an association with calcification, vulnerable plaques and arterial stiffness is advanced glycation end products (AGEs).

**Advanced glycation end products**

Advanced glycation end products (AGEs) are compounds which are non-enzymatically formed under influence of glycemic and oxidative stress on proteins. Originally, the formation of AGEs was described in the food industry. Especially heating induces the production of AGEs. In humans and animals, the body temperature contributes to the formation of AGEs.

The accumulation of AGEs can be accelerated by several diseases. For example, patients suffering from diabetes mellitus have increased AGE accumulation due to increased glycemic stress. On the other hand, patients with renal insufficiency have increased oxidative stress, and therefore have increased AGEs accumulation. Impaired excretion of free AGE and AGE peptides in renal insufficiency also contributes to increased AGE accumulation.

AGEs accumulate during healthy aging, but they also have harmful effects on the body. AGEs cause stiffness by the formation of cross-links. Stiffness of the skin causes wrinkles, which has well-known cosmetic effects. However, stiffness of the heart and arteries causes serious problems, such as heart failure or hypertension.

**Skin autofluorescence**

Assessment of AGEs can be performed noninvasively with the use of the AGE Reader™ (Figure 1). This device, developed in Groningen, The Netherlands, was first described by Meerwaldt et al. in 2004, and assesses AGEs in the skin utilizing fluorescent properties of several AGEs. Therefore the technique is called skin autofluorescence (SAF). In short, ultraviolet light with a peak excitation of 370 nm is projected on a small surface of 4 mm² of the skin of the forearm. Fluorescent emission light with a wavelength of 420-600 nm and reflection light with a wavelength of 300-420 nm is
detected with a spectrometer. SAF is calculated as the ratio between emission light divided by the reflection light.\textsuperscript{36}

The AGE Reader\textsuperscript{TM} has been used in many clinical studies, most frequently in patients with diabetes mellitus and renal insufficiency. Lutgers et al. showed increased SAF in nearly thousand patients with type 2 diabetes mellitus as compared to age-matched controls.\textsuperscript{34} In addition, SAF is an independent predictor for cardiovascular events these patients.\textsuperscript{37} Higher SAF levels, and an independent predictive value of SAF for cardiovascular disease and mortality, were also found in persons with renal failure on hemodialysis.\textsuperscript{38} Hartog et al. showed increased SAF as a predictor of graft loss in renal transplant patients.\textsuperscript{39}

Figure 1. AGE Reader\textsuperscript{TM}

In addition, Lutgers et al. showed the link between SAF and subclinical atherosclerosis.\textsuperscript{40} The results of this study showed a positive association between SAF and IMT, a noninvasive technique which measures the carotid artery for subclinical atherosclerosis.\textsuperscript{40} Moreover, SAF was also increased in clinical atherosclerosis, as shown in patients with a myocardial infarction. Both, patients with stabile coronary artery disease, as well as patients with ST-elevation myocardial infarction had increased SAF as compared to controls.\textsuperscript{41}

The first evidence for an association between widespread atherosclerosis and SAF was shown by Noordzij et al. A total of 56 patients with carotid artery stenosis were compared to age- and sex-matched controls, which resulted in increased SAF in the carotid artery group.\textsuperscript{42} A total of 46% of the carotid artery patients suffered from widespread atherosclerosis, defined as coronary artery disease or PAD. Furthermore, the most important determinants of SAF were age, smoking, diabetes mellitus, renal
function and PAD, but not coronary artery disease. The results of Noordzij et al. were the first findings of a new research line which was started at the University Medical Center Groningen (UMCG). This collaboration between the vascular surgeons and the vascular internists was created in 2006. The research question whether SAF was associated with occluding and dilating vascular disease resulted in this thesis.

Outline of the thesis

In this thesis, our aim is to evaluate the role of SAF as a marker of cardiovascular events and changes in renal function in patients with PAD and AAA. The primary focus is on patients with PAD.

AGEs are compounds that accumulate during life. The formation, accumulation, and excretion of AGEs are complex. Furthermore, measurement of AGEs is not restricted to the noninvasive SAF method. Detailed background of AGEs and the available research of AGEs in PAD is reviewed in Chapter 2. Since the start of the research collaboration in 2006, patients with vascular disease were included into a large study cohort. Almost 500 patients with PAD were included, within the period 2007 and 2011. SAF was measured in these patients, and they were compared to age- and diabetes-matched controls. Results of this study are presented in Chapter 3. A part of these PAD patients were followed for five years. During these years, cardiovascular end points, end points for local limb outcome, and end points for renal function decline were assessed. In Chapter 4, we elaborate on the association between SAF and all-cause mortality and fatal and nonfatal major adverse cardiovascular events. In Chapter 5, the association between SAF and amputation is shown in PAD patients. Chapter 6 shows the association between SAF and renal function decline in this cohort. Also a cohort of patients with an AAA were included in the same period as the PAD patients. In Chapter 7 we compare SAF between patients with an AAA and controls. As mentioned above, diabetes mellitus has paradoxical effects in PAD and AAA. Diabetes mellitus is a risk factor for PAD, while diabetes mellitus is less common in AAA and growth of the aneurysm is decreased in diabetic patients. To evaluate the role of AGEs as explanatory factor for the different effect of diabetes mellitus in occluding and dilating disease, the ARTERY study was designed. A protocol of this study is included in Chapter 8. In Chapter 9, all these chapters will be summarized, discussed and future research and possible clinical perspectives of AGEs in PAD and AAA will be reflected on.
References


General Introduction


Chapter 2

Advanced glycation end products: an emerging biomarker for adverse outcome in patients with peripheral artery disease

L.C. de Vos, J.D. Lefrandt, R.P.F. Dullaart, C.J. Zeebregts, A.J. Smit