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Mediators of cardiovascular risk in diabetes mellitus

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***Mediators of Cardiovascular Risk
in Diabetes Mellitus***

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in Diabetes Mellitus*

Cornelis J. Roos

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***Mediators of Cardiovascular Risk
in Diabetes Mellitus***

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CHAPTER I

General introduction and outline



Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disease defined by dysregulation of blood glucose levels.¹ DM is classified in type 1 DM and type 2 DM by the American Diabetes Association.² Type 1 DM is characterized by insulin deficiency as a result of destruction of insulin producing beta cells in the pancreas through an auto-immune response. Consequently, type 1 DM can be diagnosed by demonstration of antibodies.

In contrast, type 2 DM is caused by insulin resistance. Insulin resistance is raised by increasing fat mass and therefore, the risk to develop type 2 DM is higher in obese subjects.³ The presence of DM type 2 is often accompanied by one or more other cardiovascular risk factors, such as obesity, hypertension and dyslipidemia. As compared to type 1 DM, DM type 2 is more common and develops at a later age.

The global prevalence of DM is strongly increasing and has been estimated to rise from 360 million in 2011 to 552 million in 2030.⁴ Also in Europe, the prevalence of DM will increase from 53 million to 64 million.⁵

High blood glucose levels of DM induce endothelial dysfunction and atherosclerosis, causing micro- and macrovascular disease.³ Therefore, patients with DM often have retinopathy, nephropathy and neuropathy and are at increased risk for stroke, coronary artery disease (CAD) and peripheral artery disease. One of the most important complications of DM is CAD.⁶

DM and CAD

Patients with DM have an increased risk of CAD and high prevalence of cardiac morbidity and mortality. In a Dutch population cohort, an incidence rate ratio for CAD of 2.81 was demonstrated for DM patients as compared to non-DM subjects.⁷

However, identification of CAD in patients with DM is difficult, because CAD mostly progresses without symptoms. Moreover, available cardiovascular risk prediction models based on patients clinical and demographic parameters seem inadequate for the identification of diabetic patients at high risk for cardiovascular events.⁸ Consequently, CAD is not recognized and proper treatment is delayed, leading to more extensive CAD and a higher incidence of cardiovascular events. This underlines the need for sensitive diagnostic tools to improve the early identification of CAD and allow for the initiation of individually tailored therapeutic strategies. Accordingly, identified high-risk patients should receive cardiovascular medication that is effective in reducing cardiac morbidity and mortality and can be referred for further diagnostic and therapeutic procedures.

Cardiovascular risk assessment in patients with diabetes

Cardiovascular risk assessment in diabetic patients remains challenging. Risk scores to predict cardiovascular risk are widely used, but the risk scores are developed in the

general population and tend to underestimate the cardiovascular risk of DM patients. Risk scores developed in diabetic populations to estimate cardiovascular risk have demonstrated good calibration and discriminations indices. However, external validation is still needed. A recent meta-analysis which reviewed 17 risk scores showed that the predictive ability of these scores developed in diabetic populations is not superior to those scores developed in general population.⁹ Accordingly, the additional use of other biomarkers or imaging tools seems a good alternative to better risk stratify diabetic patients.

This thesis evaluates the application and performance of non-invasive cardiac imaging tests for cardiovascular risk assessment and management of DM patients. Identification of new markers of CAD derived from non-invasive cardiac imaging might result in a broader applicability of cardiovascular risk assessment. Non-invasive cardiac imaging tests might evaluate target organ damage as well as the presence, severity and extent of subclinical atherosclerosis preceding overt clinical CAD. By this means, high-risk patients for CAD can be identified and further decision making of each DM patient can be tailored in order to improve the clinical outcomes at long-term follow-up.

Outline of the thesis

The aim of this thesis is to study the application and performance of various non-invasive imaging tests in cardiovascular risk stratification and management of DM patients. **Chapter 2** provides an overview of mechanisms and mediators involved in the pathophysiology of obesity leading to CAD and the applicability of such mediators for the identification of patients at high risk of CAD. **Chapter 3** describes non-invasive cardiac imaging of coronary artery anatomy with computed tomography coronary angiography (coronary CTA) and coronary artery function with single-photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI) and clinical value of combined anatomic and functional imaging. **Chapter 4** investigates the relation of non-invasively measured arterial stiffness with stress perfusion defects on SPECT MPI.

Chapter 5 studies the relation of indices of arterial stiffness with left ventricular (LV) diastolic function as assessed with echocardiography. **Chapter 6** assesses the change in LV function after 2 years follow-up, using conventional and 2-dimensional speckle tracking echocardiography in clinically stable type 2 DM patients.

In **Chapter 7**, the feasibility of analysis of atherosclerosis in the descending thoracic aorta (DTA) on coronary CTA was evaluated in patients with suspected CAD with or without DM. Subsequently, associations of DTA atherosclerosis with CAD were determined. In **Chapter 8** analysis of DTA atherosclerosis on coronary CTA was used in DM patients to study the association of DTA atherosclerosis with indices of arterial stiffness and measures of renal function (estimated glomerular filtration rate (eGFR) and urinary albumin creatinine clearance (UACR)).

Chapter 9 compares the presence, severity and extent of CAD on coronary CTA in type 2 DM South Asian patients with matched Caucasian patients.

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CHAPTER 2

*Cardiovascular metabolic syndrome:
mediators involved in the pathophysiology from
obesity to coronary heart disease*



Cornelis J Roos, Paul HA Quax, J Wouter Jukema.

Biomark. Med. 2012

Abstract

Patients with obesity and diabetes mellitus are at increased risk for cardiovascular events and have a higher cardiovascular morbidity and mortality. This worse prognosis is partly explained by the late recognition of coronary heart disease in these patients, due to the absence of symptoms. Early identification of coronary heart disease is vital, to initiate preventive medical therapy and improve prognosis. At present, with the use of cardiovascular risk models, the identification of coronary heart disease in these patients remains inadequate. To this end, biomarkers should improve the early identification of patients at increased cardiovascular risk. The first part of this review describes the pathophysiologic pathway from obesity to coronary heart disease. The second part evaluates several mediators from this pathophysiologic pathway for their applicability as biomarkers for the identification of coronary heart disease.

Introduction

The global prevalence of obesity and diabetes mellitus (DM) is increasing.¹ Patients with obesity and DM are at high risk for cardiovascular events, because the presence and progression of coronary atherosclerosis is often asymptomatic.² Consequently, there is a delayed clinical presentation with more severe coronary heart disease (CHD) and higher cardiovascular morbidity and mortality amongst these patients. This emphasizes the importance of the early recognition of CHD, in order to start preventive medical therapy and improve the prognosis. At present, with the available cardiovascular risk prediction models, the identification of those patients who are prone to cardiovascular events is still inadequate.³ Therefore, biomarkers that improve the early identification of CHD in this population are required. For this purpose, many kinds of potential biomarkers of atherosclerosis have been evaluated for their predictive value of CHD.⁴ Accordingly, the applicability of mediators involved in the pathophysiology of atherosclerosis as biomarkers of CHD has been investigated. Such mediators can be derived from the interplay between obesity, inflammation, DM and CHD.⁵⁻¹⁰ Obesity is currently considered as a constant state of low-grade inflammation that provides a direct link with atherosclerosis. Inflammation in obesity results from an altered secretion of mediators, such as adipokines and cytokines, by the increased adipose tissue mass. Besides increasing inflammation, these mediators give rise to the development of DM and the metabolic syndrome (MetS) by inducing insulin resistance, hypertension and dyslipidemia. This further contributes to the pro-inflammatory milieu that enables the progression of atherosclerosis and development of significant CHD.^{8, 11}

Of note, this review describes only a selection of the involved mediators and does not attempt to provide a complete overview of all the factors involved. The first part of this review provides a pathophysiologic background that describes a set of important mediators involved in each clinical condition and, subsequently, how these mediators interact with the other clinical conditions. Thereafter, several of these mediators are further discussed for their applicability as biomarker of CHD. To this end, for each mediator a description is given of how it influences insulin resistance and atherosclerosis, its performance in prediction of CHD events and its impact on CHD risk prediction beyond established risk factors.

Obesity and inflammation

Adipose tissue and secretion of adipokines and cytokines

Obesity is characterized by an increased adipose tissue mass which expands the subcutaneous, visceral and perivascular fat depots. Adipose tissue is considered an endocrine organ involved in the regulation of an individual's metabolism and state of inflammation.^{7,9} Adipose tissue is made up by adipocytes and vascular stromal components, which

include macrophages, fibroblasts, lymphocytes, endothelial cells, and preadipocytes.¹² Adipocytes synthesize a large array of substances that are not only involved in metabolic processes such as energy balance, appetite, insulin sensitivity and regulation of fat mass, but also in blood pressure, coagulation, immunity and inflammation. Examples of these adipocyte products are adipokines such as adiponectin, leptin, resistin, and visfatin.^{7, 13} Moreover, cytokines, chemokines and proteins such as monocyte chemo attractant protein-1 (MCP-1), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1) are released by the stromal vascular components of adipose tissue, and are involved in local and systemic inflammation.

Adipose tissue causes inflammation by altered secretion of adipokines and cytokines

Several studies have demonstrated a dose-response-like relation between obesity and inflammation by showing that an increase in adipose tissue mass in obese individuals is associated with an increased release of proinflammatory mediators, whereas the production of adiponectin, which is an anti-inflammatory mediator, is decreased (Figure 1).^{9, 14} Besides this, the increased visceral adipose tissue mass releases more free fatty acids (FFAs) into the circulation. FFAs activate nuclear factor- κ B (NF- κ B) via Toll-like receptor-4 expressed on macrophages present in the adipose tissue. Activated NF- κ B, in turn, increases the release of TNF- α from these macrophages, which induces the transcription of intracellular adhesion molecule-1 (ICAM-1), IL-6, and MCP-1.^{14, 15} Via these factors monocytes are attracted from the blood, and their migration into the adipose tissue and differentiation into macrophages is facilitated.¹⁶ This raises the macrophages content of adipose tissue in obese subjects and causes a higher cytokine release (Figure 1).^{14, 17} Of note, adiponectin is an important anti-inflammatory mediator that counteracts all steps of this inflammatory mechanism. Consequently, obesity is regarded as a current state of low-grade inflammation originating from alterations in the secretion of adipocytes and cytokines.

Oxidative stress and inflammation

Another factor of influence on inflammation in obese subjects is oxidative stress. Oxidative stress is mediated by reactive oxygen species (ROS) which are highly reactive and cytotoxic.¹⁸ ROS are generated during the metabolic processing of high-energy substrates, such as FFAs and glucose.^{19, 20} ROS are scavenged by antioxidants, but when antioxidant defenses fail the remaining ROS can cause oxidative stress.²¹ As a consequence, lipid oxidation, expression of ICAM-1, as well as vascular cell adhesion molecule-1 (VCAM-1), apoptosis, and gene expression of NF- κ B are all increased.¹⁸ When ROS target low-density lipoprotein (LDL), oxidized LDL is formed, which, in the intima of the vascular wall, leads to foam cell formation.⁸ Foam cells are the characteristic inflammatory cells of the first stage of atherosclerosis. Oxidized LDL in the

intima of the vascular walls also induces the expression of ICAM-1 and VCAM-1 on endothelial cells. ICAM-1 and VCAM-1 contribute to inflammation by facilitating the attraction and migration of monocytes from the blood into the subendothelial space. Another effect of ROS, similar to several released proinflammatory mediators (cytokines and chemokines), is increased platelet reactivity.²² Activated platelets participate in the inflammatory process and support the atherogenic process via the release of mediators facilitating coagulation, and promoting platelet and leukocyte adhesion to the subendothelial matrix.²³

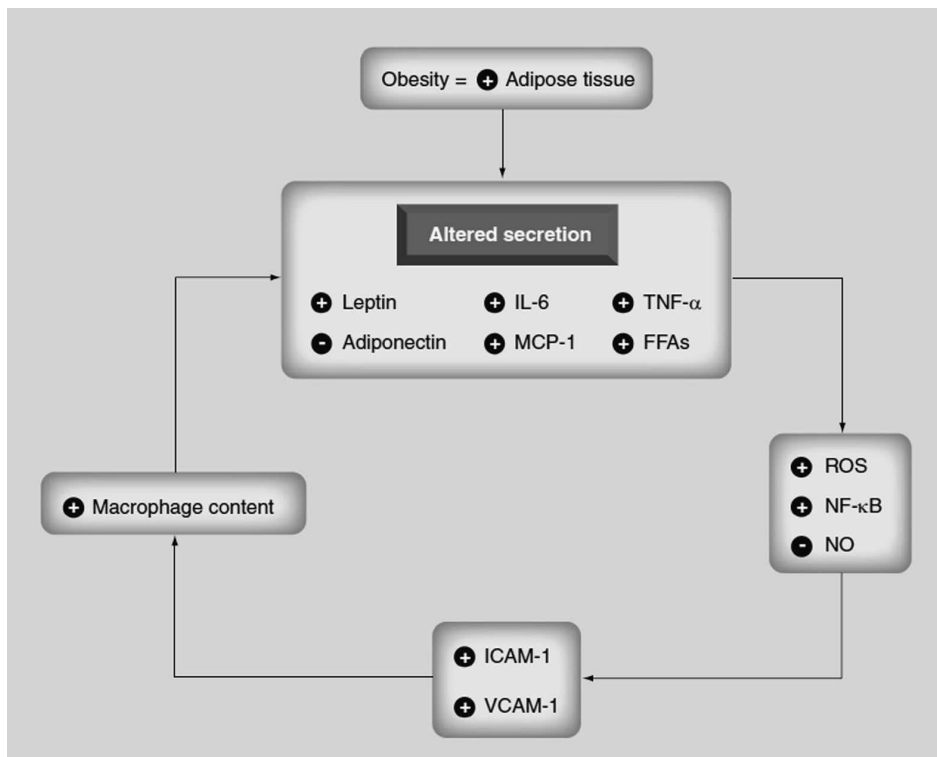


Figure 1. Development of low-grade inflammation in the adipose tissue of obese individuals
 Increased adipose tissue mass has an altered secretion, comprised of an increased release of proinflammatory adipokines, cytokines and FFAs, whereas the release of anti-inflammatory adiponectin is reduced. This induces a vicious circle, because the proinflammatory mediators facilitate the attraction and accumulation of macrophages in the adipose tissue, which in turn produce proinflammatory cytokines.
 Abbreviations +: increase; -: decrease; FFAs: free fatty acids; NO: nitric oxide; ROS: reactive oxygen species

Reduced bioavailability nitric oxide

Nitric oxide (NO) is a potent vasodilator and is produced by endothelial NO synthase (eNOS).²⁴ NO bioavailability is reduced by several mechanisms.²⁵ Oxidized LDL reduces NO production by reduction of the eNOS transcription rate. Moreover, both oxidized

LDL and TNF- α enhance the degradation of eNOS mRNA.^{26, 27} Another role player is asymmetric dimethylarginine, which is known to inhibit NO generation by eNOS.²⁸ Increased levels of asymmetric dimethylarginine are present in patients with diabetes and/or hypercholesterolemia and consequently lower their NO production. In addition, the NO concentration is diminished when NO acts as an antioxidant by binding to superoxide radicals and peroxynitrite is formed.²⁹ Vasodilation and several beneficial anti-inflammatory actions of insulin are mediated via NO pathways and are attenuated when NO bioavailability is reduced.⁵ Importantly, reduced NO bioavailability contributes to endothelial dysfunction, which refers to a state of the endothelium prone to the development of atherosclerosis.¹¹ A key feature of endothelial dysfunction is a decreased vasodilation in response to stimuli, such as acetylcholine or mechanical stress, which induce the release of endothelial vasodilators such as NO. Besides this, there are other disturbances of normal endothelium functioning that result in reduced anticoagulant properties, increased adhesion molecule expression and production of ROS from the endothelium.

Diabetes mellitus type 2

Obesity and inflammation contribute to insulin resistance

DM develops when insulin resistance increases and the adaptive increase of insulin production by pancreatic β -cells fails to overcome this resistance.³⁰ In obese patients, there are several mechanisms that enhance insulin resistance (Figure 2). First, increased concentrations of FFAs in plasma block insulin signal transduction, potentially by competitive cellular uptake and metabolism with glucose.³¹

Insulin normally blocks 'hormone sensitive lipase' in adipocytes, where this enzyme stimulates hydrolysis of triglycerides and thereby the production of FFAs. This leads to a vicious circle, as an insulin resistance state causes the concentrations of FFAs to increase.³² Second, since pancreatic β -cells have low levels of antioxidants, these cells are vulnerable to oxidative stress. As explained earlier, the metabolism of FFAs increases the production of ROS and subsequent oxidative stress. The lack of sufficient antioxidants in β -cells in the presence of high concentrations of FFAs increases pancreatic β -cell dysfunction and insulin resistance.³⁰ Third, decreased adiponectin levels in obese individuals result in the decline of insulin sensitivity, as adiponectin is known to exert an insulin-sensitizing effect, as well as an increased glucose uptake and NO production.³³ In addition, adiponectin protein expression is suppressed by Angiotensin II.³⁴ Besides an increase in angiotensin II concentration via the renin-angiotensin-aldosterone system, angiotensin II is formed locally in adipose tissue from angiotensinogen secreted by adipocytes in response to over-feeding.³⁵ Two final contributors to insulin resistance are the inflammatory markers IL-6 and TNF- α , which impair intracellular insulin signaling. IL-6 impairs insulin signaling in the liver, leading to decreased levels of glycogen synthase and decreased glucose uptake.³⁶ TNF- α inhibits insulin signaling in fat, skeletal muscle and peripheral tissues.³⁷

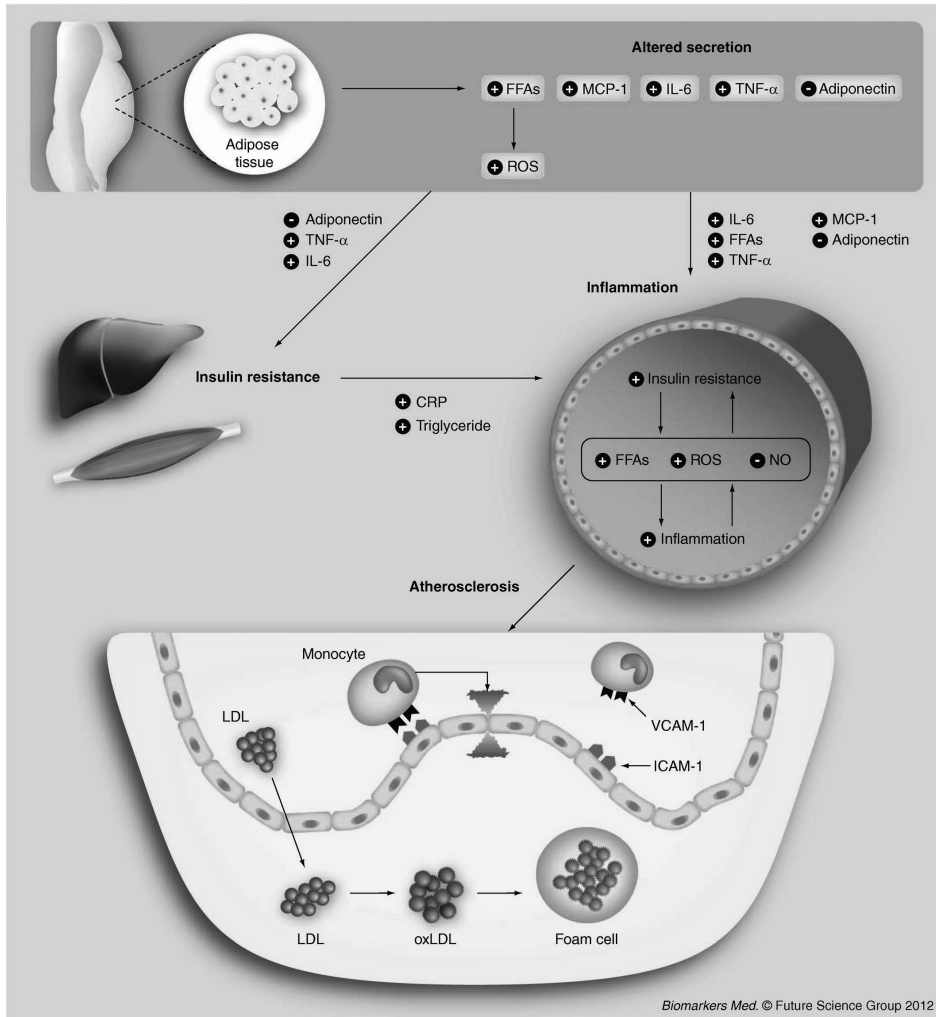


Figure 2. Obesity, inflammation, insulin resistance and atherosclerosis

The pathophysiologic pathway from obesity to atherosclerosis. Increased adipose tissue mass has an altered secretion, including an increased release of proinflammatory adipokines, cytokines and FFAs, whereas the release of anti-inflammatory adiponectin is reduced. This causes an increased insulin resistance in the liver and skeletal muscle. Circulating FFAs raise the insulin resistance in the vasculature and increase ROS, which in turn lowers NO bioavailability. This increases both insulin resistance and inflammation. The loss of insulin signaling reduces its anti-inflammatory actions and further contributes to inflammation. Endothelial cells express ICAM-1 and VCAM-1 in response to inflammatory mediators, which attract immune cells to the subendothelial space. The penetration of LDL and its oxidative modification in the intima of the arterial wall induces local inflammation and the formation of foam cells. Foam cells secrete proinflammatory mediators and express Toll-like receptors that act as pattern-recognition molecules, which, upon binding an antigen, can activate T-cells and intracellular NF- κ B.

Abbreviations +: increase; -: decrease; CRP: C-reactive protein; FFAs: free fatty acids; LDL: low-density lipoprotein; NO: nitric oxide; oxLDL: oxidatively modified LDL; ROS: reactive oxygen species.

Metabolic syndrome

The MetS is defined by the clustering of at least three out of five defined diagnostic criteria, consisting of cardiovascular risk factors associated with obesity, including obesity (defined as increased waist circumference) and DM (hyperglycemia). Therefore, besides obesity and DM, one more of the following criteria is required for the diagnosis of MetS; either increased blood pressure or dyslipidemia caused by elevated triglycerides or decreased high-density lipoprotein cholesterol.³⁸ In addition, the presence of the MetS in patients without DM strongly predicts the development of DM.³⁹

The interaction between hypertension, dyslipidemia, and obesity has been thoroughly reviewed by Chapman and Sposito.²⁵ Hypertension in obesity is the result of an increased vascular tone induced by FFAs and oxidative stress. Vasoconstriction is enhanced in these obese subjects by an increased sympathetic tone and increased angiotensin concentration, whereas insulin-mediated vasodilation is inhibited by the reduced bioavailability of NO. Dyslipidemia is induced by raised circulating levels of FFAs from visceral adipose tissue and is typically comprised of elevated triglycerides and decreased high-density lipoprotein.³²

Loss of insulin signaling promotes inflammation

The previously described factors, such as increased concentrations of FFAs, pancreatic β -cell failure, decreased levels of adiponectin and inflammatory mediators, augment insulin resistance and cause impaired insulin signaling. Insulin itself is a potent anti-inflammatory factor that suppresses the proinflammatory transcription factor NF- κ B, NF- κ B binding activity, ROS and ICAM-1.⁴⁰ Furthermore, insulin reduces platelet reactivity and has an anti-aggregating effect through a NO-dependent mechanism.⁴¹ Insulin resistance, by contrast, aggravates inflammation and augments oxidative stress, which is associated with reduction of NO and endothelial dysfunction (Figure 2).^{11, 40} Importantly, ROS activate platelets where the counteracting effect of insulin on platelet activation is reduced.^{41, 42} Thus, more platelets are activated by ROS and these platelets release pro-inflammatory mediators and ease coagulation because of their tendency to aggregate. Indeed, subjects with diabetes and hyperglycemia have increased markers of oxidative stress, as well as activated platelets.²² Moreover, oxidative stress is associated with the formation of advance glycosylation end products, which in turn increase ROS production.²¹

Perivascular adipose tissue

Perivascular adipose tissue (PAT) has gained interest because there is increasing evidence that it has a regulatory function in the microcirculation via local vasocrine signaling, and contributes to insulin resistance and blood pressure.⁴³ Obese subjects have increased PAT, which secretes adipokines and cytokines and, via vasocrine signaling, modulates

vascular tone. To this end, decreased levels of adiponectin and increased levels of TNF- α induce vasoconstriction.^{43,44} In fact, PAT augments insulin resistance through the raised TNF- α levels, which inhibit insulin signaling and thereby impair insulin's vasodilatory actions mediated through the NO pathway.⁴⁵ This hampers the ability of insulin to increase microvascular muscle perfusion for the uptake of plasma glucose. Moreover, several studies demonstrated that an increased epicardial adipose tissue volume, which directly surrounds the coronary arteries, is associated with an increased risk for CHD.⁴⁶ Further research is necessary to elucidate the physiological functions of PAT.

Coronary heart disease

The interplay between inflammation, atherosclerosis and CHD is reviewed by Hansson and is briefly discussed here.⁸ Coronary atherosclerosis precedes overt CHD and is strongly associated with inflammation. The progression of atherosclerosis is controlled by the balance between inflammatory and anti-inflammatory activity. Hence, the previously discussed pro-inflammatory mediators, oxidative stress and endothelial dysfunction, generate a favorable milieu for the development of atherosclerosis. The first stage of atherosclerosis is the formation of a fatty streak, which is composed of foam cells. These foam cells boost local inflammation by the expression of receptors that attract more inflammatory cells, which induce the activation of the adaptive immune system and the release of proinflammatory mediators. The atherosclerotic plaque develops by progressive inflammation to a characteristic vulnerable plaque with a necrotic core and thin fibrous cap. This vulnerable plaque is considered to be prone to rupture and can cause myocardial infarction.⁸

The initial step in the atherosclerotic cascade is the infiltration and retention of LDL in the intima of the arterial wall (Figure 2).²⁵ Hypercholesterolemia in obese subjects with a characteristically high LDL portion delivers a higher substrate level for this step.⁴⁷ After infiltration, LDL becomes oxidatively modified, and this oxidized LDL triggers the activation of endothelial cells and the expression of ICAM-1, VCAM-1 and inflammatory genes.^{8,48} The increased oxidative stress resulting from the metabolism of FFAs, loss of insulin sensitivity and reduced bioavailability of NO enhances the oxidative modification of LDL. Subsequently, chemokines, ICAM-1 and VCAM-1 facilitate the attraction and diapedesis of monocytes from the blood. In the subendothelial space monocytes differentiate into macrophages to internalize and destroy proinflammatory oxidized LDL particles.^{16,49} However, due to increased influx of LDL particles into the subendothelial space, LDL particles will accumulate in the macrophages and the latter change into foam cells (Figure 2). Foam cells and other attracted immune cells express Toll-like receptors, which act as pattern-recognition molecules and bind antigens. These pattern-recognition molecules bind antigens derived from oxidized LDL and, in turn, can activate intracellular T cells or NF- κ B. Activated T-cells can initiate an activation cascade, which causes the expression of more cytokines. Several more mediators contribute to increased local inflammation in the atherosclerotic plaque and promote its

progression to a vulnerable plaque.⁸ However, a complete overview of all involved mediators is beyond the scope of this review.

Biomarkers

There is a great need for a biomarker that improves the early identification of CHD in the subset of individuals at higher risk for CHD with obesity and DM. As mentioned, CHD in these individuals often has a late clinical presentation, due to asymptomatic progression of coronary atherosclerosis and inadequate risk assessment with established cardiovascular risk factors. As a result, CHD is more severe at the time of diagnosis, which implicates a worse prognosis, comprising a higher incidence of cardiovascular morbidity and mortality. The early identification of CHD is vital, because it allows early initiation of proper preventive medical therapy and thereby improves clinical outcome. Thus, a biomarker of CHD enables the prevention of cardiovascular events, mortality and morbidity by the early identification of CHD and consequent initiation of preventive medical treatment.

The ideal biomarker should enable the accurate prediction or exclusion of CHD, and also the monitoring of the effect of therapy on CHD. Besides identification of CHD, such a biomarker can be used for the guidance of medical therapy. Most of the mediators described above, involved in the pathophysiologic pathway from obesity to atherosclerosis, have been evaluated for their applicability as a biomarkers of CHD. Because of their involvement in the pathophysiology of CHD, these mediators are likely to have incremental value for CHD risk prediction beyond established cardiovascular risk factors and risk prediction models. In this section, several mediators will be more extensively discussed. For each of these mediators a description is given of how it influences insulin resistance and atherosclerosis and its performance in CHD risk prediction.

Adipocytokines

Adiponectin

Adiponectin is a protein produced by mature adipocytes. It has important favorable effects on insulin sensitivity, as well as on the atherosclerotic process.⁵⁰ Adiponectin ameliorates insulin sensitivity by stimulation of glucose uptake, oxidation of FFAs in skeletal muscle and by reduction of glucose output by the liver. This reduces the level of high-energy substrates for oxidative stress that would otherwise boost inflammation. The extensive anti-inflammatory actions of adiponectin take place on several steps of the (inflammatory) atherosclerotic cascade.⁵¹ They, for instance, interfere with NF- κ B signaling and inhibit TNF- α release after oxidative modification of LDL in the arterial wall, leading to impaired monocyte activation and reduced expression of endothelial adhesion molecules.⁵² Moreover, adiponectin reduces foam cell formation, promotes the production of NO and contributes to arterial vasodilation.⁵³

Adiponectin has a negative association with obesity and DM, meaning that plasma levels of adiponectin decrease with increasing visceral adipose tissue mass, as well as with increasing insulin resistance.⁵⁰ This occurs in response to increased levels of TNF- α and a raised proinflammatory state accompanying obesity and atherosclerosis.⁵¹ Adiponectin is, therefore, hypothesized as a marker of the MetS and inflammation. This is confirmed by the observed lower levels of adiponectin in patients with DM and CHD, and by the finding that reduced levels of adiponectin predict the development of DM.⁵⁴ With regard to CHD, the Health Professionals Follow-up Study (HPFS) in men found that a higher level of adiponectin after 6 years of follow-up was significantly associated with a lower risk of myocardial infarction.⁵⁵ By contrast, the British Women's Heart and Health Study (BWHHS), with a follow-up of 4 years, could not reproduce this relationship in women.⁵⁶ In BWHHS, adiponectin was associated with cardiovascular risk factors, but there was no association with CHD. In addition, the British Regional Heart Study (BRHS) found no association between adiponectin and CHD in men after 16 years of follow-up.⁵⁷ This study also encompassed a meta-analysis on the association of adiponectin and CHD in seven prospective studies, including the previously mentioned studies. The meta-analysis comprised 4,267 participants, of whom 1,313 experienced CHD after a mean follow-up of 9.7 years. The estimated odds ratio for CHD for the upper tertile of adiponectin compared with the lower tertile was 0.84 (95% CI: 0.70-1.01). Prospective data in the DM population are scarce. In a substudy of the HPFS in 745 men with DM type 2, after an average follow-up of 5 years, 89 subjects experienced an incident CHD event.⁵⁸ Higher levels of adiponectin were not predictive of CHD after multivariate adjustment for high-density lipoprotein. In conclusion, notwithstanding that adiponectin improves insulin sensitivity and has important anti-inflammatory actions, its association with CHD is not undisputed.

Leptin

Leptin is an adipocytokine chiefly produced by mature adipocytes. There are six different isoforms of the leptin receptor (LEPR) derived from the LEPR gene by alternate splicing.⁵⁹ The only long isoform of the LEPR is LEPRb, which is highly expressed in the hypothalamus. The first function linked to Leptin is control of body weight by its regulation of appetite, food intake and energy expenditure via these LEPRb receptors in the hypothalamus.⁶⁰ Leptin directly modulates pancreatic β -cell function and inhibits insulin secretion.⁶¹ In addition, it also protects these β -cells against oxidative stress. Insulin, in turn, stimulates leptin secretion.⁶² Therefore, leptin is closely related with obesity and DM. However, in obese individuals, endogenous leptin is unable to reduce body weight, despite its increased levels from increased adipose tissue mass. This unresponsiveness is ascribed to leptin resistance.⁶³

Leptin plays a complex ambivalent role in inflammation and atherosclerosis, anti-inflammatory as well as proinflammatory.⁶³ Thereby, a theory of 'selective' leptin resistance is embraced, which does not attenuate all of its actions. Leptin has direct effects on

immune cells, endothelial cells and platelets via its receptors.^{23, 63} The effects of leptin on immune cells are necessary for the initiation of an inflammatory response.⁶⁴ This is acknowledged by the fact that patients with leptin deficiency show immunodeficiency. In addition, activated leptin receptors on endothelial cells directly influence vascular tone and endothelial function.⁶³ Leptin enhances endothelial function by upregulating NO levels. and at higher levels might even induce NO-dependent vasorelaxation. Conversely, increased leptin levels, as seen in obese subjects, are associated with impaired NO-dependent coronary vasorelaxation.⁶⁵ The effects of leptin on inflammation are confirmed by the correlation of leptin levels with oxidative stress, levels of C-reactive protein (CRP) and expression of adhesion molecules.⁶³ Furthermore, the leptin receptors on platelets trigger platelet aggregation. Of note, the presence of leptin resistance provides relative protection against leptin-induced platelet aggregation in obese subjects by receptor desensitization.²³

The above-described associations of leptin with body weight and inflammation are verified in clinical studies. In fact, patients with obesity, DM and CHD exhibit increased levels of leptin.⁶⁶ Moreover, leptin was demonstrated to independently predict the development of DM in men, but not in women.⁶⁷ The association of leptin with CHD is investigated by several studies and systematically reviewed in 2009 by Sattar et al.⁶⁸ The two largest population based studies are the West of Scotland Coronary Prevention Study (WOSCOPS) and the BRHS. In the WOSCOPS study, elevated leptin levels predicted acute cardiovascular events after 5 years follow-up.⁶⁹ However, in the BRHS leptin was not associated with CHD, although leptin was associated with baseline cardiovascular risk factors and circulating inflammation markers.⁶⁸ The results of the studies were analyzed in a systematic review, which contained the WOSCOPS, BRHS and five other prospective studies, including 1,335 patients who developed CHD during the mean follow-up of 10 years, and 3,407 control subjects.⁶⁸ The combined adjusted risk ratio between the upper tertile and lower tertile of leptin was not significant (1.44; 95% CI: 0.95-2.16).

In patients with DM type 2, an independent association between leptin and subclinical atherosclerosis has been demonstrated by its association with the coronary calcium score (CCS), whereas its association with CHD is controversial.^{66, 70, 71} In summary, leptin proves to be a marker of obesity associated with DM, but its function as a marker of inflammation is dubious. Accordingly, at present, there is no proof that leptin can accurately be used for assessment of CHD risk.

Markers of inflammation

C-reactive protein

CRP is a sensitive and dynamic systemic marker of inflammation produced by the liver. Consequently, production of CRP directly increases in response to a variety of systemic events such as infection or tissue damage throughout the body. Besides this, mildly ele-

vated CRP levels are seen in subjects with abdominal obesity and DM.⁹ This is caused by IL-6 released from macrophages in the visceral adipose tissue and subendothelial space, which stimulates CRP secretion from the liver. CRP contributes to insulin resistance by attenuating insulin signaling.⁷² Accordingly, many studies have confirmed that elevated CRP levels are predictive of the development of insulin resistance, DM and the MetS.⁷³ With regard to CHD, there is a great body of evidence that has established the relationship between CRP and CHD as shown in prospective studies in the general population studies as well as in patients with DM and MetS.^{73, 74} Nevertheless, it remains to be elucidated whether CRP is really involved in the causal pathway of atherosclerosis or a non-specific bystander.⁷⁵ The presence of CRP in the atherosclerotic plaque is an argument for its role in atherogenesis.⁷⁶ Moreover, some mechanisms are described by which CRP promotes atherosclerosis: CRP reduces eNOS, mediates the negative effects of oxidized LDL on endothelial cells such as expression of adhesion molecules, is involved in the activation of the complement cascade and promotes foam cell formation.⁷⁷ On the other hand, some studies did not observe a difference in atherosclerotic lesion area or lesion severity in the presence of high CRP levels, compared with low levels or absence of CRP.^{78, 79} In order to further clarify whether CRP has a causal association with CHD, several genetic epidemiological studies have been performed.⁸⁰ These studies used a Mendelian randomization, which assumes that individuals with a variation in the CRP gene have a substantial change in CRP concentration, which proportionally relates to their cardiovascular risk. A genome-wide association study published in 2009 demonstrated that, despite the fact that several genetic loci were associated with changes in CRP concentration, these changes were not associated with CHD risk.⁸⁰

The association of CRP with CHD is extensively assessed in a recent meta-analysis by the Emerging Risk factors Collaboration.⁸¹ This meta-analysis included 54 studies with a total of 160,309 subjects and 10,451 CHD end points. The risk ratios (RR) for CHD, adjusted for conventional risk factors was 1.37 (95% CI: 1.27-1.48) per one standard deviation (SD) higher log CRP concentration. This demonstrated the predictive value of CRP for CHD and raised the question of whether CRP improves CHD risk prediction. In the Reykjavik study, CRP was predictive for CHD with an odds ratio of 1.45 (95% CI: 1.25-1.68) for subjects in the highest tertile compared with subjects in the lower tertile of CRP.⁸² Nevertheless, the authors concluded that CRP is a moderate predictor for CHD. Other studies have demonstrated that the addition of CRP to traditional cardiovascular risk factors improved accuracy for prediction of cardiovascular disease (CVD; defined as CHD and stroke) by reclassification of 30-50% of the patients.^{83, 84} Finally, the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial prospectively investigated the effect of lowering CRP levels on CVD events.⁸⁵ For this purpose, 17,802 men and women with low LDL (<3.4 mmol/l) and elevated high-sensitivity CRP (>2.0 mmol/l) were randomly assigned to either 20 mg daily rosuvastatin or placebo. The use of rosuvastatin after a mean follow-up of 1.9 years resulted in a reduction in LDL levels of 50% and high-sensitivity CRP levels of 37%, and was associated with a significantly reduced HR for CVD of 0.56 (95% CI: 0.46-0.69)

compared with the placebo group.

In addition, in the diabetic population, compared with other biomarkers, the value of CRP for the prediction of CHD is more extensively evaluated. Studies in the diabetic population showed that the relationship of CRP with CHD in this subgroup might be less obvious. In a substudy of the HPFS a total of 746 men with DM type 2 were enrolled, of which 103 patients had an incident CVD event after a mean follow-up of 5 years.⁷⁴ The individuals in the highest CRP quartile had a significantly increased RR for CVD events, after adjustment for baseline risk factors, of 2.62 (95% CI: 1.29-5.32). Conversely, in the Strong Heart Study (SHS), which comprised of 2,735 participants with CRP ≤ 10 mg/l, half were diagnosed with DM and 343 participants suffered from a CVD event during a median follow-up of 6.2 years.⁸⁶ Although CRP after multivariate adjustment was significantly associated with CVD events in the overall study population, in females and in the nondiabetic subgroup, no significant association was observed between CRP and CVD events in males and in the diabetic subgroup.

In conclusion, despite the fact that CRP is most likely not involved in the causal pathway of CHD, CRP is a good marker of CHD in the general population. To this end, CRP has great potential to be clinically used clinically on a large scale, due to its low cost, non-invasive assessment and its broad availability. Moreover, the JUPITER trial indicated that the combined lowering of LDL and CRP levels reduced CVD risk. The application of CRP for CHD risk discrimination among individuals with DM needs further study.

Interleukin-6

IL-6 is a cytokine mainly produced by adipose tissue, but also by monocytes, macrophages and skeletal muscle.⁸⁷ One of its functions is the regulation of the proliferation and differentiation of hematopoietic cells. IL-6 is proinflammatory and inhibits the secretion of adiponectin.⁸⁸ When secreted by adipose tissue, it can directly enter the liver through the portal vein. There, IL-6 induces the secretion of CRP, especially during an acute-phase response to inflammation or tissue injury.⁸⁹ Furthermore, in the liver, it contributes to insulin resistance by impairment of insulin signaling, which leads to decreased levels of glycogen synthase and decreased glucose uptake.³⁶ By contrast, in skeletal muscle IL-6 is secreted in response to exercise and increases glucose uptake.⁹⁰ In addition to its role in the systemic acute-phase response, IL-6 regulates local inflammation at sites of tissue injury via its direct effects on immune cells.⁹¹ Atherosclerosis is enhanced by promotion of the expression of adhesion molecules and stimulating foam cell formation. Furthermore, increased levels of IL-6 contribute to a prothrombotic state through increased platelet counts.²³

IL-6 levels should be interpreted with care; IL-6 has a short half-life and relatively greater within-person variability compared with CRP or other CVD risk factors.⁹² Several prospective studies have established that, similar as CRP, IL-6 levels are increased in subjects with obesity or DM and that raised IL-6 levels are predictive for the development of DM.⁹³ Large prospective general population studies such as the Reykjavik study,

BRHS, and WOSCOPS have recognized the predictive value of IL-6 levels for CHD.⁹²

⁹⁴ By contrast, in the HPFS in men and the Nurses' Health Study (NHS) in women, IL-6 was not predictive for CHD after multivariate adjustment for baseline risk factors.⁹⁵ This was further investigated by Danesh et al. in a meta-analysis of 17 relevant studies, including the previously mentioned studies.⁹² This meta-analysis, published in 2008, comprised a total of 24,768 individuals of whom 5,730 suffered from a CHD event after an average follow-up of 6 years. The combined multivariate adjusted odds ratio for CHD per 1 SD increase in baseline IL-6 levels was 1.26 (95% CI: 1.19-1.35). Whether IL-6 improved risk prediction was not further addressed. However, in the ARIC study, for instance, IL-6 levels did not improve the baseline CVD risk model.⁹⁶

Two substantial studies in patients with DM type 2 did not find an association between IL-6 and progression of CCS. One study was a substudy of the Veterans Affairs Diabetes Trial (VADT), with a mean follow-up of 4.6 years and was comprised of 197 patients.⁹⁷ The other study, with a follow-up of 2.5 years, comprised of 398 patients.⁹⁸ A substudy of the Estrogen and Thromboembolism Risk (ESTHER) study assessed the predictive value of IL-6 levels for CVD events in 1,072 participants with DM.⁹⁹ After 5 years follow-up, 84 patients had a CVD event. Patients in the upper tertile of IL-6 levels, compared with patients in the lower tertile, had an increased risk of CVD events and a multivariate adjusted hazard ratio of 1.90 (95% CI: 1.06-3.40). Similarly to the general population, IL-6 levels did not improve risk prediction beyond baseline risk factors.

In brief, IL-6 might not be an ideal marker because its short half-life and greater within-person variability than other markers. Although IL-6 levels predict the development of DM and CVD, current studies have not demonstrated an incremental predictive value for CVD beyond established CVD risk factors.

Tumor necrosis factor- α

TNF- α is a pro-inflammatory cytokine that is involved in the innate immune system.¹⁰⁰ It is released by monocytes, macrophages, adipose tissue and endothelial cells throughout the acute-phase response to inflammation and tissue injury. To this end, receptors of TNF- α are expressed on most cell types, except erythrocytes, and upon stimulation lead to activation of NF- κ B.¹⁰¹ Activated NF- κ B modulates the inflammatory response by increase in gene expression.¹⁰⁰ Furthermore, TNF- α increases the concentration of FFAs and insulin resistance, which leads to increases in serum triglycerides and glucose. During the acute phase response when TNF- α levels are raised, this mechanism provides extra substrates for the increased metabolic needs of cells involved in the inflammatory response.¹⁰² However, subjects with chronic inflammatory disease or obesity also have increased TNF- α levels, which promote the development of insulin resistance and atherosclerosis.

TNF- α causes insulin resistance by several mechanisms. For instance, in order to raise serum triglycerides, TNF- α counteracts insulin's antilipolytic action by inhibition of early insulin signaling.¹⁰³ As a result, lipolysis produces FFAs for triglyceride synthesis.

This mechanism demonstrates that TNF- α directly inhibits insulin signaling and by its action raises the plasma concentration of FFAs, which also inhibits insulin signaling.^{103, 104} Moreover, TNF- α increases insulin resistance through decreasing adiponectin levels and increasing leptin levels.¹⁰⁵ In fact, it was demonstrated that supplementation of TNF- α to healthy individuals reduced insulin sensitivity.¹⁰⁶

TNF- α plays an important role in the atherosclerotic process. Notably, it activates NF- κ B, which in turn can increase the production TNF- α .¹⁰⁷ ROS formation is stimulated by TNF- α in endothelial cells and also through the increase in FFAs and glucose.¹⁰⁰ On the other hand, TNF- α reduces NO bioavailability by acting on several steps of NO synthesis. Namely, by reduction of NO expression, NO activity, NO substrate availability and accumulation of asymmetric dimethylarginine.^{107, 108} Furthermore, increased scavenging of ROS further lowers the NO concentration. This results in a weakened inhibition of ICAM-1 and VCAM-1 expression by NO, whereas TNF- α supports its expression.¹⁰⁰ Thus, TNF- α leads to increased oxidative stress and reduced NO bioavailability, and thereby impairs endothelial function. This sets the stage for atherosclerosis, since endothelial dysfunction and increased serum triglycerides facilitate infiltration of LDL particles into the intima of the arterial wall. Finally, in response to oxidatively modified LDL in the intima, TNF- α is locally released and enhances foam cell formation. These cells increase inflammation by the the secretion of TNF- α , among others.¹⁰⁹

In clinical studies evaluating the predictive value TNF- α for CHD, TNF- α was measured by its plasma concentration as well as by levels of the type 1 (sTNFR-1) or type 2 (sTNFR-2) TNF- α soluble receptors. Of note, in these studies TNF- α and its soluble receptor plasma concentrations were higher in patients with DM and MetS.^{110, 111} In the Cytokines Activation and Long-Term Prognosis in Myocardial Infarction (C-ALPHA) study, including 184 patients with myocardial infarction and 45 controls, sTNFR-1 was an independent predictor of death after 406 days of follow-up.¹¹² Another study comprised of 167 patients with CHD found no association between TNF- α and the number of coronary lesions.¹¹¹ In 2003, the Aging and Body Composition (Health ABC) study reported on the predictive value of TNF- α for the 188 identified cases of CHD among 2,225 elderly individuals after 3.6 years follow-up.¹¹³ The adjusted RR for CHD per SD log (TNF- α) was 1.22 (95% CI: 1.22-1.43). Thereafter, in 2004 the HPFS and NHS assessed this association in separate analysis for men and women.⁹⁵ In men, including 529 controls and 265 cases of CHD after follow-up of 6 years, plasma concentrations of sTNFR-1 and sTNFR-2 were not associated with CHD events. By contrast, in women, comprising of 469 controls and 239 cases of CHD after 8 years of follow-up, sTNFR-1 and sTNFR-2 levels were univariately associated with CHD events, but not when multivariate adjusted.

In summary, TNF- α induces DM and CHD through its proinflammatory actions, which provoke insulin resistance and endothelial dysfunction. At present, the predictive value of TNF- α for CHD is not exactly known due to a limited number of studies; however, the largest study observed no association between TNF- α and CHD.

Chemokines

Chemokines are a large family of cytokines that are involved in the attraction and migration of immune cells from the blood into the vessel wall at sites of inflammation or injury.¹¹⁴ Chemokines and their receptors are located in vascular cells such as endothelial cells, vascular smooth muscle cells, platelets and immune cells.¹¹⁵ They are extensively involved in the atherosclerotic process. This is demonstrated in studies with transgenic mice, in which the deficiency of a chemokine or its receptor proved to reduce atherosclerotic lesions and even stabilize vulnerable plaque.¹¹⁶ For instance, deficiency of MCP-1, also known as CCL2, or its receptor CCR2 decreased atherosclerosis and macrophage infiltration.^{117, 118} As mentioned previously, inflammation and endothelial dysfunction causes the expression of cell adhesion molecules. These molecules promote the expression of chemokines on endothelial cells and smooth muscle cells, which facilitate monocyte arrest and migration into the vessel wall.¹⁶ Consequently, monocytes and macrophages accumulate in the arterial wall and augment atherosclerosis. Furthermore, the expression of several chemokines is upregulated in atherosclerotic lesions, where it enhance plaque progression.¹¹⁴ They can also activate platelets, which store large number of chemokines, which are released upon activation.¹⁶ Lastly, activation of macrophages by chemokines might induce ROS and foam cell formation.

Measurement of chemokines is difficult, since they have a short half-life and several have concentrations below the test detection limits.¹¹⁴ The *étude Prospective sur l'Infarctus du Moyarde (PRIME)* study assessed the predictive value of four different chemokines: regulated on activation normal T-cell expressed and secreted (RANTES/CCL5), interferon- γ -inducible protein-10 (IP-10/CXCL10), MCP-1, and eotaxin-1 (CCL11).¹¹⁹ This was performed as a nested case-control study in men, including 621 CHD cases and 1,242 controls, after a follow-up of 10 years. In this study, subjects with diabetes (4.4 and 1.9% of cases and controls, respectively) had similar levels of chemokines compared with the nondiabetic subjects. Moreover, there was no association between the chemokines and CHD. Whether MCP-1 is involved in the pathophysiology of CHD was investigated in the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) study.¹²⁰ This study, comprising of 1,138 patients with an incident CHD events after 6 years of follow-up, did not find an association between either genetic variants of MCP-1 or serum MCP-1 levels and CHD events. Finally, chemokines play an important role in atherosclerosis via local recruitment of immune cells and in plaque progression. The measurement of chemokines is challenging and just one chemokine is not representative for all their actions, because they function independently.¹¹⁴ At present, prospective population-based studies have not shown that chemokines can predict CHD events.

Lipoprotein-associated phospholipase A2

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme produced by circulating monocytes, T lymphocytes and mast cells, which circulates in the blood mainly

bound to LDL.¹²¹ This enzyme hydrolyzes phospholipids, predominantly LDL, in the arterial wall, which generates proinflammatory mediators such as oxidized LDL. As described previously, this is an important step in atherosclerosis, enhancing local inflammation by facilitating the attraction of monocytes, impairment of endothelial function and apoptosis in smooth muscle cells and macrophages. Therefore, Lp-PLA2 is regarded as a marker of inflammation.¹²² Lp-PLA2 levels can be measured as Lp-PLA2 mass or Lp-PLA2 activity with a good correlation. However, Lp-PLA2 mass is used in most studies and, therefore, preferred.¹²³

The relationship of Lp-PLA2 and subclinical atherosclerosis is assessed by several studies, which have displayed conflicting results. In the 1,820 participants of the Rotterdam study, Lp-PLA2 activity was not associated with extracoronary atherosclerosis.¹²⁴ By contrast, among patients with type 2 DM in the substudy of VADT, Lp-PLA2 mass was a significant predictor of progression of CCS.⁹⁷ In another study, the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study, the association between both Lp-PLA2 mass and activity with CCS progression after a mean follow-up of 2.6 years were assessed in a population comprised of 506 patients with DM type 1 and 591 subjects without diabetes.¹²⁵ This study demonstrated that in the complete study population Lp-PLA2 activity, but not Lp-PLA2 mass, independently predicts CCS progression. Conversely, in the subgroup of patients with DM type 1, Lp-PLA2 activity was not different between subjects with or without CCS progression.

The predictive value Lp-PLA2 mass and activity for CHD was evaluated by Thompson et al. in 2010 as part of a broad meta-analysis assessing CVD end-points.¹²³ Twelve studies were included for the association between Lp-PLA2 mass and CHD event, resulting in a total of 40,291 subjects and 4,361 cases. The combined adjusted RR for CHD per increase of 1 SD Lp-PLA2 mass was 1.11 (95% CI: 1.07-1.16). Analysis of Lp-PLA2 activity demonstrated a similar RR, making Lp-PLA2 a potential predictive marker to identify patients at high risk for CVD. However, this was not confirmed in the multibiomarker project Monica Risk, Genetics, Archiving and Monograph (MORGAM), where Lp-PLA2 mass and activity were not identified as an independent predictor of CVD.¹²⁶ Regarding patients with DM, the HPFS and NHS, comprised of 1,517 patients with DM type 2, of whom 324 experienced CHD after 10 years of follow-up, demonstrated that Lp-PLA2 was independently and significantly associated with incident CHD.¹²⁷ Altogether, Lp-PLA2 shows a promising association with CHD in both the general population and in patients with DM, although the evidence in the DM subpopulation is limited. Further studies are necessary to investigate whether Lp-PLA2 contributes to CHD risk stratification.

Novel biomarkers

Tumor necrosis factor-like weak inducer of apoptosis

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) belongs to the TNF superfamily, which function as transmembrane proteins as well as soluble molecules,

and has a regulatory function in inflammation and immune responses.¹²⁸ It is present at high levels in a variety of tissues and organs, for instance in the heart, liver, vasculature and adipose tissue. TWEAK acts on a single receptor, namely fibroblast growth factor-inducible 14 (Fn14). Resting tissue levels of Fn14 are low, but become rapidly upregulated during injury or inflammation and thereby sensitize the actions of TWEAK. Most actions of TWEAK are proinflammatory, although anti-inflammatory actions that attenuate the innate immune response have been described. TWEAK inhibits adipogenesis in human preadipocytes and reduces mRNA expression of adiponectin.¹²⁹ It can activate NF- κ B through the canonical pathway, similar to TNF- α , but also through noncanonical mechanisms, which give rise to a prolonged activation of NF- κ B.¹³⁰ Activated NF- κ B impairs insulin signaling and increases insulin resistance. Furthermore, TWEAK contributes to atherosclerosis by stimulating the expression of ICAM-1, MCP-1 and chemokines in endothelial cells and the expression of proinflammatory chemokines via the activation of NF- κ B in the atherosclerotic plaque.¹²⁸

Soluble TWEAK (sTWEAK) can be measured in the plasma. At present, studies on sTWEAK are scarce. Reduced concentrations of sTWEAK were found in the presence of DM and atherosclerosis.¹²⁸ Furthermore, a few studies have demonstrated that sTWEAK has predictive value for CVD in patients with chronic kidney disease.¹³¹

Pentraxin 3

Pentraxin 3 (PTX3) was first identified in various tissues as a cytokine-inducible molecule.¹³² It belongs to the superfamily of pentraxins involved in the humoral arm of the innate immune system. PTX3 is a long pentraxin which has, as compared with the short pentraxins such as CRP, an additional N-terminal. PTX3 is a pattern-recognition molecule that binds selected pathogens, such as microorganisms, and in response modulates complement activation, opsonization and glycosylation-dependent inflammation. It is expressed by dendritic cells, endothelial cells, monocytes, macrophages, fibroblasts and adipocytes, among others. These cells produce PTX3 locally upon stimulation by lipopolysaccharide, IL-1, TNF- α and oxidized LDL.¹³³ Blood levels of PTX3 rapidly increase in the presence of inflammation and have demonstrated a good correspondence with disease severity.¹³²

Recently, it has been demonstrated that, although PTX3 levels decrease with obesity, gene expression of PTX3 in visceral adipose tissue is higher in overweight and obese subjects.¹³⁴ Furthermore, PTX3 had a negative correlation with insulin secretion in response to glucose. With regards to atherosclerosis, one study found that PTX3 blood levels were not related to CVD risk factors, but its blood levels were significantly increased in patients with unstable angina pectoris compared with controls.¹³⁵ In addition, a prospective study in elderly patients found that PTX3 was associated with CVD mortality and all-cause mortality, but not with CVD events defined as angina pectoris, myocardial infarction or stroke.¹³⁶

Conclusion

The identification of CHD in individuals with obesity and DM is often delayed, causing an increased risk for cardiovascular events and a worse prognosis. Consequently, a biomarker of CHD is required to improve the early identification of CHD in these patients beyond established cardiovascular risk factors. For this purpose, many mediators involved in the pathophysiological pathway from obesity to atherosclerosis have been investigated for their applicability as biomarkers of CHD. This review discussed a select group of these mediators.

In the general population, some of these mediators have shown a good predictive value for CHD, but their incremental value for CHD risk prediction beyond established cardiovascular risk factors needs to be validated. In the subgroup of individuals with 'cardiovascular MetS', implicating an increased CHD risk, associations between these mediators and CHD have been less extensively investigated and remain unclear. Moreover, mediators with a good predictive value for CHD in the general population were not consistently associated with CHD in this subpopulation. This highlights the need for further research. New knowledge about the pathophysiology of CHD and new imaging techniques might provide opportunities for the recognition of CHD. Consequently, potential biomarkers have to be further evaluated for their predictive value for CHD.

Future perspective

The aim of future investigation should be to improve the identification of high-risk individuals who will experience a CHD event. To this end, new insights in the pathophysiology of CHD and new techniques might provide opportunities for the recognition of CHD. Furthermore, new screening strategies could be developed and further evaluated for their incremental value in the prediction of CHD and feasibility with regard to cost-effectiveness. For this purpose, currently used risk models including established CHD risk factors could be expanded with one or more validated biomarkers, or even with measurement of subclinical atherosclerosis (carotid intima media thickness, vascular stiffness or CCS). Nevertheless, further research remains necessary to identify and evaluate new biomarkers. Novel biomarkers should be validated for their predictive value of CHD and additional value to CHD risk prediction in the general population and other subgroups. This is of great importance, because novel biomarkers might not only improve the identification of individuals at increased CHD risk, but also provide new targets for pharmaceutical therapies. For example, new medical therapies are currently being investigated that target phospholipase A2 enzymes and thereby reduce Lp-PLA2.¹²² First results with these drugs of the Integrated Biomarkers and Imaging Study-2 trial in patients with confirmed CHD found a reduction of necrotic core, but not in atheroma volume.¹³⁷

Executive summary

Background

- There is a need for a biomarker of CHD to improve the identification of CHD in individuals with ‘cardiovascular metabolic syndrome’ beyond established cardiovascular risk factors
- Mediators involved in the pathophysiology of CHD induced by obesity are potential biomarkers of CHD

Obesity, inflammation, DM and CHD

- The interplay between obesity, inflammation, DM and CHD is complex and involves a great variety of mediators, signal transduction pathways and mechanisms.

Increasing adipose tissue mass contribute to inflammation

- Increased adipose tissue has an altered secretion of adipokines, cytokines and FFAs.
- Proinflammatory mediators are increased, whereas anti-inflammatory mediators are decreased.

Oxidative stress, reduced bioavailability of NO and insulin resistance

- Proinflammatory mediators increase oxidative stress and reduce the bioavailability of NO, contributing to inflammation and insulin resistance.
- Insulin resistance in turn increases oxidative stress and reduces bioavailability of NO.

DM type 2 and MetS

- Increased insulin resistance resulting from raised proinflammatory mediators promotes the development of DM type 2.
- Proinflammatory mediators and insulin resistance cause dyslipidemia and impaired vasodilation, which promote the establishment of MetS.

CHD

- The proinflammatory milieu induces the expression of cell adhesion molecules on endothelial cells and the subsequent attraction and diapedesis of monocytes.
- The infiltration of LDL in the intima of the arterial wall and its oxidative modification is a key step in the development of atherosclerosis. Increasing LDL levels in the intima cause foam cell formation.
- Local inflammation is further enhanced by foam cells through the expression of receptors and secretion of cytokines and chemokines that attract more immune cells into the atherosclerotic plaque.

Biomarkers

Biomarkers of CHD should improve CHD risk prediction beyond established risk factors.

Adipokines

- Adiponectin has anti-inflammatory properties, as opposed to leptin, which seems pro-inflammatory in obese subjects.
- Adiponectin and leptin are associated with DM and CHD. However, in meta-analysis, adiponectin and leptin were not predictive of CHD.

Inflammatory markers

- CRP, IL-6 and Lp-PLA2 are predictive for CHD and further studies are necessary to assess the incremental predictive value of these markers.
- Only a few studies report on TNF- α and CHD; the largest study did not find an association between TNF- α and CHD events.
- Chemokines were not shown to be predictive for incident CHD in population-based studies.

Novel biomarkers

- TWEAK and Pentraxin 3 are promising novel biomarkers of CHD. Their association and predictive value for CHD remains to be established in larger studies.

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CHAPTER 3

*Function and anatomy:
SPECT-MPI and MSCT coronary angiography*



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EuroIntervention 2010

Abstract

For the diagnosis of coronary artery disease (CAD), non-invasive cardiac imaging is indispensable. Myocardial perfusion imaging (MPI) by single photon emission computed tomography (SPECT) investigates the pathophysiological consequences of luminal obstructive CAD, while multislice computed tomography coronary angiography (CTA) indicates the presence, extent and location of coronary atherosclerosis. The integration of CTA and SPECT data may provide important information, which may be useful for patient management. In this manuscript the value of both techniques will be described. In addition, the feasibility and potential value of combined anatomic and functional imaging will be discussed.

Introduction

Coronary artery disease (CAD) is still one of the most prevalent healthcare problems in the industrialised world. Cardiovascular imaging plays an important role in the diagnosis of CAD. In the last decades several non-invasive functional imaging techniques, such as single photon emission computed tomography (SPECT), magnetic resonance imaging and contrast echocardiography have become readily available. SPECT myocardial perfusion imaging (MPI) in particular is generally widely used and a well-established non-invasive tool for the diagnosis of ischaemic coronary disease. Reflecting the pathophysiological consequences of luminal obstructive CAD, this technique has been used for more than 30 years and has proven to be highly accurate.^{1,2}

In recent years, non-invasive assessment of cardiac anatomy has also become possible with the introduction of multislice computed tomography coronary angiography (CTA), which allows for detection of significant CAD with a high diagnostic accuracy compared to conventional invasive coronary angiography.^{3,4} Comparative studies between SPECT and CTA have shown that a significant stenosis detected on CTA, results in a perfusion abnormality on SPECT in only approximately 50% of patients, conversely a normal SPECT was unable to rule out the presence of significant CAD or atherosclerosis in general.^{5,6} CTA and SPECT thus provide complementary information regarding the presence and haemodynamic effects of CAD. As a result the focus of non-invasive imaging has shifted towards combined assessment of both anatomy and function. In this review we will briefly describe the technique and clinical applications of SPECT and CTA, and we will describe the usefulness and the advances in combined anatomic and functional imaging.

Myocardial perfusion imaging by SPECT

The technique

The underlying principle of this technique is that under conditions of stress, territories supplied by diseased coronary arteries receive less blood flow than normal myocardium. A cardiac specific radiopharmaceutical (Technetium-99 m or Thallium-201) is administered, while the heart rate is raised (exercise or dobutamine) to induce myocardial stress or during maximal vasodilatation by adenosine or dipyridamole infusion. SPECT is a nuclear tomographic imaging technique using gamma rays, which are emitted by the injected radiopharmaceutical. SPECT imaging is performed by using a gamma camera to acquire 2-dimensional images from multiple angles. A computer is used to apply a tomographic reconstruction algorithm to the multiple projections, yielding a 3-dimensional dataset. This dataset may then be manipulated to show thin slices along any chosen axis of the body. To acquire SPECT images, the gamma camera is rotated around the patient. Projections are acquired at defined points during the rotation, typically every 3–6 degrees. In most cases, a full 360 degree rotation is used to obtain an optimal recon-

struction. The time taken to obtain each projection is also variable, but 15–20 seconds is typical. This results in a total scan time of 15–20 minutes. SPECT imaging performed after stress reveals the distribution of the radiopharmaceutical, and therefore the relative blood flow to the different regions of the myocardium. Diagnosis is made by comparing stress images to a set of images obtained at rest. The site, extent and depth of these abnormalities are assessed. Homogeneous myocardial uptake of the tracer indicates normal myocardium and perfusion. Absence of the tracer means clinically significant infarction or coronary stenosis. A defect at stress images that normalises in the rest images indicates an inducible perfusion abnormality, and generally corresponds to a significant coronary stenosis. A defect both at stress and rest images (a fixed defect) indicates an area with loss of viable myocardium, for instance myocardial infarction. With SPECT, it is possible to obtain cardiac gated acquisitions. Triggered by the electrocardiogram (ECG) to obtain differential information about the heart in various parts of its cycle, gated myocardial SPECT can be used to obtain quantitative information about myocardial perfusion, thickness, and contractility of the myocardium during various parts of the cardiac cycle. It also allows calculation of left ventricular ejection fraction, stroke volume, and cardiac output. In addition, distinction between true perfusion abnormalities and true artefacts is possible. Regions with true perfusion defects that are non-reversible will contract abnormally, while those associated with attenuation artefacts would demonstrate normal motion and thickening.

Clinical application

In clinical practice MPI-SPECT is commonly used for the following indications:

- 1: diagnosis of suspected CAD in patients with an intermediate risk of CAD
- 2: risk stratification in patients with suspected and proven CAD
- 3: risk assessment before non-cardiac surgery
- 4: detection and quantification of viability/hibernating myocardium
- 5: assessment of functional significance in patients with proven multivessel CAD
- 6: assessment of intervention effect

For the diagnosis of CAD the extent and severity of an abnormal study is commonly used for the separation of patients into high and low risk for subsequent cardiac events. Patients with a low risk scan can be treated with medical therapy and unnecessary further testing and medical costs can be avoided.⁷ On the other hand, patients with extensive and severe myocardial ischaemia have worse prognosis and are referred for invasive coronary angiography and may benefit from intervention.^{8,9}

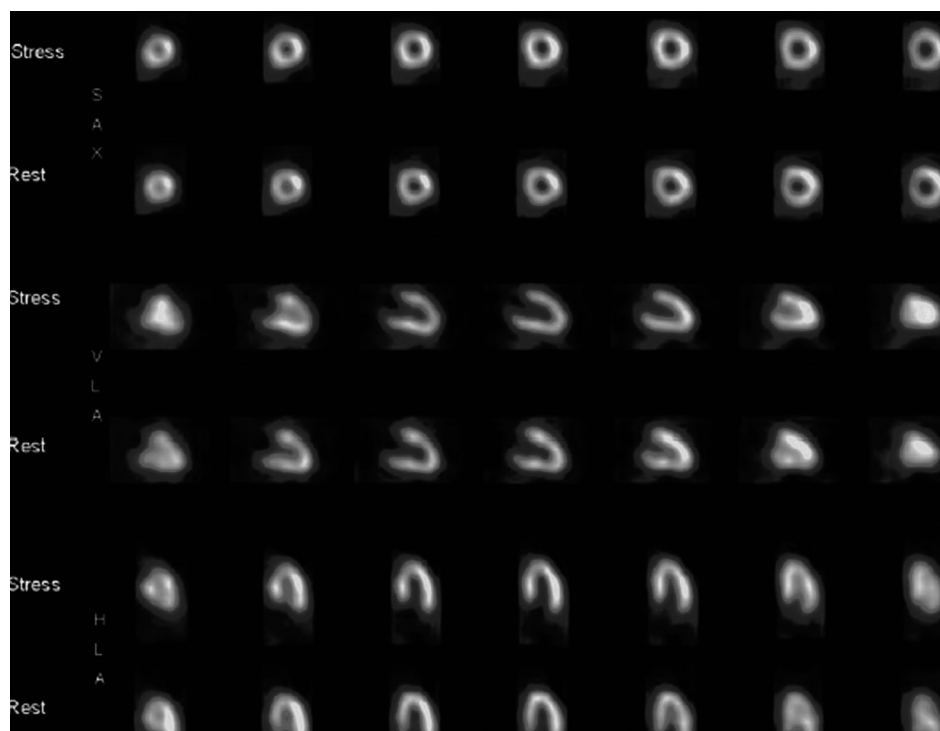


Figure 1. SPECT-MPI of a 59 year old south-Asian male with diabetes, hypertension and hypercholesterolaemia referred for screening of silent myocardial ischaemia. There is normal uptake of the tracer in the entire myocardium, without reversibility or persistent defect. ECG-gated images showed no wall motion abnormalities and good global left ventricular function at rest and post-stress. SAX: short axis; VLA: vertical long-axis; HLA: horizontal long-axis.

Computed tomography coronary angiography

The technique

Currently CTA scans are typically performed using a 64-detector row computed tomography scanner. After infusion of an iodinated contrast agent, patients are scanned during an inspiratory breath hold to counter acquisition problems arising from cardiac motion during breathing. To avoid coronary motion artefacts, acquired images are linked to the ECG in order to retrospectively select good quality images from a “motion free” phase of the cardiac cycle, typically end diastolic. Because of the need for end diastolic images of every level of the heart, and because of the limited coverage of the 64-detector row CTA scanner in the craniocaudal direction, acquisition of data is performed during multiple heartbeats. After acquisition, a dataset of the full heart is reconstructed with information obtained during the end diastolic phases of several heartbeats. Before the CTA scan patient’s heart rate and blood pressure are generally monitored to determine the need for heart rate reduction. In the absence of contraindications patients with heart

rate's exceeding 65 beats per minute are typically administered oral or intravenous beta blocking medication in order to reduce heart rate and improve image quality.

Several developments have occurred since the introduction of 64- detector row scanners. Dual-source scanners employing two X-ray tubes have been developed to increase temporal resolution resulting in improved image quality and less dependency on heart rate control.¹⁰ A further improvement has been the introduction of prospective ECG gating which allows for acquisition of images during a small predetermined "motion free" part of the cardiac phase, which substantially lowers radiation dose to approximately 1.1-3.0 mSv.¹¹ Finally, entire cardiac coverage in one heart beat can be obtained by the recently introduced 320-slice detector row CTA system.¹² This decreases artefacts from the merging of data from different heartbeats and decreases radiation dose when used in combination with prospective ECG triggering.

CTA allows for non-invasive assessment of the coronary artery tree and is used for the detection of coronary artery stenosis. In contrast to invasive coronary angiography which only visualises contrast in the lumen, CTA is able to image the vessel wall thereby directly detecting coronary atherosclerosis. A differentiation can be made between normal coronary arteries showing no signs of atherosclerosis, non-significant CAD with <50% luminal narrowing and significant CAD with $\geq 50\%$ luminal narrowing.

Clinical application

Although CTA is still a relatively new cardiovascular imaging modality, its value in the assessment of patients presenting with suspected CAD is beginning to emerge. The diagnostic accuracy of CTA has been studied extensively. In early single centre studies an average weighted sensitivity of 97.5 (95% confidence interval 96-99) and specificity of 91 (95% confidence interval 87.5-95) have been observed for the detection of significant CAD compared to invasive coronary angiography.¹³ More recently several prospective multicentre studies have been published showing similar sensitivities and specificities.^{3,4} Importantly, CTA has an especially high negative predictive value, and as a result the technique is increasingly used as a gatekeeper for further diagnostic testing. In addition, data are emerging that early identification of CAD with CTA may be useful for risk stratification. Since the first publications on the prognostic value of CTA in 2007, a number of studies have been published providing further insight into the potential value of non-invasive anatomic imaging for risk stratification.^{14, 15} These studies have shown that patients with a significant stenosis on CTA have worse outcome as compared to patients without significant CAD. An annualised event rate for the occurrence of all cause mortality and myocardial infarction ranging between approximately 1% and 5% has been observed in patients with significant CAD compared to approximately 0% to 2% in patients without significant CAD.

Combined anatomic and functional imaging

The combination of anatomic and functional imaging has the potential to improve patient management by providing complementary information for diagnosis of CAD. Assessment of the presence of coronary stenosis on CTA and its haemodynamic consequences as assessed by SPECT may improve decision making regarding referral to invasive coronary angiography and potentially revascularisation. In addition, it has been shown that CTA and SPECT provide complementary prognostic information; thus combined assessment may potentially improve risk stratification.¹⁶

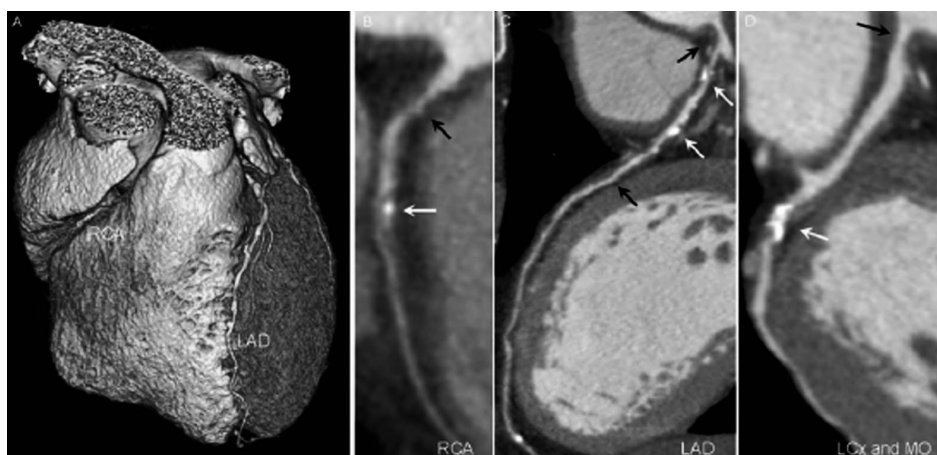


Figure 2. CTA in the same patient as Figure 1, shows diffuse coronary atherosclerosis. Panel A shows a 3D image of the heart. Panel B: Right coronary artery (RCA), proximal in the RCA - There is a non-calcified plaque (black arrow) and the mid RCA shows a mixed plaque (white arrow). Panel C: Left descending artery (LAD), with a non-calcified plaque in the left main (black arrow) with significant stenosis. Furthermore, there is a long mixed plaque from the proximal LAD till mid LAD (white arrows) with significant stenosis. Finally, there is a non-calcified plaque (black arrow) in the mid LAD. Panel D: Left circumflex artery (LCx) and the marginal obtusus (MO) branch, showing again the significant stenosis of the LM (black arrow). The patient was referred for invasive angiography, confirming the suspicion of significant three-vessel disease and significant left main stenosis. The patient underwent uneventful coronary artery bypass grafting.

Combination of CTA and SPECT data can be acquired using different approaches. Besides separate or side-by-side analysis of datasets (Figures 1 and 2), CTA and SPECT scan data can be retrospectively fused using image integration software.¹⁷ By integration of the datasets, perfusion defects may be more accurately allocated to the corresponding arteries and lesions. In a study by Gaemperli et al, the accuracy of cardiac image fusion was determined.¹⁸ An example of this is shown in Figures 3 and 4. The authors concluded that in almost one third of patients, fusion of CTA and SPECT provided additional diagnostic information compared to side-by-side analysis of SPECT and CTA, especially in functionally relevant lesions in distal segments and diagonal branches and in vessels with extensive disease or calcifications. In addition to retrospective fusion of datasets,

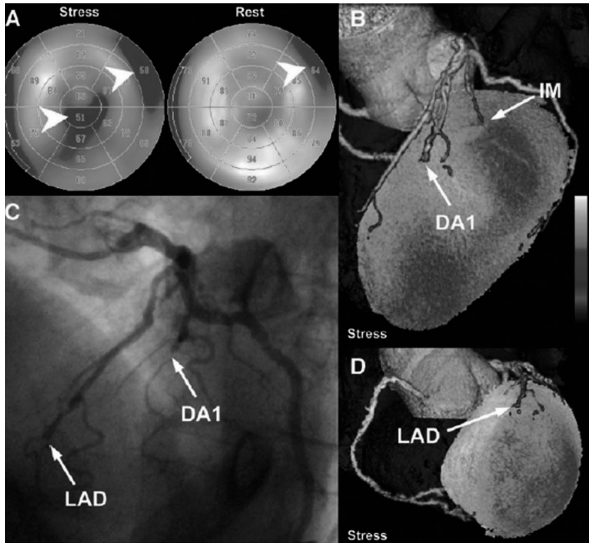


Figure 3. (A) Stress and rest perfusion polar maps of SPECT-MPI study show mixed basal anterolateral defect and reversible infero-apical perfusion defect (arrowheads). (B and D) Fused SPECT/CT images reveal total occlusion of ramus descending artery (LAD) and subtotal occlusion of first diagonal branch (DA1), which are confirmed by conventional coronary angiography (C). Anterolateral perfusion defect is caused by lesion of partially calcified small intermediary branch (IM); however, this vessel is not well visualised by conventional angiography.

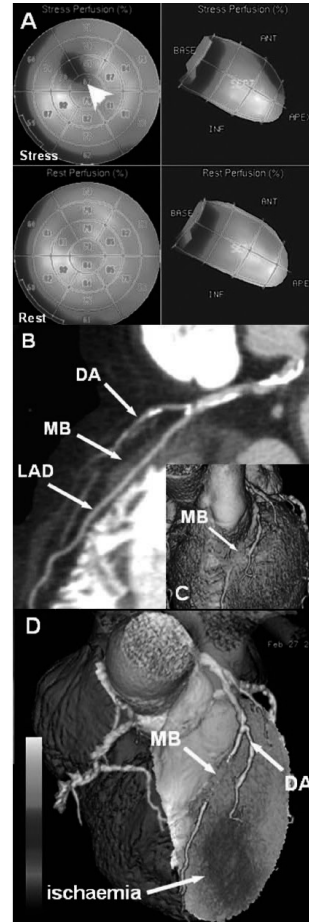


Figure 4. Perfusion polar maps at stress (dobutamine stress) and rest show reversible anteroseptal perfusion defect. (B and C) 64-slice MSCT-angiography revealed myocardial bridging (MB) of mid LAD of 0.2-cm length and calcified plaque at origin of first diagonal branch (DA). (D) Fused 3D SPECT/CT images could allocate reversible perfusion defect to DA, whereas MB seemed to be haemodynamically insignificant.

CTA and SPECT data can also be integrated by use of dedicated hybrid SPECT/CTA scanners.¹⁹ Hybrid SPECT-CT imaging first application was for the apparent reduction in tracer uptake in the anterior wall of the heart due to breast attenuation or in the inferior wall of the heart due to “diaphragmatic” attenuation, which can lead to diagnostic challenges. An example of this application is shown in Figures 5 and 6. Since the enormous progression of image quality of the coronary arteries from the CT scanners, hybrid SPECT-CT imaging can nowadays not only be used for attenuation correction but for “real” image fusion of the coronary arteries and myocardial perfusion of the left ventricle. It is unclear if the integration of CTA and SPECT using a hybrid SPECT-CTA scanner provides improved diagnostic imaging compared to retrospective fusion of separately obtained SPECT and CTA datasets. The use of a SPECT-CTA scanner may

however be advantageous from a logistic point of view as patients can be scanned during a single session in a single room.

Although, the combination of SPECT and CTA using stand alone SPECT and CTA, or by use of a hybrid scanner may provide complementary information for diagnosis and

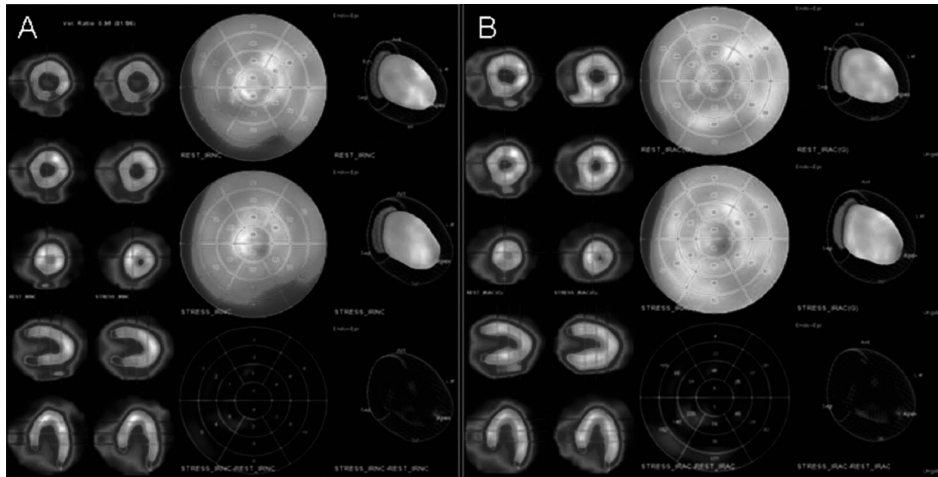


Figure 5. SPECT-MPI imaging of a 57-year old male with hypertension, hypercholesterolaemia and atypical chest pain. Panel A: Short-axis, vertical long-axis and horizontal long-axis view and polar map, without attenuation correction. Left rest, right stress images, showing a persistent defect of the inferior wall. Panel B: same patient and views with attenuation correction, showing no defect of the inferior wall.

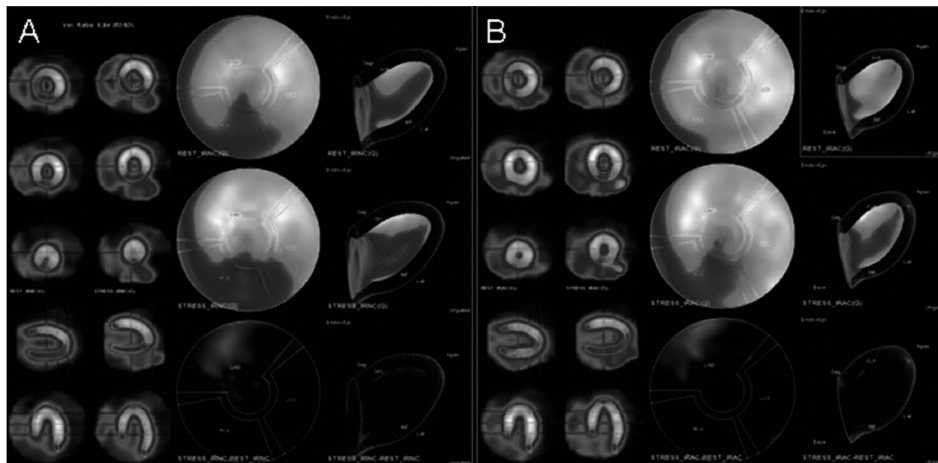


Figure 6. SPECT-MPI imaging of a 66-year old female with obesity, diabetes and chest pain one week before presentation. Panel A: Short-axis, vertical long-axis and horizontal long-axis view and polar map, without attenuation correction. Left rest, right stress images, showing a partial reversible defect of the inferior wall. Panel B: same patient and views with attenuation correction, showing the same reversible defect as Panel A.

risk stratification, it is questionable if information regarding anatomy and function is necessary in all patients referred for diagnostic imaging. In patients with a normal CTA (no evidence of coronary atherosclerosis) the likelihood of a perfusion abnormality is very low, and the survival rate is very high, suggesting that no further imaging is necessary in this subgroup. Furthermore as both CTA and SPECT are associated with ionising radiation and as most centers do not have access to a hybrid scanner, combined imaging may result in increased radiation burden and logistical problems. As a result, sequential imaging may be a more viable alternative approach. A flow chart advocating such a strategy has been recently published.²⁰ Using CTA as an initial imaging technique to rule out the presence of CAD, patients with a normal CTA can be safely discharged and do not require further testing. In patients with non-obstructive CAD (<50%) medical therapy and aggressive risk factor modification may be indicated. Patients with a significant or borderline lesion or patients with an unequivocal CTA may be referred for SPECT imaging to determine the haemodynamic effects on myocardial perfusion and to determine if revascularisation is indicated. Finally, patients with severe CAD detected on CTA may be directly referred to invasive coronary angiography. Such an approach may result in an overall reduction in mean radiation dose as was shown recently by Pazhenkottil et al.²¹ Compared to combined CTA and SPECT imaging in all patients, an individualised three tiered approach of CTA followed by stress only SPECT followed by rest SPECT only, if the preceding scan was abnormal, resulted in an approximately 40% reduction in average radiation dose.

Conclusion

Although, the first results of hybrid imaging using SPECT and CTA seems to provide additional clinical value compared to either technique alone or side-by-side analysis, more data are necessary to answer the following issues: What is the impact on treatment strategy and outcome? What is the radiation exposure to the patient? Is hybrid SPECT-CTA imaging cost-effective? Can the rapid changes in CTA technology and ultrafast MPI-SPECT be integrated in SPECT-CT machines? Although new low-dose CTA acquisition protocols with prospective ECG triggering and stress only SPECT MPI seems promising, more data are necessary to validate the clinical role of SPECT-CT.

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CHAPTER 4

Relationship between arterial stiffness and stress myocardial perfusion imaging in asymptomatic patients with diabetes



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Abstract

Arterial stiffness may potentially be used as a screening tool to identify asymptomatic patients with diabetes with abnormal myocardial perfusion. The purpose of this study was therefore to determine the association between arterial stiffness, measured in term of pulse wave velocity (PWV) and augmentation index (AIx), and abnormal myocardial perfusion imaging (MPI) in asymptomatic patients with diabetes.

Prospectively, 160 asymptomatic patients with diabetes (mean age 51 years, 87 men) underwent MPI with adenosine stress. The summed stress score (SSS) was determined in each patient according to a 17-segment and five-point score. Abnormal MPI (SSS ≥ 3) was classified as moderate (SSS 3-7) or severe (SSS ≥ 8) MPI defects. Using applanation tonometry, the carotid-femoral PWV and the radial AIx corrected to 75 beats per minute were determined noninvasively.

MPI was abnormal in 61 patients (38%), with severe MPI defects in 22 patients (14 %). Mean PWV increased with deteriorating MPI from 8.4 ± 2.2 m/s in normal MPI to 9.0 ± 2.2 m/s in moderate MPI defects ($p = 0.11$) and to 11.1 ± 2.5 m/s in severe MPI defects ($p < 0.01$). Likewise, mean AIx increased from 18.4 ± 13.4 % to 19.4 ± 10.7 % ($p = 0.66$) and towards 25.4 ± 9.0 % ($p = 0.03$). After adjustment for age and other risk factors, PWV remained a significant predictor of severe MPI defects ($p = 0.01$, OR 1.50, 95 % CI: 1.11-2.00), whereas AIx lost significance ($p = 0.20$).

Arterial stiffness measured by PWV is associated with severe MPI defects in asymptomatic patients with diabetes.

Introduction

It is considered that the global prevalence of diabetes will approximately double in the next two decades.¹ Diabetes is associated with a marked increase in the incidence of cardiovascular morbidity and mortality, mainly attributable to coronary artery disease (CAD). Moreover, the presence and progression of CAD in diabetic patients is often asymptomatic, leading to more extensive disease at the time of diagnosis.^{1,2} Since a delayed diagnosis of CAD considerably worsens the prognosis, early recognition of CAD could lead to more effectively targeted intervention and reduce morbidity and mortality in this population. Myocardial perfusion imaging (MPI) with SPECT is most commonly applied to identify patients with CAD, and can accurately identify patients at increased cardiovascular risk.¹⁻⁴ However, based on recent data, a wide ranging routine MPI screening strategy of all asymptomatic patients with diabetes would appear to be ineffective.⁵ Accordingly, a selective “prescreening” strategy using an initial test for the identification of patients with a higher likelihood of abnormal MPI, followed by referral of only these patients to MPI may be preferred. Noninvasive assessment of arterial stiffness could represent a promising tool for this purpose. In several studies, a relationship between arterial stiffness and cardiovascular disease has been observed.⁶⁻⁸ Assessment of arterial stiffness by means of pulse wave velocity (PWV) or pulse wave analysis (PWA) for augmentation index (AIx), may therefore have the potential to serve as a marker of abnormal MPI. Although PWV and AIx have been extensively studied in the general population,⁹⁻¹³ fewer data are available concerning their relationship with CAD in asymptomatic patients with diabetes.

The aim of the current study was to prospectively assess the relationship between the noninvasive measures of arterial stiffness (PWV and AIx) with the presence and extent of myocardial perfusion defects as assessed by SPECT MPI in asymptomatic patients with diabetes.

Methods

Study population

Prospectively, 160 consecutive asymptomatic patients with diabetes were recruited from a routine outpatient clinic. Patients were referred to the cardiology outpatient clinic for risk assessment and cardiovascular screening. Anginal symptoms were ruled out using a self-completed questionnaire for encountered chest pain.¹⁴ The American Diabetes association (ADA) criteria were used to identify diabetes and for further stratification into type 1 or type 2 diabetes.^{14,15} Patients were considered as having type 1 diabetes if laboratory analysis demonstrated autoantibodies to islet cells, insulin and glutamic acid decarboxylase or low levels of plasma c-peptide. Otherwise, patients were considered to have type 2 diabetes. Medical history and demographics were obtained. All patients underwent physical examination, and blood and urine laboratory testing. MPI was

performed as part of clinical work-up to determine presence and extent of myocardial perfusion defects. Additional measurements of PWV and AIx were used to assess arterial stiffness.

Cardiovascular risk factors

Cardiovascular risk factors were defined according to the following criteria: positive family history for CAD (presence of CAD in first-degree family members, male <55 years and/or female <65 years), smoking (current smoking or smoking in the last 2 years), hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive medication), body mass index, hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of cholesterol-lowering medication), and microalbuminuria (urine albumin/creatinine ratio ≥ 3.5 mg/mmol).² Plasma hemoglobin A1c (HbA1c) was determined as a measure of glycemic control.

SPECT myocardial perfusion imaging

SPECT data acquisition

ECG-gated adenosine 99mTc sestamibi (99mTc MIBI) SPECT MPI was performed using a 2-day protocol, comprising stress imaging on the first day and a rest scan on the second day.^{2, 16} Antihypertensive treatment with beta-adrenergic blocking agents or calcium antagonists was stopped and patients were instructed to abstain from caffeine-containing products 24 h prior to the stress test. Vasodilator stress was induced by intravenous infusion of adenosine 140 μ g/kg per minute for 6 min, with simultaneous hand-grip exercise. 99mTc MIBI (500MBq) was injected intravenously after the third minute. Blood pressure and a 12-lead ECG were recorded throughout the adenosine infusion. Images were acquired 2 h after injection of the radiopharmaceutical using a triple-head SPECT gamma camera (GCA 9300/HG; Toshiba, Tokyo, Japan) with low-energy, high-resolution collimators. Images were acquired using a circular 360° orbit, 60 projections and 40 s per projection, in compliance with the American Society of Nuclear Cardiology (ASNC) imaging guidelines. Images were processed to obtain the short-axis, vertical long-axis, and horizontal long-axis sections, as well as polar map formats, normalized to maximal myocardial activity.^{2, 16} Patient motion was reviewed by examining the raw cine images. No attenuation or scatter correction was used.

SPECT data analysis

For semiquantitative visual interpretation, the myocardium was divided into 17 segments according to ASNC guidelines.^{2, 16} Tracer uptake in each segment was evaluated in consensus by two expert observers blinded to patient's clinical characteristics and test results, using a five-point scoring system ranging from 0 (normal uptake) to 4 (absent uptake). The summed stress score (SSS) was determined as the sum of the 17 segmental scores of the stress images. MPI was considered normal for SSS <3. In case of abnor-

mal MPI, SSS in the range 3-7 were considered to represent moderate MPI defects, and SSS ≥ 8 to represent severe MPI defects.¹⁷ Finally, regional wall motion on gated SPECT images was evaluated to allow differentiation between true MPI abnormalities and diaphragmatic or breast attenuation artifacts.

Assessment of arterial stiffness

Measurements were derived and analyzed noninvasively by applanation tonometry using a SphygmoCor system (Atcor Medical, Sydney, Australia). All measurements were performed in the morning in a quiet, temperature-controlled clinical research laboratory by a specially trained technologist blinded to the patient's clinical characteristics and test results. The patients were instructed to omit their morning medication and continue fasting until after the test. Assessment of PWV and PWA commenced following a 10-min rest in supine position, after a state of constant heart rate and blood pressure had been reached.

Pulse wave velocity

The pulse waves were recorded at the common carotid artery and the femoral artery by sequential tonometry with simultaneous electrocardiographic gating. Pulse transit time was determined as the average of ten consecutive beats. The distance between the two sites was measured. Aortic PWV (m/s) was defined as the distance between the two recording sites traveled by the pulse wave, divided by the transit time. Using system software, aortic PWV was determined semiautomatically. The validation and reproducibility of this semiautomatic method have been previously published.^{17, 18} Measurements were performed three times in each patient and averaged to obtain the mean aortic PWV.

Pulse wave analysis

The peripheral pressure waveforms were recorded from the radial artery at the wrist with a hand-held high-fidelity tonometer (Millar Instruments, Houston, USA) and calibrated by peripheral blood pressures at the brachial artery.^{7, 19} The corresponding central aortic pressure waveform was generated by a validated generalized transfer function. The central aortic pressure waveform was analyzed to identify the first shoulder of the pressure wave, representing the incident wave, attributable to left ventricular ejection. The merging point of the incident and the reflected wave (the inflection point) was then identified on the generated aortic pressure waveform. The absolute augmented pressure was the maximum systolic pressure minus the pressure at the inflection point. Subsequently, the AIx was defined as the absolute augmented pressure divided by the pulse pressure and expressed as a percentage.^{7, 8} Finally, the AIx was normalized to a heart rate of 75 bpm (AIx@75). In each patient, three consecutive waveform recordings were averaged to obtain the mean AIx@75, which was used for statistical analysis.

Statistical analysis

Continuous variables were expressed as means $\pm$ standard deviation and categorical variables as numbers (percentages). First, associations of PWV and AIx@75 with baseline clinical risk factors were assessed using Pearson's correlation coefficient (r) or the Spearman's rank correlation coefficient (r_s) in case of dichotomous variables. Second, differences in the mean PWV and AIx@75 for each group of MPI results were evaluated with the independent T-test. Thereafter, with univariate logistic regression analysis potential predictors of severe MPI defects were identified. Subsequently, all potential predictors were analyzed in a multivariate logistic regression model to identify the independent predictors of severe MPI defects. Additionally, patients were categorized according to PWV quartiles and for each quartile the prevalence of severe MPI defects was obtained. Subsequently, global chi-square analysis was used to determine the incremental predictive value of PWV over baseline characteristics. Thereafter, using receiver operating characteristic (ROC) curve analysis two cut-off values were chosen for PWV; one for the detection of severe MPI defects with optimal sensitivity and specificity and the other for the exclusion of severe MPI defects with optimal sensitivity and negative predictive value. All statistical analyses were performed using SPSS software (version 16.0, SPSS Inc., Chicago, Illinois). All p-values <0.05 were considered statistically significant.

Results

Patient characteristics

The study population comprised of 160 asymptomatic diabetic patients. Their baseline characteristics are provided in Table 1.

Table 1 Baseline characteristics of the study population of 160 diabetic patients

Clinical factors	Mean $\pm$ SD or number (%)
Age (years)	51 $\pm$ 12
Men	87 (54%)
Type 2 diabetes	91 (57%)
Diabetes duration (years)	15 $\pm$ 13
Insulin use	125 (78%)
Family history of CAD	75 (47%)
Smoking	42 (26%)
Body mass index (kg/m ²)	28 $\pm$ 6
HbA1c (%)	8.2 $\pm$ 1.7
Hypertension	92 (58%)
Use of antihypertensive medication	76 (48%)
ACE-inhibitor use	43 (27%)
Beta-blocker use	19 (12%)
Systolic blood pressure (mmHg)	133 $\pm$ 16
Diastolic blood pressure (mmHg)	80 $\pm$ 9
Hypercholesterolemia	107 (67%)
Cholesterol lowering medication	73 (46%)
Total cholesterol (mmol/L)	4.8 $\pm$ 1.1
Micro-albuminuria	39 (24%)
Aspirin use	31 (19%)

Abbreviations: ACE: angiotensin converting enzyme; CAD: coronary artery disease; HbA1c: plasma hemoglobin A1c.

SPECT myocardial perfusion imaging

The overall mean SSS was 3.1 $\pm$ 4.1 (range 0-21). Abnormal MPI (SSS $\geq$ 3) was observed in 60 patients (38 %), including moderate MPI defects (SSS 3-7) in 38 patients (24 %) and severe MPI defects (SSS $\geq$ 8) in 22 patients (14 %).

Arterial stiffness

Pulse wave velocity

The overall mean PWV was 8.9 ± 2.4 m/s. PWV was associated with age ($r = 0.62$, $p < 0.01$), type 2 diabetes ($r_s = 0.23$, $p < 0.01$), diabetes duration ($r = 0.30$, $p < 0.01$), body mass index ($r = 0.22$, $p < 0.01$), hypertension ($r_s = 0.43$, $p < 0.01$), and microalbuminuria ($r = 0.29$, $p < 0.01$). As shown in Figure 1a, mean PWV was only slightly lower in patients with normal MPI than in patients with moderate MPI defects (8.4 ± 2.2 m/s and 9.0 ± 2.2 m/s, respectively, $p = 0.11$). However, the mean PWV was significantly higher in patients with severe MPI defects (11.1 ± 2.5 m/s, $p < 0.01$).

Association between pulse wave analysis and myocardial perfusion

The mean AIx was 21.1 ± 12.3 % in the total population. Normalization for a heart rate of 75 bpm resulted in an overall mean AIx@75 of 19.6 ± 12.4 %. A significant association was observed between AIx@75 and the following risk factors: age ($r = 0.47$, $p < 0.01$), male gender ($r_s = -0.43$, $p < 0.01$), type 2 diabetes ($r_s = 0.30$, $p < 0.01$), hypercholesterolemia ($r_s = 0.17$, $p < 0.03$), and microalbuminuria ($r = 0.26$, $p < 0.01$).

After stratification of mean AIx@75 values according to SPECT MPI results, a trend similar to that for PWV was observed. Likewise, mean AIx@75 was slightly higher in patients with moderate MPI defects than in those with normal MPI (19.4 ± 10.7 % and 18.4 ± 13.4 %, respectively, $p = 0.66$) and was significantly higher in patients with severe MPI defects (25.4 ± 9.0 %, $p = 0.03$; Figure 1b).

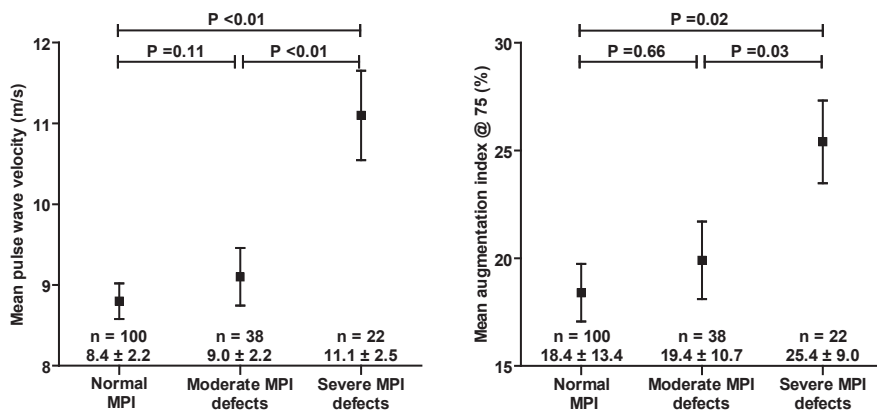


Figure 1. Relationship between parameters of vascular stiffness and the extent of MPI defects as assessed by SPECT MPI. A: Mean aortic PWV was higher in patients with abnormal MPI. The highest PWV was observed in patients with severe MPI defects. B: The relationship between mean AIx@75 and MPI shows a similar trend.

Predictors of severe myocardial perfusion defects

As illustrated in Table 2, age, gender, smoking, HbA1c, microalbuminuria and both PWV and AIx@75 were identified as potential predictors of severe MPI defects in a univariate logistic regression model. Of note, after adjustment for age, gender, smoking, HbA1c, and microalbuminuria, the PWV remained a significant predictor of severe MPI defects ($p = 0.01$), whereas the AIx@75 was no longer significant.

As demonstrated in Figure 2, the prevalence of severe MPI defects gradually increased with increasing PWV quartile. Importantly, in none of the patients in the lowest PWV quartile were severe MPI defects present. Also, only a relatively small proportion of patients (5 %) in the second PWV quartile had severe MPI defects. In contrast, the prevalence of severe MPI defects was 20 % in the third PWV quartile, and was 30 % in the fourth quartile. Moreover, the addition of PWV to a model with baseline clinical risk factors age, gender and smoking for the prediction of severe MPI defects showed a significantly improved predictive value of PWV (Figure 3).

ROC curve analysis for the detection of severe MPI defects showed the highest sensitivity and specificity (77 % and 75 %, respectively) with a PWV cut-off value of 9.8 m/s. An optimal sensitivity of 91 % with an associated negative predictive value of 98% for the exclusion of severe MPI defects was found using a cut-off value of 9.2 m/s for PWV (Figure 4).

Table 2 Predictors of severe MPI defects (SSS ≥ 8) on SPECT

Clinical characteristic	Exp β (95 % CI)	p-value	Exp β (95 % CI)	p-value
Age (years)	1.09 (1.04-1.14)	<0.01	1.06 (0.98-1.14)	0.16
Male gender	3.30 (1.15-9.45)	0.03	6.35 (1.47-27.41)	0.01
Type 2 diabetes	1.47 (0.55-3.90)	0.44		
Diabetes duration (years)	1.02 (0.99-1.06)	0.15		
Family history of CAD	1.16 (0.47-2.85)	0.75		
Smoking	3.80 (1.48-9.77)	0.01	5.74 (1.35-24.46)	0.02
Body mass index (kg/m ²)	0.99 (0.91-1.07)	0.77		
HbA1c (%)	1.28 (1.00-1.65)	0.05	1.52 (1.03-2.25)	0.03
Hypertension	1.98 (0.73-5.41)	0.18		
Hypercholesterolemia	1.65 (0.57-4.79)	0.36		
Micro-albuminuria	3.86 (1.52-9.81)	0.01	1.05 (0.26-4.27)	0.95
PWV (m/s)	1.49 (1.22-1.81)	<0.01	1.49 (1.11-2.00)	0.01
AIx@75 (%)	1.06 (1.01-1.11)	0.02	1.05 (0.97-1.14)	0.20

Abbreviations: AIx@75: Augmentation index normalized for the heart rate of 75 bpm; CAD: coronary artery disease; HbA1c: plasma hemoglobin A1c; MPI: myocardial perfusion imaging; PWV: Pulse wave velocity.

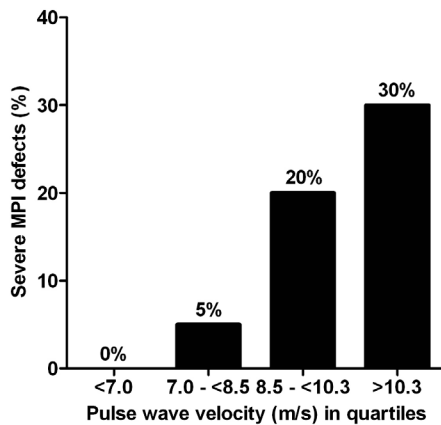


Figure 2 Prevalence of patients with severe MPI defects per PWV quartile. The prevalence of severe MPI defects increased with increasing PWV. Of note, the prevalence of severe MPI defects chiefly increased in the third and fourth PWV quartile.

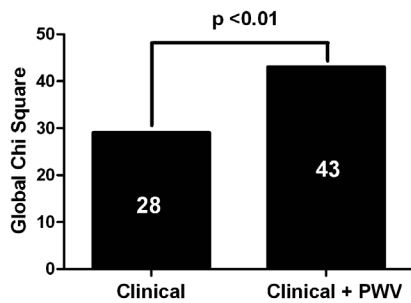


Figure 3 Incremental predictive value of PWV for the detection of severe MPI defects as shown by an increase in the value of global chi-square. Addition of PWV to a model with baseline clinical risk factors age, gender, and smoking provided a significantly improved predictive value.

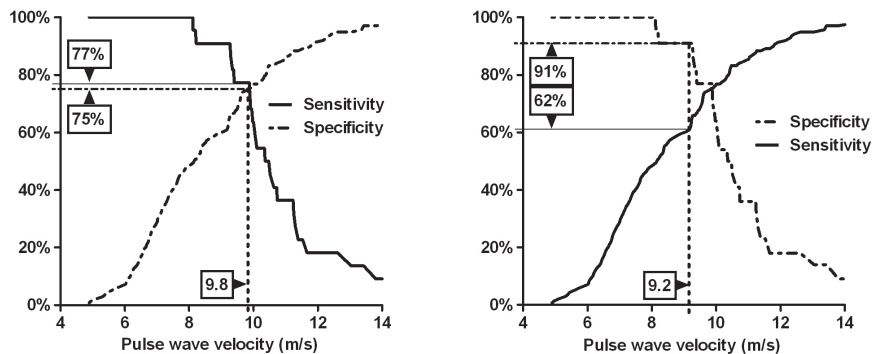


Figure 4 Detection of severe MPI defects on SPECT by PWV. A: ROC curve analysis for the detection of severe MPI defects yielded an optimal sensitivity and specificity of 77% and 75%, respectively, with a PWV cut-off value of 9.8 m/s. B: In contrast, optimization for the exclusion of severe MPI defects resulted in a cut-off value of 9.2 m/s with a sensitivity of 91% and corresponding negative predictive value of 98%.

Discussion

In the present study of asymptomatic patients with diabetes, arterial stiffness as assessed by PWV and AIx was greater in the presence of severe MPI defects. PWV was independently associated with severe MPI defects, whereas AIx was no longer significant after correction for other cardiovascular risk factors and PWV. Addition of PWV to a model of baseline clinical risk factors showed significant incremental value for the prediction of severe MPI defects. Furthermore, ROC curve analysis revealed a moderate to good sensitivity of 77 % and a specificity of 75 % for the detection of severe MPI defects, with a PWV cut-off value of 9.8 m/s. Conversely, changing the cut-off value to 9.2 m/s resulted in a high sensitivity of 91 % and negative predictive value of 98 % for the exclusion of severe MPI defects. Accordingly, the current results indicate that noninvasive evaluation of arterial stiffness may be a practical tool for prescreening asymptomatic patients with diabetes for differentiation into a higher and lower likelihood of abnormal MPI.

Arterial stiffness and relationship to CAD

In the general population the relationship between arterial stiffness and the presence of CAD has been confirmed in a considerable number of studies. Arterial stiffness measured as PWV or AIx not only is directly associated with the presence and severity of CAD on invasive coronary imaging,¹⁰ but also has incremental prognostic value for predicting cardiovascular events.^{9, 11-13} A recent meta-analysis (15,877 subjects, 17 studies, average follow-up of 7.7 years) has shown that the risk of cardiovascular events is increased twofold in patients with increased PWV.²⁰ Moreover, the predictive ability of PWV was shown to be even higher in patients with elevated baseline cardiovascular risk, supporting a role for PWV in high-risk populations, such as patients with diabetes. A few studies have specifically evaluated arterial stiffness in patients with diabetes. Cruickshank et al. evaluated the prognostic value of PWV for all-cause and cardiovascular mortality in 397 patients with diabetes with or without CAD. During a mean follow-up of 10.7 years, aortic PWV was an independent predictor for all-cause and cardiovascular mortality.²¹ Additionally, Hatsuda et al. found in 595 patients with diabetes that PWV was significantly increased in 70 patients with established CAD.²² Finally, Fukui et al. investigated 208 consecutive patients with type 2 diabetes and reported that AIx was significantly higher in 47 patients with previously confirmed CAD.²³ These observations indicate that markers of arterial stiffness may indeed be associated with CAD in patients with diabetes.

However, to our knowledge this is the first study in which PWV and AIx have been applied in asymptomatic patients with diabetes to prospectively identify the presence of CAD, defined by the presence of (severe) MPI defects. Although both PWV and AIx were higher in patients with severe MPI defects, only PWV was shown to be an independent predictor of severe MPI defects. These observations are in agreement with the previous literature, as more discrepant results have also been reported in the general

population using AIx as compared to PWV.^{24, 25}

Possibly, the more variable results with AIx may be explained by underlying methodological differences. Carotid-femoral PWV is a direct measure of arterial stiffness as determined by the intrinsic stress/strain relationship of the arterial wall and mean arterial pressure. Therefore PWV is considered as the 'gold-standard'.⁶⁻⁸ In contrast, AIx is an indirect measurement, derived from peripherally recorded pressure waveforms. Using a generalized transfer function, the corresponding central arterial waveform is generated, from which AIx is determined. Therefore, AIx is influenced by multiple factors such as PWV, heart rate, diastolic blood pressure, peripheral circulation and endothelial function.^{7, 8} Furthermore, its discriminatory value may be less in the elderly,^{24, 25} and the use of the generalized transfer function may also be inappropriate in certain populations.²⁴⁻²⁷ In fact, Hope et al. recently evaluated the validity of this method in patients with diabetes and found that estimation of central pressures was prone to substantially greater error in this population.²⁶ Similar differences in accuracy have also been reported in relation to gender, indicating that AIx might be a less representative marker of arterial stiffness than PWV.^{24, 25} Conceivably, the weaker association between AIx and CAD as compared to PWV may therefore be explained by the fact that our study was performed in patients with diabetes while also including a high percentage of female patients.

Clinical implications and perspectives

At present, screening of asymptomatic patients with diabetes for CAD remains controversial. The majority of available data are based on CAD detection using SPECT MPI.^{3, 4} In the present study, the prevalence of abnormal MPI was 38%. In contrast, the recent DIAD trial demonstrated a much lower rate of abnormal MPI with only few patients having severe MPI defects.^{5, 28} To a large extent, this discrepancy may be explained by differences in baseline characteristics of the enrolled patients. Importantly, cardiac event rates were low in the DIAD study and not significantly reduced by a MPI-based screening strategy. Nevertheless, in the small group of patients with abnormal MPI, a step-wise increase in event rates was observed with increasing MPI abnormality. Of note, hard event rates were 2% in patients with normal scans but were 12% in patients with at least moderately abnormal MPI scans. In contrast to the general asymptomatic diabetic population, these high-risk patients may benefit from screening, as also suggested by the bypass angioplasty revascularization investigation BARI 2 diabetes trial.^{29, 30} In this trial no survival benefit was shown in patients undergoing early coronary revascularization as compared to intensive medical treatment. However, among high risk patients selected for coronary artery bypass grafting, prompt revascularization was associated with a lower rate of major cardiovascular than medical therapy. Accordingly, these observations indicate that while routine screening for abnormal MPI may not be effective in asymptomatic patients with diabetes, selective screening strategies are warranted to identify the small but high-risk subgroup within this population. In this regard, our

current study may provide valuable data for the design of such strategies. Assessment of arterial stiffness by means of PWV was shown to accurately identify patients with a high risk of severe MPI defects. Accordingly, further screening in patients with elevated PWV may be recommended. On the other hand, when using a slightly lower cut-off value, PWV was also shown to have a high negative predictive value, indicating that PWV can accurately rule out severe MPI defects. Therefore, further evaluation in patients with a negative PWV study may be safely omitted.

Due to its low costs and noninvasive nature, PWV may be a practical first-line tool to differentiate patients with a higher and lower likelihood of having abnormal MPI. A number of limitations must be acknowledged in the current study. Some of the observed MPI defects may be attributed to artifact attenuation. However, regional wall motion on gated SPECT images was analyzed for optimal differentiation between true MPI defects and attenuation artifacts. Evidently, larger prospective studies are needed to demonstrate the effectiveness of this strategy in terms of costs and outcome.

Conclusions

Arterial stiffness as noninvasively assessed by PWV is related to severely abnormal myocardial perfusion in asymptomatic patients with diabetes. Accordingly, PWV could be a practical tool to identify patients at higher risk of CAD and who could benefit from further screening.

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CHAPTER 5

Relationship between left ventricular diastolic function and arterial stiffness in asymptomatic patients with diabetes mellitus



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Abstract

Left ventricular (LV) diastolic dysfunction and increased arterial stiffness are common in patients with diabetes mellitus (DM). However, the relation between these two pathophysiological factors remains unclear. The aim of this study was to investigate the relationship between LV diastolic function and arterial stiffness as assessed with applanation tonometry.

In 142 asymptomatic patients with DM (mean age 48 years, 75 (53 %) men, 72 (51 %) patients with type 2 DM) LV diastolic function was assessed with echocardiography. Arterial stiffness was evaluated measuring the aortic pulse wave velocity (PWV) whereas wave reflection was assessed measuring central systolic blood pressure (cSBP), central pulse pressure (cPP), and augmentation index (AIx) with applanation tonometry.

Mean E/A ratio, E' and E/E' ratio were 1.1 ± 0.3 , 8.1 ± 2.3 cm/s and 9.2 ± 3.3 , respectively. Mean PWV, mean cSBP, median cPP and mean AIx were 7.9 ± 2.4 m/s, 122 ± 17 mmHg, 40 [35-51] mmHg and 17.9 ± 12.1 %, respectively. PWV was independently associated with LV diastolic dysfunction grade ($\beta = 0.76$, $p = 0.03$). In contrast, measures of wave reflection, cPP, cSBP and AIx were independently related with E/A ratio, but not with the LV diastolic dysfunction grade.

Parameters of arterial stiffness and wave reflection are associated with echocardiographic indices of LV diastolic function in asymptomatic patients with DM. Therapies that prevent progression of arterial stiffness and reduce late-systolic pressure overload may help to reduce the prevalence of LV diastolic dysfunction in this population.

Introduction

Patients with diabetes mellitus (DM) have a two- to fourfold higher risk of cardiovascular events than nondiabetic patients.¹ Endothelial dysfunction, micro- and macrovascular remodeling, increased deposition of collagen and advanced glycation end products are well known pathophysiologic mechanisms that lead to accelerated arterial and myocardial stiffness in diabetic patients.² Left ventricular (LV) diastolic dysfunction is one of the first consequences of increased myocardial stiffness, contributes to 50 % of the incidence of heart failure with preserved LV ejection fraction and is associated with poor outcome.³⁻⁵ In addition, increased arterial stiffness has been associated with LV diastolic dysfunction and is an independent predictor of cardiovascular events.^{6,7} Therefore, early detection of LV diastolic dysfunction and increased arterial stiffness in patients with DM may help to identify the patients at increased risk for cardiovascular events and allow for early initiation of preventive therapeutic strategies.

So far, evaluation of the relation between LV diastolic function and arterial stiffness in patients with DM has provided conflicting results.⁸⁻¹⁰ Whereas LV diastolic function is consistently assessed by standardized parameters obtained with echocardiography according to current recommendations of the American Society of Echocardiography (ASE) and European Association of Echocardiography (EAE),¹¹ there is no uniformity in the noninvasive assessment of arterial stiffness. Based upon the methodology used to evaluate arterial stiffness, the results of previous studies investigating the relation between LV diastolic function and arterial stiffness may significantly vary.⁸⁻¹⁰ Applanation tonometry may be the preferred technique since it provides the aortic pulse wave velocity (PWV), considered the gold standard measure of arterial stiffness and is an established end-point of target-organ damage in patients with hypertension.¹²⁻¹⁴ Thus, the aim of the current study was to investigate the relation between LV diastolic function measured with echocardiography and tissue Doppler imaging and arterial stiffness assessed with applanation tonometry in asymptomatic patients with DM.

Methods

Patient population

The patient population was derived from an ongoing registry including asymptomatic patients with DM. DM was diagnosed and classified according the American Diabetes Association criteria.¹⁵ Patients with demonstrable auto-antibodies to islet cells, insulin and glutamic acid decarboxylase or low levels of plasma c-peptide in laboratory analysis were considered as having type 1 DM. Otherwise, patients were considered to have type 2 DM. Comprehensive evaluation of asymptomatic DM patients was routinely performed at the outpatient clinic of the Leiden University Medical Center.¹⁶ This evaluation included a cardiovascular risk assessment performed at the cardiology outpatient clinic consisting of structured clinical history, physical examination and blood and urine

laboratory testing. LV function and dimensions and valvular function were assessed with transthoracic echocardiography. Noninvasive assessment of arterial stiffness was performed with applanation tonometry. Clinical and echocardiographic data were prospectively collected in the departmental cardiology information system (EPD-Vision®) and echocardiographic database and were retrospectively analyzed.

Asymptomatic status was confirmed with a self-completed questionnaire on chest pain.¹⁷ Patients with significant coronary artery disease, impaired systolic function defined as LV ejection fraction (LVEF) <50 % and moderate and severe valvular heart dysfunction were excluded.

The independent associations between parameters of LV diastolic function and indices of arterial stiffness by applanation tonometry were assessed.

Cardiovascular risk factors evaluation

Overweight defined by a body mass index (BMI) ≥ 25 kg/m², family history of coronary artery disease (in first degree family members; male <55 years and/or female <65 years), smoking status (current smoking or smoking in the last 2 years), hypertension (blood pressure >140/90 mmHg or use of antihypertensive medication) and hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication) were recorded as cardiovascular risk factors. In addition, diabetes related risk factors were defined by DM duration in years, levels of hemoglobin A1c, renal dysfunction based on glomerular filtration rate and presence of microalbuminuria (urine albumin/creatinine ratio ≥ 3.5 mg/mmol).

Echocardiography

Two-dimensional transthoracic echocardiography was performed in all patients using a commercially available system (Vivid 7 and E9, General-Electric Vingmed, Horton, Norway). ECG-gated images were obtained at rest in the left lateral decubitus position using 3.5-MHz and M5S transducers in the parasternal, apical and subcostal views. Standard M-mode and two-dimensional, color, continuous and pulsed wave Doppler images were acquired during breath hold and saved in cine-loop format. The obtained images were analyzed offline, using dedicated software (EchoPac version 110.0.0 General-Electric Vingmed).

LV end-systolic volume (LVESV), end-diastolic volume (LVEDV) and LVEF were assessed using the biplane Simpson method in the apical 4- and 2-chamber views.¹⁸ LV systolic dysfunction was defined as LVEF <50 %. In addition, left atrial (LA) volume was determined with the biplane Simpson method in the apical 4- and 2-chamber views according to the ASE and the EAE guidelines.¹⁸ The LA volume index was calculated by dividing the LA volume by the body surface area. Enlarged left atrium was defined by a LA volume index ≥ 34 mL/m².¹¹

LV diastolic function assessment included the measurement of peak velocities of the

early (E) and late (A) mitral inflow and the deceleration time (DT) of the E-wave at the apical 4-chamber view, using the pulsed wave Doppler. The E/A ratio was calculated. Isovolumic relaxation time was measured from pulsed wave Doppler spectral recordings obtained in the apical 5-chamber view placing the sample volume between the aortic valve and the anterior mitral leaflet. Furthermore, systolic and diastolic pulmonary vein velocities (PVs and PVd) were measured from pulsed wave Doppler recordings at the right superior pulmonary vein in the apical 4-chamber view. Thereafter, the pulmonary vein PVs/PVd ratio was calculated. Additionally, high frame rate tissue Doppler imaging data were obtained in the apical 4-chamber view and the early peak mitral annular velocity (E') at the lateral and septal mitral annulus were measured offline. The mean E' value was obtained by averaging these measurements. Subsequently, the E/E' ratio was calculated.¹¹ Finally, patients were classified in grades of LV diastolic dysfunction, according criteria derived from the ASE guidelines.¹¹ Patients with E' ≥ 9 cm/s and LA volume ≤ 34 mL/m² were defined as having a normal diastolic function. In the remaining patients, mild diastolic dysfunction (grade I) was defined as E/A ratio < 0.8 , DT > 200 ms and E/E' ratio ≤ 8 ; moderate diastolic dysfunction (grade II) as E/A ratio 0.8-1.5, DT between 160-200 ms and E/E' ratio between 9 and 12 and severe diastolic dysfunction (grade III) as E/A ratio ≥ 2 , DT < 160 ms and E/E' ratio ≥ 13 .¹¹

Applanation tonometry

All patients underwent noninvasive evaluation of arterial stiffness with applanation tonometry using a SphygmoCor system (SphygmoCor, Atcor Medical, Sydney, Australia) with a hand-held high fidelity tonometer (Millar Instruments, Houston, TX, USA). Measurements were performed by a specially trained technologist, blinded to patient's clinical characteristics and echocardiographic results, under standardized conditions (during the morning in a quiet, temperature-controlled clinical research laboratory). Patients were instructed to abstain from their morning medication and remain fasting until the end of the test. Measurements were performed after 10-minute rest in supine position, when a state of constant heart rate and blood pressure was reached.

Pulse wave velocity

Aortic pulse wave velocity (PWV) was determined with arterial tonometry of the carotid and femoral arteries with simultaneous ECG-gating.^{6, 19} The aortic PWV was defined as the distance traveled by the pulse wave, between recording sites on the carotid and femoral artery, divided by transit time (averaged from 10 consecutive beats) and was determined semi-automatically as previously described.^{6, 19} To correct for measurement variability, three consecutive beats were measured and the average was calculated. The reference value of PWV for a healthy population aged between 40 and 49 years is 7.5 ± 2.5 m/s.²⁰

Pulse wave analysis

Central systolic blood pressure (cSBP), central pulse pressure (cPP) and augmentation index (AIx), measures of wave reflection, were derived from pulse wave analysis. Peripheral pressure wave forms were recorded on the radial artery at the level of the wrist and calibrated by peripheral blood pressures measured at the brachial artery with a cuff-sphygmomanometer. Central aortic pressure waveforms were generated from these recorded pressure waveforms with a validated generalized transfer function and used to calculate cSBP, cPP and AIx (Figure 1).^{12, 19}

cSBP was defined as the peak pressure of the aortic pressure waveform (Figure 1). cPP was calculated as the difference between central systolic and diastolic pressure (Figure 1). The aortic pressure waveform is formed by the forward pressure wave of ventricular contraction and a backward pressure wave from reflection on the peripheral arterial system.¹² With increasing arterial stiffness, the reflected wave shifts from diastole to systole and increases systolic blood pressure, which is identified on the central aortic pressure waveform by the merging point of the initial forward wave and the reflected wave (Figure 1). The AIx was defined and calculated as the percentage that the reflected wave contributes to the pulse pressure (maximum systolic pressure minus pressure at the merging point). Thereafter, AIx was normalized to a heart rate of 75 beats/min.^{12, 21} AIx was measured on 3 consecutive recordings and the average was calculated. Reference values were derived from a substudy of the Anglo-Cardiff Collaborative Trial (ACCT) on vascular aging, including 559 healthy subjects (258 men and 301 women) in the age category of 40 and 49 years.²² In this study, the observed mean cSBP, CPP and AIx in men were 113 ± 9 mmHg, 34 ± 6 mmHg and 19 ± 10 % and in women 109 ± 11 mmHg, 33 ± 8 mmHg and 28 ± 10 %, respectively.

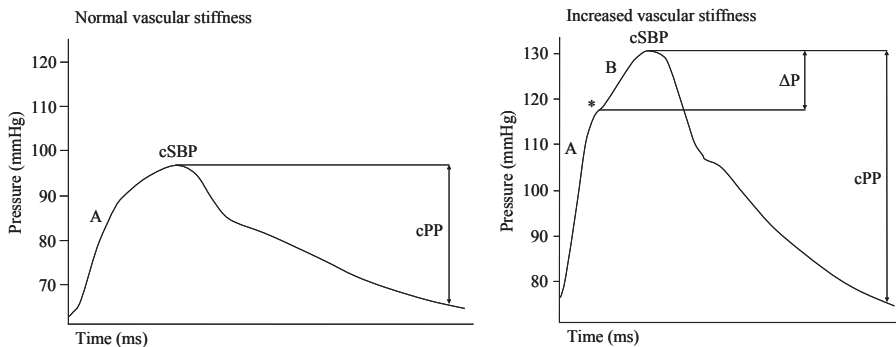


Figure 1 Central aortic pressure waveform of an individual with normal arterial stiffness (a) and an individual with increased arterial stiffness (b). Central systolic blood pressure was defined as the peak pressure of the central aortic pressure waveform. Central pulse pressure (cPP) was calculated as the pressure difference of the aortic pressure waveform. The augmentation index is calculated as the percentage pressure augmentation from the reflected wave (ΔP) to the cPP.

*: merging point of incident wave and reflected wave, ΔP : pressure augmentation from the reflected wave, A: incident wave, B: reflected wave, cPP: central pulse pressure, cSBP: central systolic blood pressure.

Statistical analysis

Normal distributed continuous variables were expressed as mean $\pm$ standard deviation, non-normal distributed variables as median (25th and 75th percentiles), and categorical variables as numbers (percentages). The Pearson's and Spearman's correlation coefficients were used to assess univariate associations between the indices of arterial stiffness and wave reflection and baseline clinical variables and echocardiographic parameters of LV diastolic function.

Multivariate linear regression analyses were performed to assess independent relations between the indices of arterial stiffness and wave reflection and parameters of LV diastolic function. These associations were assessed for each LV diastolic function echocardiographic parameter and corrected for age, gender, type and duration of DM, heart rate and BMI. All statistical analyses were performed using SPSS software (version 16.0, SPSS Inc., Chicago, Illinois). P-values <0.05 were considered statistically significant.

Results

A total of 142 asymptomatic patients with DM were evaluated. The baseline clinical variables are presented in Table 1. The mean age was 48 ± 11 years and 75 (53 %) were men. Fifty-one percent of patients had DM type 2. The mean DM duration was 15 ± 12 years and mean BMI was 28 ± 6 kg/m². Sixty-eight (48 %) patients had hypertension.

Table 1 Baseline clinical variables

Clinical variables	n=142
Age (years)	48 ± 11
Male gender, n (%)	75 (53%)
DM type 2, n (%)	72 (51%)
DM duration (years)	15 ± 12
Hemoglobin A1c (%)	7.9 ± 1.5
BMI (kg/m ²)	28 ± 6
Family history of CAD, n (%)	52 (37%)
Smoking, n (%)	35 (25%)
Diastolic blood pressure (mmHg)	80 ± 9
Systolic blood pressure (mmHg)	131 ± 16
Heart rate (beats/min)	70 ± 10
Hypertension, n (%)	68 (48%)
Hypercholesterolemia, n (%)	102 (72%)
Glomerular filtration ratio (mL/min/1.73m ²)	92 ± 21
Microalbuminuria ≥ 3.5 mg/mmol, n (%)	21 (15%)

Abbreviations: BMI: body mass index; CAD: coronary artery disease; DM: diabetes mellitus

Echocardiography

The mean LVEF and LA volume index were normal, 65 ± 9 % and 16 ± 4 mL/m², respectively (Table 2). The mean E/A ratio, E' and E/E' ratio were 1.1 ± 0.3 , 8.1 ± 2.3 cm/s and 9.2 ± 3.3 , respectively. In 15 patients an increased left ventricular filling pressure was identified by E/E' ratio ≥ 13 . Normal LV diastolic filling pattern, mild (grade I) and moderate (grade II) diastolic dysfunction were observed in 72 (51 %), 54 (38 %) and 16 (11 %) patients, respectively. Grade III diastolic dysfunction was not recorded in any patient.

Table 2 Echocardiographic parameters of left ventricular function and indices of arterial stiffness measured with applanation tonometry

Variable	n=142
Echocardiographic parameters	
LVEDV (mL)	114 ± 29
LVESV (mL)	41 ± 16
LVEF (%)	65 ± 9
LA volume index (mL/m ²)	16 ± 4
E (cm/s)	70 ± 16
A (cm/s)	65 ± 18
E/A ratio	1.1 ± 0.3
DT (ms)	226 ± 68
Isovolumic relaxation time (ms)	84 ± 12
E' (cm/s)	8.1 ± 2.3
E/E' ratio	9.2 ± 3.3
PV _s (cm/s)	50 ± 11
PV _d (cm/s)	42 ± 10
PV _s /PV _d ratio	1.2 ± 0.3
Indices of arterial stiffness	
PWV (m/s)	7.9 ± 2.4
cSBP (mmHg)	122 ± 17
cPP (mmHg)*	40 [35, 51]
AIx (%)	17.9 ± 12.1

Abbreviations: A: peak transmitral late diastolic inflow velocity, AIx: augmentation index, cPP: central pulse pressure, cSBP: central systolic blood pressure, DT: deceleration time of the E-wave velocity, E: peak transmitral early diastolic inflow velocity, E': peak early mitral annular velocity averaged from measurement in septal and lateral mitral annulus, LA volume index: left atrial volume index, LVEDV: left ventricular end-diastolic volume, LVEF: left ventricular ejection fraction, LVESV: left ventricular end-systolic volume, PVd: diastolic pulmonary vein velocity, PVs: systolic pulmonary vein velocity, PWV: pulse wave velocity.

*expressed as median (25th and 75th percentiles).

Arterial stiffness assessed with applanation tonometry

The mean PWV was 7.9 ± 2.4 m/s, similar as the reference value established in a healthy study population (Table 2).²⁰ The mean cSBP and median cPP were both slightly increased (122 ± 17 mmHg and 40 [35-51] mmHg, respectively). However, the mean AIx was within the normal range (17.9 ± 12.1 %).²²

Clinical and echocardiographic correlates of arterial stiffness

Table 3 presents the univariate correlation coefficients between indices of arterial stiffness and wave reflection and baseline clinical variables as well as echocardiographic parameters of LV diastolic function. Age was significantly correlated with all indices of arterial stiffness and wave reflection, whereas male gender was only significantly related with AIx. Hypertension and systolic blood pressure were significantly related with all indices of arterial stiffness and wave reflection. In contrast, other well-known cardiovascular risk factors or specific diabetes-related variables were not consistently related with indices of arterial stiffness or wave reflection. Importantly, echocardiographic parameters of LV diastolic function were significantly associated with all indices of arterial stiffness and wave reflection. Similarly, LV diastolic dysfunction grade was significantly associated with all indices of arterial stiffness and wave reflection.

Table 3 Correlation coefficients between indices of arterial stiffness and baseline clinical variables as well as parameters of LV diastolic function

	PWV	cSBP	cPP	AIx
Age (years)	0.34**	0.36**	0.45**	0.43**
Male gender	-0.05	-0.07	-0.07	-0.47**
DM type 2	0.11	0.23**	0.11	0.24**
DM duration (years)	0.24**	0.07	0.15	0.07
BMI (kg/m ²)	0.17*	0.19*	0.07	0.22**
Heart rate (beats/min)	0.17*	0.03*	-0.15	0.24**
Systolic blood pressure (mmHg)	0.22**	0.47**	0.31**	0.26**
Hypertension	0.39**	0.39**	0.31**	0.29**
Hypercholesterolemia	0.15	0.12	0.17*	0.22**
Microalbuminuria	0.25**	0.23**	0.13	0.14
E/A ratio	-0.35**	-0.44**	-0.39**	-0.52**
E' (cm/s)	0.32**	-0.40**	-0.38**	-0.44**
E/E' ratio	0.26**	0.30**	0.29**	0.31**
LV diastolic dysfunction	0.38**	0.37**	0.32**	0.29**

Abbreviations: A: peak transmitral late diastolic inflow velocity, AIx: augmentation index, BMI: body mass index, cPP: central pulse pressure, cSBP: central systolic blood pressure, DM: diabetes mellitus, E: peak transmitral early diastolic inflow velocity, E': peak early mitral annular velocity averaged from measurement in septal and lateral mitral annulus, PWV: pulse wave velocity.

* *p*-value <0.05, ** *p*-value <0.01

Independent associations between LV diastolic function and arterial stiffness

Independent significant associations between echocardiographic parameters of LV diastolic function and arterial indices were identified with multivariate linear regression

Table 4 Associations between echocardiographic parameters of LV diastolic function and indices of arterial stiffness.

Multivariate linear regression analysis with adjustment for age, gender, diabetes mellitus (DM) type 2, DM duration, body mass index and heart rate.

Dependent variable	Independent variable	β	95% CI	p-value
PWV	E/A ratio	-1.36	-2.86 to 0.14	0.07
	E'	-0.13	-0.36 to 0.09	0.25
	E/E' ratio	0.04	-0.10 to 0.18	0.56
	LV diastolic dysfunction	0.76	0.05 to 1.46	0.03
cSBP	E/A ratio	-18.30	-28.91 to -7.68	0.001
	E'	-2.10	-3.74 to -0.47	0.01
	E/E' ratio	0.86	-0.14 to 1.85	0.09
	LV diastolic dysfunction	4.05	-0.99 to 9.08	0.11
cPP	E/A ratio	-9.93	-17.78 to -2.07	0.01
	E'	-1.08	-2.28 to 0.12	0.08
	E/E' ratio	0.56	-0.16 to 1.29	0.13
	LV diastolic dysfunction	2.40	-1.27 to 6.07	0.20
AIx	E/A ratio	-10.38	-16.62 to -4.15	0.001
	E'	-1.05	-2.01 to -0.08	0.03
	E/E' ratio	0.04	-0.54 to 0.63	0.88
	LV diastolic dysfunction	-0.79	-3.75 to 2.19	0.60

Abbreviations: A: peak transmitral late diastolic inflow velocity, AIx: augmentation index, cPP: central pulse pressure, cSBP: central systolic blood pressure, E: peak transmitral early diastolic inflow velocity, E': peak early mitral annular velocity averaged from measurement in septal and lateral mitral annulus, PWV: pulse wave velocity.

analysis (Table 4). The multivariate linear regression models were corrected for age, gender, DM type 2, DM duration, body mass index and heart rate. PWV was independently associated with LV diastolic dysfunction grade and tended to be significantly correlated with E/A ratio. In contrast, cPP was significantly correlated with E/A ratio but not with LV diastolic dysfunction grade. Furthermore, cSBP and AIx were associated with E/A ratio and E'.

Discussion

The present evaluation demonstrated the independent associations between arterial stiffness and wave reflection parameters and LV diastolic function indices in asymptomatic patients with DM. PWV, a parameter of arterial stiffness, was independently associated with LV diastolic dysfunction grade, whereas cPP, cSBP and AIx, parameters of wave reflection, were significantly associated with E/A ratio. cSBP and AIx were also independently correlated with E'.

LV diastolic dysfunction and arterial stiffness in asymptomatic DM patients

In patients with DM, endothelial dysfunction, increased extracellular deposition of collagen and advanced glycation end products, and activation of the renin-angiotensin-aldosterone system and cytokines lead to increased arterial and myocardial stiffness.² Increased arterial stiffness has been reported in asymptomatic DM patients with LV diastolic dysfunction and preserved LVEF.^{10, 23} In addition, arterial stiffness and LV diastolic dysfunction are considered important pathophysiologic determinants of overt heart failure development and coronary heart disease events in patients with DM.^{24, 25} In a population-based study including 1,760 DM patients, the presence of LV diastolic dysfunction and increased LV filling pressures (increased E/E' ratio) was independently associated with the subsequent development of heart failure.²⁴ In addition, data from The Cardiff Diabetes Database, including 2,911 patients with type 2 DM showed that peripheral pulse pressure was the best predictor of coronary heart disease events.²⁵ This evidence suggests that parameters of LV diastolic function and arterial stiffness can be used as markers of cardiovascular disease for the identification of patients with DM at increased risk of cardiovascular events. Progressive arterial stiffening causes an accelerated systolic return of the arterial wave reflection from the peripheral arterial tree, leading to increased systolic blood pressure and reduced coronary perfusion.²⁶ These changes result in an increased systolic workload and mismatch in the myocardial supply/oxygen demand ratio, which cause LV diastolic dysfunction and at a later stage systolic dysfunction. However, the association between arterial stiffness and LV diastolic dysfunction in DM patients remains debated.

In 49 patients with new onset type 2 DM, Loimaala et al. measured arterial stiffness with whole body impedance cardiography and LV diastolic function with conventional echocardiography and tissue Doppler imaging.²⁷ PWV was only independently associated with E'. Furthermore, in 42 patients with DM, Eren and coworkers demonstrated significant correlations between aortic distensibility measured with M-mode echocardiography and E/A ratio, isovolumic relaxation time and DT.²⁸ Using magnetic resonance imaging, van der Meer et al. demonstrated a significant correlation between aortic distensibility and LV diastolic function in asymptomatic patients with type 2 DM.²³ The use of different techniques to evaluate arterial stiffness may have led to inconsistent correlations with several parameters of LV diastolic function.

Assessment of arterial stiffness with applanation tonometry and correlates of LV diastolic dysfunction

Applanation tonometry is a validated and reproducible method for the noninvasive assessment of arterial stiffness.^{19, 29} The friendly use and low costs are some of the advantages of this method. PWV measured at the carotid and femoral arteries is considered the gold standard measure of arterial stiffness.¹²

Recently, Sharman et al. evaluated wave reflection parameters (AIx and cPP) with ap-

planation tonometry in 172 patients with type 2 DM.¹⁰ cPP was independently associated with E/E' ratio and A as assessed with echocardiography. However, PWV was not evaluated. As previously described, wave reflection parameters (such as cPP and AIx) and arterial stiffness parameters (such as PWV) may reflect different aspects of arterial properties and, therefore, may correlate differently with LV diastolic function parameters. Indeed, wave reflection parameters have demonstrated to be less affected by the aging process as compared to parameters of arterial stiffness.³⁰ In addition, previous studies have reported stronger associations between PWV and LV diastolic function as compared to parameters of wave reflection.^{31, 32} The present evaluation confirms previous results by demonstrating independent associations between LV diastolic dysfunction and PWV and between E/A ratio and several indices of wave reflection. The indices derived by pulse wave analysis, cSBP, cPP and AIx, depend on the reflected wave and are also determined by LVEF.¹² This dependency of wave reflection parameters on LV function might explain their independent association with E/A ratio.

Limitations

Some limitations need to be mentioned. The cross-sectional design precluded the detection of a cause-effect relation between LV diastolic dysfunction and arterial stiffness. To confirm the causal link between these two entities, longitudinal studies are needed. The prevalence of LV diastolic dysfunction was relatively low in the present patient population including only asymptomatic DM patients. The present results may not apply to cohorts of patients with more advanced disease.

Conclusions

Indices of arterial stiffness and wave reflection are independently associated with echocardiographic parameters of LV diastolic function in asymptomatic patients with DM. PWV, parameter of arterial stiffness, was independently associated with LV diastolic dysfunction grade, whereas cPP, cSBP and AIx, parameters of wave reflection, were significantly associated with E/A ratio. Therapies that prevent progression of arterial stiffness and reduce late-systolic pressure overload may reduce the prevalence of LV diastolic dysfunction in this population.

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CHAPTER 6

Changes in multidirectional LV strain in asymptomatic patients with type 2 diabetes mellitus: a 2-year follow-up study



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Abstract

Asymptomatic patients with diabetes mellitus (DM) and normal left ventricular (LV) ejection fraction (EF) may have LV dysfunction as assessed with speckle tracking echocardiography. Whether this subtle LV dysfunction may progress or not over time remains unknown. The present evaluation assessed changes in LV function with two-dimensional (2D) speckle tracking analysis in asymptomatic clinically stable patients with type 2 DM and normal LVEF after 2-year follow-up.

A total of 112 asymptomatic patients with type 2 DM and normal LVEF (53 ± 10 years, 59 % men) were evaluated. Patients remained clinically stable between baseline and follow-up echocardiography. Conventional and 2D speckle tracking echocardiographic measurements were performed. Circumferential (CS) and longitudinal strain (LS) were measured to assess systolic function and strain rate during isovolumic relaxation time (SR IVR) and peak transmitral early diastolic inflow strain rate (SR E) to assess diastolic function.

After 2-year follow-up, a significant increase in the LV mass index and significant decrease in the E/A ratio were observed. Left ventricular ejection fraction remained unchanged (59 % to 60 %, $p = 0.4$). In contrast, 2D speckle tracking analysis demonstrated a significant impairment in CS (-19.7 ± 4.0 % to -18.9 ± 3.8 %, $p < 0.001$), LS (-17.2 ± 2.3 % to -16.9 ± 2.7 %, $p = 0.022$) and SR E (1.02 ± 0.28 S⁻¹ to 0.94 ± 0.25 S⁻¹, $p < 0.001$). After adjusting for changes in LV mass index, only changes in CS and SR E remained significant ($p < 0.001$ and $p = 0.013$, respectively).

Asymptomatic patients with type 2 DM and normal LVEF may show mild progression of subclinical LV dysfunction assessed with 2D speckle tracking echocardiography. The prognostic implications of these mild changes warrant prospective evaluation.

Introduction

Diabetes mellitus (DM) is an independent risk factor for the development of heart failure (HF).^{1,2} In addition, HF patients with DM have more severe disease and worse prognosis than patients without DM.³ The increased prevalence of coronary artery disease (CAD) and hypertension in patients with DM contribute to the increased incidence of HF.^{4,5} Furthermore, diabetic cardiomyopathy has been proposed as a primary myocardial disease in DM patients without significant epicardial CAD, hypertension or valvular heart disease. This entity is characterized by microvascular disease, altered myocardial metabolism and increased myocardial fibrosis, that lead to gradual decline in left ventricular (LV) function with impairment in LV relaxation first, and then, followed by systolic dysfunction may progress over time to congestive HF.^{6,7} Therefore, before presenting with overt HF symptoms, diabetic patients may have long-standing subclinical myocardial dysfunction. Interestingly, whether progression of subclinical LV dysfunction towards overt HF symptoms occurs in DM patients is unknown. At present, there is a lack of longitudinal evaluations concerning the presence and development of structural and functional myocardial abnormalities in patients with DM.

Two-dimensional (2D) speckle tracking echocardiography has demonstrated that type 2 DM patients without cardiovascular complications and with preserved LV ejection fraction (EF) may exhibit LV systolic and diastolic dysfunction.⁸ Further progression in LV dysfunction in asymptomatic clinically stable patients with type 2 DM has not been evaluated with this imaging technique. Therefore, the aim was to assess changes in LV function with 2D speckle tracking echocardiography in asymptomatic clinically stable patients with type 2 DM and normal LVEF.

Methods

Patient population

The population consisted of 112 asymptomatic patients with type 2 DM and complete clinical and echocardiographic follow-up at 2 years. This subgroup of patients was selected from an original cohort of patients previously described.⁹ In brief, the original cohort included type 2 DM patients who were referred for cardiovascular risk assessment as part of regular patient care. All patients underwent a structured interview, physical examination, blood and urine laboratory testing and 2D transthoracic echocardiography. Type 2 DM was diagnosed according to the American Diabetes Association criteria in the absence of demonstrable auto-antibodies to islet cells, insulin and glutamic acid decarboxylase or low plasma C-peptide levels.¹⁰ All patients were free of cardiovascular complaints as confirmed with the Rose questionnaire on chest pain.¹¹ Exclusion criteria were: angina or angina-equivalent symptoms, known CAD (defined as previous acute coronary syndrome, percutaneous or surgical coronary revascularization or angiographically documented coronary stenosis of $\geq 50\%$ luminal diameter), cardiomyopathy,

significant valvular heart disease, congenital heart disease, and heart rhythm other than sinus rhythm or conduction abnormalities.

In the present evaluation, consecutive patients with repeat echocardiography at 2-year follow-up, who remained clinically stable during the follow-up, were included. Patients who presented with major adverse cardiovascular events (acute coronary syndrome, myocardial infarction or coronary revascularization and cardiac surgery) during the follow-up were excluded. From the original 234 asymptomatic patients with type 2 diabetes mellitus, 121 patients had repeat echocardiogram at 2-year follow-up. Nine patients were excluded: 8 patients because of cardiovascular events and one because of inadequate image quality to ensure reliable speckle tracking analysis.

Clinical, demographic, and echocardiographic data were prospectively collected in the departmental electronic patient dossier information system (EPD-vision®; Leiden, The Netherlands) and retrospectively analyzed.

Echocardiography

Patients underwent 2D transthoracic echocardiography at baseline and at 2-year follow-up using a commercially available system (Vivid 7 and E9, General-Electric Vingmed, Horton, Norway) equipped with 3.5-MHz and M5S transducers. ECG-gated images were obtained in the parasternal, apical and subcostal views with the patient lying in the left lateral decubitus position. Standard M-mode, 2D, color, pulsed and continuous wave Doppler images were recorded during breath hold and saved in cine-loop format. Analyses of the images were performed offline with dedicated software (EchoPac version 112.0.1 General-Electric Vingmed).

Left ventricular end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) were measured from the apical four- and two-chamber views and calculated using the Simpson's biplane method.¹² Thereafter, LVEF was calculated as $[(LVEDV-LVESV)/LVEDV] \times 100$. LV mass was measured at end-diastole on M-mode recordings obtained in the parasternal long-axis view and calculated with the Devereux formula.¹³ The intra- and interobserver reproducibility for the measurement of LV mass, assessed in 20 randomly selected patients, was $1.4 \pm 13.2 \text{ g/m}^2$ and $-1.2 \pm 8.4 \text{ g/m}^2$, respectively. In addition, left atrial (LA) volume was calculated according to the ellipsoid method from three LA diameters measured in the apical four-chamber and parasternal long-axis views.¹² LV and LA dimensions were normalized for body surface area (BSA).

Parameters of LV diastolic function were determined from transmitral inflow velocities using pulsed wave Doppler recordings in the apical four-chamber view.¹⁴ Early (E) and late (A) peak mitral inflow velocity of LV filling and deceleration time (DT) of the E-wave were measured and the E/A ratio was calculated. Isovolumic relaxation time (IVRT) was measured from pulsed wave Doppler spectral recordings obtained in the apical five-chamber view. Systolic and diastolic pulmonary vein flow velocities (PVs and PVd) were measured from pulsed wave Doppler recordings at the right superior pulmonary vein in the apical four-chamber view and the pulmonary vein PVs/PVd ratio was

calculated. Furthermore, peak mitral annular velocity (E') was measured using tissue Doppler imaging in the apical four-chamber view. E' was measured at the septal and lateral mitral annulus and the mean E' was calculated. Subsequently, the E/E' ratio was derived.¹⁵

The diastolic dysfunction grade was determined according to the criteria proposed by the European Association of Echocardiography:¹⁴ 1. normal diastolic function when $E' \geq 9$ cm/s and LA volume ≤ 34 mL/m², 2. mild diastolic dysfunction (grade I) when E/A ratio < 0.8 , $DT > 200$ ms and E/E' ratio ≤ 8 , 3. moderate diastolic dysfunction (grade II) when E/A ratio 0.8-1.5, DT between 160-200 ms and E/E' ratio between 9 and 12 and 4. severe diastolic dysfunction (grade III) when E/A ratio ≥ 2 , $DT < 160$ ms and E/E' ratio ≥ 13 .¹⁴

2D Speckle tracking echocardiography

LV function was further assessed with 2D speckle tracking echocardiography using semi-automated software (EchoPac version 112.0.1 General-Electric Vingmed). 2D speckle tracking allows for angle-independent quantification of myocardial tissue deformation (strain) and the rate of deformation (strain rate) by analyzing frame to frame the movement of 'speckles' (myocardial acoustic markers) throughout the cardiac cycle.¹⁶ 2D speckle tracking analysis was performed offline in standard grey-scale 2D images with a frame rate of at least 40 frames per minute. LV systolic function was assessed by measuring LV systolic circumferential and longitudinal strain. As previously described, circumferential strain (CS) evaluates the myocardial shortening along the curvature of the left ventricle in the short-axis view, whereas longitudinal strain (LS) assesses the magnitude of myocardial shortening in the longitudinal direction in the apical LV views.^{17, 18} Furthermore, LV diastolic function was assessed measuring longitudinal strain rate during the IVRT (SR IVR) and at the peak early mitral inflow velocity (SR E) at the apical long-axis views.¹⁹ These variables were measured as surrogates of LV pressure decay during the IVRT and LV relaxation, respectively.

Global LV circumferential peak systolic strain was measured using the LV short-axis view at the papillary muscle level. The endocardial border was manually traced on a single end-systolic frame. Subsequently, the software automatically generated a region of interest, which was manually adjusted to include the entire myocardial wall (Figure 1). Next, the software automatically divided the region of interest in six equal segments and indicated the tracking quality for each segment. If necessary, the region of interest was adjusted to improve tracking quality. Afterwards, the software provided strain and strain rate curves for the six myocardial segments (Figure 1). In addition, a 'global' curve was provided, representing the average strain, from which global LV circumferential peak systolic strain was derived.

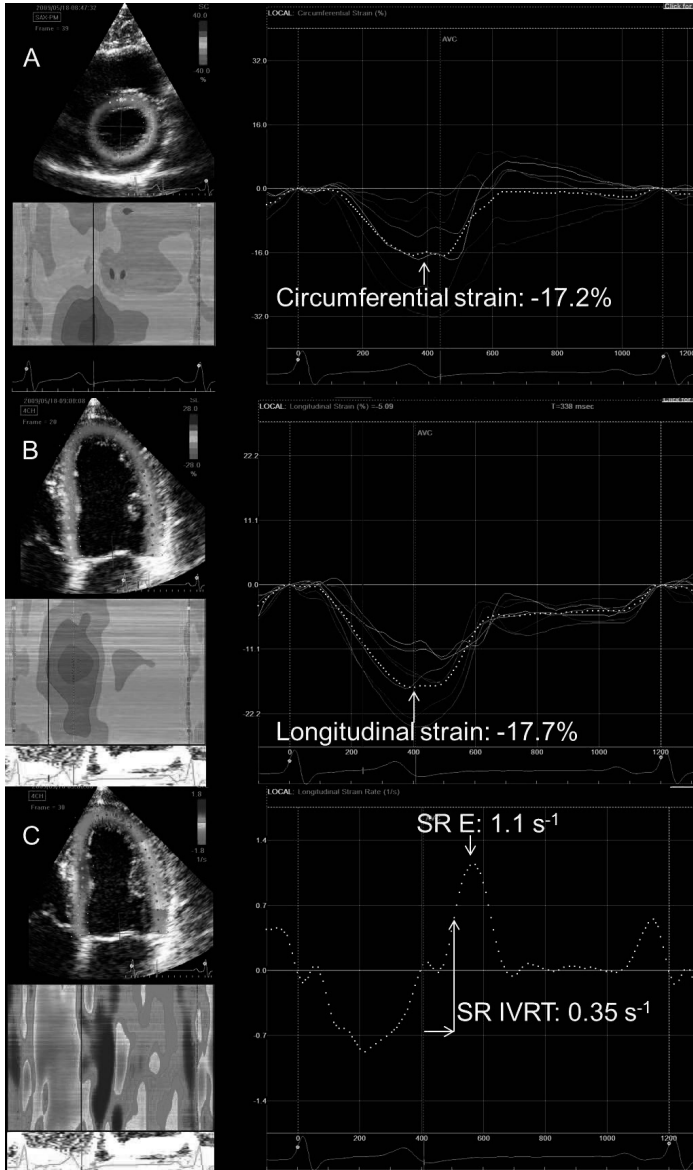


Figure 1 Assessment of left ventricular myocardial strain (A, B) and strain rate (C) using 2D speckle tracking analysis. The upper left corner of each panel shows the region of interest including the entire myocardium. Regional strain curves are presented by the software as the colored lines (A, B) and a global strain curve (A and B) or strain rate curve (C) as the white dotted line. Circumferential strain (A) was measured from the LV short axis view. Longitudinal strain (B) was measured from the three standard apical views (apical long axis, two-chamber, and four-chamber view, respectively) and the average was calculated. Strain rate (C) during isovolumic relaxation time (calculated by adding the IVRT to the aortic valve closure time which is indicated by the vertical green dotted line in the strain rate curve) and at peak early diastolic inflow velocity (defined as the first peak in global longitudinal strain after aortic valve closure time) were measured from the three standard apical views and the average was calculated.

Global LV longitudinal peak systolic strain was measured using the same method in the three standard apical views: the apical long-axis, two-chamber, and four-chamber views, respectively. The average global LV longitudinal peak systolic strain from the three apical views was calculated.

In addition, the longitudinal strain rate curves were used to assess SR IVR and SR E (Figure 1). SR IVR was defined as longitudinal strain rate during IVRT (calculated by adding the IVRT to the aortic valve closure time). SR E was defined as the first peak in global longitudinal strain rate after aortic valve closure. SR IVR and SR E were measured in the apical long-axis, two-, and four-chamber views and the average of these measurements was calculated.

Intra- and interobserver variabilities have been previously reported for global CS ($1.2 \pm 1.0\%$ and $2.3 \pm 2.4\%$) and for average global LS ($1.2 \pm 0.5\%$ and $0.9 \pm 1.0\%$).⁸

Statistical analysis

Continuous data are presented as mean $\pm$ standard deviation when normally distributed (as assessed with the Kolmogorov-Smirnov test) and as median (25th and 75th percentiles) when non-normally distributed. Categorical data are presented as frequencies and percentages.

Changes in conventional echocardiographic parameters at follow-up were determined using the paired t-test, Wilcoxon signed rank test and Friedman's test for repeated measurements, as appropriate. Changes in 2D speckle tracking strain parameters were evaluated with linear mixed models and adjusted for changes in the LV mass index during follow-up. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows (version 20.0, SPSS Inc., Chicago, IL, USA).

Results

Baseline clinical, demographic and echocardiographic characteristics

A total of 112 patients (mean age 53 ± 10 years, 66 (59%) men) were evaluated. By definition, all patients remained clinically stable and free of cardiovascular complaints during a median follow-up of 2.5 (2.3-2.8) years. The clinical characteristics of the patients are summarized in Table 1. Mean DM duration was almost 10 years and mean hemoglobin A1c was 7.6 ± 1.6 %. In addition, 72 (64 %) patients had hypertension (defined as blood pressure $>140/90$ or use of antihypertensive medication).

Echocardiographic parameters are presented in Table 2. Patients showed normal LV systolic function, based on the measurement of LVEF (59 ± 6 %) and LV volumes (mean indexed LVEDV and LVESV were 47 ± 9 mL/m² and 19 ± 5 mL/m², respectively).¹² Likewise, mean LV mass index and LA volume index were within normal range, 88 ± 18 g/m² and 18 ± 5 mL/m², respectively. In contrast, mean E/A ratio and E' were decreased and mean E/E' ratio was increased.¹⁴ Classification in grades of LV diastolic dysfunction showed that 28 % of patients had normal LV diastolic filling pattern, 41 % had mild (grade I) and 31 % had moderate (grade II) diastolic dysfunction.

When LV systolic and diastolic functions were assessed with 2D speckle tracking echocardiography, patients showed impaired global LV CS and LS (Table 3).⁸ In addition, LV diastolic dysfunction was confirmed with a reduced SR E, but normal SR IVR.^{8, 20}

Changes in conventional and 2D speckle tracking echocardiographic data at follow-up

At follow-up, conventional echocardiography demonstrated no significant changes in LV systolic function (Table 2). Indexed LV volumes and LVEF remained unchanged. Interestingly, LV mass index significantly increased (from 88 ± 18 g/m² to 95 ± 18 g/m², $p < 0.01$). In terms of LV diastolic function, the E/A ratio significantly decreased (from 1.04 ± 0.29 to 0.95 ± 0.28 , $p < 0.01$), indicating a decline in LV diastolic function. In contrast, mean E', E/E' ratio and LA volume index did not significantly change.

Changes in LV function as assessed with 2D speckle tracking echocardiography demonstrated deterioration in global LV systolic function (Table 3). Due to technical limitations, follow-up LV LS could not be assessed in one patient and LV CS analysis was not feasible in three patients. Global CS and global LS significantly impaired at follow-up (from -19.7 ± 4.0 % to -18.9 ± 3.8 %, $p < 0.001$, and from -17.2 ± 2.3 % to -16.9 ± 2.7 %, $p = 0.022$, respectively). In addition, there was a progressive decline in LV diastolic function and particularly of LV relaxation with an impairment in SR E (from 1.02 ± 0.28 S⁻¹ to 0.94 ± 0.25 S⁻¹, $p < 0.001$). In contrast, SR IVR remained unchanged. When these changes were corrected for changes in LV mass index, changes in global LS were no longer significant ($p = 0.051$), whereas changes in CS and SR E remained significant ($p < 0.001$ and $p = 0.013$, respectively) (Table 3).

Table 1 Baseline clinical, demographic characteristics.

Clinical variables	n=112
Age (years)	53 ± 10
Male gender, n (%)	66 (59%)
Diabetes duration (months)	115 ± 90
Hemoglobin A1c (%)	7.6 ± 1.6
Diabetes treatment, n (%)	
Diet only	3 (3%)
Oral glucose lowering agent	65 (58%)
Insulin	23 (21%)
Insulin and oral agent	21 (19%)
Body mass index (kg/m ²)	29 ± 5
Body surface area (m ²)	2.0 ± 0.2
Family history CAD, n (%)	60 (54%)
Smoking, n (%)	20 (18%)
Hypertension, n (%)	72 (64%)
Hypercholesterolemia, n (%)	88 (79%)
Total cholesterol (mmol/L)	4.9 ± 1.2
Triglycerides (mmol/L)	1.7 (1.2-2.6)
Creatinine (μmol/L)	78 ± 21
Glomerular filtration rate (mL/min/1.73m ²)	87 (75-106)
Urinary albumine/creatinine ratio (μg/μmol)	2.0 (0.9-7.0)
Microalbuminuria ≥3.5 μg/μmol	34 (30%)
Medication, n (%)	
Aspirin	21 (19%)
Angiotensin-converting enzyme inhibitor	38 (34%)
Angiotensin receptor antagonists	28 (25%)
Statins	61 (55%)

Abbreviations: CAD: coronary artery disease.

Family history of CAD was defined as a history of CAD in first degree family member before the age of 55 years in males or before 65 years in females. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or use of antihypertensive medication. Hypercholesterolemia was defined as a total cholesterol ≥5 mmol/L or statin use. Glomerular filtration rate (GFR) was calculated with the Modification of Diet in Renal Disease (MDRD) study equation. Patients were considered to have a normal renal function when GFR was ≥60 mL/min/1.73m² and moderate renal dysfunction when GFR was 30-59 mL/min/1.73m².

Table 2 Changes in LV function as measured with conventional echocardiography at follow-up.

Echocardiographic parameters	Baseline	Follow-up	p-value
LVEDV (mL/m ²)	47 ± 9	48 ± 10	0.5
LVESV (mL/m ²)	19 ± 5	20 ± 6	0.8
LVEF (%)	59 ± 6	60 ± 7	0.4
LV mass indexed by BSA (g/m ²)	88 ± 18	95 ± 18	<0.01
LA volume indexed by BSA (mL/m ²)	18 ± 5	18 ± 5	0.9
E (cm/s)	69 ± 15	69 ± 17	0.9
A (cm/s)	70 ± 18	75 ± 18	<0.01
E/A ratio	1.04 ± 0.29	0.95 ± 0.28	<0.01
DT (ms)	203 ± 36	217 ± 41	<0.01
IVRT (ms)	74 ± 10	74 ± 10	0.9
PVs (cm/s)	49 ± 12	48 ± 17	0.6
PVd (cm/s)	39 ± 10	37 ± 12	0.2
PVs/PVd ratio	1.31 ± 0.28	1.34 ± 0.31	0.5
E' (cm/s)	6.6 ± 2.1	6.6 ± 1.9	0.9
E/E' ratio	11.4 ± 5.1	11.3 ± 4.6	0.9
LV diastolic dysfunction			0.4
Normal function, n (%)	28%	25%	
Mild diastolic dysfunction, n (%)	41%	40%	
Moderate diastolic dysfunction, n (%)	31%	35%	

Abbreviations: A: peak transmitral late diastolic inflow velocity, BSA: body surface area, DT: deceleration time of E, E: peak transmitral early diastolic inflow velocity, E': peak early mitral annular velocity, IVRT: isovolumic relaxation time, LA: left atrium, LV: left ventricle, LVEDV: left ventricular end-diastolic volume, LVEF: left ventricular ejection fraction, LVESV: left ventricular end-systolic volume.

Table 3 Changes in left ventricular function as measured with 2D speckle tracking at follow-up.

2D speckle tracking parameters	Baseline	Follow-up	p-value	p-value*
Global circumferential strain (%)	-19.7 ± 4.0	-18.9 ± 3.8	<0.001	<0.001
Global longitudinal strain (%)	-17.2 ± 2.3	-16.9 ± 2.7	0.022	0.051
Average SR IVR (S ⁻¹)	0.39 ± 0.21	0.38 ± 0.21	0.651	0.949
Average SR E (S ⁻¹)	1.02 ± 0.28	0.94 ± 0.25	<0.001	0.013

*p-value: adjusted for changes in LV mass index.

Abbreviations: SR E: strain rate at peak transmitral early diastolic inflow velocity, SR IVR: strain rate during isovolumetric relaxation time.

Discussion

The present evaluation demonstrated a mild decline in LV function as assessed with 2D speckle tracking echocardiography in asymptomatic patients with type 2 DM and normal LVEF after a median follow-up of 2.5 years. At follow-up, deterioration of LV systolic function was shown by a significant decrease in CS and LS, whereas a decline in LV diastolic function was indicated by a significant decrease in SR E. Furthermore, a significant increase in LV mass was observed. Conversely, these changes in LV function were not detected by conventional echocardiography.

Increased prevalence of LV dysfunction in asymptomatic patients with DM

Contemporary population-based studies have shown that the incidence of congestive HF in patients with DM has increased by 3-15 times in the last years.^{6,21} Nichols et al. reported a 2.5-fold higher incidence of congestive HF in 8,231 DM patients compared with 8,845 non-DM patients who were followed-up for 5.5 years (30.9 vs. 12.4 cases per 1,000 person-years, $p < 0.001$).²¹ The presence of CAD, renal dysfunction and hypertension are strong contributors to the increased incidence of congestive HF in DM patients.^{21,22} However, DM patients may remain asymptomatic for long time and early detection of changes in cardiac structure and function due to primary myocardial disease, hypertension or asymptomatic CAD may help to identify the patients with an increased risk for developing congestive HF. A wide range of prevalence of subclinical LV diastolic dysfunction has been reported in asymptomatic patients with DM ranging from 23 to 75 %.^{6,23} In a cohort of 1,760 asymptomatic DM patients, From et al. demonstrated that the prevalence of subclinical LV diastolic dysfunction, defined by an E/E' ratio >15 , was 23 %.⁶ During 5-years follow-up, DM patients with LV diastolic dysfunction doubled the cumulative probability of incident congestive HF of DM patients without LV diastolic dysfunction (36.9 % vs. 16.8 %, $p < 0.001$). The presence of LV diastolic dysfunction was independently associated with incident congestive HF after correcting for age, CAD, hypertension, LVEF, body mass index, LA volume, LV mass and E-wave deceleration time.⁶ These findings underscore the need of sensitive diagnostic tools that permit early detection of subclinical LV dysfunction.

Prevalence of LV dysfunction assessed with speckle tracking echocardiography in asymptomatic DM patients

Conventional echocardiography is traditionally used to quantify LV systolic function by means of LVEF and diastolic function through assessment of mitral valve inflow velocity pattern and measurement of the E/A ratio and E-wave deceleration time.^{12,14} However, LVEF is an insensitive parameter to detect subtle LV dysfunction.^{24,25} In addition, the E/A ratio and E-wave deceleration time depend on preload conditions, LV relaxation and LV compliance, and cannot differentiate normal diastolic function from grade II diastol-

ic dysfunction without Valsalva manoeuvres. Accordingly, current recommendations include also the measurement of tissue Doppler imaging parameters such as the E' and the E/E' ratio, known markers of LV relaxation and LV filling pressures, respectively.¹⁴ More recently, 2D speckle tracking echocardiography has allowed the detection of sub-clinical myocardial dysfunction by measuring multidirectional LV strain and strain rate. This modality overcomes important limitations of conventional echocardiography and tissue Doppler imaging. The assessment of LV strain and strain rate is a more sensitive marker of LV dysfunction and has a better reproducibility compared to LVEF.⁸ In patients with DM and preserved LVEF, 2D speckle tracking echocardiography has demonstrated the presence of subtle LV systolic dysfunction.^{8, 26} For example, the study by Nakai and coworkers showed that patients with type 2 DM and normal LVEF had impaired LV CS and LS when compared with healthy controls (LV CS: -22.6 % in type 2 DM patients vs. -24.4 % in controls, $p < 0.005$; and LV LS: -17.6 % in type 2 DM patients vs. -20.8 % in controls, $p < 0.001$).²⁶ However, studies evaluating changes in LV mechanics over time in type 2 DM patients who remain asymptomatic are sparse.

Progression of subclinical LV dysfunction

At present, only a few studies have investigated changes of LV dysfunction over time in patients with DM.²⁷ In 27 type 2 DM patients, Vintila et al. showed progression of sub-clinical LV dysfunction after 5-years follow-up by a significant reduction in longitudinal velocities measured with TDI echocardiography (mean longitudinal systolic velocity 4.9 cm/s vs. 5.6 cm/s, $p = 0.001$).²⁸ The present evaluation confirms and extends previous observations by measuring 2D speckle tracking echocardiography derived LV systolic and diastolic parameters. Asymptomatic patients with type 2 DM showed subclinical LV systolic and diastolic dysfunction as reflected by impaired CS and LS, and reduced SR E. More important, after 2-year follow-up and despite remaining clinically stable and asymptomatic, these patients showed progression of subclinical LV dysfunction with further impairment in CS and LS, and decline in SR E together with an increase in LV mass. In contrast, conventional echocardiographic measurements did not show significant changes in LV systolic and diastolic function. Therefore, 2D speckle tracking echocardiography may be a promising tool for identification and monitoring of sub-clinical LV dysfunction in patients with DM. Early identification of type 2 DM patients with subclinical LV dysfunction allows initiation of therapeutic strategies to prevent progression to HF and improve prognosis. Moreover, the current study demonstrated mild progression of subclinical LV dysfunction after 2-year follow-up, which supports the importance of regular echocardiographic surveillance of these patients.

Limitations

Some limitations should be acknowledged. First, the present evaluation is retrospective, includes a relatively small cohort of asymptomatic type 2 DM patients and some

patients had additional cardiovascular risk factors, such as hypertension or hypercholesterolemia, which may contribute to development and progression of LV dysfunction. However, as type 2 DM often is part of a clustering of cardiovascular risk factors, the current population is a better representation of the daily clinical practice. Second, to avoid the influence of myocardial ischemia on LV function, only patients who were clinically stable were included in the study. Exercise test was not systematically performed to exclude significant coronary stenosis. However, patients who presented with angina complaints or acute coronary syndromes during follow-up were excluded. Third, previous studies have demonstrated that strain and strain rate increase from base to apex, and therefore, heterogeneity in regional strain peaks was not further evaluated in the present study.²⁹ Fourth, N-terminal pro brain natriuretic peptide (NT-proBNP) was not systematically assessed. Finally, the prognostic implications of these mild changes in LV function were not evaluated. Additional prospective studies are needed in order to elucidate whether systematic echocardiographic surveillance should be recommended in order to improve the risk stratification of this subpopulation.

Conclusions

Asymptomatic patients with type 2 DM and normal LVEF may show mild progression of subclinical LV dysfunction assessed with 2D speckle tracking echocardiography. The prognostic implications of these mild changes in LV function and recommendations on systematic echocardiographic surveillance of this subpopulation need further prospective evaluation.

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CHAPTER 7

Association of atherosclerosis in the descending thoracic aorta with coronary artery disease on multi detector row computed tomography coronary angiography in patients with suspected coronary artery disease



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Abstract

The association between atherosclerosis in the descending thoracic aorta (DTA) visualized on computed tomography coronary angiography (CTA) and coronary artery disease (CAD) has not been extensively explored. Therefore, a comprehensive analysis of DTA atherosclerosis on CTA was performed and the association of DTA atherosclerosis with CAD was evaluated in patients with suspected CAD.

A total of 344 patients (54 ± 12 years, 54 % men) with suspected CAD underwent CTA. CTA were classified based on CAD severity in no signs of atherosclerosis or minor wall-irregularities <30 %, non-significant CAD 30-50 %, or significant CAD ≥ 50 % stenosis. The DTA was divided in segments according the posterior intercostal arteries. Per segment the presence of atherosclerotic plaque (defined as ≥ 2 mm wall thickness) was determined and maximal wall thickness was measured. Plaque composition was scored as non-calcified or mixed and the percentage of DTA segments with atherosclerosis was calculated.

Significant CAD was present in 152 (44 %) patients and 278 (81 %) had DTA atherosclerotic plaque. DTA maximal wall thickness and percentage of DTA segments with atherosclerosis were 2.7 ± 1 mm and 49 ± 36 %. The presence, severity and extent of DTA atherosclerosis significantly increased with increasing CAD severity. Multivariate logistic regression analysis corrected for age and other risk factors demonstrated independent associations of DTA plaque (OR 6.56, 95 % CI 1.78-24.19, $p = 0.005$) and maximal DTA wall thickness (OR 2.00, 95 % CI 1.28-3.12, $p = 0.002$) with significant CAD.

The presence and severity of DTA atherosclerosis were independently related with significant CAD on CTA in patients with suspected CAD.

Introduction

Multi-slice computed tomography coronary angiography (CTA) is a well-established modality for the evaluation of coronary artery disease (CAD)^{1,2} and also permits visualization of the descending thoracic aorta (DTA). Interestingly, atherosclerosis in the DTA, a marker of diffuse atherosclerotic cardiovascular disease, is frequently observed.³

⁴ In addition, various imaging techniques have demonstrated the association between DTA atherosclerosis, CAD and cardiovascular events.^{3, 5-10} The advantage of CTA for assessing DTA atherosclerosis is the complementary information that can be derived from the available CTA images. Various parameters can be derived from CTA to address DTA atherosclerosis, including DTA calcification, atherosclerotic plaque and wall thickness. Although a number of studies have confirmed the independent association between DTA calcification on CTA and CAD,^{7, 9-12} other CTA derived parameters of DTA atherosclerosis have not been used extensively for this purpose.

Hence, the aim of the present study was to (1) perform a comprehensive evaluation of the presence, extent, severity and composition of DTA atherosclerosis on CTA and (2) to assess associations between DTA atherosclerosis and CAD.

Patients and methods

Patients

The cohort comprised 344 patients with suspected CAD evaluated in the Leiden University Medical Center in the period between February 2005 and December 2009. All patients underwent CTA to assess the presence of significant CAD. Contra-indications for CTA included renal failure (glomerular filtration rate <30 mL/min), known hypersensitivity to iodine containing contrast or pregnancy. Inclusion criteria were an interpretable CTA with diagnostic image quality for the evaluation of CAD and the display of the complete DTA on the CTA scan (entire DTA visualized on the axial CTA images and at least 5 or more DTA segments present on the CTA scan).

Clinical data, including demographics, symptoms and CTA results were collected in the departmental electronic patient file (EPD vision version 8.3.3.6; Leiden, The Netherlands) and retrospectively analyzed. The Institutional Review Board of the Leiden University Medical Center approved this retrospective evaluation of clinically collected data, and waived the need for written informed consent.

Cardiovascular risk factors included hypertension (defined as systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg or use of antihypertensive medication), hypercholesterolemia (defined as serum total cholesterol ≥ 230 mg/dL or use lipid lowering therapy), diabetes mellitus (defined as fasting plasma glucose level ≥ 126 mg/dL, use of oral glucose lowering medication or insulin), obesity (body mass index > 30 kg/m²), smoking status, positive family history for CAD (presence of CAD in first-degree family member at <65 years in women and <55 years in men).

The presence and severity of CAD were assessed on CTA. Furthermore, a comprehensive analysis of the DTA was performed on the CTA scan, to assess the presence, severity, extent and composition of DTA atherosclerosis.

CTA data acquisition

Patients were scanned on a 64-detector row helical scanner (Aquilion 64, Toshiba Medical Systems, Otawara, Japan) or 320-detector row volumetric scanner (Aquilion ONE, Toshiba Medical Systems, Otawara Japan). Additional scan parameters for the 64-detector row scanner were collimation 64 x 0.5 mm, gantry rotation time 400 ms, tube voltage 120-135 kV and tube current 300-350 mA as adjusted to body mass index.² For the 320-row scanner collimation was 320 x 0.5 mm, gantry rotation time 350 ms, tube voltage 100-135 kV and tube current 400-580 mA as adjusted to body mass index.¹ One hour prior to the scan, blood pressure and heart rate were measured and beta-blockers (50-100 mg metoprolol, orally) were administered to patients with a heart rate ≥ 65 beats/min without contraindication for beta-blocking agents. First, a non-enhanced calcium scan was performed with slice thickness of 3.0 mm. Thereafter, CTA was performed with simultaneous recording of the electrocardiogram for retrospective (64-detector row scanner) or prospective (320-detector row scanner) gating of the data. An amount of 60-110 mL non-ionic contrast medium (Iomeron 400, Bracco, Milan, Italy) was administered in the antecubital vein with a flow rate 5-6 mL/s, followed by a saline flush. The scan was triggered with automated peak enhancement detection with the region of interest located in the left ventricle. Data acquisition was completed within a single breath hold of approximately 10 s. A data set at 75 % of the R-R interval was then automatically reconstructed with a slice thickness of 0.5 mm and reconstruction interval of 0.3 mm for the 64-detector row scanner and 0.5 mm and 0.25 mm, respectively, for the 320-detector row scanner. In case of motion artifacts, additional reconstructions of different time points in the R-R interval were reconstructed.

The estimated mean radiation dose for the 64-row CTA and 320-row CTA in our centre have been previously described as 18.1 ± 5.9 mSv and 7.1 ± 1.7 mSv, respectively.¹³

CTA data analysis

Post-processing and evaluation of the CTA scans were performed on a remote work station with dedicated CTA analysis software (Vitrea 2 or Vitrea FX 1.0, Vital Images, Minnetonka, MN, USA). For each patient, the Agatston coronary calcium score was measured on the calcium scan. Thereafter, the CTA reconstruction with the best image quality was used for the evaluation of the coronary arteries.

Analysis of CAD

Computed tomography coronary angiography scans were evaluated by two experienced observers, including one interventional cardiologist. Discrepancies between observers were resolved by a consensus decision during a joint reading. The coronary artery system was divided into 17 segments according to the modified American Heart Association classification.¹⁴

First, the quality of the scan was classified as good, reasonable, moderate or poor, based on scan noise, motion artifacts and contrast filling of the coronary arteries.

Per segment, the presence of atherosclerotic plaque and severity of luminal stenosis were determined from the axial images and verified by the interpretation of curved multiplanar reconstruction in at least 2 orthogonal planes. Atherosclerotic plaque was defined as a structure $>1 \text{ mm}^2$ within and/or adjacent to the coronary artery lumen that could clearly be distinguished from the coronary artery lumen and surrounding tissue.¹⁵ For each segment, the severity of luminal stenosis was visually scored as follows: 1. no signs of atherosclerosis or minor wall irregularities ($<30\%$ stenosis), 2. non-significant CAD ($30\text{--}50\%$ stenosis) and 3. significant CAD ($\geq 50\%$ stenosis).¹⁶ In patients with a percutaneous coronary intervention (PCI), stented coronary segments were considered as having significant CAD.

A CTA was considered non-interpretable if the presence and severity of CAD could not be determined due to poor image quality (non-diagnostic image quality of CTA for the evaluation of CAD).

On a patient basis, patients were stratified according to the most severe stenosis as follows: 1. no signs of atherosclerosis or minor wall irregularities ($<30\%$ stenosis), 2. non-significant CAD ($30\text{--}50\%$ stenosis) and 3. significant CAD ($\geq 50\%$ stenosis).¹⁶

Analysis of DTA atherosclerosis

In addition to the coronary arteries, the CTA scan also permits visualization of the DTA. In order to visualize the entire heart, the preset scan range of the CTA reaches from the tracheal bifurcation to beneath the diaphragm. Hence, the DTA is imaged over the course from beneath the aortic arch to the abdominal aorta.

Assessment of atherosclerosis in the DTA was performed on the axial images by two experienced observers, blinded to patient's clinical characteristics and CTA results (presence and severity of CAD).

First, the Agatston calcium score of the DTA was determined on a non-contrast enhanced scan. Thereafter, the DTA was anatomically divided based on the posterior intercostal arteries in 5-8 segments per scan as shown in Figure 1. In each segment, the site of maximal wall thickness was measured perpendicularly to the center of the aorta. The presence of atherosclerotic plaque was defined as a wall thickness $\geq 2 \text{ mm}$ in at least one DTA segment.³ Per patient plaque composition was scored as no plaque, non-calcified plaque (only low-density plaque) or calcified plaque (containing high-density plaque) (Figures 1 c, d). Finally, the extent of atherosclerosis was determined by the percentage of segments with atherosclerotic plaque.

The intra- and inter-observer variability of the measurements of DTA atherosclerosis were determined by repeated analysis of 20 randomly selected patients more than 4 weeks apart, by the same observer, and by the assessment of DTA atherosclerosis in the same patient by a second independent observer.

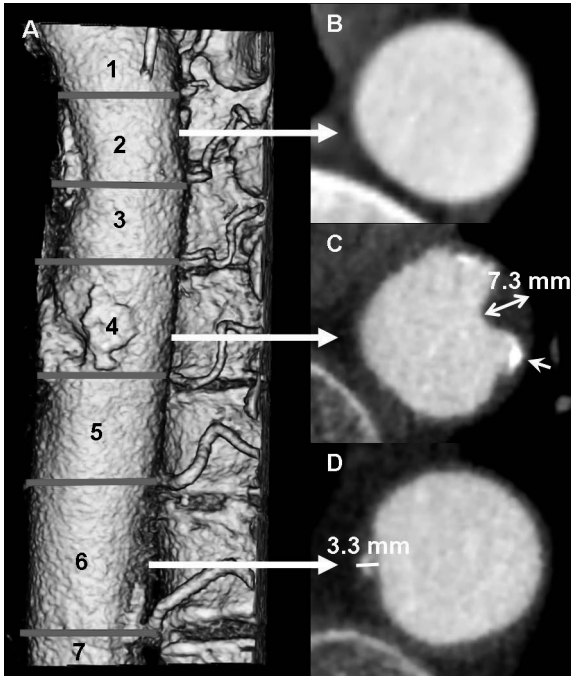


Figure 1. Evaluation of atherosclerosis in the descending thoracic aorta (DTA) on a CTA scan. Panel A demonstrates how the descending thoracic aorta was anatomically divided in segments, based on the posterior intercostal arteries (red horizontal lines). Panels B, C and D are axial images at the sight of maximal wall thickness of the corresponding DTA segments 2, 4 and 6, respectively. Panel B shows a normal DTA wall without atherosclerosis. Panel C depicts atherosclerotic plaque with calcification (indicated with arrow) and maximal wall thickness of 7.3 mm. Panel D shows atherosclerotic plaque with wall thickness of 3.3 mm.

Statistical analysis

Continuous variables with normal distribution, as assessed with the Kolmogorov-Smirnov test, are presented as mean $\pm$ standard deviation and non-normal distributed continuous variables as median (25th and 75th percentiles). Categorical variables are presented as frequencies and percentages. Comparisons between groups of continuous variables were performed with the Student t-test, one-way analysis of variance and Kruskal-Wallis test, as appropriate. Categorical variables were compared with the Chi square test.

Correlates of significant CAD amongst clinical and demographic characteristics and DTA atherosclerosis variables were identified with univariate logistic regression analy-

sis. Subsequently, independent associations of the correlates with significant CAD were evaluated with multivariate logistic regression analysis. For logistic regression analyses, non-normal distributed continuous variables were log-transformed to obtain a normal distribution. Odds ratio (OR) and 95 % confidence intervals (CI) were derived from logistic regression analysis.

Intra- and inter-observer variability of the measurements of DTA atherosclerosis were evaluated with the intraclass correlation (ICC) coefficient for continuous variables and Cohen's Kappa statistics for categorical variables. The ICC coefficient of good agreement was defined as >0.72 . Kappa values of agreement between 0.40-0.75 were defined as moderate and >0.75 as good.

For all statistical tests $p < 0.05$ was considered significant. All statistical analyses were performed with SPSS version 20.0 (SPSS, Inc, Chicago, IL).

Results

Patient's clinical and demographic characteristics

The cohort consisted of 344 patients who underwent CTA for suspected CAD. The mean age of the patients was 54 ± 12 years and 186 (54 %) were men. The clinical and demographic characteristics of all patients and of the patients with versus without DTA atherosclerotic plaques are presented in Table 1. Patients with DTA atherosclerotic plaque were significantly older and had a higher prevalence of hypertension, hypercholesterolemia and previous PCI as compared to patients without DTA atherosclerosis. Moreover, the coronary artery calcium score was significantly higher (39 [0-278] vs. 0 [0-1], $p < 0.001$) in patients with DTA atherosclerotic plaque.

Table 1. Clinical and demographic characteristics of the entire cohort and comparison between patients with and without DTA plaque.

	All patients n=344	DTA atherosclerotic plaque (≥ 2 mm wall thickness)		p-value
		No plaque n=66 (19%)	Plaque n=278 (81%)	
Age, (years)	54 ± 12	42 ± 10	57 ± 10	<0.001
Men, n (%)	186 (54%)	30 (45%)	156 (56%)	0.118
Hypertension, n (%)	158 (46%)	19 (29%)	139 (50%)	0.003
Hypercholesterolemia, n (%)	141 (41%)	19 (29%)	122 (44%)	0.034
Diabetes, n (%)	80 (23%)	16 (24%)	64 (23%)	0.758
Obesity, n (%)	73 (21%)	9 (14%)	64 (23%)	0.118
Smoking, n (%)	94 (27%)	19 (29%)	75 (27%)	0.685
Family history, n (%)	168 (49%)	32 (48%)	136 (49%)	0.896
Myocardial infarction, n (%)	43 (12%)	4 (6%)	39 (14%)	0.089
PCI, n (%)	52 (15%)	2 (3%)	50 (18%)	0.002
Coronary calcium score	10 [0-216]	0 [0-1]	39 [0-278]	<0.001

Abbreviations: DTA: descending thoracic aorta, PCI: percutaneous coronary intervention.

Analysis of CAD and DTA atherosclerosis

Analysis of CAD identified no signs of atherosclerosis or minor wall irregularities, non-significant CAD and significant CAD in 103 (30 %), 89 (26 %) and 152 (44 %) patients, respectively (Table 2).

Results of the comprehensive analysis of DTA atherosclerosis are shown in Table 2. DTA calcification (DTA calcium score > 0) was present in 183 (53 %) patients and the median DTA calcium score was 1 [0-97]. The overall mean DTA maximal wall thickness was

2.7 ± 1 mm. DTA atherosclerotic plaque was present in 278 (81 %) patients and calcified DTA atherosclerotic plaque in 87 (25 %) patients. In addition, the mean extent of DTA atherosclerosis (percentage of DTA segments with atherosclerotic plaque) was 49 ± 36 %, respectively.

DTA atherosclerosis and CAD severity

The presence, severity and extent of DTA atherosclerosis significantly increased in parallel to increasing severity of CAD (Table 2). An example of a patient with DTA atherosclerosis and CAD on CTA is presented in Figures 1 and 2.

The median DTA calcium score significantly increased over the patient groups of CAD severity, from 0 [0-2] in patients without signs of atherosclerosis or minor wall irregularities to 1 [0-98] in patients with non-significant CAD and to 22 [0-513] in patients with significant CAD ($p < 0.001$). Similarly, DTA maximal wall thickness significantly increased from 2.0 ± 0.5 mm in patients without signs of atherosclerosis or minor wall irregularities to 2.6 ± 0.8 mm in patients with non-significant CAD and to 3.2 ± 1.1 mm in patients with significant CAD ($p < 0.001$).

Table 2. Measurements of DTA atherosclerosis of the entire population and comparison between patients groups of CAD severity.

DTA variables	All patients	CAD severity			p-value
	n=344	<30 % n=103 (30%)	30-50 % n=89 (26%)	≥50 % n=152 (44%)	
Calcium score	1 [0-97]	0 [0-2]	1 [0-98]	22 [0-513]	<0.001
Maximal wall thickness, mm	2.7 ± 1	2.0 ± 0.5	2.6 ± 0.8	3.2 ± 1.1	<0.001
Atherosclerotic plaque, n (%)	278 (81%)	60 (58%)	69 (77%)	149 (98%)	<0.001
Plaque composition					<0.001
no plaque, n (%)	66 (19%)	43 (42%)	20 (22%)	3 (2%)	
non-calcified, n (%)	191 (55%)	59 (57%)	47 (53%)	85 (56%)	
calcified, n (%)	87 (25%)	1 (1%)	22 (25%)	64 (42%)	
Extent atherosclerosis, % of segments affected	49 ± 36	24 ± 28	47 ± 38	66 ± 30	<0.001

Comparisons between groups of CAD severity was performed with the one-way analysis of variance for continuous variables with normal distribution and with the Kruskal-Wallis test for non-normal distributed continuous variables. Categorical variables were compared with the Chi square test.

Abbreviations: CAD: coronary artery disease, DTA: descending thoracic aorta.

The prevalence of DTA atherosclerotic plaque significantly increased from 60 (58 %) in patients without signs of atherosclerosis or minor wall irregularities to 69 (77 %) in patients with non-significant and to 149 (98 %) in patients with significant CAD ($p < 0.001$). With respect to plaque composition, the prevalence of calcified DTA plaque significantly

increased with increasing CAD severity, whereas the prevalence of non-calcified DTA plaque did not change.

Finally, the extent of DTA atherosclerosis significantly increased from 24 ± 28 % in patients without signs of atherosclerosis or minor wall irregularities to 47 ± 38 % in patients with non-significant CAD and to 66 ± 30 % in patients with significant CAD ($p < 0.001$).

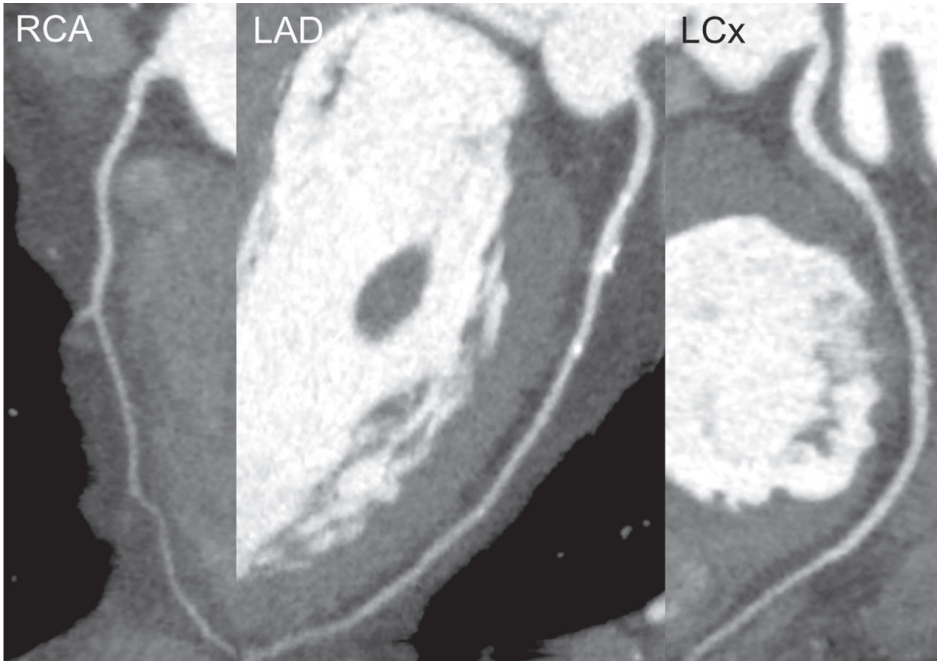


Figure 2. Multi planar reconstructions of the coronary arteries of the same patient in Figure 1. There is a left dominant coronary system with separate ostia of the LAD and LCx. The proximal segment of the LCx and mid segment of the LAD have mixed plaques (containing non-calcified plaque and calcified plaque) with non-significant coronary artery disease (30-50 % luminal stenosis).

Correlates of significant CAD

The results of univariate logistic regression analysis for the assessment of associations between clinical and demographic characteristics and measurements of DTA atherosclerosis with significant CAD are presented in Table 3. Age, male gender, hypertension, hypercholesterolemia and smoking were correlates of significant CAD. Moreover, all measurements of DTA atherosclerosis (log transformed calcium score, maximal wall thickness, presence of atherosclerotic plaque, plaque composition and extent of atherosclerosis) were correlated with significant CAD.

Table 3. Univariate associations of clinical, demographic characteristics and measurements of DTA atherosclerosis with the presence of significant CAD.

	OR	95% CI	p-value
Clinical, demographic characteristics			
Age	1.07	1.05-1.09	<0.001
Men	1.94	1.26-3.00	0.003
Hypertension	1.95	1.26-3.01	0.003
Hypercholesterolemia	2.25	1.45-3.49	<0.001
Obesity	0.64	0.37-1.09	0.102
Smoking	2.20	1.36-3.57	0.001
Family history	0.85	0.55-1.30	0.459
Diabetes	0.84	0.51-1.40	0.515
DTA variables			
Log DTA calcium score	1.89	1.55-2.31	<0.001
Maximal DTA wall thickness (mm)	3.80	2.68-5.39	<0.001
Presence of DTA plaque	24.26	7.44-79.09	<0.001
Plaque composition			
Calcified (versus non-calcified)	1.24	1.99-6.05	<0.001
Extent atherosclerosis, % of segments affected	1.03	1.02-1.03	<0.001

Abbreviations: CAD: coronary artery disease, DTA: descending thoracic aorta.

Independent associations between DTA atherosclerosis and significant CAD

The independent association between DTA atherosclerosis and significant CAD was assessed with multivariate logistic regression analysis. The multivariate model was corrected for the (univariately identified) correlates of significant CAD: age, male gender, hypertension, hypercholesterolemia and smoking. Furthermore, DTA calcium score, DTA maximal wall thickness and presence of DTA plaque were entered as parameters of DTA atherosclerosis. After multivariate correction, DTA maximal wall thickness and presence of DTA plaque remained independently associated with significant CAD (OR 2.00, 95 % CI 1.28-3.12, $p = 0.002$ and OR 6.56, 95 % CI 1.78-24.19, $p = 0.005$, respectively). Of note, the association of DTA calcium score with significant CAD lost significance after DTA maximal wall thickness was entered into the model (Table 4).

Table 4. Independent associations (on multivariate analysis) of clinical, demographic characteristics and measurements of DTA atherosclerosis with the presence of significant CAD.

	OR	95% CI	p-value
Age	1.02	0.98-1.05	0.333
Male	2.03	1.18-3.49	0.011
Hypertension	1.11	0.64-1.91	0.704
Hypercholesterolemia	1.90	1.11-3.27	0.019
Smoking status	2.11	1.13-3.92	0.019
Log DTA calcium score	1.19	0.88-1.61	0.258
Maximal DTA wall thickness (1mm)	2.00	1.28-3.12	0.002
Presence of DTA plaque	6.56	1.78-24.19	0.005

Abbreviations: CAD: coronary artery disease, DTA: descending thoracic aorta.

Intra- and inter-observer variability of DTA atherosclerosis analysis

The intra- and inter-observer variability of the analysis of DTA atherosclerosis showed good agreement between observers. The intra-observer variability of DTA maximal wall thickness and extent of atherosclerosis demonstrated a good intra-observer agreement, ICC coefficients 0.81 (0.59-0.92), $p < 0.001$ and 0.77 (0.45-0.91), $p < 0.001$, respectively. Furthermore, the agreement of the presence of DTA atherosclerotic plaque and plaque morphology was moderate, Kappa values 0.57, $p = 0.010$ and 0.57, $p < 0.001$, respectively. Similarly, the inter-observer variability showed a good agreement of DTA maximal wall thickness and extent of DTA atherosclerosis, ICC coefficients 0.89 (0.72-0.96), $p < 0.001$ and 0.92 (0.81-0.97), $p < 0.001$, respectively, and moderate agreement of the presence of DTA atherosclerotic plaque and plaque morphology, Kappa values 0.53, $p = 0.015$ and 0.59, $p < 0.001$, respectively.

Discussion

In the present study in patients with suspected CAD, a comprehensive analysis of DTA atherosclerosis on CTA was performed and independent associations of parameters of DTA atherosclerosis with CAD were assessed. A high prevalence of DTA atherosclerosis and CAD was observed in this group of patients, and the presence, severity and extent of DTA atherosclerosis increased in parallel to the increase in CAD severity. In addition, all parameters of DTA atherosclerosis were significantly correlated with significant CAD in univariate logistic regression analysis. After multivariate correction for other cardiovascular risk factors, the presence of DTA atherosclerotic plaque and DTA maximal wall thickness were identified as independent correlates of significant CAD. For this reason, there was no association between the DTA calcium score with CAD.

Assessment of atherosclerosis in the descending thoracic aorta

At present, aortic atherosclerosis has been evaluated using computed tomography (CT), magnetic resonance imaging and fluorodeoxyglucose positron-emission tomography,^{3, 17, 18} but mostly with transesophageal echocardiography (TEE).^{5, 8, 19} DTA atherosclerosis on TEE is recognized as irregular intimal wall thickening with increased echogenicity and additionally classified in simple or complex atherosclerosis, based on plaque thickness, presence of ulceration and mobile debris. In addition, CT enables the evaluation of DTA atherosclerosis through measurement of the Agatston calcium score, atherosclerotic plaque and DTA wall thickness.^{3, 20, 21}

Several general population studies have investigated DTA atherosclerosis and its association with CAD. In the Stroke Prevention: Assessment of Risk in a Community (SPARC) study including 581 subjects, the prevalence of DTA atherosclerosis as assessed with TEE was 45 % and significantly associated with known CAD.⁵ Both the Heinz Nixdorf Recall study including 4,025 participants and the Multi-Ethnic Study of Atherosclerosis (MESA) including 6,814 participants assessed DTA calcification using non-enhanced CT and demonstrated independent associations with coronary calcium.^{9, 10} The prevalence of DTA calcification in the Heinz Nixdorf Recall Study was 57 % and comparable to the present study, whereas the median DTA calcium score was higher (6.5 [interquartile range 0-59.1]). However, in the MESA study the prevalence of DTA calcification was only 28 %. These differences might be explained by the older age of the Heinz Nixdorf Recall study cohort and on the other hand, by a lower cardiovascular risk profile as indicated by a lower prevalence of diabetes and smoking in the MESA study participants, despite the older age.

Associations between atherosclerosis in the DTA and CAD

Only a few studies investigated whether CTA derived parameters of DTA atherosclerosis were independently associated with CAD. Yamamoto et al. found in 180 patients with suspected CAD that DTA wall volume derived from 7 contiguous slices on enhanced computed tomography (CT) was associated with significant CAD (defined as >75% stenosis on invasive coronary angiography).²² Okuyama and colleagues evaluated 89 patients with suspected CAD and observed that DTA atherosclerosis on CTA (calculated as the sum of DTA atherosclerosis severity grades of the upper, middle and lower DTA) was independently associated with significant CAD (defined as ≥ 50 % stenosis on invasive coronary angiography).²¹ Takasu et al. performed a comprehensive analysis of DTA atherosclerosis on electron beam CT, including aortic calcification as well as DTA wall volume, maximal wall thickness and plaque (graded from 1 to 4 based on lumen irregularity and wall thickness) in 25-30 mm of the midportion of the DTA in 97 patients with suspected CAD. Independent associations were demonstrated of aortic calcification, DTA wall volume, maximal wall thickness, and aortic plaque with significant CAD (defined as ≥ 70 % stenosis on CTA).³ Jang et al. confirmed associations of aortic calcification and DTA maximal wall thickness with significant CAD (defined as ≥ 50 % stenosis on invasive coronary angiography) in 104 patients who underwent invasive coronary angiography because of significant CAD on CTA.²³ Finally, Jeltsch et al. assessed DTA maximal wall thickness in 30 mm of the midportion of the DTA on CTA in 160 patients with suspected or known CAD and did not observe an independent association between DTA maximal wall thickness and significant CAD (≥ 75 % stenosis on CTA), although DTA maximal wall thickness was independently associated with coronary atherosclerosis.²⁰

The findings in the current study extend the previous findings by demonstrating independent associations between DTA atherosclerosis and CAD on CTA. Whereas the earlier studies evaluated only a small portion of the DTA, the present study demonstrated the feasibility of assessing DTA atherosclerosis in the entire DTA. Moreover, a comprehensive analysis of DTA atherosclerosis was proposed, including the DTA Agatston calcium score, atherosclerotic plaque and DTA wall thickness. It was demonstrated that the presence, severity, extent and calcification of DTA atherosclerosis were increased in patients with increased severity of CAD. After multivariate adjustment, DTA atherosclerotic plaque and DTA maximal wall thickness were independently associated with significant CAD, but the DTA Agatston calcium score was not. The differences in results with the previous studies might be explained by the differences in measuring DTA atherosclerosis, the different definitions of significant CAD, and approach of the multivariate analysis. Importantly, in the present study significant CAD was defined by ≥ 50 % stenosis on CTA in line with guidelines of the appropriate use for diagnostic catheterization from the American Heart Association and Society of Cardiovascular Computed Tomography.²⁴

Clinical relevance

General population studies like the MESA study and Heinz Nixdorf Recall study have already established the relationship between thoracic aortic calcification and coronary calcium.^{9, 10} Independent associations of DTA atherosclerosis with CAD indicate that atherosclerosis in the DTA is not limited to local vascular disease, but an expression of diffuse atherosclerotic cardiovascular disease. Other markers of diffuse atherosclerotic cardiovascular disease, such as the carotid intima media thickness, coronary calcium score or arterial stiffness have been confirmed as independent predictors of cardiovascular events.²⁵⁻²⁷ Furthermore, the presence of arterial calcification in different vascular beds was demonstrated to have incremental predictive value beyond established risk factors for cardiovascular events and mortality.^{28, 29}

Therefore, the detection of DTA atherosclerosis might benefit the identification of patients at higher risk of CAD and worse prognosis. DTA atherosclerosis can easily be obtained from routine CT coronary angiography through the measurement of maximal wall thickness and atherosclerotic plaque. The prognostic value of DTA atherosclerosis needs further evaluation.

Limitations

Some limitations need to be addressed. First, due to the spatial resolution of CTA, differentiation of the intima and media layers of the aortic wall is precluded. However, increasing aortic wall thickness most likely represents thickening of the intima layer, as the intima layer thickness predominantly increases with age and atherosclerosis.³⁰ Second, atherosclerosis in the ascending thoracic aorta and aortic arch was not evaluated because the ascending thoracic aorta and aortic arch were not systematically visualized on CTA. Third, the scan range of CTA was adjusted to the height of the heart, leading to a different length of DTA for the evaluation of DTA atherosclerosis. Finally, associations of DTA atherosclerosis with (cardiovascular) events were not assessed, because the event rate in the study population is low and precludes assessment of the prognostic value of DTA atherosclerosis.

Conclusions

In patients with suspected CAD, DTA atherosclerosis and CAD were common on CTA and the presence, severity and extent of DTA atherosclerosis increased with increasing severity of CAD. DTA atherosclerosis measured as the presence of DTA atherosclerotic plaque and DTA maximal wall thickness were independently associated with significant CAD.

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CHAPTER 8

Associations of Atherosclerosis in the Descending Thoracic Aorta on CTA with Arterial Stiffness and Chronic Kidney Disease in Asymptomatic Patients with Diabetes Mellitus



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Abstract

The relation between atherosclerosis in the descending thoracic aortic (DTA), arterial stiffness and chronic kidney disease (CKD) in patients with diabetes mellitus (DM) remains unclear. The current aim was to evaluate associations of DTA atherosclerosis with arterial stiffness and parameters of CKD in asymptomatic patients with DM.

A total of 213 asymptomatic patients with diabetes (mean age 52 years, 56% men) underwent cardiovascular risk assessment including multi-slice computed tomography (for non-invasive coronary angiography, from which DTA atherosclerosis can be derived), non-invasive assessment of arterial stiffness with applanation tonometry and assessment of renal function. Measurements of DTA atherosclerosis included assessment of DTA thickening and calcium score. Arterial stiffness was determined by the carotid-femoral pulse wave velocity (PWV), parameters of CKD included estimated glomerular filtration rate (eGFR) and urinary albumin-creatinine ratio (UACR).

DTA atherosclerosis was present in 180 (84%) patients. Patients with DTA atherosclerosis had increased arterial stiffness, lower eGFR and higher UACR values. After multivariate correction, DTA calcium score was independently associated with PWV ($\beta=0.18$, $p=0.04$). Furthermore, both DTA maximal wall thickness and DTA calcium score were independently associated with eGFR ($\beta=-7.37$, $p<0.001$ and $\beta=-1.99$, $p<0.003$, respectively), but not with UACR.

The increase in arterial stiffness by atherosclerosis seemed to be mediated by arterial calcification, while the DTA calcium score was independently associated with arterial stiffness, but not DTA maximal wall thickness. Furthermore, parameters of CKD in patients with DM had a distinct relationship with DTA atherosclerosis: DTA atherosclerosis was associated with eGFR but not with UACR.

Introduction

Patients with diabetes mellitus (DM) have an increased atherosclerotic burden, increased arterial stiffness and a higher prevalence of chronic kidney disease (CKD), as compared to age-matched subjects without DM.¹⁻³ Studies have shown relations between these factors.

Atherosclerosis is a diffuse cardiovascular disease which can affect the aorta. Atherosclerosis is defined by arterial wall thickening with or without calcification and can be assessed in the descending thoracic aorta (DTA) on computed tomography (CT) angiography.^{4,5}

Atherosclerosis in the aorta has been related to arterial stiffness, especially by calcification in the aorta. Several studies demonstrated that aortic calcification increases arterial stiffness, whereas the association of aortic wall thickening or non-calcified atherosclerosis with arterial stiffness showed conflicting results.⁶⁻⁸ Therefore, the first aim of the present study was to investigate whether the association between DTA atherosclerosis (as derived from CT angiography) and arterial stiffness is mediated by the amount of calcification.

In addition, DTA atherosclerosis might enhance target end-organ damage, which includes CKD, but limited data are currently available, particularly in patients with DM.⁹ CKD in patients with DM can be assessed by estimated glomerular filtration rate (eGFR) and urinary albumin-creatinine ration (UACR). An early expression of CKD in patients with DM is albuminuria, which over time progresses from microalbuminuria to macroalbuminuria and eventually reduces eGFR.¹⁰

Advancing insights in the role of atherosclerosis in the development of CKD in patients with DM may guide preventive treatment.

Consequently, the second aim of the present study was to assess associations of DTA atherosclerosis (either with or without calcification) with UACR and eGFR as markers of early and advanced CKD, respectively.

Patients And Methods

Patients

Patients were included from an ongoing registry of asymptomatic patients with DM.¹¹ Based on the American Diabetes Association criteria¹², patients were classified as having type 1 DM if they had demonstrable auto-antibodies to islet cells, insulin and glutamic acid decarboxylase or low levels of plasma c-peptide in laboratory analysis, or type 2 DM if not.

Comprehensive evaluation of asymptomatic patients with DM is routinely performed at the outpatient clinic of the Leiden University Medical Center for risk stratification.¹¹ The cardiovascular risk assessment included a structured clinical history, physical examination, blood and urine laboratory testing. Arterial stiffness was non-invasively assessed with applanation tonometry. Multi-slice CT coronary angiography (CTA) was performed to evaluate the presence and severity of coronary artery disease (CAD).

Clinical and demographic data were prospectively collected in the departmental cardiology information system (EPD-Vision version 8.3.3.6; Leiden, The Netherlands) and were retrospectively analyzed. Asymptomatic status was confirmed with a self-completed questionnaire on chest pain.¹³ The Institutional Review Board of the Leiden University Medical Center approved this retrospective evaluation of clinically collected data and waived the need for written informed consent.

For the current evaluation, the presence, severity, extent and composition of atherosclerosis in the DTA were assessed from the CTA images. Patients were included in the analysis when the entire DTA was visualized on the axial CTA images and at least 5 or more DTA segments were present on the CTA. Subsequently, potential associations of DTA atherosclerosis with arterial stiffness and CKD were studied.

CTA data acquisition

Examinations were performed on a 64-detector row CT scanner (Aquilion 64, Toshiba Medical Systems, Otawara, Japan) or 320-detector row CT scanner (Aquilion ONE, Toshiba Medical Systems, Otawara Japan). One hour prior to the scan, heart rate and blood pressure were measured in order to administer beta-blockers (50 to 100 mg metoprolol, orally) to patients with a heart rate exceeding 65 beats/min and without contraindication for beta-blocking agents. First, a non-contrast calcium scan was performed with slice thickness of 3.0 mm. Thereafter, CTA was performed with simultaneous recording of the electrocardiogram for retrospective (64-detector row scanner) or prospective (320-detector row scanner) gating of the data. Scan acquisition parameters and scan protocol have been previously described.^{14, 15} A data set at 75 % of the R-R interval was automatically reconstructed with a slice thickness of 0.5 mm and reconstruction interval of 0.3 mm for the 64-detector row scanner and 0.5 mm and 0.25 mm, respectively, for the 320-detector row scanner. Post-processing and evaluation of

the CTA scans were performed on a remote work station with dedicated CTA analysis software (Vitrea 2 or Vitrea FX 1.0, Vital Images, Minnetonka, MN, USA).

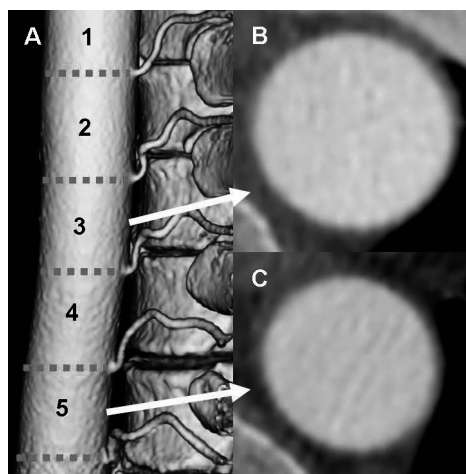


Figure 1. Evaluation of atherosclerosis in the descending thoracic aorta (DTA) on CTA of a 50 year-old man with a PWV of 7.4 m/s, eGFR of 103 ml/min/1.73m², and urinary albumin-creatinine ratio (UACR) of 0.7. Panel A shows the segments of the DTA by division based on the posterior intercostal arteries (red dotted lines). Panels B and C are axial images of the DTA at the site of maximal wall thickness of the corresponding segments 3 and 5, respectively. There is no thickening or irregularity of the arterial wall.

DTA atherosclerosis

DTA atherosclerosis was assessed on the axial CTA images by an experienced observer, blinded to patient's clinical characteristics, arterial stiffness and parameters renal function.¹⁶ The CTA scan range was adjusted to the heart to limit patients' radiation exposure. Consequently, the DTA was imaged and analyzed for atherosclerosis from beneath the aortic arch to the abdominal aorta.

First, the Agatston calcium score of the DTA was determined on the non-contrast calcium scan. Then, the DTA on CTA was anatomically divided based on the posterior intercostal arteries in 5 to 8 segments per scan as shown in Figures 1 to 3. Per segment, the site of maximal wall thickness was measured perpendicularly to the center of the aorta and evaluated for atherosclerosis (Figure 2 and 3). Any DTA atherosclerosis was defined to be present if the aortic wall thickness was ≥ 2 mm; more specifically, calcified DTA atherosclerosis was defined as aortic wall thickness ≥ 2 mm with a high-density component of atherosclerosis (Figure 3).¹⁷

Good intra- and inter-observer variability has been previously demonstrated for measurement of DTA maximal wall thickness (intraclass correlation coefficients 0.81 and 0.89) and percentage of DTA segments with atherosclerosis (intraclass correlation coefficients 0.77 and 0.92), and moderate agreement for the presence of DTA atherosclerosis (kappa 0.57 and 0.53).¹⁶

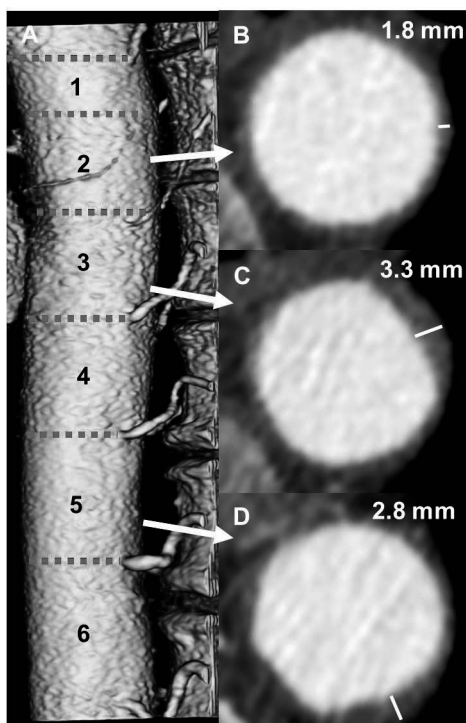


Figure 2. Evaluation of atherosclerosis in the descending thoracic aorta (DTA) on CTA of a 54 year-old woman with a PWV of 10.1 m/s, eGFR of 66 ml/min/1.73m², and urinary albumin-creatinine ratio (UACR) 0.6. Panels B, C and D are the axial images of the DTA at the site of maximal wall thickness of the indicated segments, 2, 3 and 5, respectively. Panel B reveals no signs of atherosclerosis and a maximal wall thickness of 1.8 mm. Panel C and D show the presence of atherosclerosis with a maximal wall thickness of 3.3 mm and 2.8 mm, respectively.

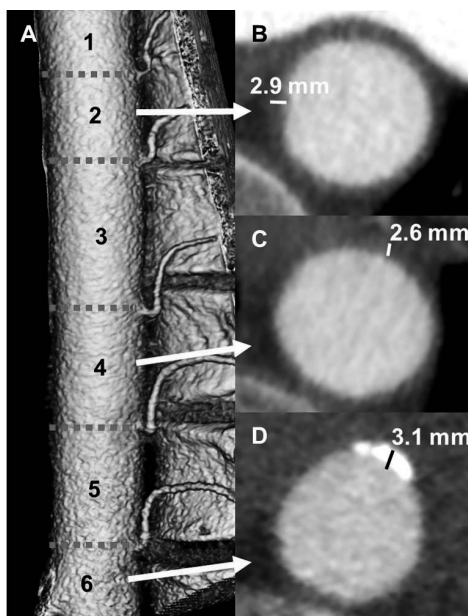


Figure 3. Evaluation of atherosclerosis in the descending thoracic aorta (DTA) on CTA of a 56 year-old woman with a PWV of 11.9 m/s, eGFR of 67 ml/min/1.73m², and urinary albumin-creatinine ratio (UACR) of 1.4. Panels B, C and D are the axial images of the DTA at the site of maximal wall thickness of the indicated segments, 2, 4 and 6, respectively. Panel B and C reveal atherosclerosis with a maximal wall thickness of 2.9 mm and 2.6 mm, respectively. Panel D shows the presence of calcified atherosclerosis with a maximal wall thickness of 3.1 mm.

Arterial stiffness

Arterial stiffness was non-invasively assessed with applanation tonometry using a Sphygmocor system (SphygmoCor, Atcor Medical, Sydney, Australia) with a hand-held high fidelity tonometer (Millar Instruments, Houston, TX, USA)¹⁸. Patients were instructed to abstain from their morning medication and remain fasting until after the test. Measurements were performed by a trained technologist, blinded to patient's clinical characteristics, under standardized conditions (during the morning in a quiet, temperature controlled clinical research laboratory, after the patient rested ten minutes in supine position and a constant heart rate and blood pressure was reached).

First, the aortic pulse wave velocity (PWV) was calculated as the distance traveled by the pulse wave between the carotid and femoral artery, divided by transit time (averaged from 10 consecutive beats) and was determined semi-automatically as previously described.¹⁹

Thereafter, pulse wave analysis was applied to obtain central aortic pressure waveforms.¹⁸ To this end, peripheral pressure waveforms were recorded on the radial artery at the level of the wrist and calibrated by peripheral blood pressures measured at the brachial artery with a cuff-sphygmomanometer. The central aortic pressure waveforms were generated from the recorded pressure waveforms with a validated generalized transfer function. The aortic pressure waveform is composed of a forward pressure wave from ventricular contraction and a backward pressure wave from reflection on the peripheral arterial system. The central systolic blood pressure (cSBP) and central pulse pressure (cPP) were semi-automatically derived from the central aortic pressure waveforms. In addition, the augmentation index (Aix), defined as the percentage that the reflected wave contributes to the pulse pressure, was assessed and normalized to a heart rate of 75 beats per minute.²⁰

Chronic kidney disease in patients with diabetes

Chronic kidney disease was assessed by albuminuria (UACR) as a marker of early CKD and creatinine clearance (eGFR) as a marker of more advanced CKD. The eGFR was calculated with the Modification of Diet in Renal Disease (MDRD) study equation.²¹ In addition, the UACR was determined from an overnight urine sample. Microalbuminuria was defined as $\text{UACR} \geq 3.5 \text{ mg/mmol}$.⁹

Statistical analysis

Normal distribution of continuous data was checked with the Kolmogorov-Smirnov test. Continuous data with normal distribution are presented as mean $\pm$ standard deviation and non-normally distributed data as median and interquartile range (25th and 75th percentiles). Categorical data are presented as frequencies and percentages. Continuous data were compared between patients with and without DTA atherosclerosis with the

Student T-test or Wilcoxon sum rank test, as appropriate. Categorical data were compared using the chi-square test.

Univariate associations of the measurements of DTA atherosclerosis with indices of arterial stiffness and parameters of renal function were assessed by Spearman's correlation coefficients. Thereafter, multivariate linear regression analysis was used to detect independent associations of DTA atherosclerosis with arterial stiffness and parameters of renal function. Non-normally distributed continuous data were log-transformed before being introduced in the multivariate analysis. The variable of arterial stiffness that had the strongest correlation with the measurements of DTA atherosclerosis was used in multivariate analyses. The independent association between each DTA atherosclerosis variable and arterial stiffness was corrected for a baseline model including age, gender, type 2 DM, hypertension and hypercholesterolemia. The independent association between each DTA atherosclerosis variable and eGFR was corrected for type 2 DM, hypertension, hypercholesterolemia and arterial stiffness, but not for age and gender because the equation of eGFR already corrects for these parameters. Finally, the independent association between each DTA atherosclerosis variable and UACR was corrected for the baseline model and arterial stiffness.

A p value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows (SPSS version 20.0, Inc., Chicago, IL, USA).

Results

Patient clinical and demographic characteristics

A total of 213 asymptomatic patients with DM were included with a mean age of 52 ± 12 years; 120 (56%) were men and 121 (57%) had type 2 DM. Clinical and demographic characteristics of the entire population and of the patients with versus without DTA atherosclerosis are summarized in Table 1. The mean DM duration was 16 ± 13 years, 132 (62%) patients had hypertension and 44 (21%) microalbuminuria.

DTA atherosclerosis

Atherosclerosis in the DTA was demonstrated in the majority of patients (Table 2). The overall median DTA calcium score and mean DTA maximal wall thickness were 3 [0-42] and 2.7 ± 0.9 mm, respectively. Any form of DTA atherosclerosis was present in 180 (84%) patients, and calcified DTA atherosclerosis was observed in 51 (24%) patients.

Patients with versus without DTA atherosclerosis

Patients with any DTA atherosclerosis were significantly older, had more often type 2 DM and had a higher BMI as compared to patients without DTA atherosclerosis (Table 1).

Furthermore, these patients had a higher prevalence of hypertension and hypercholesterolemia and a higher coronary artery calcium (CAC) score (Table 1). Moreover, patients with DTA atherosclerosis had a significantly increased arterial stiffness, reduced eGFR and higher UACR (PWV 8.7 ± 2.7 versus 6.3 ± 1.2 m/s, $p < 0.01$, eGFR 86 ± 22 versus 95 ± 21 ml/min/1.73m², $p = 0.03$, and UACR 0.9 [0.5-2.9] versus 0.6 [0.3-1.5], $p = 0.03$, respectively).

Relation between DTA atherosclerosis and arterial stiffness

All measurements of DTA atherosclerosis were significantly related with all indices of arterial stiffness (correlation coefficients provided in Table 3). Amongst the indices of arterial stiffness, measurements of DTA atherosclerosis were strongest correlated with PWV. Consequently, PWV was used as marker for arterial stiffness in the multivariate linear regression analysis.

After multivariate adjustment for baseline clinical variables, DTA maximal wall thickness was not associated with arterial stiffness (Table 4). However, the DTA calcium score remained significantly associated with arterial stiffness ($\beta = 0.18$, 95% confidence interval (CI) 0.01, 0.35, $p = 0.04$), suggesting that the increase in arterial stiffness is independently related to calcified DTA atherosclerosis, and not any DTA atherosclerosis (DTA wall thickening).

Relation between DTA atherosclerosis and chronic kidney disease

Measurements of DTA atherosclerosis were not consistently related with all parameters of renal function (Table 3). Although DTA calcium score and maximal wall thickness were significantly correlated with creatinine, the presence of DTA atherosclerosis and calcified atherosclerosis were not. In addition, all measurements of DTA atherosclerosis were significantly correlated with eGFR and UACR, but no measurement of DTA atherosclerosis was correlated with microalbuminuria. Among the parameters of renal function, measurements of DTA atherosclerosis showed the strongest relations with eGFR.

The independent associations of DTA maximal wall thickness and calcium score with eGFR or UACR were evaluated in separate multivariate linear regression models (Table 4). After multivariate correction for baseline clinical variables and arterial stiffness, maximal wall thickness and DTA calcium score remained significantly associated with eGFR ($\beta = -7.37$, 95% CI -10.93, -3.82, $p < 0.001$, and $\beta = -1.99$, 95% CI -3.27, -0.71, $p = 0.003$, respectively). In contrast, measurements of DTA atherosclerosis were not independently associated with UACR. Of note, arterial stiffness was significantly associated with both eGFR and UACR after correction for the baseline model. These findings indicate that DTA atherosclerosis is not related with all markers of CKD. DTA atherosclerosis was related with eGFR, but not with UACR. In contrast, arterial stiffness is related with both parameters of CKD.

Table 1 Clinical and demographic characteristics, indices of arterial stiffness and parameters of renal function of the entire population and comparison between patients with versus without DTA atherosclerosis.

Clinical variables	Overall n=213	No DTA atherosclerosis n=33 (16%)	DTA atherosclerosis n=180 (84%)	p-value
Age (years)	52 ± 12	37 ± 8	55 ± 10	<0.01
Male gender	120 (56%)	18 (54%)	102 (57%)	0.82
DM type 2	121 (57%)	10 (30%)	111 (62%)	<0.01
DM duration (years)	16 ± 13	13 ± 10	17 ± 13	0.1
Hemoglobin A1c (%)	8.1 ± 1.6	7.8 ± 1.7	8.1 ± 1.6	0.4
Insulin use (y/n)	151 (71%)	27 (82%)	124 (69%)	0.1
Body mass index (kg/m ²)	28 ± 6	25 ± 8	29 ± 10	<0.01
Smoking (y/n)	55 (26%)	6 (18%)	49 (27%)	0.3
Hypertension (y/n)*	132 (62%)	7 (21%)	125 (69%)	<0.01
Antihypertensive drug use (y/n)*	100 (47%)	4 (12%)	96 (53%)	<0.01
Hypercholesterolemia (y/n)*	150 (70%)	18 (54%)	132 (73%)	0.03
Statin use (y/n)	107 (50%)	10 (30%)	97 (54%)	0.01
CAC score	18 [0-203]	0 [0-0]	34 [0-287]	<0.01
PWV (m/s)	8.3 ± 2.6	6.3 ± 1.2	8.7 ± 2.7	<0.01
cSBP (mmHg)	124 ± 18	112 ± 14	126 ± 18	<0.01
cPP (mmHg)	43 [36-54]	36 [30-44]	45 [38-56]	<0.01
AIx (%)	19.5 ± 11.3	10.6 ± 15.3	21.1 ± 9.6	<0.01
Creatinine (μmol/L)	78 [68-90]	75 [69-82]	78 [68-91]	0.6
eGFR (ml/min/1.73m ²)	88 ± 22	95 ± 21	86 ± 22	0.03
UACR	0.9 [0.5-2.5]	0.6 [0.3-1.5]	0.9 [0.5-2.9]	0.03
Microalbuminuria ≥3.5 mg/mmol, n (%)	44 (21%)	3 (9%)	41 (23%)	0.07

Abbreviations: AIx: augmentation index, CAC: coronary artery calcium, cPP: central pulse pressure, cSBP: central systolic blood pressure, DM: diabetes mellitus, DTA: descending thoracic aorta, eGFR: estimated glomerular filtration rate, PWV: pulse wave velocity, UACR: urinary albumin-creatinine ratio.

* Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic pressure ≥90 mmHg or use of antihypertensive medication.

* Antihypertensive drugs; beta-blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonist and calcium antagonists.

* Hypercholesterolemia was defined as serum total cholesterol ≥5 mmol/L or use lipid lowering therapy.

Table 2 DTA atherosclerosis

DTA variable	All patients n=213
Calcium score	3 [0-42]
Maximal wall thickness (mm)	2.7 ± 0.9
Atherosclerosis (y/n)	180 (84%)
Calcified atherosclerosis (y/n)	51 (24%)

Abbreviations: DTA: descending thoracic aorta

Table 3 Correlation coefficients of the measurements of DTA atherosclerosis with indices of arterial stiffness and parameters of renal

Overall	Calcium score	Atherosclerosis	Calcified atherosclerosis	Maximal wall thickness
CAC score	0.41**	0.36**	0.36**	0.47**
Arterial stiffness				
PWV (m/s)	0.31**	0.36**	0.24**	0.39**
cSBP (mmHg)	0.25**	0.28**	0.24**	0.34**
cPP (mmHg)	0.30**	0.27**	0.34**	0.37**
AIx (%)	0.24**	0.26**	0.21**	0.26**
Renal function				
Creatinine (μmol/L)	0.15*	0.04	0.08	0.25**
eGFR (ml/min/1.73m ²)	-0.30**	-0.16*	-0.23**	-0.37**
UACR	0.25**	0.15*	0.14*	0.21**
Microalbuminuria ≥3.5 mg/mmol(y/n)	0.09	0.12	-0.02	0.13

Abbreviations: AIx: augmentation index, CAC: coronary artery calcium, cPP: central pulse pressure, cSBP: central systolic blood pressure, DTA: descending thoracic aorta, eGFR: estimated glomerular filtration rate, PWV: pulse wave velocity, UACR: urinary albumin-creatinine ratio.

* *p* value <0.05, ** *p* value <0.01

Table 4 Assessment of independent associations between each DTA atherosclerosis variable and arterial stiffness, eGFR and UACR.

DTA variable	PWV		eGFR		UACR	
	R	β (95% CI) P	R	β (95% CI) P	R	β (95% CI) P
Maximal wall thickness	0.441	0.42 (-0.09, 0.93) 0.10	0.408	-7.37 (-10.93, -3.82) <0.001	0.417	0.13 (-0.09, 0.34) 0.24
PWV		-		-1.03 (-2.15, 0.10) 0.07		0.11 (0.05, 0.17) <0.001
Calcium score	0.451	0.18 (0.01, 0.35) 0.04	0.370	-1.99 (-3.27, -0.71) 0.003	0.419	0.04 (-0.03, 0.11) 0.29
PWV		-		-1.16 (-2.30, -0.02) 0.046		0.11 (0.05, 0.16) <0.001

The multivariate linear regression models for the association of DTA maximal wall thickness and calcium score with arterial stiffness were corrected for a baseline model including age, gender, type 2 DM, hypertension and hypercholesterolemia. Multivariate models with renal function as dependent variable were adjusted for type 2 DM, hypertension, hypercholesterolemia and arterial stiffness, but not for age and gender because the equation of eGFR already corrects for age and gender. Multivariate models with diabetic nephropathy as dependent variable were adjusted for the baseline model and arterial stiffness.

Abbreviations: DTA: descending thoracic aorta, eGFR: estimated glomerular filtration ratio, PWV: pulse wave velocity, UACR: urinary albumin-creatinine ratio.

Discussion

The present study in asymptomatic patients with DM investigated associations of DTA atherosclerosis as assessed on CTA with arterial stiffness and CKD. First, this study demonstrated that the DTA calcium score, but not DTA maximal wall thickness, was independently associated with arterial stiffness suggesting that the relation between aortic atherosclerosis and increased arterial stiffness is mediated by calcified DTA atherosclerosis and not by the presence of any DTA atherosclerosis (defined as DTA wall thickening).

Second, DTA atherosclerosis was associated with CKD. After multivariate correction for baseline risk factors and arterial stiffness, both DTA calcium score and maximal wall thickness were independently associated with eGFR, a marker of advanced CKD, whereas DTA atherosclerosis was not independently associated with UACR, a marker of early CKD.

Additionally, the relationship between DTA atherosclerosis and CAC score was confirmed in asymptomatic patients with DM. Previous general population studies have demonstrated that thoracic aortic calcification is determined by similar risk factors as coronary artery disease and that thoracic aortic calcification is related with CAC score.^{22,23}

DTA atherosclerosis and arterial stiffness

The presence of DTA atherosclerosis has been related to increasing arterial stiffness, specifically, different studies have shown the relation between calcified DTA atherosclerosis and increased arterial stiffness.^{7,8} In a community-based cohort with 193 healthy volunteers increasing aortic calcification (assessed by 16-slice CT) was independently associated with increased aortic PWV.⁷ An open question is whether any DTA atherosclerosis (in the current study expressed as increased aortic wall thickness) relates to increased arterial stiffness or whether the presence of calcified DTA atherosclerosis is required to result in increased arterial stiffness. Brandts and coworkers evaluated 15 patients with essential hypertension and 15 age-matched healthy volunteers with magnetic resonance imaging (MRI); the authors demonstrated that aortic wall thickness (derived from 10 contiguous slices at the height of the 8 vertebra) was significantly related with arterial stiffness, as assessed by aortic PWV measured on MRI.⁴ In contrast, Cecelja et al. evaluated 100 postmenopausal female twins with MRI and demonstrated that aortic PWV did not increase in parallel to increasing aortic atherosclerotic plaque burden, but that there was a relation between aortic PWV and aortic calcium score on non-contrast CT images.⁶ In the present study, an independent association between DTA calcium score and aortic PWV was observed in asymptomatic patients with DM, but the presence of any DTA atherosclerosis or DTA maximal wall thickness (markers of DTA atherosclerosis independent of calcium) were not significantly associated with PWV after multivariate correction for baseline clinical risk factors. These observations suggest that increased arterial stiffness is related to calcified DTA atherosclerosis rather than any DTA atherosclerosis.

DTA atherosclerosis and chronic kidney disease

Chronic kidney disease in patients with diabetes can be measured by eGFR and UACR. These two parameters reflect different stages of CKD, with UACR preceding the reduction in eGFR. Previous work has addressed the associations between aortic atherosclerosis and eGFR or UACR.

Concerning the relation between aortic atherosclerosis and eGFR, Desai and coworkers evaluated maximal thickness of atherosclerotic plaque in the aortic arch and DTA on transesophageal echocardiography (TEE) in 200 consecutive patients clinically referred for TEE.²⁴ The thickness of atherosclerotic plaque increased in parallel to the reduction of eGFR and was independently associated with severe CKD, defined as eGFR <30 ml/min/1.73m². Another TEE study by Haruki et al. analyzed atherosclerotic plaque in the DTA in 350 consecutive patients who underwent clinically indicated 3-dimensional TEE.²⁵ Patients with DTA atherosclerotic plaque had a significantly reduced eGFR. In addition, a Japanese study investigated the association of atherosclerosis in the coronary arteries and aorta with renal function in 149 patients who underwent coronary angiography for suspected or known CAD and magnetic resonance imaging (MRI) of the aorta. The authors demonstrated that atherosclerosis in the coronary arteries was independently associated with reduced eGFR, whereas the association of atherosclerosis in the thoracic aorta with eGFR lost significance after multivariate correction.²⁶ The current findings are in line with and expand these previous studies and the novelty is that the current study used CT for imaging of aortic atherosclerosis, highlighting that non-calcified and calcified DTA atherosclerosis are related with reduced eGFR. Moreover, the association of increased arterial stiffness with reduced eGFR was weakened when DTA atherosclerosis was entered to the multivariate model.

Concerning the relation between aortic atherosclerosis and UACR, one study assessed the association of aortic atherosclerosis with UACR, while various studies investigated the association of carotid intima-media thickness (CIMT) with diabetic nephropathy. Kim et al. performed MRI in patients with type 1 DM, and atherosclerosis in the aorta was compared between 63 patients with macroalbuminuria (defined as urinary albumin excretion rate >300 mg/24 h) and 73 patients with normoalbuminuria.²⁷ However, the prevalence of atherosclerosis in the thoracic aorta was only 3% in patients with macroalbuminuria versus 0% in patients with normoalbuminuria. A Korean population study assessed the association of CIMT with UACR in 7555 participants.²⁸ The increase in CIMT was independently associated with a higher UACR. However, another Korean study including 673 patients with type 2 DM did not observe any association between CIMT or carotid atherosclerotic plaque and UACR.²⁹ Shin et al. also demonstrated that CIMT was not associated with microalbuminuria (defined as UACR of 30-300 mg/g) in 218 patients with newly diagnosed type 2 DM.³⁰

In the present study, early CKD as indicated by UACR showed no relation with atherosclerosis, whereas advanced CKD as indicated by reduced eGFR, was related with DTA atherosclerosis. How the temporal development of aortic atherosclerosis and the differ-

ent stages of CKD relate, needs further evaluation in longitudinal studies assessing both aortic atherosclerosis and CKD periodically.

Limitations

The present study has several limitations. The cross-sectional nature of the present study precludes assessment of causality of DTA atherosclerosis with arterial stiffness or CKD. The spatial resolution of CTA precludes differentiation of the intima and media layers of the aortic wall. Furthermore, aortic atherosclerosis was assessed on CTA images with the scan range adjusted to the patient's heart to reduce radiation exposure. This excludes analysis of atherosclerosis in the ascending thoracic aorta, aortic arch and abdominal aorta.

Conclusions

In the present study in asymptomatic patients with DM, the DTA calcium score was independently associated with arterial stiffness, but not any DTA atherosclerosis, indicating that arterial calcification mediates the increase in arterial stiffness by atherosclerosis. Furthermore, DTA atherosclerosis was independently associated with eGFR, a marker of advanced CKD in patients with diabetes, but not with UACR, a marker of early CKD.

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CHAPTER 9

Comparison by Computed Tomographic Angiography – the Presence of and Extend of Coronary Arterial Atherosclerosis in South Asians versus Caucasians with Diabetes Mellitus



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Abstract

South Asians in the Western world have a high prevalence of diabetes mellitus (DM) and increased risk of coronary artery disease (CAD) and mortality as compared to Caucasians. CAD in asymptomatic South Asian patients with type 2 DM has not been investigated. The aim of this observational cohort-study was to investigate CAD in asymptomatic South Asian patients with type 2 DM and compare with matched Caucasian patients. A total of 120 asymptomatic South Asian type 2 DM patients and matched Caucasian patients (mean age 52 years, 55% male) were derived from an ongoing registry of cardiovascular risk stratification in asymptomatic patients with DM. Cardiovascular risk assessment included multi-detector row computed tomography coronary angiography. CAD was assessed as the coronary artery calcium (CAC) score and classified in: no signs of atherosclerosis or minor wall irregularities <30%, non-significant CAD 30-50%, or significant CAD $\geq 50\%$ stenosis. On a patient base, CAD was scored according to severity and number of vessels and segments with significant CAD. Subsequently, CAD was assessed per coronary artery and per segment. As compared to Caucasian patients, South Asian patients had a significantly higher CAC score and higher prevalence of significant CAD (41% vs. 28%, $p=0.008$), involving more coronary vessels and segments. Significant CAD was especially more frequent in the left anterior descending coronary artery. In conclusion, asymptomatic South Asian patients with type 2 DM have a higher prevalence and extent of CAD as compared to matched Caucasian patients.

Introduction

Several studies have demonstrated that South Asian individuals in the Western world have more severe CAD and higher cardiovascular mortality as compared to Caucasian individuals.^{1, 2} However, differences in CAD between asymptomatic South Asian and asymptomatic Caucasian patients with DM type 2 have not been investigated. Multi detector row computed tomography coronary angiography (coronary CTA) is a well validated modality for the non-invasive assessment of CAD and allows assessment of the CAD and plaque composition.^{3, 4} Therefore, the aim of the current study was to assess CAD in asymptomatic South Asian patients with DM type 2 with coronary CTA and to compare with matched asymptomatic Caucasian patients with DM type 2.

Methods

South Asian patients with DM type 2 were enrolled from an ongoing registry of cardiovascular risk stratification in asymptomatic patients with DM in the Leiden University Medical Center, the Netherlands.⁵ These South Asian patients are 'Surinamese South Asian immigrants' whose ancestors were recruited from North India from 1873 until 1916 to work on plantations in Suriname.⁶ In 1975 around the independence of Suriname a large group of Surinamese South Asians settled in The Hague. Cardiovascular risk stratification contained a structured clinical history, physical examination, blood and urine laboratory testing and coronary CTA for the evaluation of CAD. South Asian ethnicity was established in the anamnesis.⁷

South Asian patients were individually matched with asymptomatic Caucasian patients with DM type 2, based on age, gender, DM duration and BMI. Since South Asian individuals have increased abdominal fat storage and higher insulin resistance at similar BMI levels, a lower obesity cut-off BMI is recommended by the World Health Organization.⁸ In line with this, South Asian patients were matched to Caucasian patients with a higher BMI.

Patients were diagnosed DM type 2 in the absence of demonstrable auto-antibodies to islet cells, insulin and glutamic acid decarboxylase or low levels of plasma C-peptide levels, according the American Diabetes Association criteria.⁹ Inclusion criteria were DM type 2, asymptomatic status as confirmed by the Rose questionnaire on chest pain,¹⁰ no previous myocardial infarction, coronary revascularization or cardiac surgery, and a coronary CTA of diagnostic image quality for the assessment of CAD.

Clinical and demographic data were prospectively collected in the departmental cardiology information system (EPD-Vision version 8.3.3.6; Leiden, The Netherlands) and were retrospectively analyzed. The Institutional Review Board of the Leiden University Medical Center approved the retrospective evaluation of clinically collected data and waived the need for written informed consent.

Coronary CTA was performed on a 64-detector row CT scanner (Aquilion 64, Toshiba Medical Systems, Otawara, Japan) or a 320-detector row CT scanner (Aquilion ONE,

Toshiba Medical Systems). Scan parameters of these CT scanners have been previously described.^{3,4}

One hour before the scan, heart rate and blood pressure were measured to administer beta-blockers (25 to 100 mg metoprolol, orally) in patients with a heart rate ≥ 65 beats/min, unless contraindicated. In addition, immediately prior to the coronary CTA scan, nitroglycerin 0.4 mg was administered sublingual.

The scan protocol commenced with a non-contrast scan of the heart with slice thickness of 3.0 mm for the coronary artery calcium (CAC) score. Subsequently, coronary CTA was started with the injection of non-ionic contrast media (Iomeron 400, Bracco, Milan, Italy) in the antecubital vein in a bolus of 60 to 110 ml with a flow rate of 5-6 mL/s (adjusted to patient's weight), followed by a saline flush. Coronary CTA image acquisition was triggered using automated peak enhancement detection with the region of interest in the left ventricle. Scanning was completed during a single breath-hold of approximately 10 seconds. Data was retrospectively (64-row scanner) or prospectively (320-row scanner) gated by recording of the electrocardiogram during the scan. An initial data set was automatically reconstructed at 75% phase of the R-R interval. In case of limited image quality or motion artifacts, additional data sets of different phases in the R-R interval were reconstructed.

Coronary CTA scans were post-processed and evaluated on a remote work station using dedicated coronary CTA analysis software (Vitrea 2 or Vitrea FX 1.0, Vital Images, Minnetonka, MN, USA). First, the Agatston CAC score was measured on the non-contrast calcium scan. Thereafter, the coronary CTA data set with the best image quality was selected for the evaluation of the coronary arteries. Coronary CTA scans were analysed by two experienced observers in consensus, blinded to patient's clinical and demographic characteristics. The coronary artery system was divided according the modified American Heart Association classification into a 17-segment model.¹¹ All segments were evaluated for the presence of atherosclerotic plaque, defined as a structure >1 mm² within and/or adjacent to the coronary artery lumen that could clearly be distinguished from the coronary artery lumen and surrounding tissue.¹² For each atherosclerotic plaque the severity of luminal stenosis was visually determined from the axial images and curved multiplanar reconstructions in at least two orthogonal planes (see Figure 1). Per segment luminal stenosis was graded based on the most severe stenosis as follows: 1. no signs of atherosclerosis or minor wall irregularities <30 % stenosis, 2. non-significant CAD 30-50 % stenosis or 3. significant CAD ≥ 50 % stenosis.¹³ In addition, the predominant atherosclerotic plaque composition was scored as non-calcified plaque (consisting of only low-density plaque), calcified plaque (consisting of only high-density plaque) or mixed plaque (consisting of both low- and high-density plaque). Local non-interpretable segments were excluded from further analysis.

Patients were stratified according to the segment with the most severe stenosis grade.¹³ Significant narrowing was assessed per coronary artery and significant one-vessel, two-vessels and three-vessels CAD. In addition the Gensini score was calculated.¹⁴ Subsequently, the location of CAD narrowing in proximal and distal coronary segments

was determined. Finally, the presence of any CAD ($\geq 30\%$ stenosis) was assessed per coronary artery and per segment.

Continuous data were checked for normal distribution with the Kolmogorov-Smirnov test. Normal distributed continuous data are presented as mean $\pm$ standard deviation and non-normally distributed continuous data as median (25th and 75th percentiles). Categorical data are presented as frequencies and percentages. Comparisons of continuous data between asymptomatic South Asian and Caucasian patients with DM type 2 were performed with the independent Student T-test, one-way analysis of variance or Mann-Whitney U test, as appropriate. Categorical data were compared with the chi-square test. A p value of <0.05 was considered statistically significant. All statistical analyses were performed with SPSS (SPSS version 20.0, Inc., Chicago, IL, USA).

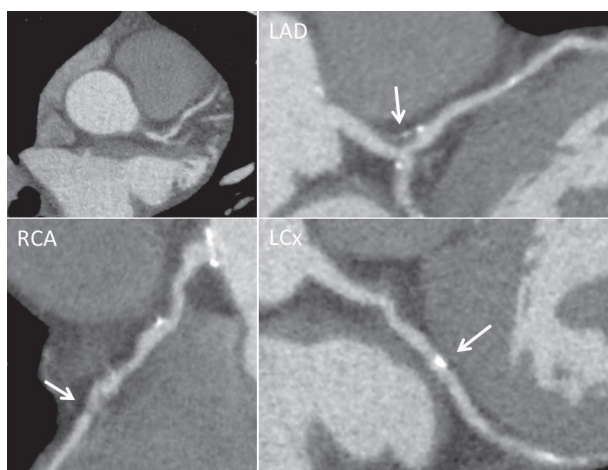


Figure 1 Example of coronary CTA in an asymptomatic South Asian patient with DM type 2, showing multivessel coronary artery disease. The left upper panel shows an axial image of the LM and proximal LAD. The other panels represent the multi-planar reconstructions of the indicated coronary arteries. The distal LM contains a mixed plaque with significant stenosis, which continues in the ostium of the LAD (indicated with the white arrow). The mid segment of the RCA contains a non-calcified plaque with significant stenosis (indicated with the white arrow). The proximal segment of the LCx contains a calcified plaque without significant stenosis (indicated with the white arrow)

Results

A total of 120 asymptomatic South Asian patients with DM type 2 were included and individually matched with asymptomatic Caucasian patients with DM type 2. The baseline clinical and demographic patient characteristics are presented in Table 1. The overall mean age was 53 ± 10 years, 55% were men and mean DM duration 11 ± 8 years. Because South Asian patients were matched to Caucasian patients with a higher BMI, they had a significantly lower BMI and waist circumference. Furthermore, South Asian patients

had a significantly higher prevalence of peripheral artery disease (21 vs. 10 %, $p=0.04$). Other baseline clinical risk factors were similar.

Coronary CTA results and p -values for comparison are presented in Table 2. South Asian patients had a significantly higher presence and extent of CAC. Moreover, the presence of significant CAD was significantly higher: 49 (41%) versus 33 (28%) patients, $p=0.008$. The presence of significant one-, two-, and three-vessel CAD was significantly higher, especially the left anterior descending coronary artery (LAD) was more frequently affected. Also, the Gensini score was significantly higher. There was no significant difference in coronary plaque composition.

On a vessel base, any CAD ($\geq 30\%$ stenosis) was significantly more frequent in the left main (LM) and LAD in South Asian patients, but was not different in the left circumflex (LCx) and right coronary artery (RCA). In addition, any CAD ($\geq 30\%$ stenosis) was more frequent in proximal coronary artery segments. On a segment base, CAD narrowing was significantly more frequent and severe in the LM coronary artery, the proximal and mid segment of the LAD and the proximal segment of the LCx.

Table 1 Baseline clinical and demographic characteristics of all patients and comparison between asymptomatic South Asian and matched Caucasian patients with DM type 2

Clinical variables	Overall n=240	South Asian n=120	Caucasian n=120	p-value
Age (years)	53 ± 10	52 ± 10	53 ± 9	0.4
Men	132 (55%)	66 (55%)	66 (55%)	1
DM duration (years)	11 ± 8	12 ± 8	11 ± 8	0.5
Body mass index (kg/m ²)	29 ± 6	28 ± 5	30 ± 6	<0.01
Waist circumference (cm)	101 ± 16	98 ± 16	104 ± 14	<0.01
Hemoglobin A1c (%)	7.9 ± 1.6	7.8 ± 1.6	7.9 ± 1.6	0.6
Insulin treatment (y/n)	112 (47%)	43 (36%)	69 (58%)	<0.01
DM complication (y/n)*	99 (41%)	53 (44%)	46 (38%)	0.4
Hypertension (y/n)*	161 (67%)	81 (68%)	80 (67%)	1.0
Systolic blood pressure (mmHg)	136 ± 18	134 ± 17	138 ± 18	0.1
Diastolic blood pressure (mmHg)	83 ± 10	83 ± 11	84 ± 10	0.5
Antihypertensive drug use (y/n)*	137 (57%)	70 (58%)	67 (56%)	0.7
Hypercholesterolemia (y/n)*	185 (77%)	91 (76%)	94 (78%)	0.8
Total cholesterol (mmol/L)	4.6 ± 1.1	4.4 ± 1.1	4.7 ± 1.1	0.09
Statin use (y/n)	161 (67%)	87 (73%)	74 (62%)	0.07
Smoking (y/n)	60 (25%)	32 (27%)	28 (23%)	0.6
Stroke or transient ischemic attack	12 (5%)	7 (6%)	5 (4%)	0.5
Peripheral artery disease	31 (13%)	21 (18%)	10 (8%)	0.04
ACE inhibitor	61 (25%)	31 (26%)	30 (25%)	0.9
AT II antagonist	66 (28%)	29 (24%)	37 (31%)	0.2
Beta blocker	26 (11%)	13 (11%)	13 (11%)	1
Calcium antagonist	25 (10%)	12 (10%)	13 (11%)	0.8
Aspirin	46 (19%)	21 (18%)	25 (21%)	0.5
Plasma creatinine (μmol/L)	78 ± 24	77 ± 29	78 ± 18	0.7
eGFR (ml/min/1.73m ²)*	93 ± 28	96 ± 33	89 ± 21	0.04

Abbreviations: ACE: angiotensin converting enzyme, AT II: angiotensin II receptor, CAD: coronary artery disease, DM: diabetes mellitus, eGFR: estimated glomerular filtration rate.

* DM complication included retinopathy, nephropathy, neuropathy and peripheral vascular disease as derived from the structured clinical history.

* Hypertension: systolic blood pressure ≥140 mmHg, diastolic pressure ≥90 mmHg or use of antihypertensive medication.

* Antihypertensive drug use included angiotensin converting enzyme inhibitors, angiotensin II receptor antagonist, beta-blockers and calcium antagonists.

* Hypercholesterolemia was defined as serum total cholesterol ≥5 mmol/L or use of statins.

* eGFR was calculated with the Modification of Diet in Renal Disease (MDRD) study equation.²⁹

Table 2 Coronary artery disease as assessed with coronary CTA of all patients and comparison between asymptomatic South Asian and matched Caucasian patients with DM type 2.

Clinical variables	Overall n=240	South Asian n=120	Caucasian n=120	p-value
Dominant right coronary	183 (76%)	90 (75%)	93 (78%)	0.6
Coronary artery calcium (CS ≥ 1)	143 (60%)	79 (66%)	64 (53%)	0.03
Total Agatston calcium artery score	16 (0-149)	26 (0-205)	3 (0-99)	0.03
Normal or wall irregularities <30%	82 (34%)	32 (27%)	50 (42%)	
Non-significant CAD 30-50%	76 (32%)	39 (33%)	37 (31%)	
Significant CAD $\geq 50\%$	82 (34%)	49 (41%)	33 (28%)	
Gensini score	6 $\pm$ 8	8 $\pm$ 9	5 $\pm$ 7	<0.01
No. of coronary arteries narrowed				0.03
1	44 (18%)	26 (22%)	18 (15%)	
2	24 (10%)	13 (11%)	11 (9%)	
3	14 (6%)	10 (8%)	4 (3%)	
Coronary artery narrowed				
left main	3 (1%)	3 (3%)	2 (2%)	0.08
left anterior descending	68 (28%)	42 (35%)	26 (22%)	0.02
left circumflex	30 (13%)	18 (15%)	12 (10%)	0.2
right	33 (14%)	19 (16%)	14 (12%)	0.3
Location of CAD narrowing				
proximal $\geq 30\%^*$	128 (53%)	76 (63%)	52 (43%)	<0.01
distal $\geq 30\%^*$	133 (55%)	71 (59%)	62 (52%)	0.2
proximal $\geq 50\%$	47 (20%)	29 (24%)	18 (15%)	0.07
distal $\geq 50\%$	68 (28%)	43 (36%)	25 (21%)	0.01
Number of segments narrowed				
$\geq 30\%$	2.7 $\pm$ 2.9	3.1 $\pm$ 3.0	2.3 $\pm$ 2.8	0.03
30-50%	1.9 $\pm$ 2.0	2.1 $\pm$ 2.1	1.7 $\pm$ 2.0	0.1
$\geq 50\%$	0.8 $\pm$ 1.4	1.0 $\pm$ 1.6	0.6 $\pm$ 1.2	0.03
Coronary plaque composition				
Non-calcified	1.4 $\pm$ 1.5	1.4 $\pm$ 1.8	1.5 $\pm$ 1.2	0.5
Mixed	1.7 $\pm$ 1.9	1.8 $\pm$ 1.8	1.7 $\pm$ 1.9	0.7
Calcified	1.0 $\pm$ 1.6	1.2 $\pm$ 1.7	0.8 $\pm$ 1.5	0.2

Abbreviations: CAD: coronary artery disease, CS: calcium score.

* Proximal CAD narrowing was defined as narrowing in the left main coronary artery or in the proximal segment of the left anterior descending coronary artery, circumflex coronary artery or right coronary artery.

* Distal CAD narrowing was defined as narrowing in segments other than proximal segments.

Discussion

In the present study, asymptomatic South Asian patients with DM type 2 had a higher presence, severity and extent of CAD as compared to individually matched asymptomatic Caucasian patients with DM type 2. More specifically, the presence and extent of CAC was higher. Significant CAD ($\geq 50\%$ stenosis) was significantly more frequent in South Asian patients, involving a higher number of coronary arteries and segments and particularly more frequently the LAD.

South Asians living in an urban or Western environment are highly susceptible to develop DM type 2. The age-standardized prevalence rate of DM type 2 in South Asian men and women in the Netherlands has been reported as high as 27.4%, approximately 4-fold higher than Caucasian men and women.¹⁵ In the Southall and Brent study, the presence of DM in 1420 South Asian men was associated with a nearly 3-fold increased risk of CAD mortality.¹ Thus, South Asian individuals have a high prevalence of DM, which contributes to their increased risk of CAD.

At present, only few studies investigated the presence, severity and extent of CAD in South Asian subjects. A recent study evaluated CAD in patients with suspected CAD using invasive quantitative coronary angiography, including 63 South Asian patients as compared to 61 Caucasian patients.² South Asian patients had more severe CAD as shown by a higher mean percentage stenosis per vessel and more patients with multivessel CAD.

Coronary CTA showed similar CAC scores in asymptomatic subjects under the age of 50 years, but above this age CAC scores were higher in South Asians.¹⁶ In a study by Tillin et al. CAC scores in the LAD of 41 South Asian patients with CAD with mean age of 61 years were comparable to 42 Caucasian patients with mean age of 67 years.¹⁷ Moreover, Koulaouzidis et al. investigated CAD with coronary CTA in 101 South Asian patients and their age and gender matched Caucasian counterparts in London.¹⁸ South Asian patients above the age of 50 years had a significantly higher coronary artery calcium score, a higher mean number of segments with CAD and more multivessel CAD.

In line with these studies, asymptomatic South Asian patients with DM type 2 in the present study had more severe and extensive CAD as compared to Caucasian patients. Nevertheless, only a few studies investigated the severity and extent of CAD in South Asians.^{2, 18} The novelty of the present study is the fact that CAD was evaluated in South Asian patients with DM type 2, who were asymptomatic. By this means, a high prevalence of significant CAD ($\geq 50\%$) of 41% was observed. As compared to available literature, the prevalence of significant CAD on CTA reported in the large diabetes cohort (n=3370) of the CONFIRM (CORonary CT Angiography EvaluationN For Clinical Outcomes: An International Multicenter Registry) study was 37%.¹⁹

In patients with suspected CAD, South Asians have been demonstrated to have significantly higher mean percentage stenosis in the proximal and distal LAD and proximal RCA as compared to Caucasians.² Another study demonstrated that CAD ($\geq 25\%$ stenosis) on coronary CTA was more frequent in all coronary arteries, with the highest

prevalence in the LAD.¹⁸ The present study extends these findings, by demonstrating that CAD was more frequent in proximal coronary artery segments, especially the LM and LAD, in asymptomatic South Asian patients. This proximal distribution of CAD has been associated with an increased risk for coronary events and worse outcome.²⁰

South Asians have a higher prevalence of CAD events at a younger age. The Southall and Brent study evaluated CAD events in 1517 South Asians and 2049 Caucasians over 20 years follow-up.²¹ South Asians had a 1.5 to 3-fold higher incidence rate of CAD events. Individual risk factors explained only a portion of the excess risk in South Asians. Other studies have demonstrated that the younger age of cardiac events in South Asian subjects was related with their increased cardiovascular risk profile at younger age.^{22,23}

In the present study, CAD was assessed in a local Dutch cohort of asymptomatic South Asian patients with DM type 2. Because local environment influences cardiovascular risk in South Asians, findings of the present study might not be generalizable to South Asian patients living in other countries in the Western world.

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CHAPTER 10

Summary and conclusions



Summary and conclusions

In **Chapter 1**, a general introduction on diabetes mellitus (DM) and the correlated increased cardiovascular risk is provided. Risk stratification remains challenging and the current risk score models have limited applicability. New markers of coronary artery disease (CAD) of non-invasive cardiac imaging might improve the predictive ability of current risk scores and allow for the initiation of patient tailored therapeutic strategies for the prevention of cardiovascular events. The aim of this thesis was to determine the potential role of non-invasive cardiac imaging in cardiovascular risk stratification and management in patients with DM.

Chapter 2 reviews the pathophysiology from obesity to CAD. First, obesity induced mechanisms and mediators involved in the development of CAD are described and subsequently several mediators are evaluated for their applicability as a biomarker for the identification of patients with CAD. Although some of these mediators showed a good predictive value for CAD, incremental value beyond established cardiovascular risk factors needs to be validated.

Chapter 3 describes non-invasive cardiac imaging of coronary artery anatomy using coronary computed tomography angiography (CTA) and ischemia assessment with single photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI). Additionally, the feasibility and potential clinical value of combined anatomic and functional imaging for patient management are discussed. The results of hybrid imaging using coronary CTA and SPECT MPI seem to have incremental clinical value, as compared to either technique alone or side-by-side analysis.

Chapter 4 investigates whether arterial stiffness could be used for the identification of patients with DM with stress perfusion defects on SPECT MPI. In 160 DM patients (mean age 51 years, 54% male and 57% type 2 DM) arterial stiffness was non-invasively measured with applanation tonometry as the carotid-femoral pulse wave velocity (PWV) and radial augmentation index (AIx). Stress myocardial perfusion defects were derived from SPECT MPI by evaluation of the summed stress score (SSS). Abnormal MPI (SSS >3) was sub-classified as moderate (SSS 3-7) or severe (SSS ≥8) MPI defects. Arterial stiffness increased in parallel with the severity of perfusion defects. After multivariate adjustment for age and other risk factors, PWV remained a significant predictor of severe MPI defects, but AIx was not. In conclusion, vascular stiffness as measured by PWV is strongly associated with severe MPI defects in DM patients. Accordingly, PWV could be a useful technique to identify patients with DM at elevated risk for CAD.

Chapter 5 studies associations between indices of arterial stiffness and left ventricular (LV) diastolic function as assessed with echocardiography in DM patients. A total of 142 patients (mean age 48 years, 53% male and 51% type 2 DM) underwent applanation tonometry and transthoracic echocardiography. Indices of arterial stiffness included PWV, central systolic blood pressure (cSBP), central pulse pressure (cPP) and AIx and parameters of LV diastolic function were E/A ratio, E', E/E' ratio and grade of LV diastolic dysfunction. PWV was independently associated with the severity of LV diastolic dysfunction, whereas cPP, cSBP and AIx were independently related with E/A ratio, but not with the LV diastolic dysfunction grade. Thus, indices of arterial stiffness are associated with echocardiographic parameters of LV diastolic function in DM patients. These observations suggest that therapies reducing arterial stiffness could help to prevent the development of LV diastolic dysfunction.

Chapter 6 assesses the change in LV function after 2 years follow-up with conventional and 2-dimensional speckle tracking echocardiography in clinically stable type 2 DM patients. A total of 112 patients with normal LV ejection fraction were included (mean age 53 years, 59% male), who underwent baseline transthoracic echocardiography and a repeated examination after at least 2 years follow-up. Circumferential (CS) and longitudinal strain (LS) were measured to assess systolic function and strain rate during isovolumetric relaxation time (SR IVR) and peak transmitral early diastolic inflow strain rate (SR E) to assess diastolic function. After 2 years follow-up, conventional echocardiography demonstrated no differences in LV volumes and ejection fraction (EF), although there was a significant increase in LV mass index and decrease in E/A ratio. In contrast, 2D speckle tracking echocardiography showed a small significant impairment in CS ($-19.7 \pm 4.0\%$ to $-18.9 \pm 3.8\%$), LS ($-17.2 \pm 2.3\%$ to $-16.9 \pm 2.7\%$) and SR E ($1.02 \pm 0.28 \text{ S}^{-1}$ to $0.94 \pm 0.25 \text{ S}^{-1}$). The changes in CS and SR E remained significant when adjusted for the change in LV mass index. In conclusion, type 2 DM patients and normal LV ejection fraction may show mild deterioration of subclinical LV function after 2 years follow-up, which can be detected by 2D speckle tracking echocardiography. The prognostic implications of these mild changes warrant prospective evaluation.

Chapter 7 performs a comprehensive analysis of atherosclerosis in the descending thoracic aorta (DTA) on coronary CTA in patients with suspected CAD, and evaluates the association of DTA atherosclerosis with CAD. A total of 344 patients were included (mean age 54 years, 54% male) with a coronary CTA of diagnostic image quality to assess CAD and complete display of the DTA. Analysis of DTA atherosclerosis included the DTA calcium score, maximal wall thickness and presence of atherosclerotic plaque. Coronary CTA were classified based on CAD severity in no signs of atherosclerosis or minor wall-irregularities <30%, non-significant CAD 30-50%, or significant CAD $\geq 50\%$ stenosis. DTA atherosclerotic plaque was observed in 81% of the patients and overall DTA maximal wall thickness was $2.7 \pm 1 \text{ mm}$. The presence, severity and extent of DTA atherosclerosis increased in parallel to the increase in CAD severity. Moreover, after

multivariate correction for age and other risk factors the presence of DTA atherosclerotic plaque and maximal DTA wall thickness were independently associated with significant CAD. In conclusion, a high prevalence of DTA atherosclerosis on coronary CTA was observed, which was independently related with the presence of significant CAD.

Chapter 8 studies whether DTA atherosclerosis assessed on coronary CTA is associated with arterial stiffness and measures of renal function. In 213 DM patients (mean age 52 years, 56% male) coronary CTA, applanation tonometry and assessment of renal function were performed for cardiovascular risk stratification. DTA atherosclerosis was present in 84% patients and overall maximal wall thickness was 2.7 ± 0.9 mm. Patients with DTA atherosclerosis had increased arterial stiffness (as shown by increased PWV and AIx) and worse renal function (as indicated by lower estimated glomerular filtration rate (eGFR) and higher urinary albumin creatinine ratio (UACR) values). After multivariate correction, DTA calcium score was independently associated with PWV. Furthermore, both DTA calcium score and DTA maximal wall thickness were independently associated with eGFR, but not with UACR. These findings suggest that atherosclerosis increases arterial stiffness via arterial calcification, while the DTA calcium score was independently associated with arterial stiffness, but not DTA maximal wall thickness. Furthermore, parameters of renal function in patients with DM had a distinct relationship with DTA atherosclerosis: DTA atherosclerosis was associated with eGFR but not with UACR.

Chapter 9 compares the presence, severity and extent of CAD in type 2 DM South Asian patients and matched Caucasian patients. South Asians in the Western world have a high prevalence of DM and an increased rate of CAD events. CAD was assessed with coronary CTA in 120 South Asian patients and matched Caucasian patients (mean age 52 years, 55% male). CAD was classified based on severity (no signs of atherosclerosis or minor wall irregularities <30%, non-significant CAD 30-50% or significant CAD $\geq 50\%$ stenosis) and scored on a patient base by the most severe stenosis and the number of vessels and segments with significant CAD. As compared to Caucasian patient, South Asian patients had a significantly higher prevalence of significant CAD (41% vs.28%), involving more coronary vessels and segments. Therefore, this chapter demonstrates that type 2 DM South Asian patients have a higher presence, severity and extent of CAD as compared to matched Caucasian patients.

Conclusions and future perspectives

New markers of CAD might benefit availability of cardiovascular risk stratification to the large population of DM patients. In addition to plasma biomarkers, non-invasive cardiac imaging can be of use for improved cardiovascular risk assessment in DM patients. The different studies in this thesis demonstrated that arterial stiffness measured as carotid-femoral PWV was independently related with calcified DTA atherosclerosis, decreased coronary artery function and target organ damage. Measurement of arterial stiffness is easy to perform, non-invasive and of low cost. Therefore, arterial stiffness measured as PWV is suggested as a good marker of CAD and may be a first-line approach for cardiovascular risk assessment of DM patients. Further studies are needed to validate the incremental clinical value in DM patients, before implementation in daily clinical practice.

In addition, DTA atherosclerosis on coronary CTA was independently associated with the presence of significant CAD and target organ damage. Further studies could compare the performance of DTA atherosclerosis for the identification of DM patients at increased risk for significant CAD versus other markers of subclinical coronary atherosclerosis.

2D speckle tracking echocardiography allows assessment of subtle changes in LV function and demonstrated mild deterioration in LV function in type 2 DM patients after 2 year follow-up. Therefore, it seems a useful modality for monitoring progression of sub-clinical LV dysfunction in order to titrate individual medication to prevent development of heart failure, but further studies are clearly needed.

Finally, South Asians patients with type 2 DM were demonstrated to have more severe and extensive CAD as compared to Caucasian patients. This observation suggests that an individual's cardiovascular risk is also determined by ethnicity and that a low threshold for cardiovascular risk assessment should be considered in type 2 DM South Asian patients.

Nederlands

Samenvatting en conclusies

Hoofdstuk 1 vormt een algemene inleiding op het onderwerp diabetes mellitus (DM) en het daarmee verbonden verhoogde risico op coronairlijden (CAD). Er is behoefte aan verbetering in risicofratificatie in patiënten met DM en de bestaande risicomodellen hebben slechts beperkte geschiktheid. Nieuwe markers van CAD verkregen met niet-invasieve beeldvormingstechnieken van het hart, al dan niet gebruikt in combinatie met de bestaande risicomodellen, zouden kunnen leiden tot verbetering in risicofratificatie. Op basis hiervan kan het medische behandelplan voor het voorkomen van een hartinfarct of hartstilstand per patiënt worden geoptimaliseerd. Het doel van dit onderzoek was om vast te stellen wat het mogelijk aandeel is van niet-invasieve beeldvormingstechnieken van het hart voor risicofratificatie op CAD en het optimaliseren van de medische behandeling in patiënten met DM.

Hoofdstuk 2 geeft een overzicht van hoe obesitas aanleiding geeft tot de ontwikkeling van CAD. Allereerst worden verschillende factoren en mechanisme besproken die door obesitas worden gestimuleerd en betrokken zijn bij de ontwikkeling van CAD. Vervolgens wordt op basis van de literatuur gekeken naar de toepasbaarheid van dergelijke factoren als biomarker voor het onderscheiden van personen met CAD. Enkele van deze factoren toonden een goede relatie met CAD. Echter, tot op heden is er geen bewijs dat het gebruik van dergelijke biomarkers leidt tot betere onderscheiding van patiënten met CAD, dan erkende risicofactoren van CAD.

Hoofdstuk 3 beschrijft niet-invasieve beeldvorming van de anatomie van de coronaire arteriën met computed tomography (CT) coronair angiografie (coronaire CTA) en ischemie (functie van de coronaire arteriën) middels myocardperfusiescintigrafie (MPI SPECT). Daarnaast wordt de klinische waarde bediscussieerd van gecombineerde beeldvorming, anatomie en functie van de coronaire arteriën, voor de behandeling van de patiënt. Huidige resultaten met een hybride opstelling van coronaire CTA en MPI SPECT tonen een klinische meerwaarde, ten opzichte van naderhand naast elkaar beoordeelde coronaire CTA en MPI SPECT.

Hoofdstuk 4 onderzoekt of arteriële stijfheid gebruikt kan worden voor de identificatie van patiënten met DM met perfusiedefecten in het myocard op de inspannings-opnames van SPECT MPI. In 160 patiënten met DM (gemiddelde leeftijd 51 jaar, 54% man en 57% DM type 2) werd de arteriële stijfheid op niet-invasieve manier gemeten met applanatie tonometrie, als de 'pulse wave velocity' (PWV) tussen de arterie carotis en arterie femoralis en 'augmentatie index' (AIX) gemeten aan de arterie radialis. Perfusiedefecten in het myocard werden beoordeeld op de inspannings-opnames van SPECT MPI met behulp van de 'summed stress score' (SSS). Afwijkende myocardiale

perfusie werd onderverdeeld in middelmatige (SSS 3-7) en ernstige (SSS ≥ 8) perfusie-defecten. De arteriële stijfheid nam toe met toename in de ernst van perfusiedefecten. Na multivariate correctie voor leeftijd en andere risicofactoren bleef de PWV een onafhankelijke voorspeller voor ernstige perfusiedefecten, maar de AIx niet. Hieruit werd geconcludeerd dat arteriële stijfheid gemeten als PWV sterk geassocieerd is met ernstige perfusiedefecten in patiënten met DM. Daarom, zou de PWV een bruikbare test kunnen zijn voor het identificeren van patiënten met DM met een verhoogd risico op CAD.

Hoofdstuk 5 bestudeert de associatie tussen arteriële stijfheid en diastolische functie van de linker kamer (LV) op echocardiografie in patiënten met DM. In 142 patiënten (gemiddelde leeftijd 48 jaar, 53% man en 51% DM type 2) werd de arteriële stijfheid gemeten met applanatie tonometrie en de LV diastolische functie werd bepaald van trans-thoracale echocardiografie. Metingen van arteriële stijfheid waren PWV, centrale systolische bloeddruk (cSBP), centrale polsdruk (cPP) en AIx. Parameters van LV diastolische functie waren de E/A ratio, E', E/E' ratio en de graad van LV diastolische dysfunctie. De PWV had een onafhankelijke relatie met de graad van LV diastolische dysfunctie en de cPP, cSBP en AIx hadden een onafhankelijke relatie met de E/A ratio. De cPP, cSBP en AIx toonden geen associatie met de graad van LV diastolische dysfunctie. Hieruit werd geconcludeerd dat in patiënten met DM, metingen van arteriële stijfheid gerelateerd zijn aan echocardiografische parameters van LV diastolische functie. Deze bevindingen onderbouwen dat medische behandeling gericht op het verlagen van de arteriële stijfheid het ontwikkelen van LV diastolisch dysfunctie zou kunnen voorkomen.

Hoofdstuk 6 bepaalt de verandering in LV functie na 2 jaar follow-up met conventionele en 2-dimensionele 'speckle-tracking' echocardiografie, in klinisch stabiele patiënten met DM type 2. In totaal werden 112 patiënten (gemiddelde leeftijd 53 jaar, 59% man) met een normale LV functie geïnccludeerd. Van deze patiënten was een trans-thoracaal echocardiogram gemaakt bij de initiële risicofactorenstratificatie en een follow-up onderzoek na 2 jaar. Metingen van 2D speckle tracking echocardiografie waren de circumferentiële strain (CS) en longitudinale strain (LS) voor LV systolische functie en de strain rate gedurende de isovolumetrische relaxatietijd (SR IVR) en gedurende E (SR E) voor diastolische functie. Na 2 jaar follow-up toonde conventionele echocardiografie geen verandering in LV volumes en ejectiefractie. Wel was er een significante toename van de LV massa geïndexeerd voor lichaamsoppervlakte en een afname van de E/A ratio. Daarentegen, werd er met gebruik van 2D speckle tracking echocardiografie een geringe significante afname gezien in CS ($-19,7 \pm 4,0\%$ naar $-18,9 \pm 3,8\%$), LS ($-17,2 \pm 2,3\%$ naar $-16,9 \pm 2,7\%$) en SR E ($1,02 \pm 0,28 \text{ s}^{-1}$ naar $0,94 \pm 0,25 \text{ s}^{-1}$). De afname in CS en SR E bleven significant na correctie voor de verandering in LV massa geïndexeerd voor lichaamsoppervlakte. Op basis van deze resultaten werd geconcludeerd dat patiënten met DM type 2 en een normale LV ejectiefractie over 2 jaar follow-up mogelijk een geringe afname van subklinische LV functie hebben. Dit kan aangetoond en gecontroleerd worden met 2D speckle tracking echocardiografie. De prognostische betekenis van deze geringe veran-

deringen in LV functie behoeft prospectieve studie.

Hoofdstuk 7 verricht een uitgebreide beoordeling van atherosclerose in de descendende thoracale aorta (DTA) op coronaire CTA in patiënten met verdenking op CAD. Vervolgens werd onderzocht of atherosclerose in de DTA was geassocieerd met CAD. In totaal werden 344 patiënten geïncludeerd (gemiddelde leeftijd 54 jaar, 54% man) met een coronaire CTA van diagnostische kwaliteit voor de beoordeling van CAD en de gehele DTA afgebeeld op de scan. Voor de beoordeling van atherosclerose in de DTA werd de DTA calciumscore gemeten, de DTA maximale wanddikte en de aanwezigheid van DTA atherosclerotische plaques. Coronaire CTA werden onderverdeeld op basis van de ernst van CAD in 'geen tekenen van atherosclerose of geringe wandonregelmatigheden <30%', 'niet-significant CAD 30-50%' of 'significant CAD $\geq 50\%$ ' vernauwing. Atherosclerotische plaques in de DTA werden aangetoond in 81% van de patiënten en de gemiddelde DTA maximale wanddikte was $2,7 \pm 1$ mm. De aanwezigheid, ernst en uitgebreidheid van atherosclerose in de DTA nam toe met toenemende ernst van CAD. Voorts bleven de aanwezigheid van DTA atherosclerotische plaques en DTA maximale wanddikte onafhankelijke geassocieerd met de aanwezigheid van significant CAD. Deze studie toonde aan dat er in patiënten met verdenking op CAD een hoge prevalentie was van atherosclerose in de DTA op coronaire CTA, welke een onafhankelijke relatie had met de aanwezigheid van significant CAD.

Hoofdstuk 8 bestudeert associaties van atherosclerose in de DTA op coronaire CTA met arteriële stijfheid en metingen van nierfunctie. In 213 patiënten met DM (gemiddelde leeftijd 52 jaar, 56% man) was in het kader van risicostratificatie op CAD een coronaire CTA, applanatie tonometrie en nierfunctiebepaling verricht. Patiënten met atherosclerose in de DTA hadden een verhoogde arteriële stijfheid en een slechtere nierfunctie. De DTA calciumscore was onafhankelijk geassocieerd met PWV na multivariate correctie. Bovendien waren zowel de DTA calciumscore en de DTA maximale wanddikte onafhankelijk geassocieerd met eGFR, maar niet met UACR. Deze resultaten laten allereerst zien dat atherosclerose in de DTA de arteriële stijfheid lijkt te verhogen door middel van arteriële verkalking, omdat de arteriële stijfheid onafhankelijke geassocieerd was met de DTA calciumscore, maar niet met DTA maximale wanddikte. Daarnaast hadden de metingen van nierfunctie een verschillende relatie met atherosclerose in de DTA, eGFR was wel geassocieerd met atherosclerose in de DTA maar UACR niet.

Hoofdstuk 9 vergelijkt de aanwezigheid, ernst en uitgebreidheid van CAD in Hindoestaanse patiënten met DM type 2 met gematchte blanke patiënten. Hindoestanen in de Westerse wereld hebben een sterk verhoogde prevalentie van DM en een verhoogd voorkomen van cardiale events. CAD werd bepaald middels coronaire CTA in 120 Hindoestaanse patiënten en gematchte blanke patiënten (gemiddelde leeftijd 52 jaar, 55% man). CAD werd onderverdeeld op basis van de ernst van CAD (geringe wandonregelmatigheden <30%', 'niet-significant CAD 30-50%' of 'significant CAD $\geq 50\%$ '

vernaauwing) en beoordeeld op patiëntniveau op ernstigste vernauwing en het aantal coronaire arteriën en segmenten met significant CAD. Ten opzichte van blanke patiënten hadden de Hindoestaanse patiënten een significant hogere prevalentie van significant CAD (41% vergeleken met 28%), waarbij meer coronaire arteriën en segmenten waren aangedaan. Hieruit werd geconcludeerd dat Hindoestaanse patiënten met DM type 2 een verhoogde aanwezigheid, ernst en uitgebreidheid hebben van CAD als vergeleken met gematchte blanke patiënten.

Conclusies en toekomstperspectief

De ontdekking van nieuwe markers van CAD kan risicostratificatie beschikbaar maken voor een grotere groep patiënten met DM. Tot zulke markers behoren naast beschikbare plasma biomarkers ook markers van niet-invasieve cardiale beeldvorming, die gebruikt kunnen worden om risicostratificatie in patiënten met DM te verbeteren. De studies in dit proefschrift tonen aan dat arteriële stijfheid gemeten als de PWV tussen arterie carotis en arterie femoralis onafhankelijk was gerelateerd met atherosclerose in de DTA, perfusiedefecten in het myocard en eindorgaanschade. Meting van de arteriële stijfheid is gemakkelijk uit te voeren, niet-invasief en goedkoop. Daarom zou arteriële stijfheid een geschikte marker kunnen zijn voor risicostratificatie op CAD in patiënten met DM in de eerstelijns. Aanvullende studies zijn nodig om de toegevoegde klinische waarde van arteriële stijfheid in patiënten met DM te valideren, voordat arteriële stijfheid ingevoerd kan worden in de dagelijkse kliniek.

Daarnaast werd aangetoond dat atherosclerose in de DTA op coronaire CTA onafhankelijk was geassocieerd met de aanwezigheid van significant CAD, alsook met eindorgaanschade. Toekomstige studies zijn nodig om het onderscheidend vermogen van atherosclerose in de DTA te vergelijken met andere markers van subklinische atherosclerose, voor het identificeren van patiënten met DM met een verhoogd risico CAD. 2D speckle tracking echocardiografie maakt het mogelijk om geringe veranderingen in LV functie te detecteren en toonde aan dat er een geringe afname was na 2 jaar follow-up in patiënten met DM type 2. Deze echocardiografische techniek lijkt daarom erg geschikt om de ontwikkeling van subklinische LV dysfunctie te controleren en aan de hand hiervan de individuele medicatie te optimaliseren om hartfalen te voorkomen. Echter, verdere studies hiernaar zijn nodig.

Tenslotte werd aangetoond dat Hindoestaanse patiënten met DM type 2 ernstiger en uitgebreider CAD hebben dan gematchte blanke patiënten. Deze observatie maakt duidelijk dat het individuele risico op CAD mede wordt bepaald door etniciteit. Dit pleit ervoor dat risicostratificatie voor CAD eerder overwogen moet worden in Hindoestaanse patiënten met DM type 2.

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Curriculum Vitae

Cornelis Jacobus Roos werd geboren op 17 juni 1981 te Velsen. In 1999 behaalde hij zijn VWO diploma aan het Noordzee college locatie Ichthus college te Driehuis. Datzelfde jaar begon hij met de studie Geneeskunde aan de Universiteit Utrecht. Tijdens zijn studie was hij betrokken bij wetenschappelijk onderzoek op de afdeling Hartziekten en Radiologie van de Universiteit Utrecht onder begeleiding van prof. dr. P.A.F.M. Doevendans en prof. dr. M. Prokop. In 2006 studeerde hij af voor Geneeskunde en was vervolgens werkzaam als arts-assistent-niet-in-opleiding op de afdeling Hartziekten van eerst het Maxima Medisch Centrum in Veldhoven en later het Kennemer Gasthuis in Haarlem. In 2009 startte hij met zijn promotieonderzoek op de afdeling Hartziekten van het Leids Universitair Medisch Centrum onder begeleiding van prof. dr. J.J. Bax en prof. J.W. Jukema. Dit promototraject werd ondersteund door het Interuniversitair Cardiologisch Instituut van Nederland en de resultaten zijn beschreven in dit proefschrift. In maart 2014 is hij gestart met zijn verder klinische opleiding tot cardioloog op de afdeling Hartziekten van het Medisch Spectrum Twente in Enschede onder begeleiding van dr. P.M.J. Verhorst.

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