Doctoral Thesis for the Degree of Doctor of Philosophy, Faculty of Medicine

Longitudinal Common Carotid Artery Wall Motion

Mechanistic, prognostic and translational studies

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Cover illustration: A conventional B-mode ultrasound image of the common carotid artery. The outlined yellow arrows in the image correspond to the velocity vectors indicating the direction and magnitude of the longitudinal tissue wall motion.

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Till Mamma och Pappa

Abstract

The longitudinal arterial movement of the common carotid artery (CCA) has been less investigated during the past compared to the radial movement. This is probably due to lack of an adequate measuring method. Velocity vector ultrasound imaging (VVI) now enables assessment of the longitudinal movement. The general aim of this thesis was to evaluate the potential use of VVI in assessment of CCA longitudinal wall motion. Furthermore, its clinical correlates, possible mechanisms and predictive value were evaluated in studies from man to mouse.

VVI was studied from standard CCA B-mode ultrasound images in healthy volunteers, patients with established coronary artery disease (CAD) and in medium to high risk patients. All images were analyzed with specific interest of the longitudinal vessel wall movement of the far wall of the CCA. Additionally, the VVI technique was used to study the longitudinal CCA wall movement in a mouse model of atherosclerosis using high frequency ultrasound.

Findings from this thesis suggest that it is possible to assess longitudinal CCA vessel wall motion with VVI technique in man with good accuracy. Patients exhibiting low total longitudinal displacement (tLoD) showed greater intima-media thickness (IMT), increased clinically determined ischemia score and area on myocardial perfusion scintigraphy examination, and decreased cardiac performance as measured by tissue velocity also determined by VVI. In patients with suspected CAD, high tLoD predicted greater one year cardiovascular (CV) event-free survival. In a separate survival analysis including patients with IMT above and below the median value, tLoD provided an incremental value above IMT in prediction of event-free survival. Finally, tLoD is also measurable in mice and low tLoD was associated with greater atherosclerotic plaque burden in mice.

This thesis concludes that VVI can be used to study the longitudinal CCA vessel wall movement in both man and mouse. The tLoD seems to reflect both vessel wall structure and cardiac performance. VVI assessed longitudinal displacement has a predictive value for future short term CV events in patients with suspected CAD.

Key words: carotid artery, longitudinal wall motion, ultrasound, velocity vector imaging, risk factor.

List of publications

The thesis is based on the following papers which are referred to in the text by their Roman numerals:

I. Sara Svedlund, Li-ming Gan.

Longitudinal Wall Motion of the Common Carotid Artery Can be Assessed by Velocity Vector Imaging

Clin Physiol Funct Imaging. 2011 Jan;31(1):32-8. Epub 2010 Sep 23.

II. Sara Svedlund, Charlotte Eklund, Sinsia Gao, Li-ming Gan.

Carotid Artery Longitudinal Displacement is Associated with Cardiac Wall Motion

Submitted

III. Sara Svedlund, Charlotte Eklund, Per Robertsson, Milan Lomsky, Li-ming Gan.

Carotid Artery Longitudinal Displacement Predicts One Year Cardiovascular Outcome in Patients with Suspected Coronary Artery Disease

2nd revision submitted

IV. Sara Svedlund, Li-ming Gan.

Longitudinal Common Carotid Artery Wall Motion is Associated with Plaque Burden in Man and Mouse

Accepted for publication in Atherosclerosis

Contents

List of abbreviations

AIx = augmentation index

BMI = body mass index

CABG = coronary artery bypass grafting

CAD = coronary artery disease

CCA = common carotid artery

CV = cardiovascular

DBP = diastolic blood pressure

ECA = external carotid artery

ECG = electrocardiogram

EF = ejection fraction

ICA = internal carotid artery

IMT = intima media thickness

LAS = large artery stiffness

LAX = long axis view

LDL = low-density lipoproteins

LV = left ventricle

MACE = major adverse cardiovascular events

MPS = myocardial perfusion scintigraphy

PCI = percutaneous intervention

PP = pulse pressure

PWV = pulse wave velocity

SAX = short axis view

SBP = systolic blood pressure

tLoD = total longitudinal displacement

TV = tissue velocity

UBM = ultrasound biomicroscopy

VAC = ventricular-arterial coupling

VVI = velocity vector imaging

Introduction

Cardiovascular disease

Cardiovascular disease is our leading cause of death in the world and continuous to be a major health issue [1, 2]. Although the concept includes diseases affecting different circulatory organs like the heart and brain, the common base for morbidity is due to atherosclerosis. Well known complications to cardiovascular disease include angina pectoris, myocardial infarction, stroke, transient ischemic attacks and peripheral artery disease. However, the majority of deaths caused by cardiovascular disease are due to coronary artery atherosclerosis[3]. Despite modification of traditional risk factors such as smoking, hypertension, diabetes mellitus and hypercholesterolemia as well as the great progress within interventional cardiology, atherosclerotic cardiovascular disease still remains the number one killer in the western world.

Atherosclerosis

In addition to hypercholesterolemia, atherosclerosis is also recognized as a slowly progressive chronic inflammatory vascular disease. The local hemodynamic environment is also known to affect atherogenesis[4]. The main factors influencing the hemodynamic environment are shear stress and tensile stress. Shear stress is the tangential force caused by the blood friction on the inner most cell layer in an artery, the endothelial cells. Flow turbulence will result in low shear stress. Tensile stress is the force the blood pressure creates circumferentially to the vessel wall and mainly affects the smooth muscle cells in the vessel wall. Atherosclerotic lesions are usually found at bifurcations, branching points and at the inner walls of curvatures [4]. The early types of lesions already present in children, the "fatty streaks", exclusively consist of inflammatory cells, i.e. macrophages and T-lymphocytes[5]. The endothelium is considered to play an important role in atherosclerosis development. Damage to the endothelium, endothelial dysfunction, represent a key step towards atherosclerotic lesions and may be initiated by several factors such as hypertension, modifications of low-density lipoproteins (LDL), circulating free radicals and diabetes mellitus which change the endothelial characteristics[6].

The continuing process which leads to atherosclerotic plaque formation is of complex nature and can be described in great detail. Briefly, endothelial dysfunction increases permeability for accumulation of lipoproteins into the intima (the inner layer of the arterial wall facing the blood stream). Increased adhesion of leukocytes and platelets activate formation of different molecules such as cytokines, different enzymes and growth factors. Attracted monocytes differentiate into macrophages which absorb the modified LDL by that time present in intima resulting in formation of foam cells. The process stimulates migration and proliferation of smooth muscle cells that integrates at the inflammatory site. The formed lesion will result in vascular wall thickening. With more advanced lesions, more leukocytes and macrophages will be recruited and eventually there will be even more vascular damage including necrosis. Formations of fibrous tissue will eventually overly the lipid core and necrotic tissue as a cap. In the course of time, an advanced lesion will protrude into the vessel lumen affecting blood flow since the vessel will not be able to compensate by dilation more than up to a certain point.

However, there are several plaque types with different histological characteristics that can cause coronary events which in most cases are found not to be due to occlusive lesions. Plaque rupture due to plaques with a thin fibrous cap and large lipid content is considered to be responsible for the majority of fatal myocardial infarctions and may be accompanied by thrombus formation. Other plaque types include erosive lesions, calcified lesions and plaques with hemorrhage. Plaques fulfilling criteria such as ongoing active inflammation, thin cap and large lipid content and being occlusive are considered “vulnerable plaques” [7]. Interestingly, one of the few prospective studies investigating local plaque feature and cardiovascular (CV) events in man, the recently published PROSPECT study, demonstrated that plaque burden, minimal lumen diameter as well as ultrasound-characterized thin-cap fibroma plaque type are all independent predictors of future CV events[8]. The clinical manifestation of a vulnerable plaque may vary from patient to patient depending on other factors such as the status of the blood and myocardium. It is therefore recommended to have a more holistic approach for improved prediction of coronary events by including local structural and global functional assessment to identify the “vulnerable patient” [9].

Imaging of atherosclerosis

Early diagnose and interventions of atherosclerotic disease in patients are highly desirable goals for both clinicians and researchers. To be able to detect atherosclerotic lesions at an early stage, risk-stratify patients, and follow potential treatment effects, a non-invasive and feasible imaging method would be highly attractive.

Several different non-invasive imaging modalities can be used for morphological imaging of atherosclerotic disease at different vascular sites. For imaging of the coronary arteries, several invasive catheter-based techniques are used for visualization of atherosclerotic lesions such as intra-vascular ultrasound (IVUS), and optical coherence tomography (OCT). Beside these invasive techniques, multidetector computed tomography (MDCT) can be used to assess the extension of calcified lesions in the coronary arteries in a non-invasive way. Different grading systems are in use to determine coronary calcium score (CCS) which has been shown to provide prognostic information in different patient populations [10-12]. Further, offering assessment of stenosis severity, coronary CT angiography (CCTA) allows visualization of non-calcified lesions and provides a prognostic value in symptomatic patients[13]. Ultrasound can be readily used for assessment of carotid artery intima-media thickness (IMT) which is known to be an accepted surrogate marker for atherosclerosis and risk factor for CV events[14].

The use of ultrasound for imaging of tissues is an established technique and widely used in different medical areas. Very basic, the ultrasound is emitted and produced from a transducer that consists of piezoelectric crystals that oscillate in response to electric current. The emitted sound interacts with tissues in the body and an echo is produced which is used for image formation. The frequency (Hz) of the ultrasound transducer is dependent on the attribute of the piezoelectric crystals in the transducer. Higher transducer frequency results in greater resolution but diminished penetration depth into the tissue. In addition to IMT and plaque area measurement, recent 3D-vascular ultrasound technique[15] as well as back-scatter ultrasound technology may introduce more accurate plaque volume measurement and also facilitate characterization of plaque composition[16].

Arterial stiffness

Another factor accompanying the atherosclerotic process is arterial stiffness. The importance of arterial stiffness with increasing degree of atherosclerosis has been addressed extensively in the literature. The term ”arterial stiffness” has no absolute definition but is a descriptive expression[17]. Several mechanisms have been suggested to determine arterial stiffness including altered structural components, smooth muscle tone and blood pressure.

Pathophysiological considerations in arterial stiffness

Following increased age, a gradual loss of elastin will occur as well as calcification and atherogenesis which will increase the stiffness of the vessel wall. The pressure wave in aorta is determined by cardiac output, peripheral vascular resistance, the stiffness of the conduit vessels and the magnitude of the returning pressure wave[18]. As the arterial tree branches and thinnest throughout the body there is a change in pressure and resistance which results in reflection of a pressure wave[19]. This reflecting wave has beneficial purposes, such as under normal circumstances the wave returns in diastole and enhances coronary perfusion. Further, the reflecting wave reduces pulsatility to microvascular beds, which reduces mechanical organ damage. Given increased aortic stiffness, the returning pressure wave will occur in systole and thereby enhance the systolic aortic pressure. Consequently, this means an increased cardiac afterload and an increased cardiac oxygen demand. The diminished elasticity of the aorta and loss of diastolic pressure enhancement gives a reduced coronary perfusion. These changes will ultimately lead to left ventricle hypertrophy and coronary ischemia[20, 21]. Cardiovascular remodeling is a process induced by hypertension or a combination of risk factors including arterial stiffness [22]. Cardiac hypertrophy has a negative impact on diastolic function and the mechanics of the septal wall during systole but also the coronary microcirculation.

Normally the diastolic blood pressure (DBP) in the aorta is influenced by two factors; first the systemic vessel resistance and secondly aortic distensibility. Presence of atherosclerosis produce a stiffer vessel and dysregulation of the balance between increased elastin break down and an overproduction of abnormal collagen[18]. Structural changes in the coronary vessels will potentially disturb the laminar flow profile and thereby accelerate the development of atherosclerotic lesions.

As is well known, the coronary arteries have its origin in the aorta and thereafter take course to the surface of the heart. The most important factor contributing to the coronary blood flow is the difference between the pressure in aorta and intraventricular pressure. This pressure difference creates forward and backward pressure waves. In systole, there is a backward flow in intramural arteries due to the compression of the myocardium. Subepicardial and epicardial arteries normally show a forward flow [23-26]. In case of advanced coronary artery disease (CAD) the DBP in the aorta will be the driving pressure for the filling of the coronary arteries which in case of increased aortic stiffness will not be enhanced to the same extent due to alteration of the returning pressure wave.

Different available non-invasive arterial stiffness methods

Arterial stiffness cannot be quantified with an all-coverage measure[27] due to the array of the arterial tree. Consequently, it must be determined indirectly and several non-invasive methods have been described to determine arterial stiffness. A common classification in the literature is to divide arterial stiffness methods into local and regional. A simplified overview of different available non-invasive methods is shown in figure 1.

Regional methods

The pulse pressure (PP), is considered the most simple surrogate measure to assess arterial stiffness and is calculated as the difference between the systolic blood pressure (SBP) and the DBP. PP is known to depend on arterial stiffness [28]. An increased PP indicates lowered buffering function of large arteries in the body. A centrally measured PP has been suggested to be more useful and has a stronger correlation to endothelial function compared to brachial PP [29]. PP measured during 24 hours is known to be a better predictor of CV mortality compared to one single measure[30].

Pulse wave velocity (PWV) is defined as the velocity of a pressure wave along an arterial segment. PWV can be measured in all arterial segments which can be palpated. The most common way is to measure carotid-femoral (aortic) or femoral-dorsalis pedis. The examination is conducted by using pressure tonometers. The distance between the two measuring points is measured. The PWV is calculated as the distance in meters divided by

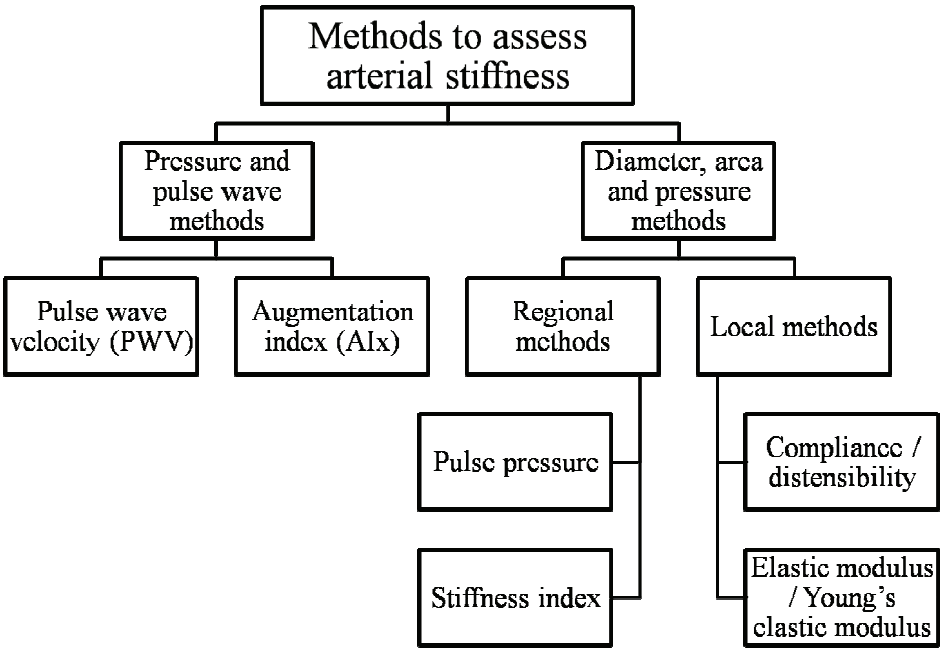

Figure 1. A simplified overview of different available non-invasive arterial stiffness methods.

mean transit time. The golden standard is to perform a carotid-femoral examination[31]. The stiffer the vessel the faster is the PWV. To give exact measure, pressure invasive techniques must be used. To avoid this, transfer functions can be used which means that the appearance of the pressure wave at the two measuring sites can be used to estimate the pressure at a third point. However, the validity of these transfer functions has been questioned since a prerequisite is that the PWV is constant along the arterial segment investigated[31].

Augmentation index (AIx) represents the timing of the peripheral reflecting pressure wave in relationship to the left ventricle (LV) systolic pressure and is calculated as the pressure difference between the peak and the notch on the systolic pressure wave and the PP. The measure can be conducted directly or by transfer functions which gives an estimate of the aortic pressure wave from the radial artery[32]. To understand the concept of AIx, pulse wave reflection is fundamental. Close to the aortic root, the initial pressure increase is fast enforced

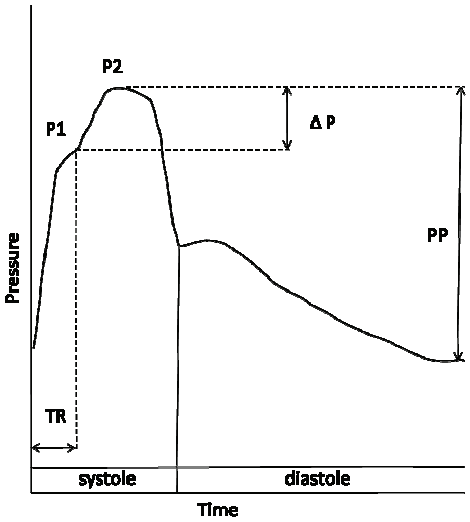

Figure 2. The aortic pressure curve. TR = time to return of reflected pressure wave; P1 = first systolic notch; P2 = peak systolic pressure; Δ P = pressure augmentation; PP = pulse pressure.

by the returning pressure wave from the peripheral sites. The start of the returning pressure wave can be detected in the wave form as an augmentation point. AIx is the mathematical calculation of this augmentation point, i.e. the change in pressure after the first systolic notch to the peak aortic pressure, i.e. the pressure augmentation which is calculated as the percentage of PP. AIx depends on the outflow of the left ventricle, the elasticity in the ascending aorta and the timing of the returning pressure wave, which in turn depends on gender, length, the amplitude of the reflecting wave and the stiffness of the vessel. Further, there is a known significant relationship between heart rate and AIx. For every ten beats increase in heart rate, the AIx decreases with four percent[33]. Therefore, several studies use a corrected AIx adjusted to a heart rate of 75 beats per minute. It has been suggested that AIx must be calculated from a central wave form[34]. If calculated from a transfer function compared to a central wave, the measures do not correlate. Figure 2 illustrates the aortic pressure curve.

Local methods

Compliance is defined as the change in volume or cross-sectional area for a given change in pressure. Compliance is calculated as Δ volume/ Δ pressure. If the pressure is measured directly compliance is known to reflect the stiffness in smaller arteries and if the pressure is indirectly measured compliance reflects the stiffness in large arteries. The vessel suggested to be the principal determinator of systolic arterial compliance is the aorta due to the vessels size and elastic properties[35].The distensibility is the fractional and relative change in volume or cross-sectional area for a given change in pressure. Distensibility is calculated as Δ volume / (Δ pressure x volume). The diameter of the vessel of interest is measured simultaneously as the blood pressure. These both measures give a local estimate of the vascular mechanical properties which can be quantified[36]. It is important to bear in mind the large variation of compliance and distensibility in the same vessel depending on the site measured. In older patients the distensibility is most decreased in the carotid bulb[37].

The elastic modulus describes the theoretical pressure needed to achieve a 100 percent distention of the resting diameter of a vessel and is the inverse of distensibility. Elastic modulus is calculated as (Δ pressure x diameter)/ (Δ diameter). Young's elastic modulus is the elastic modulus per unit area, i.e. it takes into account the wall thickness as well. It is calculated as (Δpressure x diameter)/ (Δ diameter x wall thickness).

Another described local/regional stiffness method is the stiffness index β which takes into account both vessel strain and blood pressure. It is calculated as ln (systolic blood pressure /diastolic blood pressure) x diastolic diameter/(systolic diameter-diastolic diameter), where ln is the natural logarithm. The index was first described by Kawasaki and co-workers and describes the ratio of the natural logarithm of the SBP/DBP to the relative diameter change in the vessel. This index gives a correction for the non-linear impact on lumen diameter accounted for by the blood pressure and is therefore considered to be a more reliable local method reflecting regional properties of an artery[38].

Arterial stiffness as an important predictive risk factor for cardiovascular diseases

Pulse pressure

Centrally assessed PP is known to be associated with cardiovascular outcome [39]. Pulse pressure assessed from brachial artery pressure independently predicts CV events and risk in older subjects [40-42]. Further, PP is known to be of predictive value in certain patient groups [30, 41, 43-47]. Additionally, an index calculated as the stroke volume / pulse pressure has been studied and gives a predictive measure of future CV events [48, 49].

Pulse wave velocity

Aortic stiffness measured by PWV predicts cardiovascular morbidity and mortality[50]. The use of PWV-measurements has been extensively studied in a variety of patient cohorts, there among patients with renal failure[51-53], hypertension[54, 55] and diabetes mellitus [56]. It has also been shown to be predictive of future cardiovascular events including stroke in older adults [50, 57-60]. The PWV is significantly higher in patients with established coronary artery disease compared to healthy subjects[61].

Augmentation index

Augmentation index as measured from carotid site is significantly increased with increasing cardiovascular risk scores and has been shown to correlate to cardiovascular risks[62]. Also, AIx assessed from the radial artery is associated with increased cardiovascular risk[63]. Weber et al has shown radial AIx to predict death, myocardial infarction and re-stenosis in patients who have underwent percutaneous intervention (PCI) [64]. AIx declines very little after the age of 55 years and therefore AIx has been suggested to be a more sensitive marker of arterial stiffness in a younger population and PP is suggested to be a better marker in older patients[65]. The predictive value of AIx has not been studied to the same extent as PWV [64, 66, 67]. Patients with hypercholesterolemia shows elevated AIx[68]. In younger men, AIx is increased with a high alcohol intake, smoking and increased LDL-levels[69, 70]. A combination of elevated AIx and carotid IMT gives an increased Framingham cardiovascular risk score [71]. AIx is inversely

related to endothelial function assessed by flow-mediated vasodilatation in the brachial artery [29]. A significant positive correlation has also been shown between high-sensitive CRP and AIx[72]. Additionally, in patients with renal failure a prognostic value has been shown with presence of increased AIx [73].

Local vascular stiffness measurements based on the radial diameter change have in far less extension compared to regional methods been shown to be able to provide a prognostic value for future CV events [74-78].

The concept of using velocity vector imaging for arterial movement assessment

Velocity vector imaging (VVI) is a new echocardiographic image analysis software developed for evaluation of cardiac dyssyncronies and has been shown to be useful in assessment of cardiac motion during acute myocardial infarction[79]. The technique relies on algorithms answering to multiple M-mode evaluations and speckle tracking technique, and determines properties of the myocardium from a two-dimensional gray scale image. In the echocardiographic image, guiding points are used to outline the contours of the myocardium. The movements of the guiding points create data for the magnitude and directions of the vectors produced which corresponds to the measured motion. This new method has previously not been used for evaluation of artery function in the longitudinal direction in larger clinical studies.

The Longitudinal Arterial Movement

The longitudinal vessel wall movement occurring in the cranio-caudal direction alongside vessels is less well studied and has gained less research interest during the past. Figure 3 shows a schematic illustration of the direction of the longitudinal and radial vessel wall movements. Patel et al has studied the longitudinal movement of the aorta in dogs using an electromechanical device attached externally to the aorta. They found the movement to be very small and it was mainly considered to be a breathing artifact [80, 81]. Consequently, little is known of its clinical relevance and mechanistic background. This is probably due to lack of an adequate measuring method. Person et al has developed a specialized tissue tracking

ultrasound based method with the ability to simultaneously study the longitudinal as well as the radial vessel wall movement [82]. When using this technique in healthy volunteers, Cinthio et al were able to demonstrate a longitudinal movement of the common carotid artery (CCA) of about the same magnitude as the radial[83].

Aspects on vessel wall mechanical properties

Considering arterial biomechanics, wall shear stress and the circumferential stress acting on the arterial wall traditionally account for vessel wall remodeling during pathological circumstances. Vessel wall geometry is under influence of the flowing blood [84, 85]. Further, it is also well-documented that arterial wall thickness is largely dependent on the blood pressure level it is exposed to[86-88]. The fundamental axial stress acting on the vessel in the longitudinal direction is less well studied in comparison and increasing interest is aimed at evaluation of the biaxial forces in experimental settings. However, axial stretch in vivo has been shown to be reduced with increasing wall thickness or hypertension [89, 90]. Elastin has been proposed to play a crucial role in providing an axial pre-stretch in vivo due to the fact that elastin is stretched during development [91]. Hypertension induced increased collagen deposition will result in increased wall thickness and a reduced axial retraction. This becomes evident when calculating the C:E ratio (collagen/elastin) where a larger ratio is clearly associated with lower in vivo axial stretch [90, 92]. It has been shown both theoretically and experimentally, that following vessel wall thickening, the axial stress is decreased to larger extent than the circumferential stress. These findings suggest, that compared to the traditional radial wall strain, the longitudinal wall motion might be an earlier and more sensitive measure of vascular wall remodeling [93].

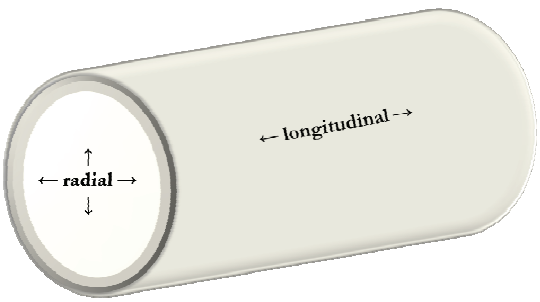

Figure 3. A schematic illustration which shows the radial and longitudinal vessel wall motions.

Aims of Thesis

The general aim of this thesis was to study clinical correlates, mechanisms and potential prognostic values of longitudinal CCA wall motion using ultrasound VVI-technique.

Specific aims:

- To evaluate feasibility and accuracy of using VVI technique in assessment of the CCA longitudinal wall movement and to study potential differences in this movement between healthy volunteers and patients with established CAD (paper I).
- To study the potential relationship between the longitudinal movement of the CCA and cardiac tissue velocity during echocardiography (paper II).
- To explore the value of longitudinal CCA wall motion for future cardiovascular events and its clinical correlates in patients with suspected coronary artery disease (Paper III).
- To validate VVI-assessed longitudinal CCA wall motion in a mouse model of atherosclerosis and explore its relationship with plaque burden in both man and mice (paper IV).

Methods

Patients and study subjects

In paper I, sixteen younger healthy study subjects without symptomatic cardiovascular disease were included in the study. As a control group with established atherosclerotic CAD, 16 patients were recruited with the inclusion criteria of a prior coronary artery bypass grafting (CABG) operation.

In paper II, a number of 400 consecutive patients referred to our department with suspected CAD for myocardial perfusion scintigraphy (MPS) examination from 2006 to 2009 were recruited for study participation. On a separate occasion, all patients underwent echocardiography and ultrasound examination of the CCA. Offline analysis of tissue velocity imaging (TV) on echocardiographic images as assessed by velocity vector imaging was successfully measured in 357 patients who formed the study population.

In paper III, four hundred and forty-one consecutive patients with clinically suspected CAD (referred for investigations due to clinical chest pain) were recruited for study participation at our department during 2006-2008.

In paper IV, fourty-six Apo E knock-out mice on C57BL/6 background (Taconic and Bom, Denmark) were included. The mice were fed high-fat diet (21% pork lard, 0.15% cholesterol; SDS, Witham, England) from the age of 8-weeks for 16 weeks. All animals and had free access to tap water and chow. Ten patients referred to our department for evaluation of cardiovascular status diagnosed with CCA plaques were included in the study together with 10 age and gender matched control subjects without CCA plaques. All included human study subjects underwent prior MPS examination and were free from significant myocardial ischemia.

In all mentioned studies the participating patients and study subjects gave their informed and written consent to study participation. Further, all studies were approved by the Local Ethics Committee in Gothenburg.

Carotid artery ultrasound examinations

Ultrasound examinations of the CCA in humans were performed according to guidelines in all included papers in this thesis [94, 95]. B-mode real-time imaging was performed to evaluate structural and functional properties of the carotid artery. Patients were placed in supine position and the Acuson Sequoia 512 ultrasound system (Siemens Medical Solutions Inc., Mountain View, USA) was used with the standard 8 MHz transducer. CINE looped images of consecutive cardiac cycles of the CCA were stored for later offline analysis. The examinations were performed including investigation of the CCA approximately 2.5 cm distal to the CCA bifurcation, the CCA bulb, the internal common artery (ICA) and the external common carotid artery (ECA). Color Doppler was used to evaluate flow velocities. Presence of stenosis was defined as a 50 % lumen narrowing and diagnosed as an elevated peak systolic velocity of >1.2 m/s in the ICA, ECA or CCA [94]. The IMT was evaluated in the CCA about 1 cm proximal to the carotid bifurcation and in the bifurcation of the CCA far wall [96]. IMT was assessed in all papers in this thesis. The presence of plaque burden was measured in long axis view as well as in short axis view in the CCA bifurcation. Presence of plaque was defined as a focal lesion >1.2 mm[78]. The vessel lumen diameter was assessed from the leading-to-leading edge from the near wall to the far wall of the CCA. The maximal systolic lumen diameter was determined visually and from the R-wave of the ECG-recording and the minimal lumen diameter was used for the diastolic diameter. In paper I, III and IV CCA strain was calculated using the formula:

$$\frac{\text{(systolic diameter – diastolic diameter)}}{\text{diastolic diameter}}$$

Carotid stiffness index β was used in paper I and III and calculated as:

$$\frac{\ln(\text{systolic blood pressure}/\text{diastolic blood pressure}) \times \text{diastolic diameter}}{(\text{systolic diameter} - \text{diastolic diameter})}$$

where ln is the natural logarithm[97].

Velocity vector imaging of the CCA

Measurements were made offline from standard B-mode ultrasound images at a workstation. Velocity vector imaging software (Research Arena 2, TomTec imaging systems GmbH, Unterschleissheim, Germany) was used with the ability to assess vessel wall velocity, strain, strain rate and displacement. In paper I, both the near- and far walls of the right and left CCA were examined. In papers II, III and IV the right CCA far wall was evaluated. The radial movement in terms of velocity and displacement was studied in paper I. In all papers, delineation of the lumen-vessel wall border was conducted from leading-to-leading edge. Measurements were outlined approximately one centimeter (cm) distal to the carotid bulb. Five guiding points were used evenly arranged (0.25 cm apart) within a 1 cm segment i.e. a total of ten measuring points were used. Using available software settings, the outlined segments corresponding to the far wall of the CCA were selected. Further, settings were manually positioned to cover peak systolic and peak diastolic displacement values during one cardiac cycle. For quantification of the longitudinal vessel wall movement, we calculated the total longitudinal displacement (tLoD) during a cardiac cycle as the sum of absolute values of maximal systolic plus the maximal diastolic displacements. The measuring concept for calculation of tLoD was used in all papers in this thesis. Figure 4 illustrates our measuring concept.

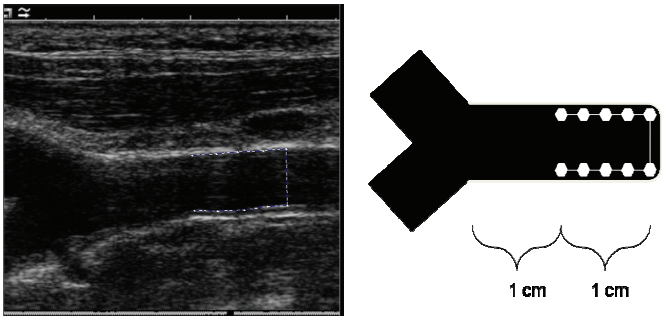

Figure 4. The left image shows delineation of the lumen vessel borders using the VVI tool in a standard CCA image. The schematic picture to the right illustrates the guiding points in the near and far wall of the CCA proximal to the carotid bulb.

Echocardiography and tissue velocity measurements

In paper II, echocardiographic examinations were conducted with the patients in the standard left lateral position using the Acuson Sequoia Ultrasound System (Siemens Medical Solutions Inc., Mountain View, USA). Images were acquired with the 3.5 MHz transducer. Left ventricular indexes were measured in Image Arena (Tomtec and Research Arena 2, TomTec imaging systems GmbH, Unterschleissheim, Germany). The Simpson's rule was used in consistency with recommended standardized protocols [98]. Tissue velocity (TV) measurements were delineated in 4-chamber and 2-chamber views at the LV endocardial border as displayed in figure 5. For acquirement of TV-data from the septal and lateral wall at the level of the mitral annulus, measurements were performed in the 4-chamber view. From the 2-chamber view, corresponding data from the posterior and anterior walls was acquired in analogy. The generated tissue velocity curves were further processed to contain peak velocities in systole and diastole. To derive mean TV, the average velocity value of the septal, lateral, posterior and anterior measuring points was calculated. In paper II, we adjusted TV-values to 66 bpm to be able to compare tLoD and cardiac tissue velocity data according to the formula:

(TV at rest/HR at rest x 66 bpm).

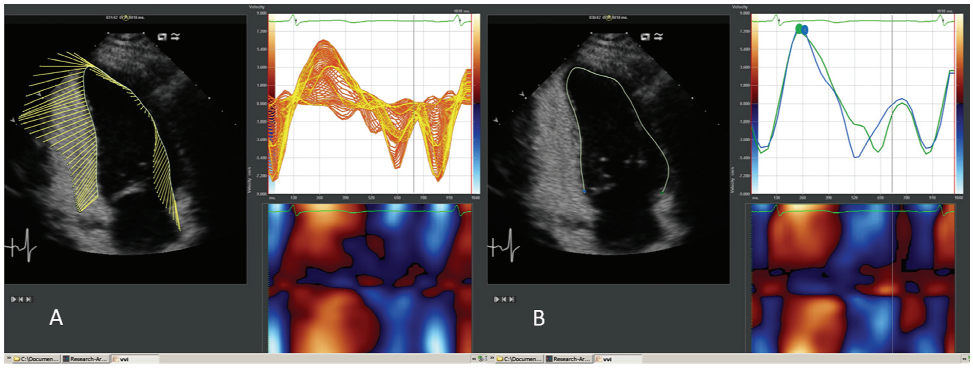

Figure 5. (A) Shows a processed measurement from a 2-chamber view. The delineation of the same is displayed in (B). TV at the mitral annulus in 2-chamber view was used to derive the posterior and anterior velocities (blue and green dots) as shown in the diagram to the right.

Myocardial perfusion scintigraphy

Myocardial perfusion scintigraphy (MPS) was performed for diagnosis of CAD in papers II and III. The examinations were performed in consistency with a clinical standard protocol used at our department. Gated-SPECT studies were conducted applying a two-day non-gated stress/gated rest 99m Tc-sestamibi protocol. Patients were subjected to either maximal exercise, symptom limited ergometry test or pharmacological stress test with adenosine. Later image acquisition was performed with two different dual-head SPECT cameras (Infinia or Millenium VG, General Electric, USA) using a low energy, high-resolution collimator. In case of patient motion during image acquisition, an automatic motion-correction program was applied for adjustments. For image interpretation, a clinical assessment with consideration to ischemia score and ischemia area was performed off-line by one experienced physician with support from the automatically generated variables from the software Emory Cardiac Toolbox. Ischemia area and severity were scored as small, medium and large with regards to extent of LV perfusion defect (< 10%, 10-19% and > 19%, score 1-3), and low, medium and high (score 1-3), respectively. Left ventricular volumes and ejection fraction (EF) were examined using Cedars-Sinai quantitative gated SPECT (QGS) program.

Laboratory analysis

In paper III, triglycerides and cholesterol in serum were measured using reagent systems from Roche (Triglycerides/GB kit No; 12146029216, Cholesterol kit No; 2016630, Roche Diagnostics GMBH, Mannheim Germany). Apolipoprotein A1 and B concentrations were measured with turbidimetric technique, using polyclonal rabbit anti-human antibodies (Q 0496 and Q 0497, Daco Cytomation, Glostrup, Denmark). C-reactive protein was measured with a high sensitivity reagent kit (CP 3847, from Randox Laboratories Ltd, Crumlin, United Kingdom.

In paper IV, blood samples in mice were collected from the left ventricle during termination. Total cholesterol levels were analyzed using commercially reagents (Cat. No. 12016630, Roche Diagnostics, Mannheim, Germany) and Cobra Mira Plus (Roche Diagnostics) with enzymatic-spectrophotometric methods. In the human study group, triglycerides, cholesterol, apolipoprotein A1 and B were measured using the same methods as in paper III.

Definition of outcome measures

In paper III, patients were followed-up one year after examinations by telephone interviews and medical records. Study endpoint was set to major adverse cardiovascular events (MACE) defined as the incidence of death from any cause, stroke, myocardial infarction and coronary arterial revascularizations (either CABG or PCI). A patient was considered to have a stroke in case of presenting focal or global neurological deficits lasting for more than 24 hours and verified by either a clinical examination by a neurologist or a CT brain scan. The definition of myocardial infarction was clinically driven and confirmed by elevation of troponin above upper normal limit in at least two consecutive samples.

CCA morphological examinations and velocity vector imaging in mice

In paper IV, ultrasound biomicroscopy (UBM) with a transducer frequency of 40 MHz was used for vascular imaging (Vevo 2100, Visualsonics, Toronto, Canada). The brachiocephalic artery and right CCA were visualized in a long-axis and short-axis views and a CINE loop of 100 frames was stored for later off-line analysis. All vascular morphological measurements were performed by one experienced operator. Lesions of the brachiocephalic artery were assessed in analogy with previously published protocol with good reproducibility of IMT and plaque area measurements [99].

For analysis of the longitudinal wall motion of the CCA in mice, Vevostrain-analysis was performed using the software Vevo 2100 (version 1.1.1, Visualsonics, Toronto, Canada) offline at a work station. The conducted measurements were delineated approximately one millimeter proximal to the CCA bifurcation in the near wall using four distributed guiding points attached in the vessel wall facing the border to vessel lumen (figure 6). For measurement of the vessel wall motion in the longitudinal direction one heart beat was selected and then processed by the software. For analysis of longitudinal CCA vessel wall displacement, data from each mouse was saved as the specific vessel wall velocity in predefined time points during the selected cardiac cycle. For calculations of longitudinal displacement, the velocity time integral based on velocity data was assessed in the longitudinal direction. The velocity in each measuring point was averaged in each predefined time point and then integrated with the specific time point.

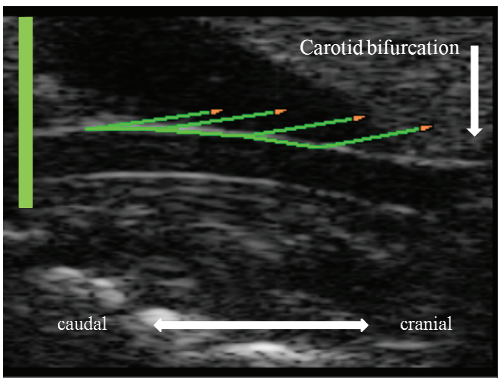

Figure 6. Example of a Vevostrain-mesurement of the CCA near wall. The green arrows (the vectors) indicate the magnitude and direction of the longitudinal wall motion. The white arrows indicate anatomical landmarks. The green scale bar = 2mm.

Statistics

All statistical analyses in this thesis were made using SPSS statistical software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Generally, a two-tailed p-value of less than 0.05 was considered significant. To determine differences between groups, a Student's paired T-test was used in all included papers.

For evaluation of intra- and inter-observer variability in paper I, the coefficients of variance were calculated using the formula:

$$\frac{SD\,(x\text{-}y)}{average\,(x,y)} \times 100\,\%$$

where x and y represent values of the first and the second measurement, respectively. This was also performed in paper IV for calculation of intra-observer variability.

Pearson's bivariate correlation coefficients between TV variables and myocardial perfusion scintigraphy data were determined in paper II.

In paper III, ANOVA were used to determine differences between groups. The Bonferroni correction was used for multiple comparisons. Kaplain-Meier survival analysis was used to explore prospective prognostic value of tLoD and incremental value of tLoD on top of IMT. For tests of linear trend significance of tLoD tertiles, the Chi-square p-value was used.

Logistic regression analysis was used to analyze the impact of various parameters on MACE in paper III and tLoD in paper II.

Due to the relatively limited human sample size in paper IV, a Mann-Whitney u-test was performed for comparisons between the human study groups.

Summary of results

Longitudinal CCA vessel wall motion in man and mice

In paper I, the 16 healthy subjects were aged 25.4 ± 5.1 years (range 20 to 38 years) and had normal blood pressure. All investigated subjects showed an evident longitudinal vessel wall motion during cardiac cycles (figure 7). No difference in tLoD between the CCA near and far wall could be seen. Further, there was no significant difference between tLoD of the right CCA (0.543 ± 0.394 mm, range 0.090 to 1.516 mm) and the left CCA (0.401 ± 0.274 mm, range 0.036 ± 0.824 mm). The magnitude of the radial vessel wall motion was of about the same as the longitudinal. Patients in the CAD-group showed significantly lower tLoD of both the near and far wall of the CCA.

In paper II, the cardiac and carotid ultrasound examinations and measurements of interest were successfully performed in 357 patients (89. 3 %). The mean age of the study group was 62.3 ± 9.1 years (range 32 to 84 years). For the statistical analysis, patients were divided in two groups of equal size by using the median tLoD value (0.090 mm) as cut-off. The mean tLoD value of the study population was found to be 0.156 ± 0.229 mm (range 0.003 to 2.129).

In paper III, the study population consisted of 441 patients aged 62.0 ± 9.0 years (range 35 to 84 years). For the data analysis, tLoD was divided into tertiles (lower tertile < 0.055 mm (n=148), middle tertile 0.056 - 0.144 mm (n=147) and higher tertile >0.145 mm (n=147)).The range of tLoD was found to be 0.002 – 2.129 mm. Radial carotid strain derived from conventional diameter measurements was lower in the lowest tertile compared to the highest. The relationship between the variables generated from the VVI-software was highly associated, low tLoD showed the lowest radial displacement, longitudinal velocity, longitudinal strain and strain rate ($p < 0.001$).

In paper IV, the CCAs were successfully visualized and tLoD could be determined in all investigated animals and humans. The median age of the human study group was 61.0 years, range 39.0 - 77.0 years (10 males aged 60.0 years, range 39.0 - 77.0 years and 10 females aged 64.5 years, range 46.0 – 76.0 years). No significant differences between the plaque and

plaque-free patients could be seen regarding ongoing medical treatments including statins, betablockers, ACE-inhibitors and aspirin.

Relationship between the longitudinal CCA wall motion and morphology

In paper I, twelve of the 16 included patients showed CCA plaques in the bifurcation. The mean plaque area in study group was determined in long-axis view and was found to be 0.151 ± 0.209 cm. Further, patients with established CAD showed significantly lower tLoD compared to healthy volunteers (0.112 ± 0.074 mm vs. 0.543 ± 0.394 mm, $p<0.0001$).

In paper II, patients in the group exhibiting lower tLoD showed greater IMT (0.066 ± 0.026 vs. 0.061 ± 0.016, $p= 0.04$).

In paper III, patients with tLoD in the lowest tertile showed the greatest IMT in a significant linear trend towards higher tLoD with the thinnest IMT ($p = 0.04$).

In paper IV, mice with low tLoD compared to higher tLoD showed greater plaque burden in the brachiocephalic artery (0.1096 ± 0.0340 mm^2vs. 0.0848 ± 0.0239 mm^2, $p = 0.007$) and greater IMT (0.2223 mm ± 0.0468 vs. 0.1948 ± 0.0324 mm, $p = 0.03$). None of the patients included in the study who were free from significant myocardial ischemia were found to have a significant stenosis in CCA, ICA or ECA. Patients with CCA plaques were found to have lower tLoD compared to controls (median value 0.062, range 0.020-0.098 mm vs. 0.100, range 0.052-0.302 mm $p=0.003$).

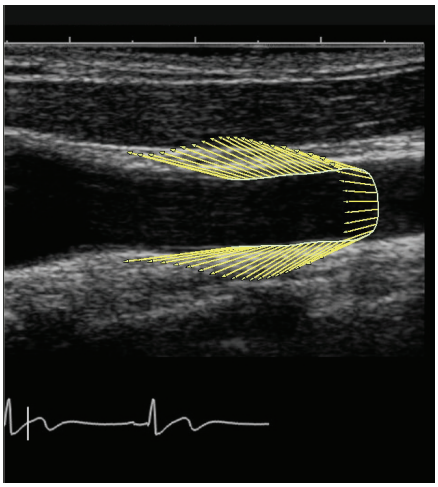

Figure 7. The image shows a processed CCA measurement. The yellow arrows indicate the direction and magnitude of the CCA wall motion. Offline analysis was performed for calculation of the total longitudinal displacement (tLoD).

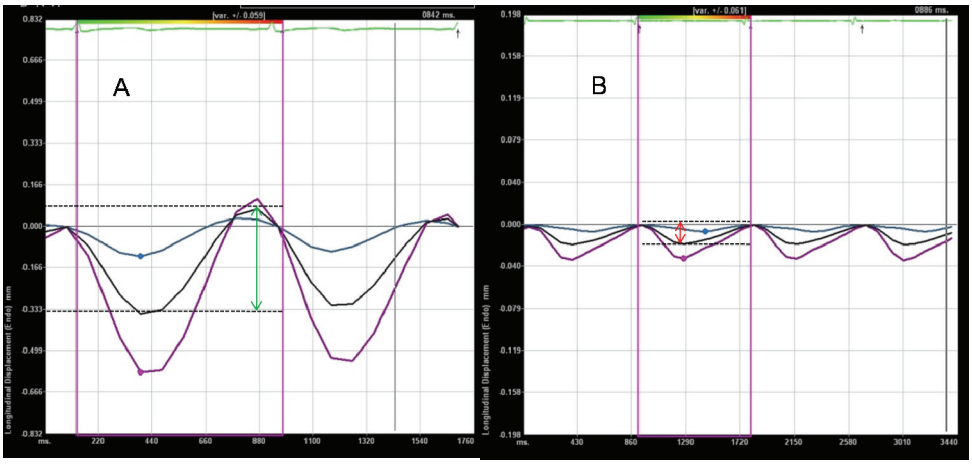

Figure 8. Illustrates detailed examples from the output of the VVI-software. Displayed are the longitudinal displacement curves during cardiac cycles. The blue and purple curves correspond to the CCA far wall, the black curve is the average of the blue and purple curves. The purple vertical lines denote the measured cardiac cycle. The ECG-recording can be seen above the displacement curves. (A) Displays a normal longitudinal wall motion of the CCA far wall in a healthy volunteer. The green arrow indicates tLoD which in this patient was found to be 0.438 mm. (B) Shows a patient with low tLoD. The red arrow indicates the tLoD to be 0.019 mm.

Relationship between the longitudinal CCA wall motion and cardiac performance

In paper II, we were able to demonstrate a correlation between tLoD and mean systolic TV at rest ($r = 0.105$, $p < 0.05$). Patients in the lower tLoD-group generally showed decreased TV in investigated cardiac segments. The lowest tLoD median showed significantly greater clinically determined ischemia score and ischemia area in comparison to the higher tLoD median (0.680 ± 0.989 vs. 0.414 ± 0.775, $p = 0.005$ and 0.622 ± 0.846 vs. 0.394 ± 0.704, $p = 0.006$, respectively). Further, mean TV in systole and diastole was reflected in clinically determined infarction score and area. In a logistic regression model predicting tLoD above and below the median value, clinical ischemia score and TV diastole anterior remained significant independent predictors after adjustment for age, gender and IMT.

In paper III, patients with lower tLoD showed a greater clinically scored MPS ischemia area and ischemia severity compared to patients with higher tLoD. The automatically generated variable, ischemia severity, was also higher in the lower tLoD-tertile. No relationship between tLoD and conventional cardiac measures such as EF or volume indexes could be seen.

Longitudinal CCA wall motion and traditional cardiovascular risk factors

Generally, no relationship between tLoD and blood pressures could be seen.

In paper I, there was no significant difference in tLoD between men and women (0.532 ± 0.460 mm vs. 0.557 ± 0.323 mm).

In paper III, studied biochemical analyses did not seem to relate to tLoD. Patients with lower tLoD did not show any differences in age or in gender. Patients with tLoD in the lowest tertile compared to patients in the middle tLoD tertile showed a greater body mass index (BMI) (26.9 ± 3.9 vs. 25.7 ± 3.6, $p=0.03$). Further, low tLoD was not associated with diabetes mellitus or patients with a smoking habit.

In paper IV, mice with lower tLoD showed higher cholesterol-levels (46.3 ± 11.5 mmol/L vs. 39.9 ± 7.8 mmol/L, p=0.04).

Reproducibility measurements of the longitudinal wall motion

In paper I, the intra-observer and inter-observer variability of tLoD was evaluated and was found to be 10.5 % and 9.1 %, respectively.

In paper IV, the intra-observer variability for tLoD measurements in mice was found to be 28.2 %.

Relationship between longitudinal CCA wall motion and cardiovascular outcome

In paper III, a number of 61 MACES occurred during a median follow-up time of 372 days. Higher tLoD predicted one year event-free survival with an OR of 1.9 when comparing the upper and lower tertile in a Kaplan-Meier survival analysis (Chi-square $p < 0.01$). The lowest tLoD tertile showed the highest frequency of arterial revascularizations (p=0.005), but also total number of MACEs (p=0.001). In a logistic regression model adjusting for age, gender, CCA IMT, hypertension and percentage reversibility mass of myocardium, low tLoD remained a significant independent predictor of MACE alongside gender and percentage reversibility mass of myocardium ($p = 0.01$). In an additional survival analysis concerning patients with IMT above and below the median value (0.06 cm), lower tLoD provided a significantly greater event-rate compared to higher tLoD and IMT in the lower median. Consequently, tLoD provides an incremental value above IMT in event-free survival prediction (figure 9).

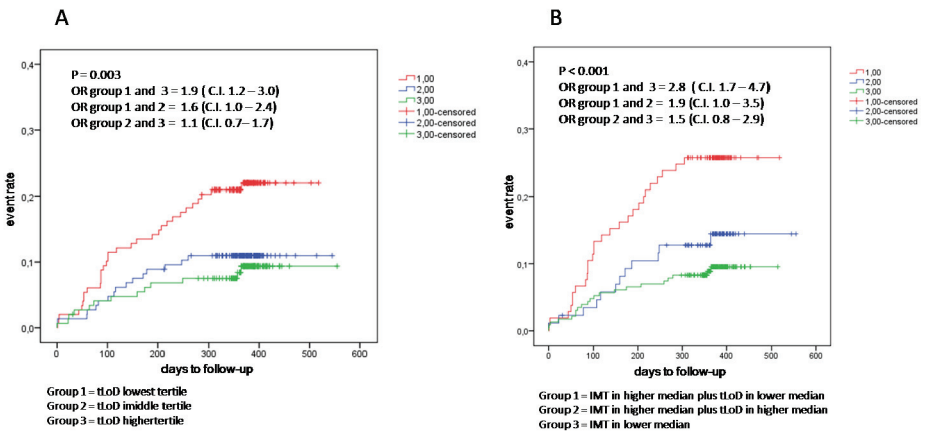

Figure 9. In a Kaplain-Meier survival analysis, tLoD provides a prognostic value for one year CV events (A). In a Kaplain-Meier survival analysis, tLoD provides an incremental value above IMT in prediction of one year CV events (B).

General discussion

This thesis shows the longitudinal wall motion of the CCA to be measurable with VVI technique in both man and mice as total longitudinal displacement (tLoD). Our data shows the longitudinal movement of the CCA near- and far walls to be of similar magnitude in healthy volunteers. Additionally, no differences in movements could be seen between the right and left CCA. Patients with CAD displayed significantly lower total longitudinal displacement compared to healthy volunteers. The intra-observer and inter-observer reproducibility of the right CCA far wall tLoD was found to be acceptable. Total longitudinal displacement of the CCA seems to be independently related to cardiac wall motion properties investigated with TV echocardiography in patients with suspected CAD. Patients with low tLoD in general displayed decreased TV of the left ventricle. We demonstrated patients presenting lower longitudinal VVI-assessed wall displacement of the CCAs to have a greater clinically scored MPS ischemia area and score. Patients presenting greater IMT in the CCA exhibited the lowest tLoD. Divided in tertiles, higher tLoD was associated with a better cardiovascular prognosis at one year follow-up. VVI-derived longitudinal displacement seems to reflect both vessel wall morphology and cardiac performance in a population with suspected CAD. Finally, tLoD is also shown to be measureable in mice and low tLoD was associated with greater brachiocephalic plaque burden.

Methodological considerations

Measuring longitudinal CCA displacement with VVI-technique

The arterial system is of complex nature and subject to different physiological conditions during cardiac cycles. Therefore, assessment of arterial stiffness is challenging and has faced practical problems. Study documentation on the longitudinal wall motion in the literature is limited but the motion is known to be very small as studied in experimental models [80, 81]. The confined research focus of the longitudinal wall motion is probably due to lack of an adequate tool to assess this small movement. Using VVI-technique we have provided a potential measuring concept by adapting a method which is readily available. In our experience, the tracking ability of the VVI-technique is good determined by visual inspection which is

confirmed by previous experimental validation [100]. By measuring VVI-derived tLoD, we suggest the longitudinal CCA movement of both the near and far walls to be of about equal size in healthy volunteers. Further, also the tLoD of the right and left CCAs are of about the same magnitude. CCA movements in healthy volunteers yield somewhat large individual variations which could be due to both physiological and methodological reasons. We therefore suggest that the measurements of this small movement must be carefully performed and conducted in a standardized manner. Using the present VVI-software version, we propose to calculate the total longitudinal displacement (tLoD) of the CCA during a heart cycle, which with given conditions seems to be the most robust read-out of CCA wall motion. Additionally, to provide a standardized measuring concept, we suggest the measurements to be performed on the far wall of the right CCA, which usually presents with best 2D image quality.

For assessment of the longitudinal wall motion using modern ultrasound techniques, Persson et al have developed a method able to simultaneously measure the magnitude of the longitudinal and radial wall motion at different depths of the arterial wall[82]. Results from their initial in vivo trial using this method showed a greater longitudinal movement of the intima-media complex in comparison to the tunica adventitia. In the mentioned study, the longitudinal movements of the anterior and posterior CCA walls were reported to be of about the same magnitude. Further studies by the same research group shows the CCA vessel wall displacement to be multiphasic, at least in healthy volunteers[83]. Even though we clearly demonstrate the VVI-technique to generate robust measurements of peak and reversed peak values, the current software version does not seem to be suitable for evaluation of the multiphasic aspect. However, advances in software development may shed light on the clinical importance of this reported phenomenon.

Furthermore, VVI-technique is able to accurately quantify tissue movements of both cardiac wall motion and tLoD of the CCA as shown in this thesis. Examination of cardiac tissue motion by ultrasound is conventionally assessed by the naked eye and semi-quantification by various scales determining wall motion score abnormalities, which have limitations [101, 102]. The development of tissue Doppler emerged which provides automatic quantification of cardiac movements [103, 104]. However, the advantage with VVI- technique is that it provides angle-independent assessment and in this research setting both allows examination of cardiac wall motion and CCA vessel wall motion.

Measuring longitudinal CCA wall motion in mice

Arterial stiffness in terms of PWV has previously been measured mice [105-109]. However, regarding local arterial stiffness measurements, to our knowledge, only the radial vessel wall motion has been measurable by means of arterial compliance. The advances in use of high resolution ultrasound allow imaging of vessel structure with high precision and with great detailing in mice. Providing a whole new dimension of vessel wall function evaluation in mice, Vevostrain allows studies of the vessel wall motion also in the longitudinal direction. Paper IV represents an initial attempt to assess the longitudinal CCA wall motion in mice with various degrees of atherosclerosis with VVI-technique. Currently the software used in this study requires manual calculation of the longitudinal displacement from the velocity time integral. Preferentially, for future applications it would be desirable to be able to quantify this aspect automatically.

Comparing reproducibility of repeated measurements for intra-observer, a coefficient of variance of 12.2 % for the CCA near wall has been reported in man. The presented study in mice shows the coefficient of variance to be greater (28.2 %). However, this might be due to several factors. First, the magnitude of the longitudinal CCA vessel wall motion is smaller in mice and consequently a more delicate measuring challenge. Second, in this study, the anatomical landmarks of the CCAs were sometimes hard to identify since the ultrasound examinations were performed with respect to optimal morphological measurements. This aspect can therefore be improved in future studies. Last, the time period selector for determination of the heart cycle duration, when not enabling an ECG-recording in mice, opens a slight possibility for subjective operator biases in this very sensitive setting.

Radial wall motion

In the context of using VVI-technique for evaluation of the radial wall movements; it provides the radial velocity and displacement. For assessment of radial wall movements by means of diameter changes, these measurements are more easily performed by established methods. As indicated by our data, the radial CCA displacement appear to be of about equal size compared to the longitudinal movement in the CCA near wall and of about twice the magnitude in the far wall. These findings confirm data from previous reported observations[82, 83, 110]. However, the nature and importance of this phenomenon still remains to be elucidated. Several local arterial stiffness methods relay on the radial diameter changes and often take blood pressure into account. Assessment of the carotid stiffness index is a well known local measure of arterial stiffness [111]. As can be confirmed in our study, patients with CAD show increased stiffness index[112].

Possible determinants of longitudinal CCA wall motion

Traditional cardiovascular risk factors

Traditionally, male gender is associated with increased arterial stiffness and CAD. However, in our studies no relationship between tLoD and gender distribution could be seen. Further, no association between tLoD and diabetes mellitus diagnosis could be established. Furthermore, hypertension is well known to be one of the most important determinants of arterial stiffness. However, tLoD does not appear to have a direct association to hypertension diagnosis or actual blood pressure levels. These findings suggest that longitudinal vessel wall movement is not directly related to traditional CV risk factors unlike conventional arterial stiffness measurement. This lack of association to known risk factors may indicate that tLoD is reflecting other aspects of arterial wall function or alternatively, a result of the specific characteristics of our patient cohorts or various ongoing treatments.

Local measurements, vessel wall structure and morphology

Interestingly, only few studies have shown prognostic values for future CV events with local stiffness measurements in the radial direction [74-78]. In paper III, local radial strain was not

associated with CV outcome. It has been shown that following vascular wall thickening in response to various CV risk factors, the axial stress (i.e. in the longitudinal direction) is decreased to larger extent than the circumferential stress which may suggest that the longitudinal wall motion might be an earlier and more sensitive measure of vascular wall remodeling in comparison to the traditional radial wall strain [93]. Indeed, the correlation between tLoD-tertiles and IMT in paper III may partially support this presumption. To avoid potential local mechanical influence from the carotid bifurcation, tLoD was measured 1 cm proximal to the bulb, with the intention to reflect mechanical properties of a systemic atherosclerotic disease. In analogy with this finding we suggest tLoD is preferentially measured approximately one cm proximal to the carotid bulb to avoid local influence of plaque occurrence.

Moreover, in patients with suspected CAD no significant relationship to plaque area could be seen in paper III. This suggests that tLoD really is reflecting local vascular properties. Plaque occurrence in the carotid bifurcation is dependent on systemic atherosclerosis as risk factor but also the local hemodynamic environment. In man, by studying subjects with normal cardiac function, tLoD is more related to plaque burden in the CCA as shown in paper IV. This finding is in analogy with our results in mice. It is also known that in Apo E KO mice on dietary interventions according to the current protocol do not develop cardiac dysfunction at this age [113]. In humans, patients with significant bifurcation atherosclerotic lesions demonstrated reduced tLoD, which is also associated with increased IMT at the site of tLoD measurement. At given magnitude of hemodynamic stress, increased wall thickness locally will result in reduced longitudinal tensile stress leading to lowered tLoD. However, how the distal bifurcation lesion may impact on tLoD from a biomechanical point of view is still a subject for future studies. In mice, despite extreme hypercholesterolemia, atherosclerotic lesions only occur at flow-turbulent vascular regions such as the brachiocephalic artery [114], while laminar flow regions are relatively protected against lesion formation. Further, lesion formation in mice always starts at the aortic root and extend toward the peripheral vasculature, and only in very aged high-fat fed mice, significant lesions develop in the carotid bifurcations. In the mouse model, it is likely that the brachiocephalic lesions proximal to the tLoD measurement site may theoretically damp the potential cardiac mechanical force transmission resulting in the longitudinal carotid wall motion. Despite theoretically distinct functional and morphological correlates to tLoD in man and mice, atherosclerotic lesion

formation still seems to be one of the common determinants of tLoD. Whether reduction in tLoD as a functional physiological consequence of atherosclerosis may impact on further deterioration of the cardiovascular system will be a highly interesting subject for future research. Paper IV consequently shows CCA morphology, both in terms of increased IMT and plaque area, to be associated with low tLoD. Thus, VVI-assessed tLoD can probably be used as a translational marker reflecting CCA morphology and biomechanical consequences of established local atherosclerotic lesions.

Relationship between cardiac and CCA wall motions

This thesis shows tLoD to be under influence of TV of the left ventricle. Although not examined in this work, a possible explanation for this may be the mechanic relationship explained by the ventriculo-arterial coupling (VAC) phenomena. The intricate mechanical interplay between the heart as a pump and the large vessels as reservoirs might play a key role for the studied functional properties. The performance of the entire cardiovascular system is decided by preload, afterload and contractility [115]. Of these reported parameters contractility is the most difficult to assess in clinical settings. However, so called single-beat methods now allows non-invasive measurements of left ventricle elastance and thereby assessment of VAC [116]. Patients with decreased cardiac performance have been shown to display an increased VAC-ratio [117]. Subject to further investigations; tLoD may be a sensitive measure in analogy with cardiac performance reflecting VAC.

Further, the tLoD magnitude is closely related to the extent of cardiac ischemia, both clinically and automatically as determined by MPS.

Potential clinical values of the CCA longitudinal wall motion

Great research efforts are made to define risk markers of early atherosclerotic disease with the ambition to predict future CV events. As demonstrated in this thesis, the CCA longitudinal wall motion could be a potential novel composite risk marker with additional informative values on top of CCA structure.

In advanced cardiovascular disease, it is conceivable that the radial as well as the longitudinal wall motion are defective. However, based on the theoretical analysis by Humphrey et al [93], the longitudinal wall motion may be more sensitive to detect early vessel wall changes as well as to investigate vascular pathology and potential treatment effects. The more pronounced differences between the longitudinal and radial wall motion seen in patients with established CAD and healthy subjects in paper I may support this hypothesis.

Considering CV risk stratification, it is important to view both cardiac and vessel status. Our studies suggest that VVI-derived longitudinal displacement of the CCA is a simple, highly feasible, non-invasive method with the advantage of being a potential integrative method reflecting both vessel and cardiac functions.

Effects of intervention on arterial stiffness

Arterial stiffness as assessed by PWV can be modified both by non-pharmacological and pharmacological approaches. Non-pharmacological approaches known to alter PWV include physical activity and dietary interventions such as weight reduction, low salt intake, moderate alcohol intake[118], fish-oil consumption and dark chocolate intake[119]. Considering pharmacological interventions, effects on PWV have been seen with different drug classes, but most frequently studied with antihypertensive drugs. Several studies have been performed in subjects with essential hypertension which shows a beneficial effect on lowering PWV and blood pressure with different antihypertensive agents. These agents include beta blockers and the typical data suggests that most beta blockers have a positive effect on arterial stiffness. For example treatment with bisoprolol has shown a reduction in carotid-femoral PWV[120]. Beneficial effects have also been seen with other beta blockers[121-124]. Further, calcium-channel blockers have also shown a generally favorable effect in lowering PWV[125-127]. However, the mentioned studies were most often performed with low doses or in comparison to another agent without control of blood pressure. Hence, there is still a matter of debate in the literature whether or not the reduction in PWV is independent of the reduction in blood pressure. Longitudinal CCA wall movement has been shown to increase in a porcine model after administration of adrenaline which, to my knowledge, represents the first study on intervention of the longitudinal CCA vessel wall movement[128]. Future studies may be directed to further elucidate possible intervention strategies regarding the longitudinal movement.

Possible future improvements

The VVI software adapted in this work generates peak systolic and diastolic values of the longitudinal CCA wall motion during a cardiac cycle. The current software version is developed for cardiac wall motion evaluation, but can most likely be further defined with specific focus on evaluation of vessel wall motion. The longitudinal CCA movement has previously been shown to be multiphasic throughout cardiac cycles [83]. At present with available VVI-software, we suggest measurement of peak systolic and diastolic values to generate the most robust read-out.

In paper III, we defined MACE as a composite measure of death, stroke, acute myocardial infarction and coronary arterial revascularization (including PCI and CABG). Considering, the short follow-up time and relatively low number of events, the difference in events in the various tLoD groups was mainly driven by arterial revascularization. However, in this setting with clinical patients undergoing MPS to diagnose presence of ischemia for potential intervention, the ability of tLoD to predict this outcome is considered most relevant. Indeed, it has been shown that MPS-verified ischemia is a great predictor of future hard MACE [129] and interventions leading to reduction in ischemia is known to save lives [130]. Future long-term follow-up will have to address its potential ability to predict spontaneous events in terms of cardiac death and non-fatal myocardial infarction. Despite this, tLoD appears to be an independent predictor of one-year event-free survival. Furthermore, whether tLoD will be a prognostic factor in a general population other than patients with suspected CAD needs to be further investigated.

Conclusions

This thesis demonstrates CCA longitudinal wall motion to be measurable with VVI-technique with acceptable accuracy. Our results suggest VVI-derived longitudinal displacement of CCA to be an integrative composite variable reflecting both local vessel wall structure and cardiac performance. Additionally, longitudinal CCA displacement has a predictive value for short term CV events in patients with suspected CAD and may serve as a possible useful surrogate marker of CAD. Furthermore, tLoD seems to provide additional prognostic value on top of morphological characteristics of the CCA. Finally, longitudinal CCA vessel wall motion can be measured with VVI-technique in mice, and low total longitudinal CCA wall motion is associated with increased plaque burden in a mouse model of atherosclerosis.

Summary in Swedish

Sammanfattning på svenska

Min avhandling visar att en ny teknik innefattande hastighetsvektorer (VVI) tillämpad på ultraljudsbilder kan användas för att studera den longitudinella halskärlsrörelsen hos både människa och mus. Total longitudinell halskärlsrörlighet reflekterar både kärlväggsstruktur och hjärtfunktion. VVI-bedömd longitudinell halskärlsrörlighet har ett prediktivt värde för kardiovaskulära händelser hos patienter med misstänkt kranskärlssjukdom.

Samtidigt som den radiella rörelse som sker när blodkärl i kroppen vidgar sig (diameter förändring) sker en longitudinell rörelse i kärlets längsriktning. Denna longitudinella rörelse är i mindre utsträckning studerad bl.a. på grund av brist på adekvat mätteknik. Det är nu möjligt att med VVI simultant bedöma en vävnads både radiella och longitudinella rörelse. Det övergripande syftet med detta avhandlingsarbete är att undersöka potentiell användbarhet av VVI-teknik för att studera longitudinell halskärlsrörlighet. Dessutom syftar arbetet till att studera eventuella samband med kliniska förhållanden, möjliga bakomliggande mekanismer samt prediktivt värde i en serie studier av människa och mus.

VVI-teknik användes på vanliga kliniska ultraljudsbilder hos friska försökspersoner, patienter med etablerad kranskärlssjukdom och hos patienter med misstänkt kranskärlssjukdom. Alla bilder analyserades för specifik bedömning av den longitudinella rörelsen över halskärlens bakre vägg. Dessutom tillämpades VVI-tekniken för analys av longitudinell halskärlsrörlighet i en musmodell av åderförkalkning.

Patienter med låg total longitudinell halskärlsrörlighet (tLoD) har en större intima-media tjocklek jämfört med patienter med hög tLoD. Vidare visar patienter med låg tLoD en mer uttalad kliniskt bedömd ischemisvårighetsgrad och en mer utberedd ischemiyta vid myokardscintigrafi. Dessutom har patienter med låg tLoD minskad hjärtfunktion relaterad till lägre vävnadshastighet vilket också undersökts med VVI-teknik. Hos patienter med misstänkt kranskärlssjukdom predikterade hög tLoD ett års händelsefri överlevnad avseende kardiovaskulära händelser. I ådersförkalkningsmusmodellen är ökad plackbörda associerat med låg tLoD.

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