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Feasibility of X-ray grating-based phase-contrast imaging for the detection of atherosclerotic plaque features and validation with histopathology.

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Contents

1 List of publications

Hetterich H[*], Fill S[*], Herzen J, Willner M, Weitkamp T, Rack A, Schüller U, Sadeghi M, Pfeiffer F, Bamberg F, Saam T. Grating-based X-ray phase-contrast tomography of atherosclerotic plaque at high photon energies. Z Med Phys. 2013 Sep; 23(3):194-203. doi: 10.1016/j.zemedi.2012.12.001.

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Hetterich H, Willner M, Fill S, Herzen J, Bamberg F, Hipp A, Schüller U, Adam-Neumair S, Wirth S, Reiser M, Pfeiffer F, Saam T. Phase-contrast CT: qualitative and quantitative evaluation of atherosclerotic carotid artery plaque. Radiology. 2014 Jun; 271(3):870-8. doi: 10.1148/radiol.14131554.

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2 Abstract

In this thesis, we examine ex-vivo human carotid arterial vessel walls and atherosclerotic plaque features by phase-contrast imaging (PCI) using a synchrotron-based as well as a lab-based set-up. We compare results from PCI to histopathology as gold standard. PCI is an X-ray-based imaging method providing better soft tissue contrast than conventional X-ray imaging techniques. Unlike conventional X-ray methods, which are based on the attenuation of the X-ray beam, PCI relies on the phase-shift of X-rays when passing through matter. A lab-based PCI set-up has the potential for future clinical application.

In the first part, we performed phase-contrast computed tomography (PC-CT) at a synchrotron facility on five carotid artery specimens at two different photon energies, 23 keV and 53 keV, where the higher photon energy is in a clinically applicable range. In healthy specimens qualitative and quantitative features of the vessel wall were assessed at both photon energies. In diseased specimens plaque features including fibrous tissue, lipid, necrotic core (NEC), intraplaque hemorrhage (IPH), inflammatory cell infiltration and calcifications (CAs) were evaluated at 53 keV concerning qualitative and quantitative characteristics including phase-contrast Hounsfield units (HU-phase). Healthy samples had the same signal characteristics at 23 keV and 53 keV with tunica intima and adventitia showing bright signal, and tunica media showing dark signal. Plaque components at 53 keV showed different signal intensities, texture and HU-phase. In summary, the potential of PC-CT for the visualization and quantification of atherosclerotic plaque at clinically applicable photon energies could be demonstrated in this study.

In the second part of the thesis two carotid endarterectomy specimens were investigated at a synchrotron-based PC-CT set-up at 23 keV and three specimens at a lab-based set-up with a conventional X-ray tube (35-40 kVp; 70 mA). During the imaging process absorption-based images were also obtained. The determination of signal-to-noise ratio (SNR) between PC-CT and absorption images showed higher SNRs in synchrotron-based images compared to laboratory-based images and superior SNRs in both PC-CT set-ups compared to absorption-based images. Two independent evaluators determined vessel dimensions in a manual measurement. All PCI-based measurements showed high correlation with significant overestimation of lumen, intima as well as vessel wall area for both set-ups, as compared with histology. Reproducibility between readers was excellent. Overall, in this study, we demonstrated that PC-CT of carotid specimens and in particular the identification of vessel wall structures is feasible with both synchrotron and conventional X-ray sources.

In the third study plaque features of seven carotid artery specimens were assessed by PC-CT using a lab-based set-up. Characteristics of the NEC, fibrous cap (FC), IPH, and CAs were established. Sensitivity, specificity and accuracy of PC-CT for plaque identification as well as the potential for quantification were evaluated. PC-CT showed good sensitivity for detection of NEC, FC, IPH, and CAs as well as excellent specificity and accuracy, with good agreement between readers. The correlations for quantitative measurements of NEC, FC, and CAs between PCI and histopathology were excellent as well as the reproducibility between readers. In this study, we demonstrated that lab-based PC-CT is suitable for identification and quantification of atherosclerotic plaque components and has potential for clinical applications.

3 Zusammenfassung

In der vorliegenden Arbeit wurden humane Carotisarterien ex-vivo bezüglich der Beschaffenheit der Wandschichten und atherosklerotischer Plaqueanteile mittels Phasenkontrastbildgebung (PCI) mit einer Synchrotronquelle und mit einer konventionellen labor-basierten Röntgenquelle untersucht. Eine histologische Vermessung der Proben diente als Goldstandard für den Vergleich der Phasenkontrastdaten. PCI ist eine röntgenbasierte Bildgebungsmethode mit verbessertem Weichteilkontrast im Vergleich zu konventionellen röntgenbasierten Techniken. PCI basiert auf der Phasenverschiebung von Röntgenstrahlen durch Materie, während konventionelle Techniken auf der Abschwächung der Röntgenstrahlung durch Absorption beruhen. Der PCI Laboraufbau birgt Potential für eine zukünftige klinische Anwendbarkeit.

In der ersten Studie wurden fünf Proben von Carotisarterien mittels Phasenkontrastcomputertomographie (PC-CT) in einem Synchrotronaufbau bei zwei verschiedenen Photonenenergien (23 keV und 53 keV) untersucht. Die höhere Photonenenergie liegt im Bereich klinischer CT Anwendungen. Es erfolgte eine qualitative und quantitative Analyse der Arterienwand bei beiden Photonenenergien. Pathologisch veränderte Proben mit Plaquestrukturen wie fibröses Gewebe, fetthaltiges Gewebe, nekrotischer Kern (NEC), Einblutung in den Plaque (IPH), Infiltration inflammatorischer Zellen und Verkalkungen (CAs) wurden bei einer Photonenenergie von 53 keV bezüglich qualitativer und quantitativer Charakteristika einschließlich Phasenkontrast Hounsfield-Einheiten (HU-phase) untersucht. Proben ohne Pathologie zeigten dieselben Charakteristika bei beiden Photonenenergien, wobei die Tunica intima und adventitia hell und die Tunica media dunkel erschien. Plaquekomponenten die bei 53 keV untersucht wurden, wiesen verschiedene Signalintensitäten, Gewebeeigenschaften und HU-phase auf. Zusammenfassend konnte in dieser Studie das Potential von PC-CT für die Darstellung und quantitative Auswertung von atherosklerotischen Plaques mit für klinische Anwendungen üblichen Photonenenergien demonstriert werden.

Im zweiten Teil dieser Arbeit wurden zwei Carotisendarterektomie-Präparate an einem Synchrotron-Aufbau mit 23 keV und drei Proben an einem Aufbau mit konventioneller Röntgenröhre (35-40 kVp; 70 mA) untersucht. Während des Bildgebungsprozesses wurden auch Absorptionsbilder aufgenommen. Es zeigten sich höhere Signal-zu-Rausch-Verhältnisse (SNR) zwischen PC-CT- und Absorptionsbildern in den Synchrontronaufnahmen im Vergleich zu Aufnahmen mit konventioneller Röntgenröhre. Die PC-CT-Aufnahmen zeigten in beiden Aufbauten höhere SNR im Vergleich zu den Absorptionsbildern. Zwei unabhängige Auswerter werteten die Arterienschnitte quantitativ aus. In allen Phasenkontrastaufnahmen zeigten die so ermittelten Messwerte eine hohe Prädiktion mit jedoch signifikanter Überschätzung im Vergleich zu den Messwerten aus den histologischen Schnittbildern sowie eine hohe Interobserver-Reproduzierbarkeit. In dieser Studie zeigte sich, dass mittels PC-CT die Identifikation verschiedener Strukturen der Arterienwand sowohl an einem Synchrotronaufbau, als auch an einem Laboraufbau mit konventioneller Röntgenröhre möglich ist.

In der dritten Studie wurden Plaquekomponenten von sieben Karotisendarterektomiepräparaten mittels PC-CT an einem konventionellen Laboraufbau untersucht. Merkmale von NEC mit einer fibrösen Kappe (FC), IPH und CAs wurden beschrieben. Untersucht wurde die Sensitivität, Spezifität und Genauigkeit von PC-CT bei der Identifikation und Quantifizierung von Plaques. PC-CT erreichte eine gute Sensitivität bei der Detektion von FC, NEC, IPH und CAs sowie eine hohe Spezifität und Genauigkeit mit guter Übereinstimmung zwischen unabhängigen Auswertern. Neben einer signifikanten Überschätzung der Lumenfläche zeigte sich eine hohe Korrelation der quantitativen Messwerten von FC, NEC, CAs zwischen PCI und Histopathologie sowie eine hohe Reproduzierbarkeit zwischen den unabhängigen Auswertern. Zusammenfassend zeigte diese Studie, dass ein konventioneller PC-CT Aufbau geeignet ist Plaque-Komponenten zu identifizieren und zu quantifizieren und daher potentiell für klinische Applikation angewendet werden könnte.

4 Motivation and aims of the thesis

Atherosclerosis is a common disease, in which the formation of plaques potentially leads to the occlusion of an arterial lumen by a thromoboembolic event, e.g. resulting in myocardial infarction or ischemic stroke. These diseases belong to the most common diseases in the world and are associated with high mortality and morbidity[1]. The identification of atherosclerotic plaques and features associated with a high risk for ischemic events still remains a challenge using conventional imaging techniques[2]. Phase-contrast imaging (PCI) is a relatively new X-ray-based imaging method which provides better soft tissue contrast compared to conventional X-ray-based imaging techniques[3]. The aim of the thesis was a preclinical ex-vivo evaluation of human carotid arteries to assess the potential of PCI for atherosclerotic plaque imaging. Using a synchrotron-based set-up, high-resolution images can be obtained to evaluate the potential of PCI for both qualitatively and quantitatively characterization of plaque features in comparison to the gold standard histopathology. However, large-scale synchrotron facilities are not suitable for transition to a future clinical application. Therefore a special focus of this thesis was the identification and evaluation of plaque using a conventional X-ray source in combination with a three-grating-based PCI (gb-PCI) set-up developed by Pfeiffer et al.[3]. For better identification of the different vessel wall and plaque components we applied phase-contrast Hounsfield units (HU-phase) to quantify the different tissues.

The idea and concept of the presented studies were developed by the doctoral candidate Sandra Fill together with Prof. Tobias Saam, Prof. Fabian Bamberg, Priv.-Doz. Dr. Holger Hetterich, Prof. Franz Pfeiffer and Prof. Julia Herzen, who also supervised the whole project. The doctoral candidate was responsible for overall coordination of the experiments and data evaluation (coordination of acquisition, imaging and histological processing and evaluation of the specimen). The preparation and performance of histopathological processing was conducted by the candidate together with a medical technical assistant. Imaging of the specimens was conducted at the European Synchrotron Radiation Facility (ESRF), Grenoble, France and at the labs of Prof. Franz Pfeiffer at the Technical University Munich. The candidate was involved in the imaging process at the Technical University Munich especially in preparation of the specimen and definition of the region to be investigated. The candidate was responsible for digitalization of the slices, matching of the phase-contrast and histological slices, qualitative evaluation and quantitative measurements of vessel wall and plaque components of the phase-contrast images and histopathological images (including qualitative signal characteristics of vessel wall and plaque components, area measurements, comparison of signal-to-noise-ratios in phase-contrast and absorption images). The candidate was also responsible for the statistical analysis, in particular consolidating different data sources and preparing them for statistical evaluation. The major part of the manuscript was written independently by the candidate. In the publication with shared-first authorship both the candidate and Priv.-Doz. Dr. Holger Hetterich contributed equally to the publication: The candidate was responsible for the above mentioned processes. The identification of relevant tissues for HU-phase measurement, data interpretation, in particular of the acquired HU-phase measurements, as well as the manuscript writing process was performed by Priv.-Doz. Dr. Hetterich in close cooperation with the candidate. Prof. Dr. Dr. h.c. Maximilian Reiser supervised and supported the project.

5 Introduction

5.1 Basic features of atherosclerosis

Atherosclerosis is a systemic multifactorial disease that affects the arterial vessel wall. Atherosclerotic lesions can lead to cardiovascular events such as cardiovascular disease and acute coronary syndrome, cerebrovascular events such as an ischemic stroke and peripheral vascular disease[2,4,5]. The complex pathomechanism of atherosclerosis is based on an imbalance of the lipid metabolism, endothelial dysfunction and an inflammatory process[4,6]. Diseases caused by atherosclerosis like ischemic heart disease and stroke account for the majority of deaths worldwide[7].

5.1.1 Physiological composition of the arterial vessel wall

The arterial vessel wall is basically composed of three parts: the tunica intima adjacent to the vessel lumen, the tunica media and the tunica adventitia. This brief introduction to the vessel wall components is based on Chapter 5 of the histology textbook by Welsch et al.[8].

Tunica intima

The tunica intima contains the endothelium, which constitutes the border to the lumen, and underlying connective tissue. The plane epithelial cells of the endothelium function as a barrier between blood and the tissue of the vessel wall. Therefore, they secrete antithrombogenic factors. They influence the vessel tonus by production of vasoconstrictive and vasodilatative factors. The endothelium also plays a role in the regulation of inflammation, cellular growth and lipid metabolism, especially the absorption of low-density lipoprotein (LDL) by LDL receptors. The connective tissue of the intima is composed of fibrocytes, collagen type III, elastic fibers and a small number of smooth muscle cells. The tunica intima is separated from the tunica media by the lamina elastica interna, an elastic lamella of the tunica media.

Tunica media

The tunica media is composed of smooth muscle cells and elastic fibers. Collagen fibers and proteoglycans can also occur. The lamina elastic externa forms the outer border to the tunica adventitia.

Tunica adventitia

The tunica adventitia is mainly composed of fiber-rich connective tissue. A large number of elastic fibers is located at the neighboring region to the media. A reticulum of collagen fibers is also part of the adventitia. Small nerves and vasa vasorum can also be found.

The systemic arteries can be divided into two histological types of arteries: arteries of the elastic type and arteries of the muscular type. Large arteries like the aorta and the A. iliaca communis are elastic arteries with a so-called windkessel effect. They have a large number of elastic fibers in the tunica media to even out blood pressure differences by the pulsatile ejection of the heart, whereas muscular arteries have a more regulatory function by their large number of smooth muscle cells in the tunica media.

5.1.2 Systemic risk factors for atherosclerosis

The systematic and long-term Framingham study has identified several risk factors for atherosclerotic lesions and accompanying diseases[9]. Factors related with endothelial dysfunction and formation of atherosclerotic lesions include modifiable factors such as hypertension, dyslipidemia, smoking, physical inactivity and hyperglycemia[4,10]. Factors which are not modifiable include age, male sex and a genetic susceptibility[5]. The weight of each risk factor for developing a certain disease entity is different. For example, the main risk factor for cardiovascular events is the elevation of LDL cholesterol blood levels[11,12]. Smoking and diabetes mellitus have the greatest impact on the risk for peripheral arterial disease[5]. The major risk factor for stroke is hypertension[5,12]. Genome-wide association studies have investigated chromosome loci associated with atherosclerosis-based diseases, such as coronary artery disease and stroke[13]. However, the results have not yet been fully reproducible and the impact and

predictive value of the identified loci are still not clear[6]. The development of atherosclerosis so far is a complex and not fully understood interaction of these individually different factors and yet unknown parameters. Therefore prediction of vascular events in individuals remains insufficient[14].

5.1.3 Local hemodynamic risk factors for atherosclerosis

As the whole arterial vessel tree is affected by systemic risk factors, for example elevated cholesterol blood levels, smoking or hypertension, one would expect atherosclerotic lesions to be randomly distributed throughout the whole vascular system[15]. However, atherosclerotic lesions cluster, where hemodynamic factors like a turbulent instead of laminar flow occur[6]. The key parameter of the hemodynamic factors is the endothelial shear stress, which is a force tangential to the endothelium. It depends on the blood viscosity and the spatial gradient of blood velocity over the cross section of the vessel[15]. Low endothelial shear stress is associated with initiation and progression of atherosclerotic lesions[5]. Atherosclerosis prone regions include branch-off points, the outer wall of bifurcations and the inner wall of curvatures[15]. Anatomically the main sites of atherosclerotic lesions are large and medium sized arteries like the abdominal aorta, coronary arteries, iliofemoral arteries and carotid bifurcations[5]. The underlying pathogenesis is not fully understood, but implies an interaction between local hemodynamic factors with the endothelium leading to a modification of the local protective nitric oxide metabolism and a following higher susceptibility of the endothelium to systemic atherogenic factors[15].

5.1.4 Pathogenesis of atherosclerosis

The initiation of atherosclerosis is to date understood as an interplay between local hemodynamic factors, differences in regional arterial development and the modification of the endothelium by atherogenic factors like smoking, hypertension and dyslipidemia[16]. These mechanical and humoral factors lead to a dysfunctional endothelium which is prone to the retention of apolipoprotein B (apoB LP)[17]. Dysfunctional endothelial cells represent a specific phenotype concerning their pattern of gene expression and mechano-activated signalling pathways[17]. The incorporation of apoB LPs in the intima triggers an aggravation of the pre-existing dysfunctionality of the endothelium[17]. These LPs bind to proteoglycans in the intima, where these lipoprotein-proteoglycan complexes have a greater susceptibility to oxidation[18]. Oxidated and aggregated apoB LPs act as triggers for an immune response by the expression of adhesion molecules, chemoattractants and growth factors in the endothelial cells[5]. Thereby monocytes get attracted and enter the vessel wall and differentiate into macrophages and dendritic cells[5]. By incorporating cholesterol from the retained lipoproteins they turn into foam cells[17]. Lesional macrophages can proliferate and by stimulating pro-inflammatory mediators they amplify the inflammatory response, which leads to a plaque progression[17]. Also other immune cells play a role in the complex pathomechanism of atherosclerosis. The role of B cells activated by T cells is not yet fully understood. There might be a atheroprotective effect of IgM and IgG[19]. T cells transmigrate into the intima, where they get activated by antigens[19]. The activated T cells act as an additional pro-inflammatory trigger. Both macrophages and T cells promote the recruitment and modulation of vascular smooth muscle cells[19]. Smooth muscle cells (SMCs) are specialized contractile cells[20]. In the vessel wall they are the major part of the tunica media. Their function is the regulation of the blood vessel tone[20]. Due to local stimuli such as growth factors, mechanical influences, inflammatory mediators and interaction with other cells and the matrix vascular SMCs can undergo a phenotypic modulation[20]. This implies a transformation from a contractile type to a proliferative type[20]. This dedifferentiated SMC type is able to produce a high amount of extracellular matrix components such as collagen, elastin, proteoglycans and fibrin[20]. The dedifferentiated SMCs migrate into the tunica intima, where they produce these components, mainly collagen type I to form a fibrous cap (FC) and stabilize the plaque[20]. The SMCs are thus a major part in protection of plaques and healing and repair after arterial injury[21]. However, in an advanced state of the plaque formation macrophages and SMCs produce matrix metalloproteases[20]. The degradation of collagen by metalloproteases and the apoptosis of SMCs lead to a destabilization of the FC and increased susceptibility to rupture[20]. The apoptosis and necrosis of macrophages and its insufficient clearance in advanced lesions leads to an accumulation of extracellular lipids and cell debris in the center of the plaque, which is also called lipid or necrotic core (NEC) covered by the FC[22]. The formation and invasion of fragile leakage prone neovessels from the adventitial vasa vasorum into the plaque is followed by intraplaque bleeding, free cholesterol deposits, immigration of inflammatory cells[5,23,24]. This leads to an expansion and destabilization of the plaque[24]. Free cholesterol can crystallize and thereby contribute to an increase of the NEC and thinning of the FC[25].

During plaque progression, the arterial wall also undergoes a remodelling process which can result in an expansion of the vessel wall without restricting the lumen size[26,27]. Constrictive remodelling leads to vessel shrinkage and narrowing of the lumen. Expansive remodelling is observed more often in rupture-prone plaques including large lipid accumulations and a high macrophage density[26,27]. The compensatory vessel wall dilating around the eccentric plaque is associated with inflammation, plaque progression and plaque rupture, but not so much with stenosis[26,27]. Constrictive remodelling by adventitial fibrosis and wall thickening leads to higher grades of stenosis[27]. However, it occurs more at stable circumferential plaques which are less prone to rupture[27].

5.1.5 The role of calcification

Vessel wall calcification (CA) can occur as intimal CA associated with atherosclerosis or as medial Mönckeberg CA or as arterial CA of the infant[28]. Each type shows a different pathogenesis and has to be considered as an own entity[28]. The role and pathogenesis of atherosclerotic intimal CA has not yet been fully understood. For the risk stratification of cardiovascular events, the amount of CA is often used as a predicting factor[28]. The degree of CA is seen as a marker for the overall plaque burden. Calcifications can appear in different morphologies. Most likely CAs originate from apoptotic SMCs and matrix vesicles secreted by macrophages as micro-CAs[23,28]. These micro-CAs often occur in plaques with large lipid cores and thin FCs[28]. The presence of micro-CAs in the FC is discussed to be associated with mechanical instability and rupture of the plaque[23]. Calcifications can progress to large plates and fill out the NEC completely[5]. Some plaques only show fibrous and calcified tissue[5]. The role of these fibrocalcific plaques remains unclear[5]. Large CA plates can also fracture and form nodules[28]. The protrusion of these CAs into the plaque lumen is associated with thrombus formation[28]. Recent investigations by Mauriello et al. have shown that stable plaques and healed plaque ruptures show higher amounts of CAs than unstable plaques[29]. The authors suggest that coronary CA quantification might rather determine the vulnerable patient than the vulnerable plaque[29]. The amount of CA shows differences concerning age, sex and race[28]. Women show less CA than men, especially in the premenopausal period[28]. 50% of patients with asymptomatic carotid atherosclerosis were reported to show CAs[28,30]. Asymptomatic patients undergoing carotid endarterectomy surgery have a higher incidence of CAs than symptomatic patients[28,31].

5.1.6 The role of stenosis

Carotid artery stenosis is accepted as a risk factor for acute events, accounting for 10-20% of strokes or transient ischemic attacks[32]. Patients with a severe stenosis (>50%) of the carotid artery show a significantly higher risk for the development of a stroke[12,33,34]. After a transient ischemic attack the risk of stroke within the next 3 months is considered to be 15-20% in the presence of carotid artery stenosis[35]. The degree of carotid artery stenosis is still a major factor in the evaluation of further treatment, namely either medical treatment or surgical or interventional carotid revascularization[35]. However, the degree of stenosis alone is an insufficient marker for future acute cardio- or cerebrovascular events[12,36]. Investigations have demonstrated that two-thirds of the patients with acute coronary events show lumen narrowing in the region of the culprit lesion of <50%[12,37,38].

5.1.7 Consequences of atherosclerosis and plaque formation

The progress of atherosclerosis is subtle and typically remains asymptomatic over decades[5]. Advanced lesions can lead to a narrowing of the vessel lumen cross section with symptomatic stenosis[5,22]. Stenotic lesions are responsible for insufficient perfusion of distal tissues, which manifests as a stable angina pectoris for example. However the majority of acute coronary events occur in vessels without critical stenosis[21,39,40]. Plaques can cause the formation of a local thrombus which suddenly occludes the lumen or lead to an embolization in distal arteries[22]. This can be followed by ischemia or infarction of the downstream tissue[25]. Clinical manifestations of atherosclerosis include myocardial infarction, stable and instable angina pectoris, sudden coronary death, stroke and transient ischemic attack, peripheral artery disease including claudication and critical limb ischemia[28].

5.1.8 Causes for thrombosis

To date a model of three pathogenic causes of thrombus formation is widely accepted. In this concept a thrombus can develop on the basis of plaque rupture, plaque erosion and calcified nodules protruding into the lumen[41]. In general, ruptured plaques account for around two-thirds, plaque erosion for around one-third of thrombotic events, while calcified nodules only play a minor role in the thrombus formation process[41]. Not all ruptured or eroded plaques are followed by an acute event.[12] They can heal without clinical manifestation[12,42,43]. Histologically, healed plaques show a pattern of loosely arranged collagen III and dens collagen I[5]. The formation of a fibrous scar is associated with constrictive remodeling[5]. Healed plaques are often associated with high-grade lumen narrowing in coronary arteries[5].

Plaque rupture

Plaque rupture describes the formation of a defect or gap in the FC which exposes the blood to the prothrombogenic cap collagen and the NEC[5,22,44]. Coagulation proteins in the blood lead to the formation of a thrombus which can occlude the vessel lumen locally or embolize in distal arteries[25]. Plaque ruptures take place at the thinnest part of the FC, which is mostly located at the shoulder region[5]. The thickness of a thin FC in coronary arteries is in most cases smaller than 65 µm and in carotid arteries smaller than 0,25 mm[5,45]. Atherosclerotic lesions with a FC thickness <65 µm or 0,25 mm respectively and a large lipid core are also referred to as thin-cap fibroatheromas[46] Plaque rupture develops mostly on the ground of SMC and collagen loss on the basis of degradation by infiltrated macrophages[5].

Plaque erosion

Thrombus formation on the basis of denudation of the endothelium is also called plaque erosion[5]. Plaque erosion is often associated with pathological intima thickening and fibroatheromas with a thick cap[5]. Eroded plaques responsible for sudden cardiac death show only few CAs, negative remodeling and less inflammation than ruptured plaques[5]. The exposed intima is mainly composed of SMCs and proteoglycans[46]. The pathogenesis of plaque erosion and the following formation of a thrombus remains unclear until today[5].

Calcified nodule

The formation of calcified nodules from broken large calcified plates in the arterial vessel wall was explained in section 5.1.5. By protrusion into the lumen and loss and/or dysfunction of overlying endothelial cells they are suggested to be able to act as thrombogenic factors[5,23,44].

5.1.9 Concept of vulnerable plaque

To identify atherosclerotic lesions of patients with a high risk for a sudden thrombotic event apart from luminal stenosis the concept of vulnerable versus stable plaque has been established[39,40]. The American cardiologist James E. Muller was the first to use the term "vulnerable plaque" in the field of coronary ischemic events in 1989[35,47]. The term vulnerable plaque comprises types of remodeled vessel walls that are prone to cause thromboembolic events[40]. The concept could be transferred from cardiovascular events to carotid vascular alterations in patients with ischemic strokes[48,49]. To distinguish vulnerable plaques from stable plaques characteristics for vulnerability have been established[40]. The following features have been identified as vulnerable: thin FC, large lipid or NEC, intraplaque hemorrhage, active inflammation with macrophage infiltration and outward remodeling[40]. There has been a recent controversy about the evidence and relevance of the concept of vulnerable plaques[14]. The impact of individual plaque features on the risk of a cardiovascular event might have been overestimated until now. The overall plaque burden of an individual might be of greater importance than expected. Also the model of the vulnerable plaque has limitations concerning profound in-vivo cause-and-effect data as well as the insufficiency of animal models due to differences in plaque composition and progression[50].

5.1.10 Atherosclerosis in cervical carotid arteries, stroke risk and treatment

Cervical carotid artery atherosclerosis is responsible for 18-25% of strokes[25,51,52]. To decide if and how a patient should be treated to prevent stroke, several criteria have been established[25]. To date the risk stratification of stroke and treatment planning is based on the degree of stenosis and patient symptom status.[25] Patients with severe stenosis (> 70 %) have a high stroke risk.[25] This relation however applies

mainly to symptomatic patients[25]. Patients with asymptomatic carotid stenosis develop strokes in less than 2-3% per year and under optimal medical treatment less than 1% annually[53]. For most of these patients medical treatment like lifestyle changes, blood pressure control and statins is the preferred choice[25,54]. For symptomatic patients with severe carotid stenosis (> 70 %), surgical therapy such as carotid endarterectomy or carotid artery stenting are the recommended therapy[55]. For symptomatic patients with lower percentage of stenosis (< 70 %) there are still controversies about the best treatment. The characterization of vulnerable plaque features has been proposed to allow for better risk stratification for cerebrovascular events[55].

5.2 Current status of clinical imaging of carotid atherosclerotic lesions

Several imaging methods are used for plaque detection[25,51,55–58]. Each of them inherits advantages as well as disadvantages. They can be divided into invasive and noninvasive methods.

Invasive imaging includes intravascular ultrasound, angioscopy, optical coherence tomography, near-infrared spectroscopy and intravascular magnetic resonance imaging (MRI). As for all invasive procedures, they carry the general risks of bleeding, infection and injury of neighboring tissue structures. Non-invasive imaging methods include ultrasound, computed tomography (CT), MRI and molecular imaging techniques, such as positron emission tomography (PET) [56]. In this summary we will focus on non-invasive imaging techniques as PCI is also a non-invasive method.

In carotid artery plaque detection several factors for vulnerable plaques are well-established[56]. These include plaque ulceration, intraplaque hemorrhage (IPH), thin or ruptured FC, lipid-rich NEC and CAs[56].

5.2.1 Ultrasound

Ultrasound is an imaging method with the advantages of low costs, high availability, few contraindications and absence of ionizing radiation[35,56]. Disadvantages include high dependence on the operator's skills, local anatomic factors and the lack of independent validation[35,56]. Ultrasound techniques include 2-dimensional (2D) B-mode in combination with color Doppler flow, 3-dimensional (3D) mode and contrast-enhanced ultrasound (CEUS).

The measurement of the carotid intima media thickness (CIMT) is used as a predicting marker for cardiovascular risk[59–61]. CIMT represents the distance between the lumen-intima interface and the media-adventitia interface which both appear as small echogenic bands divided by the hypoechogenic band of the media[62]. However, measurement methods differ in the various existing studies depending on the angle of measurement and on the exact section taken for measurement[63]. The results of different studies concerning the predicting value of CIMT are inconsistent, therefore there is no consensus on the use of this method for cardiovascular risk stratification[63]. Risk assessment for cardiovascular events can be improved by additional evaluation of plaque presence[63].

A better predictive value than CIMT can be achieved by evaluation of the plaque burden and its progression either by measuring the total plaque area by 2D ultrasound or the total plaque volume by 3D ultrasound[63]. Progression of total plaque volume has been shown to be superior in predicting cardiovascular events than total plaque area[64].

In recent years, evaluation of plaque morphology has evolved. Several sonographic features to detect vulnerable plaques have been identified as risk factors for ischemic stroke[32]. The FC can be detected by its high echogenicity. Assessment of the thickness of the FC has been reported with moderate sensitivity and specificity by the use of stratified grey-scale median (GSM)[65]. Calcification has a hyperechogenic aspect and can be assessed by the mean pixel value, the GSM and the pixel distribution with good correlation to histology[56,66–68]. Calcifications cause sound extinction behind the calcified structures, which limits the evaluation of neighboring tissue. Echolucency inside the plaque can be attributed to IPH or a lipid-rich NEC and has a strong correlation to a higher risk for ischemic stroke[56,69]. The sensitivity for detection of echolucency is high, however IPH and lipid-rich NEC cannot be differentiated[70]. Neovascularization is supposed to be an initiating factor of IPH and can be visualized by CEUS[71,72]. Neovascularization is a marker for plaque progression and vulnerability[73]. The size of the echolucency was found to be an individual risk marker, as larger lipid-rich NECs appear to be less stable[74]. Plaque

ulceration is defined as a recess of the plaque surface measuring at least 2 mm deep and 2 mm long based on a well-defined wall with an area of reversed flow at the site of the recess[75]. Ultrasound is not a reliable method to detect plaque ulceration, as a wide range of sensitivity (33-75%) and specificity (33-92%) has been reported[56]. A simplified definition by Muraki et al. has improved the accuracy of the detection of ulceration[76]. Also CEUS and 3D ultrasound have been reported to be superior in plaque surface characterization than 2D ultrasound[77,78].

5.2.2 Computed tomography

A common non-invasive imaging method for the assessment of the carotid artery is computed tomographic angiography (CTA). The application of a contrast agent is necessary to generate contrast between the vessel wall and the lumen[35]. By the use of Hounsfield units radiodensity of different tissues can be quantitatively assessed. CTA is widely used for the evaluation of the degree of stenosis of the carotid arteries and further treatment planning[79]. Several plaque components can be identified by CTA, however limitations are due to significant overlaps between radiodensities of several components[35]. Other disadvantages of CTA in general are the restrictions and potential adverse effects of the use of contrast agents, the exposure to ionizing radiation and the limited visualization of the plaque due to artifacts in the presence of heavy CAs[35].

Ulceration defined as "intimal defect larger than 1000 μm in width, exposing the NEC of the atheromatous plaque"[80] which can be detected by the extension of contrast media from the lumen into the plaque can be identified by multidetector row CTA (MDCTA) with good sensitivity and specificity[81,82]. CTA is unable to differentiate between the fibrous cap and the underlying NC. Lipid rich cores and IPH appear as hypodense areas and can be detected with good sensitivity and specificity, however due to a significant overlap in HU (Hounsfield units) differentiation between IPH and lipid-rich core is difficult[35,83–86]. Calcification appears with a high density and can be detected and quantified with high sensitivity by MDCTA[56].

5.2.3 Magnetic resonance imaging

Magnetic resonance imaging (MRI) offers high soft tissue contrast, high resolution and high reproducibility[56]. MRI currently shows the highest potential in carotid plaque imaging[56]. Mostly, serial images with different contrast weightings are generated for plaque imaging[35]. Most commonly 1.5-Tesla scanners are used[56]. However 3-Tesla scanners provide a better signal-to-noise ratio and therefore a better visualization of plaque components[56]. 3D-based MRI techniques and molecular MRI are under investigation concerning their potential for improving the evaluation of vulnerable plaques[56,87]. The disadvantages of MRI are its higher costs compared to CTA or US and its longer procedure time[56]. Additionally a consensus on the optimal clinically adaptable scan protocol is still missing[35]. The FC can be identified as a juxtaluminal band, hypo- or isointense depending on the sequence[56]. The nonexistence of the band can either be interpreted as a ruptured or thin FC[56]. A study using a 3D multiple overlapping thin slab protocol showed a high capability of discriminating the FC concerning thickness and intact versus ruptured surface[45]. The signal of Lipid-rich-NECs in T1w and T2w- images depends on whether they contain IPH or not; in general they appear hypointense on post-contrast enhanced T1w images compared to the surrounding fibrous tissue[88–92]. IPH can be identified as a T1 hyperintense intraplaque signal with high sensitivity and specificity with limitations by hemorrhage size and coexisting CA[88,93,94]. Presence of IPH was shown to be a strong predictor of cerebrovascular events[95–97]. Ulceration can be detected as a surface defect in the intimal layer and disorganized blood flow signal with moderate to good sensitivity using longitudinal black-blood cardiovascular magnetic resonance angiography[98]. Determination of CAs as a hypointense area in all sequences works with good sensitivity and high specificity, although quantifications of the calcified area of the vessel wall tend to be underestimated[88,90,92].

5.2.4 Positron emission tomography

PET is a functional imaging method which can visualize metabolic changes by application of a weakly radioactive tracer, mostly ^{18}Fludeoxyglucose (^{18}FDG)[56]. In ^{18}FDG-PET inflammation in vulnerable plaque can be identified directly[99]. Limitations are the low spatial resolution, non-specific uptake of the tracer in the surrounding tissue, high costs and low availability[56]. By combining PET with CT or MRI

limitations concerning anatomical correlations can be addressed[56]. Single photon emission computed tomography can provide additional information about molecular processes inside the plaque, however the spatial resolution is inferior to PET[56].

5.3 X-ray-based phase-contrast imaging

As described above, existing clinical imaging methods of carotid atherosclerosis are limited either by insufficient spatial resolution and accuracy [56,63] low soft tissue contrast [35] or high cost[56]. X-ray-based PCI is expected to combine high spatial resolution of X-radiation as well as sufficient contrast for soft tissue materials by sensible detection of the phase-shift of X-ray beams[100–108]. Grating-based PCI (gb-PCI), which is investigated in this thesis, is a promising PCI method suitable for future clinical practice because it can be realized with conventional X-ray sources and therefore at low cost[3,109]. The utilization of conventional X-ray sources makes gb-PCI fully compatible with the established clinical absorption radiography, because both conventional absorption images as well as phase-contrast images can be recorded simultaneously[3]. In the following basic principles of PCI are described and an outlook on potential medical applications is given.

5.3.1 Properties of X-radiation

Since the discovery of X-rays by Conrad Wilhelm Röntgen in 1895 X-ray-based imaging methods have evolved to an essential tool in medical diagnostic. X-radiation is defined as the portion of the electromagnetic wave spectrum spanning from wavelengths of 10^{-9} m to 10^{-11} m [110]. The propagation of an electromagnetic wave is described by five quantities: wavelength, frequency, velocity, amplitude and phase. The amplitude of a wave changes periodically in space and time, where the period length in space is the wavelength and the periodicity in time is described by the frequency[111]. Wavelength and frequency have a fixed relation, called the dispersion relation, which depends on the medium of propagation. For example, in vacuum, and also as a good approximation in optically dense media, the dispersion is linear, i.e. the frequency is proportional to the inverse wavelength and the velocity[110,111]. The phase of a wave refers to a certain position at a specific time of a wave period. The relation between waves can be described by their phase-difference. Coherent waves are referred to as being “in phase”. Waves with different phase are called “out of phase”. A wave can also change its phase, for example when it enters a medium with a different optical density. X-rays are characteristic for their ability to propagate through matter where the penetrated object only absorbs a small fraction of the light, meaning there is a slight decrease of amplitude of the X-ray beam[110,112]. In classical X-ray imaging one only measures the intensity, which is the squared time averaged amplitude of the wave. As a consequence, the phase information of the X-rays propagating through the object is lost[111]. The amount of intensity drop is given by the Beer-Lambert law, stating that the intensity decreases exponentially with the penetration depth and the material-dependent absorption coefficient[110,113]. The absorption coefficient is wavelength-dependent and also depends on the atomic number of the penetrated material[110,112]. In classical X-ray methods, the image contrast originates from different absorption coefficients of the materials in the sample; e.g. bone tissue has a higher absorption coefficient than soft tissue and thus produces a different intensity on the X-ray detector[113].

Either X-ray tubes[113] or particle accelerators[114,115] are used to generate X-ray light. Both methods are based on electron processes[110,112]. Characteristic for synchrotron radiation is its high brilliance. This means that there is a high number of photons per time and spatial angle for a given wavelength of the X-ray beam[115].

5.3.2 Principles of phase-contrast imaging

PCI is an X-ray-based method, which has rapidly evolved in the last decades as a new preclinical imaging approach with improved soft tissue contrast compared to conventional X-ray techniques[116,117]. X-ray PCI relies on the phase-shift of X-rays in contrast to conventional X-ray methods, which are based on the attenuation of the X-ray beam, when passing through matter[3]. In conventional X-ray images like CT contrast relies on differences in the absorption coefficients of the investigated sample[118]. High contrast is achieved for example in samples containing highly absorbing structures like bones embedded in poorly absorbing muscle tissue[118]. However, tissues with lower absorption cross sections like breast tissue, vessel walls, muscles cannot be visualized properly with this technique[116]. For the understanding

of PCI it is essential to consider the wave character of light. PCI is based on the phase-shift an X-ray beam undergoes when entering and passing through a medium[3]. Directly related to the phase-shift is an angular deviation of the wave front, which means that its propagation velocity and direction are changed by the sample, i.e. the X-rays get refracted on the interface of the sample[111]. One can describe the interaction of X-rays with the sample mathematically by the refractive index of the sample which is in general a complex number, consisting of a real and imaginary component[111]:

$$n = 1 - \delta + i\beta$$

Here, δ is the refractive index decrement of the real part of the refractive index and the imaginary component β is the extinction coefficient[111].

The real part 1- δ of the refractive index characterizes the phase-shift of the light. For X-rays the real part of the refractive index is smaller than one for most materials and therefore expressed by the decrement δ of the real part[111,113]. In vacuum and in very good approximation also in air the refractive index is one[113]. If the X-rays enter from air into a more dense medium such as liquids or solids, i.e. from a medium with a higher to a medium with a lower real part of the refractive index, their propagation direction is slightly bent away from the surface normal of the interface between the two materials[110,111]. The extinction coefficient which is the imaginary part of the refractive index n specifies the amount of attenuation of the X-ray beam during the transmission through the specimen[110,111].

In general, the refractive index depends on the atomic number Z of the material[113]. For soft tissues, which are composed of low Z materials such as hydrogen, carbon, nitrogen and oxygen the magnitude of the refractive index contrast is greater than the absorption index contrast[108,113]. This means that within the diagnostic energy range of X-rays PCI is more sensitive for soft tissue than absorption based methods[108].

Different approaches to exploit the phase-shift of the X-ray beam have been established, namely crystal-interferometry,[100,101] analyzer-based imaging,[102–104] propagation-based imaging[105–107] and grating-based interferometry[3]. All these methods except for the grating-based PCI (gb-PCI) cannot be realized with conventional X-ray sources and require highly brilliant X-ray light e.g. provided by synchrotron facilites[119]. This limits their practical implementation into clinical routine, as only a small number of these facilities exist. Gb-PCI, which is investigated in this thesis is a promising PCI method to be suitable for future clinical practice because it can be realized with conventional X-ray sources[3].

5.3.3 Grating-based phase-contrast imaging

Gb-PCI is an interferometric method where two or more gratings are utilized to detect the phase-shift[3,109]. In principle the set-up consists of an X-ray source, the sample, gratings and a detector[3]. Parts of the experiments in this thesis were carried out at a synchrotron facility. For these experiments only two gratings are required in total[3]. When using a lab-based conventional X-ray source the set-up is composed of three gratings[3]. The lab-based set-up includes a source grating G_0, a phase grating G_1 and an analyser grating G_2[3]. The source grating is placed behind the X-ray tube and induces the generation of focally coherent X-ray sources[3]. This grating is only relevant to meet the requirements of coherence for the detection of the phase-shift by interference[3]. The sample is placed between the source grating and the phase grating[3]. The phase grating and analyser grating placed behind act as a Talbot interferometer, which basically converts interference patterns due to the phase-shift into spatially separated intensity variations[3]. The intensity variations can then be recorded by a standard X-ray detector[3]. The phase grating causes a division of the incoming beam, which will interfere further downstream by formation of a fringe pattern [109]. Due to the Talbot-Effect the interference pattern created by the phase grating G_1 reoccurs in specific distances, called Talbot-distances[120]. By use of the analyser grating G_2 situated close to the detector this pattern is transformed into intensity variations at each pixel of the imaging detector[3,109]. To extract the phase information one of the gratings is stepped parallel to the other gratings and the detector[121]. Tomographic reconstructions are generated from the spatial distribution of the refractive index decrement δ(x,y,z) of the sample[122]. From the same recordings also tomographic reconstructions of the distribution of the linear attenuation coefficients can be acquired[122].

For identification of different tissues in conventional X-ray images a quantified grey scale has been established. This so-called HU (Hounsfield units) scale is a linear scale based on the linear attenuation

coefficient of the tissue with water and air as a reference[123]. Similarly, phase-contrast Hounsfield units (HU-phase) were introduced to scale the refractive index decrement δ[122].

5.3.4 Current status of phase-contrast imaging of vessel structures

Momose et al. conducted first investigations on the depiction of blood vessels by PCI[124]. They used a crystal interferometer set-up at a synchrotron source to visualize ex-vivo liver specimens freshly excised from mice[124]. The blood vessels were tied to hold the blood inside the specimen[124]. In these initial results, vessels with diameters of about 50 µm could be observed without contrast agents[124]. However, the vessel wall cannot be delineated in these images[124]. Shinohara et al. were the first to obtain tomographic phase-contrast images of vessel walls with a crystal X-ray interferometer using a synchrotron source[125]. They could visualize atherosclerotic plaques in ex-vivo vessel specimen of apolipoprotein E-deficient mice.[125] Several plaque features such as the FC and the lipid core could be identified[125]. Müller et al. could demonstrate initial results of a human coronary artery using gb-PCI at a synchrotron source[126]. The images provide enough contrast to detect the vessel wall, plaque and surrounding fatty tissue[126]. Holme et al. compared images of ex-vivo human coronary arteries obtained by laboratory µCT and gb-PC- at a synchrotron source[127]. They demonstrated a better identification of different tissue structures like foamy cells, muscles and plaques in PC-CT images than in µCT images[127]. Appel et al. compared the identification of carotid artery plaques by ultrasound, conventional radiographs and PCI[128]. In their study they investigated patients before carotid endarterectomy by ultrasound and the excised endarterectomy specimens by conventional absorption-based radiographs and by using phase-contrast at a synchrotron source[128]. Phase-contrast images were obtained by a crystal-analyzer set-up[128]. This study demonstrated that phase-contrast images were able to visualize plaque features that could not be seen in ultrasound and absorption-based images[128]. Phase images revealed regions of inflammation, NEC and lipid-rich tissue[128]. In a similar study, Appel et al. also investigated carotid plaque microstructures by analyzer-based phase-contrast imaging, where they could identify the interface between plaque and media, regions of inflammation and lipid-rich tissue[129]. In our studies we demonstrate identification of vessel wall layers and plaque components in a lab-based setting. The use of a grating-based approach with a conventional X-ray source represents an important step towards future clinical application of PCI. Additionally, we can show that HU-phase are suitable to quantify and thereby discriminate different tissues similarly to the HU scale routinely used in the attenuation-based CT images.

5.3.5 Recent advances towards medical application and outlook

A large number of organs and tissues have already been investigated by PCI, including breast[130–134], cartilage[135], lung[136137138], liver[139–141], renal[142], brain[143,144], pancreatic[145] and lymphatic[146] tissues. Currently, the technique is still at a preclinical stage at the edge towards first experimental clinical application. A big step towards future clinical tomographic phase-contrast recordings has been taken by the development of a small animal scanner[137]. Until recently, all PCI set-ups used a rotating sample for tomographic recordings[147]. In 2012, Tapfer et al. reported the first realization of a gantry-based phase-contrast set-up[147]. They were able to take a tomographic scan of a porcine rind sample and identify several tissues including the epidermis, dermis subcutis and muscle tissue[147]. Scherer et al. could optimize a lab-based phase-contrast set-up concerning dose issues and conducted first dose-compatible phase-contrast mammography[133]. Recently Gromann et al. demonstrated first in-vivo full-field chest radiographs of a larger mammal at a clinically relevant dose[148]. In summary, PCI represents a promising method for future clinical use if technical problems such as dose-issues, examination-time, cost-effective fabrication of the necessary gratings can be overcome

6 Results

6.1 Phase-contrast CT: qualitative and quantitative evaluation of atherosclerotic carotid artery plaque

Hetterich H, Willner M, Fill S, Herzen J, Bamberg F, Hipp A, Schüller U, Adam-Neumair S, Wirth S, Reiser M, Pfeiffer F, Saam T. *Radiology* 271, 870-878 (2014).

https://doi.org/10.1148/radiol.14131554

6.2 Translation of atherosclerotic plaque phase-contrast CT imaging from synchrotron radiation to a conventional lab-based X-ray source

Saam T, Herzen J, Hetterich H, Fill S, Willner M, Stockmar M, Achterhold K, Zanette I, Weitkamp T, Schüller U, Auweter S, Adam-Neumair S, Nikolaou K, Reiser MF, Pfeiffer F, Bamberg F. *PLoS One* 8, e73513 (2013).

https://doi.org/10.1371/journal.pone.0073513

6.3 Grating-based X-ray phase-contrast tomography of atherosclerotic plaque at high photon energies

Hetterich H, Fill S, Herzen J, Willner M, Zanette I, Weitkamp T, Rack A, Schüller U, Sadeghi M, Brandl R, Adam-Neumair S, Reiser M, Pfeiffer F, Bamberg F, Saam T. *Z. Med. Phys.* 23, 194–203 (2013).

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7 Abbreviations

^{18}FDG..... ^{18}Fludeoxyglucose

2D 2-dimensional

3D 3-dimensional

apoB LP ..... apolipoprotein B

CA..... calcification

CIMT ..... carotid intima media thickness

CT ..... computed tomography

CEUS..... contrast-enhanced ultrasound

CTA ..... computed tomographic angiography

ESRF..... European Synchrotron Radiation Facility

FC ..... fibrous cap

gb-PCI..... grating-based phase-contrast imaging

GSM ..... grey-scale median

IPH..... intraplaque hemorrhage

LDL ..... low-density lipoprotein

MDCTA..... multidetector row computed tomographic angiography

NEC ..... necrotic core

PCI..... phase-contrast imaging

PC-CT..... phase-contrast computed tomography

HU ..... Hounsfield units

HU-phase..... phase-contrast Hounsfield units

PET ..... positron emission tomography

SNR ..... signal-to-noise ratio

SMC..... smooth muscle cell

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