The natural history of human atherosclerosis : a histopathological approach

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CHAPTER 1

General Introduction and outline of the thesis

GENERAL INTRODUCTION

Atherosclerosis is a complex pathology of large and medium-sized arteries that constitutes the principle cause of cardiovascular disease[1,2]. Despite major clinical successes, complications of atherosclerosis remain the most common cause of death in Westernized societies[3]. Consequently there is a persistent need for better pharmaceutical strategies and improved risk stratification.

The atherosclerotic process reflects a complex interplay of metabolic, environmental, inflammatory and physical factors[1]; aspects that are extensively reviewed and continuously updated in state-of-the art reviews[4,5,6.] It is becoming more and more apparent that the clinical manifestations of atherosclerosis (*i.e.* myocardial infarction, stroke and peripheral arterial disease) relate to qualitative changes in plaque structure (*i.e.* plaque rupture) rather than to a mass (quantitative) effect. As such insight in the qualitative changes is crucial for understanding the chain of events underlying the clinical manifestations of this disease.

The qualitative changes in atherosclerotic process proceed through distinct, histological stages. These consecutive stages were first outlined by an American Heart Association (AHA) working group. This initial classification scheme from the mid-1990s was later updated in 2000[7,8,9,10] (figure 1). This AHA consensus scheme has been criticized for ignoring aspects of plaque destabilization (*viz.* vulnerable lesions) that predispose to clinical events[11]. Based on the morphology of coronary atherosclerotic lesions, Virmani *et al.*[11] proposed a more comprehensive classification scheme that incorporates both the various aspects of vulnerable lesion development as well as plaque destabilization (Table 1). An open question remains whether and how this classification based on observations from coronary arteries, translates to other vascular beds with different risk factor profiles[12,13].

Current mechanistic insight into the atherosclerotic process is largely based on static information from epidemiological studies and on (histological) evaluation of surgical specimens (*viz.* late stage and sudden coronary death victims). Dynamic insight in the disease processes essentially relies on animal (murine) studies. Although mice are indispensable tools for atherosclerosis research, mice are naturally protected from atherosclerosis by their atheroprotective lipoprotein profile[14]. As such atherosclerosis development in mice critically depends on interference with the lipoprotein metabolism along with specific dietary interventions. Consequently, atherosclerosis in these models is essentially lipid (cholesterol) driven.

The ideal animal model of atherosclerosis will mimic the pathophysiological chain of events leading to human disease, and will develop lesions and complications similar to those found in humans[15]. Available data indicate overlap

between disease development in murine atherosclerosis models and human disease[16]. However, it is acknowledged that full translation is hampered by fundamental physiologic, anatomical, inflammatory, immunologic and metabolic differences between men and mice, as well as differences between mice strains[17,18]. A further major limitation is the fact that atherosclerotic lesions in mice fail to progress to advanced unstable culprit lesions that give rise to the clinical manifestations of atherosclerosis (Figure 2). As such specific information on the sequence of events leading to plaque destabilization is missing from these models.

Experimental atherosclerosis in murine models of atherosclerosis provides an important research tool. However, the leap from experimental animal findings to human atherosclerosis and clinical application yet still presents many challenges.

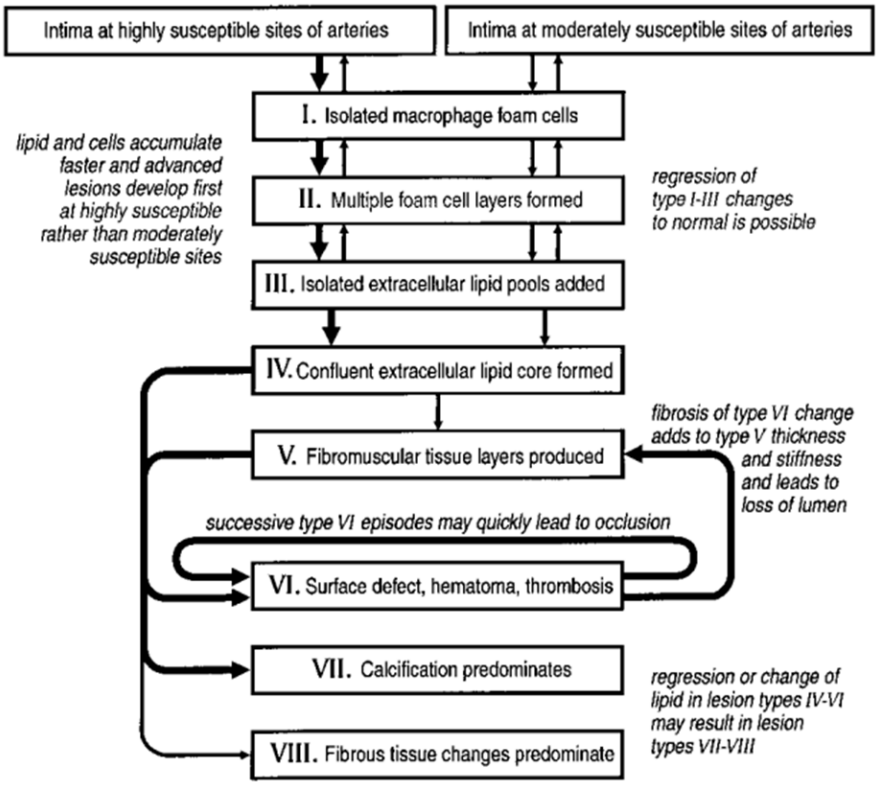
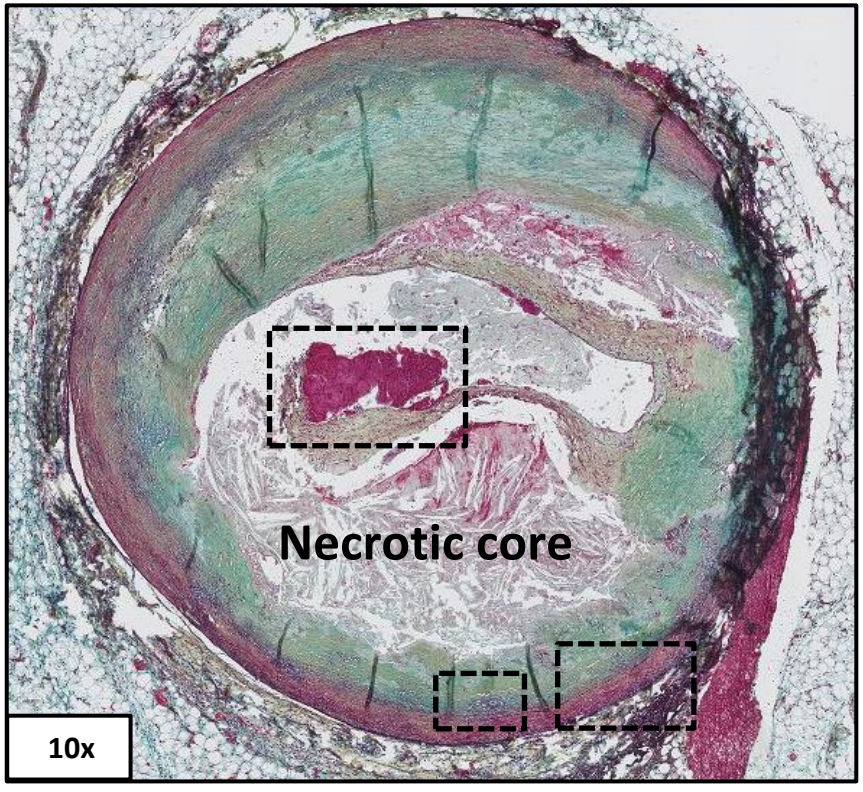


Figure 1. Outline of the sequence in the evolution of atherosclerotic lesions from type I to type IV and of the various possible subsequent pathways of progression to lesion types beyond type IV. The diagram lists the main histological characteristics of each sequential step (lesion type). Adapted from reference 10.



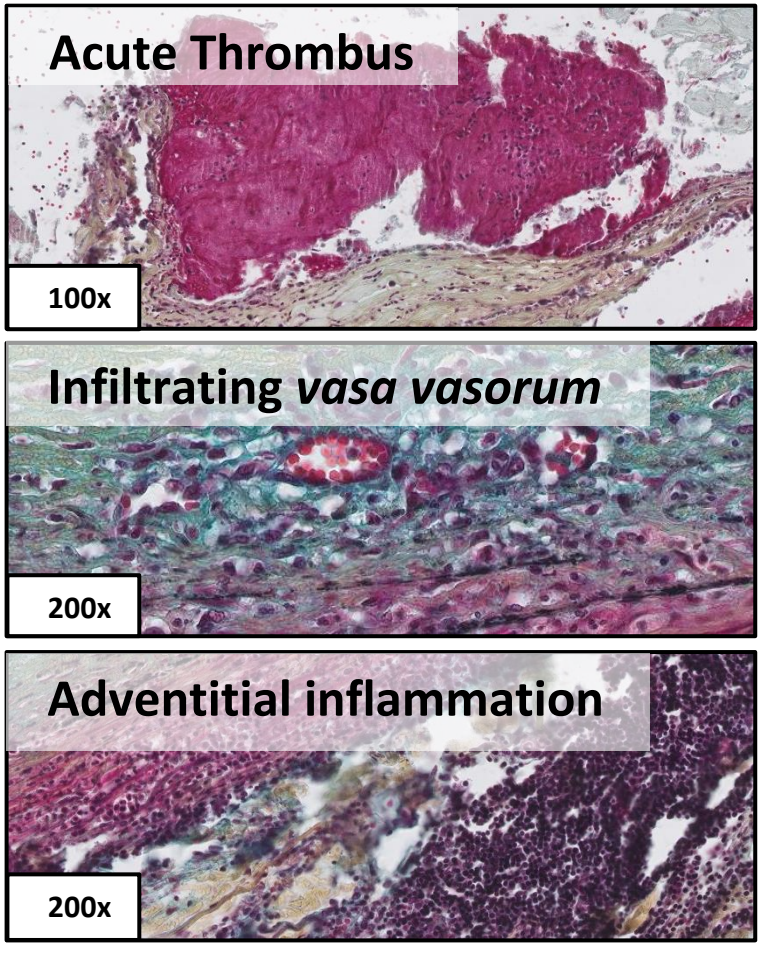


Figure 2. A typical example of an unstable culprit coronary lesion that gives rise to the clinical manifestations of atherosclerosis due to cap rupture hence exposing the large thromboembolic necrotic core.

Table 1 Modified AHA Classification Based on Morphological Description. Adapted from reference 11.

	Description	Thrombosis
Nonatherosclerotic intimal lesions		
Intimal thickening	The normal accumulation of smooth muscle cells (SMCs) in the intima in the absence of lipid or macrophage foam cells	Absent
Intimal xanthoma, or "fatty streak"	Luminal accumulation of foam cells without a necrotic core or fibrous cap. Based on animal and human data, such lesions usually regress.	Absent
Progressive atherosclerotic lesions		
Pathological intimal thickening	SMCs in a proteoglycan-rich matrix with areas of extracellular lipid accumulation without necrosis	Absent
Erosion	Luminal thrombosis; plaque same as above	Thrombus mostly mural and infrequently occlusive
Fibrous cap atheroma	Well-formed necrotic core with an overlying fibrous cap	Absent
Erosion	Luminal thrombosis; plaque same as above; no communication of thrombus with necrotic core	Thrombus mostly mural and infrequently occlusive
Thin fibrous cap atheroma	A thin fibrous cap infiltrated by macrophages and lymphocytes with rare SMCs and an underlying necrotic core	Absent; may contain intraplaque hemorrhage/fibrin
Plaque rupture	Fibroatheroma with cap disruption; luminal thrombus communicates with the underlying necrotic core	Thrombus usually occlusive
Calcified nodule	Eruptive nodular calcification with underlying fibrocalcific plaque	Thrombus usually non-occlusive
Fibrocalcific plaque	Collagen-rich plaque with significant stenosis usually contains large areas of calcification with few inflammatory cells; a necrotic core may be present.	Absent

Despite all the progress we still lack definitive evidence to show that processes such as lipoprotein oxidation, inflammation and immunity have a crucial involvement in human atherosclerosis and its complications. A systematic evaluation of the human atherosclerotic process, especially regarding plaque vulnerability is highly relevant.

This thesis systematically evaluates the critical morphological, pathophysiological and immunological aspects of the human atherosclerotic process using a unique biobank containing over 500 individual peri-renal abdominal aortic wall patches and over 600 coronary artery segments of the left coronary artery. The aortic patches were obtained during liver, kidney or pancreas transplantation and the coronary artery segments were collected from healthy human hearts that were retrieved from Dutch post-mortem donors within 24 hours after circulatory stop and brought to the National Heart Valve for heart valve donation. Contrary to other histological studies investigating mechanistic insight into the atherosclerotic process, we used tissue from a group of apparently healthy individuals with an equal age and sex distribution, thereby avoiding potential bias introduced by the use of autopsy material from coronary death victims (mostly either young or old patients) or by the use of material from patients undergoing vascular surgery (generally end-stage atherosclerotic disease).

AIM AND OUTLINE OF THIS THESIS

Of the primary locations, *i.e.* coronaries, carotids and aorta, surprisingly little is known about how atherosclerosis in the aorta, the second largest manifestation of atherosclerosis, progresses from early to more advanced, complicated lesions [19,20,21]. This lack of information is remarkable, especially considering the fact that the aorta is the reference vessel in mouse atherosclerosis models.
The primary aim of this thesis was to explore the natural history of human aortic atherosclerosis in order to gain more insight in the pathophysiology of plaque development and unstable culprit lesion formation that potentially gives rise to the clinical manifestations of atherosclerosis.

The first part of this thesis focusses on the general morphology of aortic atherosclerosis. **Chapter 2** evaluates the comprehensive morphological classification scheme as proposed by Virmani[11] using a well-documented large tissue bank of human peri-renal aortic tissue that covers the whole spectrum of atherosclerotic disease. Aortic atherosclerotic lesions follow a similar pattern of progression as seen in coronaries but grow far beyond the size of coronary

atherosclerotic lesions. Yet presumably owing to the aortic diameter plaque rupture, remains clinically silent[22].

In order to improve atherosclerosis risk prediction and to detect clinically relevant lesions, the intimal media thickness (IMT) along with coronary calcium scores (CCS) have been brought forward as epidemiological measures of atherosclerosis burden[23,24]. In **Chapter 3** we look into IMT and the coronary calcium scores and their relation to disease progression in order to assess their value as individual risk prediction tools. There is a moderate correlation between age and IMT but the influence is minimal and hardly affects the estimates.

As mentioned above, the aorta is the reference vessel in mouse atherosclerosis models. However, to what extend the available mouse models of atherosclerosis mimic the spectrum of lesions present in humans is unclear and a direct translation from the findings in mice models to the situation seen in humans is still missing. **Chapter 4** presents a morphological overview and a 1 to 1 comparison of the human coronary and aortic atherosclerotic lesions and their murine counterpart. By doing so, we aim at a detailed classification scheme for atherosclerosis in mice based on the Virmani classification that parallels the human situation since a uniform classification system for mouse lesions is missing[25,26,27].

The second part of this thesis focusses on key players in the process of human atherosclerosis. A primordial event in atherogenesis is LDL accumulation in intimal layer of the vessel wall and extensive experimental data exists defining the role of oxidation of LDL cholesterol in both progression and regression of atherosclerosis[1]. **Chapter 5** systematically describes the relationship of several oxidation specific neoepitopes and human atherosclerosis and their relationship to early atherosclerotic lesions and clinically relevant advanced, unstable, or ruptured plaques. Understanding this relationship may have significant clinical implications with the emergence in the clinical and translational arenas of oxidative biomarkers, molecular imaging, and therapeutic approaches including immune modulation.

Information on the immune response in initiation, progression and complications of atherosclerotic disease is largely based on experimental studies. It is unclear how these observations translate to the human situation. **Chapter 6** systematically evaluates the cellular components of the innate immune system (macrophages and their subtypes, dendritic cells, mast cells, natural killer cells, neutrophils and eosinophils) throughout the process of human atherosclerosis.

Chapter 7 further explores the inflammatory component that drives atherosclerosis by evaluating the adaptive immune response within the process of atherosclerotic lesion formation, progression, destabilization and stabilization. These explorative studies confirm extensive and dynamic presence of cellular

components of the innate and adaptive immunity in the human atherosclerotic process, and reveal profound changes in the inflammatory foot print immediately prior to and during the process of plaque destabilization.

Since inflammation is a canonical factor in the progression and complications of atherosclerotic disease, strategies aimed at the 'central hub' of inflammation have been brought forward as a target in limiting vascular inflammation. In **Chapter 8** we first of all histologically evaluate and acknowledge abundant activation of the proinflammatory transcription factor AP-1 (activator protein-1) in early and advanced atherosclerotic disease. This is followed by an interventional study to test whether quenching AP-1 activation improves vascular function in high-risk patients (*viz.* patients with peripheral artery disease).

The regulation of plaque and matrix homeostasis is evaluated in **Chapter 9**. Members of the TGF-β superfamily have been proposed as critical regulators of the atherosclerotic process but their role in human disease remains controversial[28,29]. By using Smad phosphorylation as a read out for TGF-β and BMP signaling and immunostaining against T-cells, monocytes and macrophages (CD68) we explore the putative association between TGF-β and BMP signaling and vascular inflammation during the initiation, progression and (de)stabilization of human atherosclerotic disease.

Finally, this thesis is completed with a summary and future perspectives in **Chapter 10**, samenvatting (Dutch summary) **Chapter 11**, list of publications and a *Curriculum Vitae* of the author.

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