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The natural history of human atherosclerosis : a histopathological approach

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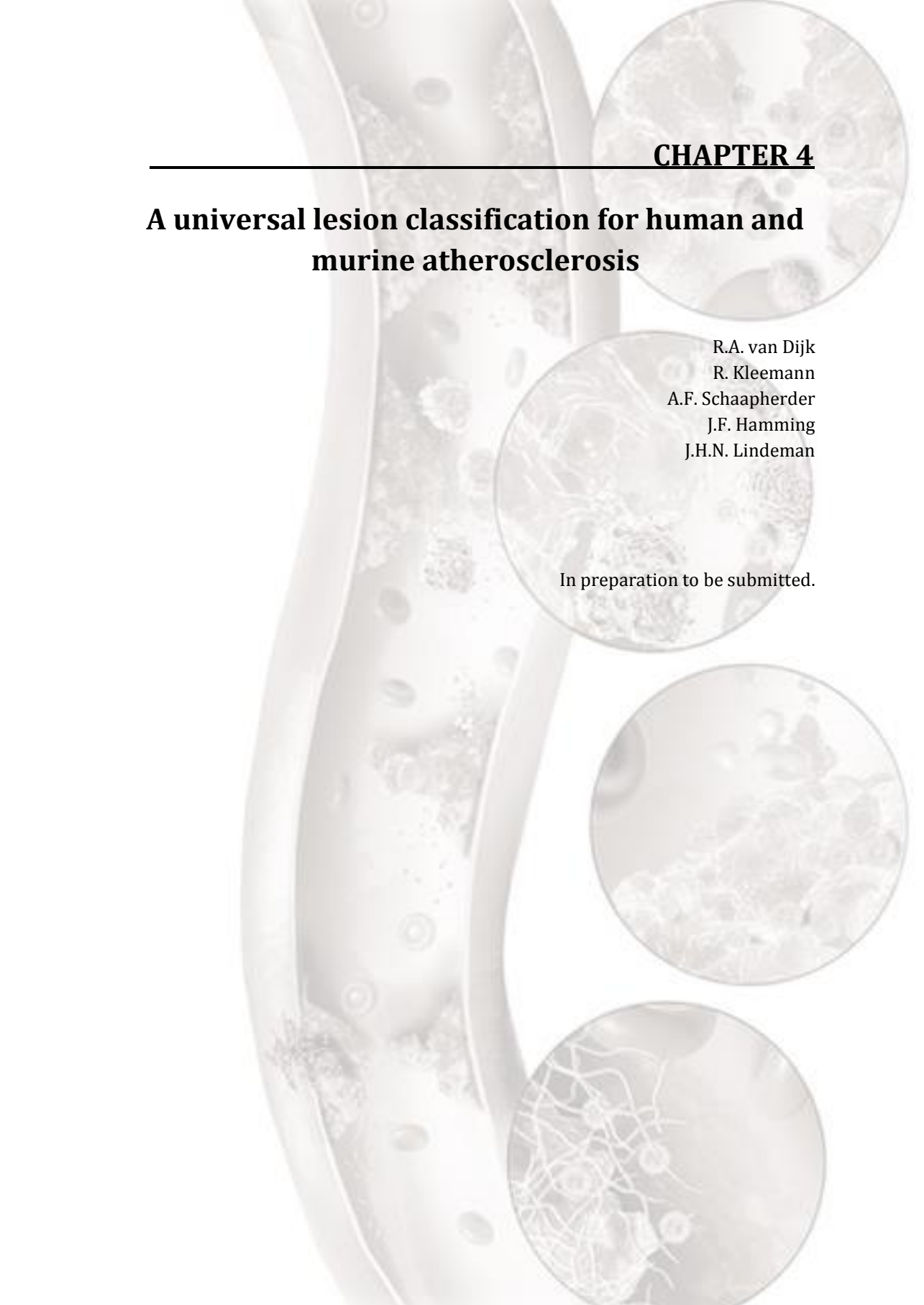


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CHAPTER 4

A universal lesion classification for human and murine atherosclerosis

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In preparation to be submitted.

ABSTRACT

Background: Qualitative characterization of the atherosclerotic process fully relies on histological evaluation and grading of plaque characteristics. An extensive morphological classification scheme exists for human coronary artery atherosclerosis and has been validated for the human aorta. However, it is unclear to what extent this classification scheme can be used to grade human aortic lesions and experimental lesions in murine models of atherosclerotic disease.

Methods: Human coronary artery and human aorta sections were collected from large tissue banks. They were analysed together with histologic sections of the aortic root from mice in accordance with the modified American Heart Association classification as recently proposed by Virmani *et al.* (Nature Reviews Cardiology; 2015).

Results: The overall histological features of (non-)progressive, vulnerable and stabilizing aortic atherosclerotic lesions in mice are remarkably similar to human coronary atherosclerosis. The early stages of atherosclerosis in mice are comparable to adaptive intimal thickening, intimal xanthoma, pathological intimal thickening, and early fibroatheroma. Importantly, the most advanced lesions in mice do not progress to the equivalent of a late fibroatheroma in humans. Therefore mice do not develop the vulnerable lesion phenotype.

Conclusion: The initial and more advanced atherosclerotic lesions in mice follow a more or less similar pattern of progression compared to coronary and aortic atherosclerosis in humans and can be classified accordingly using a uniform, detailed morphological classification scheme based on the Virmani classification. However, vulnerable lesions do not develop in mice, and therefore analysis of plaque stability in mice carries a considerable risk of over interpretation.

INTRODUCTION

Atherosclerosis is a complex pathology of the large and medium-sized arteries^{1,2}. It is well accepted that progression of atherosclerosis and its clinical consequences is related to qualitative changes in plaque characteristics. Indeed, the common insidious manifestations of the disease are thought to essentially reflect abrupt plaque destabilization and rupture rather than gradual obstruction due to quantitative changes in plaque volume³. It is now conceived that atherosclerotic disease reflects a long-term process that proceeds through well-defined sequential, morphologically distinct stages, up to the point of plaque destabilization and rupture. Plaque rupture is then followed by thrombus formation, a healing process and consolidation (*i.e.* fibrosis). At this point qualitative grading of atherosclerotic lesions fully relies on histological evaluation and classification based on plaque characteristics.

A first consensus classification scheme for grading human atherosclerosis has been introduced by the American Heart Association (AHA) working group^{4,5,6,7}. However, this classification fails to capture all clinical aspects of coronary thrombus formation and was therefore refined by Virmani and co-workers⁸. Based on their observations from autopsy material, they extended the AHA classification in order to better reflect the heterogeneity of the advanced stages of atherosclerotic disease. It describes the critical phases of plaque destabilization, rupture and subsequent healing in more detail. Furthermore, this classification better reflects the sequence of events of the disease process.

The insight in the molecular aspects of the atherosclerotic process is mainly based on extrapolation of observations from murine models, but there is no one-to-one morphological comparison of human with murine disease^{9,10,11}. It is unclear whether and how the refined human classification system by Virmani can be employed for murine lesions especially regarding the interpretation of lesion vulnerability and stability. Moreover, there is currently no uniform classification system for murine atherosclerosis. As a consequence, comparison of observations from different disease models with different diets and interventions is challenging, and extrapolation and translation of murine findings to the human situation is almost impossible.

We therefore considered a morphological comparison of human to murine atherosclerosis, and an evaluation of the Virmani classification scheme as a universal grading system for atherosclerotic disease relevant. Results of this evaluation show that this classification scheme can be applied to mouse studies, but confirm the notion that murine lesions fail to develop atherosclerotic features unique to the human vulnerable lesions that give rise to clinical symptoms.

MATERIAL AND METHODS

Human aortic tissue sampling

Tissue sections were selected from a large tissue bank containing over 500 individual abdominal aortic wall patches (AAWPs) that were obtained during liver, kidney or pancreas transplantation (*viz.* all material was from cadaveric donors). Details of this bank have been described previously by van Dijk *et al*⁹. All patches were harvested from grafts that were eligible for transplantation (*i.e.* all donors met the criteria set by The Eurotransplant Foundation) and due to national regulations, only transplantation relevant data for donation is available. Sample collection and handling was performed in accordance with the guidelines of the Medical and Ethical Committee in Leiden, Netherlands and the code of conduct of the Dutch Federation of Biomedical Scientific Societies (https://www.federa.org/sites/default/files/digital_version_first_part_code_of_conduct_in_uk_2011_12092012.pdf).

Human coronary tissue sampling

Tissue sections were selected from a large tissue bank containing over 700 individual coronary artery segments (CAS) of the left coronary artery obtained from human hearts. CAS were retrieved from Dutch post-mortem donors within 24 hours after circulatory arrest and brought to the Heart Valve Bank Rotterdam for valve donation. All donors gave permission for research, and met the criteria maintained by the Dutch Transplantation Foundation. In the donation procedure the aortic valve is removed from the donated heart. During further aortic valve preparation the adjacent tissue including the left coronary artery is trimmed according to standard procedures. The removed coronary artery segments are used in this study. This procedure does not interfere with the pathological analysis of the heart necessary for release of the harvested valves.

Characterization of the lesions and histological definitions (Table 1)

Histologic sections of AAWPs and CASs were stained by haematoxylin and eosin (H&E), and Movat pentachrome for classification of the lesions in accordance with the modified AHA classification as proposed by Virmani *et al*⁸. Classification was performed by two independent observers with no knowledge of the patient characteristics of the aortic or coronary tissue. A detailed description of plaque characterization, morphological analysis for AAWPs is provided in reference 9 and for CASs in reference 8. Plaque morphologies included adaptive intimal thickening (AIT), intimal xanthoma (IX), pathological intimal thickening (PIT), early (EFA) and late fibroatheroma (LFA), thin cap fibroatheroma (TCFA), acute plaque rupture (PR), healed rupture (HR) and fibrotic calcific plaque (FCP).

Table I. Morphological classification of aortic and coronary tissue according to the modified AHA classification proposed by Virmani *et al* ⁸.

Subtype of Lesion	Abbr.	Morphological description	Mouse equivalent morphological description
Normal	N	No signs of intimal thickening and intimal inflammation	No signs of intimal thickening and intimal inflammation
Non-progressive intimal lesions			
Adaptive intimal thickening	AIT	Natural accumulation of SMCs in the absence of lipid and macrophage foam cells	Accumulation of SMCs in the absence of lipid and macrophage foam cells
Intimal xanthoma	IX	Superficial accumulation of foam cells without a necrotic core or fibrous cap	Up to several layers of foam cells without a necrotic core or fibrous cap
Progressive atherosclerotic lesions			
Pathological intimal thickening	PIT	Plaque rich of SMCs and focal accumulation of extracellular lipids with or without the presence of macrophages	Small extracellular lipid pools with overlying or adjacent located macrophages. Intimal SMC can be identified.
Early fibroatheroma	EFA	Focal macrophage infiltration into areas of lipid pools with an overlying fibrous cap	Larger amounts of extracellular lipid with infiltrating macrophages and cholesterol clefts are visible. The core is shielded from the bloodstream by several layers of SMC. No apoptosis or necrosis.
Late fibroatheroma	LFA	Loss of matrix and extensive cellular debris with an overlying fibrous cap with or without calcification.	Macrophage accumulation within the core of extracellular lipid with distinguishable cholesterol clefts. The core is shielded from the bloodstream by several layers of SMC and macrophages.
Vulnerable atherosclerotic lesions			
Thin cap fibroatheroma	TCFA	A thin fibrous cap (<65 µm in coronary artery and <155 µm in the aorta) overlying a large necrotic core. Intraplaque haemorrhage can be present in coronary lesions	N/A
Plaque rupture	PR	Thin cap fibroatheroma with cap disruption with a luminal thrombus communicating with the necrotic core	N/A
Stabilizing lesions			
Healing rupture	HR	Healed lesion composed of SMCs, proteoglycans and collagen with or without an underlying disrupted fibrous cap. With or without calcifications.	N/A
Fibrotic calcified plaque	FCP	A fibrous lesion with large amounts of calcification without an underlying necrotic core.	N/A

Tissue samples from preclinical models of atherosclerosis

Hearts and aortic tissues were obtained from an extensive mouse tissue biobank (TNO, Leiden, The Netherlands) and atherosclerotic lesions of Ldlr^{-/-} mice, ApoE^{-/-} mice and ApoE*3Leiden mice were analysed. Lesion development in these mice was induced as reviewed in reference 10 (and references therein) involving feeding of atherogenic cholesterol-containing diets. The heart and the aortic root were collected at sacrifice, embedded in paraffin and subsequently used for preparation of aortic cross-sections as previously reported¹². The aortic tissue was stained by Movat pentachrome for classification of the lesions according to the modified AHA classification as proposed by Virmani *et al*⁸. Morphological analysis and classification of the lesions were performed by two independent observers. Plaque morphologies included adaptive intimal thickening (AIT), intimal xanthoma (IX), pathological intimal thickening (PIT) and early fibroatheroma (EFA). The mouse lesions were also analysed for presence of more advanced lesion stages.

RESULTS

The Movat pentachrome staining allows a clear visualization of the different constituents of the vessel wall. More specifically, this staining procedure identifies mucins/proteoglycans (blue), collagen (yellow), elastic fibers (black), smooth muscle cells, erythrocytes and fibrinogen (red) and nuclei (purple). Different shades of turquoise and green reflect co-localization of collagen and proteoglycans.

The obvious size differences between normal human aorta, coronary artery and the murine aortic root (*i.e.* the predilection places of atherosclerotic lesion formation) are illustrated in figure 1.

The morphology of the intima (composed of endothelial cells with sometimes focal thickened segments of extracellular matrix), is essentially similar for these three vessels (Figure 1A-C). The internal and external elastic laminae of the coronary artery and to a lesser extent of the aorta are clearly shown. The Movat stain clearly illustrates the different structures of the muscular coronary artery and the elastic aorta. This is visualized by the large number of collagen and elastin filaments in the tunica media (black elastic fibers). In terms of structure, the adventitia of the coronary artery and aorta are almost similar. Differences were found for the adventitia and the vaso vasorum in these different arteries. The vaso vasorum in the coronary artery remains confined to the adventitia. In the human aorta the vaso vasorum crosses the medial/adventitial border whereas in mice the adventitia lacks vaso vasorum at the level of the aortic root.

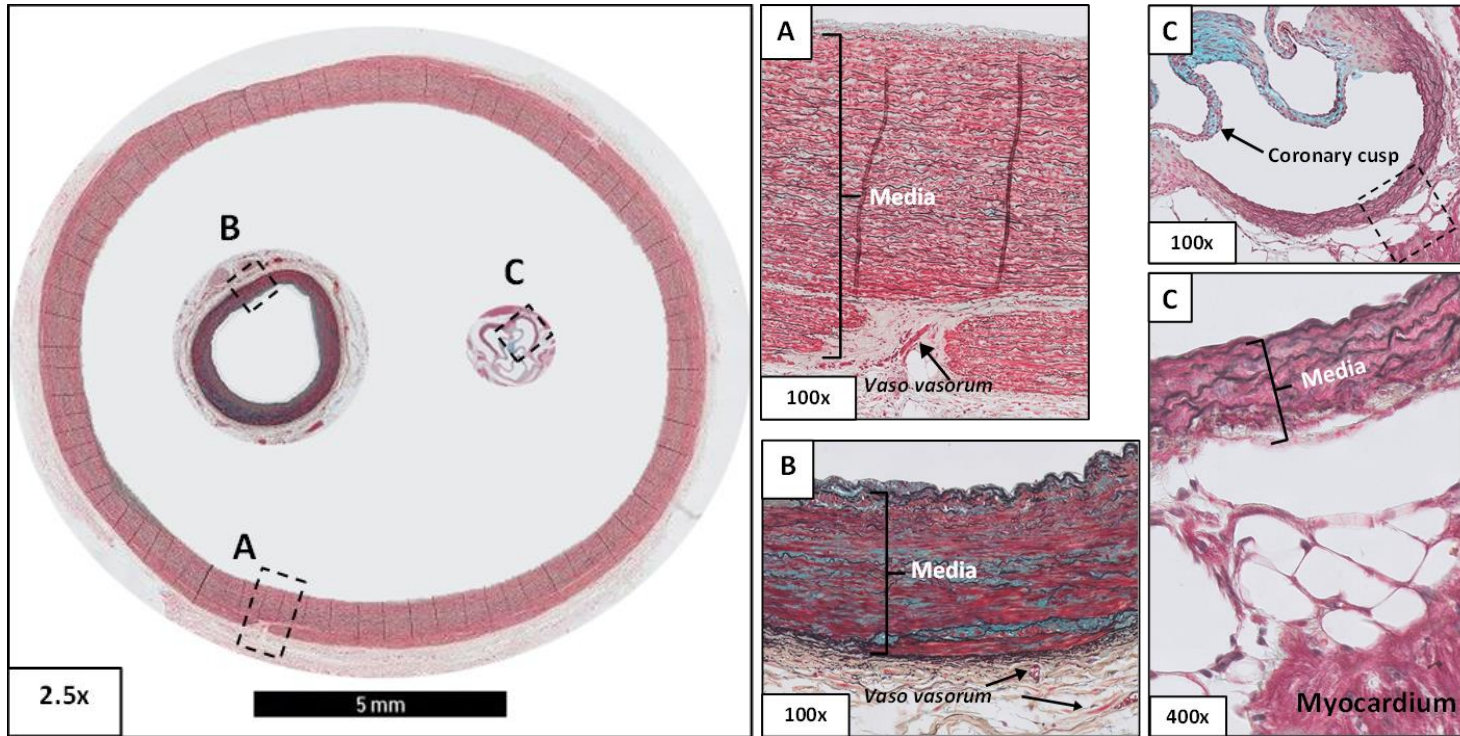


Figure 1. The normal human aorta (A), normal human coronary artery (B) and normal aortic root in mice (C). Images of a normal human peri-renal abdominal aorta, a normal human coronary artery and a sample of a normal aortic root of a mouse all taken at the same 2,5x magnification. This image clearly shows how the various vessels relate to one another. Additional high resolution images of the various vessels clearly show the concentrically arranged fenestrated elastic laminae in the media of the aorta (A) and the coronary artery (B) and to lesser extent in the aortic root (C). The medio-adventitial border of the human aorta and coronary artery consist of vasa vasorum while, by contrast, near the aortic root of mice only myocardium and fatty cells are identified. Note the absence of an intima in the aortic root.

Non-atherosclerotic intimal lesions (Figure 2 and 3)

Adaptive intimal thickening (AIT) and intimal xanthoma (IX)

Thickening of the intima (AIT) through infiltration of smooth muscle cells and deposition of a proteoglycan-rich matrix (Figure 2) is considered the earliest (and reversible) transition in the atherosclerotic process⁸. Lipid deposits, in particular foam cells, and inflammatory cells are absent. The media and adventitia remain normal. As illustrated in figure 2, the histological aspects of this stage are similar for the three vessels studied.

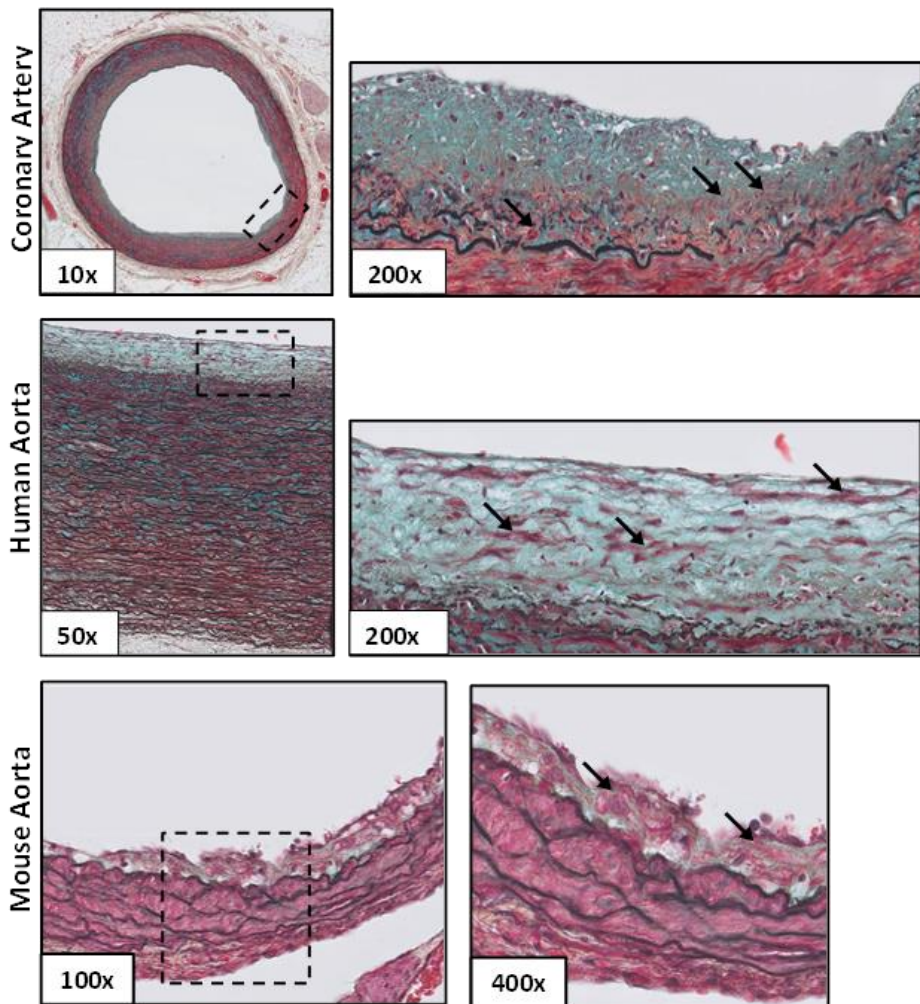


Figure 2. Adaptive intimal thickening in the human coronary artery, the human aorta and the mouse aorta. AIT is characterized by accumulating smooth muscle cells (black arrows) in a proteoglycan-rich matrix (green and turquoise background). Movat pentachrome staining.

Transition from AIT to IX (Figure 3) is hallmarked by the appearance of intimal foam cells. Significant variation is found in the number of foam cells and the size and thickness of foam cell clustering. By definition an (a-cellular) lipid pool is absent. The morphological characteristics of IX in the human arteries and the murine aortic root appear to be identical (Figure 3).

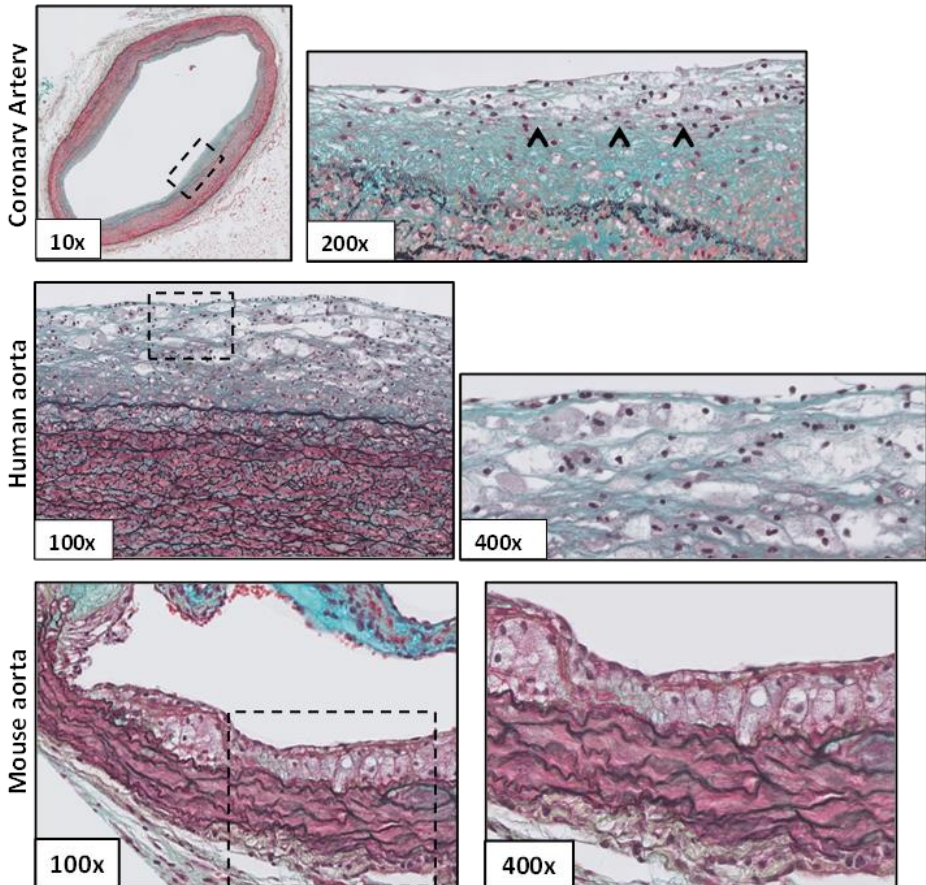


Figure 3. Intimal xanthoma in the human coronary artery, the human aorta and the mouse aorta. IX are characterized by one or several layers of infiltrating macrophage-derived foam cells (^) in the intima. Movat pentachrome staining.

Progressive atherosclerotic lesions (Figure 4 - 6).

Pathological intimal thickening (PIT), early and late fibroatheroma (EFA and LFA)

Focal acellular areas consisting of accumulated extracellular lipid within the intimal extracellular matrix (*i.e.* lipid pools; Figure 4) characterize progressive lesions. The earliest progressive lesion is referred to as PIT⁸. This phase is characterized by a matrix rich lipid pools, but absent cholesterol clefts. Lipid pools are mainly located at the medial border zone of the intima and are covered by smooth muscle cells and clusters of macrophages (Figure 4).

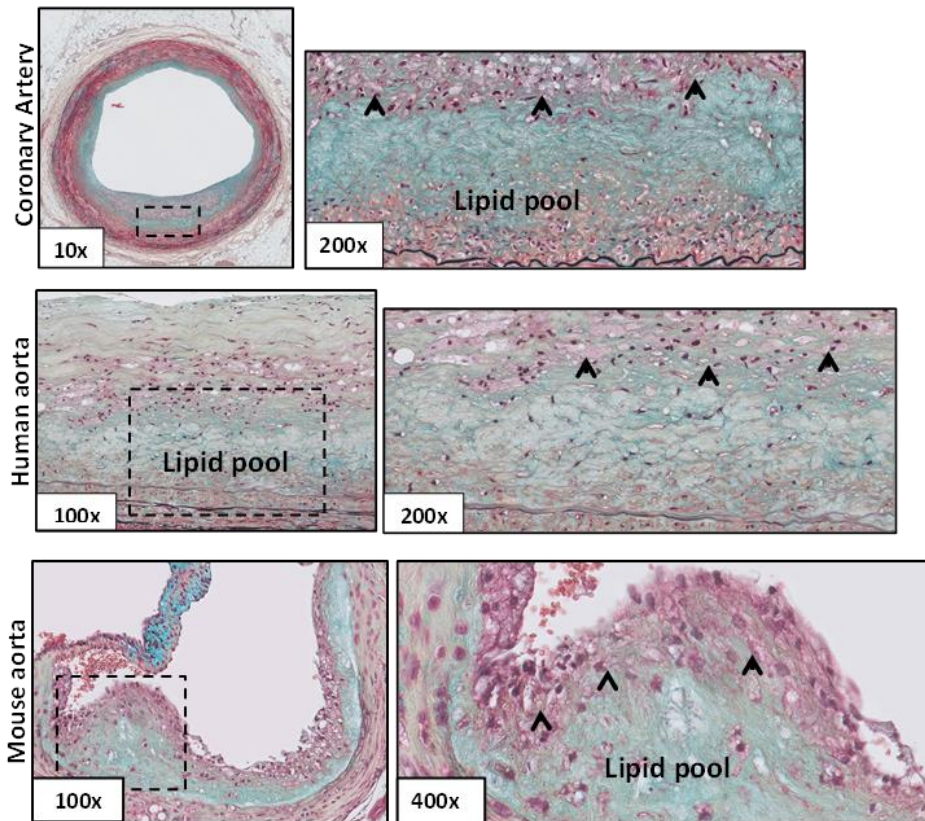


Figure 4. Pathological intimal thickening in the human coronary artery, the human aorta and the mouse aorta. PIT is characterized by the presence of lipid pools deep within the intima near the intimal medial border with overlying and infiltrating SMCs in a proteoglycan matrix with or without macrophage infiltration(^). The morphological appearance is almost identical in all the three vascular beds. Movat pentachrome staining.

A common phenomenon in the coronary artery, but not in the human and murine aorta, is disruption of the adjacent internal elastic lamina by infiltrating vasa vasora and presence of clusters of basal macrophages/foam cells in the vicinity of these infiltrating vasa vasora (illustrated in a high resolution image of the coronary artery; Figure 5). Coherence of these basal foam cells with lipid pools suggests that foam cells may contribute to lesion formation. The morphology of human and murine PIT is essentially similar, yet infiltrating vasa vasora are absent in murine lesions

Transition of PIT to an EFA is characterized by the emergence of cholesterol clefts and development of an acellular necrotic core in the existing lipid pools. Formation of this early necrotic core is thought to reflect consolidation of the existing extracellular components into one or more masses comprising large amounts of extracellular lipid, cholesterol crystals, and necrotic debris⁸. Transition from PIT to EFA in the coronary artery and human aorta is associated with an appreciable decrease in proteoglycans within the lipid pools. This, together with infiltrating macrophages ultimately undergoing necrosis or apoptosis, results in the development of an early necrotic core (Figure 4 and 5). Yet, the structure of the core remains intact. The overall morphological characteristics of the early necrotic core in the human aorta resemble that of the coronary artery with the exception of intraplaque haemorrhage which is, in the case of coronary EFA, frequently associated with infiltrating vasa vasora. Intraplaque haemorrhage is not seen within the aortic lesions.

Figure 5 shows clear gross resembles of the murine and human EFAs: the aspect of the necrotic core of advanced murine lesion bears many similarities with human EFA. However, clear differences are observed with respect to the tissue response. Mice EFA lack vasa vasora and, contrary to human EFA in which the necrotic core is covered by a multi layered fibrous cap, the core of mouse lesions is shielded from the lumen by a thin layer of SMCs (see the 400x high resolution images of the EFA in mice; Figure 5).

Transition towards a LFA is characterized by further maturation of the core that has now become mostly translucent and contains large cholesterol clefts (Figure 6). As with EFA, the necrotic core is covered by a thicker fibrous cap (*i.e.* a distinct layer of connective tissue). The thick, fibrous cap in the human coronary and human aorta consists of smooth muscle cells in a collagenous proteoglycan rich matrix, with varying degrees of infiltration by macrophage and lymphocyte infiltrates.

Importantly, the key characteristics of LFA (late necrotic core with a multi-layer fibrous cap) are absent in the murine models. Although large cholesterol clefts may occur in murine lesions, mice do not develop multi-layer thick fibrous caps.

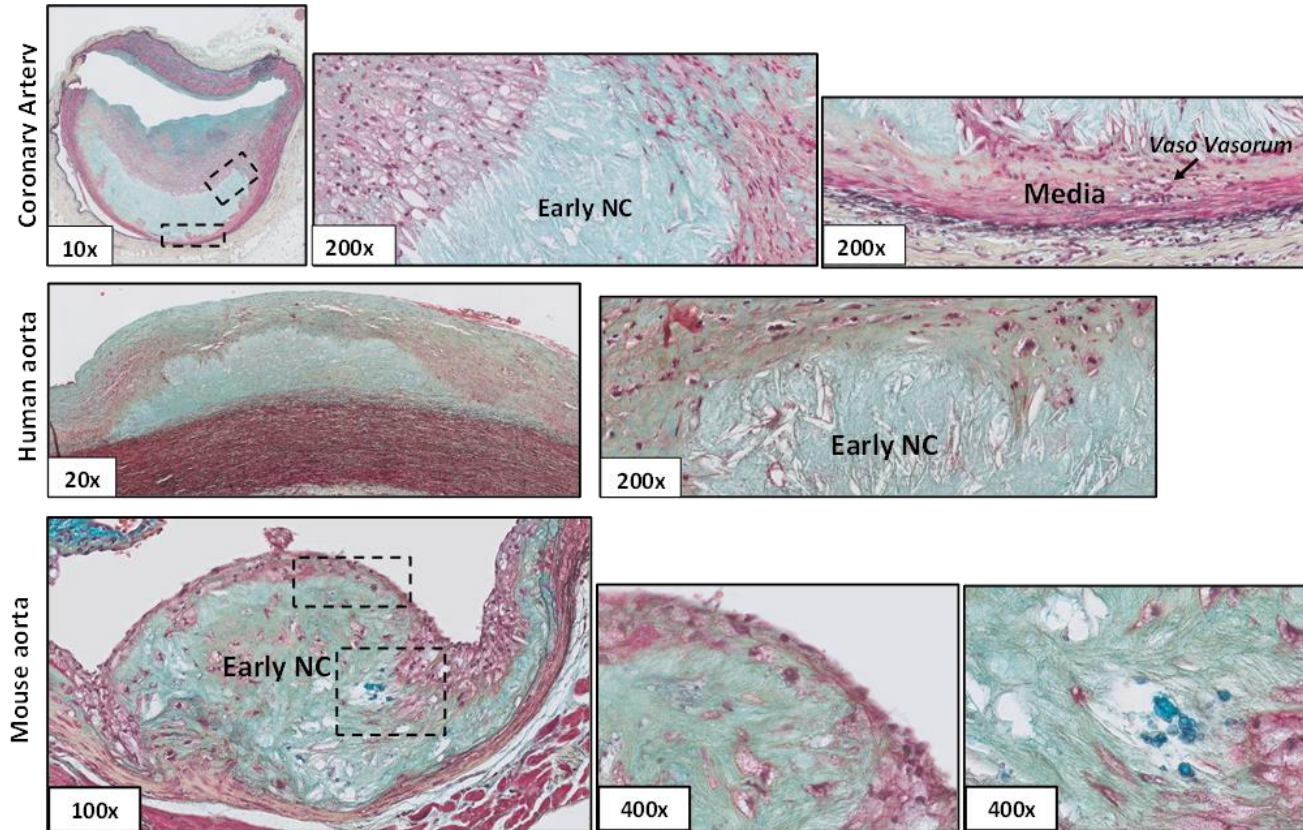


Figure 5. Early fibroatheroma in the human coronary artery, the human aorta and the mouse aorta. The EFA shows macrophage in the early necrotic core. Within the coronary artery the vaso vasorum extends through the media into the intima. This is not observed in the human aorta and in mice. Movat pentachrome staining.

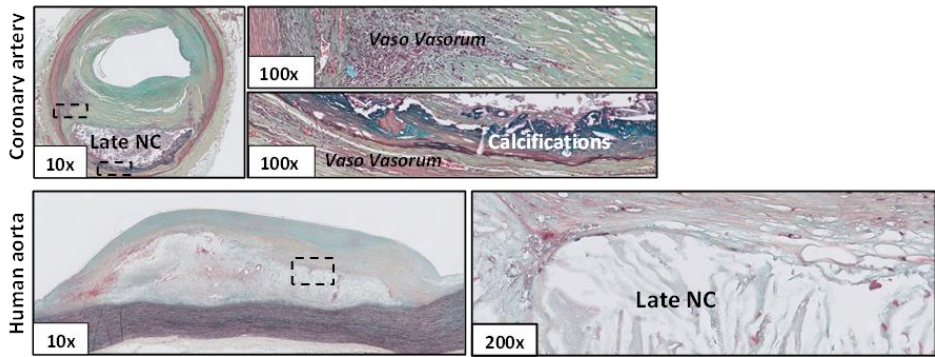


Figure 6. Late fibroatheroma in the human coronary artery and the human aorta. The larger size of the progressive lesions in the aorta compared to those identified in the coronary artery is probably the most obvious difference. The lipid pools lay deep within the intima near the intimal medial border with overlying and infiltrating macrophages (arrowheads) and areas of calcifications can easily be identified. The late necrotic cores are identical to the ones seen in the coronary artery. Unlike the coronary artery, the infiltrating vasa vasorum is limited to the intimo-medial border in the aorta. Movat pentachrome staining.

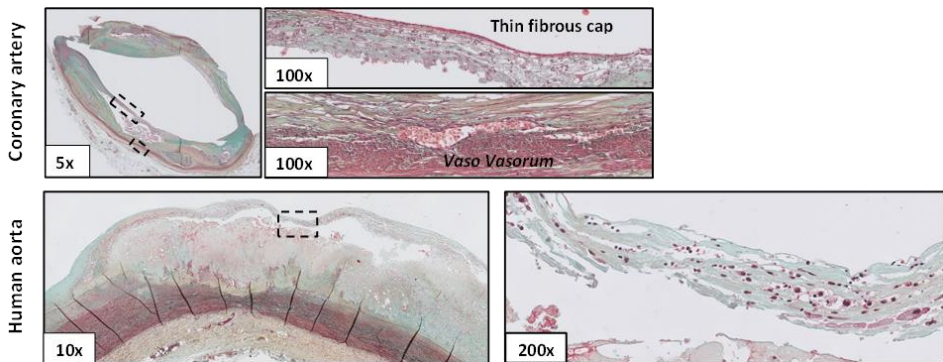


Figure 7. Thin cap fibroatheroma in the human coronary artery and the human aorta. Examples of a TCFA with 100x and 200x high resolution images of the thin fibrous cap with excessive macrophage infiltration, the progressive infiltrating vasa vasorum reaching within the necrotic core, the significant medial thinning and the abundant influx of inflammatory cells in the adventitia. Movat pentachrome staining.

Vulnerable atherosclerotic lesions (Figure 7 and 8).

Thin cap fibroatheroma (TCFA) and plaque rupture (PR)

Transition from a late progressive lesion (LFA) to a vulnerable lesion (TCFA) is defined by significant thinning of the fibrous cap (Figure 7). The necrotic core is usually larger, haemorrhage (in coronary lesions) and intraplaque vasa vasora are abundantly present in both coronary and aortic lesions (Figure 7).

PR is characterized by a disrupted fibrous cap whereby the overlying thrombus or remnants of thrombus is in continuity with the underlying necrotic core. Most

ruptured lesions have a typical large necrotic core and a disrupted fibrous cap infiltrated by macrophages and lymphocytes. The remnants of the luminal thrombus at the site of rupture are essentially composed of platelets and may be obstructive. TCFA and PR were not identified in mice.

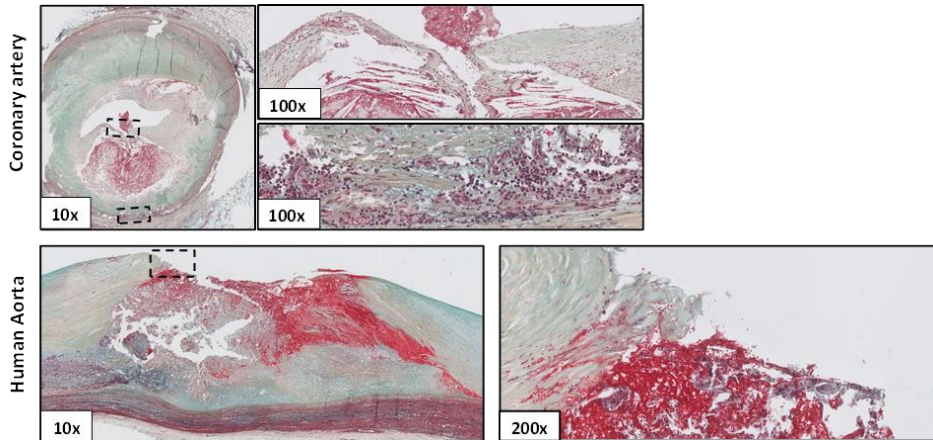


Figure 8. Plaque rupture in the human coronary artery and the human aorta. High resolution images PR with high resolution images of the thin fibrous cap and the rupture site. Movat pentachrome staining.

Stabilizing atherosclerotic lesions (Figure 9 and 10).

Healed ruptures (HR), fibrocalcific plaques (FCP)

Consolidation of a PR is characterized by a wound healing response in which a disrupted fibrous cap is covered by smooth muscle cell and proteoglycan cell-rich tissue (green/blue on Movat). The matrix within the healed fibrous cap consists of a proteoglycan-rich mass adjacent to or covering the fibrotic remnants of original cap. Lesions can contain large consolidated areas of calcification with few inflammatory cells and have a small or no necrotic core.

The FCP reflects consolidation of the healing process is associated with large condensed areas of calcification in an overall fibrotic, acellular remnant of the former necrotic core(s). Inflammatory cells are no longer present in the adventitia and there is regression of the pre-existing vasa vasora. Stacked FCPs illustrate the repetitive and chronic character of the atherosclerotic process.

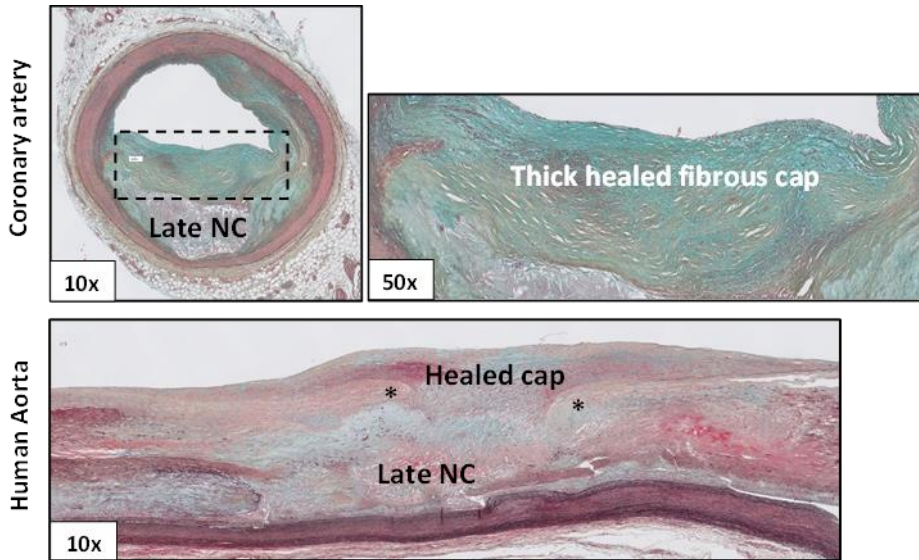


Figure 9. Healed rupture in the human coronary artery and the human aorta. The newly formed thick fibrous cap shields the remnants of the necrotic core from the lumen. The * denotes the edge of the ruptured site. This example of a FCP contains multiple calcified lesions in an almost circumferential appearance. Movat pentachrome staining.

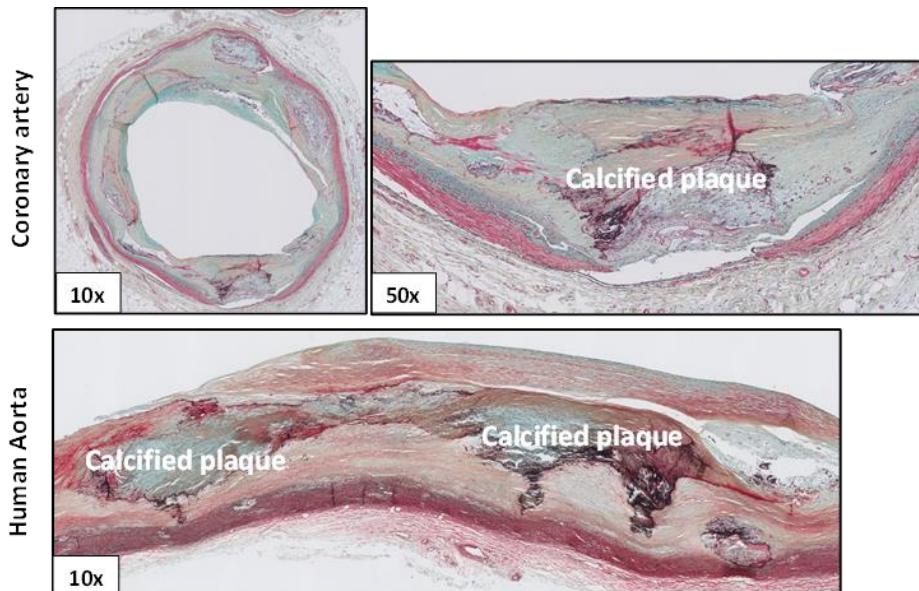


Figure 10. Fibrotic calcified plaque in the human coronary artery and the human aorta. Images of a FCP in coronary artery and the aorta. Note the relative quiescent adventitia when compared to the adventitia in the vulnerable atherosclerotic lesions (Figure 7 and 8). Movat pentachrome staining.

DISCUSSION

This systematic histopathologic comparison of human and murine atherosclerosis lesions shows that the established refined Virmani classification system for coronary atherosclerosis can be applied for grading of human aortic and murine atherosclerosis. Based on the histological characteristics the lesions in mice do not progress beyond the stage of EFA. Consequently, specific aspects of advanced and vulnerable lesion formation, and successive healing, are not present in murine experimental models. Therefore, these aspects cannot be studied in translational studies.

Staging the various phases of the atherosclerotic process and assessment of lesion vulnerability exclusively relies on a histopathological evaluation of plaque morphology. The first comprehensive classification scheme provided by the AHA involved an orderly numerical classification embedding six distinct categories⁴⁻⁷. Unfortunately this classification fails to capture several important clinical etiologies of coronary thrombus formation that are distinct from plaque rupture like plaque erosions and calcified nodules^{3, 8}. It further lacks the recognition of precursor lesions that potentially give rise to clinical events, does not reflect the dynamics of the disease process, and does not acknowledge the process of plaque healing. Based on their observations and experience, Virmani *et al.* refined the AHA classification in order to better reflect the heterogeneity of the late stages of atherosclerotic disease by staging atherosclerosis based on plaque morphology. This descriptive morphological classification incorporates both the various aspects of vulnerable lesion development and plaque destabilization, thereby better mirroring the pathophysiology of the disease⁸. Moreover, the progressive character of the classification system better reflects the natural history of the disease. Over the past years, the Virmani classification scheme has increasingly been employed in clinical research and practise but it is only sporadically employed for the evaluation in experimental models of disease^{13,14}. Our study demonstrates that the Virmani classification system allows for the analysis of morphological changes during early lesion development in humans aortic and murine samples, and we therefore advocate the use of the Virmani classification system in mice.

More specifically changes in matrix composition and tissue morphology are key elements in the atherosclerotic process¹⁵. These changes and co-localizations of proteoglycans, collagen and fibrinogen are insufficiently addressed with conventional stainings such as H&E and Sirius Red (SR). For example changes and co-localizations of proteoglycans, collagen and fibrinogen are missed with H&E staining. The Movat pentachrome staining is technically more challenging but provides a more detailed appreciation of the matrix biology of the plaque and

better distinguishes the more complex, advanced lesions from one another¹⁶. Hence, a Movat pentachrome staining provides additional information and should be preferred above a H&E and SR standard stainings. Movat staining shows that murine lesions do not develop aspects that characterize vulnerable lesions of humans. This notion contrasts with the progressive use of plaque collagen content (Sirius red (SR)) as a surrogate of plaque stability. Several studies suggest that SR staining can be used to identify the degree of plaque stabilization and destabilization in mice¹⁷. Sirius red stains ground substance and decreased SR content in mouse lesions reflects displacement of ground substance by foam cells and / or cholesterol clefts. However, the human-like fibrosis and cap formation with multiple layers is not identified in mice using the Movat staining. This reveals an important drawback in the translation of murine models of arteriosclerosis to the human situation. This is an important outcome of our study, the more so because mouse studies are often used to investigate basic molecular mechanisms or new intervention principles involving changes in lesion composition, potentially affecting lesion stability. Our comparisons of lesions in mice to vulnerable lesions in humans clearly demonstrate that there is no murine equivalent to the multi-layered cap in humans and that the morphological changes associated with plaque rupture in humans are not observed in mice. As a consequence, the ongoing discussion whether the absolute area or the lesional percentage of these three components is a good estimate of lesion stability in mice, comes into a different perspective. Furthermore SR staining solely reflects the substitution of the ground substance (*i.e.* matrix) by lipid accumulation and foam cells and does by itself not predict plaque stability. Our present study advocates a more careful interpretation of 'lesion stability data' in murine studies.

We previously validated the coronary artery-based Virmani classification for the human aorta⁹. We now extend this validation and show that this classification scheme can be directly applied to murine aortic atherosclerosis, allowing a one to one comparison of murine and human atherosclerosis at the various stages and positioning preclinical findings in the human context.

Our findings also show that the most advanced lesions identified in mice best match the morphological description of an EFA. This notion is based on the morphology of the lipid core and the fact that the lipid core is shielded from the bloodstream by one or several layers of smooth muscle cells. Compared to humans mice lesions lack an overlying cap, the hallmark of an EFA and LFA. Second, the integrity of the lipid core, with exception of the visible cholesterol clefts, remains intact. In humans the EFA precedes advanced LFA lesions, plaque destabilization, plaque rupture and subsequent plaque healing. These phenomena are all absent in the murine model of atherosclerosis, consequently prohibiting a translational analysis of plaque (de-)stabilisation in mice^{18,19}.

Our extensive histological analyses provides a number of clues why murine lesions fail to progress towards advanced lesions. In direct comparison with their human counterpart, the advancing lesions in mice are significantly smaller in size, lack ischemia and neovascularization^{20,21,22}. The relatively small lesions in mice do not progress beyond the critical 200 μm diffusion distance. As a result, the maximum diffusion distance of oxygen and nutrients in tissue is not reached and the entire atherosclerotic process can still evolve via diffusion, and an ischemic trigger is missing²³. It is thought that this ischemic trigger in human atherosclerosis is critical and eventually results in plaque neovascularization originating from the infiltrating vasa vasora²⁴. These vasa vasora are thought to further contribute to intraplaque haemorrhage due to leaky vessels and is a major contributor to plaque expansion via cholesterol influx with consequently thinning of the fibrous cap resulting in vulnerable rupture prone lesions in the coronary artery^{3,8}. Ischemia of the developing cap may also underlie the advanced fibrotic changes in humans.

Fibrosis is a notable phenomenon in advancing lesions. In fact, it is a distinct part of the atherosclerotic process in human that is minimally present in mice¹⁴. Factors not captured in this study, but need to be kept in mind interpreting the results, are the age related differences of the plaques in humans and mice and the fundamental immunological and inflammatory differences between rodents and humans^{25,26,27}.

The major contributor to lipid core expansion in mice is the excessive intimal cholesterol influx due to the required hypercholesterolaemia²⁸ as well a systemic inflammatory component involving inflammatory mediators produced locally in the aorta and in liver or adipose tissue^{29, 30}. Lesions with this type of histology lack a pronounced fibrotic component, therefore remain reversible and are rarely clinically significant except in examples of severe experimental hypercholesterolaemia, a situation that is quite atypical and does not reflect the chronic multifactorial nature or complexity of the human disease^{31,32}.

Limitations; This is an observational study with findings merely based on histological Movat Pentachrome staining of human coronary and aortic tissue, and mice tissue. Consequently, findings in this study should be considered in this context.

Conclusion; The initial and more advanced atherosclerotic lesions in mice follow a similar pattern of progression compared to coronary and aortic atherosclerosis in humans and can be classified accordingly using a uniform, detailed morphological classification scheme based on the Virmani classification. Experimental data obtained from murine studies to estimate lesion stability should be interpreted with care since morphological changes associated with instable lesions in humans are not observed in mice.

REFERENCES

- 1 Ross R. Atherosclerosis—an inflammatory disease. *The New England Journal of Medicine*. 1999;340(2):115–126.
- 2 Glass CK, Witztum JL. Atherosclerosis the road ahead. *Cell*. 2001;104:503–516.
- 3 Virmani, R., Kolodgie, F. D., Burke, A. P., Farb, A., & Schwartz, S. M. (2000). Lessons from sudden coronary death a comprehensive morphological classification scheme for atherosclerotic lesions. *Arteriosclerosis, thrombosis, and vascular biology*, 20(5), 1262–1275.
- 4 Stary, H. C., *et al.* A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, 1992, 85.1: 391.
- 5 Stary, Herbert C., *et al.* A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 1994, 14.5: 840–856.
- 6 Stary, Herbert C., *et al.* A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, 1995, 92.5: 1355–1374.
- 7 Stary, Herbert C. Natural history and histological classification of atherosclerotic lesions an update. *Arteriosclerosis, thrombosis, and vascular biology*, 2000, 20.5: 1177–1178.
- 8 Yahagi, K., Kolodgie, F. D., Otsuka, F., Finn, A. V., Davis, H. R., Joner, M., & Virmani, R. (2015). Pathophysiology of native coronary, vein graft, and in-stent atherosclerosis. *Nature Reviews Cardiology*.
- 9 van Dijk RA, Virmani R, von der Thüsen JH, Schaapherder AF, Lindeman JH. The natural history of aortic atherosclerosis: a systematic histopathological evaluation of the peri-renal region. *Atherosclerosis*. 2010;210(1):100–106.
- 10 Zadelaar, S., Kleemann, R., Verschuren, L., de Vries-Van der Weij, J., van der Hoorn, J., Princen, H. M., & Kooistra, T. (2007). Mouse models for atherosclerosis and pharmaceutical modifiers. *Arteriosclerosis, thrombosis, and vascular biology*, 27(8), 1706–1721.
- 11 Daugherty, A. Mouse models of atherosclerosis. *The American journal of the medical sciences*, 2002, 323.1: 3.
- 12 Morrison M, van der Heijden R, Heeringa P, Kaijzel E, Verschuren L, Blomhoff R, Kooistra T, Kleemann R. Epicatechin attenuates atherosclerosis and exerts anti-inflammatory effects on diet-induced human-CRP and NFκB in vivo. *Atherosclerosis*. 2014 Mar 31;233(1):149–56.
- 13 Koppa T, Sakakura K, Pacheco E, Cheng Q, Zhao X, Acampado E, Finn AV, Barakat M, Maillard L, Ren J, Deshpande M. Preclinical evaluation of a novel polyphosphazene surface modified stent. Koppa Preclinical testing of a PzF-coated stent. *International Journal of Cardiology*. 2016 Nov 1;222:217–25.
- 14 He C, Medley SC, Hu T, Hinsdale ME, Lupu F, Virmani R, Olson LE. PDGFR [beta] signalling regulates local inflammation and synergizes with hypercholesterolaemia to promote atherosclerosis. *Nature communications*. 2015 Jul 17;6.
- 15 Hansson, G. K., & Libby, P. (2006). The immune response in atherosclerosis: a double-edged sword. *Nature Reviews Immunology*, 6(7), 508–519.

- 16 Farb A, Weber DK, Kolodgie FD, Burke AP, Virmani R. Morphological Predictors of Restenosis After Coronary Stenting in Humans. *Circulation*. 105:2974-2980.
- 17 Mallat, Z., Corbaz, A., Scoazec, A., Graber, P., Alouani, S., Esposito, B., Tedgui, A. (2001). Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circulation research*, 89(7), e41-e45.
- 18 Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature*, 473(7347), 317-325.
- 19 Whitman, S. C. (2004). A practical approach to using mice in atherosclerosis research. *The Clinical Biochemist Reviews*, 25(1), 81.
- 20 Hartwig, H., Silvestre-Roig, C., Hendrikse, J., Beckers, L., Paulin, N., Van der Heiden, K. & Soehnlein, O. (2015). Atherosclerotic Plaque Destabilization in Mice: A Comparative Study. *PLoS one*, 10(10).
- 21 Maganto-Garcia, E., Tarrio, M., & Lichtman, A. H. (2012). Mouse models of atherosclerosis. *Current Protocols in Immunology*, 15-24.
- 22 Lutgens, E., Daemen, M., Kockx, M., Doevendans, P., Hofker, M., Havekes L, Wellens H, de Muinck ED. Atherosclerosis in APOE* 3-Leiden transgenic mice: from proliferative to atheromatous stage. *Circulation*. 1999; 99(2), 276.
- 23 Chauhan, V. P., Stylianopoulos, T., Boucher, Y., & Jain, R. K. (2011). Delivery of molecular and nanoscale medicine to tumors: transport barriers and strategies. *Annual review of chemical and biomolecular engineering*, 2, 281-298.
- 24 Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ, Bijmens AP. (2008). Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *Journal of the American College of Cardiology*, 51(13), 1258-1265.
- 25 Martinez FO, Helming L, Milde R, Varin A, Melgert BN, Draijer C, Thomas B, Fabbri M, Crawshaw A, Ho LP, Ten Hacken NH, Cobos Jiménez V, Kootstra NA, Hamann J, Greaves DR, Locati M, Mantovani A, Gordon S. Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. *Blood*. 2013;121(9):e57-69.
- 26 Bryant CE, Monie TP. Mice, men and the relatives: cross-species studies underpin innate immunity. *Open Biology*. 2012;2(4):120015.
- 27 Colucci F, Di Santo JP, Leibson PJ. Natural killer cell activation in mice and men: different triggers for similar weapons? *Nature Immunology*. 2002;3(9):807-813.
- 28 van der Hoorn, J. W., Kleemann, R., Havekes, L. M., Kooistra, T., Princen, H. M., & Jukema, J. W. (2007). Olmesartan and pravastatin additively reduce development of atherosclerosis in APOE* 3Leiden transgenic mice. *Journal of hypertension*, 25(12), 2454.
- 29 Kleemann R, Princen HM, Emeis JJ, Jukema JW, Fontijn RD, Horrevoets AJ, Kooistra T, Havekes LM. Rosuvastatin Reduces Atherosclerosis Development Beyond and Independent of Its Plasma Cholesterol-Lowering Effect in APOE* 3-Leiden Transgenic Mice Evidence for Antiinflammatory Effects of Rosuvastatin. *Circulation*, 2003, 108.11: 1368-1374.

- 30 Salic K, Morrison MC, Verschuren L, Wielinga PY, Wu L, Kleemann R, Gjorstrup P, Kooistra T. Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. *Atherosclerosis*, 2016, 250: 158-165.

- 31 van Dijk RA, Duiniveld AJ, Schaapherder AF, Mulder-Stapel A, Hamming JF, Kuiper J, de Boer OJ, van der Wal AC, Kolodgie FD, Virmani R, Lindeman JH. A change in inflammatory footprint precedes plaque instability: a systematic evaluation of cellular aspects of the adaptive immune response in human atherosclerosis. *Journal of American Heart Association*. 2015;4(4).

- 32 R.A. van Dijk, K. Rijs, A. Wezel, J.F. Hamming, F.D. Kolodgie, R.Virmani, A.F. Schaapherder, J.H.N. A systematic evaluation of the cellular innate immune response during the process of human atherosclerosis. *Journal of American Heart Association*. 2016.

