The natural history of human atherosclerosis : a histopathological approach

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CHAPTER 2

The natural history of aortic atherosclerosis: A systematic histopathological evaluation of the peri-renal region

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ABSTRACT

Background: Risk factor profiles for the different vascular beds (*i.e.* coronary, carotid, peripheral and aortic) are remarkably different, suggesting that atherosclerosis is a heterogeneous disorder. Little is known about the morphologic progression of atherosclerosis in the peri-renal aorta, one of the primary predilection sites of atherosclerosis.

Methods: A systematic analysis was performed in 260 consecutive peri-renal aortic patches (stained with Movat Pentachrome and H&E) collected during organ transplantation (mean donor age 46. 5 (range 5–76) years; 54% ♂;mean BMI 24. 9; 40% smokers; 20% hypertensive). Plaque morphology was classified according to the modified AHA classification scheme proposed by Virmani *et al*. (Arteriosclerosis, Thrombosis, and Vascular Biology; 2000). Immunostaining against CD68 was used to identify the distribution of intimal macrophages and monocytes in several predefined locations among various plaque types and fibrous cap thickness.

Results: There was significant intimal thickening ($p < 0.013$) and medial thinning ($p < 0.032$) with advancing age. The incidence of atherosclerotic plaques in the abdominal aorta correlated with age ($r = 0.640$, $p = 0.01$). During the first three decades of life adaptive intimal thickening and intimal xanthomas were the predominant lesions. In contrast, the fourth, fifth and sixth decades hallmarked more complicated plaques of pathological intimal thickening, early and late fibroatheromas (EFAs and LFAs), thin-cap FAs (TCFAs; cap thickness <155m), ruptured plaques (PRs), healed rupture and fibrotic calcified plaques. The mean percentage of lesional macrophages increased significantly from LFAs to TCFAs (5–17%; $p < 0.001$). Macrophage infiltration of the fibrous cap was negatively correlated with fibrous cap thickness ($p < 0.0004$); TCFAs and PRs (caps < 100μm) contained significantly more macrophages (19%) compared with caps 101–300μm (6%) and >300μm (2%). Macrophages in shoulder regions were highest in early and late FAs (~45%) followed by TCFAs (27%) and PR (20%). Further, intimal *vasa vasorum* were mostly seen adjacent to the necrotic core of advanced atherosclerotic plaques and remained confined to the intimo-medial border despite marked thickening of the intima.

Conclusion: This study shows that peri-renal aortic atherosclerosis starts early in life. Gross plaque morphologies of the peri-renal abdominal aorta are similar to coronary atherosclerosis yet indications were found for site specific differences in macrophage content and neovascularization.

INTRODUCTION

Atherosclerosis is a complex pathology of large and medium sized arteries that leads to cardiovascular disease. Although elevated LDL-cholesterol is generally accepted as the dominant and universal risk factor for atherosclerotic disease, risk factor profiles for the various predilection places are remarkable distinct[1,2]. Consequently it has been suggested that atherosclerosis is a heterogeneous disease that may proceed through different pathophysiological pathways[3].

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 [4,5,6,7,8]. This lack of information is remarkable, especially considering the fact that the aorta is generally used in rodent studies of atherosclerotic disease.

In order to establish the natural course of human aortic atherosclerotic disease we here systematically evaluate the histological progression aortic atherosclerosis in a well-documented, large tissue bank of human peri-renal aortic tissue from apparently healthy individuals (organ donors) that covers all age groups and the whole spectrum of atherosclerotic disease. Atherosclerotic lesions were classified according to adapted AHA classification[9] as proposed by Virmani *et al.*[4]. Particular attention is paid to the lesional macrophages as well as to the morphology of the fibrous cap, the critical determinant of plaque stability.

MATERIAL & METHODS

Patients and tissue sampling

Two hundred and sixty consecutive aortic patches were studied. The patches were obtained during clinical organ transplantation with grafts derived from cadaveric donors. All the aortic samples were harvested by the same surgeon at the time of transplantation or organ harvest. Two centimetres of excessive aorta proximal and distal from the ostium of the renal artery was removed and used for this study. Aneurysmal aortas (circumference > 2. 5 cm, n = 2) were excluded from the study. Demographic data concerning the causes of death are summarized in supplemental Tables I–II (data available online at http://www.atherosclerosis-journal.com). All donors met the criteria set by The Eurotransplant Foundation. Due to national regulations, only for transplantation relevant data from donors is available for research. 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 (http://www.federa.org/?s=1&m=82&p=0&v=4#827).

Histological classification of lesions

All patches were divided in parallel sections of approximately 5mm width. Movat pentachrome staining was performed for the histological evaluation of each individual section. Each section was individually classified according to the modified classification of the AHA as proposed by Virmani *et al*.[4] (Table 1, Fig. 1) by two independent observers with no knowledge of the characteristics of the aortic patch. The section showing the highest degree of atherosclerosis was used as the reference section for further studies.

In progressive atherosclerotic lesions the fibrous cap and shoulder regions were assessed for the presence of macrophages. A TCFA was defined as a fibrous cap less than 155μm thick. The 155μm thickness in cases without rupture was chosen as a criterion for thin cap because in arteries with ruptured plaque, the mean (±SEM) cap thickness was 99±27μm (95 percent of the caps measured less than154μm). Note that independent of dominant plaque type there may be several plaque components in a single section. For detailed descriptions concerning histological components of the aortic sections we refer to the online supplements.

Immunohistochemistry

The paraffin embedded sections were stained for the identification of intimal macrophages and monocytes (CD68; clone KP-1; 1:400 overnight; Dako, Denmark) as previously described and evaluated at predefined locations[8]. Smooth muscle actin (clone 1A4, 1:400; overnight; Dako, Denmark) and smooth muscle heavy chain myosin (clone 3F8, 1:1000; overnight; ABCAM, Cambridge, UK) staining were used to determine the lesional VSMC phenotype in the dominant lesion.

Morphological analysis

Morphometric and histological analyses were performed with calibrated software (IPLab, Scanalytics Inc., Rockville, MD) on all sections stained with Movat pentachrome. All measurements were performed on the dominant plaque in the tissue section showing the most advanced grade of atherosclerosis. For detailed descriptions concerning the morphological measurements of the various components of the aortic sections we refer to the online supplements.

Statistical analysis

Data in figures are presented as mean ± SEM. Mean variables between the various lesions were compared with the one-way analysis of variance (ANOVA; SPSS 16. 0; Chicago, IL) Differences between the groups were assessed by Fisher's LSD. Spearman's correlation was used to demonstrate the relationship between the age of the aortic patch and the severity of atherosclerosis and intimal thickness. Pearson 2-test was used to compare the presence of *vasa vasorum* in the intima in the various lesions. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Studied population

Data were obtained from 260 consecutive peri-renal aortic samples from organ donors in age from5 to 76 years (Supplemental Table 1, available online at http://www.atherosclerosis-journal.com). Males and females were evenly distributed as was the mean age of both genders. Within the studied population 81 subjects were considered obese (BMI > 25). One hundred and four individuals had a known history of smoking at time of death with an average of 21 and 22 pack-years (1 package of cigarettes/day/year) for males and females, respectively. One-fifth of the population had a history of hypertension (antihypertensive medication or systolic blood pressure >140mmHg and diastolic >90mmHg in the period preceding death). Only 4 patients were on statin therapy. Further details on the donor characteristics are provided in the online supplement.

Morphometric measurements

Effect of age on aortic intimal thickness (supplemental Fig. Ia)

The intimal thickness increases significantly with advancing age ($R= 0.601$, $p < 0.01$). In the third decade of life the intima of the abdominal aorta is about 0.5mm thick and almost triples in size in the upcoming years. In comparison to non-smokers, intima thickness is significantly increased in the younger smokers (31–45 years, $p < 0.003$), but no differences are found for the other age groups (46–60 and >60 years, $p < 0.203$ and $p < 0.232$ respectively; data not shown). No significant difference in intimal thickness was observed between the sexes or in the presence of (a history of) hypertension.

Effect of age on aortic medial thickness (supplemental Fig. Ib)

The medial thickness increases during the first 45 years of life but significant thinning was observed from the fifth decade and onwards ($p < 0.0001$). Smoking was associated with increased medial thinning in the age group >60 years ($p < 0.030$; data not shown). Medial thickness was not influenced by gender or by a history of hypertension.

Table 1 Histological classification of the aortic tissue according to the modified classification of the AHA proposed by Virmani *et al*. [4].

Morphological description	Associated AHA classification	Male		Female	
		N	Mean age (range)	N	Mean age (range)
Normal aorta	-	5	22.4 (6–55)	4	10.3 (4–17)
Nonprogressive intimal lesions					
Adaptive intimal thickening	I	26	31.4 (16–53)	25	40.0 (12–67)*
Intimal xanthoma	II	18	34.5 (13–62)	16	35.3 (11–57)
Progressive atherosclerotic lesions					
Pathological intimal thickening	III	19	48.7 (17–66)	17	52.2 (35–68)
Early fibroatheroma	IV	12	51.1 (36–62)	11	45.6 (36–56)
Late fibroatheroma	IV/Va	16	61.8 (50–75)	16	50.6 (37–74)**
Thin-cap fibroatheroma	-	19	56.7 (48–71)	15	53.3 (35–67)
Plaque rupture	VI	7	56.8 (48–60)	5	52.5 (43–62)
Healing rupture	VI	6	64.0 (57–73)	3	55.3 (42–67)
Fibrotic calcified plaque	Vb,c, VII	12	63.2 (47–76)	8	56.6 (48–72)

* $p < 0.023$ in AIT between mean age among gender.

**$p < 0.013$ in LFA between mean age among gender.

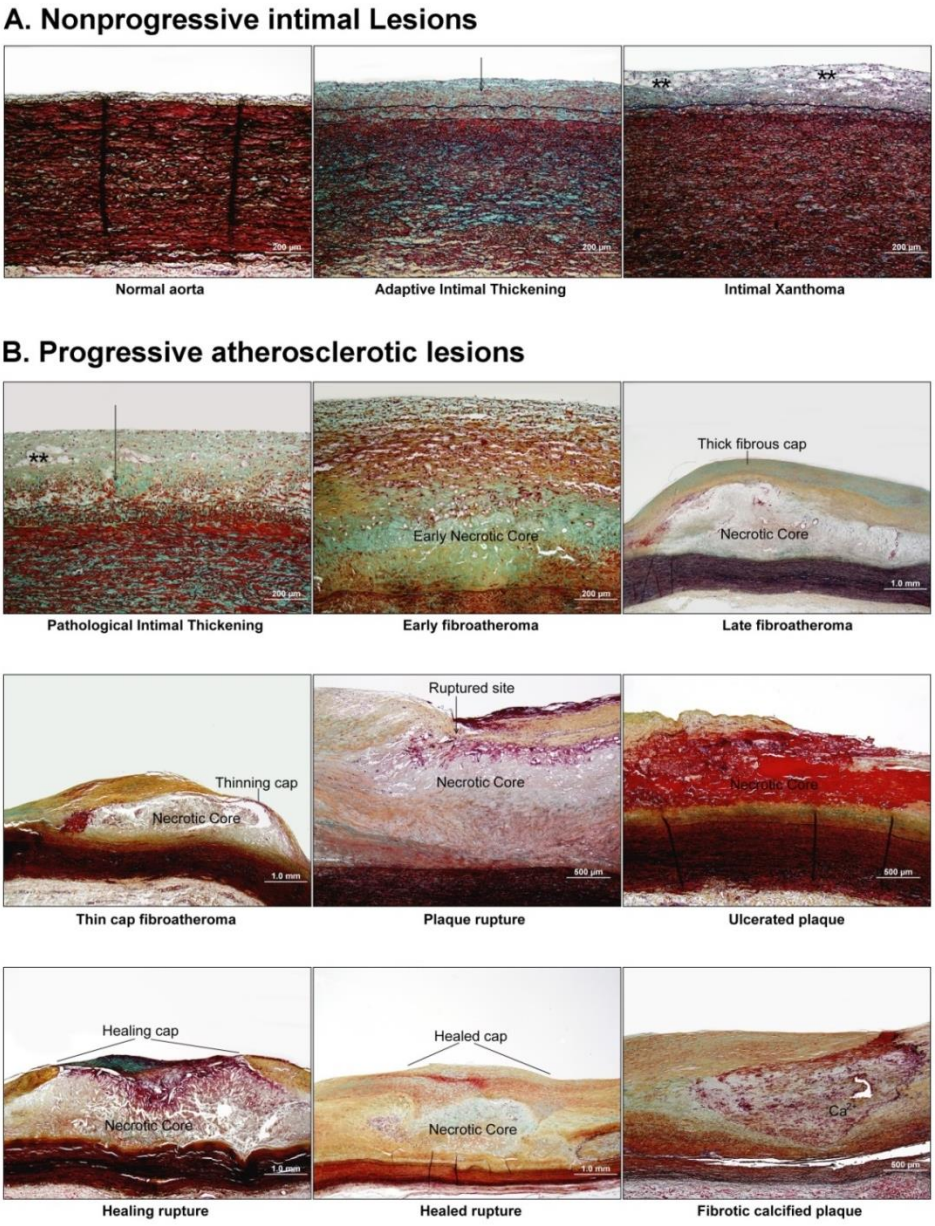

Figure 1. *Nonatherosclerotic intimal lesions (Normal, AIT and IX) and progression prone atherosclerotic lesions (PIT, early- and late-FA, TCFA, PR, ulcerated plaque and HR).* (**A**) Adaptive intimal thickening consists mainly of smooth muscle cells in a proteoglycan-rich matrix (black arrow). Intimal xanthomas were characterized by infiltrating macrophage-derived foam cells (*) in the intima. (Movat pentachrome stain). (**B**) Pathological intimal thickening is characterized by the presence of lipid pools deep within the intima near the intimal medial border with overlying SMCs (black arrow) in a proteoglycan matrix with or without macrophage infiltration (*). The early FA shows macrophage in the early necrotic core. After a rupture the plaque can become ulcerated and of the 260 studied aortas, only one lesion met that criterion. (Movat pentachrome stain).

Effect of age on lesion progression

Pre-atherosclerotic lesions (nonprogressive intimal lesions) (Figs. 1a, 2 and Table 1)

Age and gender distribution of the 260 histologically classified lesions of the aortic samples are shown in Table 1 and Fig. 2. Nine specimens were classified as normal aortic tissue as no signs of thickening of the intima were detected. Aortic tissue with intimal thickening consisting mainly of SMCs with an increase in proteoglycan-rich matrix and are referred to as AIT. As soon as macrophage-derived foam cells become evident in the intima the lesion are referred to as IX which are known as "fatty streaks". These lesions are seen within all age groups but were most predominant during the first three decades of life.

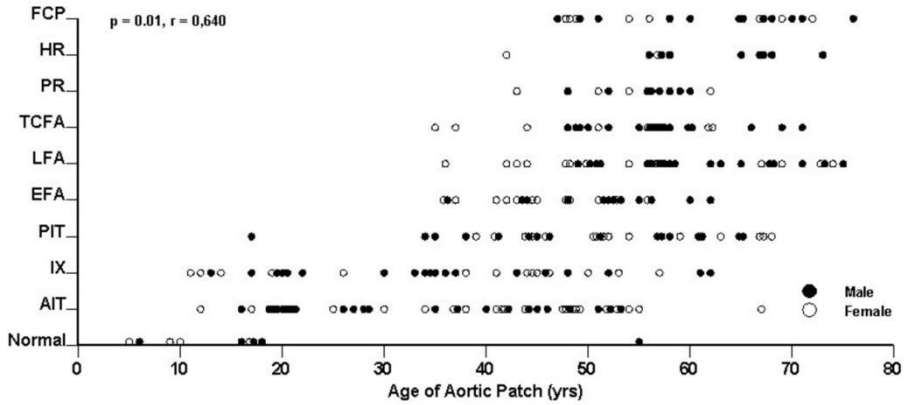

Figure 2. *Histological classification of human aortic atherosclerotic lesion type and the relationship to age.* Two hundred sixty aortic patches were characterized for the type of atherosclerosis according to Virmani *et al*.4 and plotted in relation to the age of the donor. There is a significant correlation of lesion type with age (R = 0.640, p = 0.01). For abbreviations see histological classification in Material and Methods section.

Progressive atherosclerotic lesions (Figs. 1b and 2 and Table 1)

A strong positive correlation (R = 0.640, $p < 0.01$) was found between the age of the donors and the degree of atherosclerosis when classified according to pathologic subtypes (Table 1 and Fig. 2). The fourth, fifth and sixth decade of life showed progressive advancement of the atherosclerotic lesions.

Lesions are evenly distributed among both genders. Pathological intimal thickening was frequently observed in young individuals and in the male population. However, females tend to have more advanced lesions at a younger age (from EFA to more advanced plaques) with the mean age for FA being significantly lower in comparison to men ($p < 0.013$).

Despite the noticeable thinning of the media during the evolution of the atherosclerotic plaque the internal elastic lamina remained grossly intact.

Advancing atherosclerotic lesions were associated with a significant increase in intimal *vasa vasorum* ($p < 0.001$; Fig. 3) at the intimo-medial border. The same phenomenon was seen within the inner third of the media with the presence of *vasa vasorum* correlating with advancing plaques (data not shown).

With 34 cases of TCFAs, 12 PRs and 9HRs the incidence of vicious lesions is remarkably high in this apparently healthy population. In PR the area of the fibrous cap disruption with an overlying thrombus showed continuity with the underlying necrotic core and was located near the centre of the plaque in the majority of cases. The remnants of the fibrous cap and the large necrotic core are rich in macrophages.

The nine cases of healing or healed ruptures show a remarkable proteoglycan and SMC rich cap between the remnants of the ruptured cap (Fig. 1b). The necrotic core is separated from the lumen and still consists of macrophages and lymphocytes. The healed cap however shows a reduction in the amount of infiltrated macrophages and an increase of SMCs when compared to TCFAs and PRs.

The FCP partly matches the criteria of a HR, but the necrotic core is largely 'missing'. If present at all it was usually small and contained extensive amounts of calcium.

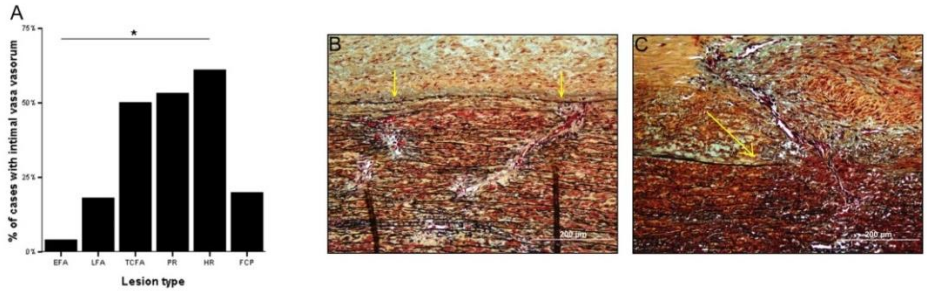

Figure 3. Percentage of cases with intimal *vasa vasorum* in relation to type of atherosclerotic plaque. (**A**) The number of cases with intimal *vasa vasorum* increased significantly with advancing atherosclerotic lesion (*$p < 0.001$). (**B**) High power magnification (200×) of *vasa vasorum* in the inner third of the media with intact internal elastic lamina (yellow arrows) in a case of early fibroatheroma. (**C**) High power magnification (200×) of *vasa vasorum* in the intima in the intimo-medial border showing disrupted internal elastic lamina (yellow arrow) in a case of plaque rupture. Bars represent 200m. For abbreviations see histological classification in Material and Methods section.

SMC phenotype, intimal and medial thickness in the atherosclerotic lesions (supplemental Figs. II–IV)

The intima thickens during lesion progression (PIT to early FA) but decreases during development of TCFAs and PRs. There is a trend towards increased intima thickness during healing of the ruptured plaque but thickness decreases again in FCPs with deposition of large amounts dense collagen.

A decrease in medial thickness was found when the lesion progresses into a late fibroatheroma and beyond. (supplemental Fig. IIb).

Alpha-SMC-actin was abundantly expressed in the intimal and medial layers. No relation was found between plaque progression and α-SMC-actin expression. Progression of atherosclerotic lesions was associated with reduced intimal SMC myosin heavy chain (SMC-MHC) expression in the shoulder and cap regions. SMC-MHC in the medial layers decreased with advancing lesions and was confined to the medio-adventitial border in the more advanced lesions (supplemental Figs. III and IV).

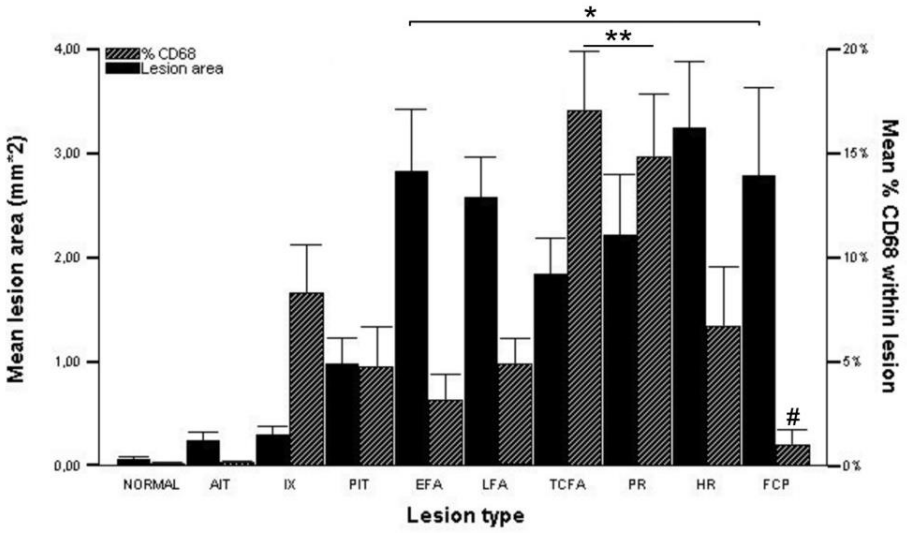

Figure 4. Total lesion area in relation to the atherosclerotic lesion type plotted with adjoining mean percent of CD68 within the total lesion type. The total lesion area (solid bars) show significant increase in lesion size in the early fibroatheroma and more advanced lesions (*$p < 0.027$). From the fatty streak to progression of advanced lesion types, the amount of macrophages in the lesions (striped bars) is significantly increased ($p < 0.023$). The mean percent of macrophages were maximum in the TCFA and PR (**$p < 0.003$) and thereafter decreasing in HR and FCP (#$p < 0.030$). Solid bars represent mean values (±SEM), striped bars represent mean percent values (±SEM). For abbreviations see histological classification in Material and Methods section.

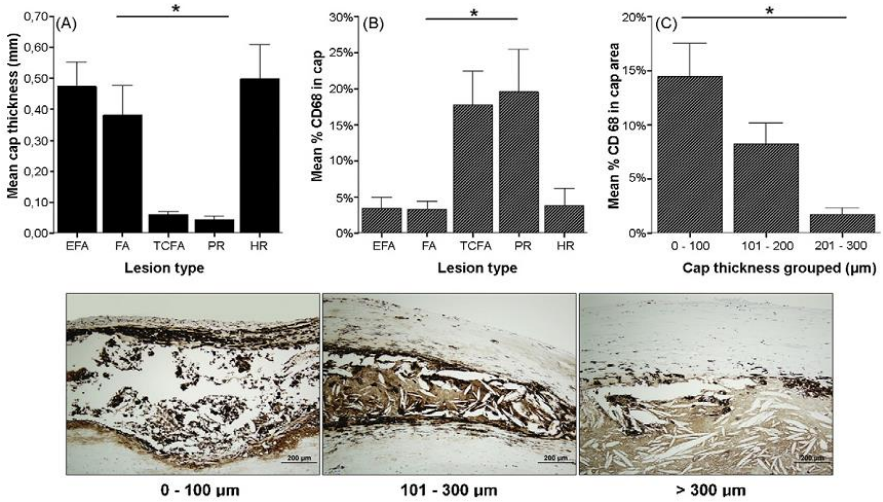

Figure 5. Fibrous cap thickness in relation to the atherosclerotic lesion type and macrophage distribution. (**A**) There is significant thinning of the fibrous cap in the thin cap fibroatheroma and ruptured plaque (*p < 0.003). (**B**) A significant increase in macrophages infiltration was observed in thin-cap fibroatheroma and ruptured plaques (*p < 0.003). Note that the fibrous cap in the healed ruptures shows a remarkable increase in thickness and decrease in macrophages infiltration. (**C**) The amount of macrophages in cap areas >300µm is significantly lower when compared to thickness <100µm (*p < 0.0004). Solid bars represent mean values (±SEM), striped bars represent mean percent values. Bars represent 200µm. For abbreviations see histological classification in Material and Methods section.

Macrophage infiltration with plaque progression

Lesional macrophages (Fig. 4)

Aortic IX are small (average size 0.3mm2) and are characterized by profuse macrophage infiltration. With advancing atherosclerotic lesions, lesion size increases and the relative percentage of macrophages decreases. The mean percent of macrophages however increases significantly in the TCFA and PR ($p < 0.003$) and thereafter decreasing in HR and FCP ($p < 0.03$).

Relationship of cap thickness to plaque type and CD68 infiltration (Fig. 5)

There is a significant thinning of the cap with advancing fibroatheromas and remarkable thickening of the fibrous cap in HRs. Only 3–5% of the area in the EFA and LFA consists of macrophages, but 45% of those macrophages reside in the shoulder, 45-49% is core related and <5% is located in the cap. In the TCFA 20% of the total amount of macrophages are located in the shoulder regions and 18% in the cap ($p < 0.003$). In PRs the percentage of macrophages related to capsize is similar (20%). In HRs only 4% of macrophages are located in the cap ($p < 0.003$). A negative correlation is found between cap thickness and macrophage infiltration, with greater density of macrophage seen in caps <100µm and significantly less as the cap thickens ($p < 0.0004$) (Fig. 5c).

DISCUSSION

In the present study we evaluated the natural history of human aortic atherosclerosis at a fixed, lesion prone location[10,11]. Unlike previous histological studies, 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[4,12]) or by the use of material from patients undergoing vascular surgery (generally end-stage atherosclerotic disease[13]).

Age is an important factor in the atherosclerotic process and is related with the significant change in the physiological properties of the afflicted vessel[14,15]. Our study demonstrates that advancing age is associated with pronounced intimal thickening and medial thinning of the peri-renal aorta. These findings are consistent with the autopsy-based studies of perfusion-fixed human aortas that have shown that wall thickening is mostly confined to the intima and is a well-known effect of atherosclerosis[6,16]. It has also been shown that the increase in intimal thickness with age is maximal in the abdominal region compared with other locations and is the predominant factor for the well-known age related increase in total wall thickness of the aorta[6,17]. Our study extends these findings and shows a clear relationship between intimal thickness and plaque characteristics in aortic atherosclerosis. This observation indicates that intimo-medial thickness measurements in conjunction with the used classification scheme provide a useful method for the overall evaluation of the presence of atherosclerosis[6].

Proliferation and migration of SMCs is an integral part of atherosclerotic plaque progression[18]. We observed stable α- actin but reduced intimal SM-MHC expression with progression of atherosclerosis. This observation may indicate a phenotypical change in SMC during the progression of atherosclerosis, or alternatively that advanced atherosclerotic lesions are dominated by myofibroblasts rather than by SMCs[19].

Our findings indicate rapid progression of peri-renal aortic atherosclerosis as early as the fourth decade of life. This observation is remarkable and, if also valid for the other predilection places, suggests that preventive conservative measures and pharmaceutical interventions should be initiated at a much younger age than commonly thought[6,7,13].

This study shows that advanced atherosclerotic disease is common in people over 45. Our data suggests that aortic atherosclerosis advancement occurs at a younger age in women than in men, an observation that concurs with clinical observations showing that peripheral artery disease develops earlier and at a relatively high rate in women[20,21,22]. Although this observation may appear

counterintuitive, it is important to realize that the male dominance in atherosclerotic disease is limited to the coronary bed[1,2,4].

Our data show that aortic atherosclerotic lesions follow a similar pattern of progression as in coronary tissue but grow far beyond the size of coronary atherosclerotic lesions in our apparently healthy population. Owing to the size of the aorta all plaques remained clinically silent. We observed numerous ruptured and healed plaques (21 (8%) in total) in this apparently healthy population, suggesting that plaque rupture is more common than generally thought and at least for the aorta does not necessary result in clinical events. A similar phenomenon has also been reported in coronary atherosclerosis[23].

Intraplaque haemorrhage and plaque neovascularization are considered key factors in atherosclerotic plaque growth and destabilization in coronary and carotid arteries[24,25,26]. Similar to the other vascular beds we found an increasing number of *vasa vasorum* with advanced atherosclerotic lesions. Yet, unlike the coronaries and carotids, the *vasa vasorum* in the aorta atherosclerotic lesions remain confined to the disrupted intimo-medial border and plaque neovascularization remains minimal despite to prominent intimal thicknesses and necrotic core sizes. These observations are remarkable and suggest that plaque neovascularization plays a less prominent role in aortic atherosclerotic plaque destabilization than reported for the other vascular beds[16].

Monocytes are key players in the atherosclerotic process. Monocyte accumulation in intimal layer of the vessel wall is a primordial event in atherogenesis[27] and is thought to be primarily driven by presence of high amounts of oxidized LDL[28,29]. Apart from their role in foam cell formation, it is generally assumed that macrophages, through release of matrix metalloproteinases directly contributed to destabilization. This process is well studied in the coronary and carotid tissue[4,30,31,32], but remains underreported and appreciated in the aortic vascular bed[32,33]. Analysis of the fibrous cap in this study reveals significant increase in macrophage infiltration in the thinned cap and a significant decrease in healing ruptures.

Conclusion

This study demonstrates that plaque morphologies of the perirenal abdominal aorta are similar to coronary atherosclerosis with macrophage and foam cells seemingly playing a larger role than plaque neovascularization in the evolution of the necrotic core, plaque progression and destabilization in our relatively young and healthy population.

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SUPPLEMENTARY DATA

Methods

Plaque Processing

All specimens were collected at the origin of the renal artery and fixed in formalin for 24 hrs and subsequently decalcified in Kristensen fluid during 5 days. Depending on their size, the aortic patches were divided into 2 or more sections prior to paraffin embedding. The sections were cut into 5 μm thick slices and mounted on aminopropylethoxysilane (APES)-coated slides and dried overnight at 37 degrees Celcius. All aortic sections were stained with hematoxylin and eosin (H&E) and Movat pentachrome method as previously described[1]. The section displaying the highest grade of atherosclerosis was used for further evaluation.

Histological Definitions

The dominant plaque type per section were defined as adaptive intimal thickening (AIT), intimal xanthoma (IX), pathological intimal thickening (PIT), early fibroatheroma (EFA), late fibroatheroma (LFA), thin cap fibroatheroma (TCFA), acute plaque rupture (PR), healed plaque rupture (HR) and fibrotic calcified plaque (FCP) (table 1, figure 1).

Intimal xanthomas were defined as areas of foam cell macrophages in the absence of significant extracellular lipid[2]. Those lesions prone to lipid accumulation were further classified as lipid pool and necrotic core. Lipid pools within PIT denoted localized areas of loss of smooth muscle cells (SMC) within the fibrous plaque usually adjacent to the media, with absence of macrophage cell death, fibrin, and hemorrhage. These lipid pools were often surrounded by macrophage foam cells, especially towards the lumen, or SMC rich areas. The distinction between early and late fibroatheroma was made as previously defined[3] namely, the complete loss of matrix with extensive cellular breakdown in the latter. Necrotic core denoted central areas of necrosis, often infiltrating the lipid pool, with apoptotic macrophage debris, prominent cholesterol crystals, presence of fibrin, and partial or complete loss of matrix.

Morphological Analysis

The intimal thickness was defined as the greatest distance between the internal elastic lamina and the lumen. The medial thickness was measured at the thinnest portion between the external and internal elastic lamina at the location of the lesion. The media adjacent to the plaque was visually divided into an outer, middle and inner third and together with the intimo-medial border each area was observed for the presence of *vasa vasorum*. The fibrous cap thickness was measured at the thinnest portion of the early and late fibroatheroma and at the

remnant site of the ruptured plaque. The lesions were measured over a distance of 1mm and grouped by cap thickness as followed: 0-100μm, 101-300μm and >300μm. The lesion area was defined as the area between the endothelium and internal elastic lamina over a distance of 1mm. In the presence of a necrotic core the distance was expanded another 500μm on both sides of the necrotic core to include the adjacent shoulder region.

Table I. Demographic data of the 260 studied aortic samples

	Male	**Female**
Distribution	140 (53.8%)	120 (46.2%)
Mean age in years	47.3 (6 – 76)	45.4 (5 – 74)
Mean BMI (kg/m^2)	24.3 (8.3 – 32.9)	23.9 (13.1 – 37.2)
Number of patients with known history of nicotine abuse	59	55
Number of patients with known history of hypertension	31	22
Number of patients with known diabetes	0	1
Cause of Death:		
Severe head trauma	31	17
CVA / SAB	26 / 24	19 / 35
Basilar artery thrombosis	0	3
Myocardial infarction	1	0
Cardiac arrest	7	0
Suicide	4	0
Other	47	47
Medication		
Anti-hypertensives	19	12
Statins	3	1
Anti-coagulants	6	6

* CVA; Cerebral vascular accident, SAB; Subarachnoid bleeding

Threshold imaging

The percentage of area occupied by macrophages in the cap, core, shoulders and total lesion was quantified by digital color threshold imaging (IPLab). The regions of interest were selected from each lesion in the Movat Pentachrome stained section and matched with the immunohistochemically stained section. The percent CD68 positive cells within the lesion or cap were calculated byCD68 positive staining area in lesion or cap / total plaque or cap area*100.

Additional information concerning the studied population

The majority of patients died from a severe head trauma (18.5%), cerebral vascular accidents (*i.e.* hemorrhage (17.3%) or subarachnoid bleeding (22.7%)). Only one patient died as a result of myocardial infarction. The four cases that committed suicide were strangulations. The remaining causes of death (n=94) were non-traumatic and not cardiovascular related and include meningitis and respiratory insufficiency. Patients who received anti-coagulants were known to have either coronary stents, an artificial aortic valve or cardiac arrhythmias. Only four patients in our database received lipid lowering agents and only one was known to have hypercholesteremia (total cholesterol >240 mg/dL).

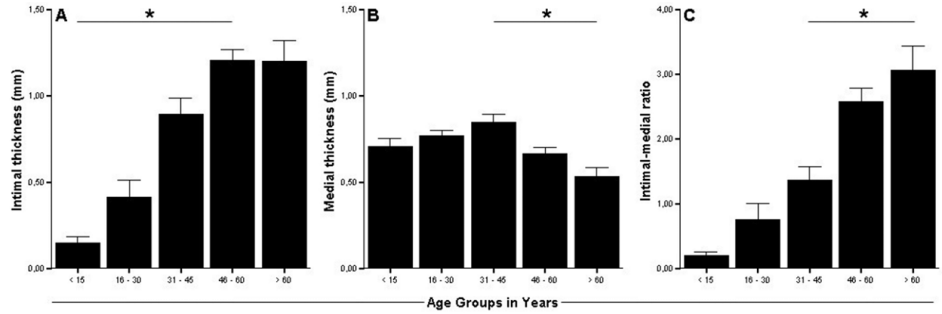

Figure I. Aortic intimal and medial wall thickness and intimal-medial ratio in the abdominal aorta in five age groups at 15 years interval. **A.** Significant difference in intimal thickening were observed between < 15 years, 16 to 30, 31 to 45 years and 46 to 60 years (*p<0,013). **B.** Medial thinning occurred between the age group 31 to 45 years vs. 46 to 60 years (* p<0,0001) and between age group 45 to 60 years and > 60 years (* p<0,032). **C.** The intimal-medial ratio showed significant increase from the age group 31 to 45 years in relation to the 46 to 60 years and > 60 years age groups (* p<0,005). Bars represent mean values (±SEM). (ANOVA across all age groups in all figures p<0.0001).

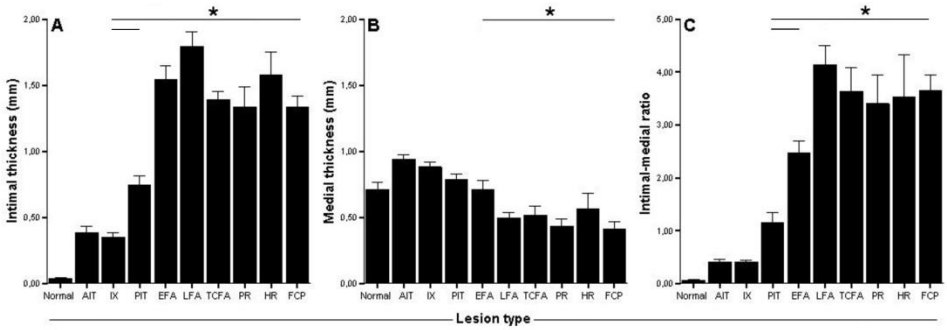

Figure II. Aortic intimal and medial wall thickness and intimal-medial ratio in the abdominal aorta in relation to characteristics of the atherosclerotic plaque type. **A.** The intimal thickness increased significantly with pathological intimal thickening, early fibroatheroma and with progression to advanced lesion types (*p<0,0001). **B.** There is significant medial thinning of the abdominal aorta in plaques which are more advanced than early fibroatheroma (*p<0,03). There is no significant difference in medial thickness between the healed ruptures and the atherosclerotic lesions preceding the late fibroatheroma. **C.** The intimal-medial ratio increased significantly from pathological intimal thickening, early fibroatheroma and with progression to advanced lesion types (* p<0,019). Bars represent mean values (±SEM). (ANOVA across all lesion types in all figures p<0.0001). For abbreviations see histological classification in methods section.

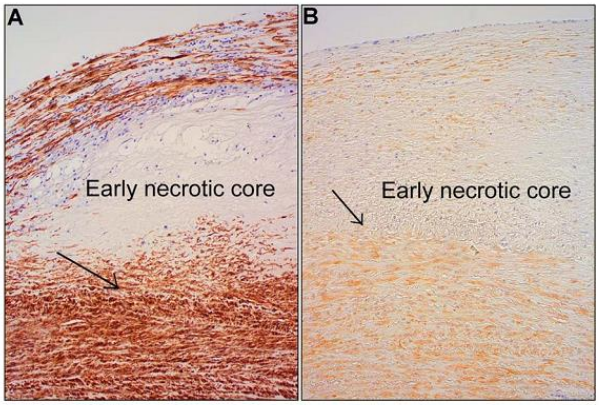

Figure III. Alpha-Smooth muscle cell (α-SMC) actin and myosin heavy chain (SMC-MCH) expression in early fibroatheroma (in adjacent sections). **A.** α-SMC-actin is abundantly expressed in the cap and shoulder region in the early fibroatheroma. Note the SMC infiltration towards the necrotic core adjacent to the internal elastic lamina (black arrow). **B.** SMC-MCH staining in the adjacent section. In comparison to α-SMC –actin, reduced number of SMC-MHC positive cells is found in the shoulder and cap regions. (100X).

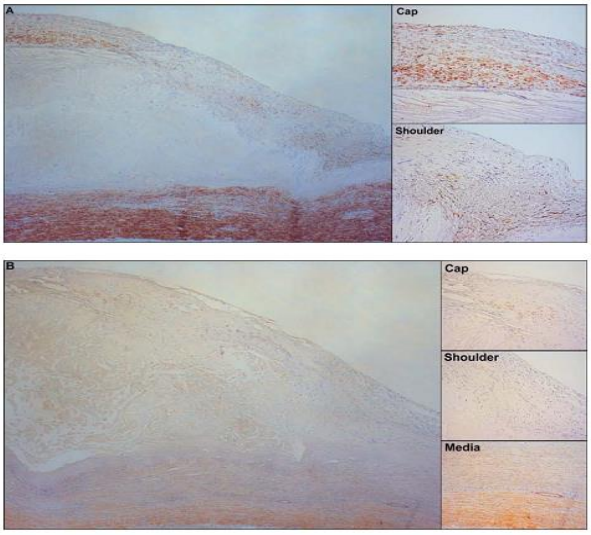

Figure IV. Alpha-Smooth muscle cell (α-SMC) actin and myosin heavy chain (SMC-MCH) expression in the healed ruptures (in adjacent sections). **A.** α-SMC -actin expression regresses in the shoulder and cap region of the more advanced plaques but not in the medial layer. **B.** Progression of atherosclerotic lesions was associated with a further reduction in intimal SMC-MHC positive cells the shoulder and cap regions. SMC-MHC positive cells in the medial layers decreased with advancing lesions and was confined to the medio-adventitial border in the more advanced lesions. (Overview shown at 40x; cap, shoulder and media regions at 100x).

Table II. The cause of death related to the histopathology of the 260 studied aortic samples.

	Cause of Death (N)						
	Severe head trauma	CVA/SAB*	Basilar artery thrombosis	Myocardial infarction	Cardiac arrest	Suicide	Other
Morphological descriptions							
Normal	5	0/2	-	-	-	-	2
Adaptive intimal thickening	22	6/10	-	-	1	1	11
Intimal xanthoma	12	6/4	1	-	-	-	11
Pathological intimal thickening	1	8/19	1	1	-	1	15
Early fibroatheroma	1	4/6	-	-	-	-	12
Late fibroatheroma	3	8/5	1	-	2	-	13
Thin cap fibroatheroma	1	7/9	-	-	1	2	15
Plaque rupture	1	0/2	-	-	1	-	8
Healing rupture	1	2/0	-	-	-	-	6
Fibrotic calcified plaque	1	4/2	-	-	2	-	11
Total:	**48**	**45/59**	**3**	**1**	**7**	**4**	**104**

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1 Farb A, Weber DK, Kolodgie FD, Burke AP, Virmani R. Morphological Predictors of Restenosis After Coronary Stenting in Humans. Circulation. 105:2974-2980.

2 Kolodgie FD, Narula J, Burke AP, Haider N, Farb A, Hui-Liang Y, Smialek J, Virmani R. Localization of Apoptotic Macrophages at the Site of Plaque Rupture in Sudden Coronary Death. American Journal of Pathology. 2000;157:1259-1268.

3 Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arteriosclerosis, Thrombosis, and Vascular Biology. 2000;20:1262-1275.