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Histological evaluation disqualifies IMT and calcification scores as surrogates for grading coronary and aortic atherosclerosis

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ABSTRACT

Background/objectives: Carotid intimal media thickness (IMT) and coronary calcium scores (CCS) are thought to reflect atherosclerotic burden. The validity of this assumption for IMT is challenged by recent meta-analyses; for CCS by absence of a relationship between negative scores, and freedom of future events. As such, we considered evaluation of the relationship between tissue IMT and CCS, and extend of atherosclerotic disease relevant.
Methods: Analyses were performed on donor aortas obtained during renal graft procurement, and on coronary arteries collected during heart valve procurement for tissue donation. Movat pentachrome and Hematoxylin staining was performed, and the degree of atherosclerosis histologically graded. IMT and presence of calcium deposits were quantified on graded tissue sections.
Results: 304 aortas and 185 coronary arteries covering the full atherosclerotic spectrum were evaluated. Aortas and coronaries showed similar relationships between tissue IMT and degree of atherosclerosis, with gradual increase in tissue IMT during earlier phases of atherosclerosis ($r = 0.68$ and $r = 0.30$, $P < 0.00001$ for aorta and coronaries respectively), followed by plateauing of the curve in intermediate and advanced stages. Results for tissue IMT reveal high variability, resulting in wide confidence intervals. Results for CCS are similar for aorta and coronaries, with calcium depositions limited to advanced lesions.
Conclusions: Histological IMT measurements for the aorta and coronaries show large variations around the trend and plateauing of, and possibly reductions in IMT in late stage atherosclerotic disease. These observations for the aorta and coronaries may (partly) explain the limited benefit of including carotid IMT in risk prediction algorithms.

1. Background/objectives

Although the value of conventional cardiovascular risk factors for individual risk assessment has been firmly established, most cardiovascular events occur in patients not classified at increased risk by using current risk algorithms. As a consequence, there is a strong need for complementary individual risk prediction tools.

Carotid intimal media thickness (IMT) and coronary calcium scores (CCS) emerged as epidemiological measures of atherosclerotic burden [1,2]. Yet, these measures are also progressively advocated as individual risk prediction tools [3,4] and changes in carotid IMT are now used as surrogate endpoints in clinical trials aimed at stabilizing or reversing the atherosclerotic disease [5,6].

Paradoxically, a number of prominent meta-analyses fail to demonstrate an additional benefit of including carotid IMT in existing risk algorithms, thereby challenging the value of carotid IMT as an individual risk marker [6–9]. Studies on the validity of CCS as a personalized risk stratification tool point to a low predictive value of a negative CCS; [10] a limitation thought to reflect absence of an association between calcification and plaque characteristics [11].

In this context, it is important to note that manifestations of atherosclerotic disease (i.e. myocardial infarction and stroke) are caused by qualitative changes in the plaque structure (plaque rupture) [12,13], rather than by quantitative changes in plaque volume. In fact, it has long been established that the degree of stenosis poorly relates to future events [14,15]. As such the value of the quantitative imaging tool IMT and the presumably late qualitative marker CCS critically depend on

Abbreviations: IMT, intimal media thickness; CCS, coronary calcium scores; AHA, American Heart Association; H&E, hematoxylin and eosin stain; AIT, Adaptive Intimal Thickening; IX, Intimal Xanthoma; PIT, Pathological Intimal Thickening; EFA, early fibroatheroma; LFA, late fibroatheroma; TCFA, thin cap fibroatheroma; PR, plaque rupture; HR, healed rupture; FCP, fibrous calcified plaque.

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their ability to flag the dynamic aspects of the atherosclerotic process [13,16].

Given the emerging limitations of carotid IMT and CCS for individual risk prediction, we considered a direct validation of an association between IMT and CCS, and qualitative aspects of atherosclerotic disease progression relevant. Such an evaluation is obviously not feasible in the clinical setting in which there is no access to the actual tissue. In order to test a putative association between the grade of atherosclerosis, and IMT and calcium scores we directly assessed IMT and CCS on arterial wall sections from two unique vascular tissue banks that cover the full spectrum of aortic and coronary atherosclerosis.

2. Materials and methods

2.1. Patients and tissue sampling

Sample collection and handling was performed in accordance with the guidelines of the Medical and Ethical Committee of the Leiden University Medical Center, the Netherlands, and the code of conduct of the Dutch Federation of Biomedical Scientific Societies. Due to national regulations, only for transplantation relevant data from donors was available for research (http://www.federa.org/codes-conduct).

This study includes data from 304 consecutive aorta samples from as many donors collected during kidney transplantation, and from 185 consecutive left coronary artery samples from as many donors collected during aortic valve harvesting for tissue donation. Due to the implied age restrictions maximum age of the aortic valve donors was 65 years. No formal age restrictions apply to kidney donations, as such this group also includes older patients.

Aortic patches (from the level of the renal artery) were obtained during kidney transplantation procedures. During the donation procedure the donor kidney including the renal artery is removed along with a large part of the adjoining aorta. Prior to transplantation the aorta is removed and the renal artery trimmed to the required length. The donor-derived aortic segments are not required for transplantation and used for further studies. Aneurysmal aortas (circumference > 2.5 cm, n = 2) were excluded from the study. All donors met the Eurotransplant criteria [17].

The left coronary artery segments were collected from healthy human hearts that were retrieved from Dutch postmortem donors within 24 h after circulation stop and brought to the Heart Valve Bank Rotterdam for heart 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 left coronary artery is trimmed according to standard procedures. Small segments of the left coronary artery were collected especially for this study, without hampering the pathological analysis of the heart necessary for release of the harvested valves.

2.2. Histological classification of lesions

All material was formaldehyde fixed and decalcified (Kristensen's solution). All aorta patches and coronary arteries were divided in consecutive 5 mm segments, paraffin embedded, and 4 μm sections were prepared from each segment. Sections were Movat pentachrome and H&E stained and classified according to the revised classification of the American Heart Association (AHA) as proposed by Virmani cs. [16,17] by two independent observers with no knowledge of the donor characteristics. Although all samples were decalcified in order to allow processing of the samples. Decalcification does not interfere with CCS scoring as footprints of earlier calcium deposits can easily be recognized in H&E staining (dark purple deposits) or Movat staining (brown and dark purple deposits) (Fig. 1). For each individual the tissue section showing the most advanced degree of atherosclerosis was used as reference section for further studies, viz. each sample in the study is from a different individual.

A thin cap fibroatheroma (TCFA) was defined as a fibrous cap less than 155 μm thick (aorta) [17] or less than 65 μm thick (coronary) [18]. Note that multiple lesions and lesion types may be present in a single section. Because of the low number of TCFAs, Plaque Ruptures (PR) and Healed Ruptures (HR) in the coronaries, we also included readings from these stages from sections in which more advanced stages were also present.

2.3. Morphological and histological analysis

Morphometric and histological analysis were performed on Movat pentachrome stained sections with Image J calibrated software (http://imagej.nih.gov/ij/) [17]. Tissue IMT was measured perpendicular to the lumen on 4 locations in the area showing the maximum IMT. A positive CCS (H&E staining) was defined by presence of a calcified area of minimal 200 μm. This lower cut-off value was chosen as it reflects the resolution of high-end CT scanning.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 20 (IBM, Amsterdam, the Netherlands). Data in figures is presented as medians with IQR. Spearman's rho test was used to demonstrate the relation between IMT and the atherosclerotic stage. To that end the lesion type was reclassified in a numeric score (normal = 0…, FCP = 9). ANOVA and Kruskal-Wallis followed by post-hoc analysis (LSD and Mann-Whitney respectively) were used to test potential differences in respectively IMT and Calcium Scores, and the individual stages of atherosclerosis. ANOVA and co-variance analysis was performed to test the influence of age and sex on the IMT readings. P values < 0.05 were considered significant.

3. Results

3.1. Studied population

Details on the study populations are provided in Table 1 (donor characteristics of the kidney donor (aortic patch)) and Table 2 (aortic valve donor (left coronary artery)).

3.2. Classification of atherosclerotic disease

Representative images illustrating the atherosclerosis grading system are shown in Fig. 1. The distribution of most advanced lesion types, and the relationships with donor age and sex are shown in Fig. 2 (aorta) and Fig. 3 (coronary artery). Clear differences were found between aortic and coronary segments with respect to the distribution pattern of the dominant lesion type (Figs. 2 and 3), with regard to a low prevalence of so-called (pre)vulnerable lesions (viz. Late Fibro Atheroma, TCFA and PRs) and a high prevalence of so called

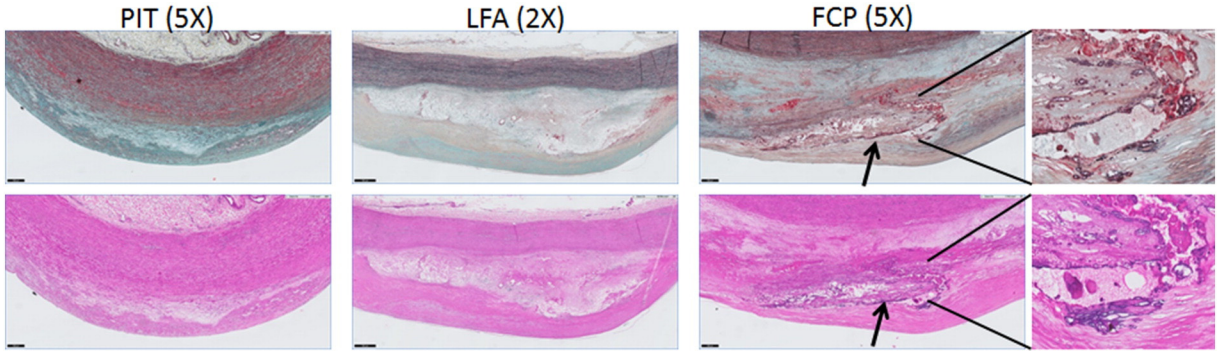

Fig. 1. Movat Pentachrome (top) and Hematoxylin Eosin (H&E) staining (bottom) of atherosclerotic lesions in different aortic lesions. Representative images illustrating aspects of the Virmani classification system for atherosclerosis. Pathological Intimal Thickening (PIT) is characterized by presence of a an acellular lipid pool in the outer intima. Late Fibroatheroma (LFA) presents with a large necrotic containing multiple cholesterol crystals and that is covered by a fibrous cap. A Fibrous Calcified Plaque (FCP) represent represents a fibrotic lesion with a single, condensed calcified area [16]. Decalcification does not interfere with CCS scoring as footprints of earlier calcium deposits can easily be recognized in H&E staining (dark purple deposits) or Movat staining (brown and dark purple deposits in the detail (20-fold, right)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Donor characteristics (aorta, mean (sd)).

	Male N = 156	Female N = 145
Age (yrs)	45.2 (17.5)	44.6 (15.7)
BMI (kg/m^2)	24.8 (3.6)	23.5 (4.4)
Cause of death (n)		
Cerebrovascular accident	34	29
Subarachnoid hemorrhage	32	56
Head trauma	47	21
Cardiac arrest	7	4
Myocardial infarction	1	0
Suffocation	5	3
Pulmonary embolism	0	1
Other/not specified	30	31
Antihypertensive use	20	18
Statin use	6	1

stabilized lesions (i.e. Fibrous Calcified Plaque, FCP) in the coronary artery group.

3.3. Distribution of tissue IMT and stages of atherosclerosis

Fig. 4 shows the relationship between tissue IMT and the aortic lesion type. A positive relationship is found during lesion initiation and early progression ($r = 0.68$, $P < 0.00001$), but the curve plateaus in the advanced, vulnerable lesions (TCFA, PR and HR) and the stabilized lesions (HR and FCP). Results for the coronary artery segments (Fig. 5) follow those of the aorta and show an increase in tissue IMT in the early phases ($r = 0.30$, $p < 0.00005$) of the disease, plateauing of tissue IMT in the advanced and stabilized lesions. Along these lines clear differences were found between the individual stages of atherosclerosis (ANOVA: Aorta: $P < 2.1\ 10^{-37}$; Coronary: $P < 2.9\ 10^{-16}$, differences between the individual stages are shown in Tables 3A and 3B). The variation around the trend is high, thus resulting in wide confidence intervals (confidence intervals are indicated in Figs. 4 and 5).

3.4. Distribution of calcium scores

Calcium deposits are absent in normal arteries and the early phases of atherosclerotic disease. Relevant calcium deposits start to appear in a minority of the LFAs and the prevalence rapidly increases thereafter (Kruskal-Wallis: Aorta: $P < 6.5\ 10^{-29}$; Coronary: $P < 2.5\ 10^{-16}$), and relevant calcification deposits are found in 75% ($P < 0.002$) and 96% ($P < 0.00006$) of the aortic and coronary FCPs respectively. Although calcified plaques are often considered stabilized lesions, we observed signs of instability such as penetrating calcified lesions (i.e. eroded calcified lesions (Fig. 6A) or newly forming lesions over old calcified lesions, Fig. 6B).

Table 2
Donor characteristics (left coronary artery, mean (sd)).

	Male N = 99	Female N = 87
Age (yrs)	48.8 (12.4)	53.4 (9.6)
BMI (kg/m^2)	25.9 (3.3)	25.5 (4.3)
Cause of death (n)		
Cerebrovascular accident	18	11
Sub-archnoidal hemorrhage	8	22
Head trauma	16	11
Cardiac arrest	12	4
Myocardial infarction	27	19
Suffocation	7	9
Pulmonary embolism	4	5
Other/not specified	9	6
Antihypertensive use (n)	29	40
Statin use (n)	19	15

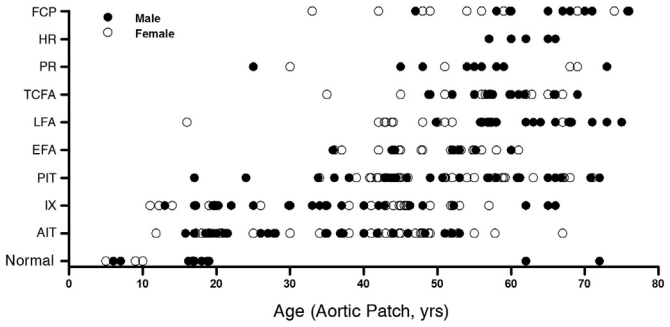

Fig. 2. Histological classification of human aortic atherosclerotic lesion type, and the relationship to age and sex. Three hundred and four aortic patches were characterized for the type of atherosclerosis according to Virmani et al. [16] and classified on basis of the highest degree of atherosclerosis present. The graph shows the highest grade of aortic atherosclerosis in relation to the age of the donor. There is a significant correlation of lesion type with age ($r = 0.640$, $P = 0.01$). Abbreviations; AIT: Adaptive Intimal Thickening, IX: Intimal Xanthoma, PIT: Pathological Intimal Thickening, EFA: Early Fibroatheroma, LFA: Late Fibroatheroma, TCFA: Thin Cap Fibroatheroma, PR: Plaque Rupture, HR: Healing Rupture, FCP: Fibrotic Calcified Plaque.

3.5. Ageing and IMT

Age is a co-variant of the atherosclerotic disease and has been brought forward as a potential confounder of IMT. We used to two different strategies to test the effect of age on tissue IMT in the aortic samples. In the first approach we sought for an association between tissue IMT and age in adult patients (with non-pathological intimal thickening (i.e. normal, adaptive intimal thickening and intima xanthoma) through simple regression analysis. The analysis showed a moderate correlation between age and tissue IMT ($r = 0.335$; adjusted $r^2 = 0.083$) and borderline significance in the ANOVA ($P = 0.057$). The second analysis was based on co-variant analysis on the full cohort. This approach showed that correction for age and sex minimally influences the estimates (Table 4).

4. Conclusions

This study evaluates putative associations between IMT and calcium scores, and atherosclerotic disease progression (histological plaque characteristics defined on the modified AHA classification) in vessel material obtained during organ/tissue donation, viz. material from the donor. Results confirm an association between tissue IMT and plaque progression, but also show plateauing and even reductions in tissue

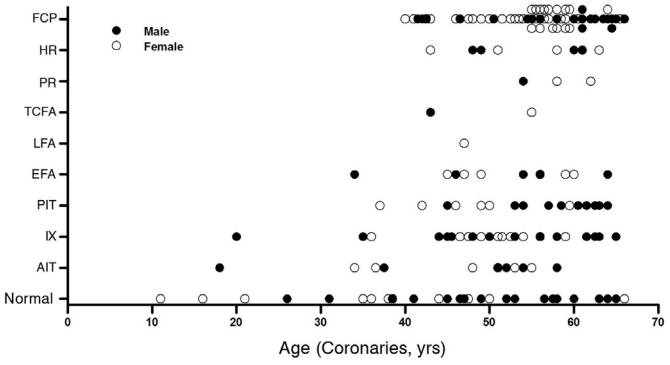

Fig. 3. Histological classification of human coronary artery atherosclerotic lesion type and the relationship to age and sex. One hundred eighty five coronary samples were characterized for the type of atherosclerosis according to Virmani et al. [16] and classified on basis of the highest degree of atherosclerosis present. The graph shows the highest grade of aortic atherosclerosis in relation to the age of the donor. There is a significant correlation of lesion type with age ($r = 0.30$, $P = 0.00005$). Abbreviations are defined in the legend of Fig. 1.

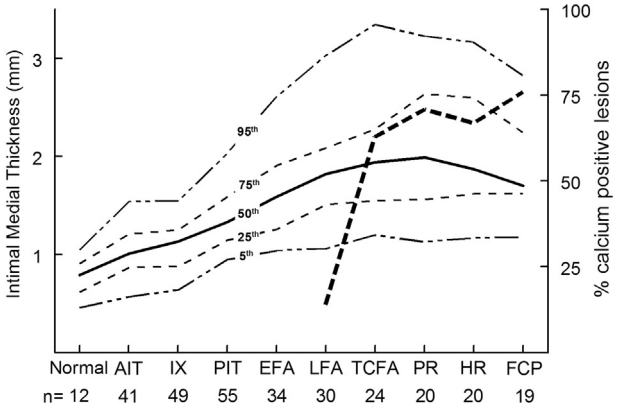

Fig. 4. Aortic IMT and CS versus the grade of atherosclerosis. Relations between histological IMT (the thick line represents the median IMT, dispersion around the median is shown by the 5th, 25th, 75th and 95th percentile lines). Presence of detectable (defined by presence of at least one or more calcium aggregates >200 μm) is indicated by the robust dashed line; and the dominant aortic atherosclerotic lesion type). Abbreviations are defined in the legend of Fig. 2.

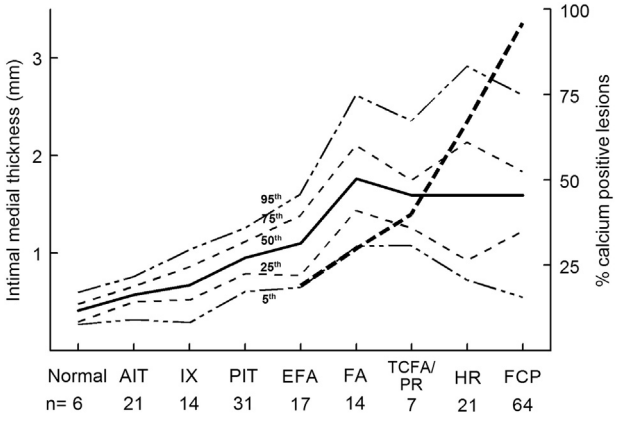

Fig. 5. Coronary IMT and CS versus the grade of atherosclerosis. Relations between histological IMT (the thick line represents the median IMT, dispersion around the median is shown by the 5th, 25th, 75th and 95th percentile lines). Presence of detectable (defined by presence of at least one or more calcium aggregates >200 μm in the lesion; indicated by the robust dashed line), and the dominant atherosclerotic lesion type. Abbreviations are defined in the legend of Fig. 2.

IMT readings in the advanced stages of the disease. Findings also show a large variability in tissue IMT, thereby resulting in wide confidence intervals around the trend. Results for the CCS confirm data from Mauriello et al. [11] and characterize CCS as a marker of advanced and end-stage atherosclerotic disease.

Carotid IMT is now widely accepted as a screening tool to detect atherosclerosis in the early and asymptomatic stages of the disease. Indeed IMT has been adopted by the AHA as a surrogate marker for coronary artery disease [1]. In addition to its use as a screening tool, carotid IMT evolved as a surrogate endpoint in trials monitoring the effectiveness of pharmaceutical interventions aimed at reducing atherosclerotic progression and cardiovascular risk [5,6]. Notwithstanding the broad recognition of carotid IMT as a complementary risk prediction tool, large epidemiological evaluations shed doubt on the validity of carotid IMT as an individual risk prediction tool [7–9]. In fact these recent evaluations only indicate a modest or fail to show a benefit of including IMT in risk prediction algorithms. In fact, a large meta-analysis concluded that "The addition of common CIMT (carotid IMT) measurements to the Framingham Risk Score was associated with small improvement in 10-year risk prediction of first-time myocardial infarction or stroke, but this improvement is unlikely to be of clinical importance" [8]. Along similar lines a 2012 AHA position statement denounced the use of CCS and carotid IMT for screening the asymptomatic adult population for CHD [10]. These observations are remarkable and imply that the added value of IMT is less than commonly thought and that individualized use of IMT is challenged.

A possible explanation for the limited additional benefit of including IMT in established risk algorithms is the fact that IMT is a pure quantitative measure, and therefore may not necessarily reflect the atherosclerotic disease process. Moreover, it has been pointed out that increases in IMT are a consequence of the aging process, and thus that increased IMT could simply reflect physiologic, ageing-related thickening of the intimal-medial layers [19]. On the other end of the spectrum, it is well established that the degree of luminal stenosis caused by plaque growth poorly predicts future cardiovascular events [14,15]. In this context it is important to note that atherosclerosis is a multi-stage disease process that evolves through successive stages of intimal lipid accumulation, plaque formation and progression, followed by plaque destabilization and plaque rupture, and finally plaque stabilization [12,13]. It is now assumed that plaque destabilization is the pivotal factor in the development of insidious manifestations of the disease.

At this point it is unclear how and to what extent IMT readings translate to the process of plaque evolution, destabilization and subsequent stabilization. In order to assess such a relationship, we considered a systematic evaluation of a possible relationship between IMT and plaque characteristics relevant. To that end we performed a systematic evaluation of a putative association between intimal media thickness directly measured on tissue slides (tissue IMT) and histological grading in two large biobanks of human vascular tissue.

In the ideal world situation such a study would be performed on carotid tissue. Yet, available biobanks of carotid tissue are all based on surgical specimens of carotid endarteriectomies; hence representing end-stage atherosclerotic disease and, depending on the depth of the -tomy missing segments of the medial layer. In order to be able to cover the full atherosclerotic spectrum, and the obvious need for an intact media (hence full thickness samples) we decided for a study on material collected during transplantation procedures (i.e. intact arterial tissue).

The first series of studies was performed on donor material from a biobank of aortic segments [17]. These donor aortic segments come with the kidney graft and are (partially) removed during

Table 3A
P-values for a comparing of aortic IMT between the different stages (LSD post-hoc test).

	Normal	AIT	IX	PIT	EFA	LFA	TCFA	RP	HR
AIT	0,051462								
IX	0,013681	0,450608							
PIT	7,43E−06	9E−05	0,000966						
EFA	2,18E−09	1,74E−09	2,71E−08	0,004753					
LFA	2,9E−12	3,98E−13	6,24E−12	6,59E−06	0,0658				
TCFA	2,51E−15	2,65E−17	4,17E−16	2,64E−09	0,000907	0,14849			
RP	5,48E−15	1,96E−16	2,91E−15	6,81E−09	0,000975	0,132691	0,912142		
HR	1,49E−15	3,2E−17	4,76E−16	1,55E−09	0,000403	0,082835	0,735055	0,825458	
FCP	3,63E−12	1,5E−12	2,06E−11	6,9E−06	0,042112	0,767626	0,281548	0,251151	0,171564

Table 3B
P-values for a comparing of coronary IMT between the different stages (LSD post-hoc test).

	Normal	AIT	IX	PIT	EFA	LFA	TCFA/RP	HR
AIT	0,529884							
IX	0,34549	0,622489						
PIT	0,030391	0,016838	0,11385					
EFA	0,010435	0,004541	0,034858	0,39813				
LFA	1,68E−06	4,6E−09	6,15E−07	1,33E−05	0,001224			
TCFA/RP	0,00019	4,36E−05	0,000438	0,006783	0,048938	0,524844		
HR	8,67E−06	1,53E−08	3,24E−06	7,48E−05	0,007026	0,392468	0,998496	
FCP	1,75E−06	1,25E−11	8,33E−08	5,52E−07	0,001492	0,302046	0,979117	0,969555

transplantation. The peri-renal aorta constitutes a predilection place for atherosclerotic disease; although its large local diameter generally precludes clinical manifestations [17]. An obvious question is whether these observations from an elastic artery such as the aorta are also valid for other arterial beds. We therefore validated the findings for the aorta on material from a second biobank based on donor coronary arteries that were collected during aortic valve procurement for tissue donation. Although this material is from an obviously more relevant arterial bed, material in this bank is more circumscribed due to stricter age restrictions (maximum age for aortic valve donation is 65), and the fact that hearts from younger/healthier donors are more likely to be allocated for whole organ donation. Hence, it's likely that material in this biobank is less representative to the general population than the material in the aorta-based biobank.

We classified all donor material in our study according to the modified AHA classification as proposed by Virmani et al. [16]. This classification is thought to better differentiate between the more advanced stages of the atherosclerotic process, and unlike the AHA classification it has a continuous (progressive) association between disease progression and score. Classification was performed on basis of the Movat staining as this staining allows for clear differentiation between the different advanced lesions (in particular late fibroatheroma and fibrous calcified lesion).

A systematic evaluation in the aortic tissue bank shows that atherosclerotic disease is very common among those over 40 years of age, and that unstable plaques and healed plaques (FCP) are present in 7% of the donors. Results for the coronary artery segments roughly followed those for the aorta, although the very limited availability of material from donors under the age of 30 interferes with firm conclusions on a possible

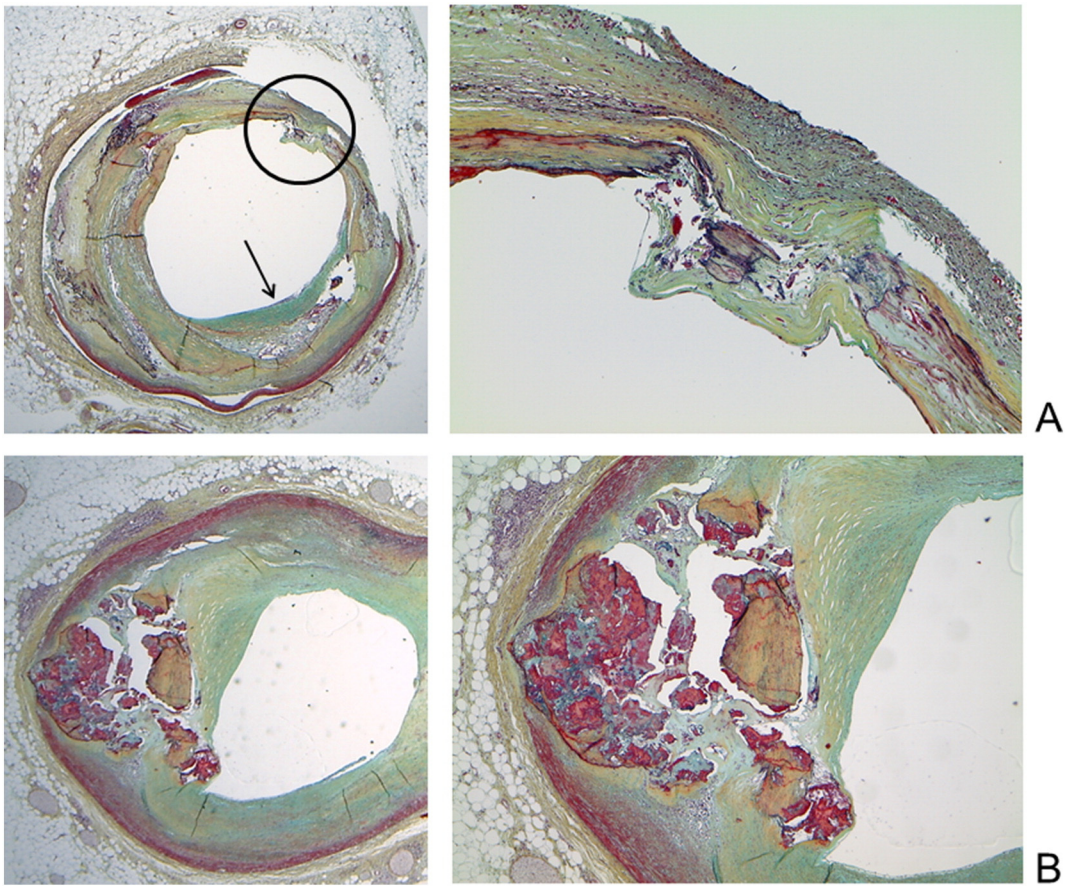

Fig. 6. Instable lesions in calcified coronaries. A: Movat pentachrome staining showing a ruptured fibrous cap overlying a small calcified nodule (circle). Note presence of multiple lesions in this single cross section and the formation of a new lesion (neo atherosclerosis, greenish proteoglycan/collagen rich area, arrow) (12.5 fold (overview), and 100 times magnification (detail). The defect in the upper right corner reflects a cutting artifact. B: Movat Pentachrome staining showing a penetrating calcium nodule. Note the complete loss of media in the underlying area and an extremely thin, a-cellular overlying cap (12.5 fold (overview), and 40-fold magnification (detail)). Note: color legend for the Movat staining: blue: proteoglycans, yellow: collagen, black: elastin, red: smooth muscle cells and fibrinogen, purple: nuclei. Green reflects co-localization of proteoglycans and collagen. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Plain regression coefficients and corrected (age and sex) regression coefficients in adult donors (age > 20 years) for the association between IMT and the stage of atherosclerosis.

	Uncorrected	Corrected
AIT	0.27 (0.14)	0.24 (0.14)
IX	0.33 (0.13)	0.31 (0.14)
PIT	0.61 (0.13)	0.55 (0.15)
EFA	0.87 (0.14)	0.81 (0.16)
LFA	1.07 (0.15)	1.02 (0.17)
TCFA	1.25 (0.15)	1.22 (0.17)
RP	1.26 (0.15)	1.22 (0.17)
HR	1.29 (0.15)	1.26 (0.18)
FCP	1.11 (0.15)	1.01 (0.18)

age relationship. A remarkable observation is the very low prevalence of classical lesions (early and late fibroatheroma) and instable lesions, and the high prevalence of fibrous calcified lesions in coronary arteries (32% of the specimens were classified as a FCP). Although lack of longitudinal data interferes with firm conclusions, these observations may indicate that plaque destabilizing in the coronary arteries is a relative rapid process and that asymptomatic plaque rupture and subsequent healing, as reflected by the high prevalence of FCPs, is a very common phenomenon, even in individuals as young as 40–50 years.

IMT measurements performed on the tissue slices confirm a positive relationship between tissue IMT and the histologic classification of atherosclerosis. Yet, the data also show flattening, and possibly even a decline in tissue IMT in the vulnerable and particularly stabilized lesions. This phenomenon challenges the use of sequential IMT measurements as surrogate end-point in interventional studies as disease regression or progression may occur in the absence of changes in IMT, or alternatively that reductions in IMT can reflect regression or progression. Such a conclusion is supported by clinical evidence failing to characterize IMT as a dynamic marker [20].

The number of tissue samples in our study also allowed for the establishment of confidence intervals, which indicate a marked variation in tissue IMT around the trend. These wide confidence intervals profoundly interfere with the interpretation of individual measurements, thus challenging the use of IMT for personalized care and (partly) explaining the limited value of including IMT in risk algorithms.

Results for histological assessment of CCS herein follow the conclusions of Mauriello et al. [11] and show that calcium deposits reflect the later stages of the disease, and are particularly prominent in the so called stabilized lesions. This observation supports the notion that calcium deposits are associated with end stage, stabilized lesions. Yet, we found indications that penetrating ulcers can develop in the fibrous cap overlying a calcium nodule (Fig. 5). Moreover, new lesions can develop on top of extinguished lesions, resulting in a repetitive process of plaque healing, followed by formation and destabilization of a new plaque over the existing pacified lesion (Fig. 5). Formed buried lesions show that calcium deposits persist for extended periods of time, and hence that calcium scores largely reflect the past.

All in all, results for calcium deposits suggest that CCS should be considered a retrospective marker, identifying patients with manifest atherosclerotic disease, whereas a negative calcium score does not rule out presence of atherosclerotic disease. The apparent persistence of calcium implies a very low clearance rate, hence disqualifying calcium scores as a dynamic marker.

Limitations: For practical reasons outlined above we were unable to evaluate a relation between carotid IMT and qualitative measures of carotid atherosclerosis. Yet, given the established relation between carotid IMT and coronary readings we consider our findings from the aorta and coronary arteries relevant for the carotid bed [1]. A second limitation is that measurements are performed on paraffin embedded tissue sections. As such, we were able to use clear anatomical land marks for the tissue IMT. It is unknown how these actual landmarks translated to the contrast-based defined landmarks used in B-mode ultrasound [21]. Another point is that the process of tissue preparation for histology involves tissue shrinkage due to intrinsic tissue contractility as well as due to the dehydration process required for paraffin embedding [22]. As such measures in the study are relative, and do not translate one to one with the real-life situation. Moreover, we cannot exclude that shrinkage may be less in more fibrotic tissues such as the FCP lesion type. As such our data may underestimate the apparent decline in IMT during plaque stabilization. Finally, we observed a low number of vulnerable lesions in our set of coronary arteries; hence conclusions for this stage should be interpreted with caution.

In conclusion, this histological evaluation shows a weak association between histological IMT and the atherosclerotic process. Extremely wide confidence intervals interfere with the interpretation of individual data, whereas flattening or possible even declining IMT values may severely compromise interpretation of sequential IMT in the context of intervention studies. Consequently, on basis of our observations for the aorta and coronaries IMT cannot be recommended as a qualitative measure of atherosclerosis. Findings for the calcium deposits show that calcium deposition occurs late in the process and the calcium deposits are stable. As such, CCS do not qualify as a dynamic marker and largely reflect the past. Consequently, positive CCS identify those patients with manifest advanced atherosclerotic disease.

Conflict of interest

None.

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