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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

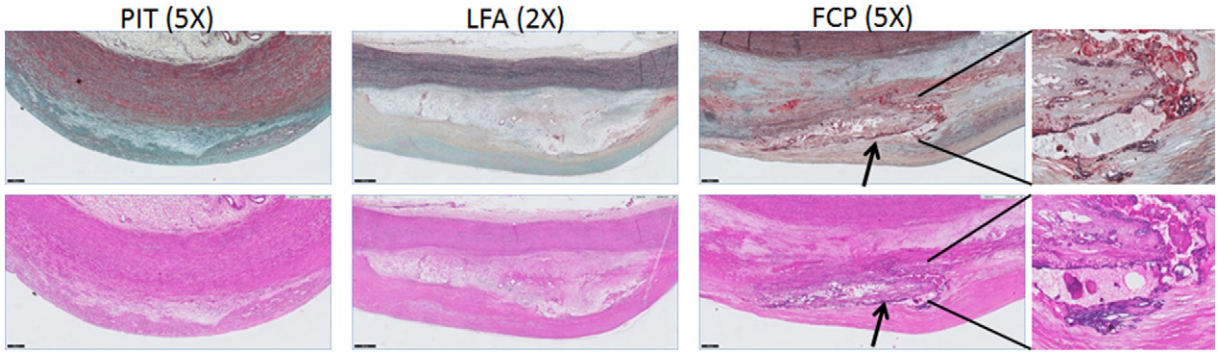

Fig. 1. Movat Pentachrome (top) and Hematoxylin Eosin (H&E) staining (bottom) of atherosclerotic lesions in different aortic lesions. Representative images illustrating aspects of the Virmani classification system for atherosclerosis. Pathological Intimal Thickening (PIT) is characterized by presence 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)).