Advances in Medical Sciences 60 (2015) 236–239

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Histomorphological evaluation of atherosclerotic lesions in patients with peripheral artery occlusive disease

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ARTICLE INFO

Article history:
Received 30 July 2014
Accepted 23 March 2015
Available online 4 April 2015

Keywords:
Atherosclerosis
Femoral artery
Inflammatory cells
Histological classification
Peripheral arterial occlusive disease

ABSTRACT

Purpose: Peripheral arterial occlusive disease (PAOD) is mainly caused by atherosclerosis of the vessel wall. These pathological changes are classified into different stages and are well described for carotid and coronary vessels, but not for PAOD. The aim of our study was to analyze plaque morphology of femoral arteries in patients with intermittent claudication and critical limb ischemia.

Patients and methods: In this retrospective study 85 atherosclerotic plaques (common and superficial femoral artery) of 71 patients with a clinical symptomatic PAOD were analyzed, by histology (01/2009–07/2010). Atherosclerotic lesions were classified according to Stary (type I–VIII). For further characterization, plaques were evaluated for the presence of collagen, elastin, calcifications, smooth muscle cells, macrophages, leucocytes, and cellularity.

Results: The majority (91%) of atherosclerotic lesions were of advanced types according to Stary (V–VII). Atherosclerotic lesion type VI was associated with significant higher amount of inflammatory cells in comparison to all other atherosclerotic plaque types (CD45: $p < 0.001$; CD68: $p = 0.013$). In addition, atherosclerotic plaques with a pronounced neovascularization contained a higher amount of CD45 ($p = 0.015$; $rho = 0.273$) and CD68 ($p = 0.016$; $rho = 0.275$) positive cells.

Conclusion: Atherosclerotic lesions of femoral arteries show similar morphological changes as coronary or carotid arteries. But inflammatory cells had a higher impact on plaque progression and destabilization than any other factor.

1. Introduction

The incidence and severity of atherosclerotic lesions accelerate with increasing age. Atherosclerosis is a heterogeneous disease initiated by different pathophysiological pathways in the vessel wall, affecting almost all parts of the body [1]. Especially diseased arterial regions are the coronary vessels, carotid arteries, and the arteries of the lower extremities. Worldwide, more than 20% of individuals over 75 years suffer from peripheral arterial occlusive disease of the lower extremity (PAOD) [2,3]. Although almost three-quarters of these patients are asymptomatic, the prevalence of intermittent claudication (IC) in the PAOD population is about 25–30%, and the prevalence of critical limb ischemia (CLI) is 1–3%. Current clinical findings of an one-year prognosis indicate 25% mortality in patients with CLI, 20% with continuing CLI, 30% amputation and 25% with a resolved CLI [4].

Despite this devastating prognosis little is known about the plaque composition of arteries of lower extremities in patients with PAOD. Several studies have already characterized carotid and coronary atherosclerotic lesions, proposing that luminal thrombus formation due to rupture or erosion of the plaque surface are the most important mechanisms resulting in acute coronary syndrome or cerebral infarction [5–7]. Atherosclerotic changes in peripheral arteries are believed to be similar to those evaluated in carotid and coronary arteries [8]. However, only vascular segments of the peri-renal region and lower limb amputations were evaluated so far, showing similar plaque morphologies to coronary or carotidal atherosclerosis [8,9]. A detailed characterization of plaque composition in patients with all symptomatic stages of PAOD has not yet been performed.

Therefore, the aim of our study was to analyze plaque morphology of the common or proximal superficial femoral artery in terms of calcification, lesional inflammatory cells, collagenous and soft plaque content, plaque stability and rupture in patients with IC and CLI.

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http://dx.doi.org/10.1016/j.advms.2015.03.003
1896-1126/

2. Patients and methods

2.1. Patients

In this retrospective study, we analyzed in total 85 atherosclerotic lesions of 71 patients in symptomatic stages of PAOD (IC or CLI), who underwent open surgical revascularization procedures between 01/2009 and 07/2010. All specimens were collected from the common or proximal superficial femoral artery. The local ethics committee of our university hospital approved the study (approval number: 2799) and written informed consent was given by all patients. The study was performed according to the Guidelines of the World Medical Association Declaration of Helsinki. Data on sex, age, clinical status (according to Rutherford), and concomitant diseases were extracted from the medical records of each patient.

2.2. Histological analysis

The atherosclerotic plaques excised during surgery were directly processed for histological examination. The plaques were evaluated for the presence of collagen, elastin, calcifications, smooth muscle cells, macrophages, leucocytes, and cellularity.

Tissue samples were fixed with formalin, segmented to pieces of 3–4 mm, and embedded in paraffin. Each segment was cut in slides of 2–3 μm, stained by Hemalaun–Eosin (HE) and Elastica-van-Gieson (EvG) in order to assess plaque morphology, cellularity, and the content of elastic and collagenous fibers. Digital photographs were taken of each stained sample and computer analyzed. For the clinical relevance and comparison between the groups, the most advanced atherosclerotic areas were used. The degree of atherosclerosis was determined in accordance with the American Heart Association using Stary's classification [10–12]. This classification grades atherosclerotic plaques in eight types from initial, moderate and stable atherosclerotic lesions (I–IV) to unstable/advanced and complicated (V–VII) and VIII as fibrotic lesion. Plaques were defined as ruptured when they contained intra-plaque thrombus and/or incomplete cap layer (type VI).

For cellular characterization, femoral artery samples were treated with antibodies against vascular smooth muscle cells (anti-SMA, Dako, Glostrup, Denmark) and endothelial cells (anti-von Willebrand Factor (anti-vWF) = factor VIII, Dako). To evaluate the severity of inflammation and to identify the inflammatory cells, paraffin embedded sections were stained with CD68 for monocytes/macrophages (anti-CD68, Dako) and with CD45 for leukocytes (anti-CD45, Dako), as previously described [13]. Following primary antibody incubation the individual factors were visualized by using either APAAP ChemMate Detection Kit (Dako) or LSAB ChemMate Detection Kit (Dako) according to the manufacturer's instructions.

All histological sections were independently examined by two observers (S.S. and J.P.), who were blinded to the patients' clinical data. In case of disagreement, a final consensus examination was performed. For the evaluation of all histological features a semi-quantitative scoring ranging from (−) for no staining (no appearance) to (+++++) for maximal positive staining (maximal appearance) of the individual factors was used.

2.3. Statistical analysis

All statistical analyses were performed by using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between groups are shown as a mean ± standard deviation (SD) for continuous variables or as a percentage for categorical variables. Normal distribution was excluded using the D'Agostino–Pearson test. For the correlation between diabetes and type VI unpaired *t*-test was applied. The Mann–Whitney-*U* test was used for two independent groups and the Kruskal–Wallis test for more than two independent groups was used to compare the level of quantitative data. Correlations between continuous variables were quantified by using Spearman's rank correlation coefficient. Values of $p \leq 0.05$ were considered statistically significant.

3. Results

Demographic data of the study patients ($n = 71$) are summarized in Table 1. The mean age of the study population was 69 years (standard deviation: 10; range: 43–96 years); 48 patients were male (68%), and 23 patients were female (32%). Forty-five (63%) of these patients were active smokers or past smokers. Fifty-nine patients (83%) had hypertension and 28 (39%) patients had diabetes. Forty-two of these patients were classified as Rutherford stage 3 (59%), 15 patients as stage 4 (21%), and 14 as stage 5 and 6 (20%). This means that 29 out of 71 patients (41%) were diagnosed with CLI. All patients were on statin medication therapy.

The majority of our study patients had clinically relevant advanced type V–VII plaques (Fig. 1). Detailed histological and morphological characterization of the 85 atherosclerotic plaques according to Stary (AHA classification) showed following results: type III ($n = 2$), type IV ($n = 1$), type V ($n = 9$), type VI ($n = 34$), type VII ($n = 34$), type VIII ($n = 5$) (Figs. 1 and 2). Subgroup analysis of atherosclerotic lesion type VI displayed in 5 cases disruption of the surface (type VIa; 15%), in 3 cases hematoma or hemorrhage (type VIb; 9%), in 14 cases thrombosis (type VIc; 41%) and in 12 cases all of these features were observed (type VIabc; 35%) (Fig. 3).

No significant differences were found between type of lesion and age ($p = 0.811$), smoking ($p = 0.804$), sex ($p = 0.644$), diabetes ($p = 0.855$), clinical stage ($p = 0.935$) or cellularity ($p = 0.811$). The semi-quantitative analysis of plaque cellularity revealed a continuous decrease with the increasing age ($p = 0.009$; $rho = -0.285$). Furthermore, smoking ($p = 0.003$) and male patients ($p = 0.012$) showed a higher plaque cellularity than none-smokers and female patients. Regarding inflammatory cells, the number of CD45 as well as CD68 positive cells was significantly increased in plaques of patients with CLI in contrast to individuals suffering from IC ($p = 0.025$ and $p = 0.020$, respectively).

In addition, atherosclerotic plaques with a pronounced neovascularization contained a higher amount of CD45 ($p = 0.015$; $rho = 0.273$) and CD68 ($p = 0.016$; $rho = 0.275$) positive cells.

Atherosclerotic lesion type VI was associated with a significant higher amount of inflammatory cells in comparison to all other atherosclerotic plaque types (CD45: $p < 0.001$; CD68: $p = 0.013$). Further analysis of the individual subtypes of VI (Via–VIabc) revealed no significant differences in the occurrence of CD45 ($p = 0.083$) or CD68 ($p = 0.139$) positive cells.

No significant correlation was seen for calcification and clinical or histological data.

Furthermore, we observed a significant relationship between the content of smooth muscle cell in atherosclerotic lesions and collagen ($p = 0.028$; $rho = 0.248$) and elastin ($p = 0.001$; $rho = 0.366$). Elastin

Table 1
Demographic and clinical characteristics of the study population ($n = 71$).

	n (%)
Mean age	69 years
Male/female	48/23 (68/32)
Smoking	45 (63)
Hypertension	59 (83)
Hyperlipidemia	42 (59)
Diabetes	28 (39)
Stadium IC/CLI, *n* (%)	42/29 (59/41)