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Abdominal aortic aneurysm and histological, clinical, radiological correlation

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ABSTRACT

To date, the pathogenesis of abdominal aortic aneurism (AAA) still remains unclear. As such, the aim of this study was to evaluate changes of the aortic structure during AAA. We analysed the microscopic frame of vessels sections, starting from the *primum movens* leading to abnormal dilatation. AAA samples were collected and processed through various staining methods (Verhoeff-Van Gieson, Masson Goldner, Sirius Red). Subsequently, the vessel morphology and collagenic web of the *tunica media* and *adventitia* were determined and the amount of type I and type III collagen was measured. We also applied immune-histochemistry markers for CD34 and PGP 9.5 in order to identify vascular and nerve structures in the aorta. Immune-positivity quantification was used to calculate the percentage of the stained area. We found increasing deposition of type I collagen and reduced type III collagen in both *tunica media* and *adventitia* of AAA. The total amount of *vasa vasorum*, marked with CD34, and *nerva vasorum*, marked with PGP 9.5, was also higher in AAA samples. Cardiovascular risk factors (blood pressure, dyslipidemia, cigarette smoking) and radiological data (maximum aneurism diameter, intra-luminal thrombus, aortic wall calcification) increased these changes. These results suggest that the *tunica adventitia* may have a central role in the pathogenesis of AAA as clearly there are major changes characterized by rooted inflammatory infiltration. The presence of immune components could explain these modifications within the framework of the aorta.

1. Introduction

Abdominal aortic aneurysm (AAA) indicates an irreversible dilatation of the terminal aortic segment. Indeed, the word aneurysm originates from the ancient Greek ἀνεύρυσμα (aneurysma). Normally, the diameter of the abdominal aorta is between 1,8 cm and 2 cm. whereas in AAA it is more than 3 cm. The incidence of the disease is increasing in the general population and reaches a maximum of 4% in people more than 60 years old. When the balloon-like dilation ruptures, mortality is about 85% of cases. It is estimated that 1–3% of deaths in the male population is caused by this pathology (Lederle et al., 2001).

Male sex, age, arterial hypertension, diabetes, dyslipidemia and cigarettes are cardiovascular risk factors, which play an important role in the development of AAA (Landenhed et al., 2015; Lemaître et al., 2011; Vardulaki et al., 2000). Furthermore, genetic alterations connected to collagen synthesis and familiarity are also risk factors specific to AAA (Kuivaniemi et al., 1991). Although the involvement of these factors is recognized, the *primum movens* has not yet been identified. It has been demonstrated that, according to Laplace's Law, the increased diameter of the aneurysm enhances surface tension on the aortic wall, producing an expansion of the lesion as a final effect until it ruptures.

The pathogenesis of abdominal aortic aneurysms is complex and needs further investigation. Different theories have been proposed, but none have been accepted as fully established or accepted (Pearce and Shively, 2006; Tanweer et al., 2014). It would seem that the interaction of several factors may determine changes in the vessel thus causing dilatation. The alteration of the connective tissue present in the aortic wall play an important role in the development of aneurysms given that elastic and collagen fibres are the main determinants of the mechanical properties of the aorta. Elastin is stabilized by a series of cross-links between molecules and can be degraded by specific proteases that have elastase properties. The

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elastic fibres associated with smooth muscle cells tend to be more abundant in the *tunica media* of the aortic wall. Collagen, on the other hand, is an important component of the *tunica media* and also of the *tunica adventitia*. In particular two types of collagen, type I and type III, oppose the tensive force of blood pressure and give support in maintaining the structural integrity of the arterial wall.

One of the most important histological features of aneurysmal tissue is the fragmentation of elastic fibres and the decreased concentrations of elastin during aneurysm growth until its eventual rupture (Sakalihasan et al., 1993; Swaminathan et al., 2014). The loss of elastic fibres can lead to an initial step toward the formation of aneurysm (Dobrin and Mrkvicka, 1994). The degradation of fibres seems to be caused by inflammatory components, which lead to the production of cytokines. This in turn attracts other immune cells which arrive at this site via the bloodstream (Curci and Thompson, 2004). The alterations of elastin and collagen are the consequence of proteases production by arterial wall cells, such as smooth muscle cells of the *tunica media, adventitia fibroblasts* and cells constituting inflammatory infiltrates. Proteases and in particular metallo-proteases (MMP), are closely linked with aneurysm (Ciavarella et al., 2015; Longo et al., 2002; Tromp et al., 2004).

Some research has reported the morphology of AAA, in particular focusing on the *tunica media* and inflammatory infiltrates (Curci and Thompson, 2004; Dobrin and Mrkvicka, 1994; Holmes et al., 1995; Mäyränpää et al., 2009). To date, however, less is known about the nervous structures present. The aim of this study was to establish the collagenic framework, the *vasa vasorum* and *nerva vasorum* in the *tunica media* and *tunica adventitia* of the aortic wall of AAA, focusing on the quantitative and qualitative changes of these structures between aneurismatic aorta and aorta without aneurismatic pathologies. It would also be useful to demonstrate whether there is a link between the increased alteration of the aortic wall and several risk factors in AAA. Moreover, we analyzed the information provided by Computed Tomography Angiography in order to find any correlation between radiographic data and morphological data.

2. Material and methods

2.1. Sample collection

The study was performed in samples of 26 Caucasian patients admitted to the Surgery Department of "Spedali Civili" hospital in Brescia, Northern Italy, between 2013 and 2015, for abdominal aortic aneurysms resection. Demographic and clinical characteristics of the patients were collected, in particular eighteen patients had arterial hypertension in therapy with different drugs (angiotensin-converting-enzyme inhibitor, angiotensin receptor blockers and calcium channel blockers), eleven patients had dyslipidemia treated with statin and thirteen patients had smoking habits. The age of the patients treated varied between 61 and 89-years old with an average of 74.6 years old (SD 7.2). The study was approved by institutional ethic committee (PEVEL01, Vascular and linfatic endothelial purification). Participants did not receive any form of financial compensation.

During elective open AAA repair, histological specimens of anterior part of aortic wall were obtained where the aneurysm riches the maximum pathological diameter, after placing the prosthesis. This tissue was immediately fixed in formalin in the operating room according to the protocol. An equal distance between the different specimens that were designated for histology was maintained.

In this study were collected aneurysmatic walls with *tunica media* and *tunica adventitia*, thanks to the Unit of Vascular Surgery, Department of General Surgery, University of Brescia—Azienda Spedali Civili of Brescia (Italy). The risk factors of each patient during the days of hospitalization were obtained.

Furthermore the analysis of CT-angiographies of the patients allowed obtaining information of the structure of AAA such as maximum diameter, presence of the thrombus in anterior position, calcification or ulceration of thrombus, calcification of aortic wall.

We collected also 5 segments of abdominal aorta without aneurysmatic pathology which are included in the study (mean age 77.4 years old, SD 7.9). These 5 elements are extracted from Caucasian corpses in Human Anatomic Department of University of Vienna.

All the samples were rapidly collected, washed briefly in phosphate buffer (0.2 M, pH 7.4) and immersion fixed in 10% neutral buffered formalin, before being embedded in paraffin wax using routine procedures.

2.2. Histopathological examination

Paraffin embedded specimens were serially sectioned at 5 μm (for standard stainings and immunohistochemical analysis) and 7 μm (for Sirius Red staining) using a microtome. These sections were deparaffinized, rehydrated and then stained with haematoxylin and eosin (H&E), Sirius Red staining, Verhoeff Van Gieson and Masson Goldner. After Sirius Red staining, collagen fibres were detected by polarized light microscopy (Olympus, Hamburg, Germany); under these conditions, type I collagen (newly formed) fibers appear yellow/red, whereas type III collagen (constitutive) fibres appear green (Junqueira et al., 1978; Rizzoni et al., 2006).

2.3. CD34 and PGP 9.5 immunohistochemistry

For the immunohistochemical study, sections were deparaffinized and rehydrated by routine protocol and immersed in 3% hydrogen peroxide (H_2O_2) in methanol for 30 min to rinse the endogenous peroxidase. Sections obtained from all samples were incubated for 1 h, side by side, at room temperature in normal serum (diluted 1:5 Dako Cytomation, Glostrup, Denmark). The samples were then incubated with primary antibody directed against the specific antigen CD34 (Novocastra, Code: NCL-END), diluted 1:50 Tris buffer (0.1 M, pH 7.4; TBS) or with with ubiquitin carboxyterminal hydrolase L1, known as protein gene product 9.5 (PGP 9.5) (AnaSpec Code: 53772) diluted 1:40 in TBS for 2 h at room temperature. Sections were washed in TBS, then incubated with a biotinylated secondary antibody diluted 1:50 for 1 h and then with the avidin–biotin horseradish peroxidase complex (ABC Kit; Dakopatts, Milan, Italy), prepared according to the manufacturer's instructions for a further 1 h. Sections were immersed in a solution of 0.05% 3.3-diamino-benzidine tetrahydrochloride (DAB) and 0.03% H_2O_2 for 10 min, dehydrated, cleared and mounted according to routine protocols.

Control reactions were performed without in the absence of primary antibodies but with isotype-matched IgGs.

The samples mentioned were also evaluated by two observers blinded to ensure the experimental reproducibility. Representative fields scattered in the preparation are analyzed objectively by investigators unaware of the group assignment. For quantitative analysis, immunopositive area was evaluated at a final X200 magnification using an optical microscope (Olympus, Hamburg, Germany). Digitally fixed images were analyzed using an image analyzer (Image Pro-Plus, Milan, Italy) by researchers unaware of the group assignment and were calculated as percentage of positive area (per-area) measuring five fields with the same area for each section.