Original Article
Relevance of inflammation and matrix remodeling in abdominal aortic aneurysm (AAA) and popliteal artery aneurysm (PAA) progression

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Abstract: Aneurysm is a multifactorial degenerative disease characterized by focal dilatation of blood vessels. Although abdominal aortic (AAA) and popliteal aneurysms (PAA) are the most common dilatative vascular diseases and share some features, a comparison between the different anatomical sites and the relative pathophysiological differences has not been established. In order to gain deeper insights to AAA and PAA, we have characterized the role of matrix remodelling, vascular cells phenotype depletion and the inflammatory process in both diseases. Results show a more extensive presence of T-cell, B-cell and monocyte-macrophage infiltration in AAA with respect to PAA. Concurring with this aspect, IL-6, IL-8 and MCP-1 are 10-fold increased in AAA. Moreover, MMP-9, and metalloproteinase inhibitor 3 (TIMP3) resulted up-regulated in AAA tissues. Regarding the catalytic activity, which is tightly related to the oxidative stress, we found an up-regulation of superoxide dismutase [Mn] mitochondrial (SODM), glutathione peroxidase 3 (GPX3) and peroxiredoxin-1 (PRDX1). Histological analyses clearly showed a massive elastin fragmentation in AAA. This may enhance the inflammatory response, which has a prevalent role in AAA, while PAA is mainly guided by a loss of the contractile phenotype. These findings suggest insight in these potentially devastating diseases in term of their progression, aiming to identify potential specific markers respectively for AAA and PAA treatment.

Keywords: Abdominal aortic aneurysm, popliteal aneurysm, vascular diseases, vascular remodeling

Introduction

Aneurysm is a pathological dilatation occurring in blood vessels. Aorta belongs to elastic arteries, the diameter of the abdominal tract is 1.8-2.0 cm, therefore the threshold for defining an abdominal aortic aneurysm (AAA) is an artery diameter larger than 3 cm [1]. The prevalence of AAA is 4-8% in men over 55, whereas women are affected in a lower percentage, which ranges between 1-2% [2, 3].

AAAs account for the 80% of all aortic aneurysms, which may have an atherosclerotic origin. Other less common causes include inflammatory, tuberculous and syphilitic or infectious aneurysms. AAA usually remains asymptomatic until the rupture occurs. Rupture of AAA and its associated catastrophic pathological insult carries the overall mortality more than 80%, and 2% of all deaths are AAA-related. Major risk factors for the occurrence of AAA are male gender, age, smoking, race, and pre-existent cardiovascular affections, such as hypertension and atherosclerosis [4].

Popliteal artery aneurysms (PAA) are the most frequent peripheral aneurysm, accounting for 80% of all of the peripheral aneurysms. Also for PAA, there is an increased incidence related to
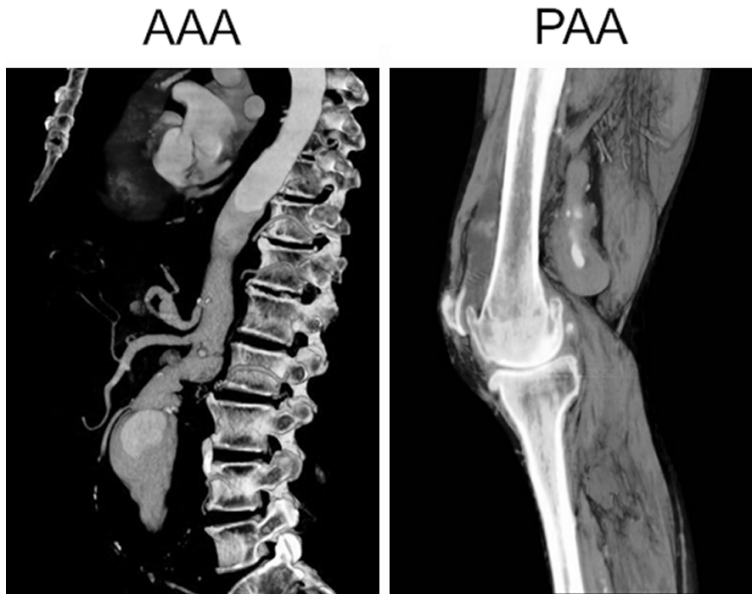
Figure 1. Computed tomography (CT) of abdominal aortic aneurysm (AAA) and popliteal aneurysm (PAA). Figure shows evident dilatation of the arteries involved, in AAA close to spinal column and in PAA behind the knee. Courtesy of Dr. Casella F.

Table 1. Clinical features of AAA and PAA patients enrolled in the study

	AAA	PAA
Age (mean ± SD)	75 ± 6	72 ± 12
Sex	Male (89%)	Male (100%)
DA (mean ± SD)	5.1 ± 0.5 cm	3.8 ± 0.9 cm
Hypercholesterolemia (%)	44%	33%
Smoking (%)	66%	25%
Hypertension (%)	89%	62.5%
CAD	56%	12.5%
Statins (%)	55%	25%

Table 1 shows demographical data (age, sex) and cardiovascular Risks (DA aneurysm diameters, hypercholesterolemia, smoking, hypertension, CAD coronary artery disease).

age and a strong male preponderance (male to female rate is 9:1, even higher than AAA which is about 4:1) [5]. The popliteal artery belongs to muscular arteries, in continuity with the superficial femoral artery. Anatomic landmarks include the tendinous insertion of the adductor magnus muscle in the distal femur superiorly, and the bifurcation of the popliteal artery into the anterior tibial artery and tibioperoneal trunk at the level of the tibial tuberosity inferiorly. The anatomical site together with the mechanical stress due to repeated flexion and extension of the lower limb may also concur to aneurysms

formation. The popliteal artery has a diameter of 7 mm, therefore the threshold for defining a popliteal aneurysm is an artery larger than 1 cm. PAA is often bilateral (60% of cases); moreover, there is a strong association with aneurysms of the abdominal district: 5% of people presenting AAA have a PAA, whereas about 20% of people diagnosed with a PAA have a concomitant AAA [6].

There is a remarkable strong clinical association between AAA and PAA, although the rupture is rare in PAA [7]. Distinctive feature of aneurysm is represented by the loss of the vessel wall architecture, in terms of extracellular matrix (ECM) fragmentation of the media layer and depletion of vascular smooth muscle cells (vSMCs). Two classes of proteases are associated with ECM fragmentation: cathepsin [8] and matrix metalloproteases (MMPs) [9]. MMPs are secreted by both vascular (endothelial cells, vSMCs, and fibroblast) and inflammatory cells (macrophages) [10-12]. MMP-2 and MMP-9 are the most commonly enzymes associated with aneurysm disease. They both degrade elastin and collagen. MMP-2 is constitutively expressed in small aneurysm, suggesting a role in aneurysm initiation, whereas MMP-9 is more prevalent in large diameter aorta, indicating a main participation in aneurysm progression and expansion [13].

ECM represents a dynamic structure that supports the arterial wall, driving its functions [14]. While ECM remodelling is strictly regulated under physiological conditions, abnormal expression and activity of the main ECM remodelling mediators are characteristic of several vascular disorders. Elastin fragmentation represents an early event in aneurysm formation, causing wall weakening [15]. Degradation of fibrillar type I and III collagen occurs in the late phase of the aneurysm expansion, leading to the loss of aortic tensile strength and causing the aortic rupture. vSMCs, in presence of aneurysm, reduce the expression of contractile markers, including smooth muscle cell myosin heavy

chain (MYH11) and smooth muscle actin (ACTA2). Moreover, vSMCs undergoing phenotypic switching [16] can also acquire macrophage markers and properties in atherosclerosis [17]. Based on these facts, improving the knowledge of aneurysm pathophysiology could lead to underline different potential targets involved in vascular wall degeneration. Thus, the purpose of this study is to investigate the differences in aortic versus popliteal aneurysms in term of matrix remodeling, vascular cells phenotype maintaining, inflammatory response and proteolytic pathways involved in the two distinct aneurysm diseases.

Materials and methods

Human sample collection of AAAs and PAAs

Human atherosclerotic AAAs (n = 10) and atherosclerotic PAAs (n = 10) were obtained during open surgical repair (**Figure 1**); control aortic and popliteal artery normal wall tissues were obtained from autopsies, excluding any cardiovascular pathology. As the aim of the study was to clarify the pathological mechanisms involved in the two different contexts, patients with concurrent PAA and AAA were excluded from the study.

Table 1 shows the clinical features for AAA and PAA patient recruitment. Informed consent was obtained from all the patients included in the study, according to the local institute's regulations and policies.

Histological and immunohistochemical analyses

AAA and PAA samples were fixed in neutral buffered formalin for 24 hours, and 5 µm-thick sections were cut from paraffin-embedded tissues. Sections were stained with hematoxylin/eosin, Weigert staining for elastic fibers, and Masson's trichrome. Collagen content was quantified by ImageJ software, and the percentage of collagen fibers was evaluated in cross-section area of the vessel walls, whereas elastin content was identified by immunofluorescence staining. Samples were incubated with anti-elastin (DBS, Italy) antibodies. Subsequently, they were incubated with FITC-conjugated secondary antibody (Vector, Ca, US), and DAPI (4',6-diamidino-2-phenylindole, Sigma Aldrich, Italy) was used for nuclear staining.

Immunohistochemistry analysis using CD4, CD8, CD20, lysozyme and CD68 (PGM1 and KP1) for inflammatory cells and alpha SMA for vascular smooth muscle cells phenotype (prediluted antibodies, Roche-Ventana, US) was performed. For alpha-SMA and inflammatory cells quantification ImageJ software has been used. Inflammatory cells quantification was obtained by the sum of infiltrated cells in the tissue under 20× objective (200×) of five random fields. Thus, a five-point grading system was used: from 0 to 15 positive cells = 0; from 16-30 = 1; from 31-45 = 2; 46-60 = 3; 61-75 = 4, more than 75 = 5.

Cytokines arrays kit

After removing the intraluminal thrombus, tissues were lysated and 500 µg of total protein were assayed for a wide panel of cytokines using a human antibody array (AAH-CYT-1, Ray-Biotech, Italy) following the manufacturer's protocol. Cytokines panel was visualized using enhanced chemiluminescence ECL detection reagents in a chemosensitive visualizer (VersaDoc, BioRad, Italy).

Western blot and zymography assay

After removing the intraluminal thrombus, tissues were lysated in RIPA buffer (1% Triton X100, 0,5% sodium deoxycholate, 0,1% sodium dodecyl sulfate (SDS), 150 mM sodium chloride, 2 mM EDTA, 50 mM sodium fluoride and 50 mM TRIS, pH 7.4) supplemented with protease inhibitors (0.2 mM sodium orthovanadate, 1 mM phenylmethyl sulfonyl fluoride and protease inhibitors cocktail P8340, all from Sigma, Italy). Proteins concentration was determined using the bicinchoninic acid assay (Pierce, Rockford, IL, USA). SDS-page was performed on 50 µg total proteins in sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.02% bromophenol blue and 62.5 mM Tris-HCl, pH 6.8), and proteins were transferred to a nitrocellulose membrane (Amersham Biosciences, Buckinghamshire, UK). Ponceau red was used as loading control. Membranes were incubated overnight with the primary antibody at 4°C: anti-MMP-9, fibulin V (Millipore, Italy), anti-MMP-2, EMILIN, and anti-alpha SMA (all from Abcam, Italy). Proteins were revealed with secondary antibody peroxidase-conjugates (Perkin-Elmer, UK). Protein bands were visualized using ECL (Perkin-Elmer, Western lightning
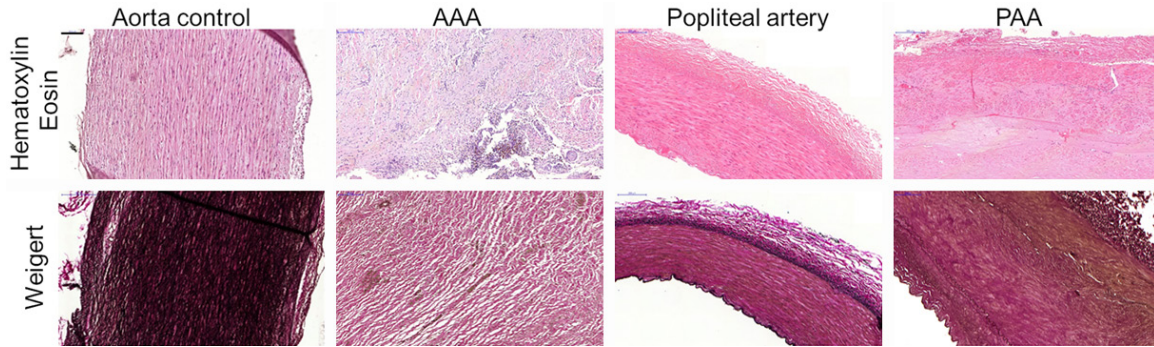
Figure 2. Histological analysis on human AAA and PAA and relative controls. Representative images of hematoxylin/eosin, showing the wall microstructure of the arteries tissues and the inflammatory infiltration, while Weigert staining displays the elastic fibers content. Figures show that AAA microstructure is severely degraded, while PAA microstructure is almost preserved. Scale bar 200 µm.

PLUS-ECL, UK) detection reagents in a chemo-sensitive visualizer (VersaDoc, BioRad, Italy). In order to assess MMP-2 and MMP-9 activity, zymography assay was performed. 50 µg of non-reduced proteins were separated on 10% of acrylamide gels with 0.2% of gelatin (Sigma, Italy). After washing, gels were incubated with developing solution (1 mM CaCl$_2$, 15 mM NaCl, 50 mM TRIS HCl, pH 7.4) overnight at 37°C. After fixation, gels were stained with Coomassie blue (0.05% Coomassie Brilliant blue R, 25% methanol and 10% acetic acid).

Proteomic analysis

Protein lysates, obtained from different tissues, were digested using the following protocol: samples were subjected to denaturation with 2,2,2-trifluoroethanol (TFE), to reduction with 1,4-dithiothreitol (DTT) 200 mM, alkylation with 2-iodoacetamide (IAM) 200 mM and the complete protein trypsin digestion with 2 µg of trypsin/lys-C (Promega, Madison, WI, US). The peptide digests were desalted on the Discovery® DSC-18 solid phase extraction (SPE) 96-well Plate (25 mg/well) (Sigma-Aldrich Inc., St. Louis, MO, US). Peptides were dried by Speed Vacuum until the analysis.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analyses were performed on digests using a micro-LC Eksigent Technologies (Dublin, US) system with a stationary phase of a Halo Fused C18 column (0.5×100 mm, 2.7 µm; Eksigent Technologies, US). The injection volume was 4.0 µL and the oven temperature was set at 40°C. The mobile phase was a mixture of 0.1% (v/v) formic acid in water

(A) and 0.1% (v/v) formic acid in acetonitrile (B), eluting at a flow-rate of 15.0 µL min^{-1} at an increasing concentration of solvent B from 2% to 40% in 30 min. LC system was interfaced with a 5600 + TripleTOF system (AB Sciex, Concord, Canada) equipped with a DuoSpray Ion Source and CDS (Calibrant Delivery System). The relative abundance of proteins was obtained using the label-free quantification. Samples were subjected to data-dependent acquisition (DDA): the mass spectrometer analysis was performed using a mass range of 100-1500 Da (TOF scan with an accumulation time of 0.25 s), followed by a MS/MS product ion scan from 200 to 1250 Da (accumulation time of 5.0 ms) with the abundance threshold set at 30 cps (35 candidate ions can be monitored during every cycle). The samples were, then, subjected to cyclic data independent analysis (DIA) of the mass spectra, using a 25-Da window: the mass spectrometer was operated such that a 50-ms survey scan (TOF-MS) was performed and subsequent MS/MS experiments were performed on all precursors [18, 19]. The MS data were acquired with Analyst TF 1.7 (AB SCIEX, Concord, Canada). Three instrumental replicates for each sample were subjected to the DIA analysis.

Protein identification was performed using Mascot v. 2.4 (Matrix Science Inc., Boston, USA), the digestion enzyme selected was trypsin, with 2 missed cleavages and a search tolerance of 50 ppm was specified for the peptide mass tolerance, and 0.1 Da for the MS/MS tolerance. The charges of the peptides to search for were set to 2+, 3+ and 4+, and the search was set on monoisotopic mass. The instrument
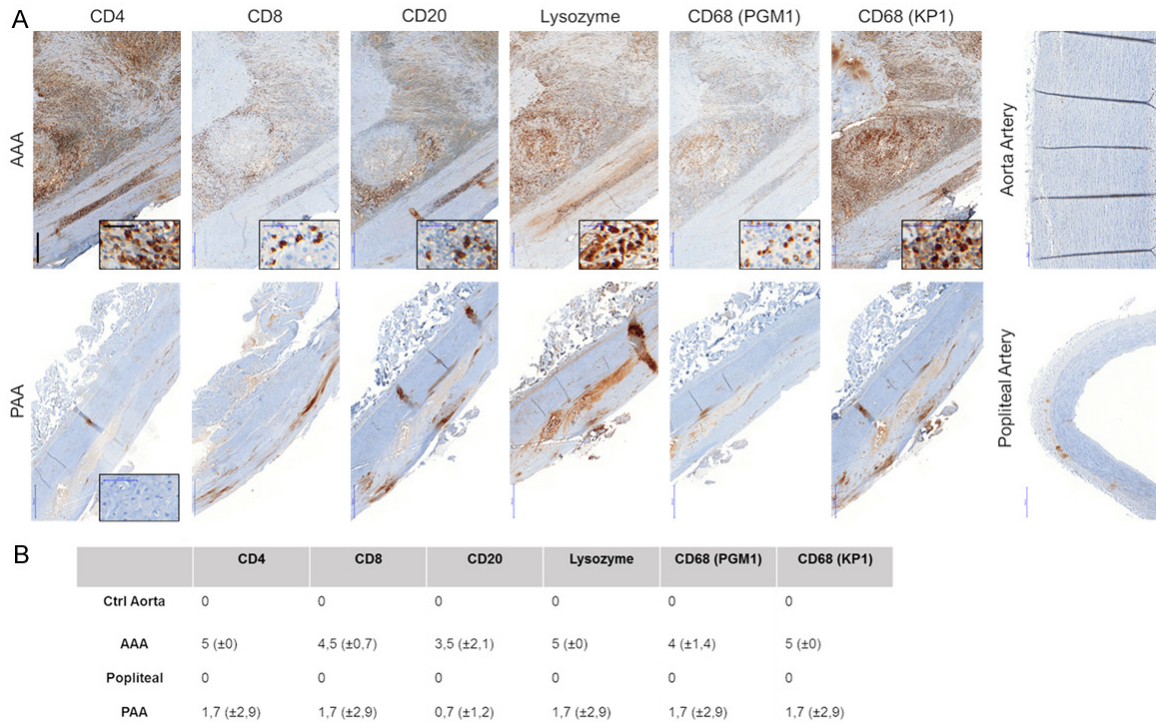
Figure 3. Inflammatory cell infiltration in AAA and PAA. A. Representative immunohistochemistry for anti CD4, CD8, CD20, lysozyme, PGM1 and KP1 staining. CD4+ is performed for T-helper lymphocytes, CD8+ for T-killer, CD20+ for B-cell, lysozyme, PGM1 and KP1 for monocytes-macrophage. Healthy popliteal and aorta, represented on the right, are negative for inflammatory cells infiltration (scale bar 100 μm). B. Score for inflammatory cells infiltration shows that in PAAs the inflammatory cells were significantly less represented, while in AAA T-cells and macrophages are the main inflammatory population in the medial layer.

was set to ESI-QUAD-TOF and the following modifications were specified for the search: carbamidomethyl cysteines as fixed modification and oxidized methionine as variable modification. The UniProt Swiss-Prot reviewed database containing human proteins (version 2015.07.07, containing 42131 sequence entries) was used and a target-decoy database search was performed. False Discovery Rate was fixed at 1%. The label-free quantification was carried out with PeakView 2.0 and MarkerView 1.2. (ABSCIEX, Concord, Canada). The up and down regulated proteins were selected using P value < 0.05 and fold change > 1.5.

Statistical analyses

All the experiments were repeated three times. All data are expressed as mean values ± standard deviation. Student's t-test was performed to determine the statistical significance. *Indicates P ≤ 0.05.

Results

Immuno-histological analyses

In presence of AAA, the wall microstructure was significantly altered, and the three layers could not be distinguished. In contrast, in PAA the microstructure was almost preserved. AAA showed moderate- to severe inflammatory infiltration almost along all the wall thickness, with defined areas particularly representative. On the contrary, only rare inflammatory cells were found in PAAs' wall, as shown by hematoxylin/eosin staining (**Figure 2**) and following confirmed in **Figure 3**. AAAs are characterized by an extensive medial elastolysis, as observed in Weigert staining (**Figure 2**). Indeed, elastic fibers in the medial layer were absent, or irregularly arranged and, frequently, fragmented. PAA showed a preserved internal elastic lamina, showing a less extensive medial elastolysis.
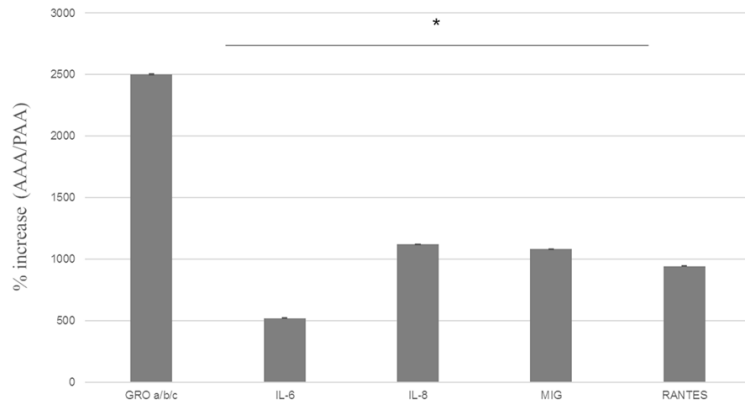
Figure 4. Cytokines assay on AAA and PAA. Data reported as AAA/PAA ratio, where AAAs were normalized respect to healthy aortas and PAA respect to healthy popliteal arteries. Cytokines expression analysis shows a significant up-regulation of Gro a/b/c, IL-6, IL-8, MIG and RANTES in AAA compared to PAA. *indicates P ≤ 0.05.

Inflammatory population in AAA and PAA tissue

In order to characterize the inflammatory cell population infiltrating AAA and PAA, immunohistochemistry for CD4, CD8, CD20, lysozyme, and CD68 was performed and quantified (**Figure 3**). As expected, there was a predominant inflammation in AAA, characterized by the presence of T-cells (helper and cytotoxic) and B-cells, as well as monocytes. Specifically, CD4 positive cells were found in all section area of the forming follicles in the aortic wall. In addition, CD8+ T-cells and B-cell (CD20) were significantly represented in aortic aneurysms. AAA showed also a considerable macrophage population (lysozyme-, PGM1- and KP1-positive cells). On the other hand, in PAAs the inflammatory cells were significantly less represented, as shown in **Figure 3B**.

Cytokines in aneurysm tissues

Cytokines are crucial mediators of the inflammatory response and regulator of immune and non-immune cells in AAA. We observed the same panel of inflammatory mediators expression in AAA and PAA. These cytokines resulted significantly upregulated in AAA with respect to PAA. In particular, GRO a/b/c resulted 25-fold more expressed in AAA, while IL-6, IL-8, MIG and RANTES were respectively increased of 5-, 10-, 10-, 10-fold (**Figure 4**).

ECM remodeling and fibrosis

Gelatinase (MMP-2 and MMP-9) involvement in pathological remodeling contextually to aneurysm, was verified by western blot and zymography assays. MMP-9 expression resulted significantly upregulated in AAA tissues with respect to healthy aortas, while MMP-2 expression was not significantly modified with respect to control. No significant differences were observed in PAA tissues with respect to healthy popliteal tissues both for MMP-2 and MMP-9 expression (**Figure 5A**). Anyway, MMP-9 activity resulted upregulated in all pathological tissues (**Figure 5B**).

As expected, in AAAs the wall damage is balanced by significant collagen increase, indicating a repairing process. On the contrary, in PAAs collagen content did not result significantly modified (**Figure 5C**).

Proteomic analyses

Proteomic analysis of aortic and popliteal aneurysms tissues was performed with LC-MS, in order to investigate the modulation of proteins related to inflammation, ECM remodeling and oxidative stress pathways. **Table 2** reports the identities, the modulation and the biological functions of eight proteins resulted over or under expressed in the AAA with respect to the PAA. Comparing the aortic control and popliteal arteries, these proteins were not regulated, so they can be considered specific of the pathological state and not of the different type of vessel. Metalloproteinase inhibitor 3 (TIMP3) resulted up-regulated in AAA tissues (fold change of 2.26), confirming the pathological ECM remodeling previously highlighted in AAA. Complement C1q subcomponent subunit B (C1-QB), immunoglobulin heavy constant gamma 1 (IGHG1) and immunoglobulin heavy constant gamma 2 (IGHG2) are related to inflammatory processes: our data showed an overexpression of C1QB and a down-regulation of IGHG1 and IGHG2 in AAA samples (fold change of 4.20, 0.43 and 0.50 respectively). Regarding the cat-
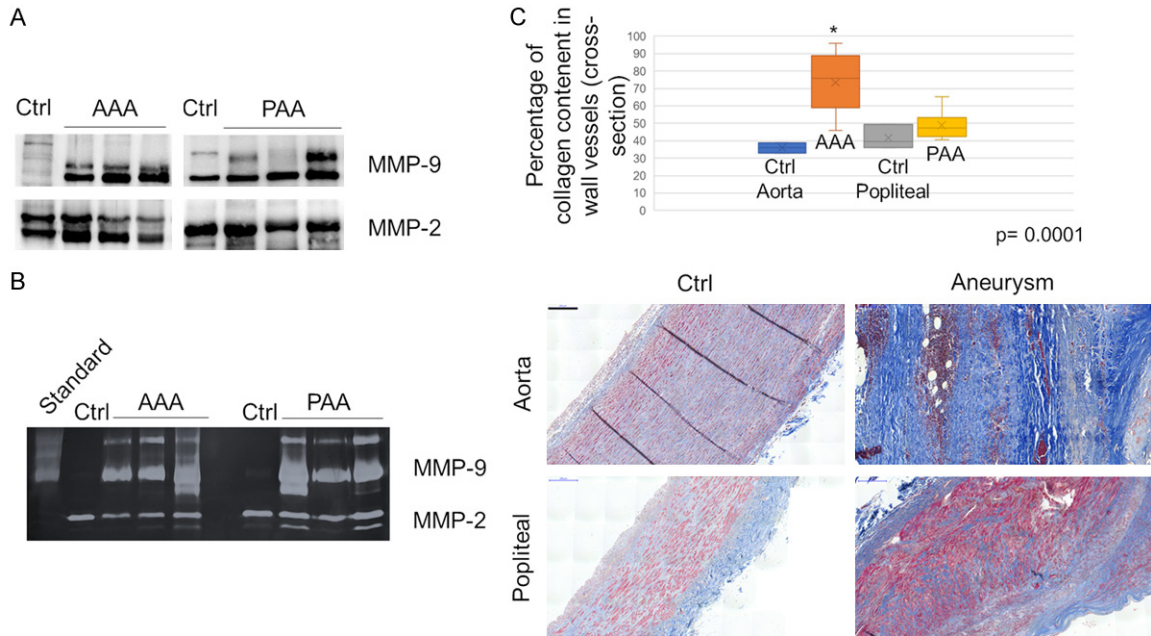
Figure 5. Matrix remodeling and fibrosis event in presence of aneurysms. A. Immunoblotting for MMP-2 and MMP-9 on representative sample of controls, AAA, and PAA. B. Gelatin zymography assay on AAA and PAA lysate and relative controls, peripheral blood was used a standard sample. MMP-9 activity resulted upregulated in all pathological tissues. C. Representative Masson trichrome for collagen visualization. Scale bar 200 μm. Percentage of collagen in cross-section area of vessel wall. Collagen content significantly increased in AAA respect to healthy aorta, while no significant difference is observed between PAA and healthy popliteal artery. *Indicates P ≤ 0.05.

Table 2. Regulated proteins in AAA and PAA

Protein	Accession name	Fold Change (*p*-value < 0.05) AAA/PA	Biological function
Metalloproteinase inhibitor 3	TIMP3_HUMAN	2.26	Extra cellular matrix remodeling
Complement C1q subcomponent subunit B	C1QB_HUMAN	4.20	Inflammatory response
Immunoglobulin heavy constant gamma 1	IGHG1_HUMAN	0.43	Inflammatory response
Immunoglobulin heavy constant gamma 2	IGHG2_HUMAN	0.50	Inflammatory response
Superoxide dismutase [Mn], mitochondrial	SODM_HUMAN	2.18	Oxidative stress
Glutathione peroxidase 3	GPX3_HUMAN	2.55	Oxidative stress
Peroxiredoxin-1	PRDX1_HUMAN	2.80	Oxidative stress

Regulated proteins in AAA and PAA after comparison to relative healthy tissues (aorta and popliteal artery) by proteomic analysis. The up- or downregulated proteins were selected using *P* value < 0.05.

alytic activity, which is tightly related to the oxidative stress, we found an up-regulation of superoxide dismutase [Mn] mitochondrial (SODM), glutathione peroxidase 3 (GPX3) and peroxiredoxin 1 (PRDX1) (fold change of 2.18, 2.55 and 2.80 respectively).

Elastic and muscular component

The involvement of the elastic and muscular tone loss was investigated in presence of AAA and PAA. As shown by **Figure 6A**, the normal elastin structure is observed in control sections (aortic and popliteal tissues). Specifically, elastin structure was completely lost in AAA, while it was preserved in PAA tissues, confirming data obtained by Weigert staining (**Figure 2**). In addition, EMILIN and fibulin V were quite preserved in presence of popliteal aneurysms, but strongly down regulated in AAA. Finally, AAA and PAA were characterized by a loss of vSMCs compared to their controls, as shown by western blot and immunohistological analyses in **Figure 6C** and **6D**. Cells in the medial layer were extensively positive to alpha-SMA antibody in control tissues, while in PAA tissues, alpha-SMA was
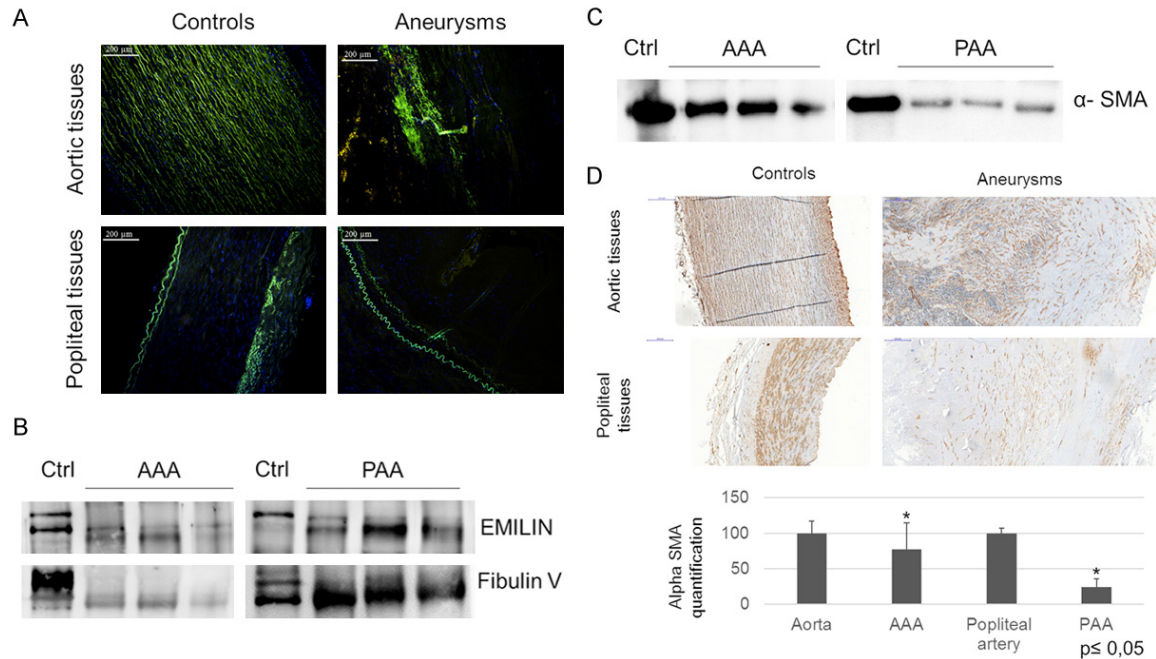
Figure 6. Elastic and muscular component. A. Representative image showing the structure of elastin in the cross-sections of AAA, PAA and relative controls. Elastin is marked in green, and nuclei were detected in blue by DAPI staining. Elastin fibers disorganization and fragmentation is more evident in the AAA than in PAA. B. Immunoblot for EMILIN and fibulin V. Both proteins were quite preserved in presence of PAA, but strongly down regulated in AAA. C. Immunoblot for alpha-SMA. AAA and PAA were characterized by the loss of alpha-SMA protein respect to healthy arteries. D. Representative immunohistochemistry for anti-alpha-SMA staining (scale bar 200 µm) and relative quantification with respect to healthy controls. Alpha-SMA level is lower in PAA than in AAA. *Indicates P ≤ 0.05.

significantly less represented than in AAA. A quantitative analysis confirmed the immunohistochemical data (**Figure 6D**). Thus, briefly, alpha-SMA was significantly more represented in AAA with respect to PAA, while elastic fibers were more preserved in PAA compared to AAA.

Discussion

Abdominal aorta and popliteal artery have significantly different microanatomical structures, as well as the anatomical sites are functionally different. In fact, as subjected to very high systolic pressures, aorta displays elastic characteristics, counteracting the internal forces while maintaining the elastic recoil necessary to the blood flow. From a functional point of view popliteal arteries are required to possess a muscular feature in order to guarantee an adequate peripheral bloodstream. Distally, internal pressures progressively decrease, while other mechanical stresses are present, for instance related to the lower limb mobility. Despite these relevant morpho-functional differences, aortic and popliteal aneurysms are characterized by

common features, like ECM degradation and loss of vSMCs [20, 21], thus leading to expansive remodeling of the wall with progressive loss of arterial functions. In AAA, the vessel wall microstructure is significantly damaged, with a severe inflammatory infiltration. Humoral inflammatory factors such as cytokines, chemokines, and reactive oxygen species could be secreted by these infiltrating cells. In wall vessels, ECM remodeling is a physiological process, necessary for the maintaining of tissue homeostasis and functionality. This process is mediated by proteolytic enzymes, among which MMPs are the most characterized and studied. However, MMPs are also responsible for ECM degradation during tissue remodeling in several pathologic conditions. MMP-9 strongly increases in presence of several cardiovascular diseases, like hypertension, atherosclerosis and myocardial infarction [20]. MMP-9 is absent in healthy aortas, whereas it is up-regulated in AAA. In popliteal artery wall (healthy and pathologic), there is no significant difference in expression of MMP-9. Instead, in both pathologic conditions, MMP-2 is similarly overex-

Table 3. Summary of obtained data

	Aortas	AAA	Popliteal arteries	PAA
Tissue microstructure	Preserved	Altered	Preserved	Partially altered
Functionality				
Elastic	Preserved	Altered	-	-
Muscular	-	-	Preserved	Altered
MMP-9	Absent	High expression	Expressed	High expression
		High activities	Not active	High activities
Inflammation	Absent	High	Absent	Moderate
Oxidative stress	Absent	High	Absent	Absent

pressed with respect to healthy arteries. The proteolytic activity in tissues is a highly dynamic process, regulated by the interplay of MMPs with TIMPs and other inhibitors: in agreement with a previous study [21], the proteomic analysis revealed an up-regulation of TIMP3 protein in AAA with respect to PAA. In particular, TIMP3 seems to exert a major role on MMP-2 activity in AAA. TIMP3 downregulation in PAA resulted in an increased activity of the both expressed gelatinases. Moreover, TIMP3 promotes the apoptotic death of vSMCs [22]. Since the tissue damage is demostrated in both pathological tissues, a scarring process was expected in both pathological tissues. Surplisingly, collagen increasing was present only in AAA tissues. This change in ECM proteins strongly modifies the vessel wall mechanical properties, leading to a plastic deformation when subjected to blood preassure. These processes are not present in PAAs, where ECM content resulted substantially not modified. Thus, causes of PAA progression have to be find somewhere else.

Unlike PAA, AAA is associated with a strong inflammatory infiltration in medial and adventitial layers [23]. Inflammatory cells are probably recruited by massive elastin fragmentation [24]. Indeed, this process releases soluble peptides exerting a chemotactic effect that recruits inflammatory cells, which further secrete proteolytic enzymes, thus amplifying the ECM degradation. Although elastolysis is present in both pathologies, our data indicate that it is significantly more extensive in AAA. Due to the elastic morpho-functional characteristics of the aorta, this tissue damage results in a significant loss of functionality. In fact, the wall weakening and loss of the elasticity in this case leads to a progressive inefficient pressure counteraction, with a plastic deformation of the vessel wall.

Our findings concerning AAA wall inflammatory infiltrate revealed the presence of CD4 and CD8 positive T-cells, representing respectively T-helper and cytotoxic T-lymphocytes, of CD20, representing B-cells, CD68 (KP1 and PG-M1) and lysozyme for macrophages. Most represented inflammatory cells in AAA are T-helper lymphocytes and macrophages. Inflammatory cells activate a Reaction cascade increasing inflammation environment and exacerbating the proteolysis process. Macrophages accumulating in the aneurysmatic tissues have a key role in AAA's pathogenesis. Monocytes recruitment and macrophages accumulation are modulated by cytokines and chemokines. We observed the same cytokines and chemokines panel for both aneurysms examined, but in AAA there was a strong increase of GRO a/b/c (CXCL1), IL-6, IL-8, MIG, RANTES (CCL5) with respect to PAA.

The overexpression of the complement fragment C1QB in AAA samples confirms the role of inflammation and of the complement cascade in the aortic aneurysm [25]. Moreover, there is a modulation (down-regulation) of IGHG1 and IGHG2, two inflammatory markers, in AAA [26]. Macrophages actively participate to parietal pathological remodelling and maintaining of the inflammatory conditions: immunohistochemistry and in-situ hybridization data revealed an intensive MMP-9 expression by aneurysm-infiltrating macrophages [27]. More recent papers demonstrated the macrophage-mediated release of cytokines, such as IL-1beta, TNF-α, IL-8, thus stimulating B-cell and cytotoxic T-cell differentiation, cytokine and protease synthesis [28]. The proteomic analysis showed a strong involvement of proteins related to oxidative stress and catalytic activity: PRDX1, SODM and GPX3 were overexpressed in aortic samples. Reactive oxygen species and oxidative stress are enhanced in the intraluminal thrombus: an upregulation of peroxiredoxins was already highlighted in ruptured aneurysmal wall tissue [29].

Taken together, these results show as the common outcome for AAA and PAA in terms of extensive ECM remodeling, weakening, and

loss of functionality is driven by substantially different processes. Inflammation and related oxidative stress remain the main features of AAA pathophysiology, however they seem to have only a limited role in PAA progression, where vSMCs phenotype depletion seems to drive the pathological outcome. A summary of our findings is reported in **Table 3**.

In conclusion, focal dilatation, caused by a loss of bio-mechanical properties, is the common feature of AAA and PAA. This process is mediated by the dysfunction of two different targets: medial elastin for elastic arteries, such as aorta, and muscular tissue in muscular arteries, like popliteal artery. Indeed, as a consequence of the altered environment, elastin resulted destroyed in the medial layer in presence of AAA, whereas it is quite preserved in PAA. In contrast, alpha-SMA is more represented in AAA with respect to PAA, where the muscular component resulted significantly deregulated.

Acknowledgements

Disclosure of conflict of interest

None.

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