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NEW INSIGHTS INTO EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF ABDOMINAL AORTIC ANEURYSMS

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Walter Friedrich Alfred Osswald

A ti, Pedro

Aos meus pais

À minha irmã

Em memória dos meus avôs

CONTENTS

ABBREVIATIONS

95% CI – 95 per cent confidence interval

AAA – Abdominal aortic aneurysm

AAC – Abdominal aortic calcification

ACEI/ARA – Angiotensin converting enzyme inhibitors/ angiotensin receptor antagonists

ACI – Aortic calcification index

CHSA – Portuguese Central Health System Administration / *Administração Central do Sistema de Saúde (ACSS)*

ACT – Angiocomputed tomography

AIOD – Aortoiliac occlusive disease

AS – Agatston score

BMI – Body mass index

C/EBPb – CCAAT-enhancer-binding protein b

CD – Carotid artery disease

CHUSJ – Centro Hospitalar Universitário de São João / São João University Hospital Center

CINTESIS – Centro de Investigação em Tecnologias e Serviços de Saúde

CT – Computed tomography

CVD – Cardiovascular diseases

DGS – Direcção Geral da Saúde

DM – Diabetes mellitus

DRGs – Diagnosis-Related Groups / Grupos de Diagnósticos Homogéneos (GDH)

ECM – Extracellular matrix

EU-28 – European Union-28

EVAR – Endovascular aneurysm repair

FMUP – Faculdade de Medicina da Universidade do Porto / Faculty of Medicine of the University of Porto

HU – Hounsfield Units

iAAA – Intact abdominal aortic aneurysm

ICC – Intraclass correlation coefficient

ICD-CM 9/10 – International Classification of Diseases – Clinical Modification 9/10

IL – Interleukin

ILT – Intraluminal mural thrombus

INE – Instituto Nacional de Estatística

IQR – Interquartile range

KLF-5 – Kruppel like factor 5

MCP-1 – Monocyte chemoattractant protein-1

MDCT – Multidetector computed tomography

MMPs – Matrix metalloproteinases

MS – Mass spectrometry

NF-κB – Factor nuclear factor kappa B

NHS – National Health Service

NMR – Nuclear magnetic resonance

OR – Odds ratio

OS – Open surgery

OSR – Open surgical repair

PAD – peripheral artery disease

PAI-1 – Plasminogen activator inhibitor-1

PAOD – Peripheral aortic occlusive disease

PPAR-g – Peroxisome proliferator-activated receptor g

PVAT – Perivascular adipose tissue

rAAA – Ruptured abdominal aortic aneurysm

RCTs – Randomized control trials

SAT – Subcutaneous adipose tissue

SD – Standard deviation

SMCs – Smooth muscle cells

SNS – Sistema Nacional de Saúde

TIMP-1 – Metallopeptidase inhibitor 1

UnIC – Unidade de Investigação e Desenvolvimento Cardiovascular / Cardiovascular Research Unit

US – Ultrasound

VAT – Visceral adipose tissue

WHO – World Health Organization

ABSTRACT

Introduction: Abdominal aortic aneurysm is a chronic condition that leads to aortic dilatation and rupture, a potentially lethal event. This disease has experienced an epidemiological transition in Western countries. Although the prevalence and mortality increased throughout the 20th century, it seems now in decline, even if this has not been synchronized among all countries neither between gender or age groups. The decline has been related to better control of the cardiovascular risk factors such as decreased smoking habits and more widespread use of cardioprotective drugs. The effect of improvements in AAA treatment as the introduction of EVAR on such decrease in mortality remains under debate. Unfortunately, while considerable achievements have been made in the devices field, with new and improved endografts dedicated to end-stage repair of AAAs, less effort seems to be invested in stopping or declining the progression of AAA. A deeper view to the pathophysiology of AAA may offer the possibility to re-think the disease itself, its biologic players and offer better therapies.

Aims: The two major aims of this thesis are: (a) To assess the trends in iAAA and rAAA rates of admission, repair and mortality in Portugal (Part I); (b) To explore the roles of AAC and aortic PVAT in AAA pathophysiology (Part II).

Methods: To accomplish the first aim, we resorted to the retrospective national administrative hospital database that contains a registration of all hospitalizations (consecutive case entry) occurring in public hospitals in mainland Portugal and to the causes of death database provided by INE. For the second aim, we used a CT-based approach to study the AAC (single-center retrospective study) and PVAT (multicenter case-control study) in patients with AAA. For PVAT, measures were compared to PAOD patients and normal controls. Human aortic PVAT and SAT were further collected during open surgery from patients suffering from AAA, PAOD and from post-mortem renal transplantation donors (controls). SAT and PVAT from AAA were used for *ex vivo* culture with SMC retrieved from non-pathologic aortas.

Results: As to the epidemiologic assessment, the rate of iAAA repair continues to grow, especially in patients ≥ 75 years old, even if the absolute numbers still lag behind those described in other European countries. A reduction in global and EVAR operative mortality was achieved, but mortality after OSR increased in the recent years. Differently from iAAA, admission due to rAAA reached a peak and have been recently decreasing. The global rAAA incidence, including cases that presented to the hospital and those that died before, has recently reached a plateau. This plateau corresponded to a parallel increase in deaths outside the hospital, decrease in patient turndown rate and stable global operative mortality in recent years. In our department a gradual increase in EVAR adoption occurred along with decreased in AAA repair mortality.

As to the second aim, in patients with iAAA, we found that the amount of AAC measured by the CT-based method ACI had a high correlation to that measured using the AS, was the most reproducible and was more often associated to demographic and clinical variables in the dataset that were associated with the AS. The main finding of our multicenter case-control image study about PVAT is that AAA patients had a higher PVAT density around the aneurysm sac than the healthy neck. This presented a positive correlation with aortic dimensions, after adjustment for other fat compartments, body mass index, sex, and age. Furthermore, PVAT of AAA patients has smaller adipocytes with higher interstitial fibrosis, presents with higher inflammation and increased extracellular matrix degradation. Adipose tissue of patients suffering from AAA was able to induce inflammation in healthy SMC.

Conclusion: (Part I) Our nationwide analysis is consistent with a middle stage in the AAA epidemiologic transition, meaning that a decline in overall iAAA admissions and iAAA repairs might be expected in upcoming years. Operative mortality after OSR both for iAAA and rAAA remains a concern, as well as deaths from rAAA outside the hospital. (Part 2) We propose that PVAT plays an important role in the onset and progression of AAA and underlying pathways through PVAT might be a novel promising therapeutic target in both prevention and conservative treatment of AAA.

INTRODUCTION

Definition

Aneurysm is a dilatation or widening of an artery, most commonly being fusiform in shape. This pathological dilation, should the patient live long enough, leads to rupture of the aorta, a potentially lethal event. The most widespread definition of AAA is based on the diameter of the abdominal aorta: an abdominal aortic diameter of 3.0 cm or more in either anteroposterior or transverse planes, which usually is more than 2 standard deviations above the mean diameter for men, is considered to be aneurysmal (1, 2). It can also be defined when the maximum diameter is ≥50% greater than the suprarenal diameter (1).

Unless an aneurysm is ≥5cm in women or ≥5.5cm in men upon diagnosis, current guidelines (1) do not recommend surgery given the lack of benefit against the risk of rupture, that is considered to be low in those cases. A lower threshold might be more appropriate in women and some Asian populations. Patients with symptomatic aneurysms and whose aneurysms increase in diameter by 1 cm or greater within one year should also undergo repair, regardless of aneurysm diameter (1).

Risk factors

Risk factors for AAA include the usual occlusive atherothrombosis risk factors such as male sex, ageing, smoking and dyslipidemia, but not diabetes mellitus (3-7). The presence of AAA in a patient is a marker of atherothrombotic disease elsewhere (8), and aortic diameter a predictor of all-cause and cardiovascular mortality (9).

The prevalence of AAA increases with advancing age by 2% to 4% per decade (10, 11). Smoking is the major AAA risk factor (12-14). The prevalence of AAA in tobacco smokers is more than four times that in life-long nonsmokers (12). A systematic review of 10 studies revealed that the relative risk associated with smoking was three-fold greater than the risk for developing coronary artery disease and nearly five-fold greater than the risk for cerebrovascular disease (15). Among lipid markers, low high-density lipoprotein level is the most sensitive predictor of AAA (16). This finding has been related not only to the impact of hypercholesterolemia on the initial step of atheroma in the aorta, but also to the low levels of α1-antitrypsin conveyed by high-density lipoprotein in human AAA (17).

The familial clustering of AAA has drawn attention to non-environmental risk factors for the disorder. Genetic determinants of susceptibility to AAA have been approached through investigation of familial aggregation of the disease, and more recently by genome-wide association studies in populations. A study based on the Swedish twin register showed that concordant pairs of AAA were higher in monozygotic compared with dizygotic twins, indicating genetic effects. The monozygotic twin of an AAA patient had a AAA risk that was 71 times that of the monozygotic twin of an unaffected person (18). Genome-wide association studies have been able to identify additional AAA risk loci. These include: the sequence variant rs10757278-G on 9p21 (OR of 1.31), adjacent to the genes CDKN2A/CDKN2B, which are important regulators of cell growth and survival (19); the A allele of rs7025486 on 9q33 (OR of 1.21), located within the DAB2IP gene that encodes an inhibitor of cell growth and survival (20). More recently, through a meta-analysis of 6 genome-wide association study data sets and a validation study totaling 10 204 cases and 107 766 controls, 4 new AAA risk loci were identified: 1q32.3 (SMYD2), 13q12.11 (LINC00540), 20q13.12 (near PCIF1/MMP9/ZNF335), and 21q22.2 (ERG). Network analyses identified ERG, IL6R, and LDLR as modifiers of MMP9, with a direct interaction between ERG and MMP9 (21).

Acquired and morphologic factors positively associated with AAA expansion include: smoking (22), large baseline AAA diameter and large AAA thrombus load, and possibly male gender and age (23), but not hypertension (24) or COPD(25) despite their association with AAA presence. Factors that have been negatively associated with AAA expansion include diabetes mellitus (26-28), and possibly peripheral artery disease (29, 30), aortic calcification (31, 32) and coronary artery disease (33).

Aneurysm diameter and rapid AAA expansion is the most prominent predisposing factor for rupture (34). The UK Small Aneurysm Trial (35) found that the risk of rupture was independently associated with female sex (females had a threefold increase in rupture risk compared with males), larger initial AAA diameter, smoking, lower forced expiratory volume during the first second, and higher mean blood pressure. Risk of rupture was not independently associated with age, BMI, serum cholesterol, or the ankle/brachial index.

Epidemiology

AAA prevalence is routinely reported in articles to emphasize the importance and express the burden of the disease. However, the epidemiology of AAA is changing, and although AAA prevalence and mortality increased throughout the 20th century, it seems now on the decline (36-42). In a systematic review, the prevalence of AAA was reported to be in a range from 1.7% to 12.7% (43). Although the lowest incidence identified was 1.7% in 65-year-old men from the aneurysm screening program, 0.5% of men were known to have an AAA and therefore were not invited to screening, resulting in a prevalence of 2.2% in the population (44).

Population-based mortality from rAAA was calculated in another systematic review that included 24 retrospective cohort studies, published between 1977 and 2012 (45). The estimated pooled total mortality rate was 81% (95% CI of 78-83). As expected by the assumption of an epidemiologic transition in AAA, a decline in mortality was observed over time, being 86% (95% CI 83-89%) before 1990, compared with 74% (95% CI 72-77%) since 1990.

When we consider only the cases that were submitted to repair, both elective and emergent repair must be considered. VASCUNET is an international collaboration of registries, consisting of national (Australia, Denmark, Hungary, Iceland, New Zealand, Norway, Sweden, Switzerland, UK), regional (Finland), and multicenter (Germany) databases. In a VASCUNET report, data were analyzed overall and per treatment modality to address peri-operative mortality as a primary outcome. The overall peri-operative mortality for rAAA repair was 28.8% (95% CI 27.9-29.8), being 32.1% (31.0-33.2) for open aortic repair and 17.9% (16.3-19.6) for EVAR (46). Peri-operative mortality of elective repair fell from 3.0% in 2005-2009 to 2.4% in 2010-2013. Mortality for EVAR decreased from 1.5% to 1.1%, but the outcome worsened for open repair from 3.9% to 4.4% (47).

National assessment

It seems clear that a reliable national assessment of incidence/prevalence, admission and mortality associated to iAAA and rAAA might give insight into the expected health care costs associated with the treatment of this disease.

The assessment of the incidence/prevalence of iAAA in the population must resort to screening. To study admissions and repairs due to iAAA or rAAA, retrospective administrative hospital databases or prospective AAA registries are often used. Finally, evaluation of mortality due to rAAA that occurs outside the hospital relies on the cause of death databases, its accuracy depending on the autopsy rate. When we started this thesis, a national prospective AAA registry was not available, and a formal screening had never been implemented in Portugal.

The retrospective administrative hospital database available for this purpose is the NHS administrative database, the hospital morbidity database, formerly designated as DRGs database. This database was provided by the ACSS and contains a registration of all hospitalizations (consecutive case entry) occurring in public hospitals in mainland Portugal, where most of iAAA repairs are performed. The main diagnosis was used for cases' selection. Like any patient classification system, DRG require the collection of a minimum set of data, and the main diagnosis (which, after study of the patient, was found to be responsible for admission to the hospital), the diagnosis (all other diagnoses associated with the patient's clinical condition, which may lead to complications or comorbidities), the procedures performed, the patient's age and gender, the destination after discharge (transferred, out of medical opinion, deceased) and birth weight (for newborns).

The Portuguese health system is characterized by three co-existing and overlapping systems: the NHS; special public and private insurance systems for certain professions or companies (health subsystems); and private voluntary health insurance. All residents in Portugal have access to health care provided by the NHS, mainly financed through taxation. Public hospitals are funded through global budgets, but with an increasing role of DRGs, and private insurers and health subsystems providers. Private health care providers mainly fulfil a supplementary role to the NHS rather than providing a global alternative to it. Currently, the private sector mainly provides diagnostic, therapeutic and dental services, as well as some ambulatory consultations, rehabilitation and hospitalization (48).

Each registered hospital episode includes information about diagnoses and medical or surgical procedures, both coded using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) and International Classification of Diseases, Ninth Revision, *Procedure* Coding System (ICD-9-PCS). Internal audits are performed by auditing doctors that are part of the coding services that exist at each institution. External audits are performed by the ACSS to verify the accuracy of the clinical coding performed at NHS hospitals. The episode presents no disconformity when it respects ICD-9, Coding Clinic and National Consensus. The report that compared the public hospitals was in 2010 when a total of 4,510 episodes were audited in 44 hospital institutions. The results indicate that nonconformities among diagnoses, procedures and other administrative and demographic variables in general did not affect the respective DRGs grouping (nonconformities mean: 13.7%, 26.5% maximum, 2.1% minimum) (49).

INE has provided information on mortality by causes of death since 1969. This information has changed over time. It started in paper, with the entries of deaths sent to INE by the Civil Registry. In 2006, a significant part of the information about deaths, has already been sent to INE electronically, culminating in 2007 with total dematerialization of the information. Since 2007, the information on deaths from causes of death, collected by the Conservatories and registered at the Civil Registration and Identification Integrated System is sent to INE by the Institute of Technologies from the Ministry of Justice, by electronic file, under the collaboration protocol signed between INE and the referred institute.

The death database sent to INE is shared by two statistical operations:

a. Death Statistics - area of demographics
b. Causes of death - area of health

Death data sent to INE is based on the transcript of the death certificate by the Conservatories. It is received and validated

at INE by the area of demography. It is subsequently made available to the DGS as an online program with a restricted number of variables about death/causes of death, and currently this entity codifies the causes of death according to the International Classification of Diseases (ICD 10) of the World Health Organization (50). Completeness of statistics on cause of death in Portugal, that is number of deaths for which cause of death is registered to the civil registration system, is fairly good as among the member states of the World Health Organization, Portugal was among the 42% of the WHO member states that provided data completeness of 70% to 100% (51).

"A silently ticking time bomb"

In perspective, if a 50-years-old individual is diagnosed with an aneurysm less than 5.5 cm, all he can do is wait and comply with a surveillance program. However, he also has to live with the fact that a ruptured AAA is a surgical emergency with high mortality rates if not managed immediately. Although surgical repair is recommended for aneurysms ≥ 5cm in women or ≥ 5.5cm in men, almost 6% of all ruptured AAAs are < 55 mm in diameter, and hence a detailed examination of the aneurysm morphology is crucial for risk prediction (52).

While considerable achievements have been made in the field of the endografts, with new and targeted devices dedicated to end-stage repair AAAs, less effort seems to be invested in stopping or declining the progression of the AAA. A deeper view in the pathophysiology of AAA may offer the possibility to re-think the disease itself, its biologic players and offer better therapies for AAA. To slow the AAA progression might lead not only to avoidance of a surgical correction, but also make it more successful.

A defective medial layer

Several etiologies might cause abdominal aortic aneurysmal dilatation, but few AAA are the direct consequence of specific causes. These include trauma, acute infection (brucellosis, salmonellosis), chronic infection (tuberculosis), inflammatory diseases (Behçet and Takayasu disease) (53, 54) and connective tissue disorders (Marfan Syndrome, Ehlers-Danlos type IV) (55). Therefore, most AAA are non-specific degenerative or atherosclerotic, the former term being preferred by most authors to avoid the unproven causative relationship between aneurysms and typical atherosclerosis (56, 57). Since not all patients with atherosclerosis develop an AAA, different local responses to atherosclerosis in the abdominal aorta in human beings may play a role.

The different etiologies share a common histopathological aspect characterized by thinning of the media, fragmentation of the elastic fibers and decreased concentration of elastin (58-60) (Figure 1). Differently, plaque deposits without media thinning, without loss of elastic lamellae, and without artery wall dilation may predispose the aorta, in the event of continuing plaque accumulation, to the development of lumen stenosis (56). Classically, the occurrence of aortic aneurysm is associated with alterations of the connective tissue in the aortic wall. Elastin and associated proteins form a network of elastic fibers responsible for the viscoelastic properties. The loss of elastic fibers seems to be an early step in aneurysm formation (61).

Collagen is also a significant component of the media and the surrounding fibrous adventitia, providing tensile strength and help maintaining the structural integrity of the vascular wall. The contribution of the collagen to the resistance of the aorta is emphasized in the absence of medial elastin. Increased collagen turnover has been reported in AAA (62, 63), and collagen degradation is taken as the ultimate cause of rupture (61).

The impact of the rigidity of the aortic wall in AAA progression is suggested by the negative association between AAC and AAA progression. Calcium plaque density was considerably higher both in the supra and infrarenal segments in the non-aneurysmatic group, whereas in the infrarenal segments in aneurysmatic aortas they were thinner and smaller than in normal-sized (31). Moreover, the mean annual growth rate was significantly lower in men with higher US-assessed AAC, even after multivariate linear regression analysis adjusting for age, smoking and aspirin use (32).

The referred aortic wall defects and damage are dependent on the production of proteases by different cells including medial SMC and adventitial fibroblasts from the aortic wall, and by inflammatory cells infiltrate in the media and adventitia, coming from the aortic blood and from medial neovascularization (64-66). The neural structures are also increased in aneurysms, in particular in the tunica adventitia (67). Compared to nonruptured aneurysms, angiogenesis is more obvious in the external two thirds of the media tissue in ruptured AAA (68). These neovessels are described as incomplete, leaky, and rupture easily, resulting in vascular remodeling and aortic wall weakening by damaging its structural integrity (69). Furthermore, marked intimal hyperplasia of the adventitial *vasa vasorum* was found in the AAA sac (70). Further immunohistology studies revealed proliferation of SMCs, which caused luminal stenosis of the *vasa vasorum* and decreased HemeB level in the sac wall, suggesting low perfusion of the sac.

The fibers of elastic and collagen are degraded by proteolytic enzymes mostly represented by MMP locally activated by either other MMP or by plasmin generated by plasminogen activators (71-76). Elastin degradation fragments are thought to promote leucocyte recruitment into the aortic wall as well as proinflammatory cytokines, chemokines, and prosta-

glandin derivates produced by both the resident mesenchymal cells and the inflammatory cells themselves (77, 78). The hypothesis that leucocyte recruitment is due to innate or autoimmunity to extrinsic antigens was also raised. Based on previous studies, it is in fact possible that AAA formation is triggered by innate or autoimmunity to extrinsic antigens that may share molecular motifs with those on the aortic wall (79). Along with the destruction of the ECM in the medial layer, the key event in the development of AAA is the reduction in the density of SMC (80). Besides apoptosis, a phenotypic shift of SMC was noted in human AAA (81). SMC were larger than non-aneurysmal SMC and exhibited reduced proliferation, greater apoptosis and increased senescence. It has been demonstrated that inhibition of the mTOR cascade attenuates AAA progression by preserving or restoring SMC contractile phenotype versus a synthetic phenotype (82). The phenotypic skewing of SMC to a synthetic phenotype causes increased secretion of MMPs, while production of ECM is reduced (83). More recently, using novel methodology, low SMC contraction in vitro was demonstrated in a sub-group of patients with sporadic AAA, and a link between impaired contractility of SMCs of AAA patients and current tobacco smoking, suggesting a possible connection between the effects of present-day smoking and SMC dysfunction (84).

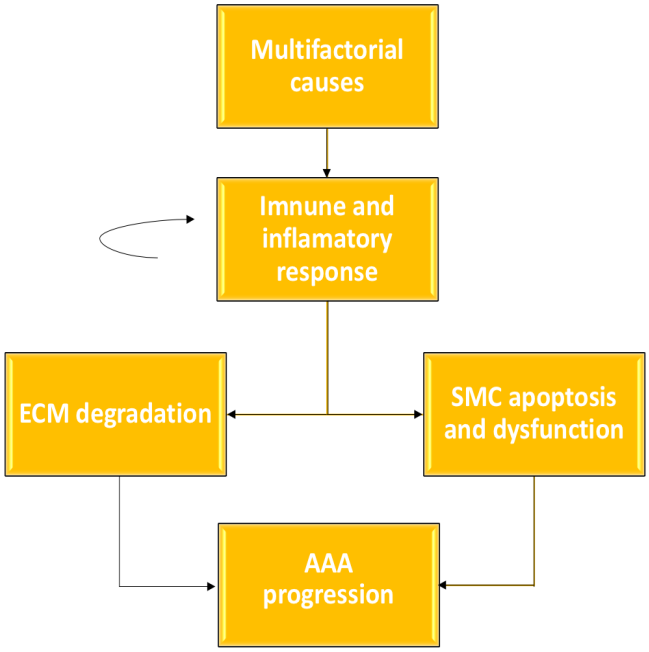

Figure 1 The pathophysiological processes underlying the progression of AAA (adapted from (85)).

The inner side

Evidence for Endothelial Dysfunction

As mentioned before, AAAs share the usual risk factors with occlusive atherothrombosis, except for diabetes mellitus. In the Atherosclerosis Risk in Communities Study cohort, circulating biomarkers of endothelial dysfunction (white blood cell count, fibrinogen, D-dimer, troponin T, N-terminal pro-brain natriuretic peptide, and high-sensitivity C-reactive protein) were strongly positively associated with AAA incidence (86).

Besides the clinical evidence, animal studies gradually drew the attention of AAA research into the endothelium. Endothelial cells participate in several biological processes that maintain the vessel wall structure and function. It is possible that the pathological changes of endothelial cells are earlier than those of media and adventitia in AAA formation process.

Franck G et al (87) demonstrates the potential of restoring the endothelial lining to control AAA dynamics. Rat aortic endothelial cells endovascularly seeded prevented AAA formation and stabilized formed AAAs through the reestablishment of the endothelial lining, the suspension of proteolysis, and the reconstitution of new aortic wall rich in SMCs and ECM. Transplanted rat aortic endothelial cells did not participate directly in aortic wall repair. Their healing properties implicated paracrine mechanisms involving the upregulation of endothelium-derived stabilizing factors and the recruitment of resident vascular cells. The importance of endothelial nitric oxide synthase (eNOS) was tested in the apolipoprotein--knockout mice (88). Male animals with eNOS-double knockout accelerated arteriosclerosis, AAA and aortic dissection formation, and ischemic heart disease. The coupling status of eNOS is determined by its cofactor (HB4). HB4 deficiency in a mouse model was characterized as having eNOS uncoupling and endothelial dysfunction, leading to a high risk of

AAA with Ang II infusion (89). Restoration of dihydrofolate reductase expression by oral administration of folic acid or overexpression of dihydrofolate reductase completely prevented AAA formation in the Ang II-infused mice model of HB4 deficiency, while attenuating progressive uncoupling of eNOS, vascular remodeling and inflammation characterized by medial elastin breakdown and augmented matrix metalloproteinase 2 activity and activation of matrix metalloproteinase 9, as well as macrophage infiltration.

The endothelium was also hypothesized as the mediator of the hemodynamic influences on AAA development and progression. Blood flow is in direct contact with endothelium and can produce shear stress. On the other side, shear stress regulates the expression and activity of proteases secreted by endothelial cells. High unidirectional laminar shear stress reduces the inflammation and development of AAA via direct regulation of intimal macrophage adhesion, transmural migration or survival (90), while in conditions of oscillatory shear stress, the activity of MMPs and cathepsin increase and the expression of TIMP3 descreases compared to unidirectional laminar shear stress (91-94). Other endothelial pathways mediating pro-aneurysmatic reactions in the aortic wall include: the transcription factor NF-κB (95), P-selectin (96), reactive oxygen species (97) and cyclophilin A (98).

In the proposed role of the endothelium in AAA formation, shear stress produced by blood flow influences the endothelium to cause endothelial dysfunction via uncoupling of eNOS and oxidative stress induction. Endothelial dysfunction activates NF-κB, cyclophilin A and other pathways to further initiate and amplify the inflammatory reaction via overproduction of cytokines and chemokines. More inflammatory cells infiltrate and exacerbate oxidative stress to form a vicious cycle, where the balance of TIMP/MMP is tipped, and proteolysis is promoted. Eventually, the aortic wall is damaged as a result of SMCs apoptosis and ECM degradation due to proteolytic degradation.

AAA ILT

The development of AAA is also associated with ILT in most patients. Different from atherosclerosis that leads to arterial occlusive diseases, blood flow is maintained in aortic aneurysms allowing a persistent remodeling activity of the thrombus itself. The eccentric distribution of ILT was associated with continuous expansion (99), and aortic mural thrombus volume is associated with AAA growth (100-102) as mentioned before.

The increasing thickness of the ILT leads to local hypoxia at the inner layer of the media, which can induce increased medial neovascularization and inflammation (103). There is a significant positive correlation of ILT thickness with active MMPs concentration in the adjacent AAA wall (73, 104). By trapping polymorphonuclear leukocytes and adsorbing MMP9 and other plasma components, ILT could also act as a source of proteases in aneurysms that plays a critical role in enlargement and rupture (105-110).

Proteomic analysis of the thrombus itself and the assessment of plasma ILT-derived markers have been used in the identification of novel proteins related to ILT biological activities. The aneurysmal diameter was correlated with plasma markers of ILT turnover such as of fibrin formation and degradation (111), the circulating complex plasmin-α 2-anti-plasmin (112), thioredoxin (113) and peroxiredoxin-1 (114). The presence of AAA increased the hemorphin 7 concentration in the serum relative to controls, and their levels were positively correlated with the volume of the thrombus (115).

The outer side

Adventitia

As pointed before, the determining cause of the first inflammatory activation and the initial recruitment of immune cells in the aortic wall is yet to be understood. The production of proteases by resident vascular wall cells (medial SMC and adventitial fibroblasts) and lymphomonocytic infiltrate cells can be seen as a central process in which adventitia plays a major role. The infiltrate cells include a varying number of macrophages in the inner part of the adventitia, capable of phagocytosing iron originating from ILT hemoglobin, and adventitial tertiary lymphoid organ formation, consisting mainly of lymphocytes, monocytes, plasma cells, and sparse mast cells, usually on a background of fibrous tissue (116), providing evidence of the shift from innate to adaptive immunity.

Inflammatory cells could reach the aorta using the *vasa vasorum* structure (vide supra) as a bridge between the patients' immune system and the inner layers of the vessel wall (117, 118). Indeed, clusters of immune cells were observed in the outermost portion of the aortic wall, mostly in the tunica adventitia and occasionally between the SMCs of the middle layer (67). The higher concentration of immune cells was founded in the peri-vascular spaces in the tunica adventitia.

In *in vitro* studies, adventitial fibroblasts secreting MCP-1 have also been shown to recruit monocytes that then promote the proliferation of more fibroblasts to amplify the inflammatory response (119). Fibroblasts also play a critical role in the adventitial response to injury, since they can proliferate and secrete procollagen-1, leading to perivascular fibrosis and resistance to rupture. Transforming growth factor-*β*, synthesized by polarized M2 macrophages and immune cells, is probably the main molecular link between inflammation and the peri-aortic fibrotic healing process (120).

In contrast to non-specific degenerative AAA, the inflammatory variant is characterized pathologically by marked thickening of the aneurysm wall, fibrosis of the adjacent retroperitoneum, and rigid adherence of the adjacent structures to the anterior aneurysm wall (121). An extraordinary expansion of the adventitia due to inflammation also distinguishes inflammatory from atherosclerotic AAA.

Adipose tissue

Although adipocytes in the AAA wall have not been recognized as a major pathological feature of AAA, recent studies using human AAA samples have underlined its importance. Histologic evaluation of AAAs, popliteal artery aneurysms, and control aorta shows extensive medial (popliteal artery aneurysms) and transmural fibrosis (AAA), and reveals abundant adventitial adipocytes aggregates as an exclusive phenomenon of AAAs (122). The meaning of these findings is still unclear. It is possible that these adipocyte deposits play an active role in the pathological processes occurring in the media during AAA progression since adventitial proteolytic (MMP9) and inflammatory factors (TNF-α, IL-1β) were higher in the adventitial fat of diseased vessels compared with healthy tissues in samples from human abdominal aneurysms (123). More intriguingly, microarray analysis of the aortic wall tissue identified genes essentially associated with processes related to impaired tissue remodeling, such as angiogenesis and adipogenesis that appear to converge at activation of HIF-1α signaling in mesenchymal cells (124). In vitro differentiation tests indicated a sharply increased adipogenic potential of AAA adventitial mesenchymal cells (122). Furthermore, ruptured AAA are enriched with adipocytes and adipocyte-related genes pointing to an association between the extent of fatty degeneration and rupture. The association between the adipocyte content and higher wall vulnerability was also verified in the analysis of the changes in the microstructure, histology and mechanics to link them to AAA disease progression (125).

In the rat hypoperfusion model of AAA, the administration of triolein increased the AAA rupture rate and this was along with the number of adipocytes was increased in ruptured vascular walls compared to non-ruptured walls (126). In the ruptured group, macrophage infiltration and the protein levels of MMPs 2 and 9 were increased in the areas around adipocytes, while collagen-positive areas were decreased in the areas with adipocytes compared to those without adipocytes. In the same study, the administration of fish oil, which suppresses adipocyte hypertrophy, decreased the number and size of adipocytes, as well as decreased the risk of AAA rupture compared to the triolein administered group. Accordingly, Niestrawska JA et al were able to show significant differences in elastin content, collagen orientation, adipocyte contents, and also a deposition of newly formed collagen forming a neoadventitia, being able to discriminate two types of remodeled walls: (i) potentially safe and (ii) possibly vulnerable associated with inflammation and a higher number of adipocytes.

The mechanisms underlying the appearance of the ectopic adipocytes remain poorly characterized (Figure 2). Fatty degeneration has been observed in chronic degenerative conditions, such as severe limb ischemia, muscle wasting dystrophies, and recurrent rotator cuff lesions (127-129). The phenomenon is thought to reflect dysregulated repair processes that also include (trans)differentiation of mesenchymal cells into adipocytes (130).

Abundant presence of C/EBPb, KLF-5, and PPAR-g was shown in the mesenchymal cells of the outer media and adventitia of the aneurysm wall (122), showing the transcriptional machinery required for fatty degeneration is present within mesenchymal cells of the AAA wall and colocalizes with the adipocyte clusters. The process of fatty degeneration further depends on the ability of the resident mesenchymal cell population to undergo transdifferentiation into adipocytes. Appropriately, Kugo H at al (131) showed that CD44+CD90+ mesenchymal stem cells express adipogenic transcription factors in the AAA wall of a hypoperfusion-induced AAA model.

PVAT is a specific depot of adipose tissue that adjacently surrounds blood vessels. Recent studies have highlighted the relation between that PVAT and the CVD. Indeed, several reviews have already summarized the relationships between PVAT and common CVD (132-135). Besides acting as a support tissue, protecting vessels against neighboring tissues, PVAT is also an endocrine organ that releases a wide range of biologically active molecules that may have profound influence in the adjacent vasculature (136).

Experimental studies in mice show that the thoracic PVAT displays characteristics of the brown adipose tissue phenotype, with high vascularization and multiple small lipid droplets and numerous mitochondria in adipocytes (137), whereas abdominal PVAT displays an intermediate phenotype between white and brown adipose tissue (138), being more prone to inflammation, as indicated by more markers of immune cell infiltration and greater expression of inflammatory genes in abdominal PVAT than for those in thoracic PVAT.

When having the PVAT removed from vascular wall, the number of CD44+CD90+ cells and adipocytes in the AAA wall significantly decreased. Intriguingly, the AAA diameter significantly decreased in the PVAT-removed vascular wall compared with that in the vascular wall with PVAT (131). The role of PVAT for aneurysm progression was further emphasized during the study of Interleukin-18 in the AAA development (139). Interleukin-18 uses both receptors, IL18r and NCC, to promote AAA formation. Lesion adipocytes and PVAT contribute to AAA pathogenesis by releasing leptin and FABP4 that induce the expression of IL18, IL18r and NCC and promote IL18 action.

Taking all together, a potential mechanism underlying the ectopic appearance of adipocytes in the AAA wall might impli-

cate hypoperfusion in the aortic wall, having possibly the obstruction of the *vasa vasorum* as a trigger (70) or the presence of the ILT. This resulted in the appearance of CD44+CD90+ mesenchymal stem cells in the aortic wall. The increased CD44+CD90+ mesenchymal stem cells differentiated into abnormal adipocytes in the vascular wall with the expression of adipocyte differentiation regulatory factors. The appearance of adipocytes can cause a weakness of the vascular wall. This process seems to depend also on the contribution of PVAT, since the lack of PVAT diminished the number of mesenchymal cells with adipocyte-differentiation potential and the adipocytes in the AAA wall, but the mechanisms are yet unknow.

To challenge this hypothesis, the use of a biomaterial-guided periadventitial delivery of adipose tissue-derived stromal cells for treatment of experimental AAA was shown to prevent the dilation of the aorta, to suppress the loss of SMC and the degradation of elastin and to reduce influx of macrophages in rats (140). Adipose tissue-derived stromal cells migrated from the loaded patches into the arterial wall and showed a homogeneous distribution in the aortic media, and in the adventitia. Bare patches could not prevent aortic dilation. On the other hand, loaded patches did prevent aortic dilation, which suggests that these cells influenced the aneurysm development and progression.

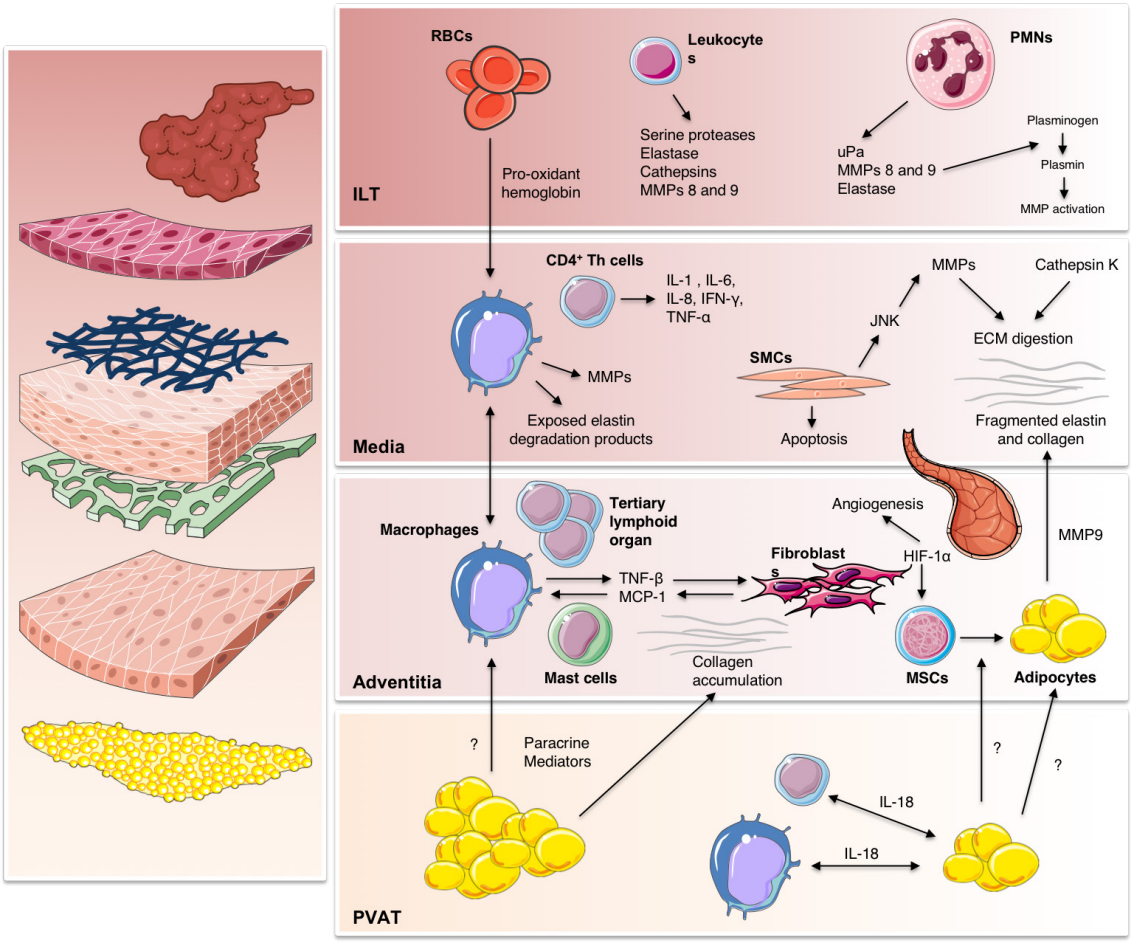

Figure 2 Schematic diagram demonstrating the molecular complexity of AAA pathogenesis. The role of PVAT in the AAA development remains unclear.

THESIS OVERVIEW

The aim of this thesis is to create more insight into epidemiologic (Part I) and pathophysiologic (Part II) features of AAA.

Regarding the **first part** of the thesis, **Chapter 1** exhibits a narrative review on the epidemiologic transition of AAA. This topic is further explored at the institutional level in **Chapter 2** by providing a retrospective analysis of consecutive case entries admitted to iAAA repair and submitted to EVAR or OSR, in a single center. In **Chapter 3**, a nationwide analysis of the incidence and mortality after iAAA repair is provided based on the retrospective hospital administrative database. **Chapter 4** focuses on rAAA incidence and mortality, resorting to the retrospective hospital administrative database but also the prospective cause of deaths database.

Regarding the **second part** of the thesis, **Chapter 5** offers a narrative review about the putative impact of AAC on aneurysmal progression, rupture and after endovascular correction, emphasizing the challenges in the study of AAC in AAA. **Chapter 6** describes the correlation between three semiquantitative and one computerized method based on Agatston Score, when measuring AAC in AAA patients. We hypothesized that one reason for the great heterogeneity of the impact of AAC in AAA might be the use of different methods of assessing its severity. The establishment of a fast and easy-to-use method of assessing AAC can be of value in research settings, ultimately leading to a better understanding of the clinical implications of AAC in AAA.

In **Chapter 7** we discuss the PVAT as a putative intervenient in the pathologic pathways occurring in AAA and/or aortic atherosclerosis in a narrative review. **Chapter 8** is a multicenter retrospective case control study to compare the PVAT density in patients with AAA, with aortoiliac occlusive disease and individuals without aortic pathology using a CT-based approach. In **Chapter 9**, we explore the putative role of PVAT in inflammation of the aortic wall in AAA in a prospective translational study.

SPECIFIC AIMS

PART I

Chapter 1

1. To provide a narrative review focusing on:
   1.1 The magnitude of the AAA epidemiological change
   1.2 Possible underlying causes

Chapter 2

2. To compare outcomes after iAAA repair in a single center focusing on the differences between EVAR and OSR
   2.1 Demographic and clinical differences
   2.2 Length of hospital stay
   2.3 Survival and freedom from aortic-related mortality
   2.4 Vascular reintervention
   2.5 Time trends

Chapter 3

3. To assess trends in the rate of iAAA repair, demographics and outcomes over a 16-year interval in Portugal

Chapter 4.

4. The assess the trends in rAAA rates of admission, repair and mortality over a 16-year interval in Portugal

PART II

Chapter 5

5. To provide a narrative review focusing on:
   5.1 The impact of ACC in the progression, rupture and repair of AAA
   5.2 The challenges of the ACC research in AAA

Chapter 6

   6.1 To assess the performance of three semiquantitative methods compared to a computerized one in scoring AAC
   6.2 To access differences in AAC upon clinical variables, when different methods of calcium scoring are used

Chapter 7

7. To provide a narrative review focusing on:
   7.1 The association between global and specific adiposity and AAA
   7.2 The association between global and specific adiposity and PAD
   7.3 Molecular markers of PVAT versus other abdominal aortic deposits

Chapter 8

   8.1 To develop a new method for CT-assessment of abdominal PVAT
   8.2 To evaluate the distribution of abdominal fat deposits including PVAT in patients with AAA compared with AIOD and patients with healthy aortas

Chapter 9

9. To investigate the mechanisms underlying the role of PVAT in the occurrence of AAA
   9.1 To compare the histological aspect of PVAT from AAA and PAOD patients and controls
   9.2 To address expression of inflammatory genes in PVAT from AAA and PAOD patients and controls
   9.3 To performed live *ex vivo* stimulation of SMC using SAT and PVAT from AAA patients and controls

PART I SPECIFICITIES OF THE EPIDEMIOLOGICAL TRANSITION OF ABDOMINAL AORTIC ANEURYSMS

CHAPTER 1 A TRANSIÇÃO EPIDEMIOLÓGICA NO ANEURISMA DA AORTA ABDOMINAL | EPIDEMIOLOGICAL TRANSITION IN ABDOMINAL AORTIC ANEURYSM

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ARTIGO REVISÃO

A TRANSIÇÃO EPIDEMIOLÓGICA NO ANEURISMA DA AORTA ABDOMINAL

EPIDEMIOLOGICAL TRANSITION IN ABDOMINAL AORTIC ANEURYSM

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RESUMO

O aneurisma da aorta abdominal (AAA) tem exibido uma transição epidemiológica nos países ocidentais. O padrão do século XX caracterizado por elevada incidência e mortalidade tem dado lugar a uma incidência e mortalidade decrescentes por AAA. A diminuição das mortes por AAA observada desde os anos 90 parece ter sido motivada pela diminuição da exposição a factores de risco, à maior utilização de fármacos cardioprotetores, ao aumento da disponibilidade de meios de diagnóstico e à melhoria das modalidades de tratamento. Esta transição epidemiológica poderá ter impacto na estratégia de rastreio (quer este seja organizado ou oportunista) bem como na gestão de recursos relacionados com o tratamento do AAA. O objetivo desta revisão é fazer um levantamento da magnitude destas modificações epidemiológicas, das possíveis causas que lhe estão subjacentes e do seu potencial impacto.

Palavras-chave
aneurisma da aorta abdominal; epidemiologia; incidência; mortalidade

ABSTRACT

Abdominal aortic aneurysm (AAA) has shown an epidemiological transition in Western countries. The standard of the XX century characterized by increasing incidence and mortality has been replaced by decreasing incidence and mortality due to AAA. The decrease in deaths from AAA observed since the 90s seems to have been motivated by reduced exposure to risk factors, increased use of cardioprotective drugs, increased availability of diagnostics and improved treatment modalities. This epidemiological transition could have an impact on the screening strategy (both in organized or opportunistic screening) as well as in the management of resources related to the treatment of AAA. The objective of this review is to scrutinize the magnitude of these epidemiological changes, the possible underlying causes and its potential impact.

Keywords
abdominal aortic aneurysm; epidemiology; incidence; mortality

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(1)INTRODUÇÃO

O aneurisma da aorta abdominal (AAA) tem experimentado uma transição epidemiológica nos países ocidentais, variando de um padrão determinado por elevada incidência e mortalidade para outro, mais recente, caracterizado por diminuição da incidência e da mortalidade por AAA. Esta alteração epidemiológica não se tem verificado sincronamente em todos os países e nem mesmo entre géneros ou grupos etários [2].

O desconhecimento deste facto tem levado a que os dados de prevalência e mortalidade reportados em diferentes estudos, como forma de salientar a importância do tema em estudo, sejam frequentemente incorretos [3], sendo que a maioria dos autores tende a reportar em excesso o ónus desta doença. Adicionalmente, uma avaliação nacional fidedigna quando às tendências de admissão e de mortalidade por AAA permite ajustar programas de rastreio e contribuir para a gestão de recursos no que diz respeito ao tratamento desta doença.

O objetivo desta revisão é fazer um levantamento das recentes modificações epidemiológicas no AAA, bem com das possíveis causas que lhe estão subjacentes.

1. ADMISSÕES E MORTALIDADE POR AAA NO SÉCULO XX

O século XX testemunhou um aumento da prevalência e mortalidade por aneurisma da aorta abdominal nos países desenvolvidos. Entre 1951 e 1995, os estudos epidemiológicos revelavam que o AAA era uma doença em crescimento. No Reino Unido, Filipovic e colaboradores documentaram aumentos da taxa de mortalidade de 13 para 25 por milhão em mulheres e de 80 para 115 por milhão em homens e aumento da taxa de admissão de 3 para 22 por milhão/ano em mulheres e de 52 para 149 por milhão/ano em homens, entre 1979 e 1999 [4]. Apesar da significativa melhoria da fatalidade nos doentes operados por AAA íntegro durante esse período (diminuição de 25,8% para 9,0%), nos doentes com AAA em rotura essa diminuição foi ligeira (69,9% para 54,4%) e salientava-se a necessidade de detecção e tratamento do AAA antes da ocorrência de rotura.

Nos Estados Unidos, de 1951 a 1968, a mortalidade anual por aneurisma aórtico, sendo o abdominal o mais comum nesta análise, registou um aumento tanto em homens como em mulheres, caucasianos ou não caucasianos, tendo-se verificado atenuação e discreta diminuição de 1968 a 1981 [5]. Este aumento correspondeu a um aumento no número total de óbitos por aneurisma aórtico de aproximadamente 2500 para 14000, com média de aumento anual de 17% para homens caucasianos, 12% para mulheres caucasianas, 14% para homens não caucasianos e 15% para mulheres não caucasianas.

2. ADMISSÕES E MORTALIDADE POR AAA NO SÉCULO XXI

A evidência mais recente proveniente de diversos países ocidentais sugere que uma reversão da epidemiologia tem ocorrido durante o século XXI, com diminuições marcadas na incidência e mortalidade por AAA.

O estudo de Sidlof e colaboradores [2] corresponde à maior análise de base populacional sobre mortalidade por AAA até à data e confirma o declínio da mortalidade na maior parte do 19 países da Organização Mundial da Saúde *(World Health Organization)* que foram incluídos, apesar da heterogeneidade nas tendências de mortalidade padronizada para a idade. As diferenças geográficas evidenciadas nesse estudo são apresentadas na Figura 1A (homens) e na Figura 1B (mulheres). Nas Figuras 1A e 1B torna-se evidente que as exceções à tendência global de diminuição da mortalidade incluem aumentos nos seguintes grupos: homens da Hungria (2,7%) e da Roménia (1,7%), e mulheres da Hungria (3,5%), da Roménia (1%), da Dinamarca (2,2%) e da Áustria (0,5%).

Figura 1A e 1B *Variação anual da mortalidade padronizada para a idade por aneurisma da aorta abdominal em homens (1A) e em mulheres (1B). Países representados: Austrália, Áustria, Canada, Dinamarca, Finlândia, França, Alemanha, Hungria, Islândia, Israel, Japão, Holanda, Nova Zelândia, Noruega, Roménia, Espanha, Suécia.*
Legenda: verde escuro – diminuição estatisticamente significativa, verde claro – diminuição sem significância estatística, amarelo – variação próxima de zero, vermelho claro – aumento sem significância estatística, vermelho escuro – aumento estatisticamente significativo. Baseado no estudo de Sidloff e colaboradores [1].

As maiores reduções na taxa de mortalidade em homens verificaram-se nos EUA, no Reino Unido e na Austrália e foram, respectivamente, 6,7%, 6,2% e 6,2% por ano. As maiores reduções na taxa de mortalidade em mulheres foram observadas nos Reino Unido e nos EUA: 4,0% e 3,9% por ano, respetivamente. Estes dados evidenciam ainda uma diferença de género, sendo a taxa de declínio menor nas mulheres do que nos homens.

Para além do estudo supramencionado, vários autores descreveram mais detalhadamente dados nacionais relativos a admissões hospitalares, à reparação e à mortalidade por AAA nomeadamente nos EUA, Austrália, Nova Zelândia, Reino Unido, Holanda e Itália. Os principais resultados destes estudos encontram-se na Tabela 1.

Especula-se que o declínio da incidência de AAA na Austrália Ocidental possa ter precedido o dos EUA e da Europa[6, 7] devido à mais precoce modificação de factores de risco e alteração do estilo de vida, incluindo a redução do consumo tabágico (ver adiante).

3. POSSÍVEIS CAUSAS

A diminuição das mortes por AAA observada desde os anos 90 parece ter sido motivada pela diminuição da exposição a factores de risco, à maior utilização de fármacos cardioprotetores, aumento da disponibilidade de meios de diagnóstico e à melhoria das modalidades de tratamento.

3.1 REDUÇÃO DA EXPOSIÇÃO AOS FACTORES DE RISCO

Uma análise das tendências nos factores de risco cardiovascular entre 1946 e 2010 em vários países, predominantemente da Europa, Austrália e América do Norte, mostrou que estes se correlacionam de forma independente com as tendências de mortalidade por AAA[2]. Neste estudo demostrou-se uma associação linear positiva entre as tendências globais de prevalência de pressão arterial sistólica ($p \leq 0.03$), de colesterol ($p \leq 0.03$) e de consumo tabágico ($p \leq 0.02$) em ambos os géneros. Curiosamente, o índice de massa corporal demonstrou uma associação linear negativa com a mortalidade por AAA ($p \leq 0.007$), enquanto o nível de glicose em jejum não mostrou qualquer associação.

À semelhança do AAA, a incidência de doença coronária e doença cerebrovascular tem diminuído nas últimas décadas[8] e esta tendência tem sido atribuída a alterações nos factores de risco cardiovasculares[9]. Como os factores de risco dominantes do AAA são semelhantes aos da aterosclerose[10] (com exceção da diabetes mellitus), as tendências no AAA podem ter sido simplesmente mais tardias.

Tabela 1 Principais resultados de estudos primários sobre a evolução da incidência de admissão, reparação e mortalidade por aneurisma da aorta abdominal ao longo do tempo.
Legenda: AAA – aneurisma da aorta abdominal.

Referência	País/Período	Principais resultados
Semmens,1998	Australia Ocidental, 1985 a 1994	Declínio na incidência de ambos os procedimentos de emergência e eletivos para AAA após 1992. Enquanto a taxa de mortalidade por AAA roto também caiu desde 1991 e a taxa de letalidade global de AAA roto caiu apenas 1,3%.
Norman, 2011	Australia, 1999 a 2008	Queda significativa nas taxas de admissão hospitalar e na mortalidade por AAA em rotura e por AAA íntegro em ambos os géneros desde 1999.
Sandiford, 2011	New Zeland, 1994 a 2009	Diminuição da mortalidade, da admissão hospitalar e da taxa de mortalidade hospitalar por AAA (não separa AAA íntegro de AAA em rotura).
Anjum, 2011	Inglaterra, País de Gales e Escócia, 1950 a 2009	Diminuição da admissão por AAA em rotura (em todas as idades, excepto >85 anos). Aumento das admissões para reparação de AAA íntegro entre 1989 e 1996 e apenas aumento modesto entre 1997 e 2009 (superior em homens e em idades >75 anos e inferior na faixa de 55-64 anos). Diminuição da mortalidade a partir de 1997 sobretudo à custa dos AAA em rotura (mais em homens e em idades inferiores a 75 anos).
Choke, 2011	Inglaterra e País de Gales, 2000 a 2009	Diminuição no número de admissões e de reparações por AAA em rotura (em ambos os géneros e para idades < ou > a 75 anos). Manutenção do número de admissões por AAA íntegro (mais em homens e idades superiores a 75 anos e menos em mulheres e em idades <75 anos). Aumento do número de reparações por AAA íntegro (mais nos doentes com idade >75 anos). Diminuição da mortalidade por AAA (mais no AAA em rotura do que no AAA íntegro, mais em homens e em todas as idades).
Sensi, 2013	Itália, 2000 a 2011	Diminuição significativa das taxas de hospitalização quer para AAA em rotura quer para AAA íntegros. As taxas de mortalidade a 30 dias não variaram significativamente ao longo do tempo em nenhum dos grupos.
Nelissen, 2015	Holanda, 1980 a 2010	A mortalidade total por AAA aumentou de 1980 até 1995, seguida por um declínio até 2010 (mais proeminente nos homens). A mortalidade por AAA íntegro mostrou um aumento no período de 1980 até 2010. A idade da morte por AAA foi maior nas mulheres do que nos homens; esta diferença diminuiu à medida que aumentava a idade de morte por AAA em homens. A diminuição da mortalidade por AAA foi observada pela primeira vez no grupo etário mais jovem (55-69 anos) e só posteriormente em grupos etários consecutivamente superiores.

Dados específicos provenientes de diversos países suportam esta hipótese, sendo o tabaco o factor de risco mais frequentemente evocado por diversos motivos: (a) a correlação entre tabagismo e formação de AAA já foi demostrada [11], sendo este o mais importante factor de risco para o desenvolvimento de AAA; (b) estima-se que aproximadamente um quinto de todas as mortes por AAA poderiam ser evitadas por redução tabágica [12]; o tabagismo aumenta a taxa de rotura [13]. A prevalência de consumo tabágico na Austrália caiu de 35% em 1980 para 23% em 2001, sendo para os adultos com mais de 60 anos de apenas 10% em 2001 [14].

Assume-se que sucesso do movimento antitabágico da Austrália tenha contribuído para uma diminuição da mortalidade por cancro do pulmão no género masculino desde meados do anos 80 [6] e que uma tendência semelhante para o AAA possa ser o resultado da coorte de não fumadores ir atingindo a sua sétima ou oitava década de vida. Na Holanda, em 1970, mais de 75% dos homens adultos e menos de 25% das mulheres são fumadores [15]. Neste país a diminuição no consumo de tabaco em homens cursa em paralelo com o declínio na mortalidade por AAA. De forma semelhante, em mulheres, quer o consumo de tabaco, quer a mortalidade por AAA não se alteraram nos últimos 40 anos. Na Inglaterra, após 1982, a taxa de declínio do tabagismo abrandou com quedas de prevalência de apenas 1% por cada 2 anos até aos anos 90 [16]. Desde 2000, a queda tem sido ainda menor e estimada em 0,4% ao ano e entre 2007 e 2008 a prevalência estagnou em 21%. Para os adultos com 60 anos, a taxa de tabagismo na Inglaterra era de 16% em 1998 em comparação com os 10% registados na Austrália em 2001. Curiosamente, a mais rápida queda de consumo tabágico na Austrália em relação à Inglaterra pode explicar a discrepância na taxa de admissões eletivas por AAA que está a diminuir na Austrália, mas não na Inglaterra. Por fim, especula-se que a diminuição do consumo tabágico possa ainda servir para atrasar o início da doença nos indivíduos geneticamente predispostos, o que explicaria a diminuição da prevalência de AAA em homens com 65 anos submetidos a rastreio de AAA [7].

3.2 MAIOR UTILIZAÇÃO DE FÁRMACOS CARDIOPROTETORES

O papel dos fármacos cardioprotetores na prevenção do crescimento do AAA não está bem definido [17, 18]. Tem sido sugerido que as estatinas podem ter efeitos pleiotróficos na expansão aneurismática [19]. Um dos primeiros documentos de consenso a sugerir o uso de agentes anti-dislipidémicos, entre outras recomendações, para a prevenção secundária no AAA foram as *guidelines* da *American College of Cardiology/American Heart Association* de 2005 [19, 20]. É provável que a diminuição da incidência de AAA em rotura documentadas em vários países seja ocresultado do aumento do uso de estatinas [21]. Dados específicos da Nova Zelândia mostraram que menores níveis de lípidos séricos e um aumento dramático no uso de estatinas foram atingidos no género masculino desde 1986-1988 [22], o que pode estar relacionado com a diminuição da incidência de rotura, de admissões e de mortalidade por AAA documentada no período de 1994 a 2009. Outro factor eminentemente relacionado com a rotura é a pressão arterial [23]. Da mesma forma, a proporção de homens da Nova Zelândia com pressão arterial acima de 150/90 mmHg sem tratamento diminuiu de cerca de 20% em 1986-1988 para 3,3% em 2002-2003 [22].

3.3 MAIOR DIAGNÓSTICO

Para além da existência de programas de rastreio do AAA em países como o Reino Unido e a Suécia [24], o uso disseminado de técnicas de imagiologia abdominal pode ter levado a uma maior referenciação de doentes com AAA e promovido a sua reparação eletiva.

Por outro lado, a consciencialização pública em relação a esta patologia pode ter levado mais homens a procurarem proactivamente o rastreio, nos países em que o mesmo não está implementado de forma organizada [15].

3.4 MAIOR REPARAÇÃO ELETIVA E MELHORES CUIDADOS PERI-OPERATÓRIOS

Uma importante mudança desde o início dos anos 90 foi a introdução da reparação do AAA por via endovascular (EVAR). Esta introdução trouxe consigo uma mudança de paradigma que é crescente na medida em que a modalidade endovascular apresenta cada vez melhores resultados, como menor taxa de migração, menos *endoleak* tipo I e menos rotura [25]. A par da maior taxa de diagnóstico, a utilização do EVAR pode assim ser responsável pelo aumento do número de reparações de AAA em doentes que de outra forma não seriam candidatos a tratamento como, por exemplo idosos [14,26]. A mudança para um tratamento menos invasivo pode assim ter contribuído para a diminuição da mortalidade no tratamento eletivo de AAA, ainda que, à luz da evidência atual, não possa ser atribuído ao EVAR menor mortalidade aos 90 dias por AAA em rotura versus cirurgia aberta [27].

Contudo, pode-se especular que o aumento da taxa de cirurgia eletiva pode ter atingido níveis suficientemente altos para reduzir o número de AAA em risco de rotura, com consequente estabilização ou queda das reparações e da mortalidade por AAA em rotura [6]. Adicionalmente, a publicação recente de dados provenientes do registo Vascunet demonstra que a mortalidade intra-hospitalar após reparação de AAA em rotura tem diminuído significativamente de 42,5% em 2005 para

28,5% em 2009 no Reino Unido [28]. A combinação da carga de reparação eletiva, de melhor seleção de casos e de melhores resultados peri-operatórios podem assim contribuir para a diminuição da mortalidade por AAA em rotura.
Por fim, o aumento da cirurgia eletiva num determinado período de tempo pode ter resultado no estabelecimento de um novo patamar, mais baixo, na taxa de reparações eletivas, a par da menor taxa de reparação de AAA em rotura [6].

4. IMPLICAÇÕES DA TRANSIÇÃO EPIDEMIOLÓGICA

O impacto da transição epidemiológica pode ser discutido a dois níveis: na estratégia de rastreio do AAA; e na gestão de recursos relacionados com o tratamento do AAA.

4.1 RASTREIO DE AAA

A meta-análise de Costford e colaboradores demonstrou que o rastreio é efetivo em diminuir a mortalidade por AAA em homens com idades entre 65 e 79 anos avaliados por ecografia [29]. Este estudo da Cochrane incluiu quatro estudos clínicos randomizados, no total de 127891 homens e 9342 mulheres e o AAA foi detectado em 5% a 10% dos homens com idade entre 65 e 79 anos. Apesar deste resultado ser provavelmente válido para uma larga faixa de taxas de prevalência, o número de homens que precisam de ser rastreados para salvar uma vida (ou anos de vida) aumenta à medida que a mortalidade cai. Assim, o custo-efetividade é necessariamente inferior em padrões de baixa mortalidade, o que pode desafiar o custo-efetividade do rastreio [7, 30], quer este seja de base populacional e organizado ou oportunista.
Por outro lado, pode ser ainda mais imperativo optimizar o público-alvo do rastreio.
Se estas tendências de mantiverem, Lederle e colaboradores [11] advogam que os programas dirigidos a populações de alto risco [31], como homens mais idosos e com história de consumo tabágico, possam ser mais efetivas. No entanto, esta estratégia deve ser contrabalançada pela redução do ganho em anos de vida inerente ao rastreio de um coorte de idade mais avançada [7].

4.2 GESTÃO DE RECURSOS RELACIONADOS COM O TRATAMENTO DO AAA

Uma meta-análise de estudos que investigam a relação entre o volume de procedimentos cirúrgicos e os respetivos resultados [32] mostra que maiores volumes operatórios anuais estão associados de forma estatisticamente significativa a menor mortalidade, tanto na reparação de AAA eletiva como na rotura. Na a reparação eletiva o odds ratio (OR) foi de 0,66 (0,65 a 0,67) para um limiar de 43 AAA por ano e na reparação de AAA em rotura o OR foi de 0,78 (0,73 to 0,82) para um limiar de 15 AAA por ano, em favor das instituições de maior volume. Perante a diminuição da incidência da doença pode-se tornar ainda mais pertinente que a reparação seja realizada apenas em centros com elevado volume de casos.
Por fim, se se espera uma alteração demográfica no sentido de doentes cada vez mais idosos, pode-se especular que estes serão mais frequentemente candidatos a cirurgia endovascular do que a cirurgia aberta. Este dado, aliado ao contínuo desenvolvimento da técnicas endovasculares (no sentido de uma maior aplicabilidade), salienta a importância de uma adequada e atempada formação e preparação dos centros cirúrgicos para este tipo de estratégia.

CONCLUSÃO

Apesar da transição epidemiológica ter sido verificada na maior parte dos países ocidentais, desconhece-se até à data qual o estadio atual de Portugal em relação a este assunto. Este levantamento de dados epidemiológicos sobre o AAA em Portugal parece ser necessário sobretudo porque os dados provenientes do Sul da Europa são igualmente escassos. A importância deste levantamento prende-se não só com a definição do custo-benefício e de grupos-alvo do rastreio (organizado ou oportunista) do AAA em Portugal, mas também com a gestão dos recursos relativos ao tratamento desta patologia.

REFERÊNCIAS

1. Summaries for patients. Screening for abdominal aortic aneurysm: recommendations from the U.S. Preventive Services Task Force. Annals of internal medicine. 2005 Feb 1;142(3):I52. PubMed PMID: 15684202.
2. Sidloff D, Stather P, Dattani N, et al. Aneurysm global epidemiology study: public health measures can further reduce abdominal aortic aneurysm mortality. Circulation. 2014 Feb 18;129(7):747-53. PubMed PMID: 24249717.
3. Stather PW, Sidloff DA, Rhema IA, et al. A review of current reporting of abdominal aortic aneurysm mortality and prevalence in the literature. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2014 Mar;47(3):240-2. PubMed PMID: 24368205.
4. Filipovic M, Goldacre MJ, Roberts SE, et al. Trends in mortality and hospital admission rates for abdominal aortic aneurysm in England and Wales, 1979-1999. The British journal of surgery. 2005 Aug;92(8):968-75. PubMed PMID: 16034842.
5. Lilienfeld DE, Gunderson PD, Sprafka JM, et al. Epidemiology of aortic aneurysms: I. Mortality trends in the United States, 1951 to 1981. Arteriosclerosis. 1987 Nov-Dec;7(6):637-43. PubMed PMID: 3689207.
6. Semmens JB, Norman PE, Lawrence-Brown MM, et al. Population-based record linkage study of the incidence of abdominal aortic aneurysm in Western Australia in 1985-1994. The British journal of surgery. 1998 May;85(5):648-52. PubMed PMID: 9635813.

7. Choke E, Vijaynagar B, Thompson J, et al. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? Circulation. 2012 Apr 3;125(13):1617-25. PubMed PMID: 22361325.
8. Beaglehole R. International trends in coronary heart disease mortality, morbidity, and risk factors. Epidemiologic reviews. 1990;12:1-15. PubMed PMID: 2286214.
9. Knuiman MW, Jamrozik K, Welborn TA, et al. Age and secular trends in risk factors for cardiovascular disease in Busselton. Australian journal of public health. 1995 Aug;19(4):375-82. PubMed PMID: 7578538.
10. Reed D, Reed C, Stemmermann G, et al. Are aortic aneurysms caused by atherosclerosis? Circulation. 1992 Jan;85(1):205-11. PubMed PMID: 1728451.
11. Lederle FA. The rise and fall of abdominal aortic aneurysm. Circulation. 2011 Sep 6;124(10):1097-9. PubMed PMID: 21900095.
12. Anjum A, von Allmen R, Greenhalgh R, et al. Explaining the decrease in mortality from abdominal aortic aneurysm rupture. The British journal of surgery. 2012 May;99(5):637-45. PubMed PMID: 22473277.
13. Brown LC, Powell JT. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance. UK Small Aneurysm Trial Participants. Annals of surgery. 1999 Sep;230(3):289-96; discussion 96-7. PubMed PMID: 10493476. Pubmed Central PMCID: 1420874.
14. Norman PE, Spilsbury K, Semmens JB. Falling rates of hospitalization and mortality from abdominal aortic aneurysms in Australia. Journal of vascular surgery. 2011 Feb;53(2):274-7. PubMed PMID: 21055899.
15. Nelissen BG, Herwaarden JA, Pasterkamp G, et al. Shifting abdominal aortic aneurysm mortality trends in The Netherlands. Journal of vascular surgery. 2015 Mar;61(3):642-7 e2. PubMed PMID: 25720926.
16. Jarvis MJ. Monitoring cigarette smoking prevalence in Britain in a timely fashion. Addiction. 2003 Nov;98(11):1569-74. PubMed PMID: 14616183.
17. Sweeting MJ, Thompson SG, Brown LC, et al. Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. The British journal of surgery. 2012 May;99(5):655-65. PubMed PMID: 22389113.
18. Hurks R, Hoefer IE, Vink A, et al. Different effects of commonly prescribed statins on abdominal aortic aneurysm wall biology. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2010 May;39(5):569-76. PubMed PMID: 20226696.
19. Schouten O, van Laanen JH, Boersma E, et al. Statins are associated with a reduced infrarenal abdominal aortic aneurysm growth. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2006 Jul;32(1):21-6. PubMed PMID: 16520071.
20. Hirsch AT, Haskal ZJ, Hertzer NR, et al. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation. 2006 Mar 21;113(11):e463-654. PubMed PMID: 16549646.
21. Van Kuijk JP, Flu WJ, Witteveen OP, et al. The influence of statins on the expansion rate and rupture risk of abdominal aortic aneurysms. The Journal of cardiovascular surgery. 2009 Oct;50(5):599-609. PubMed PMID: 19741575.
22. Metcalf P, Scragg RK, Schaaf D, et al. Trends in major cardiovascular risk factors in Auckland, New Zealand: 1982 to 2002-2003. The New Zealand medical journal. 2006;119(1245):U2308. PubMed PMID: 17146483.
23. Choke E, Cockerill G, Wilson WR, et al. A review of biological factors implicated in abdominal aortic aneurysm rupture. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2005 Sep;30(3):227-44. PubMed PMID: 15893484.
24. Svensjo S, Bjorck M, Gurtelschmid M, et al. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. Circulation. 2011 Sep 6;124(10):1118-23. PubMed PMID: 21844079.
25. Dua A, Kuy S, Lee CJ, et al. Epidemiology of aortic aneurysm repair in the United States from 2000 to 2010. Journal of vascular surgery. 2014 Jun;59(6):1512-7. PubMed PMID: 24560865.
26. Schermerhorn ML, Bensley RP, Giles KA, et al. Changes in abdominal aortic aneurysm rupture and short-term mortality, 1995-2008: a retrospective observational study. Annals of surgery. 2012 Oct;256(4):651-8. PubMed PMID: 22964737. Pubmed Central PMCID: 3507435.
27. Sweeting MJ, Balm R, Desgranges P, et al. Individual-patient meta-analysis of three randomized trials comparing endovascular versus open repair for ruptured abdominal aortic aneurysm. The British journal of surgery. 2015 Sep;102(10):1229-39. PubMed PMID: 26104471. Pubmed Central PMCID: 4744980.
28. Mani K, Lees T, Beiles B, et al. Treatment of abdominal aortic aneurysm in nine countries 2005-2009: a vascunet report. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2011 Nov;42(5):598-607. PubMed PMID: 21775173.
29. Cosford PA, Leng GC. Screening for abdominal aortic aneurysm. The Cochrane database of systematic reviews. 2007 (2):CD002945. PubMed PMID: 17443519.
30. Sandiford P, Mosquera D, Bramley D. Trends in incidence and mortality from abdominal aortic aneurysm in New Zealand. The British journal of surgery. 2011 May;98(5):645-51. PubMed PMID: 21381003.
31. Force USPST. Screening for abdominal aortic aneurysm: recommendation statement. Annals of internal medicine. 2005 Feb 1;142(3):198-202. PubMed PMID: 15684208.
32. Holt PJ, Poloniecki JD, Gerrard D, et al. Meta-analysis and systematic review of the relationship between volume and outcome in abdominal aortic aneurysm surgery. The British journal of surgery. 2007 Apr;94(4):395-403. PubMed PMID: 17380547.

CHAPTER 2 IMPACT OF GRADUAL ADOPTION OF EVAR IN ELECTIVE REPAIR OF ABDOMINAL AORTIC ANEURYSM RETROSPECTIVE COHORT STUDY FROM 2009 TO 2015

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Abstract

Introduction: The recommendations about the preferred type of elective repair of abdominal aortic aneurysm (AAA) still divides guidelines committees, even nowadays.
Aim: To assess outcomes after AAA repair focusing on differences between endovascular (EVAR) and open repair (OSR).
Methods: Observational retrospective cohort study of consecutive patients submitted to elective AAA repair at a tertiary centre, 2009-2015. Exclusion criteria: non-elective cases or complex aortic aneurysms. Primary outcomes were postoperative complications, length of hospital stay (LOS), survival, freedom from aortic-related mortality and vascular reintervention. Time trends were assessed along the period under analysis.
Results & Discussion: From a total of 211 included patients, those submitted to EVAR were older (74±7 vs. 67±9 years; p<.001), presented higher prevalence of hypertension (83.5% vs. 68.5%, p=.004), obesity (28.7% vs. 14.3%, p=.029), previous cardiac revascularization (30.5% vs. 14.7%, p=.005), heart failure (17.2% vs. 5.2%, p=.013) and COPD (32.8% vs. 13.3%, p=.002). Patients were followed during a median of 46 months. EVAR resulted in a significantly shorter LOS (median 4 and interquartile range 3 vs. 8 (9); p<.001), lower 30-days complications (10.6% vs. 22.8%, p=.017) and aortic-related mortality after adjustment with a propensity score. Along the time under analysis, EVAR became the predominate type of repair (p=.024) and both proportion of complications (p=.014) and 30-day mortality (p=.035) were lower in the most recent years. Although EVAR was offered to patients with more comorbidities, better and durable outcomes were achieved after EVAR, favoring its adoption for elective AAA repair.

Introduction

Abdominal aortic aneurysm (AAA) is defined as a focal dilatation at least 50% larger than the expected normal diameter [1], with elective surgical repair being recommended for patients diagnosed with AAA ≥ 55 mm due to the elevated risk of rupture. The decision to operate a patient with an AAA is complex, as it depends on the risk of aneurysm rupture, the risk associated with its repair and the patient's life expectancy. Ultimately, the patient's personal preference is also a factor to consider. Once the necessity for AAA repair is established, the choice of operative strategy will need to be made between conventional open surgery repair (OSR) and endovascular aortic aneurysm repair (EVAR).

Although conventional OSR proved to be a successful and durable correction for aortoiliac aneurysmal disease [2, 3], endovascular aortic aneurysm repair (EVAR) emerges in the 1990s as a less-invasive alternative to the standard OSR, not requiring abdominal laparotomy nor long periods of aortic clamping. In contemporary practice, endovascular techniques are gradually replacing OSR for the treatment of AAAs, supported by several observational studies reporting its short-term advantages [4], with low rates of morbidity and mortality in high risk patients as well as lower systemic complication rates. In fact, the development of surgical techniques, perioperative management and the introduction of endovascular exclusion strategies contributed to lower the elective surgical mortality to less than 5% in most series [5].

In Portugal, the analysis of the national hospitals administrative database revealed that the age-standardized rate of AAA repair increased consistently across the time periods under analysis from 3.6±0.6/100,000/year in 2000-2004, to 5.6±0.4/100,000/year in 2005-2009 and to 7.1±0.9/100,000/year in 2010-2015 ($p<0.001$) [6], with a growing preference for EVAR over OSR. Nevertheless, analysis of administrative databases lacks long-term follow-up.

The aim of this study was to assess complications, length of hospital stay (LOS), survival and vascular reintervention after AAA repair in a retrospective cohort of patients focusing differences upon endovascular and open repair.

Methods

Study design and sample

This is an observational retrospective cohort study of all patients consecutively submitted to elective AAA surgery repair at a tertiary academic center between 2009 and 2015. The exclusion criteria were non-elective cases, aortic intervention due to diagnosis other than infrarenal AAA and complex aortic aneurysms such as juxta-renal, thoraco-abdominal or thoracic aneurysms. Patients who did not meet the inclusion criteria or with any of the exclusion criteria mentioned above were excluded from the study.

Centro Hospitalar de São João is a tertiary teaching center in the North of the Portugal, serving 0.7 million people. Both open and endovascular elective repair of AAA are performed either by consultant vascular surgeons or by residents during their vascular training under the direct supervision of an experienced consultant vascular surgeon. EVAR is performed in the operating theatre using a mobile C-arm. A gradual preference has been given to local/regional anesthesia and percutaneous closure for EVAR, although the decision depends on the individual case. For OSR, a midline transperitoneal approach is preferred, and aorto-aortic or aortobiiliac reconstruction is performed depending on the presence of concomitant iliac aneurysms.

Follow up after ORS repair is by yearly clinical assessment complemented by ABI and/or ultrasound if need and abdominopelvic computed tomographic angiography (CTA) every five years, as recommended [7]. Follow up after EVAR started as an abdominopelvic CTA 1, 6, 12 months after surgery and then annually. Recently, our protocol was modified to adapt to the most recent recommendations of the European Society for Vascular Surgery [7], including a CTA at least every 5 years and annual ultrasound to assess the annual diameter if a type II endoleak is present.

The study complies with the STROBE statement [8].

Data collection

The characterization of the sample was carried out by consulting the electronic health processes. Clinical and demographic characteristics of patients were collected according to the reporting standards for infrarrenal AAA repair [9]. The medication undertaken by the patients refer to the chronic drug medication at the time of the first observation in the hospital. After consultation patients are generally

prescribed with antiplatelet therapy and statins, unless there is a contraindication.

The main outcomes were 30-day and late survival after repair. Secondary outcomes included cause of death, vascular primary reintervention(s), cause of reintervention(s), postoperative medical complications and length of hospital stay.

Definitions

Body mass index corresponded to the weight (kg) divided by the square height (m^2). Obesity was defined as a body mass index $\geq$ 30 kg/m^2. Early mortality was defined by in-hospital mortality or death within 30 days of surgery. Major complications were defined as one of the following: respiratory as pneumonia, prolongation of endotracheal intubation, need for a tracheostomy; acute myocardial infarction with PCI, CABG or no intervention, decompensated heart failure or cardiac arrest; cerebrovascular including transient ischemic attack or stroke; renal failure as rise in plasma creatinine to > 3 mg/dL with or without the need for dialysis; abdominal including intestinal obstruction, ischemia or evisceration. The vascular reinterventions included any open or endovascular procedure performed due to lower limb ischemia or any access-related or graft-related complication. Elective revascularization of the lower limbs due to femoral or popliteal aneurysms were not considered as reinterventions.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS Inc., Chicago IL, USA), version 23.0 for Mac®.

Descriptive statistics are presented as absolute frequencies (n) and relative (%) for categorical variables whereas continuous variables are expressed as mean $\pm$ standard deviation (SD) when normally distributed as median and interquartile range (IQR) when skewed.

Differences between both groups of AAA repair were studied by Chi-square/Fisher's exact test if variables were proportions. For continuous variables, t-test was used when normally distributed and Mann-Whitney U test for two independent variables when normality could not be assumed. Time-dependent variables were analyzed by Kaplan-Meier curves. Cases were censored on the date of death or the last recorded clinical follow-up, ensuring accurate information collection. Median time of follow up was estimated by the reverse Kaplan-Meier method.

Missing data among the clinical variables of interest varied from 6% to 13%, except for carotid disease that was 46% since this is not a mandatory evaluation before surgery in the institution. Demographic and clinical variables that differ among groups of AAA repair in univariate analysis were introduced in a logistic regression model to predict the risk of undergoing EVAR repair, after multiple imputation being performed for missing clinical data. Variables that remained significant after this multivariable analysis were used to calculate a propensity score that was introduced in the Cox regression model for adjustment.

For time trends, the variables of interest were tested for homogeneity of proportions among the years under analysis if categorical or with ANOVA if continuous.

The tests were considered statistically relevant for a significance level (p-value) of less than 0.05 and the confidence interval was 95%.

Ethical issues

The study protocol was approved by the local Ethics Committee and respected the Declaration of Helsinki. Patient informed consent was not required and the anonymity and confidentiality of all the participants was assured by the investigators.

Results

Demographics and clinical features

Between January 2009 and December 2015, a total of 221 patients were included in the analysis, of which 201 (95.3%) were male with a mean and SD age of 71 $\pm$ 9 years. EVAR patients were older than OSR patients (74 ± 7 vs. 67 ± 9 years; p<.001). Among clinical variables, a higher prevalence of hypertension (83.5% in EVAR vs. 68.5% in OSR, p=.004), obesity (28.7% in EVAR vs. 14.3% in OSR, p=.029), history of cardiac treatment (30.5% in EVAR vs. 14.7% in OSR, p=.005), congestive heart failure (17.2% in EVAR vs. 5.2 in OSR, p=.013), chronic obstructive pulmonary disease (COPD) (32.8% in EVAR vs.13.3 in OSR, p=.002) was found in the EVAR group. Accordingly, EVAR patients achieved higher American Society

of Anesthesiologist' (ASA) classification (p=.004) and were more often medicated with anti-hypertensive drugs such as calcium channel blockers (p=.005) and beta blocking agents (p=.016) as well as with antiplatelet therapy at the time of the first observation at the hospital (p=.044). The remaining clinical and demographic data are reported in Table 1.

Multivariate analyses demonstrated that older age and COPD remained significant predictors of EVAR instead of OSR (Figure 1).

Operative details

Aneurysm repair was performed in 79 patients by open surgery, of which 40 (50.6%) were tubular aortic interpositions, 24 (30.4%) had at least one anastomosis to the femoral arteries and 15 (19.0%) had distal anastomosis to the iliac arteries (Table 2). All open interventions were performed under general anesthesia.

In the endovascular group, femoral cutdown was performed for aortobiiliac EVAR in 100 cases and a total percutaneous access was used in 27. Five cases (3.8%) were aortouniiliac EVAR with femorofemoral bypass. In the EVAR group, only 18 (14.5%) cases were performed under general anesthesia. Most cases resort to regional anesthesia (91 cases, 73.4%) and 15 (12.1%) to local anesthesia with/without sedation.

In-hospital complications

Specific medical complications at 30 days are presented in Table 3. Both respiratory (15.4% vs. 1.5%, p<.001) and abdominal complications (7.6% vs. 0.8%, p=.012) were more common in OSR versus EVAR group. A composite outcome of all medical complications at 30 days was higher for OSR versus EVAR (22.8% vs. 10.6%, p=.017).

LOS

EVAR resulted in a significantly shorter LOS when compared to OSR (p<.001). The EVAR group had a median hospital stay of 4 days, with an interquartile range of 3 days (minimum and maximum values of 1 and 105 days, respectively). Regarding de OSR group, a median hospital stay of 8 days was observed, with an interquartile range of 9 days (minimum and maximum values of 4 and 153 days, respectively).

Mortality

A total of 42 patients died during the follow-up period. Median follow up was 49 months (95% CI 43-54 months).

Overall survival (Figure 2) in the EVAR group was higher than in the OSR group at 30 days (100% vs. 94.9 $\pm$ 2.5%), at 12 months (95.9 $\pm$ 1.8% vs. 93.5 $\pm$ 2.8) and at 24 months (94.1 $\pm$ 2.2 vs. 90.6 $\pm$ 3.4). Global survival was similar at 36 months (89.0 $\pm$ 3.0 in EVAR vs. 88.9 $\pm$ 3.7 in OSR). Survival after EVAR was lower than after OSR thereafter (80.0 $\pm$ 4.2 vs. 84.6 $\pm$ 4.6% at 48 months, respectively). These differences did not present statistical significance nor before (OR for EVAR vs. OSR 1.598 95% CI 0.817-3.127, p=.171), neither after the introduction of the propensity score in the Cox regression model (adjusted OR 0.803 95% CI 0.588-1.099, p=.170).

Aortic-related mortality

There were 3 aortic-related deaths in the OSR group. Two of them were early deaths, that is, occurring in the first 30 days: one was an intra-operatory death due to uncontrollable hemorrhage and the second occurred in the post-operative period due to shock and multiorgan disfunction. The later death occurred 23 months after the index procedure due to aortic blow out syndrome, after removal of the infected abdominal prothesis.

There were 4 aortic-related deaths in the EVAR group, all related to persistent endoleaks and sac enlargement: at 11 months after the index procedure, one death occurred during a reintervention where a fenestrated EVAR was planned due to proximal endoleak, where proximal migration of the first endoprosthesis occurred with visceral vessels occlusion; at 47 months, one death was attributed to the compression of the bile ducts possibly related to aneurysm sac enlargement; at 73 and 79 months, two patients presented with rupture and died.

Survival free from aortic-related mortality (Figure 3) showed no significant difference between groups (p=.090), however, after adjustment with the propensity score, EVAR was a protective risk factor for aortic mortality versus OSR (OR 0.177 95% CI 0.092-0.338, p<.001).

Vascular reintervention

In the OSR, 7 primary vascular interventions were performed in the first 30 days, mostly due to limb ischemia (3 embolectomies, 1 major amputation, and 2 bypasses to restore inflow to the limb). In one case of early graft infection, the patient was submitted to removal of the aortic graft and revascularization by an axillobifemoral bypass.

In the EVAR group, early reinterventions were due to ischemia (1 embolectomy, 1 axillounifemoral bypass and 1 axillobifemoral bypass) or endoleak (extension of the iliac coverage due to type Ib endoleak).

Freedom from aortic reintervention was similar in the EVAR group versus OSR both before (OR for EVAR vs. OSR 1.036 95% CI 0.753-1.426, p=.828) and after the introduction of the propensity score in the Cox regression model (adjusted OR 1.148 95% CI 0.801-1.645, p=.452), Figure 4.

Time trends

Throughout the years under analysis, the proportion of EVAR among the repaired cases were not homogenous (p=.024). The higher proportions were registered in the most recent years (2014 and 2015), where EVAR was performed in more than 80% of the AAA cases (Figure 5). Complications (p=.014) and the 30-day mortality (p=.035) of the repaired cases were not homogenous throughout the years. The highest value was in 2009, where 31.8% of the cases had one or more complications, while lower proportions of complications occurred recently in 2014 and 2015 (6.9% and 6.1%, respectively). Similarly, the higher 30-day mortality was registered in 2010, where it reached 9.5%. Early mortality was null in 2011 and during the last 3 years under analysis (Figure 5).

When addressing time trends of 30-day mortality per type of repair, 30-day mortality after EVAR was 0% in the whole period under analysis. After OSR, mortality was not homogenous throughout the years (p=.035), dropping from 12% from 2009-2012 to 0% in 2013-2015.

Variation of early reintervention did not reach statistical significance (p=.220). Other morphologic and clinical features such as maximum aortic diameter, age and propensity score did not vary significantly across time.

Discussion

This is a retrospective cohort of 211 patients submitted to AAA repair in a tertiary referral center, that covers seven years of experience, where EVAR gradually became the predominant type of repair, reaching 82% in 2015. Differences between EVAR and OSR among patients' backgrounds are usually present in every-day practice, since EVAR started to be offered to older or physically frail patients in contrast to OSR patients, mostly preferred for younger patients with low operative risk. Despite these differences, EVAR resulted in significantly shorter hospital length of stay, lower respiratory and abdominal complications and similar non-adjusted mortality, aortic-related mortality and reintervention. After adjustment with a propensity score, to minimize the impact of the selection bias regarding the differences in patients' demographic and clinical features might have in the studied outcomes, EVAR presented lower aortic-related mortality versus OSR. Accordingly, in the longitudinal analysis, the gradual EVAR adoption occurred along with lower complications and lower early mortality post AAA repair.

Four major randomized trials – EVAR-1 [10], DREAM [11] and ACE [12] performed in Europe and OVER [13] trial in the USA - compared open and endovascular repair of AAAs. Three of them evinced marked advantage of EVAR with respect to 30-day mortality [10, 11, 13], while the ACE trial [12] revealed similar risk between both surgical techniques. This lower 30-day operative mortality in the EVAR group contributed to an early survival advantage in the first 6 months. However, this early benefit in total mortality of EVAR is gradually lost, namely after 1-2 years in the DREAM trial [11], 2 years in the EVAR-1 trial [10] and 3 years in the OVER trial [13]. In the pooled analysis of the trials, the total mortality benefit in the EVAR group gradually decreased over time and by 5-years there was no difference between groups, with an estimated survival rate of 73.6% in both [14]. A more recent quantitative synthesis including **s**even RCTs reporting a total of 2983 patients [15] confirmed that EVAR compares favorably with OSR during the first six months but it carried an increased risk of aneurysm related mortality after eight years. The risk of secondary intervention (HR 2.13; 95% CI 1.69-2.68), aneurysm rupture (OR, 5.08; 95% CI 1.11-23.31), and death due to rupture (OR, 3.57; 95% CI 1.87-6.80) was significantly higher after EVAR, but the risk of death due to cancer was not significantly different between EVAR and open repair (OR, 1.03; 95% CI 0.84-1.25). These surprising results underlie the need for local reports with both short and long-term outcomes after AAA repair, reflecting the local decision making and cases selection, the focus of this study.

A stratification of patient's co-morbidities is crucial for favorable clinical outcomes, since aspects such as age and ASA physical status can be regarded as risk factors and strong predictors of survival [16]. Before emerging as a first line option in the treatment of AAA [7], EVAR was first seen as an alternative for patients unfit for OSR. Even nowadays, the Society for Vascular Surgery Guidelines does not make any recommendation on the preferred method, emphasizing that information should be given to patients contemplating OSR or EVAR and their Vascular Quality Initiative perioperative mortality risk score [17]. This is evident in historical observational studies like this one, where EVAR patients are usually not only older [18] but also carry a higher incidence of concomitant disorders [19-22], that are more often deprecated for OSR. Despite clinical discrepancies, a composite outcome including all medical complications at 30 days revealed a higher index in the OSR versus EVAR. Recent observational studies reported significantly lower postoperative complications among patients who underwent EVAR, namely lower rates of acute bowel ischemia, renal and respiratory insufficiency [19] but also fewer rates of myocardial infarction and dysrhythmia in addition to inferior rates of acute kidney injury and respiratory failure [20]. AAA repair is a high-risk procedure which results in a severe surgical stress response. It is plausible that the lower rates of systemic complications after EVAR are due to a significantly higher incidence of systemic inflammatory response in OR than in EVAR patients [23]. Levels of IL-6, IL-8, cortisol and epinephrine are higher after OR especially when compared with those of EVAR. Accordingly, the median LOS was significantly shorter after EVAR than after OSR, in accordance with those reported in several observational studies. In fact, a single centre study conducted by Choi K. et al [18] reported a total LOS of 6.5 ± 6.8 in the EVAR group vs. 11.5 ± 13.1 days in the OSR group ($p<.001$). Similarly, a German study [19] presented a median post-operative LOS of 6 days (IQR 5-8) in the EVAR group versus 11 (9-15) days in the OSR group, which was observed in both older [21] and younger patients [20].

Consistent with these results, in many centers the strategy of risk-benefit assessment has been replaced by an EVAR-first approach, if the AAA is anatomically suitable. This strategy is corroborated by the recent guidelines from the ESVS recommend that in most patients with suitable anatomy and reasonable life expectancy, EVAR should be considered as the preferred treatment modality, being reasonable to suggest an OSR first strategy in younger, fit patients with a long life expectancy > 10-15 years [7]. In our center, the gradual and more frequent use of EVAR occurred along with decreased complications and early mortality after AAA repair.

ESVS guidelines received strong opposition from the NICE guidelines draft that propose not to offer EVAR to people with an unruptured infrarenal AAA if open surgical repair is suitable, even if the same guideline does recommend the treatment of ruptured AAA by EVAR, especially in women and men over the age of 70 years [24]. The argumentation for the OSR-first approach points out three uncertainties to EVAR.

One of these uncertainties is that the increased use of EVAR could lead to higher reintervention. Reintervention rates after AAA repair vary widely in the literature, mostly because the report of the procedures considered as reinterventions is very heterogeneous, even among trials. In this study, freedom from primary vascular reintervention showed no differences among groups. Similar findings were reported in recent observational studies, including propensity weighted analysis [20, 21, 25]. Even so, significantly higher reintervention rates in the EVAR group were also described [18, 22]. On the other hand, a recent observational study showed a significantly lower rate of secondary intervention in the EVAR group (11% vs. 27%; $p=.001$), with type II endoleak repair being the most common reintervention after EVAR and incisional hernia repair the most common procedure after OSR [26]. Lastly, a meta-analysis of the four multicenter randomized trials of EVAR versus OSR reported higher overall rates of reintervention related to AAA in the EVAR group, mainly due to type II and type I endoleaks, [14]. Long term analysis, however, was based on few patients, was not statically powered, and did not appropriately capture re-interventions for open repair. Early endografts have shown a propensity toward device-related complications, such as material fatigue and migration, in extended follow-up. The current generation of endografts have been in use only for the past decade, but the long-term durability of these devices seems to have a clear dependence on adherence to the device instructions for use [27]. Many of the patients who were enrolled in the trials 15-20 years ago would probably be offered very different technical solutions, materials, and techniques today, retaining the early mortality/morbidity advantage and promising better durability [28]. Finally, the little evidence supporting the efficacy of secondary intervention for type II endoleaks after EVAR, lead to a marked reduction in this type of reintervention [29].

Another evoked uncertainty is what happens in the long-term. The median length of observation was almost 4 years, and during this time, there were no differences in global mortality, while adjusted

aortic-related mortality was lower in the EVAR group. Despite this, all aortic deaths in the EVAR group were late deaths attributed to endoleaks with sac enlargement. Only one late death occurred in the OSR group due to aortic "blow out" syndrome. In all late EVAR deaths, different degrees of failure in the compliance with the follow up imaging plan were identified. In one case, the aneurysm sac enlargement was identified but the female patient was deemed unfit for an elective attempt of endograft explant. The adoption of a simplified tailored follow up protocol in our department is recent and is not reflected yet in the present results. However, the optimization of the surveillance protocols into less troublesome but equally safe ones is expected to improve compliance and provide better long-term security, by identifying a large proportion of patients who may require less intensive or more rigorous follow up.

Finally, a gradual adoption of EVAR is feared to impact on the mortality after OSR. First, cases that are not fit for EVAR may also perform worse after open repair [30, 31]. Patients with wider neck diameters may warrant a more aggressive management of their medical comorbidities to improve survival, as they are associated with a relative excess of cardiovascular mortality [31]. This was not verified in this study. In fact, the higher mortality rates after OSR were seen in the first half of the period and improved in the later, that is, gradual adoption of EVAR occurred along with reduction in 30-day mortality after OSR. This might be because, in certain violations of the instructions for use (IFU) during EVAR, like necks with severe angulation or high thrombus load in the proximal neck, results of outside IFU EVAR seem to match the results of inside IFU [32]. Also, patients with IFU violations were shown to have higher overall long-term survival with open surgery compared with EVAR [33]. Second, teams may be less prepared for OSR in the EVAR era. In a series from 1982 through 2016 covering 1572 repairs for AAA, Ammar AD et al demonstrated that the introduction of EVARs has not negatively impacted the in-hospital mortality for elective OSR for one vascular surgeon who completed training before EVARs became available [34]. In the period covered by this study, OSR were performed by teams that always included at least one vascular surgeon who completed training before EVARs became available. The impact of completing training of OSR in the EVAR era in the outcomes of OSR remains to be addressed in our center.

Limitations

Study limitations must be considered in interpreting these results and include the retrospective data collection and its inherent dependence on medical records. This is a single-center study narrowing the external validity of the results. Although attempts were made for adjustment based on relevant clinical and demographic features, a possible selection bias among patients who underwent OSR versus EVAR must be considered. Open surgical and endovascular repair groups may present conditioned comparability regarding aortic anatomy, as open surgical candidates are likely to be patients who have unfavorable aortic anatomy for EVAR [33]. Only vascular reinterventions were considered in the analysis. Abdominal hernia repairs were not considered because, despite 7% (7 out of 79) of OSR patients having abdominal hernia repair, these procedures were often performed in subsidiary hospitals and the precise date could not be ascertained which precluded the use of survival analysis. Likewise, access complications different from ischemia such as lymphocele were not considered as well, despite 0.8% (1 out of 132) EVAR patients presenting that complication, implying that in the OSR group, vascular reintervention is expected to be an under-estimation of the global reintervention rate.

Conclusion

In this retrospective cohort study EVAR was offered to older patients with amassed comorbidities. Still, better and durable outcomes were achieved after EVAR, favoring its continuous adoption for elective AAA repair. Tertiary referral centers must include open and endovascular surgery for AAA repair. EVAR is by no means a gold standard treatment for the majority of patients while OSR has an important role in the management of several subgroups.

References

[1] Johnston KW, Rutherford RB, Tilson MD, et al. (1991) Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. J Vasc Surg 13:452-8.

[2] Hallett JW, Jr., Marshall DM, Petterson TM, et al. (1997) Graft-related complications after abdominal aortic aneurysm repair: reassurance from a 36-year population-based experience. J Vasc Surg 25:277-84; discussion 285-6.
[3] Conrad MF, Crawford RS, Pedraza JD, et al. (2007) Long-term durability of open abdominal aortic aneurysm repair. J Vasc Surg 46:669-75.
[4] Cronenwett J, Johnston, K. W., *Rutherford's Vascular Surgery*, ed. t. Edition. Vol. 2. Saunders.
[5] Heller JA, Weinberg A, Arons R, et al. (2000) Two decades of abdominal aortic aneurysm repair: have we made any progress? J Vasc Surg 32:1091-100.
[6] Dias-Neto M, Mani K, Leite-Moreira A, et al. (2020) Nationwide analysis of intact abdominal aortic aneurysm repair in Portugal from 2000-2015. Ann Vasc Surg.
[7] Wanhainen A, Verzini F, Van Herzeele I, et al. (2019) Editor's Choice - European Society for Vascular Surgery (ESVS) 2019 Clinical Practice Guidelines on the Management of Abdominal Aorto-iliac Artery Aneurysms. Eur J Vasc Endovasc Surg 57:8-93.
[8] von Elm E, Altman DG, Egger M, et al. (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Int J Surg 12:1495-9.
[9] Ahn SS, Rutherford RB, Johnston KW, et al. (1997) Reporting standards for infrarenal endovascular abdominal aortic aneurysm repair. Ad Hoc Committee for Standardized Reporting Practices in Vascular Surgery of The Society for Vascular Surgery/International Society for Cardiovascular Surgery. J Vasc Surg 25:405-10.
[10] Greenhalgh RM, Brown LC, Kwong GP, et al. (2004) Comparison of endovascular aneurysm repair with open repair in patients with abdominal aortic aneurysm (EVAR trial 1), 30-day operative mortality results: randomised controlled trial. Lancet 364:843-8.
[11] Prinssen M, Verhoeven EL, Buth J, et al. (2004) A randomized trial comparing conventional and endovascular repair of abdominal aortic aneurysms. N Engl J Med 351:1607-18.
[12] Becquemin JP, Pillet JC, Lescalie F, et al. (2011) A randomized controlled trial of endovascular aneurysm repair versus open surgery for abdominal aortic aneurysms in low- to moderate-risk patients. J Vasc Surg 53:1167-1173 e1.
[13] Lederle FA, Freischlag JA, Kyriakides TC, et al. (2012) Long-term comparison of endovascular and open repair of abdominal aortic aneurysm. N Engl J Med 367:1988-97.
[14] Powell JT, Sweeting MJ, Ulug P, et al. (2017) Meta-analysis of individual-patient data from EVAR-1, DREAM, OVER and ACE trials comparing outcomes of endovascular or open repair for abdominal aortic aneurysm over 5 years. Br J Surg 104:166-178.
[15] Antoniou GA, Antoniou SA, Torella F (2019) Endovascular vs. Open Repair for Abdominal Aortic Aneurysm: Systematic Review and Meta-analysis of Updated Peri-operative and Long Term Data of Randomised Controlled Trials. Eur J Vasc Endovasc Surg.
[16] Boult M, Maddern G, Barnes M, et al. (2007) Factors affecting survival after endovascular aneurysm repair: results from a population based audit. Eur J Vasc Endovasc Surg 34:156-62.
[17] Chaikof EL, Brewster DC, Dalman RL, et al. (2009) The care of patients with an abdominal aortic aneurysm: the Society for Vascular Surgery practice guidelines. J Vasc Surg 50:S2-49.
[18] Choi K, Han Y, Ko GY, et al. (2018) Early and Late Outcomes of Endovascular Aortic Aneurysm Repair versus Open Surgical Repair of an Abdominal Aortic Aneurysm: A Single-Center Study. Ann Vasc Surg 51:187-191.
[19] Behrendt CA, Sedrakyan A, Riess HC, et al. (2017) Short-term and long-term results of endovascular and open repair of abdominal aortic aneurysms in Germany. J Vasc Surg 66:1704-1711 e3.
[20] Liang NL, Reitz KM, Makaroun MS, et al. (2018) Comparable perioperative mortality outcomes in younger patients undergoing elective open and endovascular abdominal aortic aneurysm repair. J Vasc Surg 67:1404-1409 e2.
[21] Majd P, Ahmad W, Galas N, et al. (2018) Patients Older Than 80 Years Can Reach Their Normal Life Expectancy After Abdominal Aortic Aneurysm Repair: A Comparison Between Endovascular Aneurysm Repair and Open Surgery. J Endovasc Ther 25:247-251.
[22] Symonides B, Sliwczynski A, Galazka Z, et al. (2018) Short- and long-term survival after open versus endovascular repair of abdominal aortic aneurysm-Polish population analysis. PLoS One 13:e0198966.
[23] Moris DN, Kontos MI, Mantonakis EI, et al. (2014) Concept of the aortic aneurysm repair-related surgical stress: a review of the literature. Int J Clin Exp Med 7:2402-12.
[24] Liapis CD, Avgerinos ED, Eckstein HH (2019) Proposed NICE Abdominal Aortic Aneurysm Repair Guidelines: Swinging the Pendulum too Far? Eur J Vasc Endovasc Surg 58:637-638.

[25] Sugimoto M, Koyama A, Niimi K, et al. (2017) Long-term Comparison of Endovascular and Open Repair of Abdominal Aortic Aneurysms: Retrospective Analysis of Matched Cohorts with Propensity Score. Ann Vasc Surg 43:96-103.
[26] Krishnamoorthi H, Jeon-Slaughter H, Wall A, et al. (2018) Rate of Secondary Intervention After Open Versus Endovascular Abdominal Aortic Aneurysm Repair. J Surg Res 232:99-106.
[27] Speziale F, Sirignano P, Setacci F, et al. (2014) Immediate and two-year outcomes after EVAR in "on-label" and "off-label" neck anatomies using different commercially available devices. analysis of the experience of two Italian vascular centers. Ann Vasc Surg 28:1892-900.
[28] Siracuse JJ, Schermerhorn ML, Meltzer AJ, et al. (2016) Comparison of outcomes after endovascular and open repair of abdominal aortic aneurysms in low-risk patients. Br J Surg 103:989-94.
[29] Ultee KHJ, Buttner S, Huurman R, et al. (2018) Editor's Choice - Systematic Review and Meta-Analysis of the Outcome of Treatment for Type II Endoleak Following Endovascular Aneurysm Repair. Eur J Vasc Endovasc Surg 56:794-807.
[30] Kontopodis N, Tavlas E, Ioannou CV, et al. (2020) Prognosis Systematic Review and Meta-Analysis of Outcomes of Open and Endovascular Repair of Ruptured Abdominal Aortic Aneurysm in Patients with Hostile vs. Friendly Aortic Anatomy. Eur J Vasc Endovasc Surg.
[31] Oliveira NFG, Ultee K, van Rijn MJ, et al. (2019) Anatomic predictors for late mortality after standard endovascular aneurysm repair. J Vasc Surg 69:1444-1451.
[32] Oliveira-Pinto J, Oliveira N, Bastos-Goncalves F, et al. (2017) Long-term results of outside "instructions for use" EVAR. J Cardiovasc Surg (Torino) 58:252-260.
[33] Charbonneau P, Hongku K, Herman CR, et al. (2019) Long-term survival after endovascular and open repair in patients with anatomy outside instructions for use criteria for endovascular aneurysm repair. J Vasc Surg.
[34] Ammar AD (2019) Mortality for Open Abdominal Aortic Aneurysm Repair before and after Endovascular Aortic Repair (EVAR). Am Surg 85:1341-1344.

Table 1. Clinical and demographic data.

Characteristics, n (%)		OSR (n=79)		EVAR (n=132)		Total (n=211)		p
Gender (male)		74	93.7	127	96.2	201	95.3	.400
Age, years		67	9	74	7	71	9	<.001
Tobacco	No	20	26.7	38	31.1	58	29.4	.774
	Former smoker	25	33.3	34	27.9	59	29.9	
	Active smoker	30	40.0	50	41.0	80	40.6	
Diabetes mellitus	No	66	85.7	102	81.0	168	82.8	.215
	Diet or oral medication	11	14.3	20	15.9	31	15.3	
	Adult insulin dependent	0	0.0	4	3.2	4	2.0	
Hypertension	No	23	31.5	20	16.5	43	22.2	.004
	Regulated by monotherapy	12	16.4	23	19.0	35	18.0	
	Regulated by 2 drugs	28	38.4	38	31.4	66	34.0	
	Regulated by >2 drugs	10	13.7	40	33.1	50	25.8	
Obesity		9	14.3	35	28.7	44	23.8	.029
BMI, kg/m^2		26.70	2.93	27.15	3.70	27.00	3.45	.532
Coronary artery disease		15	21.1	39	32.0	54	28.0	.106
History cardiac treatment	No	64	85.3	82	69.5	146	75.6	.005
	PCI	8	10.7	17	14.4	25	13.0	
	CABG	3	4.0	19	16.1	22	11.4	
Congestive heart failure		4	5.2	21	17.2	25	12.6	.013
Carotid artery disease	No	30	85.7	65	83.3	95	84.1	.463
	Asymptomatic significant stenosis	1	2.9	1	1.3	2	1.8	
	History of TIA	3	8.6	4	5.1	7	6.2	
	Ischemic stroke	1	2.9	8	10.3	9	8.0	
Preoperative plasmatic creatinine, mg/dL		1.28	1.52	1.09	.40	1.15	.97	.169
Chronic renal failure*	No	68	93.2	115	89.1	183	90.6	.882
	Mild	2	2.7	11	8.5	13	6.4	
	Severe	0	0.0	2	1.6	2	1.0	
	Terminal	3	4.1	1	0.8	4	2.0	
Peripheral arterial disease	No or asymptomatic	54	77.1	107	87.0	161	83.4	.077
	Intermittent claudication or Critical limb ischemia	16	22.9	16	13.0	32	16.6	
COPD		10	13.3	40	32.8	50	25.4	.002
ASA	2	31	47.0	33	28.0	64	34.8	.005
	3	33	50.0	74	62.7	107	58.2	
	4	2	3.0	11	9.3	13	7.1	
Anticoagulants		5	6.8	19	15.1	24	12.1	.086
Antiplatelet		35	48.6	81	63.3	116	58.0	.044
Digoxin or lanoxin		2	2.8	3	2.5	5	2.6	.892
Vasodilators		6	8.3	19	15.4	25	12.8	.152
ACE inhibitors		25	34.7	50	41.0	75	38.7	.387

Diuretics	23	32.4	46	38.0	69	35.9	.433
Calcium channel blockers	10	13.9	39	32.2	49	25.4	.005
Beta blocking agents	18	25.0	51	42.1	69	35.8	.016
Statin	42	62.7	91	75.8	133	71.1	.059
Ever had abdominal surgery	14	18.9	34	29.6	48	25.4	.101
Maximum transverse aortic diameter, cm	6.27	1.59	6.13	1.53	6.18	1.55	.537

Legend: ACE – angiotensin converting enzyme; ASA – American Society of Anaesthesiology's Physical Status Classification System; BMI – body mass index; CABG – coronary artery bypass graft; COPD – chronic obstructive pulmonary disease; EVAR – endovascular repair; OSR – open surgery repair; PCI – percutaneous coronary intervention.

*Chronic renal failure classes were defined as Mild if Serum Creatinine <2.10 µmol/L, severe if creatitine 2.20-5.20 µmol/L, and terminal if Serum creatinine >5.20 µmol/L or dialysis/kidney transplantation dependent.

Table 2. Details of aneurysm repair.

Characteristics		OSR (n=79)	EVAR (n=132)
Surgical Technique, n (%)	Tubular aortic interposition	40 (50.6)	-
	Aortofemoral bypass	24 (30.4)	-
	Aortobiiliac bypass	15 (19.0)	-
	EVAR with femoral cutdown	-	100 (75.8)
	Percutaneous EVAR	-	27 (20.4)
	Aorto-Uni-Iliac EVAR with FF bypass	-	5 (3.8)
Type of Anesthesia, n (%)	Local with/without sedation	0	15 (12.1)
	Locoregional	0	91 (73.4)
	General	79 (100)	18 (14.5)

Legend: EVAR – endovascular aneurysm repair; FF – femoro-femoral; OSR – open surgical repair.

Table 3. Medical complications 30 days after repair.

Characteristics, n (%)	OSR		EVAR		Total		P value
	(N=79)		(N=132)		(N=121)		
AMI	3	3.8	5	3.8	8	3.8	1.00
w PCI	0	0.0	2	1.5	2	1.0	
w CABG	0	0.0	1	0.8	1	0.5	
wt intervention	3	3.8	2	1.5	5	2.4	
CHF	0	0.0	3	2.3	3	1.4	.296
Cardiac arrest	0	0.0	1	0.8	1	0.5	1.00
Stroke/TIA	1	1.3	4	3.0	5	2.4	.653
Respiratory failure	12	15.4	2	1.5	14	6.7	<.001
Renal failure	6	7.6	4	3.0	10	4.7	.181
Abdominal	1	8.9	1	0.8	8	3.8	.005
Composite outcome	19	24.1	14	10.6	32	15.6	.009

Legend: AMI – acute myocardial infarction, CABG – coronary arterial bypass grafting, CHF – congestive heart failure, PCI – percutaneous coronary intervention, TIA – transient ischemic attack, w – with, wt – without.

Figure 1. Logistic regression model to predict the risk of undergoing EVAR repair including demographic and clinical variables that differ among groups of AAA repair in univariate analysis. Legend: AAA – abdominal aortic aneurysm, CHF – congestive heart failure, COPD – chronic obstructive pulmonary disease, EVAR – endovascular repair, OSR – open surgical repair, PCI/CABGB – percutaneous coronary intervention/coronary bypass graft.

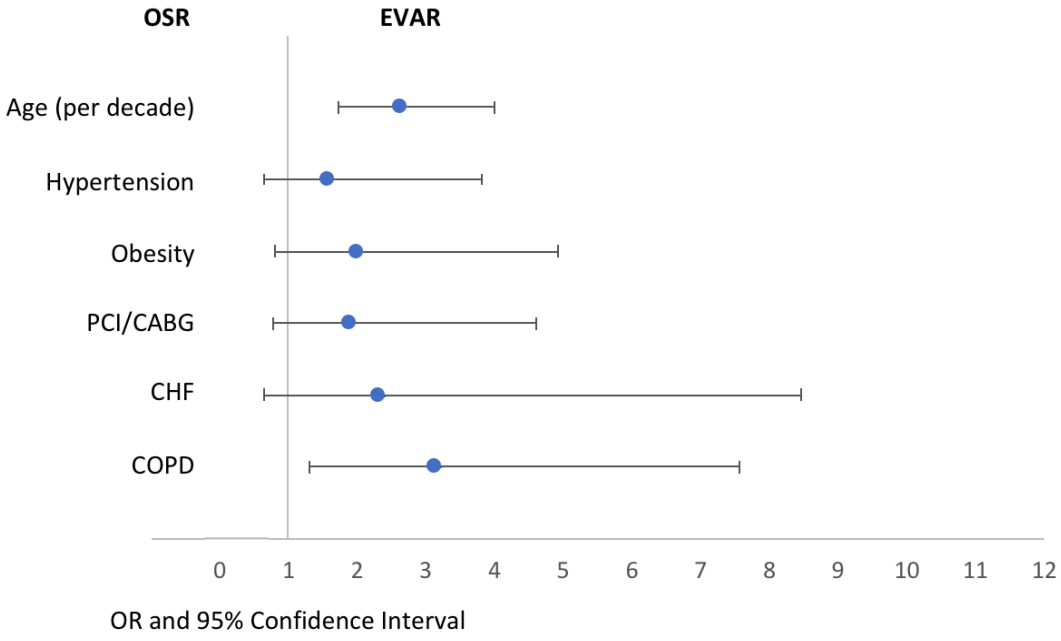
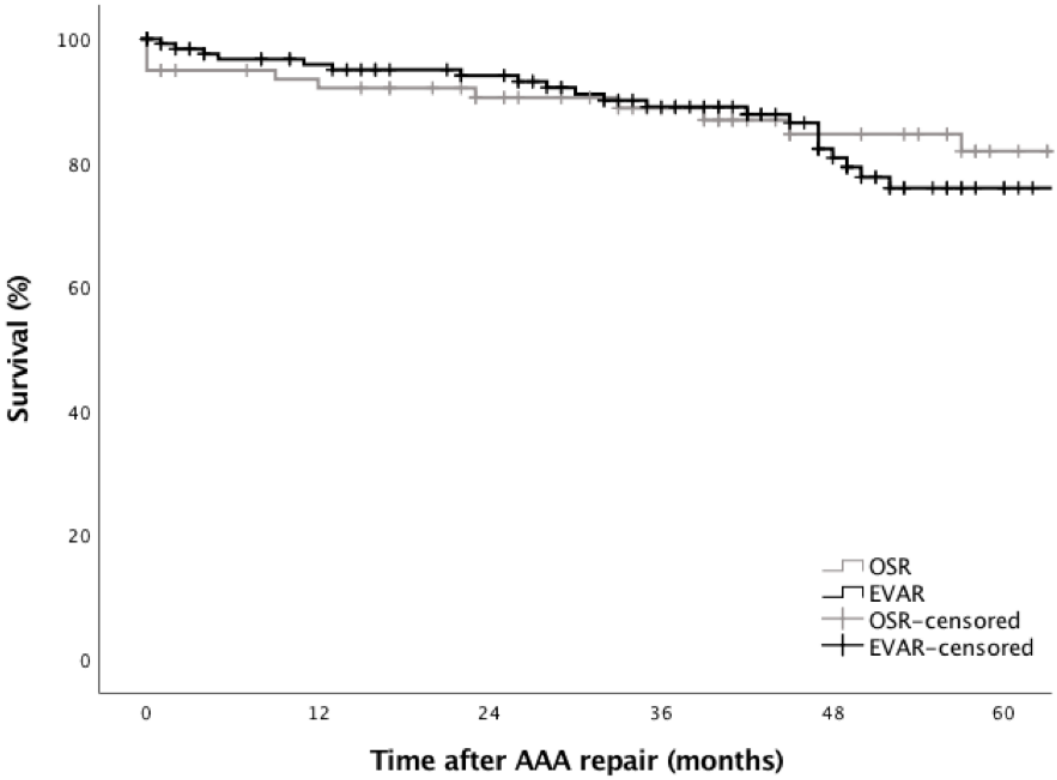
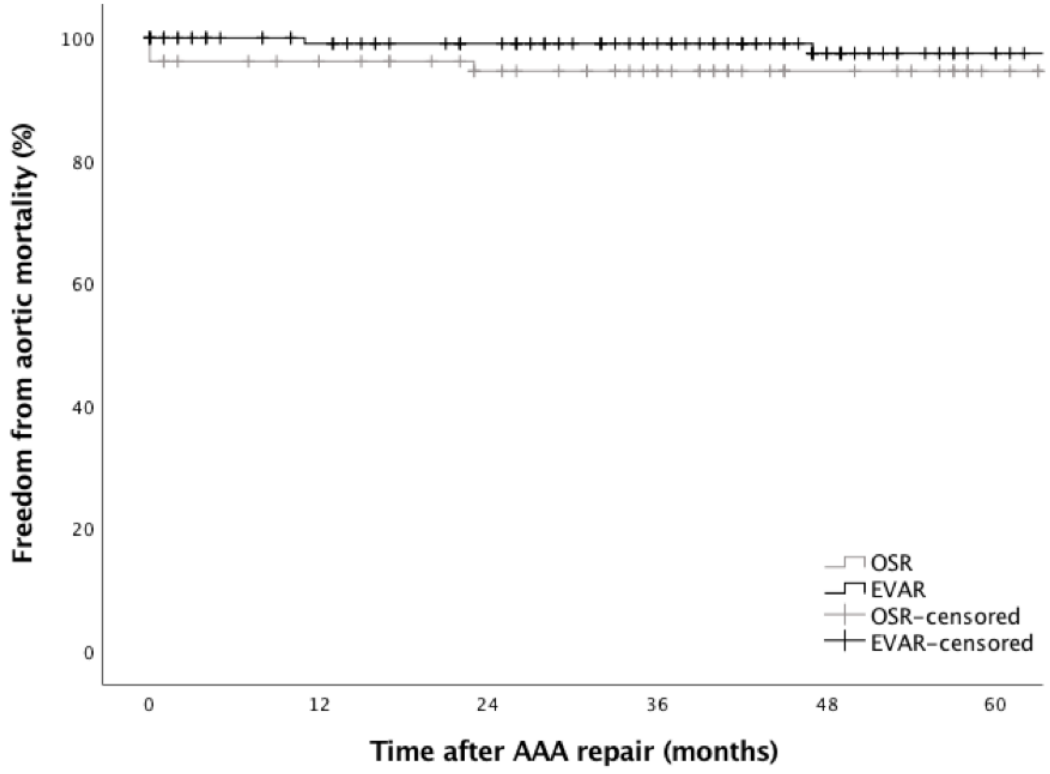


Figure 2. Overall survival after AAA repair. Legend: AAA – abdominal aortic aneurysm, EVAR – endovascular repair, OSR – open surgical repair.



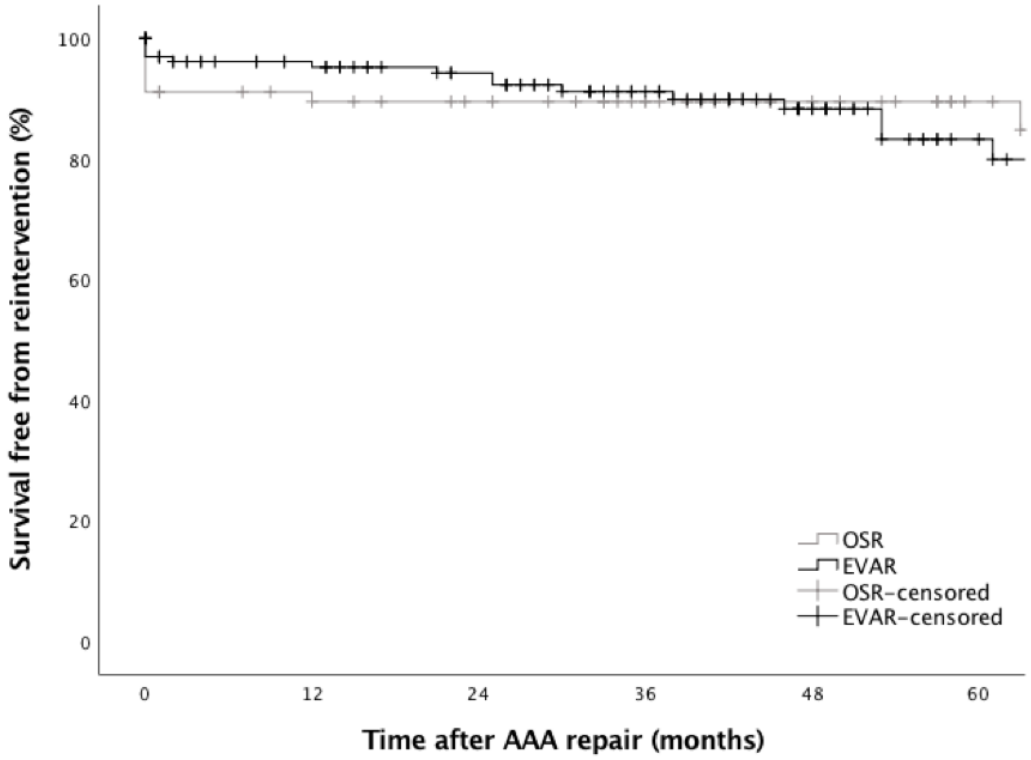
Time after repair (months)		1	12	24	36	48
OSR	Survival estimate (%)	94.9	93.5	90.6	88.9	84.6
	Standard error (%)	2.5	2.8	3.4	3.7	4.6
	Number at risk	79	66	57	49	35
EVAR	Survival estimate (%)	100	95.9	94.1	89.0	82.3
	Standard error (%)	-	1.8	2.2	3.0	4.0
	Number at risk	132	111	100	82	56

Figure 3. Freedom from aortic mortality after AAA repair. Legend: AAA – abdominal aortic aneurysm, EVAR – endovascular repair, OSR – open surgical repair.



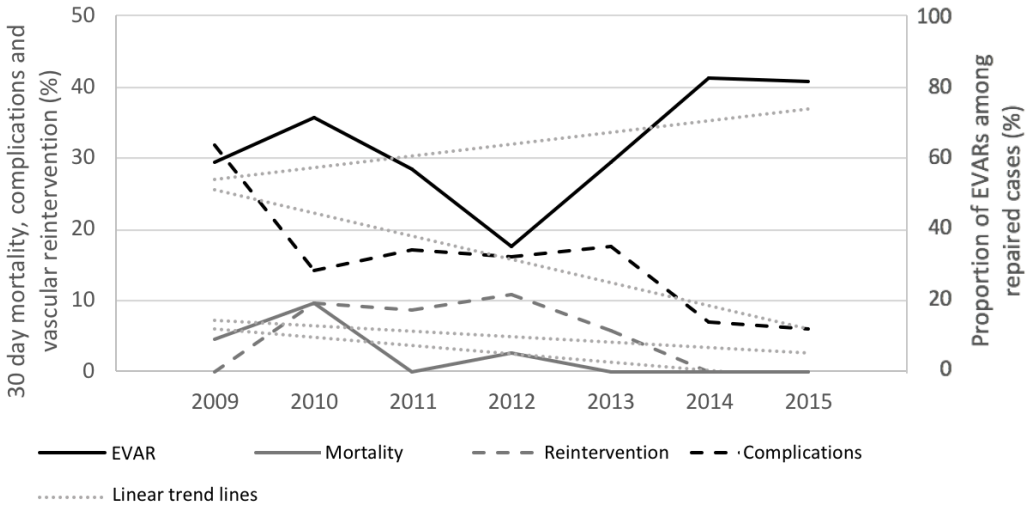
Time after repair (months)		1	12	24	36	48
OSR	Survival estimate (%)	96.2	96.2	94.6	94.6	94.6
	Standard error (%)	2.2	2.2	2.6	2.6	2.6
	Number at risk	79	66	57	49	35
EVAR	Survival estimate (%)	100	99.1	99.1	99.1	97.5
	Standard error (%)	-	0.9	0.9	0.9	1.8
	Number at risk	132	111	100	82	56

Figure 4. Freedom from reintervention after AAA repair. Legend: AAA – abdominal aortic aneurysm, EVAR – endovascular repair, OSR – open surgical repair.



Time after repair (months)		1	12	24	36	48
OSR	Survival estimate (%)	91.1	89.5	89.5	89.5	89.5
	Standard error (%)	3.2	3.5	3.5	3.5	3.5
	Number at risk	79	57	51	43	29
EVAR	Survival estimate (%)	97.0	95.3	94.3	91.2	88.3
	Standard error (%)	1.5	1.9	2.1	2.7	3.3
	Number at risk	132	107	95	75	48

Figure 5. Time trends in 30-day mortality, complications and vascular reinterventions and well in the proportion of EVAR among repaired cases of AAA form 2009-2015. Legend: AAA – abdominal aortic aneurysm, EVAR – endovascular repair.



CHAPTER 3 NATIONWIDE ANALYSIS OF INTACT ABDOMINAL AORTIC ANEURYSM REPAIR IN PORTUGAL FROM 2000-2015

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Abstract

Objective: Results on the management of infrarenal abdominal aortic aneurysm (AAA) from Mediterranean countries are scarce. The aim of this study was to evaluate trends in rate of and mortality after repair of intact AAA (iAAA) in Portugal.
Methods: iAAA repairs registered in the hospitals administrative database of the National Health Service from 2000 to 2015 were retrospectively analyzed regarding demographics (age and gender) and type of repair (open surgery [OS] or endovascular repair [EVAR]). Rate and mortality were compared among three time periods: 2000-2004, 2005-2009 and 2010-2015.
Results: Age-standardized rate of iAAA repair increased consistently across the time periods under analysis from 3.6±0.6/100,000/year in 2000-2004, to 5.6±0.4/100,000/year in 2005-2009 and to 7.1±0.9/100,000/year in 2010-2015 ($p<0.001$). The percentage of EVAR among all iAAA repairs rose steeply from 0 to 21±19% and then to 58±7% ($p<0.001$). The rate of OS also increased from the first to the second period, but there was a decrease in the third period ($p<0.001$). The in-hospital mortality after iAAA repair decreased from 7.5±1.3% to 6.6±1.6% and then to 5.1±1.9% ($p<0.001$). This variation corresponded to a decrease in in-hospital mortality after EVAR (from 4.0±3.5% to 2.8±0.9%, $p<0.001$) and increased in-hospital mortality after OS (7.5±1.3% to 7.4±1.1% to 8.3±3.7%, $p<0.001$). Low-volume centers (<15 repairs/year) did not present higher mortality rates. The number of EVARs per year in a centre presented a positive association with EVAR mortality (Spearman correlation of 0.696, $p=0.004$).
Conclusion: The rate of repair of iAAA continues to grow, especially in patients ≥ 75 years old, and did not reach an inflection point yet. This is happening along with decreased repair mortality mainly due to the increased use of EVAR. Hospital mortality for iAAA repair is still a matter of concern, warranting further investigation and planning of vascular surgical services.

Introduction

Abdominal aortic aneurysm (AAA)[1] is a potentially lethal condition responsible for significant mortality, morbidity and cost to society. The current indications for treatment of AAA are well defined in the guidelines of the Society for Vascular Surgery (2018) and the European Society for Vascular Surgery (2019), that recommend elective repair if the maximum diameter is ≥5.5 cm in men and ≥5 cm in women [2,3].
The AAA has experienced an epidemiological transition in Western countries, ranging from a former pattern consisting of high incidence and mortality to a more recent, characterized by a decrease in incidence and mortality by AAA [2,4]. This epidemiological change has not been synchronized among all countries either between gender or age groups [4]. As a result, the prevalence data reported in different studies are often varying [5]. Most important, the rates of AAA repair per 100,000 population are significantly more variable and its relationship with the disease frequency is not clear[6].
Important changes have had major impact on the management of AAA of the disease [7-9]. The introduction of endovascular repair has extended the treatment to older patients[10] and to patients who were not candidates to open surgery, despite the ongoing discussion of its cost-effectiveness. Additionally, improved perioperative care with more frequent use of locoregional anaesthesia and of percutaneous access improved the outcomes of AAA repair. A putative change in cardiovascular risk factors such as decreased smoking habits and more widespread use of cardioprotective drugs might also contribute [4,11-13] to better results after repair.
A reliable national assessment of trends of admission and mortality associated to AAA repair might give insight into the expected health care costs associated with the treatment of this disease.
The objective of this manuscript is to assess trends in the rate of intact AAA (iAAA) repair, demographics and outcomes over a 16-year interval in Portugal.

Methods

All iAAA interventions performed during the period 2000 to 2015 were retrospectively identified in a National Health Service (NHS) administrative database, the hospital morbidity database, formerly designated as Diagnosis-Related Groups (DRGs) database. This database was provided by the Portuguese Central Health System Administration (CHSA) and contains a registration of all hospitalizations (retrospective consecutive case entry) occurring in public hospitals in mainland Portugal, where more than 90% of iAAA repairs are performed. The Portuguese health system is characterized by three co-existing and overlapping systems: the NHS; special public and private insurance schemes for certain professions or companies (health subsystems); and private voluntary health insurance. All residents in Portugal have access to health care provided by the NHS, financed mainly through taxation. Public hospitals are funded through global budgets, but with an increasing role of DRGs, and private insurers and health subsystems pay providers. Private health care providers mainly fulfil a supplementary role to the NHS rather than providing a global alternative to it. Currently, the private sector mainly provides diagnostic, therapeutic and dental services, as well as some ambulatory consultations, rehabilitation and hospitalization [14].
Each episode includes information about diagnoses (primary and secondary diagnoses) and medical or surgical procedures, both coded using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9). External audits are performed by the CHSA in order to verify the accuracy of the clinical coding performed at the NHS hospitals. The episode presents no disagreement when it respects ICD-9, Coding Clinic and national Consensus. The last one was in 2010 when a total of 4,510 episodes were audited in 44 hospital institutions. The results indicate that most disagreements among diagnoses, procedures and other administrative and demographic variables did not affect the respective grouping in the DRGs (mean percentage of disagreement was 13.7%, maximum of 26.5% and a minimum of 2.1%).
All admissions considered have a primary diagnosis ICD-9 codes of 441.4 (Abdominal aneurysm without mention of rupture) or 441.9 (Aortic aneurysm of unspecified site without mention of rupture) for non-ruptured AAA. The primary diagnosis of an episode represents the main condition investigated or treated during that hospital stay. Unspecified site of aortic aneurysms were included, in line with other studies [11,15], to increase the chance that all abdominal lesions were captured in the analysis, while thoracoabdominal aneurysms were not, since they are more often related to genetic disorders which might interfere differently with their epidemiologic evolution. The rate of the repair was calculated based on the episodes of care with the above-mentioned disease ICD-9 codes plus the ICD-9 procedure codes suitable for AAA repair (Supplemental Table I). Admissions with a primary diagnose of iAAA that lacked an

ICD-9 procedure code suitable for AAA repair were excluded, assuming that no repair was performed during that hospital stay. General indication for iAAA repair in Portugal are a maximum transverse diameter ≥5.5cm in men and ≥5.0 cm in women, as recommended by guidelines [2,3].
The number of deaths accounted for all patients that fail to be discharged alive from the hospital admission where the repair took place, irrespective to the direct cause of death. The number of admissions for repair in which the patient died divided by the number of all admissions for repair was used as a proxy of *in-hospital mortality* due to iAAA. The number of the resident population in each year under analysis was provided by the National Institute of Statistics (INE) from 2000 to 2015. Resident population per year was used to calculate the rate of iAAA repair and the death associated with iAAA repair (thereafter mentioned as *mortality* to distinguish from the above-mentioned in-hospital mortality). In both databases, patients <50 years of age were excluded.
The effect of the centre volume on outcome was assessed by a sensitivity analysis, where the impact of different cut-off numbers of iAAA repairs per year was tested in all repair, endovascular (EVAR) and open surgery (OS) mortalities.

Ethics
This study follows the principles of the Declaration of Helsinki (this research study involving is registered in ClinicalTrials.gov with the Identifier number NCT04085003). Because of the retrospective nationwide administrative nature of the data used in this study, Institutional Review Board approval was waived, and patient informed consent was not obtained.

Statistics
Data were calculated overall and for three time periods (2000-2004, 2005-2009 and 2010-2015) to assess the variations over time. Subgroups analysis was performed to investigate trend differences upon gender and age (< or ≥ 75 years old) as previously performed [11].
To account for changes in the age structure of the population over time, age direct standardization was performed (using the *World Health Organization*, WHO, world standard population[16]) for iAAA rate and mortality. This option also makes the results comparable with international data that also used WHO standardization.
Proportions were compared using the chi-square test. Changes in proportions over time were assessed using the chi-square test for trend. Normally distributed data were compared using one-way ANOVA. To adjust for multiple testing due to subgroup analysis $p < 0.010$ was considered significant. All statistical analysis was performed by using SPSS for Mac version 24 (SPSS, Armonk, NY: IBM Corp).

Results

Rate of iAAA repair
A total of 3991 repairs of iAAA were identified in patients 50 years old or more, from 2000 to 2015. This corresponds to mean and standard deviation of age-standardized repair rate 5.5 ±1.6 per 100,000 inhabitants per year. Demographic characteristics are shown in Table I. The age-standardized rate of iAAA repair increased consistently across the time periods under analysis from 3.67±0.6 per 100,000 inhabitants per year in 2000-2004, to 5.6±0.4 in 2005-2009 and to 7.1±0.9 in 2010-2015 ($p<0.001$) (Figure 1).
Ninety-three per cent of the iAAA repair was performed in men (3722 of 3991) and this ratio did not change across time ($p=0.771$). The increasing rate of iAAA repair was verified in both gender subgroups being significant in men ($p<0.001$) but not in women ($p=0.024$).
The percentage of patients ≥ 75 years old increased markedly from 30±2% in the first period to 39±3% and 43±3% in the second and third periods ($p<0.001$). In the more extreme age-band of equal or above 80 years old, the percentage doubled from 10% in the first period to 20% in the last period ($p<0.001$).
The increasing of iAAA repair was significant in both age subgroups (< and ≥ 75 years old). The rise in the age-standardized rates of iAAA repair in patients ≥ 75 years old was more evident, with specific rates varying from 6.1±1.3 per 100,000 inhabitants per year in 2000-2004 and 15.5±1.3 per 100,000 inhabitants per year in 2010-2015 ($p<0.001$).
EVAR increased significantly across the two time periods ($p<0.001$). Due to lack of episodes coded for EVAR before 2006, the rate was set to null in 2000-2004. The rate of open surgery also increased from the first to the second period. However, there was a decrease in the third period, where the repairs went

down below those found in the first period (p<0.001). Indeed, the percentage of endovascular repairs among all iAAA repairs rose steeply from 21±19% to 58±7% (p<0.001).
The preferred type of repair among the age bands < 75 and ≥ 75 years old per year of analysis are depicted in Figure 2.

Mortality after iAAA repair per 100,000 inhabitants per year
Despite more patients being operated on, mortality after repair experienced a non-similar variation (Table II and Figure 1). The age-standardized mortality after repair increased and then stabilized from 0.25±0.04 per 100,000 inhabitants per year in 2000-2004, to 0.34±0.07 in 2005-2009 and to 0.32±0.06 in 2010-2015 (p<0.001). Due to the use of standardization, this variation cannot be attributed to changes in the age bands of the Portuguese population over the period under analysis. Along with increasing use of EVAR, age-standardized mortality after EVAR increased from 0.03±0.03 per 100,000 inhabitants per year in 2005-2009 to 0.10±0.03 per 100,000 inhabitants per year in 2010-2015 (p<0.001). On the other side, age-standardized mortality due to open repair increased from 0.25±0.04 per 100,000 inhabitants per year in 2000-2004 to 0.31±0.09 per 100,000 inhabitants per year in 2005-2009 and then decreased to 0.22±0.06 per 100,000 inhabitants per year in 2010-2015 as, in recent years, less and less patients are treated by open surgery.
In subgroup analysis, the variation of mortality after repair in males followed the global mortality among repaired cases with increasing and stabilization (p<0.001). In females, there was no change in the mortality over the three periods (p=0.218).
Patients ≥ 75 years old also followed the increasing and stabilization pattern. However, in ages <75 years old, mortality decreased in recent years, after a former increase (p=0.002).

In-hospital operative mortality of iAAA
There was a decrease in in-hospital mortality from 7.5±1.3% to 6.6±1.6% and then to 5.1±1.9% due to iAAA repair (p<0.001). This variation corresponded to a decrease in the in-hospital mortality after EVAR (from 4.0±3.5% to 2.8±0.9%, p<0.001) and to an increased in-hospital mortality period after OS in the third time (7.5±1.3% to 7.4±1.1% to 8.3±3.7%, p<0.001) (Figure 1).
In males, the in-hospital mortality follows the global trend with reductions from 7.3±1.5% in 2000-2004 to 6.4±1.5% in 2005-2009 and to 4.9±2.2% in 2010-2015 (p<0.001).
In-hospital mortality in female patients also follows the global trend from 10.9±6.4% in 2000-2004, to 10.2±5.4% in 2005-2009 and 7.6±4.1% in 2010-2015 (p=0.008). However, in-hospital mortality in females was significantly higher than in males (p<0.001) (Figure 3A).
The rates of in-hospital mortality in ages <75 years old were 6.9±2.3%, 5.5±1.8% and 4.0±0.7% (p<0.001). In the age band ≥ 75 years old, the rates of in-hospital mortality were 8.9±2.6%, 8.3±3.7% and 6.4±3.5% (p<0.001), remaining above the figures from younger patients (p<0.001) (Figure 3B).

The volume of the centre
The association between iAAA repair mortality and volume centre revealed that centres with ≥ 15 repairs per year presented higher rates of EVAR mortality (Figure 4). When addressed specifically, number of EVARs per year presented a positive association with EVAR mortality (Spearmen correlation of 0.696, p=0.004) (Figure 5).

Discussion

This study provides an assessment of the epidemiology of iAAA repair rate and treatment in a country where a AAA screening program is not formally implemented, offering a glimpse on the evolution of the disease beyond what is attributed to screening.
In Portugal, the rate of repair of iAAA continues to grow, especially in patients ≥ 75 years old, and did not reach an inflection point yet. This is happening along with decreased repair mortality, in both age-bands <75 years old and ≥ 75 years old mainly due to the increased use of EVAR.
The NHS administrative database that was provided by the Portuguese CHSA contains a registration of all hospitalizations occurring in public hospitals in mainland Portugal. Clinical codification at the hospital-level is not generally undertaken by the surgeons who perform the cases. Instead, independent physicians with specific preparation for coding undertake that task independently, virtually eliminating selection bias. Furthermore, this database has funding purposes to the hospitals. This funding is attributed to hospitals according to number of hospitalizations, adjusted by their case-mix index that is DRGs-weighed.

Notably, this specificity also happens to ensure that no surgery remains to be included. Accuracy of the codification is guaranteed by proper training of the staff and external audits.

The first question raised by the present data is why iAAA repair keeps rising if not fed by a screening program. These findings of increased repair are in line with other papers investigating the evolution of AAA epidemiology in European countries where screening is implemented: while in Australia and New Zealand, a decline in admissions and mortality due to iAAA have been documented since the nineties [8,15], in England and Wales decreased mortality was described along with increased iAAA admissions for repair [11]. The authors concluded that it was likely that the trends in England and Wales were trailing behind those of Australia and New Zealand, and, after this plateau stage, it could be anticipated that there would be a decline in overall iAAA repairs. In Sweden, a marked increase in the iAAA repair rate up to till 2010 was verified [17]. This was followed by stabilization, or even a decrease (if AAAs detected by screening are excluded) attributed to the reduced prevalence of the disease.

If not due to screening, two reasons might contribute to the results obtained in this paper. First, increased availability of imaging techniques may contribute toward an increased diagnosis of incidental iAAA [11]. Second, the increased use of EVAR in patients that otherwise would be denied for open surgery and treatment of an increasing number of patients with ≥ 75 years old. These figures, however, still lag behind those described in other European countries, even if a catching up effect is noticeable. While the mean standardized rate of iAAA repair was 5.5 per 100,000 inhabitants ≥ 50 years old (ranging from 3.6 in 2000-2004, 5.6 in 2005-2009 and 7.1 in 2010-2015), the corresponding mean rates from Swedish Vascular Registry for iAAA repair were 19.1 per 100,000 inhabitants ≥ 50 years old per year in 2000-2004, 22.6 in 2005-2009 and 27.3 in 2010-2014 [17]. The workload for iAAA repair detected is this analysis is thus approximately four times less, being a low rate of iAAA detection the most probable reason for that.

A population screening initiative in men with age ≥ 65 years old with an eligible population of about 900 males that took place in Portugal[18] yielded a prevalence of 2.1%, that is well below of that described in the clinical trials supporting screening programs (prevalence of 4-7%) [19,20]. However, the value found was also superior to the lowest prevalence found in the literature such as National Abdominal Aortic Aneurysm Screening Program established across the United Kingdom that reported 1.18%[21] and the Swedish screening that detected a AAA prevalence of 1.7%[22], showing a putative benefit of a national screening program.

These findings might instead/additionally result from a lower prevalence of the AAA in the Portuguese population. Notably, data from INE shows that the diseases of the circulatory system cause relatively fewer deaths in Portugal than in the European Union-28 (EU-28)[23]. In 2015, the proportion of deaths due to these diseases was 29.8% in Portugal, and 36.7% in the EU-28. In contrast, in Portugal, there are relatively more deaths due to diseases of the respiratory system (in 2015, 12.4% of deaths in Portugal and 8.5% of deaths in the EU-28) and, especially, due to diabetes mellitus (4.0% in Portugal vs. 2.3% in the EU-28 in 2015). In the population ≥ 15 years-old, 19.58% were smokers in 2005 and 19.85% were smokers in 2014 (INE reports). This hypothesis warrants further investigation since diabetes mellitus is a well-known protective factor for AAA presence and progression [24]. Worth of note is that important AAA events may be reduced in patients with diabetes who are prescribed metformin, but not those with diabetes receiving other treatments, something that could not be addressed in this paper [25].

Along with the increased repair, mortality associated with repair has been consistently decreasing. Both decrease open repair and increased endovascular repair contributed to decrease in mortality and hospital mortality. The lowest hospital mortality associated with iAAA repair was registered in 2010-2015 corresponding to 5.1±1.9%. This value is still above than what was reported for the international Vascunet Database, where iAAA peri-operative mortality was described at 2.0-5.0% for all repairs, 1.8-5.5% for open repair and 0.3-3.0% for EVAR [26]. A greater concern is open surgery, where an increasing in operative mortality was verified in the last period, probably due to the selection of cases to fit EVAR, resorting EVAR non-suitable patients to open repair. Patients with violations of the EVAR instructions for use might perform worse in terms of short-term perioperative mortality [27] when submitted to open surgery. As to EVAR, the recent decreasing in operative mortality suggests that the "learning curve", of the procedure itself and the patients' selection (at first more unfit patients or those that fall outside the indications for use), that potentially impacted the initial mortality has been overcome.

Two subgroups of patients presented higher hospital mortality rates – those that are ≥ 75 years old and females. Despite presenting a downward trend, patients ≥ 75 years old presented higher mortality associated with both OS and EVAR. Notwithstanding, in the last period, differences between EVAR in patients ≥ 75 years old and in patients <75 years old were minimal and the improvements in the hospital mortality were due to EVAR as mortality related to open repair actually increased in the last period in

these subgroups of patients. This is in favour of a survival benefit for older patients treated with EVAR, as previously demonstrated [10]. Recent European Society for Vascular Surgery AAA guidelines reporting on recent large population based registry studies from Europe and the USA also point out the sustained increased utilization of EVAR with a continued decrease in mortality and morbidity, despite older and more comorbid patients being treated by EVAR [2,3].
The increased mortality detected in female patients compared to male patients was reported before in a systematic review and meta-analysis [28], with a pooled 30-day mortality of 2.3% after EVAR and 5.4% after OS, indicating that the management of AAA in women needs critical improvement.
It has recently been shown that after EVAR, hospital volume is minimally associated with perioperative mortality, while after open AAA repair, surgeon and hospital volume are both strongly associated with mortality [29]. Centres with a higher number of EVARs per year presented an unexpected positive association with EVAR mortality, possibly due to broader case selection for EVAR, with more severely diseased patients. Contrarily, small volume centres might be performing more straight forward cases, transferring complex cases to high-volume hospitals. This hypothesis deserves further investigation as this data cannot address directly the question.

Limitations
The NHS administrative database used in this paper is not of a prospective nature. Instead, it is a retrospective registry with reimbursement purposes fed by independent trained physicians, representing a well-defined image of the real-world situation. This database covers all hospitalizations occurring in public hospitals, but not in private hospitals. However, the number of repairs performed outside the public hospitals is <10% of all iAAA repairs, due to elevated costs associated with AAA treatment in the private setting. These cases are more often non-complex and presumably associated to equal or better outcomes. Besides demographic data, the databased did not capture some clinical information that could be relevant (such as the anatomy of the aneurysm). To overcome this limitation a hard outcome, mortality, was chosen for being less susceptible to miscoding. The code for EVAR was only created in October 2000 and it is possible that it took some time to be used, resulting in underestimation of AAA repair (mainly in the first part of the period under analysis); this implies that the increased repair might have been even more pronounced. Only the first admissions were considered, where the episode had both a primary diagnose code for AAA associated with a repair code corresponding to open or endovascular surgery. For that reason, readmissions for reinterventions such as endoleak repair were excluded in this analysis and our numbers do not reflect reinterventions but primary repairs only. Furthermore, ICD data was used rather than patient-level data which precluded adjustment of the results for the patients' co-morbidities and medication. Finally, the present data cannot tell if the changes seen during this time period had any effect on total aneurysm-related mortality as no data on ruptures is presented.

Conclusion

In Portugal, the repair rate of iAAA continues to grow and an inflection point is not apparent yet. These figures lag behind those described in other European countries but a catching up effect is noticeable. The low number of iAAA repairs relates to a low detection rate, and a lower prevalence of AAA due to a high prevalence of diabetes cannot be discarded and warrants further investigation. This is happening along with decreased repair mortality mainly due to the increased use of EVAR, even if centres with a higher number of EVARs per year presented an unexpected positive association with EVAR mortality. Despite recent improvements, operative mortality for iAAA repair is still a matter of concern, justifying planning of the vascular surgical services.

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References

1. Johnston KW, Rutherford RB, Tilson MD, Shah DM, Hollier L, Stanley JC. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. *J Vasc Surg* 1991;**13**:452-8.
2. Wanhainen A, Verzini F, Van Herzeele I, Allaire E, Bown M, Cohnert T, et al. Editor's Choice - European Society for Vascular Surgery (ESVS) 2019 Clinical Practice Guidelines on the Management of Abdominal Aorto-iliac Artery Aneurysms. *Eur J Vasc Endovasc Surg* 2019;**57**:8-93.
3. Chaikof EL, Dalman RL, Eskandari MK, Jackson BM, Lee WA, Mansour MA, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg* 2018;**67**:2-77 e2.
4. Sidloff D, Stather P, Dattani N, Bown M, Thompson J, Sayers R, et al. Aneurysm global epidemiology study: public health measures can further reduce abdominal aortic aneurysm mortality. *Circulation* 2014;**129**:747-53.
5. Stather PW, Sidloff DA, Rhema IA, Choke E, Bown MJ, Sayers RD. A review of current reporting of abdominal aortic aneurysm mortality and prevalence in the literature. *Eur J Vasc Endovasc Surg* 2014;**47**:240-2.
6. Castro-Ferreira R, Lachat M, Schneider PA, Freitas A, Leite-Moreira A, Sampaio SM. Disparities in Contemporary Treatment Rates of Abdominal Aortic Aneurysms Across Western Countries. *Eur J Vasc Endovasc Surg* 2019;**58**:200-5.
7. Semmens JB, Norman PE, Lawrence-Brown MM, Bass AJ, Holman CD. Population-based record linkage study of the incidence of abdominal aortic aneurysm in Western Australia in 1985-1994. *Br J Surg* 1998;**85**:648-52.
8. Norman PE, Spilsbury K, Semmens JB. Falling rates of hospitalization and mortality from abdominal aortic aneurysms in Australia. *J Vasc Surg* 2011;**53**:274-7.
9. Dua A, Kuy S, Lee CJ, Upchurch GR, Jr., Desai SS. Epidemiology of aortic aneurysm repair in the United States from 2000 to 2010. *J Vasc Surg* 2014;**59**:1512-7.
10. O'Donnell TFX, Wade JE, Liang P, Li C, Swerdlow NJ, DeMartino RR, et al. Endovascular aneurysm repair in patients over 75 is associated with excellent 5-year survival, which suggests benefit from expanded screening into this cohort. *J Vasc Surg* 2019;**69**:728-37.
11. Choke E, Vijaynagar B, Thompson J, Nasim A, Bown MJ, Sayers RD. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? *Circulation* 2012;**125**:1617-25.
12. Nelissen BG, Herwaarden JA, Pasterkamp G, Moll FL, Vaartjes I. Shifting abdominal aortic aneurysm mortality trends in The Netherlands. *J Vasc Surg* 2015;**61**:642-7 e2.
13. Jarvis MJ. Monitoring cigarette smoking prevalence in Britain in a timely fashion. *Addiction* 2003;**98**:1569-74.
14. Simões J AG, Fronteira I, Hernández-Quevedo C. Portugal: Health system review. *Health Systems in Transition* 2017;**19(2):**:1–184.
15. Sandiford P, Mosquera D, Bramley D. Trends in incidence and mortality from abdominal aortic aneurysm in New Zealand. *Br J Surg* 2011;**98**:645-51.
16. Bray F, Guilloux A, Sankila R, Parkin DM. Practical implications of imposing a new world standard population. *Cancer Causes Control* 2002;**13**:175-82.
17. Lilja F, Mani K, Wanhainen A. Editor's Choice - Trend-break in Abdominal Aortic Aneurysm Repair With Decreasing Surgical Workload. *Eur J Vasc Endovasc Surg* 2017;**53**:811-9.
18. Castro-Ferreira R, Barreira R, Mendes P, Couto P, Peixoto F, Aguiar M, et al. First Population-Based Screening of Abdominal Aortic Aneurysm in Portugal. *Ann Vasc Surg* 2019;
19. Ashton HA, Buxton MJ, Day NE, Kim LG, Marteau TM, Scott RA, et al. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. *Lancet* 2002;**360**:1531-9.
20. Norman PE, Jamrozik K, Lawrence-Brown MM, Le MT, Spencer CA, Tuohy RJ, et al. Population based randomised controlled trial on impact of screening on mortality from abdominal aortic aneurysm. *BMJ* 2004;**329**:1259.
21. Benson RA, Poole R, Murray S, Moxey P, Loftus IM. Screening results from a large United Kingdom abdominal aortic aneurysm screening center in the context of optimizing United Kingdom National Abdominal Aortic Aneurysm Screening Programme protocols. *J Vasc Surg* 2016;**63**:301-4.

22. Svensjo S, Mani K, Bjorck M, Lundkvist J, Wanhainen A. Screening for abdominal aortic aneurysm in 65-year-old men remains cost-effective with contemporary epidemiology and management. *Eur J Vasc Endovasc Surg* 2014;**47**:357-65.
23. Estatística INd. Causas de morte - 2017. 2019;
24. De Rango P, Farchioni L, Fiorucci B, Lenti M. Diabetes and abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2014;**47**:243-61.
25. Golledge J, Morris DR, Pinchbeck J, Rowbotham S, Jenkins J, Bourke M, et al. Editor's Choice - Metformin Prescription is Associated with a Reduction in the Combined Incidence of Surgical Repair and Rupture Related Mortality in Patients with Abdominal Aortic Aneurysm. *Eur J Vasc Endovasc Surg* 2019;**57**:94-101.
26. Mani K, Venermo M, Beiles B, Menyhei G, Altreuther M, Loftus I, et al. Regional Differences in Case Mix and Peri-operative Outcome After Elective Abdominal Aortic Aneurysm Repair in the Vascunet Database. *Eur J Vasc Endovasc Surg* 2015;**49**:646-52.
27. Charbonneau P, Hongku K, Herman CR, Habib M, Girsowicz E, Doonan RJ, et al. Long-term survival after endovascular and open repair in patients with anatomy outside instructions for use criteria for endovascular aneurysm repair. *J Vasc Surg* 2019;
28. Ulug P, Sweeting MJ, von Allmen RS, Thompson SG, Powell JT, collaborators S. Morphological suitability for endovascular repair, non-intervention rates, and operative mortality in women and men assessed for intact abdominal aortic aneurysm repair: systematic reviews with meta-analysis. *Lancet* 2017;**389**:2482-91.
29. Zettervall SL, Schermerhorn ML, Soden PA, McCallum JC, Shean KE, Deery SE, et al. The effect of surgeon and hospital volume on mortality after open and endovascular repair of abdominal aortic aneurysms. *J Vasc Surg* 2017;**65**:626-34.

Table 1. Patients characteristics and repair rate of intact abdominal aortic aneurysm (iAAA) across the three time periods.

	2000-2015	2000-2004	2005-2009	2010-2015	trend	p
All cases (n)	3991	715	1226	2050	-	-
Cases/year (n)	249	143	245	342	-	
EVAR, n (%)	1462 (36.6)	-	270 (22.0)	1192 (58.1)	↗	<0.001
Men, n (%)	3722 (93.2)	673 (94.1)	1134 (93.2)	1915 (93.4)	→→	0.771
≥ 75, n (%)	1574	217 (30.0)	483 (39.4)	874 (42.6)	↗↗	<0.001
≥ 80, n (%)	681	74 (10.3)	186 (15.2)	421 (20.5)	↗↗	<0.001
Standardized rate per 100,000/y, mean (SD)	5.52 (1.65)	3.57 (0.63)	5.55 (0.40)	7.13 (0.90)	↗↗	<0.001
EVAR, standardized rate per 100,000/y, mean (SD)	1.82 (1.86)	0	1.49 (1.09)	3.91 (0.64)	↗	<0.001
OR, standardized rate per 100,000/y, mean (SD)	3.70 (0.83)	3.57 (0.63)	4.41 (0.73)	3.22 (0.69)	↗↘	<0.001
Men, standardized rate per 100,000/y, mean (SD)	11.86 (3.58)	7.66 (1.34)	11.86 (0.87)	15.36 (2.00)	↗↗	<0.001
Women, standardized rate per 100,000/y, mean (SD)	0.59 (0.20)	0.36 (0.16)	0.65 (0.14)	0.72 (0.09)	↗↗	<0.001
< 75 yo, standardized rate per 100,000/y, mean (SD)	4.56 (1.27)	3.15 (0.53)	4.28 (0.31)	5.77 (0.91)	↗↗	<0.001
≥ 75 yo, standardized rate per 100,000/y, mean (SD)	11.39 (4.18)	6.10 (1.31)	11.82 (1.25)	15.46 (1.30)	↗↗	<0.001

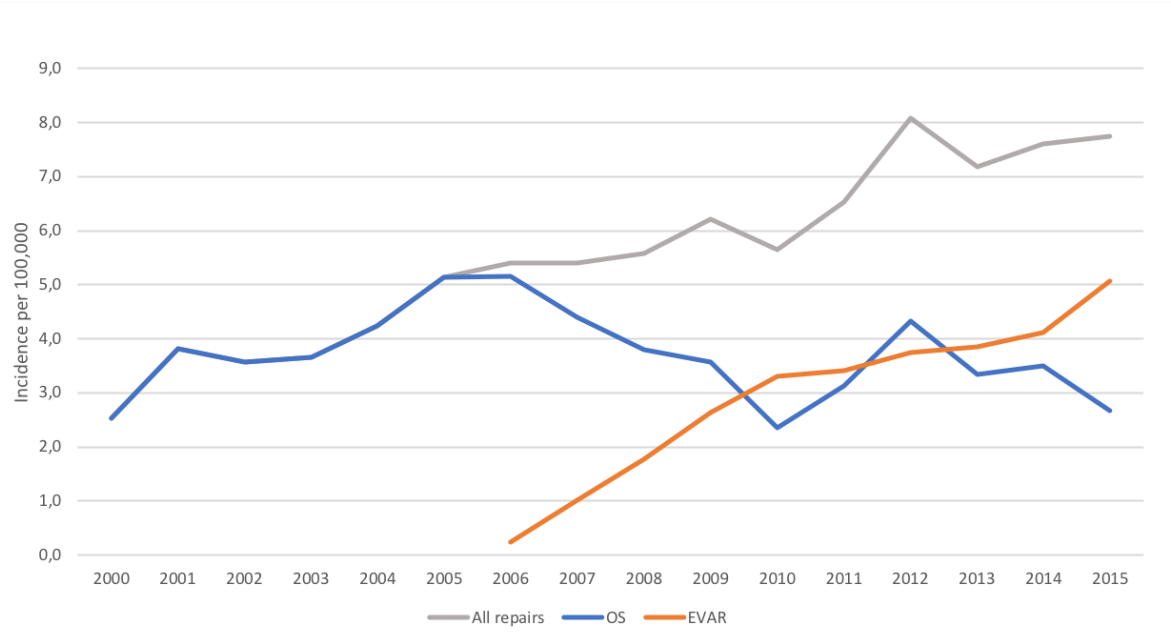
Table 2. Age-standardized rates of mortality per 100,000 inhabitants ≥ 50 years old per year, after repair of intact AAA (iAAA) across three time periods.

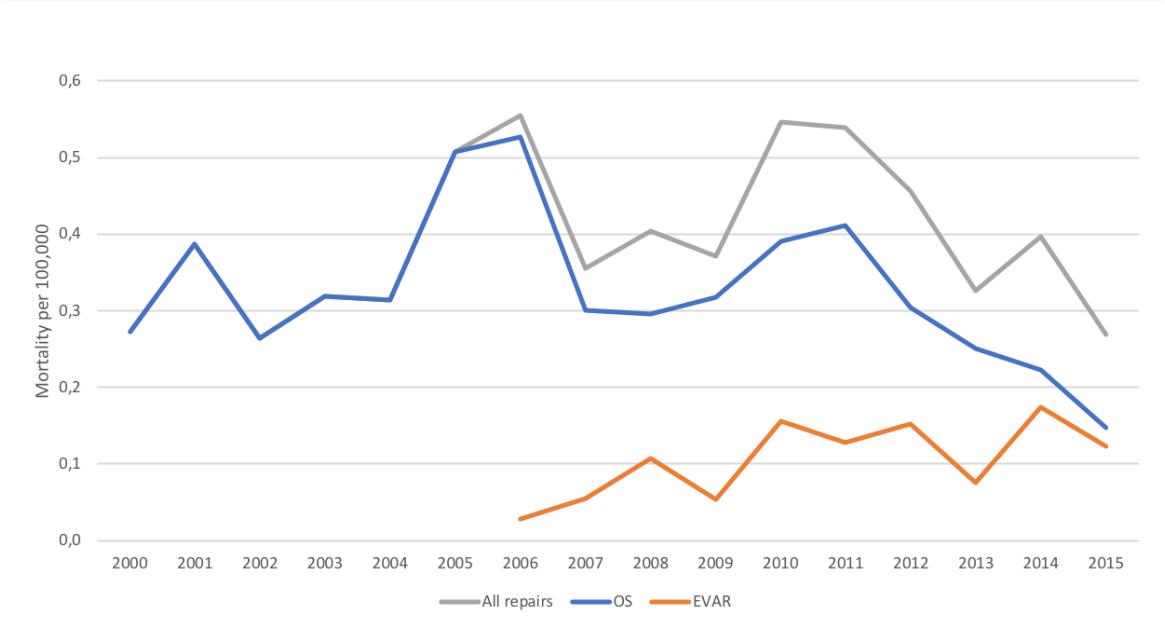
	2000-2015	2000-2004	2005-2009	2010-2015	trend	p
All deaths (n)	233	53	80	100	-	-
Deaths/year (n)	14	11	16	17	-	-
Rate per 100,000/y, mean (SD)	0.39 (0.10)	0.31 (0.05)	0.44 (0.09)	0.42 (0.11)	↗→	<0.001
Standardized rate per 100,000/y, mean (SD)	0.30 (0.07)	0.25 (0.04)	0.34 (0.07)	0.32 (0.06)	↗→	<0.001
EVAR, standardized rate per 100,000/y, mean (SD)	0.05 (0.05)	0	0.03 (0.03)	0.10 (0.03)	↗	<0.001
OS, standardized rate per 100,000/y, mean (SD)	0.26 (0.07)	0.25 (0.04)	0.31 (0.09)	0.22 (0.06)	↗↘	<0.001
Men, standardized rate per 100,000/y, mean (SD)	0.64 (0.16)	0.54 (0.07)	0.72 (0.14)	0.67 (0.20)	↗→	<0.001
Women, standardized rate per 100,000/y, mean (SD)	0.05 (0.03)	0.03 (0.02)	0.05 (0.03)	0.06 (0.04)	→	0.279
< 75 yo, standardized rate per 100,000/y, mean (SD)	0.22 (0.05)	0.20 (0.05)	0.24 (0.07)	0.21 (0.02)	↗↘	0.002
≥ 75 yo, standardized rate per 100,000/y, mean (SD)	0.83 (0.41)	0.56 (0.26)	0.95 (0.40)	0.96 (0.46)	↗→	<0.001

Table 3. In-hospital mortality after repair of intact AAA (iAAA) across three time periods. Percentages are depicted as mean ± standard deviation of yearly percentages of the period.

	2000-2004	2005-2009	2010-2015	trend	p
All repairs, %	7.5±1.3	6.6±1.6	5.1±1.9	↘↘	<0.001
EVAR, %	-	4.0±3.5	2.8±0.9	↘	<0.001
OS, %	7.5±1.3	7.4±1.1	8.3±3.7	→↗	<0.001
Men, %	7.3±1.5	6.4±1.5	4.9±2.2	↘↘	<0.001
Women, %	10.9±6.4	10.2±5.4	7.6±4.1	↘↘	0.008
< 75 yo, %	6.9±2.3	5.5±1.8	4.0±0.7	↘↘	0.002
≥ 75 yo, %	8.9±2.6	8.3±3.7	6.4±3.5	↘↘	<0.001

Figure 1. Age-standardized incidence of repair of iAAA (A) and of deaths (B) over time per 100,000 residents ≥ 50 years of age. Percentage of hospital mortality among different types of iAAA repair over time (C).





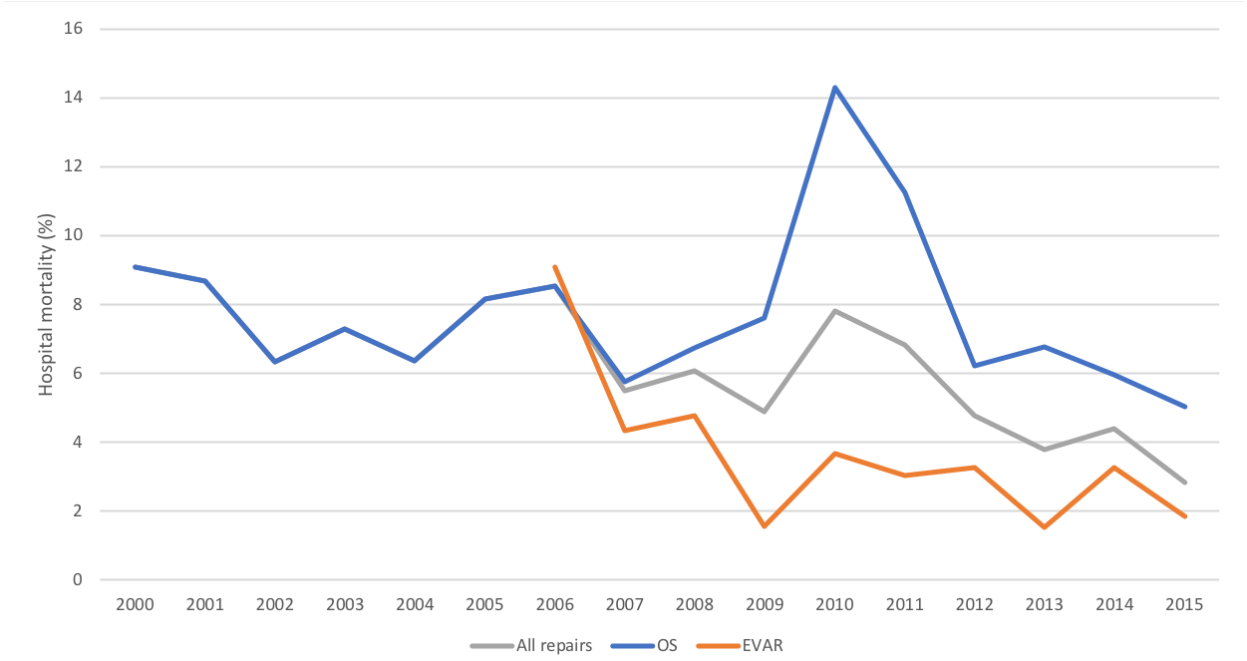

16
14
12
10
8
6
4
2
0
Hospital mortality (%)
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
All repairs
OS
EVAR

Figure 2. Age-standardized incidence of open (OS) and endovascular (EVAR) repair of iAAA over time per 100,000 inhabitants ≥ 50 years of age in two age groups of patients < 75 (A) and ≥ 75 years old (B).

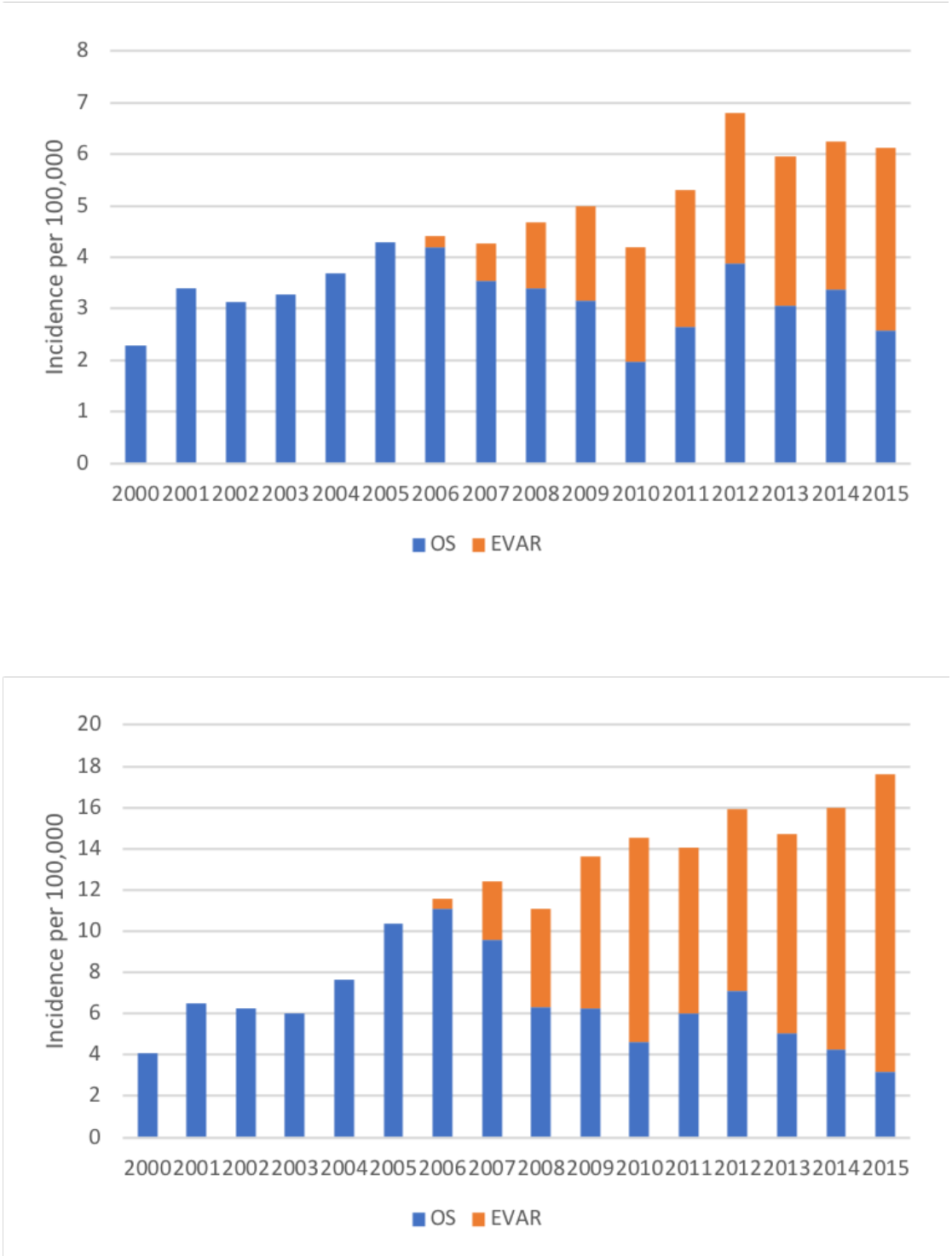
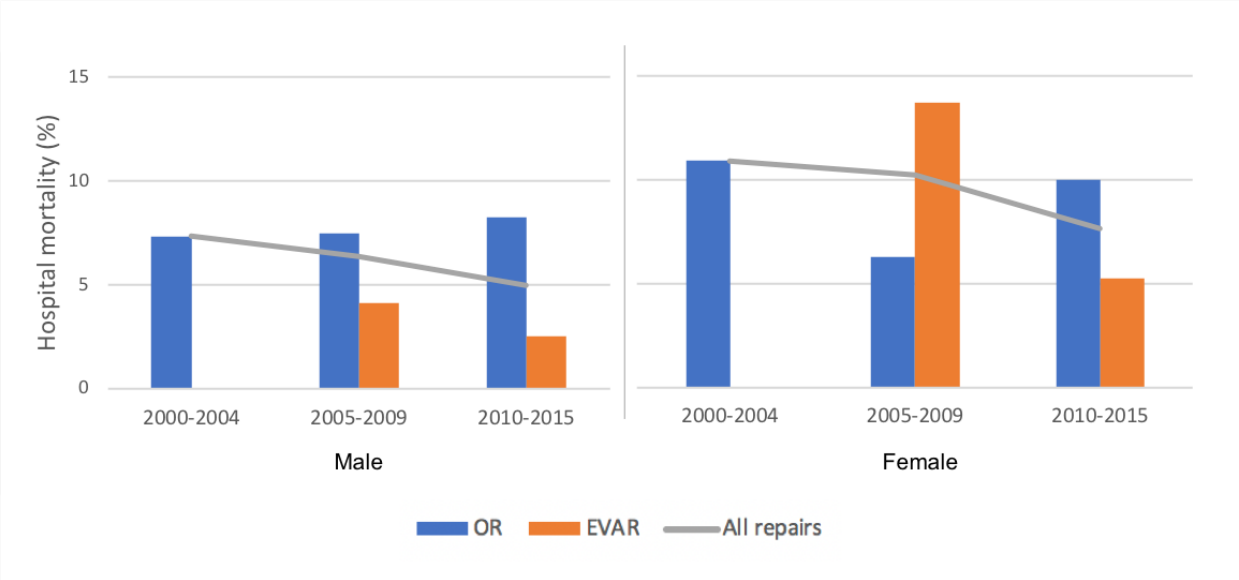


Figure 3. Percentage of hospital mortality among different types of iAAA repair per gender (A) and per age bands bands <75 and ≥ 75 years old (B) over time.



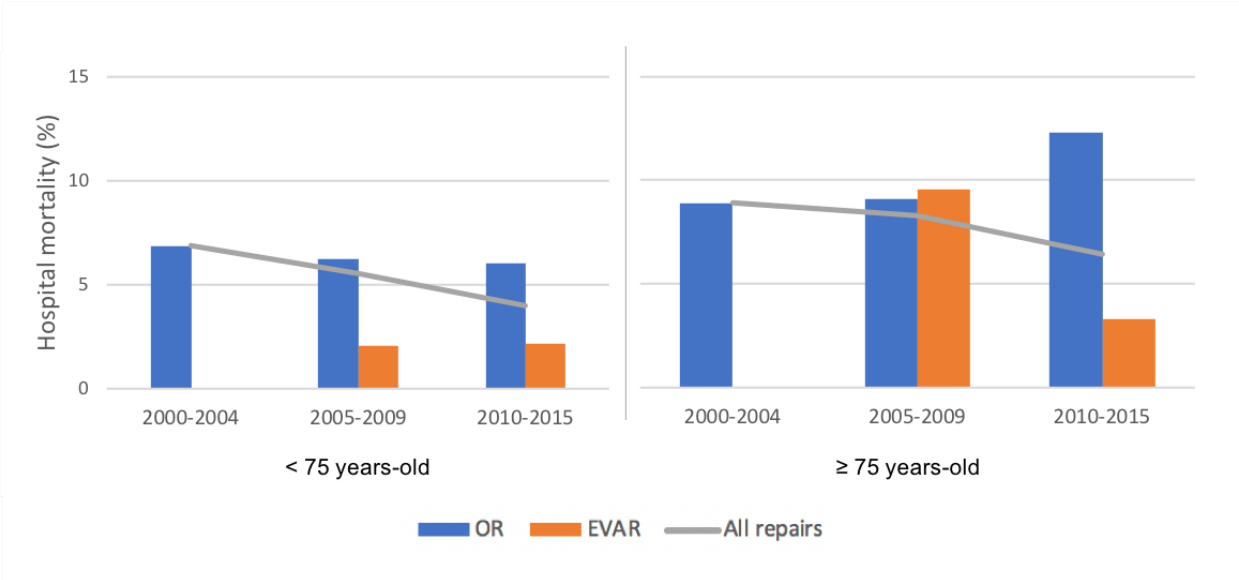


Figure 4. Association between iAAA repair rates (cases per year) in a center and repair mortality: all repairs (A), EVAR (B) and OS (C).

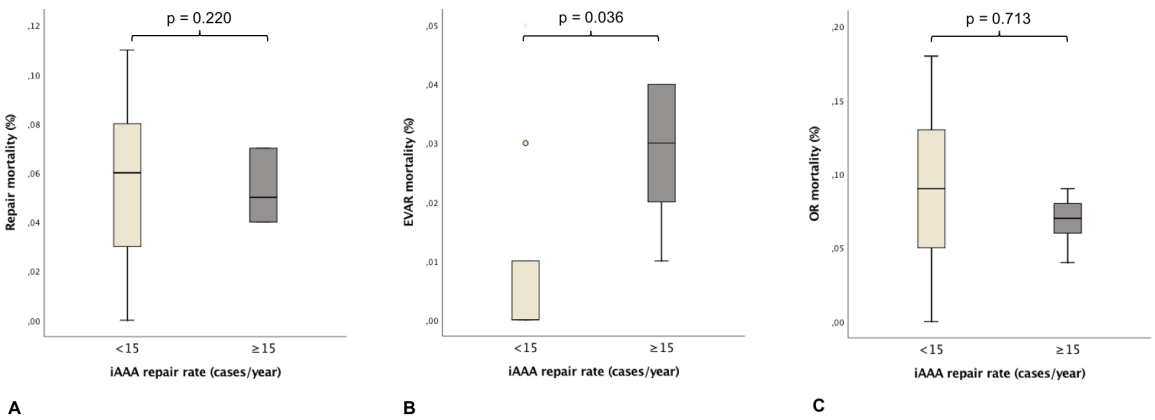
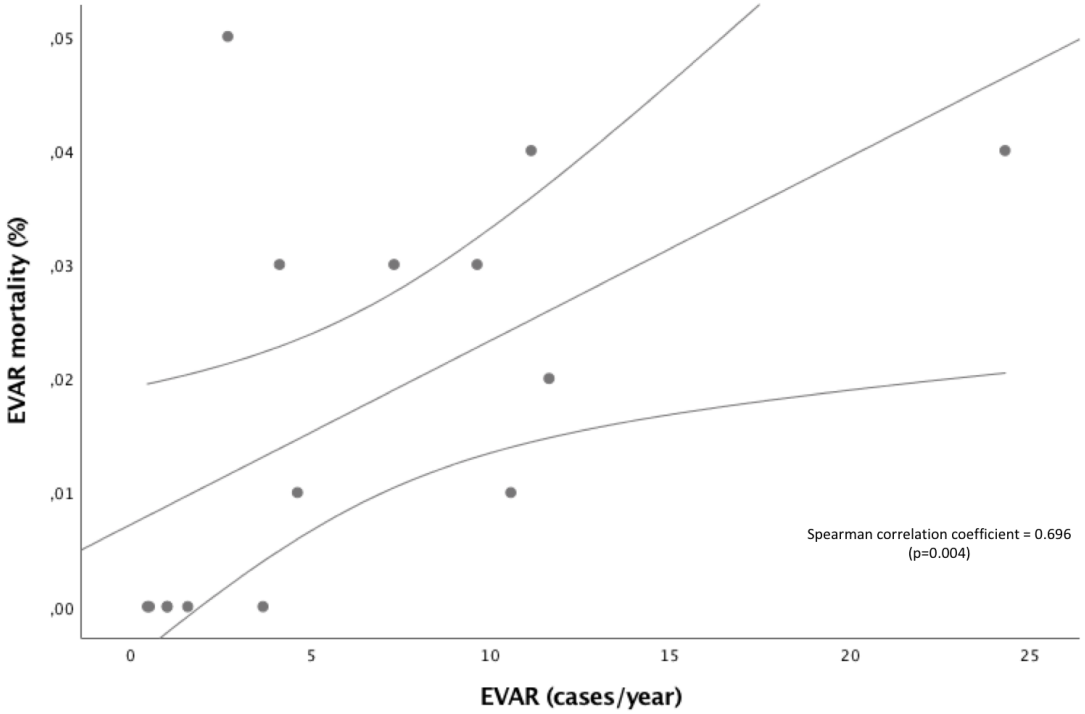


Figure 5. Correlation between the rate of EVAR per year in a center and EVAR mortality.



Supplemental Table 1. Details of the Portuguese health system.

Portuguese health system	The Portuguese health system is characterized by three co-existing and overlapping systems:
a) National Health System (NHS)	All residents in Portugal have access to health care provided by the NHS, financed mainly through taxation.
b) Health subsystems	Special public and private insurance schemes for certain professions or companies.
c) Private voluntary health insurance	Mainly fulfil a supplementary role to the NHS rather than providing a global alternative to it. Currently, the private sector mainly provides diagnostic, therapeutic and dental services, as well as some ambulatory consultations, rehabilitation and hospitalization.

Supplemental Table 2. Details of the hospital morbidity database formerly designated as Diagnosis-Related Groups (DRGs) database.

Former name	Diagnosis-Related Groups (DRGs) database
Provider	Portuguese Central Health System Administration (CHSA)
Content	Registration of all hospitalizations occurring in public hospitals in mainland Portugal.
Registry	Retrospective consecutive case entry.
Coding system	International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), Coding Clinic and national Consensus
Who undertakes coding	Clinical codification at the hospital-level is undertaken by independent physicians with specific preparation for coding.
External audits	Performed by the CHSA in order to verify the accuracy of the clinical coding performed at the NHS hospitals. The episode presents no disagreement when it respects International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), Coding Clinic and national Consensus. The last one was in 2010 when a total of 4,510 episodes were audited in 44 hospital institutions. The results indicate that most disagreements among diagnoses, procedures and other administrative and demographic variables did not affect the respective grouping in the DRGs (disagreement mean:13.7%, 26.5% maximum and 2.1% minimum).
Main aim	Reimbursement is attributed to hospitals according to number of hospitalizations, adjusted by their case-mix index.

Supplemental Table 3. ICD-9-CM diagnostic and procedure codes suitable but not specific for intact abdominal aortic aneurysm.

Diagnostic Codes	441.4 Abdominal aneurysm without mention of rupture 441.9 Aortic aneurysm of unspecified site without mention of rupture
Procedure Codes	
Open repair	38.34 Resection of vessel with anastomosis, aorta, abdominal 38.40 Resection of vessel with replacement, unspecified site 38.44 Resection of vessel with replacement, aorta, abdominal 38.60 Other excision of vessel, unspecified site 38.64 Other excision of vessel, aorta, abdominal 39.25 Aorta-iliac-femoral bypass 39.51 Clipping of aneurysm 39.52 Other repair of aneurysm 39.56 Repair of blood vessel with tissue patch graft 39.57 Repair of blood vessel with synthetic patch graft
Endovascular repair	39.71 Endovascular implantation of graft in abdominal aorta 39.79 Other endovascular repair (of aneurysm) of other vessels

CHAPTER 4 NATIONWIDE ANALYSIS OF RUPTURED ABDOMINAL AORTIC ANEURYSM IN PORTUGAL (2000-2015)

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What this study adds

The overall mortality of ruptured abdominal aortic aneurysm (rAAA) depends both on prevalence of disease, as well as mortality before and after hospital admission. In the current paper, the mortality related to rAAA was studied in Portugal based on national registries. Admission, repair and mortality due to rAAA seem to have reached a peak and have been recently decreasing (2010-2015). At the same time, a gradual increased adoption of endovascular repair for ruptures was verified. Mortality outside the hospital is still a matter of concern, warranting further planning of streamlined transfer networks and vascular surgical departments.

Abstract

Objective: Ruptured abdominal aortic aneurysm (rAAA) is a lethal condition that requires acute repair to prevent death. This analysis aims to assess the nationwide trends in rAAA admission, repair and mortality in a country without national screening for AAA, Portugal.
Methods: rAAA registered in the hospital administrative database of the National Health Service, as well as all nationally registered deaths due to rAAA based on death certificate data were analyzed. Three-time periods (2000-2004, 2005-2009 and 2010-2015) were compared in patients ≥50 years-old to assess the variations over time.
Results: A total of 2275 patients ≥50 years-old with rAAA were identified in the two databases from 2000 to 2015. The age-standardized incidence of rAAA was 2.78±0.24/100,000/year in 2000-2004, 3.17±0.39/100,000/year in 2005-2009 and 3.21±0.28/100,000/year in 2010-2015 (p<.001).
When comparing the time periods 2000-2004 to 2005-2009, the age-standardized rate of admission (n=1460) increased from 1.57±0.25/100,000/year to 2.24±0.32/100,000/year (p<.001). The operative mortality rates decreased during this time period (from 55.3±4.7% to 48.8±4.7%, p<.001). In 2010-2015, the age-standardized rate of admissions due to rAAA decreased (1.98±0.22/100,000/year). Operative mortality remained stable (48.9±6.2%).
The rate of patients that died outside the hospitals decreased from the first to the second period (1.21±0.10/100,000/year and 0.93±0.29/100,000/year, respectively) but had a later increase (1.14±0.22/100,000/year). This resulted in a higher overall rAAA-related mortality in Portugal in the third period (2.20±0.18/100,000/year, 2.21±0.27/100,000/year and 2.26±0.26/100,000/year in 2000-2004, 2005-2009 and 2010-2015, respectively, p<.001).
Conclusion: Overall, the incidence of rAAA in Portugal is stable over the past 10 years. The rates of admission, repair and mortality due to rAAA repair seems to have reached an inflection point and are now decreasing. Mortality outside the hospital is still a matter of concern, warranting further planning of streamlined transfer networks and vascular surgical departments.

Introduction

Despite recent technical advances, ruptured abdominal aortic aneurysm (rAAA) still accounts for significant mortality [1]. Rupture is often the presenting feature, and without emergent surgery, mortality is close to 100%. Overall mortality after repair is estimated at 28.8% (27.9-29.8), 32.1% (31.0-33.2) for open surgical repair (OSR) and 17.9% (16.3-19.6) for endovascular repair (EVAR) [2]. Being mostly asymptomatic before rupture, its detection relies on incidental findings on image studies performed for other reasons. Population screening for abdominal aortic aneurysm (AAA) has been shown to effectively reduce rAAA mortality, and national screening programs for AAA are currently implemented in Sweden, the United Kingdom, and the United States [3-5]. An association between screening programs and decrease mortality due to AAA has been demonstrated [3,6-8], but its efficacy might depend on certain conditions like the subpopulation screened, the healthcare and reimbursement systems [9,10].

Epidemiological studies from United Kingdom, Finland and Sweden suggest that a decrease in the prevalence of the disease itself may have contributed to the drop in mortality rates to AAA even in absence of screening [11-13]. This decrease in the prevalence of the disease has been mainly credited to reduced smoking rate and change in cardiovascular risk factors as well as more widespread use of cardioprotective drugs [13-16]. The potential effect of EVAR on rAAA mortality remains under debate. While randomized controlled trails have not shown short-term survival benefit of EVAR for ruptures[17], a possible advantage might come from the fact that older patients are more often treated with EVAR in the endovascular era resulting in a reduction in turn-down rate[12], with better short-term survival than after OSR in registry studies[18].

Whilst most of the studies of AAA epidemiology are performed in the Northern European and US setting, data suggest that disease burden related to AAA has significant geographical variations[14]. There is a paucity of data regarding the epidemiological transition in South of Europe, where factors affecting AAA prevalence such as smoking habits vary compared to Northern Europe[19].

At country-level, a comprehensive national assessment of trends in AAA epidemiology may identify the need for further protocoled approaches and give insight into the expected health care costs associated with the treatment of this disease. The objective of this analysis is to assess the trends in rAAA rates of admission, repair and mortality in Portugal.

Methods

Study design

All rAAA admissions and interventions performed during the period 2000 to 2015 were retrospectively identified in a National Health Service (SNS) administrative database formerly designated as Diagnosis-Related Groups (DRGs) database. This database was provided by the Portuguese Central Health System Administration (ACSS) and contains a registry of all hospitalizations (retrospective consecutive case entry) occurring in mainland Portugal public hospitals, where all rAAA repairs are performed. This is a mandatory registry for hospital reimbursement. All Portuguese residents have access to health care provided by the SNS, financed mainly through taxation. Private health care providers mainly fulfil a supplementary role to the SNS rather than providing a global alternative to it, providing mainly diagnostic, therapeutic and dental services, as well as some ambulatory consultations, rehabilitation and hospitalization [20].Over the period of the study, fourteen centers provided rAAA repair, mainly located in the North and Center of the coastal Western regions of Portugal.

Each registered hospital episode includes information about diagnoses and medical or surgical procedures, both coded using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). External audits are performed by the ACSS to verify the accuracy of the clinical coding performed at SNS hospitals. The episode presents no disconformity when it respects ICD-9-CM, Coding Clinic and National Consensus. The last one was in 2010 when a total of 4,510 episodes were audited in 44 hospital institutions. The results indicate that nonconformities among diagnoses, procedures and other administrative and demographic variables in general did not affect the respective DRGs grouping (nonconformities mean: 13.7%, 26.5% maximum, 2.1% minimum) [21]. The manuscript follows the RECORD statement[22], extended from the STROBE statement [23].

Data collection

All admissions considered to have a primary diagnosis with specific ICD-9-CM code for rAAA (Supplemental Table I) were included in this analysis. The primary diagnosis of an episode represents the main condition investigated or treated during that hospital stay. Aortic aneurysms at unspecified site were

included, in line with other studies [13,24], to increase the chance that all abdominal lesions were captured in the analysis. Thoracoabdominal aneurysms were excluded. The repair rate was calculated based on the episodes of care with the above-mentioned ICD-9-CM disease codes plus the ICD-9-CM procedure codes suitable for AAA repair (Supplemental Table I). Due to lack of the specific ICD9-CM code for EVAR before 2000, the first period of the analysis was set to null to avoid the early years of the EVAR code use.
Admissions with a primary diagnosis of rAAA that lacked an ICD-9-CM procedure code suitable for AAA repair that were discharged alive were confirmed as transfers between hospitals. Admissions with a primary diagnosis of rAAA that lacked an ICD-9-CM procedure that were discharged as deceased were considered as patients that died at arrival to the hospital prior to repair attempt, or that were turned down for surgery.
The number of admissions with repair in which the patient died divided by the number of all admissions with repair was considered as operative mortality due to rAAA. All hospital deaths for patients with a rAAA diagnosis were considered as rAAA-related deaths, irrespective to the direct cause of death. The hospital mortality, however, is an underestimation of the national rAAA-related mortality as it only includes patients presenting alive to a hospital. To overcome this limitation, death certificate with rAAA-specific mortality was provided from the National Institute of Statistics (INE) from 2000 to 2015. INE database used ICD-9-CM classification from 2000 to 2001 and the Tenth Revision (ICD-10) thereafter. Both databases present episode-related data retrievable through ICD codes. In INE data, the episode is death and includes deaths outside the hospital as well as those certified at the hospitals. In SNS administrative database, each episode refers to a hospitalization. The resident population in each year under analysis was also provided by the INE from 2000 to 2015. Resident population per year was used to calculate the rate of rAAA admission, repair and mortality. In both databases, individuals <50 years of age were excluded. Gender subgroups were provided only for patients presenting to the hospital.

Statistical Analysis

Data were calculated overall and for three periods (2000-2004, 2005-2009 and 2010-2015) to assess variations over time. Besides the frequency of episodes/cases per year. gender and age were available in the SNS database; age but not gender was available in INE database. There were no missing cases. Subgroup analysis was performed to investigate trend differences based on sex and age (< or ≥75 years-old) as previously performed [13].
To account for changes in the age structure of the population over time, age direct standardization was performed (using the *World Health Organization* world standard population[25]). All rates of admission and mortality were presented as age-standardized data. Age-standardized rate of admission refers to all rAAA that arrived at the hospital for one year, including those that die without an attempt to repair plus those with an attempt to repair, divided by the nationwide population during that year. Age-standardized incidence refers to all know cases of rAAA for one year, that is, all rAAA that arrive to the hospital (as defined above) plus all rAAA cases that die without reaching the hospital, divided by the nationwide population during that year.
Continuous variables are presented as mean ± standard deviation, after histogram inspection. Proportions were compared using the chi-square test. Changes in proportions over time were assessed using the chi-square test for trend. Normally distributed data were compared using one-way ANOVA. Bonferroni correction was used to adjust for multiple testing. Furthermore, to adjust for subgroup analysis p<.01 was considered significant. All statistical analysis was performed using SPSS for Mac version 24 (SPSS, Armonk, NY: IBM Corp).

Results

A total of 2275 patients ≥50 years-old with rAAA were identified in the two databases from 2000 to 2015 (Figure 1), after removal of the duplicates.

Incidence of rAAA

The age-standardized incidence of rAAA increased from 2000-2004 to 2005-2009, and remained stable after that in 2010-2015 (p<.001) (Table 1 and Figure 2). In the subgroup of patients <75 years-old, the age-standardized incidence declined in the third period (p<.001) while it increased in patients ≥75 years-old (p<.001). A total of four hundred three (18%) cases were <65 years old, i.e. below the recommended age for AAA screening.

rAAA presenting to the hospital

A total of 1460 hospital admissions due to rAAA were identified in patients ≥50 years old (Table 1). The age-standardized admission rate per 100,000 inhabitants increased from 2000-2004 to 2005-2009 and then decreased in 2010-2015 (p<.001) (Figure 2).

Fifty per cent of admitted patients (735) were ≥75 years, while 426 (29%) were ≥80 years. The proportion of patients with ≥75 years-old arriving to hospital presented a marked and significant increase in the third period (p<.001) (Table 1).

rAAA undergoing repair

From all the admissions, the age-standardized rate of non-intervention also followed a rise-fall pattern (Figure 2). Overall, 22±4% of the admitted patients did not undergo repair (Table 1). The proportion was 35.4±17.4% in women, and 19.8±4.0% in men (p<.001). The proportion was also higher in patients ≥75 years versus <75 years-old (27.5±7.6% vs. 15.7±5.6%, p<.001). Over time, the proportion of patients not undergoing repair increased (<.001).

rAAA mortality

Overall, 75.2±4.7% patients having a rAAA died before reaching the hospital or during the hospitalization (Table 2). The rAAA age-standardized mortality was stable between the first and second time period, and increased slightly in the third period (Table 2). In a subgroup analysis, the increase in age-standardized mortality in the third period occurred among ≥75 years-old (Table 2). This increase was primarily related to an increase in rAAA deaths outside the hospital, (Table 2 and Supplemental Figure 1). The age-standardized mortality for patients admitted to hospital (repaired or not) however decreased in the final time period, Table 2.

Operative mortality after rAAA repair decreased from 55±5% in 2000-2004 to 49±5% in 2005-2010 (p<.001) and then a stabilized in 2010-2015 with 49±6% (Supplemental Figure 1). This later stabilization phase corresponded to an increase in operative mortality due to OSR (55±5% to 48±5% to 54±6%, p<.001) compensated by a decrease in operative mortality after EVAR (from 75±24% in the second to 29±11% in the third period, p<.001) (Supplemental Figure 2).

In the subgroup of males, the operative mortality dropped from 55±4% in 2000-2004 to 47±7% in 2005-2009 (p<.001) and remained stable in 2010-2015 (48±6%, p=.034). Operative mortality in female patients did not vary significantly during the time but global figures in females were significantly higher than in males (50±6% vs 53±20%, p<.001) (Figure 3).

The rates of operative mortality in ages <75 years old decreased from 47±6% to 38±6% (p<.001) and then remained constant (38±8%). In the age band ≥ 75 years old, the rates of operative mortality were 65±6%, 61±10% and 61±8% (p<.001). Mortality in the elderly cohort was thus higher than in patients <75 years old (p<.001) (Supplemental Figure 3).

Discussion

In this study, a nationwide evaluation was performed on the incidence, admission, repair and mortality due to rAAA over 16 years. This study found that, overall, the incidence of rAAA in Portugal increased from 2000-2004 to 2005-2009, and stabilised thereafter. The decreased incidence of disease verified in Western countries [11-13,26] is still offset by an increased incidence of the elder population. Overall, the number of rAAA cases per year has increased. Despite an improved perioperative mortality, and an increased repair rate among younger patients presenting with rAAA to hospital, the overall death rate per 100,000 inhabitants has increased over the past years. This is due to the fact that an increasing number of deaths due to rAAA are registered among patients not presenting to hospital.

A direct comparison of the rates of rAAA admission among other reports is made more difficult by the fact that studies tend to report non-standardized rates, representing different subgroups of age and gender. Nevertheless, the rates reported in this study lag behind those described in other reports as previously noted[27]. This might be because a great number of patients do not reach the hospital, due to a lower prevalence of the disease in Portugal or related to external validity of the databases. A population screening initiative in men ≥65 years-old with an eligible population of about 900 males that took place in Portugal [28] yielded a prevalence of 2.1%. In countries where national screening is formally implemented, prevalence rates were as low as 1.2% in England[29] and 1.7% in Sweeden[10]. An increasing number of elective operations, due to increased use of imaging resulting in the incidental detection of AAA [3,6,7], may partly explain the decrease in rAAA incidence. Nevertheless, countries with higher rates of elective AAA

treatment may not have fewer ruptured AAA repairs[27]. In a recent systematic review, the country with the lowest number of elective treatments (Hungary) also has the lowest rate of emergency repairs (0.5 per 100,000) and the country with the highest rate of intact repairs (Germany) was also one of the countries with a higher number of ruptured AAAs (2.7 per 100,000). Further reduction of rAAA mortality might require the identification and surgical treatment of more asymptomatic patients before AAA rupture through screening, although formal studies in the Portuguese population are needed to address its benefit.

One might hypothesize that the widespread dissemination of the endovascular technology in older patients in the emergent setting might have contributed to improved survival among patients undergoing repair [30], nevertheless further evidence is needed to address that. In a meta-analysis that included twenty-four adjusted observational studies and 4 RCTs enrolling a total of 56,826 patients with rAAA[32], pooled analysis demonstrated a statistically significant 49% reduction in perioperative all-cause mortality with EVAR relative to OSR in observational studies and no statistically significant difference between EVAR and OSR in RCTs. Like in other observational studies, the use of EVAR was associated to lower operative mortality both in male and female patients in the current cohort. Interestingly, during the introduction of EVAR for rAAA in Portugal, the mortality of this procedure was excessively high. It is possible that this corresponds to wider use of the new minimally invasive technique mainly in patients that were unable to tolerate OSR due to comorbidities, or due to the learning curve of the new technology, as in cases of EVAR, a significant decrease both in the in-hospital mortality and length of stay with experience accumulated at the hospital level was observed for EVAR [33]. Over time, however, operative mortality of EVAR for rAAA has stabilized at a low level, while the operative mortality after OSR has been increasing in the third period. Data from the American College of Surgeons' National Quality Improvement Program database showed that for all rAAAs with hypotension, OSR had increased mortality compared to EVAR ($p<.0004$)[34]. It is possible that higher mortality with OSR was due to selection bias where patients with hypotension or more complex are more prone to undergo OSR. Despite the widespread use of endovascular technology in elective cases, EVAR was used only in 11% of all emergent cases, or 1 EVAR to 7.5 OSR cases. These odds are clearly below those found in elective repair over the same period (1 EVAR to 1.7 OSR in Portugal, data from the SNS administrative database, not published). This suggests that although surgeons became increasingly comfortable using EVAR in a controlled setting, this does not translate directly into the emergent use [34]. The offer of EVAR in the emergent setting comes with a higher complexity that involves not only the surgeon's expertise but also the availability of a wide range of endografts sizes and ancillary material on the shelf, a suitable operation room, and an autonomous team to plan and perform the case, all during 24 hours per day and 7 days a week. Efforts to accommodate for increase of EVAR for rAAA in Portugal, including vascular training focused on EVAR on hypotensive patients and the use of resuscitative endovascular balloon occlusion of the aorta (REBOA) may contribute to a further gain in rAAA mortality in the future.

The overall mortality burden of rAAA is significantly affected by turndown rate for surgery among patients who present to hospital with a rAAA diagnosis. The turndown rate of rAAA has previously been estimated at 40 (33 to 47)% in a meta-analysis of 24 retrospective cohort studies[26]. In the present report, the average percentage from 2000-2015 was 22%. Cases admitted but not operated on showed a drop after adjusting for age variation of the population through standardization. This suggests that gains obtained in mortality do not seem to be due to selection bias of better cases for repair.

Finally, a full understanding of the epidemiology of rAAA requires a look into patients that die without reaching the hospital, estimated in 32 (27 to 37)% in the same meta-analysis[26]. In the present study, on average 36% of patients died outside the hospital from 2000-2015. This percentage rose in the third period after a decrease from the first to the second periods. The rate of patients that die outside the hospital can be related to several factors. First, these ratios may reflect the time of transportation to vascular departments that can deal with aortic rupture. Vascular departments that receive rAAA patients are located in the North and Center of the coastal Western regions of Portugal. Thus, populations from South and Eastern regions of the country do not have access to a nearby specialized vascular surgery department. In what extent the existence of a wider distribution of centres that offer rAAA repair versus the benefits of centralization in high volume centres is yet to be assessed in Portugal [35].

It is important to underline, however, that the registration of rAAA as cause of death among patients not reaching hospital may be affected by other factors than prevalence of disease. In patients that die without reaching the hospital the cause of death is diagnosed based on clinical aspects and previous medical history, as well as autopsy if deemed necessary. Completeness of statistics on cause of death in Portugal, that is number of deaths for which cause of death is registered to the civil registration system, is fairly

good as among the member states of the World Health Organization, Portugal was among the 42% of the WHO member states that provided data completeness of 70% to 100% [36].

Despite providing data from two different and complementary databases, this study has some limitations. Contrary to the death certificate database from INE that includes prospective registration, the SNS administrative database contains a retrospective registration of all hospitalizations occurring in public hospitals in mainland Portugal. It is fed by independent trained physicians, representing an image of the real-world situation. Accuracy of the codification is guaranteed by proper training of the staff and external audits [21]. Because of the previous-mentioned features of the Portuguese health system[20], the amount of rAAA referred and treated in the private sector is null or, at least, neglectable in Portugal and not a realistic source of relevant selection bias. A second limitation of this study is the limited follow-up as only data associated with a specific hospital episode is available in the SNS database. Since an emergent situation (that is, rAAA) is the focus of this paper, it is expected that most rAAA-related casualties occur at the time of the first hospitalization. However, later deaths may occur and are these are not captured in this analysis. Third, ICD data was used rather than patient-level data which precluded adjustment of the results for co-morbidities, the type of anesthesia used or the etiology of the AAA (degenerative versus mycotic). Fourth, admission, repair and mortality due to rAAA seem to have reached a peak and have been recently decreasing. The authors are not aware of events happening globally or in Portugal that could have affected treatment decisions differentially over these time periods. Furthermore, these shifts follow what has been observed in the epidemiological evolution of the disease in other countries. However, the pattern of significantly increase and then decrease (or vice versa) might rather be random fluctuation in the data that are hard to discern without a longer time period of data collection. Further studies with longer time period of data collection might confirm these results. Fifth, like in other countries, autopsy rates are declining, from 6.7±0.3% of all deaths in 2000-2005 to 6.4±0.2% in 2005-2009 and to 5.5±0.8% in 2010-2015. The proportion of patients dying outside a hospital setting is prone to error owing to misdiagnosis of the cause of death if no autopsy is performed and no previous diagnosis of AAA exists. Likewise, patients with known AAA might be wrongly diagnosed as rAAA death.

Conclusion

Nationwide evaluation of rAAA from 2000 to 2015 showed that overall, the incidence of rAAA in Portugal is stable over the past 10 years. Whilst the incidence of rAAA admissions, as well as mortality among patients presenting to hospital are decreasing in the recent years, the overall mortality related to rAAA in Portugal increased. This was due to an increasing incidence of rAAA-related deaths registered among individuals who died outside hospitals, showing that mortality outside the hospital is still a matter of concern.

References

1. Collaborators GBDCoD. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017;**390**:1151-210.
2. Budtz-Lilly J, Bjorck M, Venermo M, Debus S, Behrendt CA, Altreuther M, et al. Editor's Choice - The Impact of Centralisation and Endovascular Aneurysm Repair on Treatment of Ruptured Abdominal Aortic Aneurysms Based on International Registries. *Eur J Vasc Endovasc Surg* 2018;**56**:181-8.
3. Wanhainen A, Hultgren R, Linne A, Holst J, Gottsater A, Langenskiold M, et al. Outcome of the Swedish Nationwide Abdominal Aortic Aneurysm Screening Program. *Circulation* 2016;**134**:1141-8.
4. Glover MJ, Kim LG, Sweeting MJ, Thompson SG, Buxton MJ. Cost-effectiveness of the National Health Service Abdominal Aortic Aneurysm Screening Programme in England. *Br J Surg* 2014;**101**:976-82.
5. Bird AN, Davis AM. Screening for abdominal aortic aneurysm. *JAMA* 2015;**313**:1156-7.
6. Ying AJ, Affan ET. Abdominal Aortic Aneurysm Screening: A Systematic Review and Meta-analysis of Efficacy and Cost. *Ann Vasc Surg* 2019;**54**:298-303 e3.
7. Ali MU, Fitzpatrick-Lewis D, Kenny M, Miller J, Raina P, Sherifali D. A systematic review of short-term vs long-term effectiveness of one-time abdominal aortic aneurysm screening in men with ultrasound. *J Vasc Surg* 2018;**68**:612-23.
8. Guirguis-Blake JM, Beil TL, Senger CA, Coppola EL. Primary Care Screening for Abdominal Aortic Aneurysm: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2019;**322**:2219-38.

9. Alund M, Mani K, Wanhainen A. Selective screening for abdominal aortic aneurysm among patients referred to the vascular laboratory. *Eur J Vasc Endovasc Surg* 2008;**35**:669-74.
10. Svensjo S, Mani K, Bjorck M, Lundkvist J, Wanhainen A. Screening for abdominal aortic aneurysm in 65-year-old men remains cost-effective with contemporary epidemiology and management. *Eur J Vasc Endovasc Surg* 2014;**47**:357-65.
11. Laine MT, Laukontaus SJ, Sund R, Aho PS, Kantonen I, Alback A, et al. A Population-Based Study of Abdominal Aortic Aneurysm Treatment in Finland 2000 to 2014. *Circulation* 2017;**136**:1726-34.
12. Gunnarsson K, Wanhainen A, Bjorck M, Djavani-Gidlund K, Mani K. Nationwide Study of Ruptured Abdominal Aortic Aneurysms During Twenty Years (1994-2013). *Ann Surg* 2019;[Epub ahead of print].
13. Choke E, Vijaynagar B, Thompson J, Nasim A, Bown MJ, Sayers RD. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? *Circulation* 2012;**125**:1617-25.
14. Sidloff D, Stather P, Dattani N, Bown M, Thompson J, Sayers R, et al. Aneurysm global epidemiology study: public health measures can further reduce abdominal aortic aneurysm mortality. *Circulation* 2014;**129**:747-53.
15. Nelissen BG, Herwaarden JA, Pasterkamp G, Moll FL, Vaartjes I. Shifting abdominal aortic aneurysm mortality trends in The Netherlands. *J Vasc Surg* 2015;**61**:642-7 e2.
16. Jarvis MJ. Monitoring cigarette smoking prevalence in Britain in a timely fashion. *Addiction* 2003;**98**:1569-74.
17. Sweeting MJ, Balm R, Desgranges P, Ulug P, Powell JT, Ruptured Aneurysm T. Individual-patient meta-analysis of three randomized trials comparing endovascular versus open repair for ruptured abdominal aortic aneurysm. *Br J Surg* 2015;**102**:1229-39.
18. Lilja F, Mani K, Wanhainen A. Editor's Choice - Trend-break in Abdominal Aortic Aneurysm Repair With Decreasing Surgical Workload. *Eur J Vasc Endovasc Surg* 2017;**53**:811-9.
19. Marcon A, Pesce G, Calciano L, Bellisario V, Dharmage SC, Garcia-Aymerich J, et al. Trends in smoking initiation in Europe over 40 years: A retrospective cohort study. *PLoS One* 2018;**13**:e0201881.
20. Simões J AG, Fronteira I, Hernández-Quevedo C. Portugal: Health system review. *Health Systems in Transition* 2017;**19(2):**:1–184.
21. ACSS. Relatório anual de auditoria externa à codificação clínica - Ano 2010. *Administração Central do Sistema de Saúde (ACSS)* 2011;
22. Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. *PLoS Med* 2015;**12**:e1001885.
23. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Int J Surg* 2014;**12**:1500-24.
24. Sandiford P, Mosquera D, Bramley D. Trends in incidence and mortality from abdominal aortic aneurysm in New Zealand. *Br J Surg* 2011;**98**:645-51.
25. Bray F, Guilloux A, Sankila R, Parkin DM. Practical implications of imposing a new world standard population. *Cancer Causes Control* 2002;**13**:175-82.
26. Reimerink JJ, van der Laan MJ, Koelemay MJ, Balm R, Legemate DA. Systematic review and meta-analysis of population-based mortality from ruptured abdominal aortic aneurysm. *Br J Surg* 2013;**100**:1405-13.
27. Castro-Ferreira R, Lachat M, Schneider PA, Freitas A, Leite-Moreira A, Sampaio SM. Disparities in Contemporary Treatment Rates of Abdominal Aortic Aneurysms Across Western Countries. *Eur J Vasc Endovasc Surg* 2019;**58**:200-5.
28. Castro-Ferreira R, Barreira R, Mendes P, Couto P, Peixoto F, Aguiar M, et al. First Population-Based Screening of Abdominal Aortic Aneurysm in Portugal. *Ann Vasc Surg* 2019;**59**:48-53.
29. Benson RA, Poole R, Murray S, Moxey P, Loftus IM. Screening results from a large United Kingdom abdominal aortic aneurysm screening center in the context of optimizing United Kingdom National Abdominal Aortic Aneurysm Screening Programme protocols. *J Vasc Surg* 2016;**63**:301-4.
30. Roosendaal LC, Kramer GM, Wiersema AM, Wisselink W, Jongkind V. Outcome of Ruptured Abdominal Aortic Aneurysm Repair in Octogenarians: A Systematic Review and Meta-Analysis. *Eur J Vasc Endovasc Surg* 2020;**59**:16-22.
31. Roosendaal LC, Kramer GM, Wiersema AM, Wisselink W, Jongkind V. Outcome of Ruptured Abdominal Aortic Aneurysm Repair in Octogenarians: A Systematic Review and Meta-Analysis. *Eur J Vasc Endovasc Surg* 2019;

32. Takagi H, Umemoto T, Alice. A meta-analysis of adjusted observational studies and randomized controlled trials of endovascular versus open surgical repair for ruptured abdominal aortic aneurysm. *Int Angiol* 2016;**35**:534-45.
33. Varabyova Y, Blankart CR, Schreyogg J. The Role of Learning in Health Technology Assessments: An Empirical Assessment of Endovascular Aneurysm Repairs in German Hospitals. *Health Econ* 2017;26 Suppl 1:93-108.
34. Melillo AM, Trani JL, Gaughan JP, Carpenter JP, Lombardi JV. Assessing trends, morbidity, and mortality in ruptured abdominal aortic aneurysm repair with nine years of data from the National Surgical Quality Improvement Program. *J Vasc Surg* 2019;
35. Patterson BO, O'Donnell TFX, Kolh P, Holt PJE, Schermerhorn M. Improved Outcomes for Ruptured Abdominal Aortic Aneurysm Through Centralisation. *Eur J Vasc Endovasc Surg* 2018;**56**:159-60.
36. Mahapatra P, Shibuya K, Lopez AD, Coullare F, Notzon FC, Rao C, et al. Civil registration systems and vital statistics: successes and missed opportunities. *Lancet* 2007;**370**:1653-63.

Table 1. Patients characteristics and rates of incidence, admission and repair of ruptured AAA (rAAA) across three time periods (2000-2004, 2005-2009 and 2010-2015) in Portugal.

Cases	2000-2015	2000-2004	2005-2009	2010-2015	p
All cases	2275	582	723	970	-
Cases/year	142	116	145	162	<.001
Proportions of					
≥75 yo	50.2±4.1	48.2±4.3	48.2±1.6	53.5±3.7	<.001
Standardized rate per 100,000/y					
All cases	3.07 (0.35)	2.78 (0.24)	3.17 (0.39)	3.21 (0.28)	<.001
<75 yo	2.18 (0.29)	1.96 (0.23)	2.34 (0.34)	2.23 (0.20)	<.001
≥75 yo	8.27 (0.83)	7.81 (0.84)	8.28 (0.80)	8.64 (0.79)	<.001
Cases presenting to hospital	1460	335	508	617	-
Cases/year	91	67	102	103	<.001
Proportions of					
≥75 yo	50.4±6.2	49.7±6.9	47.3±4.2	53.6±6.4	<.001
Men	87.8±3.6	88.2±4.1	88.4±3.5	87.0±3.9	<.001
Not operated on	22.0±3.6	20.9±4.4	21.5±3.4	23.0±4.2	<.001
OSR	69.8±9.3	79.0±4.4	73.1±5.3	59.4±3.9	<.001
EVAR	13.0±6.9	-	7.6±4.9	17.6±4.7	<.001
Proportion of not operated on					
In patients <75 yo	15.7±5.6	18.0±2.6	18.8± 6.6	11.4±4.0	<.001
In patients ≥75 yo	27.5±7.6	23.4±8.8	24.9±2.7	33.2±6.5	<.001
In men	19.8±4.0	19.5±3.0	19.5±5.4	20.4±4.0	.004
In women	35.4±17.4	29.1±23.8	37.4±17.2	39.2±12.4	.007
Proportion undergoing repair					
In patients <75 yo	84.3±5.6	82.0±2.6	81.2±6.6	88.6±4.0	<.001
In patients ≥75 yo	72.5±7.6	76.6±9.3	75.1±2.7	66.8±6.5	<.001
In men	80.2±4.0	80.5±3.0	80.5±5.4	79.6±4.0	.004
In women	64.6±17.4	70.9±23.8	62.6±17.2	60.8±12.4	.007

Data are presented as n, proportions or mean ± standard deviation. Proportions are presented as the mean and standard deviation of all annual percentages for each time period. P-values were calculated resorting to ANOVA after adjustment for multiple comparison. Legend: yo – years old, SD – standard deviation, OSR – open surgery repair, EVAR – endovascular repair.

Table 2. Age-standardized mortality rates referring to nationwide deaths for ruptured AAA repair (rAAA) across three time periods (2000-2004, 2005-2009 and 2010-2015) in Portugal.

	2000-2015	2000-2004	2005-2009	2010-2015	p
All deaths	*n* = 1710	*n* = 463	*n* = 519	*n* = 728	-
Deaths/year	107	93	104	121	<.001
Proportions					
≥75 yo	55.1±4.9	51.0±3.8	53.6±1.7	59.7±3.8	<.001
Mortality among all incident cases	75.2±4.7	79.6±3.7	71.7±3.1	74.9±3.9	<.001
Standardized rate per 100,000/y					
All deaths	2.22 (0.23)	2.20 (0.18)	2.21 (0.27)	2.26 (0.26)	<.001
<75 yo	1.48 (0.19)	1.48 (0.15)	1.49 (0.19)	1.47 (0.26)	.549
≥75 yo	6.79 (0.72)	6.58 (0.80)	6.61 (0.91)	7.12 (0.42)	<.001
Deaths outside the hospital	815	247	215	353	-
Deaths/year	51 (47.7)	49 (53.3)	43 (41.4)	59 (48.5)	<.001
Proportions					
≥75 yo	50.8±5.8	46.7±5.2	51.0±7.1	54.1±3.1	<.001
Standardized rate per 100,000/y					
Deaths outside hospital	1.10 (0.23)	1.21 (0.10)	0.93 (0.27)	1.14 (0.22)	<.001
<75 yo	0.78 (0.19)	0.88 (0.14)	0.68 (0.23)	0.82 (0.19)	<.001
≥75 yo	2.95 (0.58)	3.21 (0.19)	2.52 (0.75)	3.10 (0.51)	<.001
Deaths at the hospital	895	216	304	375	-
Deaths/year	56	43	61	62	<.001
Proportions					
≥75 yo	59.6±7.1	56.2±5.6	56.0±3.2	65.4±7.4	<.001
Men	85.6±6.4	87.5±7.0	84.9±8.0	84.5±5.2	.003
In-hospital mortality among all cases presenting to hospital	61.5±5.6	64.5±5.6	59.7±5.2	60.6±6.0	<.001
Standardized rate per 100,000/y					
Deaths at the hospital	1.13 (0.23)	0.99 (0.15)	1.28 (0.24)	1.11 (0.24)	<.001
<75 yo	0.68 (0.19)	0.60 (0.09)	0.82 (0.18)	0.64 (0.23)	<.001
≥75 yo	3.84 (0.66)	3.38 (0.70)	4.09 (0.65)	4.02 (0.50)	<.001
Men	2.36 (0.52)	2.08 (0.20)	2.64 (0.67)	2.35 (0.50)	<.001
Women	0.22 (0.11)	0.18 (0.14)	0.25 (0.10)	0.23 (0.09)	.018
Deaths after repair	573	146	195	232	-
Deaths /year	36	29	39	39	<.001
Proportions					
≥75 yo	57.1±7.0	56.3±4.2	57.3±9.1	58.1±9.1	.180
Men	57.3±5.6	60.9±5.7	56.3±6.3	55.2±4.3	<.001
EVAR	7.3±4.1	-	5.6±3.5	8.7±4.4	.010
Operative mortality					
In all repaired cases	50.9± 5.8	55.3±4.7	48.8±4.7	48.9±6.2	<.001
In patients <75 yo	41.0±7.5	46.8±6.4	38.4±6.1	38.5±8.2	<.001
In patients ≥75 yo	62.3±7.4	65.1±5.7	61.1±9.7	61.0±7.7	<.001
In men	50.2±6.2	55.1±3.6	47.4±6.8	48.4±5.7	<.001
In women	53.4±20.3	53.2±22.7	52.3±27.1	54.3±15.5	.912
After EVAR	50.0±29.6	-	75.2±24.0	29.0±11.1	<.001
After OSR	52.6±6.0	55.3±4.7	47.8±5.4	54.5±5.9	<.001

Data are presented as n, proportions or mean ± standard deviation. Proportions are presented as the mean and standard deviation of all annual percentages for each time period. P-values were calculated resorting to ANOVA after adjustment for multiple comparison. Legend: yo – years old, SD – standard deviation, OSR – open surgery repair, EVAR – endovascular repair.

Figure 1. Outcomes of absolute numbers of episodes due to rAAA in Portugal, 2000–2015. Legend: EVAR – endovascular aneurysm repair, OSR – open surgical repair.

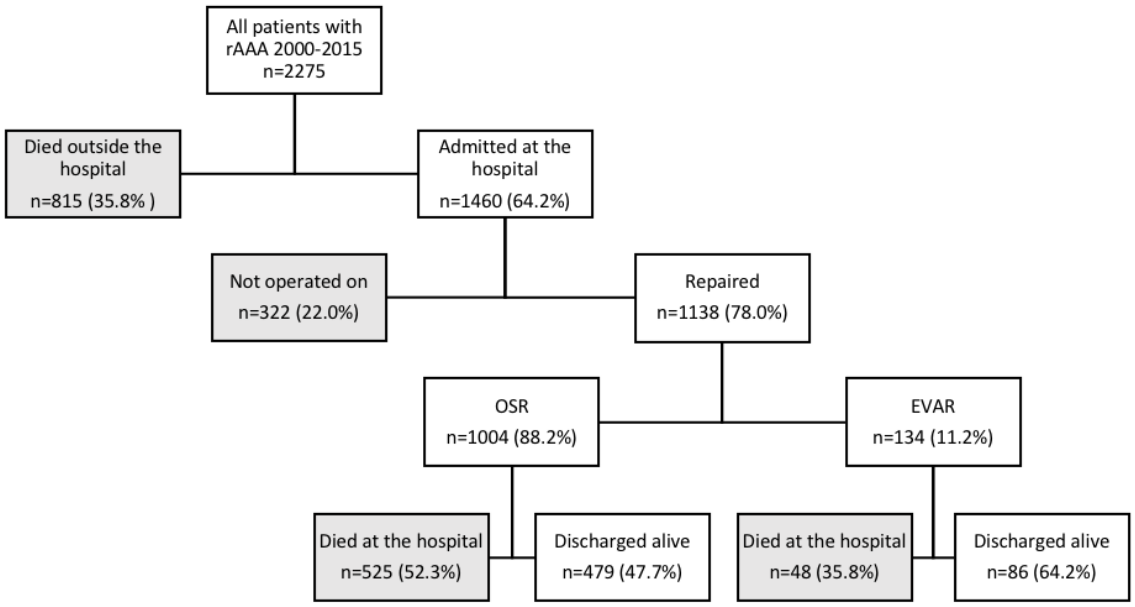


Figure 2. Age-standardized rate of admissions and repair due to rAAA per 100,000 inhabitants ≥ 50 years-old per year (2000-2015) in Portugal. The p-value (ANOVA) for the comparison of the periods 2000-2004, 2005-2009 and 2010-2015 was <.001 for all cases, admissions, not operated on, OSR and EVAR. Legend: EVAR – endovascular aneurysm repair, OSR – open surgical repair.

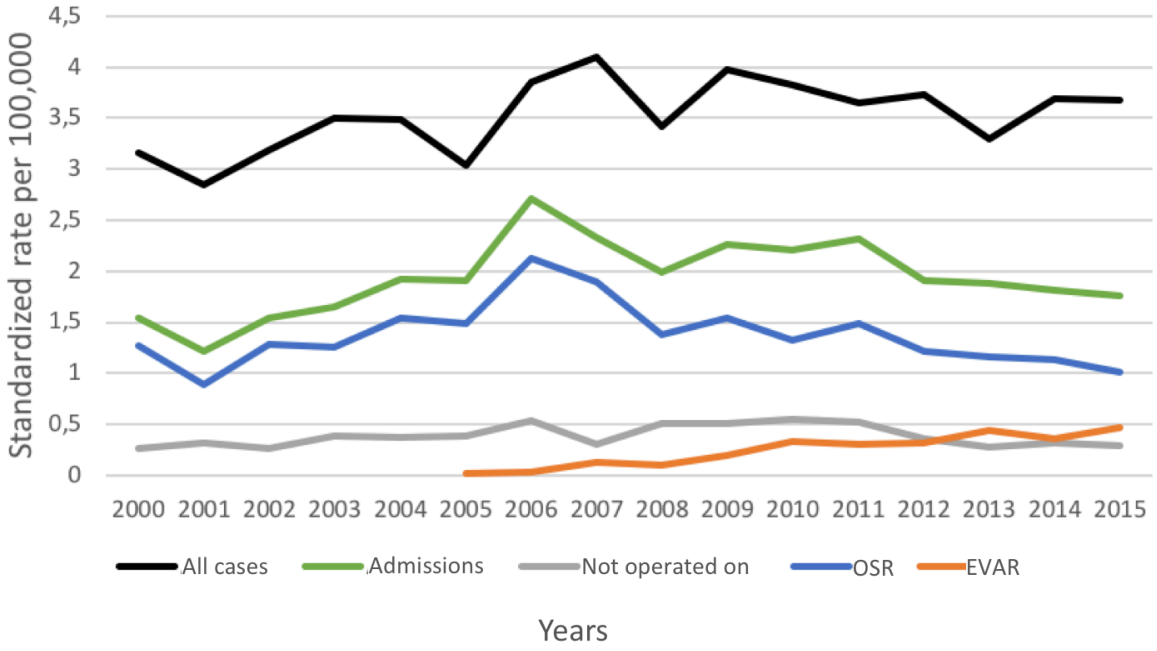
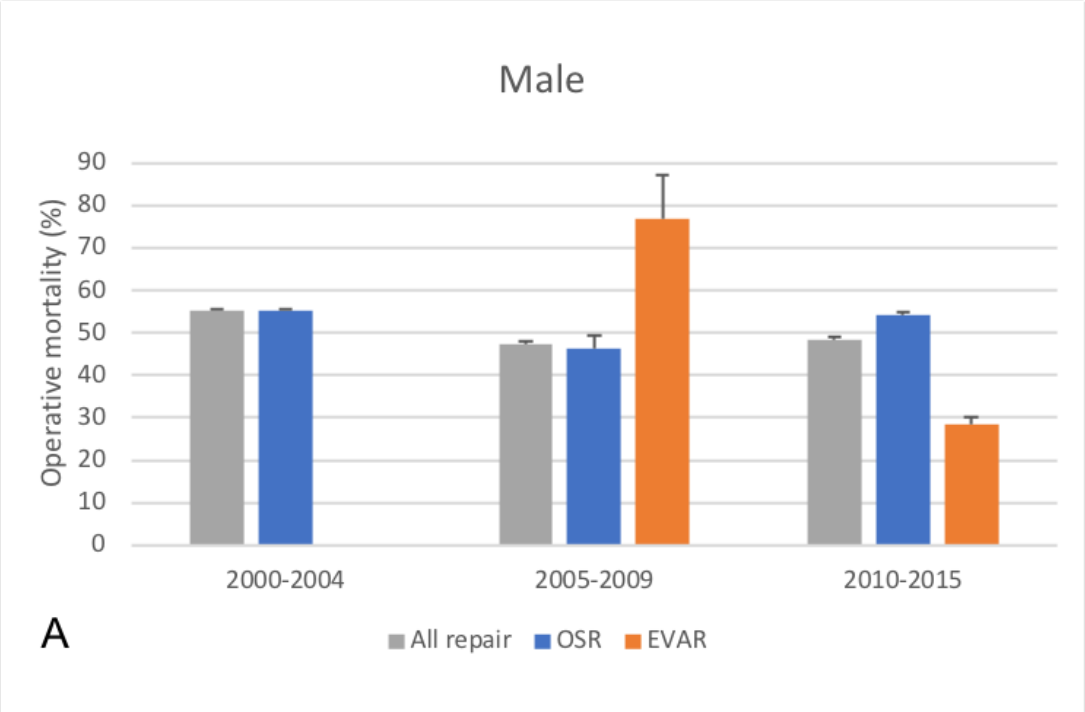
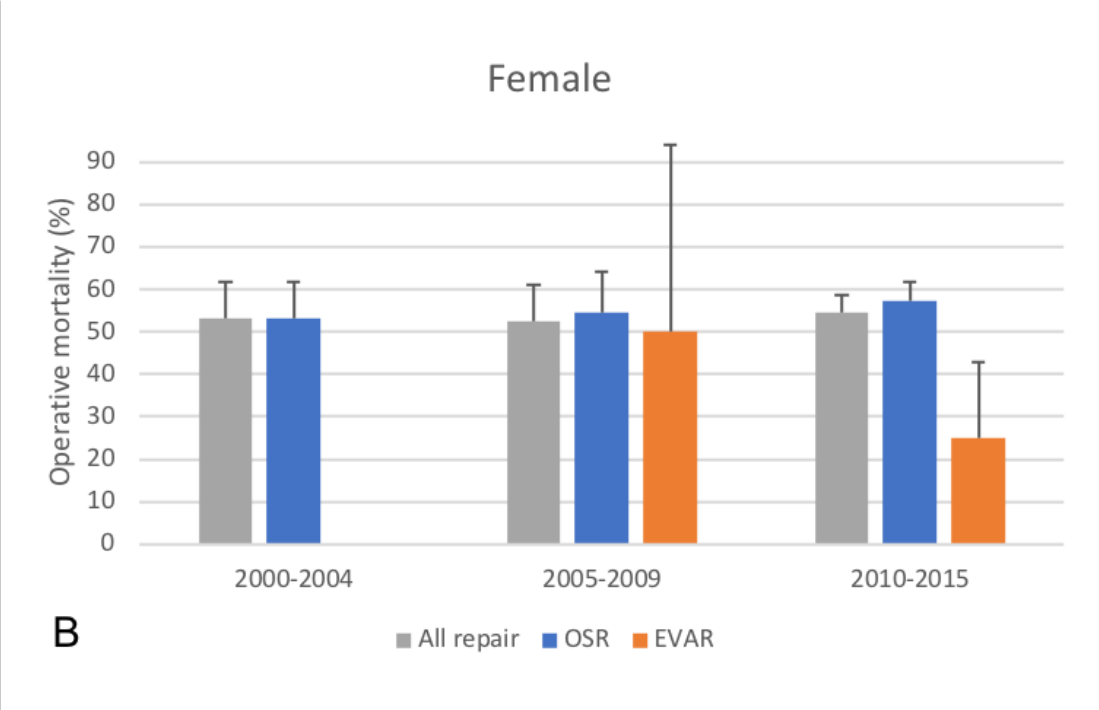
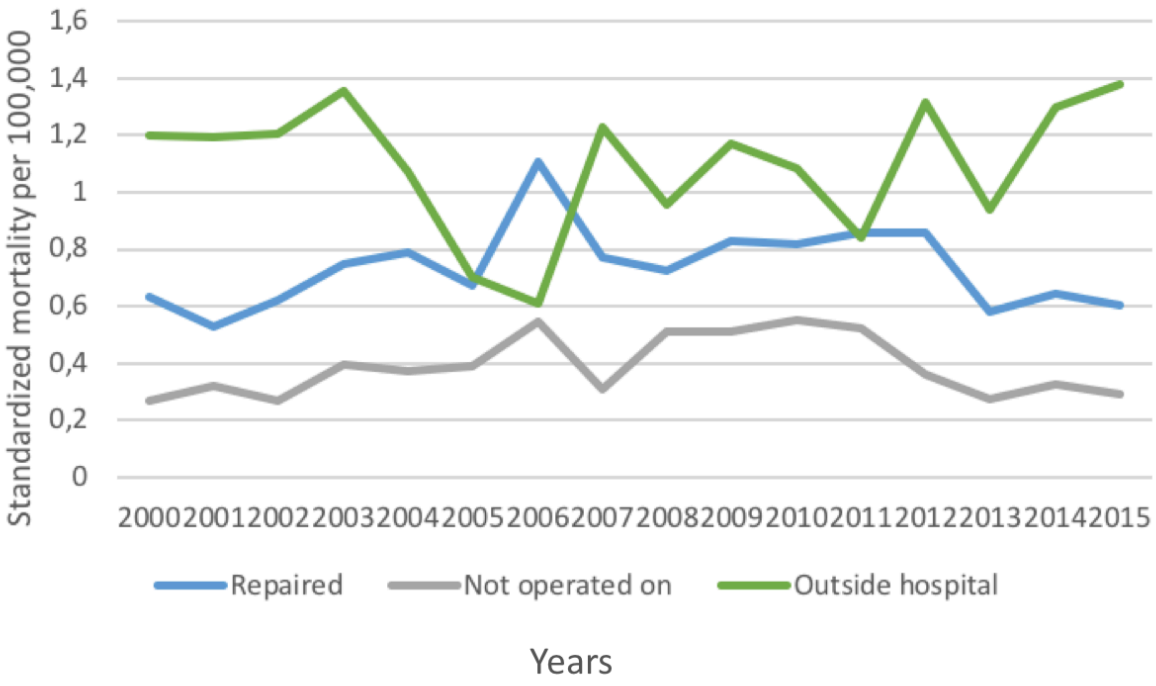


Figure 3. Percentage of operative mortality among different types of rAAA repair per gender male (A) and female (B) among three time periods (2000-2004, 2005-2009 and 2010-2015) in Portugal. Legend: EVAR – endovascular aneurysm repair, OSR – open surgical repair.

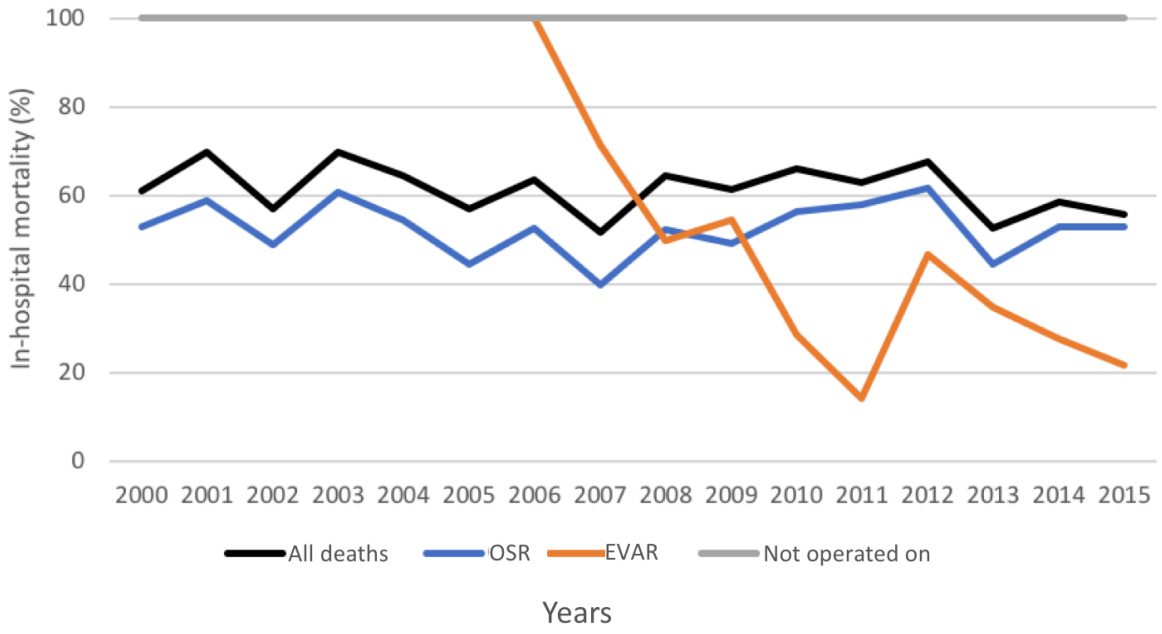




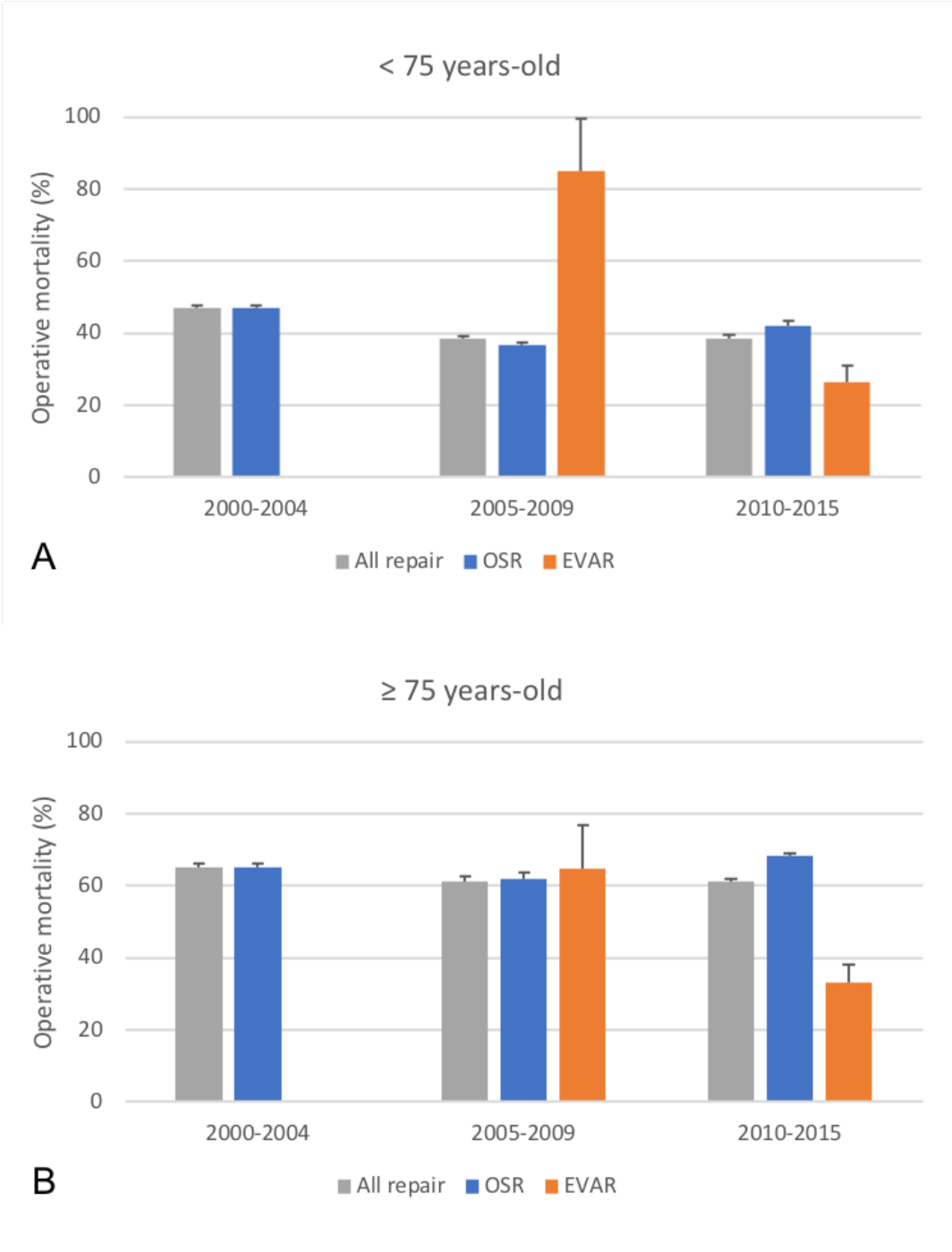
Supplemental Figure 1. Age-standardized mortality due to rAAA per 100,000 inhabitants ≥ 50 years-old per year (2000-2015) among patients with admission and repair, patients with admission but not operated on and patients dying outside the hospital, in Portugal. The p-value (ANOVA) for the comparison of the periods 2000-2004, 2005-2009 and 2010-2015 was <.001 for repaired, not operated on and outside hospital deaths.



Supplemental Figure 2. Percentage of operative mortality among different types of rAAA repair of patients ≥ 50 years per year (2000-2015) in Portugal. The p-value (ANOVA) for the comparison of the periods 2000-2004, 2005-2009 and 2010-2015 was <.001 for all deaths, OSR and EVAR. Legend: EVAR – endovascular aneurysm repair, OSR – open surgical repair.



Supplemental Figure 3. Percentage of operative mortality among different types of rAAA repair per age bands <75 (A) and ≥ 75 years old (B) among three time periods (2000-2004, 2005-2009 and 2010-2015) in Portugal. Legend: EVAR – endovascular aneurysm repair, OSR – open surgical repair.



PART II NEW PATHOPHYSIOLOGICAL FEATURES OF ABODMINAL AORTIC ANEURYSMS

IIA RESEARCH CHALLENGES IN ABDOMINAL AORTIC ANEURYSM CALCIFICATION

CHAPTER 5 PERSPETIVAS E DESAFIOS DO ESTUDO DA CALCIFICAÇÃO NO ANEURISMA DA AORTA ABDOMINAL | PERSPECTIVES AND RESEARCH CHALLENGES IN ABDOMINAL AORTIC ANEURYSM CALCIFICATION

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PERSPETIVAS E DESAFIOS DO ESTUDO DA CALCIFICAÇÃO NO ANEURISMA DA AORTA ABDOMINAL

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Resumo

O aneurisma da aorta abdominal (AAA) permanece uma causa relevante de mortalidade nos países ocidentais. É premente a contínua identificação de fatores de risco de progressão aneurismática bem como de preditores de resposta ao tratamento na otimização da estratégia terapêutica a oferecer a estes doentes.

A calcificação vascular tem sido estudada em diversos leitos capilares como um fator de prognóstico cardiovascular. Contudo, a importância da calcificação da aorta abdominal (CA) no AAA permanece incompletamente esclarecida, sendo a prova científica disponível dispersa e heterogénea.

O objetivo desta revisão é descrever o eventual impacto da CA na progressão e rutura aneurismática, bem como na resposta à correção endovascular do AAA. Salienta-se que o estabelecimento de um método validado, rápido e fácil de usar para avaliar a CA seria de grande utilidade clínica e/ou investigacional.

Abstract

Perspectives and research challenges in abdominal aortic aneurysm calcification

Abdominal aortic aneurysm (AAA) remains a relevant cause of mortality in Western countries. There is a need for continuous identification of risk factors for aneurysmal progression and predictors of treatment response to optimize the therapeutic strategy to be offered to these patients.

Vascular calcification has been studied in several capillary beds as a cardiovascular risk factor. However, the importance of abdominal aortic calcification (AC) in AAA remains incompletely clarified, and the available evidence is scattered and heterogeneous.

The objective of this review is to describe the possible impact of AC on aneurysmal progression and rupture, as well as on the response to endovascular correction. It should be noted that the establishment of a validated, quick and easy to use method for assessing AC would be of great clinical and/or research utility.

INTRODUÇÃO

O aneurisma da aorta abdominal (AAA) é uma causa major de mortalidade nos países ocidentais.[1] A elevada mortalidade após rutura e a ausência de estratégias que evitem a progressão aneurismática justificam a contínua tentativa de identificação de fatores de risco para a presença e a progressão do AAA, bem como de preditores de resposta à sua correção.

A calcificação vascular tem merecido a atenção de diversos autores como um fator de risco de doenças cardiovasculares. Este ponto tem sido particularmente estudado na

doença coronária, onde foi possível demonstrar uma associação entre a calcificação das artérias coronárias e eventos cardiovasculares, nomeadamente enfarte agudo do miocárdio.[2,3] Mais ainda, a calcificação vascular parece influenciar a própria resposta à intervenção terapêutica: na doença arterial periférica, a extensão da calcificação contribui para uma elevada taxa de falência técnica durante a recanalização percutânea de oclusões femoro-poplíteas crónicas.[4,5]

À semelhança da calcificação das artérias coronárias, verificou-se que também a calcificação aórtica (CA) estava associada a eventos cardiovasculares num estudo que utilizou um sistema de pontuação subjetivo para avaliar a CA em radiografias lombares de indivíduos do Framingham Heart Study.[6] Neste estudo longitudinal, mais de 2500 indivíduos foram acompanhados por mais de 22 anos. Os resultados indicaram que os indivíduos no tercil mais elevado de CA apresentavam um aumento superior a 50% no risco de eventos cardiovasculares. Apesar do reconhecido impacto negativo no desfecho cardiovascular global, permanece incompletamente esclarecido se a CA tem alguma relevância específica no AAA (Figura 1). O objetivo deste trabalho é descrever a evidência disponível sobre a importância da CA na progressão e rutura aneurismática bem como na resposta ao tratamento endovascular do AAA.

quantificar CA. Nas radiografias simples de perfil, o mais conhecido e estudado foi descrito por Kauppila e colaboradores,[7] o AAC-24. Neste método são traçados segmentos de reta a separar as 4 primeiras vértebras lombares que se prolongam anteriormente para dividir a aorta abdominal em 4 segmentos; a calcificação da parede anterior e posterior do vaso é avaliada em cada um desses segmentos e expressa numa escala de 0-3, num total máximo de 24 pontos. A utilidade deste método como estratificador de risco de mortalidade e eventos cardiovasculares em doentes com doença renal crónica terminal é apoiada por diversos estudos.[8,9] A ecografia abdominal foi usada especificamente no AAA por Lindholt e colaboradores[10] para estudar a relação entre a CA e o crescimento de aneurismas com diâmetro máximo $<$50 mm. A reprodutibilidade intra-observador reportada foi de 84% (Intervalo de Confiança a 95% de 70–93%). No que diz respeito à imagiologia por TC, são de destacar particularmente o *score* de Agatston[11] e o método volumétrico em MDCT descrito por Jayalath e colabodores.[12] O score de Agatston considera como calcificação uma região do espaço com $\geq$1mm^2 e $>$130 Hounsfield Units e o seu valor é em função da área de calcificação e da densidade da mesma. Pode ser calculado de forma computorizada em diversos *softwares* de análise de imagem (Figura 1). Tem como principal

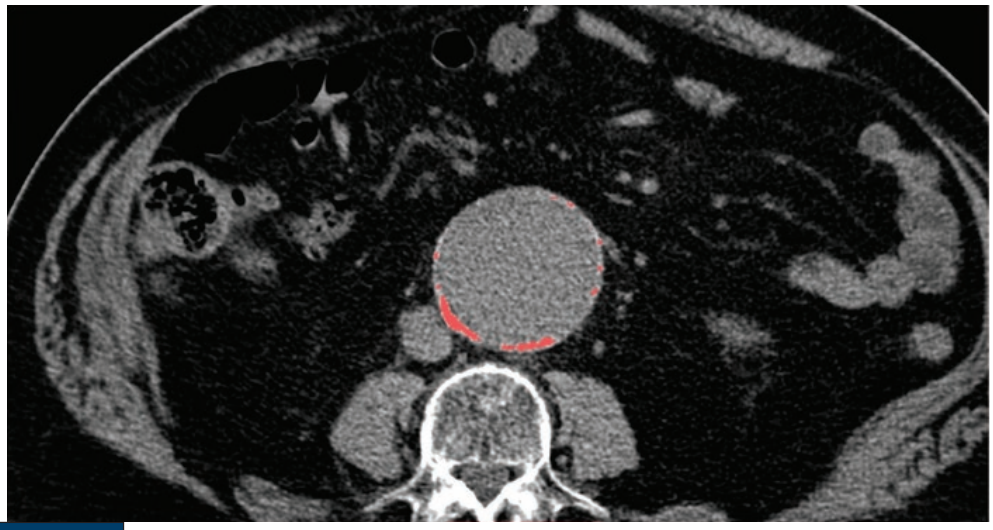

Figura 1 *Determinação computorizada do score de Agatston através do software Osirix® ou Horos®. As regiões de calcificação são selecionadas manualmente seguindo-se a atribuição automática do valor em função da área de calcificação e da densidade das mesmas.*

Medição da calcificação da aorta abdominal (CA)

Para avaliação da CA estão descritas na literatura quatro modalidades imagiológicas: a radiografia simples, a ecografia abdominal, a tomografia computorizada (TC) (MDCT, *Multiple detector computed tomography* e EBCT, *Electron Beam Computed Tomography*) e a absorciometria radiológica de dupla energia (DXA). Dentro de cada uma destas modalidades, vários métodos têm sido utilizados para desvantagem não poder ser aplicado em angiotomografia computorizada (ATC), pelo facto de a presença de contraste intravascular interferir com o cálculo do score. O método volumétrico por MDCT é aplicado com recurso a uma workstation dedicada e tem a vantagem de poder ser realizado de forma reprodutível tanto em TC como em ATC. É de salientar que este método foi utilizado no estudo do papel da calcificação aórtica na progressão de AAAs com diâmetro máximo $\leq$55 mm.

É também possível aplicar métodos semiquantitativos em imagem de TC. A este respeito, é de destacar o Índice de Calcificação Aórtico (ACI, *aortic calcification index*) descrito por Kabaya e colaboradores.[13] Neste método, a área de interesse está compreendida entre a emergência da artéria renal mais inferior e o plano imediatamente superior à bifurcação aórtica. São selecionados, em sentido proximal, 10 planos transversais da aorta, separados entre si por 10 mm tendo início no plano mais inferior da região de interesse. Em cada plano, a aorta é dividida em 12 setores radiais e a calcificação é expressa como o número total de setores em que esta esteja presente, num total máximo possível de 120 setores radiais calcificados. Este método tem como vantagem poder ser aplicado tanto em TC como ATC. À semelhança dos métodos da radiografia abdominal simples, o ACI tem sido largamente utilizado na insuficiência renal crónica.[14-16]

Por fim, importa referir que uma derivação do método AAC-24 utilizada em DXA foi aplicada em ATC para estudar a relação entre a calcificação do AAA e o seu risco de rutura.[1] Esta derivação constitui o método AAC-8, apresentado por Schousboe e colaboradores.[17] Neste método são utilizados igualmente os 4 segmentos de aorta anteriormente referidos no AAC-24 e é também avaliada a calcificação das paredes anterior e posterior, sendo que a atribuição de pontos difere face ao AAC-24. Neste caso, a calcificação não é expressa tendo como referência o comprimento da aorta em cada segmento, mas sim tendo como referência o tamanho de uma vértebra do doente. Isto é, se a calcificação total da parede anterior/posterior for inexistente, atribui-se o valor de 0; valor de 1 se ≤1 vértebra; 2 se >1 mas ≤2 vértebras; 3 se >2 mas ≤3 vértebras e 4 se >3 vértebras. O máximo por cada parede será de 4, num total possível de 8.

Atualmente, nenhuma modalidade foi aceite como *gold standard* para a medição da CA, consequentemente, a avaliação da sensibilidade e especificidade das diversas técnicas tem constituído uma lacuna na literatura. Os estudos que avaliaram o impacto da CA no AAA não são exceção na medida em que a CA foi avaliada através de diferentes métodos, muitas vezes produzindo resultados divergentes, dificultando a extrapolação do papel da CA na fisiopatologia do AAA. O estabelecimento de um método válido, rápido e fácil de usar para avaliar a calcificação aórtica seria pois de grande utilidade.

Progressão aneurismática

O papel da CA no crescimento do AAA ainda é controverso, com alguns estudos a sugerir uma associação negativa entre CA[10,18] e o crescimento do AAA e outros onde essa associação não foi verificada.[19,20] A evidência disponível é proveniente de estudos de imagem, maioritariamente baseados em MDCT.

No estudo de Heilmaier e colaboradores,[18] foram avaliados scores de cálcio para toda a aorta abdominal, bem como separadamente para os segmentos supra e infra-renal. Adicionalmente, os padrões das placas foram avaliados e classificados de acordo com sua espessura e tamanho. Apesar dos scores totais de cálcio terem sido similares em doentes com e sem AAA, a densidade e o tamanho das placas que foi consideravelmente menor nos segmentos supra e infrarrenais no grupo aneurismático (n=34) em comparação com o não aneurismático (n=33). No estudo de Lindholt e colaboradores[10] foi realizada uma avaliação da calcificação do aneurisma por ecografia (tal como supramencionado) em AAA com diâmetro inicial de 30-49 mm, sendo depois monitorizado o crescimento aneurismático durante um tempo de seguimento médio de 6 anos. A taxa de crescimento anual médio foi significativamente menor nos homens com uma calcificação da parede AAA > 50%, mesmo após ajuste para idade, tabagismo e uso de aspirina em regressão linear multivariada. Estes estudos parecem apontar para um efeito protetor da CA na progressão do AAA.

Por outro lado, este efeito protetor não foi validado nos estudos de Parr e colaboradores[20] e Hendy e colaboradores,[19] muito embora tenha sido utilizado um método diferente (avaliação volumétrica da CA medida por ATC). No estudo de Parr e colaboradores[20] não foi encontrada uma associação entre o volume inicial de calcificação e o crescimento de AAAs com diâmetro <50 mm (n=32) ao fim de uma mediana de seguimento de 18 meses. No trabalho de Hendy e colaboradores[19] o volume de calcificação da aorta infra-renal não previu o crescimento de AAA com diâmetro <55mm. Admite-se que esta técnica seja mais objetiva do que as medidas de ecografia, embora, para avaliar completamente, ambas as técnicas precisariam de ser repetidas e comparadas na mesma coorte de doentes.

Em suma, a evidência disponível, baseada em métodos diversos e não validados, com resultados conflituosos, impede a definição segura do papel da CA na progressão do AAA, não se podendo excluir um efeito protetor da CA na progressão do AAA.

Rutura aneurismática

O panorama em relação ao eventual impacto da CA na rutura do aneurisma reveste-se da mesma ambiguidade. Este tema tem sido estudado por análise *in vitro* das propriedades da parede do AAA e recorrendo a simulações computorizadas, para além dos tradicionais estudos de imagem.

O estudo de Buijs e colaboradores[1] é favorável a associação positiva entre o grau de calcificação e a rutura/pré-rutura de AAA. Os autores aplicaram o método AAC-8[21] em ATC de doentes com AAA e demonstraram que os aneurismas sintomáticos (n=28) e em rutura (n=73) apresentavam maiores scores de calcificação em comparação com aneurismas assintomáticos (n=233).

Uma análise de elementos finitos realizada pré-operatoriamente foi utilizada para identificar áreas da parede aórtica de elevado e de reduzido índice de risco de rutura.[22] Concordantemente, as regiões de parede AAA com índice risco de rutura mais elevado apresentaram desintegração histológica avançada em comparação com regiões com inferior risco de rutura no mesmo AAA: menos células musculares lisas, menos fibras elásticas, mais placas de colesterol e mais placas calcificadas. A quantidade de placas calcificadas apresentou uma correlação positiva com o stress máximo da parede determinada pela análise de elementos finitos.

No entanto, num outro estudo *in vitro*, amostras de parede aórtica colhidas de AAA de doentes submetidos a correção foram divididas em dois grupos de acordo com as suas características macroscópicas: em fibrosas e em parcialmente calcificadas.[23] Foi demonstrado que as propriedades mecânicas de falência do tecido parcialmente calcificado são significativamente menores em comparação com o tecido fibroso; a análise dos locais de falência através de microscopia eletrónica de varredura e de espectroscopia de raios X de dispersão de energia para investigar os possíveis motivos de falência, sugeriu que a junção entre um depósito de calcificação e a matriz fibrosa é altamente suscetível à falência. Uma visão unificadora destes trabalhos é a de que a calcificação possa ser eventualmente preditora de rutura na dependência não da calcificação em si mesmo, mas das regiões de charneira entre a calcificação e tecido fibroso adjacente.

Apesar deste corpo de evidência, um aumento geral do risco de rutura com a calcificação também foi posto em causa. A já mencionada análise de elementos finitos permite levar em consideração a morfologia específica do doente e a interação de diferentes constituintes do AAA, como o trombo intraluminal, a calcificação e a parede arterial, proporcionando uma previsão mais precisa do risco individual de rutura. Ao estudar o efeito da inclusão da calcificação numa análise computacional do esforço da parede de AAAs específicos em comparação com a simulação contendo apenas o trombo intraluminal e a parede arterial, Maier e colaboradores descobriram que, em dois de três pacientes, o stress máximo na parede diminui quando se toma em consideração as calcificações.[24] Em função destes achados, seriam os resultados diferentes se fosse possível ter em consideração no modelo de elementos finitos os locais de junção entre a calcificação e matriz fibrosa? Em função dos estudos *in vitro* debatidos acima, seria de esperar que a replicação do método de Maier e colaboradores tendo em conta os locais de junção entre a calcificação e a matriz fibrosa mostrasse estas zonas como locais de stress aumentado na parede em comparação com os locais onde apenas existe calcificação.

Resposta ao tratamento endovascular

A quantificação da CA no AAA tem sido realizada também no âmbito da previsão de complicações após o tratamento endovascular (EVAR). Um dos fatores mais limitantes para o EVAR é a anatomia adversa do colo proximal do aneurisma.[25] Nesse sentido, várias características do colo proximal têm sido estudadas como preditoras de *endoleak* ou de migração após EVAR tais como o comprimento, a angulação e a conicidade. A avaliação destas características tem-se revestido de graus variáveis de heterogeneidade, tendo já sido proposto um esquema de classificação dos fatores modificadores de *outcome* após EVAR.[26]

Particularmente na avaliação da calcificação do colo proximal, os métodos descritos são habitualmente baseados em ATC. A definição de colo calcificado varia desde a presença de "calcificação severa" avaliada qualitativamente,[27] à percentagem de calcificação circunferencial na zona de selagem[28] ou à proporção de volume de cálcio no segmento do colo em relação com o volume total desse segmento (método volumétrico).[29] Finalmente, alguns estudos reportam conjuntamente a presença de calcificação e de trombo.[30]

A presença de calcificação na zona de fixação proximal da endoprótese pode prejudicar a fixação entre a endoprótese e a parede do vaso, levando potencialmente a *endoleaks* e migração ou aumentando o risco de trombembolismo.[31] No entanto, os resultados destes trabalhos são por vezes conflituosos. No estudo de Bastos Gonçalves e colaboradores[30] (n=1263 doentes submetidos a EVAR), a presença de calcificação/trombo >50% no colo proximal, a par do colo <10mm, foi identificada como um fator de risco independente capaz de aumentar cerca de 5 vezes o número de eventos adversos intra-operatórios (*endoleak* de tipo 1a, conversão, complicação relacionada com implantação/recuperação ou cobertura renal não intencional) mas não de eventos pós-operatórios (*endoleak* de tipo 1a ou migração). Uma limitação deste resultado é que o efeito da calcificação não pode ser diferenciado do efeito do trombo uma vez que foram avaliados conjuntamente. Num outro estudo que incluiu 217 doentes submetido a EVAR seguidos durante 3,6 anos,[29] a presença de calcificação do colo avaliada pelo método volumétrico correlacionou-se com maiores taxas de eventos adversos. Neste estudo, a carga de trombo e de cálcio foram independentemente avaliadas no segmento de fixação. Curiosamente, o trombo parecia ter um efeito protetor na região proximal; foi proposto pelos autores deste trabalho que o trombo hipoteticamente por poderia atuar como uma textura em forma de gesso, resultando em uma adesão mais firme entre a endoprótese e a parede do vaso, enquanto que as calcificações irregulares impediriam a aproximação exata da endoprótese, algo que permanece por confirmar. Em dois outros estudos com metodologia semelhante não foi demonstrável uma associação entre a calcificação do colo e o aumento de complicações após EVAR.[27,32]

Para além da calcificação do colo, o efeito da calcificação na dinâmica do saco aneurismático após EVAR tem merecido atenção crescente (dados ainda não disponíveis sob a forma de publicação).

Implicações clínicas

As implicações clínicas do estudo da CA no AAA são diversas. A confirmação da associação entre a calcificação e o crescimento aneurismático ou a rotura poderá permitir uma personalização dos protocolos de seguimento imagiológico dos doentes que têm AAA de pequenas dimensões ainda não candidatos a correção. A importância da calcificação denuncia ainda que o uso de medidas biomecânicas adicionais poderá ajudar a alcançar uma melhoria na precisão da predição de ruptura do AAA.[33] O valor prognóstico da presença de calcificação no colo do aneurisma após EVAR poderá condicionar o processo de decisão terapêutica quanto à escolha da melhor estratégia de reparação (cirurgia aberta *versus* endovascular).

CONCLUSÃO

A evidência disponível é favorável a uma associação negativa entre a CA e o crescimento do aneurisma embora em alguns estudos essa associação não tenha sido demostrada. A CA parece associar-se ao evento de rutura, no entanto é possível que esta esteja relacionada com a região de charneira entre a calcificação e tecido fibroso adjacente e não na dependência da calcificação em si mesmo. A presença de calcificação no colo proximal associa-se a aumento dos eventos adversos após EVAR. A manifesta heterogeneidade na definição de calcificação e a multiplicidade de métodos disponíveis salienta a necessidade de clarificar e comparar os métodos disponíveis para a avaliação da CA no AAA.

REFERÊNCIAS

1. Buijs RV, Willems TP, Tio RA, Boersma HH, Tielliu IF, Slart RH, et al. Calcification as a risk factor for rupture of abdominal aortic aneurysm. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2013;46(5):542-8.
2. O'Malley PG, Taylor AJ, Jackson JL, Doherty TM, Detrano RC. Prognostic value of coronary electron-beam computed tomography for coronary heart disease events in asymptomatic populations. Am J Cardiol. 2000;85(8):945-8.
3. Alexopoulos D, Toulgaridis T, Davlouros P, Christodoulou J, Sitafidis G, Hahalis G, et al. Prognostic significance of coronary artery calcium in asymptomatic subjects with usual cardiovascular risk. Am Heart J. 2003;145(3):542-8.
4. Scheinert D, Laird JR, Jr., Schroder M, Steinkamp H, Balzer JO, Biamino G. Excimer laser-assisted recanalization of long, chronic superficial femoral artery occlusions. J Endovasc Ther. 2001;8(2):156-66.
5. Scheinert D, Braunlich S, Scheinert S, Ulrich M, Biamino G, Schmidt A. Initial clinical experience with an IVUS-guided transmembrane puncture device to facilitate recanalization of total femoral artery occlusions. EuroIntervention. 2005;1(1):115-9.
6. Wilson PW, Kauppila LI, O'Donnell CJ, Kiel DP, Hannan M, Polak JM, et al. Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. Circulation. 2001;103(11):1529-34.
7. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. Atherosclerosis. 1997;132(2):245-50.
8. Okuno S, Ishimura E, Kitatani K, Fujino Y, Kohno K, Maeno Y, et al. Presence of abdominal aortic calcification is significantly associated with all-cause and cardiovascular mortality in maintenance hemodialysis patients. Am J Kidney Dis. 2007;49(3):417-25.
9. Verbeke F, Van Biesen W, Honkanen E, Wikstrom B, Jensen PB, Krzesinski JM, et al. Prognostic value of aortic stiffness and calcification for cardiovascular events and mortality in dialysis patients: outcome of the calcification outcome in renal disease (CORD) study. Clin J Am Soc Nephrol. 2011;6(1):153-9.
10. Lindholt JS. Aneurysmal wall calcification predicts natural history of small abdominal aortic aneurysms. Atherosclerosis. 2008;197(2):673-8.
11. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15(4):827-32.
12. Jayalath RW, Jackson P, Golledge J. Quantification of abdominal aortic calcification on CT. Arterioscler Thromb Vasc Biol. 2006;26(2):429-30.
13. Kabaya T, Nitta K, Kimura H, Kawashima A, Narusawa K, Nihei H. Increased aortic calcification index in hemodialysis patients. Nephron. 1999;81(3):354-5.
14. Kinsella S, Murphy K, Breen M, O'Neill S, McLaughlin P, Coyle J, et al. Comparison of single CT scan assessment of bone mineral density, vascular calcification and fat mass with standard clinical measurements in renal transplant subjects: the ABC HeART study. BMC Nephrol. 2015;16:188.
15. Ohya M, Otani H, Kimura K, Saika Y, Fujii R, Yukawa S, et al. Improved assessment of aortic calcification in Japanese patients undergoing maintenance hemodialysis. Intern Med. 2010;49(19):2071-5.
16. Akamatsu M, Ogawa T, Fujiu A, Matsuda N, Nitta K. Clinical assessment of atherosclerotic parameters and cardiac function in chronic hemodialysis patients. Clin Exp Nephrol. 2009;13(6):651-8.
17. Schousboe JT, Wilson KE, Kiel DP. Detection of abdominal aortic calcification with lateral spine imaging using DXA. J Clin Densitom. 2006;9(3):302-8.
18. Heilmaier C, Koester A, Moysidis T, Weishaupt D, Kroger K. Abdominal aortic calcification and its distribution in normal-sized and aneurysmatic abdominal aortas. Vasa. 2014;43(2):132-40.
19. Hendy K, Gunnarsson R, Cronin O, Golledge J. Infra-renal abdominal aortic calcification volume does not predict small abdominal aortic aneurysm growth. Atherosclerosis. 2015;243(1):334-8.
20. Parr A, McLaughlin S, McLaughlin M, McHir JG. Aortic calcification and abdominal aortic aneurysm expansion. Atherosclerosis. 2009;202(2):350.
21. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PWF. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. Atherosclerosis. 1997;132(2):245-50.
22. Erhart P, Grond-Ginsbach C, Hakimi M, Lasitschka F, Dihlmann S, Bockler D, et al. Finite element analysis of abdominal aortic aneurysms: predicted rupture risk correlates with aortic wall histology in individual patients. J Endovasc Ther. 2014;21(4):556-64.
23. O'Leary SA, Mulvihill JJ, Barrett HE, Kavanagh EG, Walsh MT, McGloughlin TM, et al. Determining the influence of calcification on the failure properties of abdominal aortic aneurysm (AAA) tissue. J Mech Behav Biomed Mater. 2015;42:154-67.
24. Maier A, Gee MW, Reeps C, Eckstein HH, Wall WA. Impact of calcifications on patient-specific wall stress analysis of abdominal aortic aneurysms. Biomech Model Mechanobiol. 2010;9(5):511-21.
25. Stather PW, Sayers RD, Cheah A, Wild JB, Bown MJ, Choke

E. Outcomes of endovascular aneurysm repair in patients with hostile neck anatomy. Eur J Vasc Endovasc Surg. 2012;44(6):556-61.
26. Mackie CM, Elliot L, Thomson DA, Bigrigg A, McAllister KF. Public and private collaboration in establishing a young person's sexual health clinic in a commercial setting. J Fam Plann Reprod Health Care. 2002;28(4):201-3.
27. Albertini J, Kalliafas S, Travis S, Yusuf SW, Macierewicz JA, Whitaker SC, et al. Anatomical risk factors for proximal perigraft endoleak and graft migration following endovascular repair of abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2000;19(3):308-12.
28. Smeds MR, Jacobs DL, Peterson GJ, Peterson BG. Short-term outcomes of the C3 excluder for patients with abdominal aortic aneurysms and unfavorable proximal aortic seal zones. Ann Vasc Surg. 2013;27(1):8-15.
29. Wyss TR, Dick F, Brown LC, Greenhalgh RM. The influence of thrombus, calcification, angulation, and tortuosity of attachment sites on the time to the first graft-related complication after endovascular aneurysm repair. J Vasc Surg. 2011;54(4):965-71.
30. Bastos Goncalves F, Hoeks SE, Teijink JA, Moll FL, Castro JA, Stolker RJ, et al. Risk factors for proximal neck complications after endovascular aneurysm repair using the endurant stentgraft. Eur J Vasc Endovasc Surg. 2015;49(2):156-62.
31. Gitlitz DB, Ramaswami G, Kaplan D, Hollier LH, Marin ML. Endovascular stent grafting in the presence of aortic neck filling defects: early clinical experience. J Vasc Surg. 2001;33(2):340-4.
32. Rai D, Wisniowski B, Bradshaw B, Velu R, Tosenovsky P, Quigley F, et al. Abdominal aortic aneurysm calcification and thrombus volume are not associated with outcome following endovascular abdominal aortic aneurysm repair. Eur Radiol. 2014;24(8):1768-76.
33. Barrett HE, Cunnane EM, Hidayat H, O'Brien JM, Moloney MA, Kavanagh EG, et al. On the influence of wall calcification and intraluminal thrombus on prediction of abdominal aortic aneurysm rupture. J Vasc Surg. 2017;67(4):1234-46.

CHAPTER 6 ABDOMINAL AORTIC ANEURYSM CALCIFICATION: TRYING TO IDENTIFY A RELIABLE SEMIQUANTITATIVE METHOD

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ORIGINAL ARTICLE
VASCULAR SECTION

Abdominal aortic aneurysm calcification: trying to identify a reliable semiquantitative method

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ABSTRACT

BACKGROUND: The main objective of this study was to assess the correlation between three semiquantitative and one computerized method based on Agatston Score (AS), when measuring abdominal aortic calcification (AAC) in abdominal aortic aneurysm (AAA) patients. Secondary aim was to access differences in AAC upon clinical variables, when different methods of calcium scoring are used.
METHODS: This was an observational, retrospective, cross-sectional study. A database of AAA patients consecutively submitted to elective repair between 2008 and 2015 was used. Patients were excluded if they did not have preoperative imaging or presented scans incompatible with at least one of the whole set of calcification methods tested. Calcification measures were performed using AS, Aortic Calcification Index (ACI), AAC-8 and AAC-24 methods. The Pearson's correlation was used for primary analysis.
RESULTS: Study population comprised 102 patients, 95% males, with a median age of 71 (interquartile range, IQR 66-76) years. AAAs presented median aortic diameter of 60 (54-70) mm. Pearson's correlation with AS was 0.816 for ACI, 0.703 for AAC-8 and 0.648 for AAC-24. ACI also presented the highest ICC for intraobserver agreement (0.972) and for interobserver agreement (0.966). ACI was associated more often to demographic and clinical variables in the dataset that associated with the computerized method.
CONCLUSIONS: ACI is suggested as a fast and easy-to-use method of assessing AAC in AAA patients. Its use should be encouraged to study AAC in AAA over other semiquantitative methods, in research settings.

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KEY WORDS: Aortic aneurysm, abdominal; Vascular calcification; Tomography, X-ray computed; Observational study.

Abdominal aortic aneurysm (AAA) is a major cause of mortality in Western countries.[1] The high mortality after rupture and the current lack of strategies to prevent aneurysmal progression justify the continuous attempt to identify risk factors for the presence and progression of AAA, as well as predictors of outcome after treatment.

Abdominal aortic wall calcification (AAC) has been recognized as an independent predictor of cardiovascular mortality for a long time.[2] Nevertheless, its role in the pathogenesis and outcome of AAA is still controversial.

AAC has been studied as a risk factor for AAA expansion and rupture. The role of AAC in AAA growth is still conflicting, with some studies suggesting a negative association[3, 4] between AAC and AAA growth while others report lack of such association.[5, 6] The available evidence comes from imaging studies, mostly based on multi-detector computed tomography (MDCT), previously used for quantification of coronary artery calcification.

The possible impact of AAC in AAA rupture has been studied by in vitro analysis of AAA wall properties and

using computer simulations (finite element analysis) in addition to traditional imaging studies. Computed tomographic angiography (CTA)-based studies[1] showed that symptomatic and ruptured aneurysms had increased calcification scores when compared to asymptomatic aneurysms. In vitro studies evidenced that regions of the aortic wall with high rupture risk index present advanced histological disintegrity with increased amount of calcified plaques.[7] However, assessment of failure properties using mechanical tests were significantly reduced in partially calcified tissue from AAA compared to fibrous tissue[8] and an overall increase in the risk of rupture with calcification has also been challenged.[9] AAC has also been studied as a predictor of outcome after EVAR. Neck calcification has been recognized as an independent predictor of complications during and after EVAR,[10, 11] while other studies failed to detect such an association.[12, 13]

One reason for the great heterogeneity among AAC impact in AAA might be the use of different methods of assessing its severity instead of a simple, validated method. The establishment of a fast and easy-to-use method of assessing AAC can be of value in research settings, ultimately leading to a better understanding of the clinical implications of AAC in AAA. The aim of this study was to assess the performance of three semiquantitative methods compared to a computerized one in scoring AAC. A secondary goal was to access differences in AAC upon clinical variables, when different methods of calcium scoring are used.

Materials and methods

Study design and sample

This is a cross sectional study of patients undergoing elective AAAs repair at the Department of Angiology and Vascular Surgery of São João Hospital Center, between March 2008 and December 2015. Patients were consecutively included unless a preoperative CT/CTA) scan was not available or was incompatible with at least one of the whole set of calcification methods tested. Otherwise, respective clinical data and CT/CTA parameters were analyzed.

Ethics

The study was approved by the Ethics Committee of São João Hospital Center.

Data collection

Patient data were collected from the database at our home institution. The following clinical characteristics of the included patients were recorded: age (defined as patient age at the date of the last CT/CTA scan), sex, smoking status (defined as either "non-smoker" or "past or current smoker"), diabetes mellitus (DM) (defined as either "no" or "under oral medication or insulin medication"); hypertension (defined as either "no" or "controlled with one or more drugs"), carotid artery disease (CD) (defined as either "no" or "yes" including asymptomatic significant stenosis (stenosis >50%), history of transient ischemic attack or history of ischemic stroke), coronary heart disease (defined as either "no" or "yes" comprising unstable angina, myocardial infarction or previous endovascular or open coronary revascularization), preoperative plasma creatinine, chronic obstructive pulmonary disease (defined as either "no" or "yes" based on spirometry results when available or in the medical records), obesity (defined as a Body Mass Index >30 kg/m^2), congestive heart failure, peripheral arterial disease (PAD) (defined as either "absent" or "asymptomatic" and "intermittent claudication (Fontaine IIa/b or Rutherford 1-3)" or "critical limb ischemia (Fontaine III/IV or Rutherford 4-6)"), and routine medication with statins.

CT/CTA imaging protocol

All subjects underwent CT/CTA scanning in a supine position using a 64-slice MDCT. Scan parameters were determined by the Right Dose indicated by manufacturer, that aims at finding the right dose for every individual patient, balancing between image quality and radiation dose. Slice thickness of the available scans was 5mm. Osirix Medical Imaging Software was used for measurements. Contrast enhancement was adjusted with a windows level (contrast) of 143 Hounsfield Units (HU) and a windows width (brightness) of 411 HU.

Assessment of maximum AAA diameter and AAA volume

The main axis of each AAA was determined and maximum AAA diameter was obtained as the maximum length between opposite walls of the AAA in a plane perpendicular to that main axis. For the calculation of the AAA volume, the region located immediately below the emergence of the lowest renal artery and immediately above the aortic bifurcation was considered. In each slice, a region of interest was manually selected and the volume was automatically calculated using a dedicated software (Osirix® v.8.0.2, Bernex, Switzerland).

Assessment of abdominal aortic calcification

AAC was assessed using 4 different methods. The computerized AS[14] modified to account for slice thickness

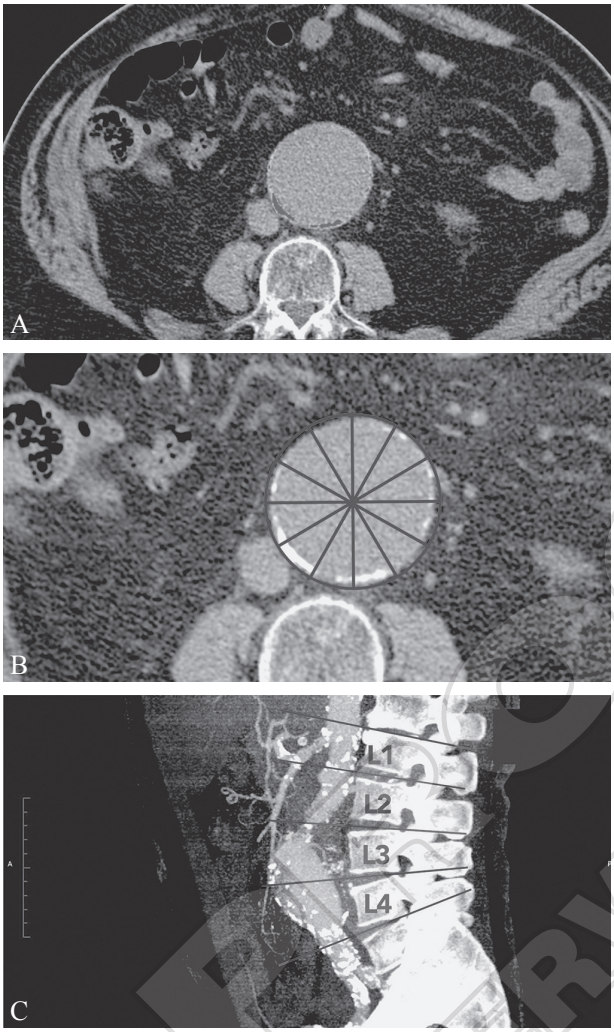

Figure 1.—CT scan view of an AAA on the transversal plane, depicting the calcium scoring through the Agatston Score (A) and Aortic Calcification Index (B) methods. An example of a maximum intensity projection on the sagittal plane used for calcium scoring through the AAC-8 or AAC-24 methods (C).

was considered our gold standard (Figure 1A). The Osirix plugin "calcium scoring" was used for this determination. Calcification was considered as a region of ≥1mm² with a density of >130 HU in each slice from the level of the lowest renal artery to the aortic bifurcation. For this study, a slice thickness of 5mm was considered as standard. Nevertheless, the score obtained on CT scans of different thickness may be adjusted using the following formula: adjusted score =original score × (slice thickness/5.0). Because a 5mm slice thickness was considered in this study, the number of slices analyzed varied from 17 to 28 (depending on the total length of infra-renal aortas). This method is computerized, therefore less prone to observer bias and provides a continuous outcome; nevertheless, it is more time-consuming since it requires manual selection of the calcified lesions (5 to 10 minutes per patient) and contrast interferes with calcium scoring preventing its use in ACT.

Calcification was also semiquantitatively measured using the Aortic Calcification Index (ACI),[15] the AAC-8[16] and AAC-24[17] scores. To apply the ACI score, 10 slices of the aorta were obtained at 10 mm intervals moving uphill from the aortic bifurcation, each slice was divided in 12 equal radial sectors and ACI was expressed as the number of calcified sectors divided by 120 (Figure 1B). ACI is faster in trained hands (takes about 5 minutes per patient) and provides an ordinal outcome that varies from 0 to 120 or from 0 to 1 (depending on using 120 as a denominator or not). In patients with CT/CTA scans whose thickness was 3 mm or 4mm or in short length aortas it was not possible to apply the ACI as defined above. The AAC-24 and AAC-8 methods were applied on sagittal profiles of the aorta. For this, on each CT/CTA image, a maximum intensity projection was selected, segments were drawn between the planes of the first 4 lumbar vertebrae and the aortic calcification contained in those resulting 4 segments was measured (Figure 1C). For the AAC-24 method the score is attributed according to the length of the aortic calcification (anterior or posterior wall) in each of the 4 segments in front of the lumbar vertebrae. If calcification in a segment is nonexistent the score is 0; if it occupies ≤1/3 of the aortic wall length the value is 1; if it's >1/3 but ≤2/3, the value is 2; finally, if it's >2/3 the value is 3. For the AAC-8 method the score is attributed according to the calcification length relative to vertebral height. If calcification is nonexistent, the value is 0; if it's ≤1 vertebra, the value is 1; if it is >1 but ≤2 vertebrae, the value is 2; if it is >2 but ≤3 the value is 3; finally, if calcification is >3 vertebrae in length, the value is 4. These methods are also fast in trained hands (takes about 3 minutes per patient) and provides an ordinal outcome that varies from 0 to 8 in AAC-8 or from 0 to 24 in AAC-24. Extreme aortic tortuosity might prevent both ACC-24 and ACC-8 calculation.

Reproducibility

To express how consistent each observer was within their own calcification scores (intraobserver agreement), intraclass correlation coefficient (ICC) was calculated based on two analyses performed by a single analyst in a subset of 30 randomly selected patients. To express how one ob-

server correlates with other observers performing the same technique interobserver agreement), ICC was used to compare the analyses performed by two independent analysts in another subset of 30 randomly selected patients.

Statistical analysis

Continuous variables were expressed as mean±standard deviation (SD) when normally distributed and as median and interquartile range (IQR) when skewed. Categorical variables were expressed as percentages. Pearson's correlation test was used to assess the comparison between the three semiquantitative methods for quantification of AAC and the computerized one because a linear relation was assumed. Finally, in the secondary analysis, the association between AAC and clinical variables was studied using AS, ACI, AAC-8 and AAC-24 using a non-parametric Mann Whitney U Test.

Results

Between March 2008 and December 2015, a total of 196 patients were admitted undergoing elective AAA repair. Patient flowchart is shown in Figure 2.

We included 102 patients in the analysis, 97 (95.1%) men and 5 (4.9%) women, with a median age of 71 years and IQR of 10 years. The baseline clinical characteristics are shown in Table I. Aneurysm proprieties and calcification scores of studied patients are depicted in Table II. ICC for intraobserver agreement were 0.999 (P<0.001) for AS, 0.972 (P<0.001) for ACI, 0.827 (P<0.001) for ACC-8 and 0.723 (P<0.001) for ACC-24; for interobserver agreement the coefficients were 0.996 (P<0.001) for AS, 0.966 (P<0.001) for ACI, 0.902 (P<0.001) for ACC-8 and 0.875 (P<0.001) for ACC-24.

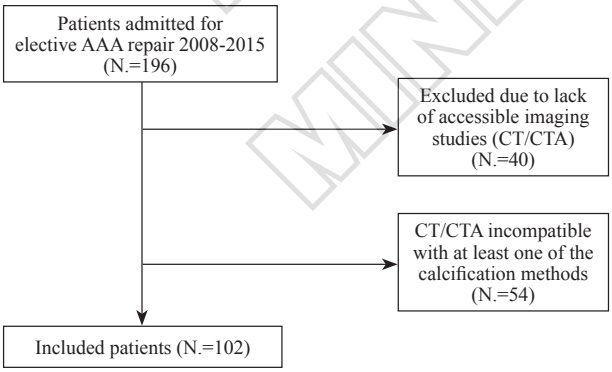

Figure 2.—Study flowchart.

TABLE I.—*Clinical characteristics of 102 included patients.*

Clinical characteristics	Values[a]
Age (years)	71 (66-76)
Male	97/102 (95%)
Female	5/102 (5%)
Medical history	
Smoking	
Former smoker	31/92 (34%)
Current smoker	36/92 (39%)
Diabetes	18/97 (19%)
Hypertension	71/93 (76%)
Obesity	23/97 (24%)
On statin medication	63/81 (79%)
Coronary heart disease	29/95 (30%)
Chronic heart failure	15/91 (17%)
Carotid artery disease	14/95 (15%)
Chronic renal disease	9/95 (10%)
Preoperative creatinine (mg/dL)	0.98 (0.80-1.25)
Peripheral artery disease	18/91 (20%)

aValues are median (interquartile range) or numbers (%) of observations.

TABLE II.—*Aneurysm proprieties and calcification scores of 102 patients.*

Aneurysm proprieties	Values[a]
Maximum diameter (mm)	60 (54-70)
Aortic volume (cm³)	175 (136-259)
Aortic calcification	
Agatston score (units)	2512 (991-4312)
ACI (units)	38 (23-57)
AAC-8 (units)	3 (2-4)
AAC-24 (units)	7 (5-10)

aValues are median (interquartile range) or numbers (%) of observations. Abbreviations: ACI, aortic calcification index.

ACI had the highest correlation with AS (0.816, P<0.001), followed by AAC-8 (0.703, P<0.001) and AAC-24 (0.648, P<0.001) (Figure 3A, B, C).

Using the computerized method AS, AAC was higher in older patients (Spearman's correlation 0.210, P=0.034) and in females (6122 (5174-10027) *vs.* 2457 (979-4077), P=0.003). ACI method could detect differences in gender (86 (45-86) in women *vs.* 37 (22-54) in men, P=0.010). No other differences were detected using ACI, AAC-24 or AAC-8 upon demographic variables.

When assessing clinical variables, AS calcification was higher in patients with CD (3640 [2493-6606] *vs.* 2263 [860-3765], P=0.027) and with PAD (4340 [2304-5942] *vs.* 2457 [979-3594], P=0.023). When using ACI, differences were apparent in PAD and chronic heart failure, being higher in diseased patients (Table III). Additionally, ACI was 49 (43-62) in patients with CD *vs.* 36 (22-54) in patients without, P=0.055.

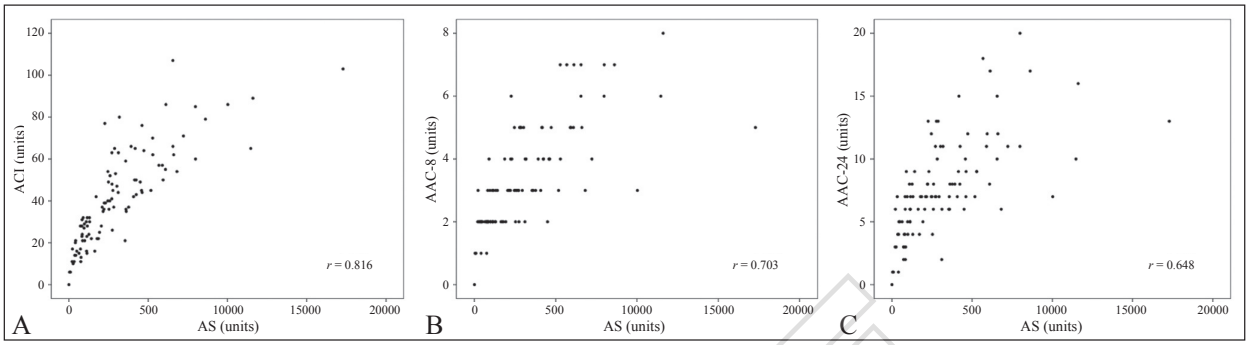

Figure 3.—Scatterplots for the correlation between different measures of aortic calcification: Agatston Score (AS) and Aortic Calcification Index (ACI) (A), AS and AAC-8 (B) and AS and AAC-24 (C). Pearson's correlation are presented (P<0.001, N.=102 patients).

TABLE III.—*Secondary analysis of calcification differences upon clinical characteristics using different methods: AS, ACI, AAC8 and AAC24.*

Clinical characteristics		AS	ACI	AAC8	AAC24
Age (years)	Spearman	0.210	0.127	0.137	0.162
	P	0.034	0.207	0.168	0.104
Gender	Female	6122 (5174-10027)	86 (45-86)	4 (3-7)	10 (7-16)
	Male	2457 (979-4077)	37 (22-54)	3 (2-4)	7 (5-9)
	P	0.003	0.010	0.068	0.067
Smoking (present or past)	No	2705 (956-4178)	39 (27-50)	3 (3-5)	7 (7-10)
	Yes	2531 (1131-4250)	37 (22-55)	3 (2-4)	7 (5-9)
	P	0.850	0.878	0.171	0.236
Diabetes	No	2457 (860-4178)	37 (22-55)	3 (2-4)	7 (5-9)
	Yes	2655 (1131-4250)	43 (32-49)	4 (2-4)	8 (6-11)
	P	0.414	0.449	0.454	
Hypertension	No	2714 (2099-4238)	41 (35-54)	3 (3-4)	7 (6-8)
	Yes	2459 (830-4581)	36 (21-57)	3 (2-4)	7 (5-11)
	P	0.426	0.286	0.254	0.870
Obesity	No	2495 (995-4581)	39 (24-57)	3 (2-4)	7 (5-9)
	Yes	2493 (830-4077)	32 (16-49)	3 (2-5)	7 (5-11)
	P	0.576	0.105	0.440	0.848
On statin medication	No	2590 (860-4146)	40 (30-55)	3 (3-4)	7 (6-8)
	Yes	2493 (979-4611)	37 (22-54)	3 (2-4)	7 (5-10)
	P	0.991	0.654	0.547	0.859
Coronary heart disease	No	2557 (901-4146)	37 (22-53)	3 (2-4)	7 (5-9)
	Yes	2457 (1131-4501)	44 (28-63)	3 (2-5)	7 (6-11)
	P	0.668	0.314	0.806	0.384
Chronic heart failure	No	2493 (901-4238)	37 (22-54)	3 (2-4)	7 (5-9)
	Yes	2778 (2157-6555)	47 (35-85)	3 (3-6)	11 (7-13)
	P	0.128	0.048	0.050	0.016
Carotid artery disease	No	2263 (860-3765)	36 (22-54)	3 (2-4)	7 (5-9)
	Yes	3640 (2493-6606)	49 (43-62)	4 (3-5)	8 (7-12)
	P	0.027	0.055	0.107	0.045
Chronic renal disease	No	2476 (1097-4238)	37 (22-54)	3 (2-4)	7 (5-9)
	Yes	3030 (2304-5679)	47 (39-57)	5 (4-5)	12 (9-13)
	P	0.253	0.245	0.041	0.022
Peripheral artery disease	No	2457 (979-3594)	36 (22-53)	3 (2-4)	7 (5-9)
	Yes	4340 (2304-5942)	51 (39-64)	4 (2-5)	9 (6-12)
	P	0.023	0.031	0.073	0.078
Maximum aortic diameter (cm)	Spearman	0.063	-0.68	-0.054	-0.144
	P	0.528	0.500	0.593	0.149

aValues are median (interquartile range) or numbers (%) of observations.

Using ACC8 revealed higher calcification in patients with chronic renal disease. AAC-24 differences were apparent in patients with CD, with chronic heart failure and with chronic renal disease (Table III).

Discussion

In this study, we assessed the correlation between three semiquantitative methods and one computerized method of measuring AAC in the specific field of AAAs. We found that, in elective AAA patients, the amount of aortic calcification measured by the ACI method has a high correlation to that measured using the AS. Furthermore, of the three semiquantitative methods used, ACI was the most reproducible and was associated more often to demographic and clinical variables in the dataset that associated with the AS. Notably, different methods led to identification and valorization of different clinical variables, which agrees to the authors assumption that the use different methods to assess AAA AAC might contribute to conflicting reports noticed in the literature.

To the extent of our knowledge, only one study applied one of the abovementioned methods in the study of AAA calcification using CT/CTA, more specifically, the AAC-8. Furthermore, there are no studies comparing the performance of different methods available for this purpose.

We chose the Agatston method as our gold standard to measure AAC mainly because it is a computerized quantitative method. Its major application is quantification of coronary artery calcium[18, 19] and the amount of research using it to quantify AAC is relatively smaller and on non-aneurysmatic aortas.[20, 21] The fact that the observer had to manually select calcified regions of the aorta on two different occasions probably explains why the ICC for intraobserver agreement was not 1. However, the values obtained have been considered good regarding the reproducibility of an abdominal aortic calcium measurement methods[20, 22, 23]

The correlation between AS and ACI was 0.816 despite not addressing the exact same aortic segments. While AS was calculated from the level of the lowest renal artery to the aortic bifurcation, ACI was used as described in the literature and accounted for 10 cm of the aorta starting at the level of the aortic bifurcation and moving uphill. We speculate that correlation might have been even better if a modified method of ACI would have been used, that is, covering the whole infrarenal aortic segment. Segment adjustment might have to be taken into consideration in future studies if regional aortic study is warranted.

Our patient database contained preoperative CT and/or CTA scans of AAA patients. Nowadays, the preferred method to study AAAs is CTA. The Agatston method is inapplicable in CTA imaging because the signal generated by the intravascular contrast medium interferes with that generated by the calcification of the aortic wall. This might explain the general lack of studies concerning the use of the Agatston method in studying AAA calcification, when compared to its common use in studying non-aneurysmatic AAC. Since the preferred imaging modality used to study AAAs is CTA, it may be questionable to perform both imaging modalities (CT/CTA) and submit patients to a double radiation exposure to study their AAAs. The semiquantitative methods that we used in this study, namely ACI, offer an advantage over the AS due to their applicability both in CT and CTA imaging. Likewise, modern workstations use volumetric methods to score AAA calcification and can be used in both imaging modalities. Nevertheless, its use is cumbersome and not readily available in most settings.

The research implication of this issue should be reinforced. Other authors have reported on the considerable variation in the methods used by different investigators in assessing AAA calcification, making interpretation of these studies difficult.[13] Taking studies that assess the effect of proximal neck calcification after EVAR as an example, methods used to quantify calcification are most often ACT-based; definition of calcified neck among those studies include: qualitative assessment of the presence of "severe calcification,"[12] percentage of circumferential calcification in CT slices that are considered representative of the sealing zone,[24] proportional volume of calcium compared to total aortic volume in a specific aortic segment (neck);[11] in some studies, the presence of calcification and thrombus are reported jointly.[10] The presence of calcification at the proximal sealing zone might prevent optimal adherence between the endoprosthesis and the aortic wall, leading to *endoleak*s and migration or might increase the risk of thromboembolism during the procedure.[25] In the study of Wyss *et al.*,[11] the presence of neck calcification assessed by the volumetric method correlated with higher adverse event rates. Notwithstanding, the study of Albertini *et al.*[12] where a qualitative assessment was used, such association was not demonstrated. It is possible that that variation in the methods used may, at least partially, justify this discrepancy.

The impact of AAC upon clinical variables were different when different methods of calcium scoring were used. This seems to be in agreement with our hypothesis that the heterogeneity in calcium scoring might be a con-

tributing factor to the apparent incoherence between different studies. When using AS, AAC was higher in older patients and in female versus males, in patients with CD and PAD. Abdominal aorta was reported as the commonest site of vascular calcification in women.[26] While calcification seem to progress at similar rates in both genders, in women a significant correlation between bone loss and the rate of increase in aortic calcification has been described.[27] In this series, females were older than males (median of 78 years and IQR of 15 years in females *vs.* 70 and 10 years in males) which may, at least in part, justify the gender differences in AAC. Besides its putative direct effects in aortic pathology, AAC is seen as a marker of atherosclerotic burden.[28] Positive correlation with PAD was already documented in non-aneurysmatic aortas.[29, 30] To the extent of the authors knowledge, the positive correlation of AAC with CD was addressed for the first time in AAA patients, but this finding requires further adjustment for confounding (this study was not powered enough to accomplish that).

Limitations of the study

There are some limitations to our study. First, data was collected retrospectively and collection of demographic and clinical variables was dependent on medical records. Nevertheless, the focus of this work is based on CT/CTA analysis that was not compromised by a retrospective inclusion of patients. Second, the method we used as our gold standard was only applicable in CT scans and not on CTA scans, the most used method in the study of AAAs. Third, a great number of patients was not included in the study and it is not possible to know if their inclusion would change our results. Fourth, the main analysis was performed by a single analyst. As reproducibility analysis in a subset of 30 patients was excellent, both for intraobserver and for interobserver agreement, the impact of replicating the analysis in the entire sample (reporting the average of the measurements by two analysis) would be neglectable.

Conclusions

The role of AAC in AAA has been assessed using different and varied methods often yielding diverging results. The establishment of a fast and easy-to-use method of assessing AAC can be of value both in AAA clinical and research settings. We found that, in AAA patients, the severity of aortic calcification measured by the semiquantitative ACI has a high correlation to that measured using the computerized method based on AS. ACI was also the most reproducible and the semiquantitative method that associated more often to demographic and clinical variables that associated with the computerized method. These findings suggest that ACI is a very reasonable tool to measure ACC in AAA and its use should be encouraged instead of other semiquantitative methods in research settings. This paper also underlines the upcoming need for automation in scoring calcification and other aortic proprieties, ultimately leading to a clearer definition of the clinical implication of AAC in AAA.

References

1. Buijs RV, Willems TP, Tio RA, Boersma HH, Tielliu IF, Slart RH, *et al.* Calcification as a risk factor for rupture of abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 2013;46:542–8.

2. Witteman JC, Kok FJ, van Saase JL, Valkenburg HA. Aortic calcification as a predictor of cardiovascular mortality. Lancet 1986;2:1120–2.

3. Heilmaier C, Koester A, Moysidis T, Weishaupt D, Kröger K. Abdominal aortic calcification and its distribution in normal-sized and aneurysmatic abdominal aortas. Vasa 2014;43:132–40.

4. Lindholt JS. Aneurysmal wall calcification predicts natural history of small abdominal aortic aneurysms. Atherosclerosis 2008;197:673–8.

5. Hendy K, Gunnarsson R, Cronin O, Golledge J. Infra-renal abdominal aortic calcification volume does not predict small abdominal aortic aneurysm growth. Atherosclerosis 2015;243:334–8.

6. Parr A, Mclaughlin S, Mclaughlin M, Mchir JG. Aortic calcification and abdominal aortic aneurysm expansion. Atherosclerosis 2009;202:350.

7. Erhart P, Grond-Ginsbach C, Hakimi M, Lasitschka F, Dihlmann S, Böckler D, *et al.* Finite element analysis of abdominal aortic aneurysms: predicted rupture risk correlates with aortic wall histology in individual patients. J Endovasc Ther 2014;21:556–64.

8. O'Leary SA, Mulvihill JJ, Barrett HE, Kavanagh EG, Walsh MT, McGloughlin TM, *et al.* Determining the influence of calcification on the failure properties of abdominal aortic aneurysm (AAA) tissue. J Mech Behav Biomed Mater 2015;42:154–67.

9. Maier A, Gee MW, Reeps C, Eckstein HH, Wall WA. Impact of calcifications on patient-specific wall stress analysis of abdominal aortic aneurysms. Biomech Model Mechanobiol 2010;9:511–21.

10. Bastos Goncalves F, Hoeks SE, Teijink JA, Moll FL, Castro JA, Stolker RJ, *et al.* Risk factors for proximal neck complications after endovascular aneurysm repair using the endurant stentgraft. Eur J Vasc Endovasc Surg 2015;49:156–62.

11. Wyss TR, Dick F, Brown LC, Greenhalgh RM. The influence of thrombus, calcification, angulation, and tortuosity of attachment sites on the time to the first graft-related complication after endovascular aneurysm repair. J Vasc Surg 2011;54:965–71.

12. Albertini J, Kalliafas S, Travis S, Yusuf SW, Macierewicz JA, Whitaker SC, *et al.* Anatomical risk factors for proximal perigraft endoleak and graft migration following endovascular repair of abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2000;19:308–12.

13. Rai D, Wisniowski B, Bradshaw B, Velu R, Tosenovsky P, Quigley F, *et al.* Abdominal aortic aneurysm calcification and thrombus volume are not associated with outcome following endovascular abdominal aortic aneurysm repair. Eur Radiol 2014;24:1768–76.

14. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–32.

15. Kabaya T, Nitta K, Kimura H, Kawashima A, Narusawa K, Nihei

H. Increased aortic calcification index in hemodialysis patients. Nephron 1999;81:354–5.

16. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. Atherosclerosis 1997;132:245–50.

17. Schousboe JT, Taylor BC, Kiel DP, Ensrud KE, Wilson KE, McCloskey EV. Abdominal aortic calcification detected on lateral spine images from a bone densitometer predicts incident myocardial infarction or stroke in older women. J Bone Miner Res 2008;23:409–16.

18. Martin SS, Blaha MJ, Blankstein R, Agatston A, Rivera JJ, Virani SS, *et al.* Dyslipidemia, coronary artery calcium, and incident atherosclerotic cardiovascular disease: implications for statin therapy from the multi-ethnic study of atherosclerosis. Circulation 2014;129:77–86.

19. Wing JJ, August E, Adar SD, Dannenberg AL, Hajat A, Sánchez BN, *et al.* Change in Neighborhood Characteristics and Change in Coronary Artery Calcium: A Longitudinal Investigation in the MESA (Multi-Ethnic Study of Atherosclerosis) Cohort. Circulation 2016;134:504–13.

20. Liu J, Musani SK, Bidulescu A, Carr JJ, Wilson JG, Taylor HA, *et al.* Fatty liver, abdominal adipose tissue and atherosclerotic calcification in African Americans: the Jackson Heart Study. Atherosclerosis 2012;224:521–5.

21. Forbang NI, McClelland RL, Remigio-Baker RA, Allison MA, Sandfort V, Michos ED, *et al.* Associations of cardiovascular disease risk factors with abdominal aortic calcium volume and density: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2016;255:54–8.

22. Jayalath RW, Jackson P, Golledge J. Quantification of abdominal aortic calcification on CT. Arterioscler Thromb Vasc Biol 2006;26:429–30.

23. Ichii M, Ishimura E, Shima H, Ohno Y, Ochi A, Nakatani S, *et al.* Quantitative analysis of abdominal aortic calcification in CKD patients without dialysis therapy by use of the Agatston score. Kidney Blood Press Res 2013;38:196–204.

24. Smeds MR, Jacobs DL, Peterson GJ, Peterson BG. Short-term outcomes of the C3 excluder for patients with abdominal aortic aneurysms and unfavorable proximal aortic seal zones. Ann Vasc Surg 2013;27:8–15.

25. Gitlitz DB, Ramaswami G, Kaplan D, Hollier LH, Marin ML. Endovascular stent grafting in the presence of aortic neck filling defects: early clinical experience. J Vasc Surg 2001;33:340–4.

26. Allison MA, Criqui MH, Wright CM. Patterns and risk factors for systemic calcified atherosclerosis. Arterioscler Thromb Vasc Biol 2004;24:331–6.

27. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. Calcif Tissue Int 2001;68:271–6.

28. Jayalath RW, Mangan SH, Golledge J. Aortic calcification. Eur J Vasc Endovasc Surg 2005;30:476–88.

29. Niskanen LK, Suhonen M, Siitonen O, Lehtinen JM, Uusitupa MI. Aortic and lower limb artery calcification in type 2 (non-insulin-dependent) diabetic patients and non-diabetic control subjects. A five year follow-up study. Atherosclerosis 1990;84:61–71.

30. Mitchell JR, Adams JH. Aortic size and aortic calcification. A necropsy Study. Atherosclerosis 1977;27:437–46.

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IIB THE ROLE OF PERIAORTIC ADIPOSE TISSUE IN ABDOMINAL AORTIC ANEURYSMS

CHAPTER 7 NOVOS CONCEITOS SOBRE ADIPOSIDADE NA PATOLOGIA DA AORTA ABDOMINAL | NEW CONCEPTS IN ADIPOSE TISSUE AND ABDOMINAL AORTIC PATHOLOGY

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ARTIGO DE REVISÃO

NOVOS CONCEITOS SOBRE ADIPOSIDADE NA PATOLOGIA DA AORTA ABDOMINAL

NEW CONCEPTS IN ADIPOSE TISSUE AND ABDOMINAL AORTIC PATHOLOGY

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RESUMO

O tecido adiposo perivascular tem tido cada vez mais reconhecimento como um importante produtor de fatores vasoativos e de citocinas que podem ter um efeito parácrino direto na vasculatura devido à sua inerente proximidade. A este respeito, a interação tecido adiposo perivascular-vaso pode ser identificada como um novo alvo terapêutico de doenças vasculares.
Contrariamente a outros depósitos adiposos específicos, cuja importância tem sido crescentemente divulgada, as propriedades do tecido adiposo periaórtico abdominal permanecem obscuras. As substâncias produzidas por este compartimento adiposo podem desempenhar um papel nas vias patológicas que ocorrem em doenças como o aneurisma da aorta abdominal e/ou a aterosclerose aórtica. Abordagens para esclarecer a sua importância são passos fundamentais na definição de estratégias terapêuticas inovadoras que enderecem a adventícia em vez da íntima danificada das artérias.

Palavras-chave
Aneurisma da aorta abdominal, doença arterial periférica, tecido adiposo visceral, tecido adiposo periaórtico.

ABSTRACT

Perivascular adipose tissue has been increasingly recognized as an important producer of vasoactive factors and cytokines that may have a direct paracrine effect on the vasculature due to the inherent proximity. In this regard, perivascular adipose tissue-vessel interaction might be identified as a novel therapeutic target of vascular diseases. Conversely to other specific adipose depots, whose characteristics have been disclosed, proprieties of abdominal periaortic adipose tissue remain obscure. Substances produced by abdominal periaortic adipose tissue may play a role in the pathologic pathways occurring in abdominal aortic aneurism and/or aortic atherosclerosis. Approaches to address this topic are fundamental steps towards innovative therapeutic strategies that address adventitia instead of the damaged intima of peripheral vessels.

Keywords
Abdominal aortic aneurysm, peripheral arterial disease, visceral adipose tissue, periaortic adipose tissue.

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INTRODUÇÃO

Nas últimas décadas foram investidos tempo e esforço na identificação de fatores de risco que podem contribuir para a presença e para a progressão das doenças vasculares. A contínua pesquisa por novos fatores tem como objetivo atingir medidas mais sensíveis de estratificação de risco ou oferecer novos alvos terapêuticos para a prevenção e o tratamento das doenças vasculares.

O excesso de adiposidade associa-se ao aumento da incidência de doenças cardiovasculares.[1] Os mecanismos explicativos incluem alterações na pressão arterial, na regulação da glicemia, no metabolismo lipídico e inflamação sistémica global.[2] Além do armazenamento de energia, o tecido adiposo comporta-se como um órgão endócrino, afetando o metabolismo da glicose e a biologia vascular. A adiposidade visceral tem maior atividade endócrina do que a gordura subcutânea, modulando as concentrações de adipocinas, como a adiponectina e a resistina (marcadores de resistência à insulina), ou como o fator de crescimento transformante α e a interleucina 6 (mediadores inflamatórios). O tecido adiposo perivascular tem sido cada vez mais reconhecido como importante produtor de fatores vasoativos e de citocinas que podem ter um efeito parácrino direto na vasculatura devido à sua inerente proximidade[3] (Figura 1).

A evidência atual é favorável a que o risco cardiovascular não esteja simplesmente ligado à **quantidade** de tecido adiposo, mas também às suas próprias **características** (elementos celulares e secretoma), bem como à **localização** em que este se acumula.[4] Por exemplo, o tecido adiposo visceral presente no tronco e no abdómen parece conferir maior risco cardiovascular do que o tecido adiposo subcutâneo presente na periferia. Consequentemente, a medida da razão perímetro da cintura-anca constitui um melhor preditor de doença cardiovascular do que o tradicionalmente utilizado índice de massa corporal.[5]

Finalmente, há cada vez mais trabalhos que suportam uma associação entre **depósitos específicos** de tecido adiposo e as doenças cardiovasculares. No campo da doença arterial coronária, isto tem sido detalhadamente explorado. A gordura epicárdica foi descrita como um marcador de risco quantificável e independente de doenças cardiovasculares. O seu papel ativo no desenvolvimento e vulnerabilidade da placa da artéria coronária nas artérias que rodeia foi igualmente demostrado[4]. A tabela 1 exibe outros tecidos adiposos específicos e a sua associação com resultados cardiovasculares.

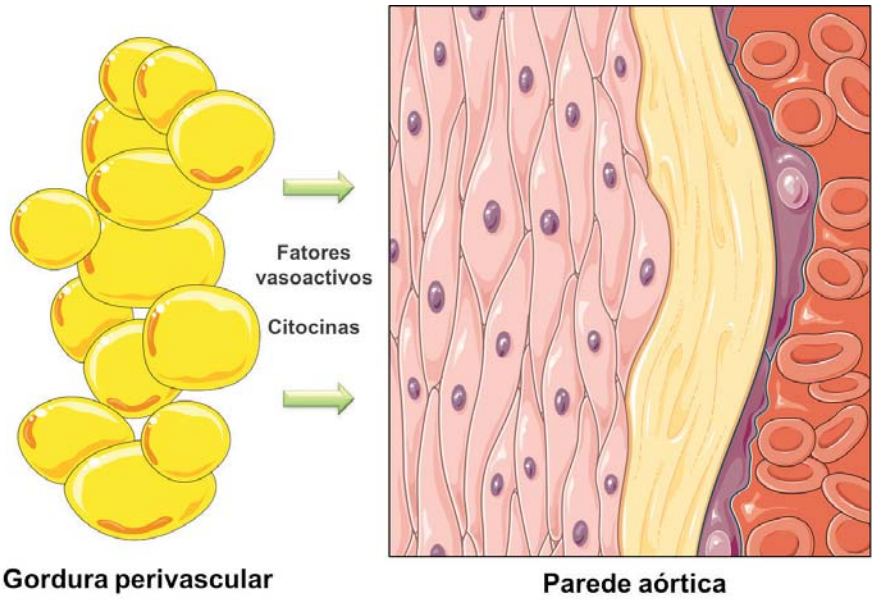

***Figura 01** : O tecido adiposo periaórtico abdominal como produtor de fatores vasoativos de citocinas que podem ter efeito na parede aórtica subjacente.*

Tabela 1 *Estudos que abordam a associação entre depósitos específicos de tecido adiposo e resultados cardiovasculares.*

Referências	Depósitos específicos de tecido adiposo	Participantes	Resultad
Brinkley TE, 2014[20]	Gordura pericárdica, gordura periaórtica da aorta ascendente e do arco aórtico, GV e gordura subcutânea	Adultos com (n = 385, 69 ± 8 anos, 52% mulheres) e sem (n = 50, idade média de 69 anos, 58% mulheres) fatores de risco de eventos CV	Participantes com fatores de risco CV exibir maior quantidade de gordura pericárd periaórtica e GV em comparação com participantes sem fatores de risco. Quar emparelhados individualmente por ida género, raça/etnia e IMC, a gordura periórt foi maior nas pessoas com do que nas pess sem fatores de risco
Britton KA, 2012[21]	GPT e GV	Participantes do Framingham Heart Study (n=3246, 48% mulheres, idade média 51,1 anos)	Após ajuste para a idade e para GV, mulhe e homens com elevada GPT na ausência GV elevada eram mais velhos e apresentav maior prevalência de doenças CV em comp ção com aqueles sem GPT elevada. Os hom neste grupo eram mais propensos a fumadores, enquanto as mulheres eram m propensas a ter baixo colesterol H
Britton KA, 2013[22]	GV, gordura pericárdica e GPT	Participantes do Framingham Heart Study (n= 3,086; 49% mulheres; idade media de 50,2 anos)	A GV foi positivamente associada a doen CV e a cancro. A adição de GV a um mod multivariado que incluiu o IMC melhora mod tamente a previsão de risco

Legenda: GV – gordura visceral, GPT – gordura periaórtica torácica, CV – cardiovascular, IMC – índice de massa corporal.

Tecido adiposo e aneurisma da aorta abdominal (AAA)

A contribuição do tecido adiposo como fator de risco de AAA permanece conflituante. O índice de massa corporal é um reconhecido marcador de adiposidade global. Uma meta-análise da associação entre obesidade e a presença de AAA incluiu 29.120 indivíduos com AAA e 3.163.575 sem AAA[6]; não se verificaram diferenças estatisticamente significativas quanto ao índice de massa corporal no grupo AAA em relação ao grupo de controlo (diferença de médias 0.46 kg/m²; intervalo de confiança a 95%, IC95% -0.07 a 1.00 kg/m²; p=0.09). A obesidade não foi associada a um aumento significativo da prevalência de AAA (odds ratio 1.07; IC95% 0.94 a 1.22; p=0.30).

A utilização da adiposidade visceral ou medidas de aproximação à adiposidade visceral como o perímetro da cintura revelou resultados mais controversos. O estudo de caso-controlo de Cronin O e colaboradores[7] avaliou a associação entre adiposidade visceral (medida através de tomografia computorizada) e a presença bem como o crescimento de AAA, incluindo doentes com (n=196) e sem AAA (n=181). A razão volume de tecido adiposo abdominal visceral-total não foi significativamente associada com o AAA após ajuste para outros fatores de risco. Além disso, a adiposidade visceral não foi associada com o crescimento do AAA. Contrariamente, o trabalho de Stackelberg O e colaboradores[8] incluiu 597 doentes com AAA identificados por cruzamento de duas bases de dados a *Swedish Inpatient Register* e o *Swedish Vascular Registry*. Em análise multivariada, doentes com elevado perímetro da cintura tinham um risco 30% superior de AAA (risco relativo, RR 1.30, IC95% 1.05 a 1.60) em comparação com os que tinham perímetro abdominal normal. O risco de AAA aumentou 15% (RR 1.15, IC95% 1.05 a 1.26) por cada aumento de 5 cm no perímetro abdominal até um nível de 100 cm para o homem e 88 cm para a mulher. Não se verificou associação entre o índice de massa corporal e o risco de AAA.

Finalmente, a diabetes mellitus parece reduzir a prevalência e o crescimento do AAA.[9][10] Sendo conhecida por induzir um desequilíbrio dos produtos vasoativos derivados do tecido adiposo perivascular, será este impacto no desenvolvimento aneurismático mediado pelas alterações ao nível do tecido adiposo?

Para além das abordagens gerais utilizando o índice de massa corporal, a adiposidade visceral ou o perímetro de cintura, os autores desconhecem estudos que avaliem a relação de outros depósitos específicos de tecido adiposo em relação com o AAA. Contudo, a relação da gordura periaórtica torácica com o diâmetro da aorta torácica e abdominal foi estudada nos participantes do *Framingham Heart Study* [11]. Neste contexto, Thanassoulis G e colaboradores demostraram que a gordura periaórtica torácica, quantificada por tomografia computorizada, estava positivamente associada com as dimensões da aorta torácica. A área de interesse para a gordura periaórtica torácica incluiu a região imediatamente ao redor da aorta torácica, definida anteriormente por uma linha traçada horizontalmente ao nível do esôfago, e posteriormente pelo corpo vertebral. O tecido adiposo dentro desta região de interesse, definida como pixels com unidades Hounsfield entre -195 e -45, foi considerado gordura periaórtica. A associação persistiu após ajuste para idade, género e fatores de risco cardiovasculares, incluindo o índice de massa corporal e tecido adiposo visceral. Os resultados para a associação da gordura periaórtica torácica e as dimensões da aorta abdominal foram semelhantes. O ajuste adicional para adipocinas (resistina e adiponectina) não teve impacto significativo nessas associações.

Em suma, contrariamente aos marcadores de adiposidade global, que não parecem demonstrar associação com o AAA, os marcadores de depósitos adiposos específicos como o perímetro da cintura (adiposidade visceral) ou o volume de tecido adiposo periaórtico sugerem que a influência local/regional do tecido adiposo na fisiopatologia do AAA deve ser considerada.

Tecido adiposo e doença arterial periférica (PAD)

Embora a associação entre o índice de massa corporal e PAD seja inconsistente, a literatura sugere que a composição corporal, particularmente pessoas com elevada adiposidade central, pode conferir risco acrescido de PAD. [12] A obesidade central, mas não o índice de massa corporal, já foi previamente associada com PAD numa coorte de homens idosos.[13] Da mesma forma, nos participantes do estudo das Fraturas Osteoporóticas em Homens, a razão perímetro da cintura-anca, mas não o índice de massa corporal, foi associada a um menor índice tornozelo-braço.[14] A obesidade também já foi anteriormente associada à gravidade da PAD.[15]

O tecido adiposo periaórtico torácico e o tecido adiposo visceral foram estudados neste âmbito e ambos parecem correlacionar-se com pior função vascular. Britton KA e colaboradores [16] estudaram a associação entre o volume de tecido adiposo periaórtico torácico ou o tecido adiposo visceral e diferentes avaliações da função vascular nos participantes do *Framingham Heart Study*. Foi demostrado que o volume de tecido adiposo periaórtico torácico e de tecido adiposo visceral permaneciam negativamente associados com o tónus arterial periférico e com a velocidade da onda de pulso carótido-femoral, mesmo após ajuste para o índice de massa corporal. O volume de tecido adiposo periaórtico torácico foi ainda negativamente associado com a velocidade de fluxo médio de hiperémia. Num outro trabalho, o volume de tecido adiposo periaórtico torácico foi associado com menores valores do índice de tornozelo-braço e com claudicação intermitente.[12] Na regressão logística multivariada, por cada 1 desvio-padrão de aumento no tecido adiposo periaórtico torácico, o odds ratio para PAD foi de 1.52 (p=0.004). Estes resultados mantiveram-se após ajuste para índice de massa corporal ou tecido adiposo visceral, enquanto que nenhuma associação foi observada para o tecido adiposo visceral. Da mesma forma, por cada aumento de desvio-padrão no índice de massa corporal ou no perímetro da cintura, nenhuma associação foi observada após a ajuste para o tecido adiposo visceral (índice de massa corporal, p=0.35; perímetro da cintura, p= 0.49).

Assim, à semelhança do que foi referido na seção anterior, são os depósitos adiposos específicos, sobretudo os perivasculares os que mais se correlacionam com a PAD.

Expressão molecular do tecido adiposo periaórtico

Um reduzido número de estudos concentrou-se na identificação de vias moleculares presentes em depósitos específicos de tecido adiposo e na demonstração do seu efeito na vasculatura periférica adjacente.

Spiroglou SG e colaboradores [17] utilizaram métodos de coloração imunohistoquímica para adipocinas em amostras de tecido adiposo periaórtico abdominal, tecido pericoronário e tecido epicárdico apical (n=41). A aterosclerose aórtica foi correlacionada positivamente com a quemerina, a vaspina, a visfatina e a leptina do tecido adiposo periaórtico abdominal. A aterosclerose coronária foi correlacionada positivamente com a expressão de gordura pericoronária de quemerina e visfatina. A expressão de adiponectina do tecido adiposo foi correlacionada negativamente com a aterosclerose em ambos os locais. A expressão de adipocinas na gordura epicárdica apical não se associou com a aterosclerose.

Para avaliar o perfil inflamatório de diferentes depósitos de tecido adiposo abdominal (gordura subcutânea e três depósitos de tecido adiposo visceral: mesentérico, omental e periaórtico), amostras dos respetivos tecidos foram colhidas em homens submetidos a cirurgia da aorta abdominal (n=28)[18]. Foi demonstrado que o tecido adiposo periaórtico continha os adipócitos mais pequenos, a maior densidade capilar e quantidades abundantes segregadas de adipocinas, sendo estes achados favoráveis uma eventual contribuição para as doenças vasculares.

Finalmente, Folkesson M e colaboradores [19] compararam o tecido adiposo periaórtico proveniente de doentes com aneurisma da aorta abdominal com doentes sem doença arterial conhecida proveniente de dadores de órgãos. A imunohistoquímica revelou neutrófilos, macrófagos, mastócitos e células T envolvendo adipócitos necróticos. A análise da expressão de genes mostrou que mais neutrófilos, mastócitos e células T foram encontrados no tecido adiposo periaórtico em comparação com a parede do AAA, bem como catepsina K e S. A concentração de ceramidas no tecido adiposo periaórtico apresentou correlação com o conteúdo de células T nesse tecido. Este estudo mostra que os adipócitos que cercam a aorta podem ser uma ótima fonte de leucócitos inflamatórios que são atraídos por adipócitos submetidos à necrose e por ceramidas pró-inflamatórias. Será viável o desenvolvimento de estratégias que impeçam a formação de tecido adiposo perivascular e que enderecem a inflamação do lado da adventícia?

Em suma, o tecido adiposo abdominal periaórtico assume uma diferente morfologia em relação a outros depósitos adiposos abdominais, verificando-se ainda uma expressão diferencial de adipocinas e de moléculas inflamatórias em função da patologia arterial adjacente. Estes resultados laboratoriais apresentam-se, pois, em linha com os achados provenientes de estudos de imagem mencionados nas secções prévias.

CONCLUSÃO

A evidência recente tem demonstrado uma associação entre depósitos específicos de tecido adiposo e diversas doenças vasculares periféricas. Embora a gordura pericoronária, a periventricular e a periaórtica torácica tenham sido amplamente caracterizados em contextos saudáveis e patológicos, pouco se sabe sobre compartimentos abdominais específicos como o tecido adiposo periaórtico abdominal e em que extensão pode afetar a fisiologia e fisiopatologia das doenças aórticas. A comprovar-se a importância do tecido adiposo periaórtico abdominal, poderá seguir-se o desenho de estratégias terapêuticas inovadoras para as doenças da aorta. Por outro lado, uma vez que condições como a obesidade, diabetes mellitus, tabaco e hipertensão arterial se encontram associadas à desregulação do tecido adiposo perivascular, estes dados reforçam a importância do controlo destes fatores, sobretudo enquanto as hormonas e citocinas produzidas pelo tecido adiposo ainda não forem um alvo terapêutico disponível.

BIBLIOGRAFIA

1. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW, Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med. 1999;341:1097-105.
2. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444:875-80.
3. Meijer RI, Bakker W, Alta CL, Sipkema P, Yudkin JS, Viollet B, et al. Perivascular adipose tissue control of insulin-induced vasoreactivity in muscle is impaired in db/db mice. Diabetes. 2013;62:590-8.
4. Talman AH, Psaltis PJ, Cameron JD, Meredith IT, Seneviratne SK, Wong DT. Epicardial adipose tissue: far more than a fat depot. Cardiovasc Diagn Ther. 2014;4:416-29.
5. Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. Br Med J (Clin Res Ed). 1984;288:1401-4.
6. Takagi H, Umemoto T. A meta-analysis of the association of obesity with abdominal aortic aneurysm presence. Int Angiol. 2015;34:383-91.
7. Cronin O, Liu D, Bradshaw B, Iyer V, Buttner P, Cunningham M, et al. Visceral adiposity is not associated with abdominal aortic aneurysm presence and growth. Vasc Med. 2014;19:272-80.
8. Stackelberg O, Bjorck M, Sadr-Azodi O, Larsson SC, Orsini N, Wolk A. Obesity and abdominal aortic aneurysm. Br J Surg. 2013;100:360-6.
9. De Rango P, Farchioni L, Fiorucci B, Lenti M. Diabetes and abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2014;47:243-61.

10. Lederle FA. The strange relationship between diabetes and abdominal aortic aneurysm. Eur J Vasc Endovasc Surg. 2012;43:254-6.
11. Thanassoulis G, Massaro JM, Corsini E, Rogers I, Schlett CL, Meigs JB, et al. Periaortic adipose tissue and aortic dimensions in the Framingham Heart Study. J Am Heart Assoc. 2012;1:e000885.
12. Fox CS, Massaro JM, Schlett CL, Lehman SJ, Meigs JB, O'Donnell CJ, et al. Periaortic fat deposition is associated with peripheral arterial disease: the Framingham heart study. Circ Cardiovasc Imaging. 2010;3:515-9.
13. Planas A, Clara A, Pou JM, Vidal-Barraquer F, Gasol A, de Moner A, et al. Relationship of obesity distribution and peripheral arterial occlusive disease in elderly men. Int J Obes Relat Metab Disord. 2001;25:1068-70.
14. Vogt MT, Cauley JA, Kuller LH, Hulley SB. Prevalence and correlates of lower extremity arterial disease in elderly women. Am J Epidemiol. 1993;137:559-68.
15. Golledge J, Leicht A, Crowther RG, Clancy P, Spinks WL, Quigley F. Association of obesity and metabolic syndrome with the severity and outcome of intermittent claudication. J Vasc Surg. 2007;45:40-6.
16. Britton KA, Wang N, Palmisano J, Corsini E, Schlett CL, Hoffmann U, et al. Thoracic periaortic and visceral adipose tissue and their cross-sectional associations with measures of vascular function. Obesity (Silver Spring). 2013;21:1496-503.
17. Spiroglou SG, Kostopoulos CG, Varakis JN, Papadaki HH. Adipokines in periaortic and epicardial adipose tissue: differential expression and relation to atherosclerosis. J Atheroscler Thromb. 2010;17:115-30.
18. Kranendonk ME, van Herwaarden JA, Stupkova T, de Jager W, Vink A, Moll FL, et al. Inflammatory characteristics of distinct abdominal adipose tissue depots relate differently to metabolic risk factors for cardiovascular disease: distinct fat depots and vascular risk factors. Atherosclerosis. 2015;239:419-27.
19. Folkesson M, Vorkapic E, Gulbins E, Japtok L, Kleuser B, Welander M, et al. Inflammatory cells, ceramides, and expression of proteases in perivascular adipose tissue adjacent to human abdominal aortic aneurysms. J Vasc Surg. 2016;

CHAPTER 8 HIGH DENSITY OF PERIAORTIC ADIPOSE TISSUE IN ABDOMINAL AORTIC ANEURYSM

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High Density of Periaortic Adipose Tissue in Abdominal Aortic Aneurysm

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WHAT THIS PAPER ADDS

A multicentre study was conducted to show differences in the distribution of abdominal fat deposits (including perivascular adipose tissue, PVAT) among patients with abdominal aortic aneurysm (AAA), aortoiliac occlusive disease, and patients without aortic disease. The presence of an AAA was an independent predictor of higher PVAT density around the aneurysm sac than the healthy neck. This intra-individual PVAT difference was positively associated with aortic volume. The findings suggest that PVAT contributes to AAA pathophysiology via local mechanisms.

Objectives: Perivascular adipose tissue (PVAT) is currently seen as a paracrine organ that produces vasoactive substances, including inflammatory agents, which may have an impact on the vasculature. In this study PVAT density was quantified in patients with an aortic aneurysm and compared with those with a non-dilated aorta. Since chronic inflammation, as the pathway to medial thinning, is a hallmark of abdominal aortic aneurysms (AAAs), it was hypothesised that PVAT density is higher in AAA patients.

Methods: In this multicentre retrospective case control study, three groups of patients were included: non-treated asymptomatic AAA ($n = 140$), aortoiliac occlusive disease (AIOD) ($n = 104$), and individuals without aortic pathology ($n = 97$). A Hounsfield units based analysis was performed by computed tomography (CT). As a proxy for PVAT, the density of adipose tissue 10 mm circumferential to the infrarenal aorta was analysed in each consecutive CT slice. Intra-individual PVAT differences were reported as the difference in PVAT density between the region of the maximum AAA diameter (or the mid-aortic region in patients with AIOD or controls) and the two uppermost slices of infrarenal non-dilated aorta just below the renal arteries. Furthermore, subcutaneous (SAT) and visceral (VAT) adipose tissue measurements were performed. Linear models were fitted to assess the association between the study groups, different adipose tissue compartments, and between adipose tissue compartments and aortic dimensions.

Results: AAA patients presented higher intra-individual PVAT differences, with higher PVAT density around the aneurysm sac than the healthy neck. This association persisted after adjustment for cardiovascular risk factors and diseases and other fat compartments ($\beta = 13.175$, SE 4.732, $p = .006$). Furthermore, intra-individual PVAT differences presented the highest correlation with aortic volume that persisted after adjustment for other fat compartments, body mass index, sex, and age ($\beta = 0.566$, 0.200, $p = .005$).

Conclusion: The results suggest a relation between the deposition of PVAT and AAA pathophysiology. Further research should explore the exact underlying processes.

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INTRODUCTION

Perivascular adipose tissue (PVAT) is a special depot of adipose tissue that surrounds the blood vessels. Besides acting as a supportive tissue, PVAT is currently seen as a paracrine and endocrine organ that produces a wide range of biologically active molecules, which may have a profound influence on the vasculature.[1,2] Excessive accumulation of

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inflamed or dysfunctional PVAT has been proposed to be a major risk factor for cardiovascular diseases.[3]

Pathophysiological involvement of the adventitial layer in atherosclerosis has already been described in 1989[4] and in vitro studies have shown that cytokines in supernatants of human PVAT aorta caused migration of granulocytes, monocytes, and T cells. This demonstrates that PVAT has properties that may influence the process of vascular wall inflammation, an important step in atherogenesis.[5] Since AAA and atherosclerosis share most of the risk factors, accordingly the importance of the PVAT derived outside to inside signalling in AAA pathophysiology might be questioned.

Aside from the well known association with visceral adipose tissue,[6] imaging studies in humans have also correlated specific fat deposits with cardiovascular diseases.[7–9] The thoracic aortic PVAT quantity, clinically examined by computed tomography (CT), was found to be positively associated with abdominal aortic calcification[10] and negatively with the ankle brachial index and intermittent claudication.[11] Although the association between visceral adiposity and aortic dilation is conflicting,[12,13] aortic PVAT was positively associated with aortic diameter in individuals from the Framingham Offspring and Third Generation cohorts.[14] This association persisted after adjustment for age, sex, and cardiovascular risk factors including body mass index (BMI) and visceral adipose tissue. However, the extent of this contribution in the setting of AAA and the evolved mechanisms under this association remain unclear.

It is hypothesised that in AAA, PVAT quantity is different from peripheral artery disease and non-diseased aortas. Differences may already be seen on CT angiography (CTA). The aim of this study was to evaluate the distribution of abdominal fat deposits including PVAT in patients with AAA compared with aortoiliac occlusive disease (AIOD) and patients with healthy aortas.

METHODS

Ethics statement

The study was approved by the local ethics committee of São João Hospital Centre and by the local ethics committee of the VU Medical Centre. All experiments were carried out in accordance with approved guidelines.

Study design

In this multicentre retrospective case control study, patients followed in the outpatient setting with AAA or thoracoabdominal aneurysm were retrospectively consecutively included between 2009 and 2016 in the two institutions if CT was available. AAA patients presented with aneurysm sizes close to the indication for elective repair and most CTAs consisted of pre-operative imaging. Additionally and identically, patients who underwent CTA because of symptomatic AIOD and patients older than 50 years who underwent CT scanning because of urinary stones (controls) were included. Patients were excluded if they had ruptured AAA, symptomatic AAA, inflammatory AAA (corresponding to aneurysm with an exaggerated inflammatory component which often incites a surrounding fibrotic reaction), clinical symptoms suggesting aortic inflammation or inflammatory aspects on CTA, previous abdominal aortic intervention, active neoplasia, active infection, or incomplete CT scans.

Pre-operative CT scans were used to measure different abdominal adipose tissue compartments: PVAT (including intra-individual PVAT differences), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT). Comparisons were made among AAA, AIOD, and control patients.

Demographic variables

Clinical baseline characteristics for all patients were collected. BMI was defined as the weight (in kg) divided by squared height (in m^2). Diabetes was defined as fasting plasma glucose $\geq$ 126 mg/dL or hypoglycemic treatment. Cigarette smoking was categorised as current, former, or never. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg, or antihypertensive therapy. Carotid artery disease was defined as either absent, asymptomatic significant stenosis (stenosis $>$ 50%), history of transient ischaemic attack, or of ischaemic stroke; coronary heart disease was defined as either "no", stable angina, unstable angina, or myocardial infarction; previous coronary invasive intervention included previous endovascular or open coronary revascularisation; congestive heart failure was based on clinical and echocardiogram findings; chronic renal insufficiency was defined as absent, mild if plasma creatinine $<$ 2.15 mg/dL, severe if creatinine 2.15—5.00 mg/dL, or terminal if creatinine $>$ 5.00 mg/dL or dialysis or kidney transplant; chronic obstructive pulmonary disease was defined as either "no" or "yes" based on spirometry results, when available, or in the medical records; peripheral arterial disease was defined as either "absent", "asymptomatic", "intermittent claudication (Fontaine IIa/b or Rutherford 1—3)" or "critical limb ischaemia (Fontaine III/IV or Rutherford 4—6)"); routine medication included statins, antihypertensive drugs, antiaggregants and/or anticoagulants, and digoxin/lanoxin.

CT/CTA imaging protocol

CT/CTA scanning in a supine position using a 64 slice multiple detector CT was performed in all patients in the study population. Scan parameters were determined by the Right Dose indicated by manufacturer, which aims to find the right dose for every individual patient, balancing between image quality and radiation dose. Slice thickness of the available scans varied from one mm to five mm. Measurements were performed at intervals of 5—6 mm. Osirix Medical Imaging Software (OsiriX Foundation, Geneva, Switzerland) was used for measurements. Contrast enhancement was adjusted with a windows level (contrast) of 143 Hounsfield units (HU) and a windows width (brightness) of 411 HU.

Assessment of maximum AAA diameter and AAA volume

The main axis of each AAA was determined and maximum AAA diameter was obtained as the maximum length between opposite walls of the AAA in a plane perpendicular to that main axis. For the calculation of the AAA volume, the regions located immediately below the emergence of the lowest renal artery and immediately above the aortic bifurcation were considered. In each slice, the region of interest was manually selected according to protocol and the volume was automatically calculated using Osirix.

Adipose tissue measurements

The CT attenuation in absolute HU corresponds to tissue properties; a HU range from −195 to −45 HU was applied to identify pixels containing adipose tissue according to Schlett et al.[15] Previous literature shows that −107 ± 8 HU is the mean of the range where the attenuation borders of adipose tissue are starting, varying until +45 HU. Therefore, in the literature a "halfway method" is used.[16] While in early publications a halfway upper boundary of −30 HU is used as the cut off point, more recent literature indicates −45 HU to be the upper boundary.[17,18] Therefore, this range was used in this study. Pixels within this range were dichotomously converted into a "1", while pixels outside this range were converted into a "0". Afterwards, the adipose tissue pixels were summed up and measured per cm^2.

In order to study visceral and subcutaneous adipose tissue, measurements were performed in a specific CT slice, as shown by Demerath et al.[19] to be the most accurate level at approximating the VAT/SAT ratio for the entire abdomen; the axial slice 60 mm cranial to the L4/L5 intervertebral disc (Figure S1A). The VAT/SAT ratio was represented as the number of fat pixels in the visceral adipose tissue area divided by the number of fat pixels in subcutaneous adipose tissue area.

Measurements of PVAT were based on the method described by Schlett et al.[15] Following the close relationship between abdominal aortic PVAT and the aortic diameter, measurement of this fat depot is performed using concentric rings calibrated to the vessel diameter. In order to study PVAT in a standardised manner, a region of interest (ROI) in each slice was defined with a diameter of 10 mm circumferential to the outer contour of the aorta. The predefined ROI was centred over the aorta. This standardisation enabled the definition of a hollow cylinder of aortic PVAT. Aortic area, the area of the circumferential ring around the aorta, and the number of fat pixels in that ring were measured in contiguous slices, starting with the first slice distal to the lowest renal artery and ending at the aortic bifurcation (Figure S1B).

The aortic bifurcation was considered as the slice immediately above the visualisation of two separate common iliac arteries or the distal slice where the transverse and antero-posterior aortic diameters did not differ more than 1.5 times. Analyses of 10 mm thick ROIs in the vertebral bone and intervertebral discs nearest to the adjacent aorta has shown that these structures do not significantly contain adipose tissue, according to above-mentioned HU for adipose tissue. Hence, vertebral bones or intervertebral discs were not excluded from the ring of PVAT included during measurements. These measurements enabled the calculation of PVAT density in each slice as the ratio between the number of fat pixels inside the ring and the area of the ring, representing a proxy for aortic PVAT density.

The average density of PVAT pixels among all slices, the average abdominal aortic PVAT density, was compared between AAA patients and the two control groups. Additionally, PVAT density in two aortic regions of the same patient (i.e., intra-individual PVAT) were compared: the two uppermost slices of infrarenal aorta (Region 1) and the region of maximum aortic diameter in patients with infrarenal AAA or the cranio-caudal mid-aortic region in patients with AIOD and in controls (Region 2). Intra-individual PVAT difference was reported as the difference between the mean PVAT in Region 2 minus the mean PVAT in Region 1 (Figure S1C). Intra-individual PVAT assessment was performed to explore regional differences in PVAT density within the same individuals by comparing diseased and non-diseased aortic segments. Patients with an infrarenal neck < 1 cm (juxtarenal or thoraco-abdominal aneurysms) were excluded from this specific analysis, since no healthy neck measurements could be performed. To adjust for baseline anatomical differences in PVAT density distribution along the aorta, these differences were compared with AIOD and control patients.

Reproducibility assessment

Inter-observer reproducibility of adipose tissue measurements was assessed by two independent analysts in 50 randomly selected patients.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation when normally distributed and as median and interquartile range when skewed. Categorical variables were expressed as percentages.

Univariable analysis of putative determinants of each adipose tissue compartment was performed by one way ANOVA. Separate linear regression models were fitted to model each adipose tissue compartment. The multivariable linear regression models included the covariables of age, sex, risk factors, cardiovascular diseases, and current medication. Variables were introduced in the model if they showed statistic relevance in univariable analysis ($p < .25$) or based on subject matter consideration for known or thought to be important variables. A hybrid forward selection and backward elimination approach was undertaken and a final model was chosen based on the maximum adjusted R^2 and the minimum standard error of the estimate. In a secondary analysis, linear regression models were further fitted to assess the association between adipose tissue compartments and aortic dimensions.

Furthermore, 50 CT scans were independently assessed by two analysts. The intraclass coefficient index was used to report the inter-observer reproducibility of VAT, SAT, and PVAT measures. All analyses were performed with SPSS version 24.0.0.0. A p value $< .05$ was considered statistically significant.

RESULTS

Patient characteristics

The present study comprised infrarenal AAA patients and patients with abdominal aneurysms extending above the renal arteries (such as thoraco-abdominal aortic aneurysms Crawford Type II, III, or IV) ($n = 140$, from which 32 were thoraco-abdominal or AAA with less than 1 cm of healthy infrarenal neck [i.e., juxtarenal]), patients with AIOD ($n = 104$), and controls without aortic disease ($n = 97$) with a mean age of 72 ± 8 years, 63 ± 10 years, and 64 ± 11 years, respectively. Patient characteristics are summarised in Table 1 and Table S1.

Aneurysm and PVAT characteristics

AAA patients presented with a maximum aortic diameter of 6.1 ± 1.4 cm. Average maximum aortic area was 29.5 ± 14.4 cm^2 in the AAA group, 3.5 ± 1.4 cm^2 in the AIOD group, and 3.1 ± 0.8 cm^2 in the control group. Mean maximum aortic volume, VAT/SAT ratio, PVAT, and intra-individual PVAT differences are described in Table 1.

Adipose compartments

Analysis of the adipose tissue compartments among the groups showed that there were disparities in the distribution of fat among the adipose tissue compartments (Fig. 1A–C).

Visceral obesity

Compared to other groups, AAA patients presented higher visceral obesity expressed as VAT/SAT ratio in unadjusted analysis ($p = .012$). However, this association did not persist after adjustment for cardiovascular risk factors and diseases in a multivariable regression model (Table 2).

PVAT density

The patients with AIOD showed lower PVAT density than other groups ($p < .001$). Nevertheless, further analysis using a linear regression model led to loss of statistical significance (Table 2).

Intra-individual PVAT difference

In the control group, the intra-individual PVAT between Region 2 and Region 1 was not statistically different from 0 (mean difference −4.72, 95% CI −10.74 to 1.29; $p = .123$), which means that PVAT density showed no difference between the regions. Patients with AIOD presented more PVAT density in Region 1 than in Region 2 (mean difference −5.59, 95% CI −10.22 to −0.96; $p = .018$) and, inversely, patients with AAA present more PVAT density in Region 2 than in Region 1 (mean difference 7.97, 95% CI 3.31 to 12.63; $p = .001$).

Comparison of the intra-individual PVAT difference within each patient among the three groups showed a significantly higher intra-individual PVAT difference in patients with AAA than their counterparts, with a higher PVAT density in the sac than in the neck. Intra-individual PVAT differences from AIOD were not statistically different from controls. The

Table 1. Clinical, aortic and adipose tissue characteristics of overall sample.

	AAA		AIOD		Control		p
N	140		104		97		
Clinical characteristics							
Gender Male	113	80.7%	78	75.0%	50	51.5%	< .001
Age	71.57	± 8.21	63.31	± 9.65	64.26	± 11.36	< .001
BMI	25.84	± 3.79	25.27	± 5.23	27.92	± 4.07	.001
Aortic dimensions							
Maximum aortic diameter (cm)	6.1	± 1.4	2.1	0.4	2.0	0.2	< .001
Maximum transverse area (cm^2)	29.47	± 14.40	3.49	± 1.35	3.13	± .79	< .001
Infrarenal aortic volume (cm^3)	208.77	± 174.24	22.82	± 9.42	21.81	± 7.05	< .001
Adipose tissue characteristics							
VAT/SAT ratio	1.49	± 0.75	1.21	± .79	1.23	± .73	.006
PVAT density (units)	81.68	± 31.68	60.21	± 32.91	84.11	± 33.41	< .001
Intra-individual PVAT differences (units)	7.97	± 24.43	−5.59	± 23.80	−4.72	± 29.86	< .001

Note. Values represent means ± standard deviation or count and percentage where otherwise specified. AAA = abdominal aortic aneurysm; AIOD = aortoiliac occlusive disease; BMI = body mass index; VAT/SAT = visceral adipose tissue/subcutaneous adipose tissue; PVAT = perivascular adipose tissue.

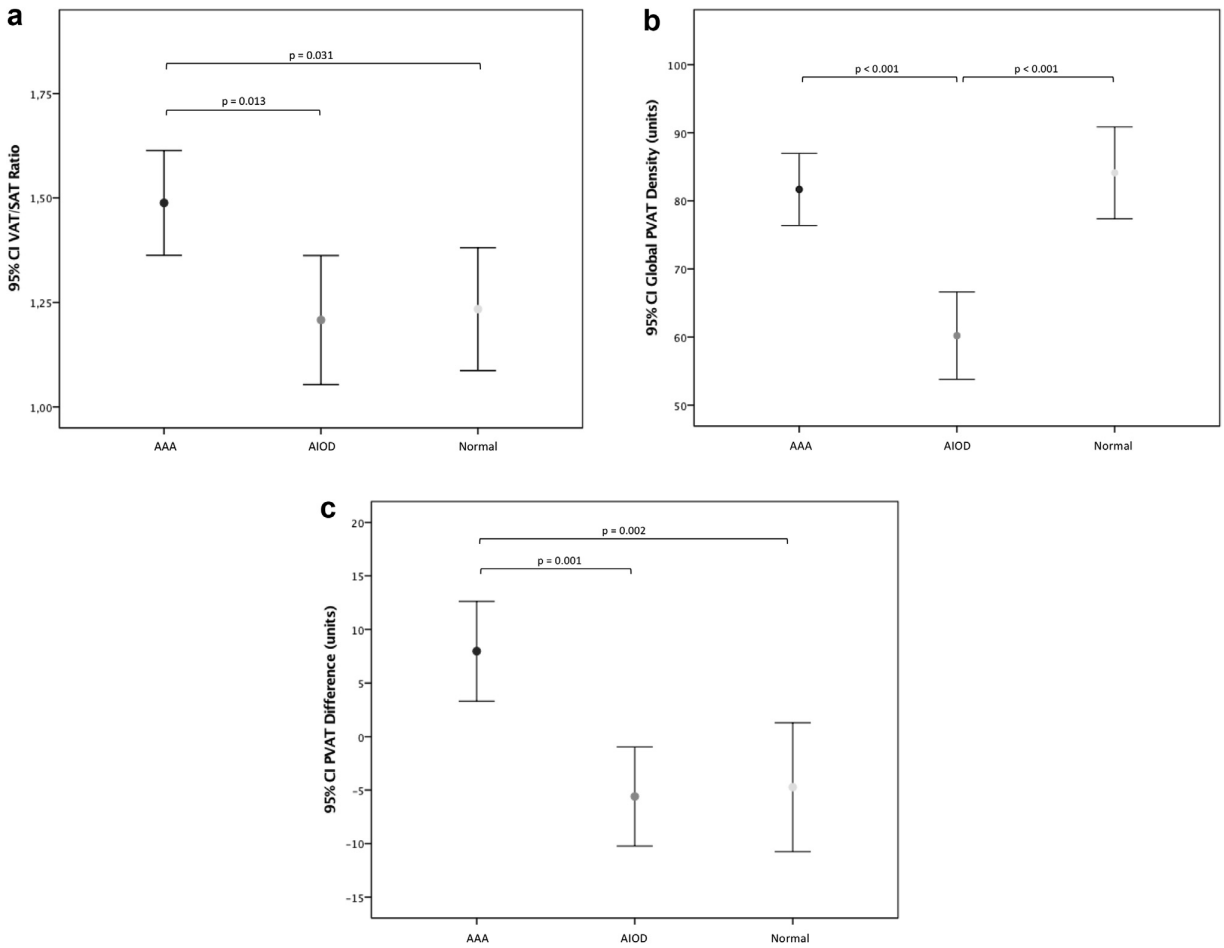

Figure 1. Distribution of adipose tissue compartments among groups in univariable analysis: visceral adipose tissue (A), PVAT density (B) and intra-individual PVAT differences (C). AAA = abdominal aortic aneurysm; AIOD = aortoiliac occlusive disease; PVAT = perivascular adipose tissue; SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue.

Table 2. Association between adipose tissue compartments and presence of aortic diseases: abdominal aortic aneurysm or aortoiliac occlusive disease.

Adipose tissue compartments (units)		Unadjusted		Risk factors and cardiovascular diseases adjusted		+ VAT/SAT and PVAT adjusted	
		β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
VAT/SAT ratio	AAA	0.254 (0.100)	.012	−0.035 (0.147)[a]	.814	—	—
	AIOD	−0.026 (0.107)	.807	−0.312 (0.189)[a]	.101	—	—
PVAT density	AAA	−2.434 (4.301)	.572	2.605 (9.630)[b]	.788	—	—
	AIOD	−23.905 (4.596)	< .001	0.403 (25.842)[b]	.988	—	—
Intra-individual PVAT differences	AAA	12.869 (3.645)	.001	13.051 (4.625)[c]	.005	13.175 (4.732)	.006
	AIOD	−0.869 (3.678)	.813	6.678 (6.093)[c]	.274	7.111 (6.376)	.266

AAA = abdominal aortic aneurysm; AIOD = aortoiliac occlusive disease; BMI = body mass index; ACEI/ARA = angiotensin converting enzyme inhibitors/angiotensin receptor antagonists; VAT/SAT = visceral adipose tissue/subcutaneous adipose tissue; PVAT = perivascular adipose tissue.

[a] Adjusted for the following covariables: gender, diabetes, hypertension, smoking (current, former, or never), coronary heart disease, peripheral artery disease, BMI, anticoagulation, antiplatelet therapy, vasodilator, IECA/ARA, diuretics, calcium channel blockers, beta blockers, statins.

[b] Adjusted for the following covariables: gender, age, diabetes, hypertension, smoking (current, former, or never), coronary heart disease, history of cardiac treatment, peripheral artery disease, BMI, anticoagulation, antiplatelet therapy, vasodilator, IECA/ARA, diuretics, calcium channel blockers, beta blockers, statins.

[c] Adjusted for the following covariables: gender, age, hypertension, coronary heart disease, history of cardiac treatment, peripheral artery disease, vasodilator, IECA/ARA, statins.

association persisted after adjustment for cardiovascular risk factors and diseases and other fat compartments. According to the model, the presence of an AAA increased patients' intra-individual PVAT differences by 13.2 units (standard error of 4.7, $p = .006$) (Table 2).

Aortic volume

To explore these findings, the relation between BMI, SAT/VAT, and PVAT properties with aortic dimensions was studied. As shown in Table 3 and Fig. 2, all adipose tissue compartments showed a correlation with aortic dimensions. Intra-individual PVAT differences presented the highest positive correlation with aortic dimensions, meaning that the higher PVAT density in Region 2 (region of maximum aortic diameter in patients with infrarenal AAA or the cranio-caudal mid-aortic region in patients with AIOD and in controls), the larger the aortic diameter. Further modelling revealed that this association was maintained after adjustment for other fat compartments, BMI, sex, and age. According to this model, for every additional unit in intra-individual PVAT differences, the aortic volume increased by a mean of 0.6 (standard error 0.2, $p = .005$) (Table 4).

Assessment of reproducibility of adipose tissue measurements

The intraclass correlation coefficient and the respective 95% CI of PVAT, VAT, and SAT densities were, respectively, 0.97 (0.95—0.99), 0.98 (0.96—0.99), and 0.94 (0.89—0.97).

DISCUSSION

In this multicentre, case control study, the main finding was that AAA patients had higher intra-individual differences in PVAT density, with a higher PVAT density around the aneurysm sac than the healthy neck. With respect to the primary research question, the hypothesis that on CTA, differences in PVAT quantity exist for patients with AAA, was supported. It is interesting to note that this difference was observed within individual AAA patients when the healthy neck was compared with the aneurysmal part of the aorta, therefore correcting for inter-individual differences.

Previous literature has shown that inflammation plays a critical role in the pathophysiology of AAA.[20] Also, it is now well established from a variety of studies that PVAT exhibits adventitial encroachment to the adjacent vessel and is interspersed with vasa vasorum,[21,22] being a good candidate for paracrine signaling. Furthermore, there is a growing body of literature that recognises PVAT as key player in atherosclerosis pathophysiology by "outside in" signalling[23,24] or that a communication between adipose tissue and the vessel wall might be bidirectional.[25] Potentially, adipose tissue adjacent to the aorta may have similar local effects on aortic wall remodelling and dilatation in AAA because of inflammatory crosstalk. It is considered, therefore, that increased density in PVAT represents more PVAT adipocytes and, subsequently, a greater inflammatory burden.

Interestingly, it is also possible that higher PVAT density in the sac region may partly reflect locally increased fat pixels in the aortic wall itself. Recently, adventitial presence of adipocyte clusters connected by intertwining strands of matrix were proposed as a dysregulated repair mechanism of AAA disease, a process seen in several chronic diseases.[26—28]

Measurements of abdominal aortic PVAT have been hampered by the inability to resolve the concerns related to the retroperitoneal lining and the inherent correlations between abdominal aortic diameter and abdominal PVAT volume.[14] To address the difficulties related to the volumetric measurements in a population prone to aortic diameter changes (that is, AAA patients), a density approach was used as a proxy for abdominal aortic PVAT quantity. Considering the ratio between the fat pixels and the area of a circle that extends from the outer contour of the aorta by 10 mm, in each slice, the close relationship between abdominal PVAT and the aortic diameter is obviated; independent from changes in aortic diameter. Therefore, previously reported measurement problems because of differences between transverse and antero—posterior diameter[15] were not a problem in the protocol.

Another important feature of the protocol was that PVAT densities were assessed in two separate regions of the aorta, allowing the comparison of healthy and aneurysmal segments individually within the same patient. This self matching helps to control for factors that are stable over time in the same person, such as gender and established comorbidities.

The reported levels of antiplatelets and statins in patients with symptomatic AIOD were relatively low. A possible explanation for these results may be the time of data collection, which was the same time as CTA. Consequently, there was no diagnosis yet, and patients were not pharmacologically treated.

These data must be interpreted with caution because of the cross sectional and observational retrospective nature of this study. Also, other clinical characteristics that were not analysed might impact the outcomes of the different

Table 3. Correlations of body mass index and adipose tissue compartments with abdominal aortic dimensions.

Adipose tissue compartments	Maximum aortic diameter		Maximum aortic area		Aortic volume	
	Pearson's correlation	*p*	Pearson's correlation	*p*	Pearson's correlation	*p*
BMI	−.064	.322	−.066	.313	−.071	.233
VAT/SAT ratio	.157	.004	.143	.008	.131	.015
PVAT density	.151	.005	.136	.012	.146	.007
Intra-individual PVAT difference	.227	< .001	.200	< .001	.219	< .001

BMI = body mass index; VAT/SAT = visceral adipose tissue/subcutaneous adipose tissue; PVAT = perivascular adipose tissue.

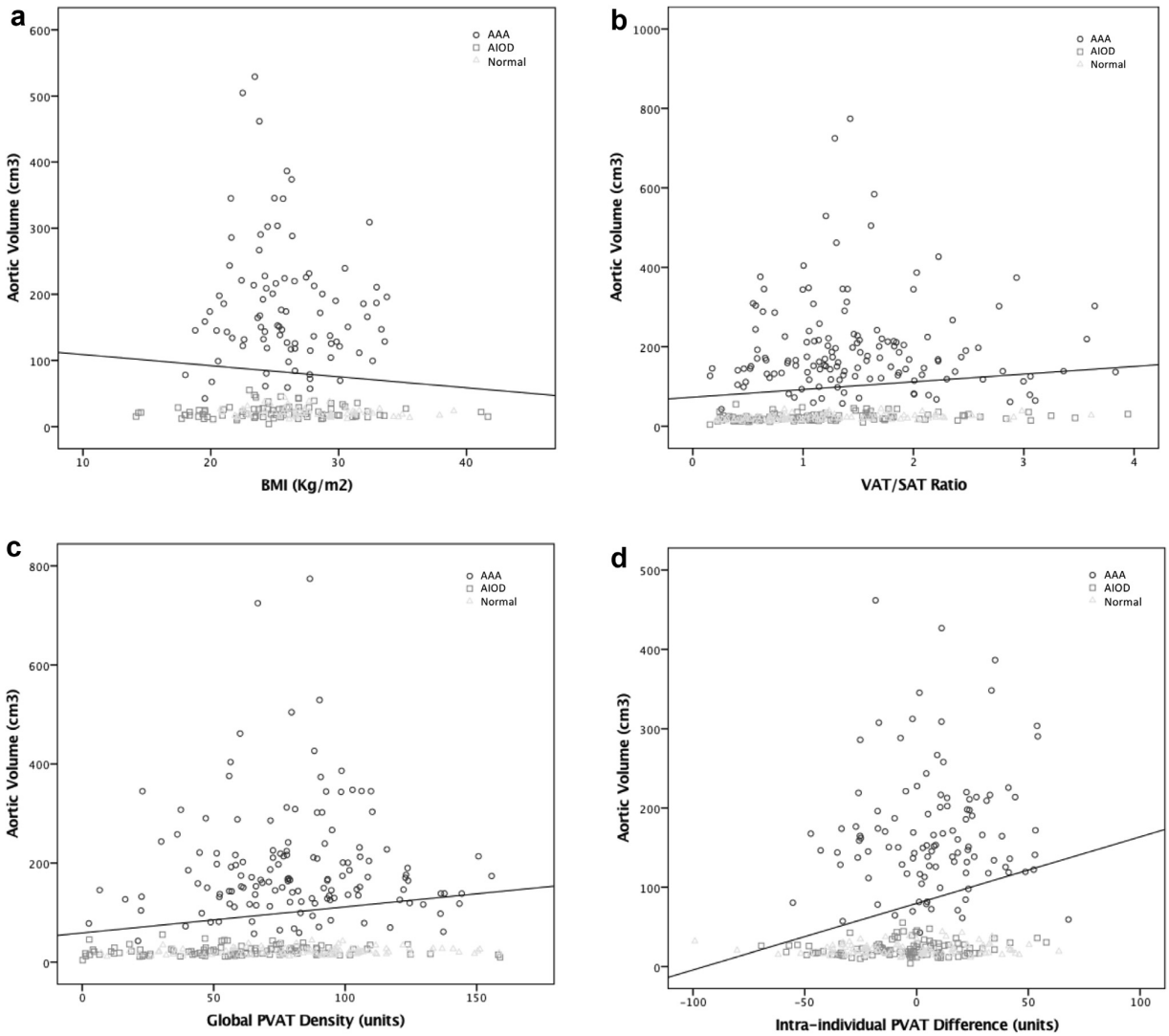

Figure 2. Correlations between (A) aortic volume and BMI, (B) aortic volume and VAT/SAT ratio, (C) aortic volume and PVAT density, and (D) aortic volume and intra-individual PVAT difference. AAA = abdominal aortic aneurysm; AIOD = aortoiliac occlusive disease; BMI = body mass index; PVAT = perivascular adipose tissue; SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue.

groups. Furthermore, the patient sample was predominantly of white Europeans and therefore results may not apply to other racial groups. Nevertheless, included patients are from two multicultural clinics in different parts of Europe, conferring ethical diversity.

These findings may also be somewhat limited by the measurement protocol. The root of the mesentery, which also consists of adipose tissue, is partly in the adipose tissue area studied circumferentially to the aorta. This adipose tissue, however, is separated from PVAT and aortic adventitia by a dual layer of peritoneum, preventing paracrine signaling. Also, within this spectrum of HU for adipose tissue, no inflamed or dysfunctional adipose tissue could be identified. It is important to bear in mind that the present study only shows data provided from CT scans. Tissue analyses on PVAT across aortic pathologies could provide

Table 4. Association of intra-individual difference in PVAT with maximum aortic diameter.

Adipose tissue compartments (units)	Unadjusted		+ Fat compartments adjusted[a]		+ Age and gender	
	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
Intra-individual PVAT difference	0.242 (0.059)	< .001	0.220 (0.067)	.001	0.173 (0.065)	.008

BMI = body mass index; VAT/SAT = visceral adipose tissue/subcutaneous adipose tissue; PVAT, perivascular adipose tissue.
[a] Adjusted for the following covariables: BMI, VAT/SAT ratio and PVAT density.

additional insights into the pathophysiological role of PVAT. Lastly, only patients with advanced (large AAA and symptomatic AIOD) were included, precluding assessment of differences in the PVAT parameters in small versus large AAA patients, which might further support the pathophysiological involvement.

CONCLUSION

In this study, the presence of an aneurysm was an independent predictor of increased intra-individual PVAT differences. Furthermore, intra-individual PVAT difference was associated positively with aortic volume, suggesting a novel correlation for AAA. These findings raise intriguing questions regarding the nature and extent of the PVAT contribution to AAA pathophysiology via local mechanisms, a novel concept of crosstalk between adipose tissue and aorta. Further research focusing on the expression of PVAT should be undertaken to investigate the exact interplay between PVAT and aorta in both healthy and in aneurysmal conditions.

CONFLICT OF INTEREST

None.

FUNDING

None.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejvs.2018.07.008.

REFERENCES

1 Chang L, Milton H, Eitzman DT, Chen YE. Paradoxical roles of perivascular adipose tissue in atherosclerosis and hypertension. *Circ J* 2013;**77**:11–8.
2 Meijer RI, Bakker W, Alta CL, Sipkema P, Yudkin JS, Viollet B, et al. Perivascular adipose tissue control of insulin-induced vasoreactivity in muscle is impaired in db/db mice. *Diabetes* 2013;**62**:590–8.
3 Iozzo P. Myocardial, perivascular, and epicardial fat. *Diabetes Care* 2011;**34**:S371–9.
4 Prescott MF, McBride CK, Court M. Development of intimal lesions after leukocyte migration into the vascular wall. *Am J Pathol* 1989;**135**:835–46.
5 Henrichot E, Juge-Aubry CE, Pernin A, Pache JC, Velebit V, Dayer JM, et al. Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? *Arterioscler Thromb Vasc Biol* 2005;**25**:2594–9.
6 Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *Br Med J (Clin Res Ed)* 1984;**288**:1401–4.
7 Brinkley TE, Leng X, Chughtai HL, Nicklas BJ, Kritchevsky SB, Ding J, et al. Periaortic fat and cardiovascular risk: a comparison of high-risk older adults and age-matched healthy controls. *Int J Obes (Lond)* 2014;**38**:1397–402.
8 Britton KA, Pedley A, Massaro JM, Corsini EM, Murabito JM, Hoffmann U, et al. Prevalence, distribution, and risk factor correlates of high thoracic periaortic fat in the Framingham Heart Study. *J Am Heart Assoc* 2012;**1**:e004200.
9 Talman AH, Psaltis PJ, Cameron JD, Meredith IT, Seneviratne SK, Wong DT. Epicardial adipose tissue: far more than a fat depot. *Cardiovasc Diagn Ther* 2014;**4**:416–29.
10 Lehman SJ, Massaro JM, Schlett CL, O'Donnell CJ, Hoffmann U, Fox CS. Peri-aortic fat, cardiovascular disease risk factors, and aortic calcification: the Framingham Heart Study. *Atherosclerosis* 2010;**210**:656–61.
11 Fox CS, Massaro JM, Schlett CL, Lehman SJ, Meigs JB, O'Donnell CJ, et al. Periaortic fat deposition is associated with peripheral arterial disease: the Framingham heart study. *Circ Cardiovasc Imaging* 2010;**3**:515–9.
12 Cronin O, Liu D, Bradshaw B, Iyer V, Buttner P, Cunningham M, et al. Visceral adiposity is not associated with abdominal aortic aneurysm presence and growth. *Vasc Med* 2014;**19**:272–80.
13 Stackelberg O, Bjorck M, Sadr-Azodi O, Larsson SC, Orsini N, Wolk A. Obesity and abdominal aortic aneurysm. *Br J Surg* 2013;**100**:360–6.
14 Thanassoulis G, Massaro JM, Corsini E, Rogers I, Schlett CL, Meigs JB, et al. Periaortic adipose tissue and aortic dimensions in the Framingham Heart Study. *J Am Heart Assoc* 2012;**1**:e000885.
15 Schlett CL, Massaro JM, Lehman SJ, Bamberg F, O'Donnell CJ, Fox CS, et al. Novel measurements of periaortic adipose tissue in comparison to anthropometric measures of obesity, and abdominal adipose tissue. *Int J Obes (Lond)* 2009;**33**:226–32.
16 Sjostrom L, Kvist H, Cederblad A, Tylen U. Determination of total adipose tissue and body fat in women by computed tomography, 40K, and tritium. *Am J Physiol* 1986;**250**:E736–45.
17 Maurovich-Horvat P, Massaro J, Fox CS, Moselewski F, O'Donnell CJ, Hoffmann U. Comparison of anthropometric, area- and volume-based assessment of abdominal subcutaneous and visceral adipose tissue volumes using multi-detector computed tomography. *Int J Obes (Lond)* 2007;**31**:500–6.
18 Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* 2007;**116**:39–48.
19 Demerath EW, Shen W, Lee M, Choh AC, Czerwinski SA, Siervogel RM, et al. Approximation of total visceral adipose tissue with a single magnetic resonance image. *Am J Clin Nutr* 2007;**85**:362–8.
20 Shimizu K, Mitchell RN, Libby P. Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* 2006;**26**:987–94.
21 Kwon HM, Sangiorgi G, Ritman EL, Lerman A, McKenna C, Virmani R, et al. Adventitial vasa vasorum in balloon-injured coronary arteries: visualization and quantitation by a microscopic three-dimensional computed tomography technique. *J Am Coll Cardiol* 1998;**32**:2072–9.
22 Gossl M, Herrmann J, Tang H, Versari D, Galili O, Mannheim D, et al. Prevention of vasa vasorum neovascularization attenuates early neointima formation in experimental hypercholesterolemia. *Basic Res Cardiol* 2009;**104**:695–706.
23 van Dam AD, Boon MR, Berbee JFP, Rensen PCN, van Harmelen V. Targeting white, brown and perivascular adipose tissue in atherosclerosis development. *Eur J Pharmacol* 2017;**816**:82–92.
24 Quesada I, Cejas J, Garcia R, Cannizzo B, Redondo A, Castro C. Vascular dysfunction elicited by a cross talk between periaortic adipose tissue and the vascular wall is reversed by pioglitazone. *Cardiovasc Ther* 2018;**36**:e12322.

25 Antonopoulos AS, Margaritis M, Coutinho P, Shirodaria C, Psarros C, Herdman L, et al. Adiponectin as a link between type 2 diabetes and vascular NADPH oxidase activity in the human arterial wall: the regulatory role of perivascular adipose tissue. *Diabetes* 2015;**64**:2207–19.
26 Doderer SA, Gabel G, Kokje VBC, Northoff BH, Holdt LM, Hamming JF, et al. Adventitial adipogenic degeneration is an unidentified contributor to aortic wall weakening in the abdominal aortic aneurysm. *J Vasc Surg* 2017;**67**:1891–900.
27 Rodeheffer MS. Tipping the scale: muscle versus fat. *Nat Cell Biol* 2010;**12**:102–4.
28 Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat Cell Biol* 2010;**12**:153–63.

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COUP D'OEIL

Coronary and Abdominal Aortic Aneurysms

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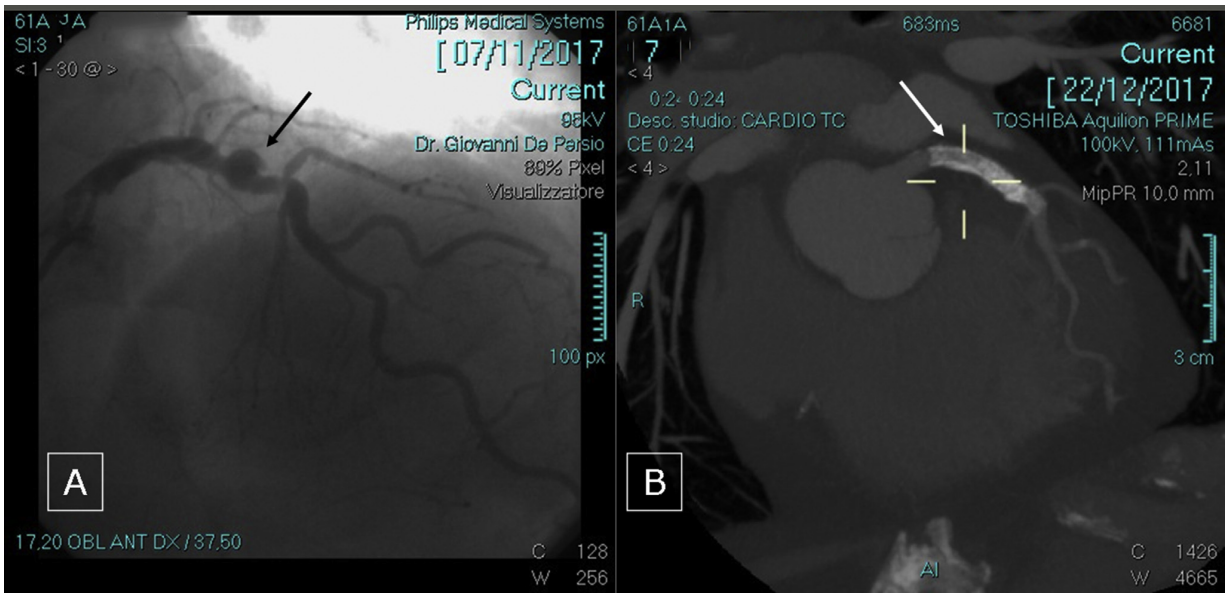

In a 61 year old male candidate for abdominal aortic aneurysm (AAA) repair, pre-operative coronary angiography showed an aneurysm of the proximal left anterior descending artery (8.4 × 9 mm; panel A). Coronary aneurysm exclusion was performed by implantation of a double layer of drug eluting stents (Resolute Onyx, Medtronic plc, Minneapolis, USA; panel B), under local anaesthesia and radial access. Endovascular AAA repair was performed one month later. Asymptomatic coronary artery aneurysms can be found in 16% of AAA patients. Percutaneous coronary aneurysm exclusion can be a safe intervention that might prevent potential future complications during AAA repair.

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CHAPTER 9 INFLAMMATORY ACTIVITY OF HUMAN PERIVASCULAR ADIPOSE TISSUE IN ABDOMINAL AORTIC ANEURYSMS

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Abstract

Background: Perivascular adipose tissue (PVAT) contributes to vascular homeostasis and is increasingly linked to vascular pathology. PVAT density and volume are associated with abdominal aortic aneurysm (AAA) presence and dimensions in imaging techniques. However, the mechanisms underlying the role of PVAT in the occurrence of AAA have not been clarified. Our study aims to explore differences in PVAT off AAA when studying histology, gene expression and functional tests.

Materials & Methods: Human aortic PVAT and control subcutaneous adipose tissue was collected during open surgery from patients suffering from AAA. Histology, gene analyses and functional tests were performed. Two control groups were composed, consisting of peripheral aortic occlusive disease (PAOD) and healthy aorta from non-living renal transplant donors. Histological analyses including adipocyte size and fibrosis in PVAT were examined to clarify previous imaging findings in density. Gene expression tests were performed to study genes potentially involved various inflammatory processes and AAA related genes. Subcutaneous adipose tissue (SAT) and PVAT from AAA were used for *ex vivo* co-culture with smooth muscle cells (SMC) retrieved from non-pathologic aortas.

Results: Adipose tissue was harvested from 36 AAA patients, 9 PAOD patients and 8 control patients. Adipocyte size was smaller in PVAT from patients with AAA compared to controls or PAOD patients (3.1 vs. 3.8 and 3.7 μm^2, p<.001). Furthermore, there was a higher proportion of fibrosis in PVAT from patients with AAA compared to controls or PAOD patients (0.03% vs. 0.01% and 0.02%, p=.026). Next, an increased inflammatory gene expression of *PTPRC* (p=.008), *CXCL8* (p=.033), *LCK* (p=.003), *CCL5* (p=.004) and an increase in extracellular matrix-down-breaking *MMP9* (p=.016) was found in AAA compared to controls. Also, there was a decreased anti-inflammatory gene expression of PPARG in AAA compared to controls (p=.040). Co-cultures of SMC from non-pathologic aortas with PVAT from AAA showed increased *MMP9* (p=.033) and *SMTN* (p=.008) expression and SAT increased *SMTN* expression in these SMC.

Conclusions: Our data reveal that PVAT from AAA shows smaller adipocytes and increased fibrosis, which are known characteristics of inflamed adipose tissue. Furthermore, an increased proinflammatory and matrix metallopeptidase gene expression and decreased anti-inflammatory gene expression were found in PVAT from AAA. Lastly, increased expression of genes involved in aneurysm formation was found in healthy SMC co-culture with PVAT of AAA patients. Therefore, PVAT from AAA contributes to inflammation of the adjacent aortic wall and hereby plays a role in the pathophysiology AAA, and could be a new therapeutic target in AAA treatment.

Introduction

Cardiovascular diseases are persistently listed among the diseases with highest morbidity and mortality [1]. Abdominal aortic aneurysm (AAA) is an irreversible pathology where dilation of the aorta can lead to fatal rupture [2]. Pathogenesis of AAA, and the role of peripheral aortic occlusive disease (PAOD) therein, remains unclear. Although PAOD is an independent risk factor for AAA development, AAA also often arise without presence of atherosclerosis [3]. Recent literature has shown an increased interest in the role of inflammation in both PAOD and AAA [4,5]. Therefore, inflammation is studied as a promising target for medical therapy to attenuate growth and prevent rupture of AAA [6–9].

Perivascular adipose tissue (PVAT) surrounding the conduit arteries was until recently thought to function merely as mechanical support. However, there is a growing body of literature that support PVAT as a tissue interacting with arteries in a paracrine fashion, with a pathogenic role in cardiovascular diseases [10–12]. It has been observed that PVAT volume positively associates with both thoracic and abdominal aortic dimensions [13]. We have recently found that patients with AAA present a higher PVAT density at the region of the maximum AAA diameter, compared to the healthy neck of the aneurysm. [14]. This was; however, not previously correlated to histologic findings.

Similar to other adipose tissues, PVAT at different anatomical sites secretes different biologically active substances (adipokines including cytokines, chemokines and growth factors), which can prevent, decelerate, induce or exacerbate atherosclerosis [15–19]. For example, the protective role of adiponectin in the onset of cardiac atherosclerosis is not found in aortic atherosclerosis [16]. Furthermore, expression levels of IL-1β, IL-6, and Leptin were higher in PVAT of atherosclerotic coronary arteries than in PVAT of internal thoracic arteries from the same patients [20]. To date, only a limited number of studies have reported on changes of PVAT in AAA. In the available literature, increased occurrence of necrosis, inflammation, fibrosis and proteolysis was found, potentially leading to macrophage infiltration and altered vascular smooth muscle cell function [21–23].

As proposed by Kakisis in the commentary to our previous study[24], our CT scan results in that study raised follow-up questions regarding histopathological alterations and underlying pathways, answers to which might lead to new prevention or conservative treatments in AAA. In the current study, we investigated the mechanisms underlying the role of PVAT in the occurrence of AAA, and tested the hypothesis that PVAT plays a role in inflammation of the aneurysmal abdominal aortic wall. We examined histological alterations in PVAT of AAA patients, we studied the RNA expression of PVAT in non-pathologic aortas and AAA and performed live *ex vivo* stimulation of smooth muscle cells (SMC) using SAT and PVAT.

Methods

Ethical considerations, study design and human tissue samples

The present study was approved by the Medical Ethical Committee of the Amsterdam University Medical Centers, Location VUmc (Amsterdam, the Netherlands) and local approval was obtained from the Medical Ethical Committees at Dijklander Ziekenhuis (Hoorn, the Netherlands) and São João University Hospital Center (Porto, Portugal). Informed consents were signed by patients and in case of acute surgery for ruptured aneurysm repair, delayed informed consent was received in the postoperative period as soon as a stable health situation was achieved in patients. Post-mortem tissue of anonymous donors was obtained in accordance with guidelines of Medical Ethical Committee of the Amsterdam University Medical Centers, Location VU Medical Center. All experiments were performed in accordance with relevant guidelines and regulations.

This was a prospective translational study in which human aortic PVAT was collected during open surgery from patients suffering from AAA, PAOD, and from post mortem renal donation (control tissue). **Figure 1** shows a full schematic overview of the study setup. The tissue was collected at the operating theatre in three hospitals (Amsterdam University Medical Centers, Location VU Medical Center, Amsterdam, the Netherlands, Dijklander Ziekenhuis, Hoorn, the Netherlands and São João University Hospital Center, Porto, Portugal) according to the same protocol and quality standards. Tissue samples were directly cut and I) fixated in formalin, II) snap frozen in liquid nitrogen and consecutively stored in -80 °C freezers until analysis or III) cultured.

Demographic variables

Clinical characteristics of all patients included in this study were collected from the electronic health records. These included: gender, age, AAA diameter, body mass index (BMI) (calculated as weight [in kg] divided by squared height [in m^2]), diabetes mellitus, statin use and smoking habits.

Histology

To avoid the degradation of the tissue as a result of under-fixation and fixative procedures that lead to variable tissue "shrinkage", a standard procedure was strictly followed for adipose tissue fixing and dehydration. After collection, the depot of interest was covered with >10-fold volume (mL) of 10% formalin to tissue (g) and kept at 4ºC for a minimum of 72 h. Preserved tissues were then paraffin processed and embedded tissue samples were faced off using a high-profile microtome blade. Three micrometre slides were stained with haematoxylin and eosin for quantification of the area of the adipocytes and Masson's Trichrome for assessment of fibrosis [25,26].

For quantification of the area of PVAT adipocytes, 100 adipocytes were counted to obtain an accurate estimation of adipocyte sizes within an adipose tissue depot; according to previous publications counting of 100 adipocytes also results in a similar distribution of adipocyte sizes compared to 300, 500, and 1000 adipocytes [25]. Fibrosis was assessed in photos depicting sections filled with adipocytes and that lacked blood vessels.

Using a Zeiss inverted microscope at least five representative photos of each section were taken with a 10 × and 20 × objective using a monochrome AxioCAM MrC camera (Carl Zeiss, Oberkochen, Germany). A semiautomated custom image analysis protocol was developed using ImageJ image analysis software (v.152, National Institutes of Health, Bethesda, MD, USA) to quantify the area of the individual adipocytes and the proportional area of fibrosis in each photo.

RNA Isolation and Quantitative PCR

Tissue pieces of 2 × 2 × 2 mm were homogenized in 2.0 mL RNase-free Microfuge Tubes (Thermo Fisher Scientific Inc., Waltham, MA, USA) using either 300 µl of lysis buffer (Zymo Research, Irvine, CA, U.S.A.) and bead lyser and homogenizer (TissueLyser II, Qiuagen, Hilden, Germany). Sequentially, total RNA was extracted from the homogenized tissue solutions using Quick-RNA ™ MiniPrep Plus (Zymo Research). According to manufacturer's instructions for use, first strand cDNA synthesis was performed using the VILO kit (Thermo Fisher Scientific Inc.). As housekeeping genes *YWHAZ*, *TBP* and *HPRT* were used and by use of Light Cycler SYBR Green I Master (Roche Applied Science, Penzberg, Germany) gene expression was analyzed by the LightCycler 480 Instrument II (Roche Applied Science).

In a 10µl solution (consisting of 1µl forward/reverse primer solution [10 pmol/µl], 5µl Light Cycler Mastermix [Light Cycler 480 SYBR Green I Master, Roche Applied Science], 2µl of five times diluted cDNA and 2µl PCR-grade H2O) qPCR reactions were prepared.

Absolute gene expression was determined using LightCycler software (RocheApplied Science), based on a standard curve of five serial reference dilutions (ranging from 10ng to 80pg of cDNA derived from human reference RNA (Agilent Technologies, Santa Clara, CA, USA). According to the Fit Points Method, PCR efficiency was automatically assessed and gene expression results were excluded if PCR efficiency was outside range of 1.85-2.0. Relative gene expression of all genes was based on normalization factor of housekeeping genes *YWHAZ*, *TBP* and *HPRT*. Therefore, normalization factor was calculated using geometric mean of housekeeping genes and individual genes were divided by normalization factor simultaneous to our previous publications. [27,28]

An initial set of primers was designed based on available literature on AAA and PAOD. The set included the following primers: of IL6, PTPRC, *CXCL8*, *MCP1*, *PLIN1*, *SAA1*, *TIMP1*, *TIMP2*, *MMP9*, *MMP2*, *ADIPOQ*, *LEP*, *ICAM1*, *ICAM3*, *RARRES2*, *CCR7* and *PPARG*. Following results of the initial RNA analysis, Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, ELIXIR, Cambridgeshire, United Kingdom) was consulted to predict potential interacting genes. By using STRING, new suggested directions for future experimental research can be explored [29]. A second RNA analysis was performed testing these additional genes. Forward and reverse primer sequences of all genes can be found in Supplementary Table 1.

Smooth muscle cell stimulation

Ex vivo stimulation of control aortic SMC without known aortic pathology was performed using PVAT and SAT. The non-pathologic aortic wall was retrieved from the operating theater of post-mortem kidney donors. Live aortic wall was transported in ice cold sterile Custodiol® (Dr. Franz Köhler Chemie GmbH, Alsbach-Hähnlein, Germany) and in a sterile Petri dish, intimal and adventitial layers were removed. Subsequently, the remaining medial layer was cut into 15 pieces of 1 mm^2 and transferred into a culture flask of 25 cm^2, which contained 1.5 ml of M231 culture medium (Thermo Fisher Scientific, Walthem, MA, USA), supplemented with Penicillin/Streptomycin (PS, 25,000 U) and 5% Smooth Muscle Growth Supplement (SMGS, Gibco, Life Technologies, Carlsbad, CA, USA). Cells were incubated for 10 days in a dark humidified atmosphere at 37 °C in 5% CO_2 and culture media were replaced biweekly. When a subconfluent cell population was established, cells were transferred into a culture flask of 80 cm^2. Within a total of 6 weeks, a confluent population of cells was generated and primary SMC used in the experiments were between passage one and eight.

SAT and PVAT of five patients were collected during AAA repair, and transported in ice cold sterile Custodiol®. According to a sectioning protocol our research group previously published to cut live aortic tissue sections [30], live SAT and PVAT sections of 200 µm were generated and preserved in supplemented M231 medium. Concurrently, in three cell culture plates (twelve wells, Sigma-Aldrich, Saint Louis, MO, USA), smooth muscle cells were seeded in six wells per plate with each 500 µL of M231 medium and allowed to adhere for 24 hours. Next, SAT sections (n=6) were added to the SMC in one plate, PVAT sections (n=6) to the second SMC plate, and the third plate with SMC functioned as a negative control. The SMC in six wells plates were then incubated for seven days in a dark humidified atmosphere at 37 °C in 5% CO_2 and the medium was changed once after three days.

After seven days, adipose sections were removed. Remaining SMC in the six wells plates were washed using PBS and consequently 300 µl of lysis buffer (Zymo Research) was repeatedly pipetted in and out to lyse the cells; cell lysates were subsequently transferred to Microfuge Tubes. Tubes containing lysis buffers with SAT, PVAT and SMC were stored at -80 °C for RNA Isolation and Quantitative PCR, following the above described protocol for aortic tissue. The following genes were assessed: *IL6*, *PTPRC*, *CXCL8*, *MCP1*, *PLIN1*, *SAA1*, *TIMP1*, *TIMP2*, *MMP9*, *MMP2*, *ADIPOQ*, *LEP*, *ICAM1*, *ICAM3*, *RARRES2*, *CCR7*, *MKI67*, *CNN1*, *ACTA2*, *SMTN*, *CXCR2*, *CD44*, *PTAFR*, *PPARG*, *JUN*, *CCL5*, *TNF*. To complete the analysis, a mean of the six wells was calculated. Next a ratio was calculated for PVAT (PVAT-co-cultured SMC/non-co-cultured SMC) and SAT (SAT- co-cultured SMC /non- co-cultured SMC) to compare adipose tissue stimulation outcomes of the healthy SMC.

Statistical Evaluation

Data were analyzed with IBM SPSS Statistics v22 (Chicago, IL, USA). Non normally distributed raw data are given as median with interquartile range (IQR), which are presented graphically in error bars. To assess multiple groups comparing continuous variables with nonparametric distribution, the Kruskal-Wallis test was used with Bonferroni correction for multiple pairwise comparison. Fibrosis results were highly right-skewed resembling a log-normal distribution. For that reason, the corresponding log-transformation was used for hypothesis testing. Because log-transformation followed a normal distribution, ANOVA was used, also with Bonferroni correction for multiple pairwise comparison. To compare two groups, Mann-Whitney U test was used. Tests were considered statistically significant at $p \leq 0.05$.

Results

Patient characteristics

Demographics and PVAT were collected for a total of 53 patients (n[control]=8, n[PAOD]=9, n[elective AAA]=27 and n[acute AAA]=9). In the control group 100% were male, in the PAOD group 100% were male and in the AAA group 75.0% were male. Median age of all controls was 62.0 years (IQR 8.3), median age of all PAOD patients was 61.0 years (IQR 8.0), and median age of all AAA patients was 70.0 years (IQR 12.0). Median maximum AAA diameter of all AAA patients was 64.0 mm (IQR 18.3). In the control group, 8.3% was using statins, and this was the case for 100% of the study population from PAOD group and 79.4% from the AAA group. In the control group 91.7% was currently or formerly smoking, in the PAOD group this was the case for 44.4% of the study population, and in the AAA group this was the case for 78.2% of the study population. A complete overview of the baseline variable can be found in Table 1.

Quantification of PVAT adipocyte area and fibrosis

An average of 170 adipocytes were measured per patient (n[control]=5, n[PAOD]=7 and n[AAA]=9). PVAT adipocytes from AAA patients were smaller than those derived from controls and PAOD patients (3.1 [IQR 2.0] vs. 3.8 [IQR 2.8] and 3.7 [IQR 2.0] μm^2, p<.001) (Figure 2A and B).

Also, fibrosis assessment was performed per patient (n[control]=5, n[PAOD]=7 and n[AAA]=7). PVAT from AAA patients presented higher amounts of fibrosis versus control and PAOD patients (0.03% [IQR 0.28%] in AAA versus 0.01% [IQR 0.09%] in controls and 0.02% [IQR 0.09%] in PAOD, p=.006 Figure 2C and D).

Gene expression of perivascular adipose tissue in AAA and controls

qPCR was used to quantify gene expression (n[control]=5, n[PAOD]=3 and n[AAA]=22). A significant increase in *PTPRC,* that encodes the enzyme PTPRC (also CD45), was found in PVAT of AAA compared to controls (1.43 [IQR 0.22] vs. 0.32 [IQR 2.93], p=.008). Furthermore, a significant increase in *CXCL8,* that encodes the chemokine CXCL8 (also interleukin 8), was found in PVAT of AAA compared to controls (1.58 [IQR 2.69] vs. 0.21 [IQR 0.92], p=.033). Also *MMP9,* that encodes the matrix metallopeptidase 9 was higher in PVAT of PAOD compared to controls (96.54 [IQR 133.36] vs. 9.74 [IQR 10.52], p=.016), higher in PVAT of PAOD compared to AAA (96.54 [IQR 133.36] vs. 19.59 [IQR 24.91], p=.048) and higher in PVAT of AAA compared to controls (19.59 [IQR 24.91] vs.

9.74 [IQR 10.52], p=.016). Additionally, a significant decrease in *PPARG*, that encodes the protein peroxisome proliferator-activated receptor gamma (also glitazone receptor) was found in PVAT of AAA compared to controls (3.23 [IQR 6.94] vs. 7.82 [IQR 7.93], p=.040).

Next, based on the statistically significant differences found in the primary analysis, these genes were tested in STRING Search Tool for the Retrieval of Interacting Genes/Proteins, which revealed predicted interactions of: *LCK*, *CD44*, *PTAFR*, *CXCR2*, *JUN*, *CCL5* and *FYN*. For these genes, we measured mRNA expression levels by qPCR. A significant increase in *LCK* was found in PVAT of AAA compared to controls (0.31 [IQR 0.69] vs. 0.04 [IQR 0.03], p=.003) and a significant increase in *CCL5* was found in PVAT of AAA compared to controls (2.48 [IQR 4.39] vs. 0.72 [IQR 0.68], p=.004). **Figure 3** shows the strength of interaction between these genes and the box plots of the statistically significant different genes.

Live PVAT and SAT co-culture changes in gene expression profile of aortic SMC

SAT and PVAT of patients suffering from AAA were collected (n=5). After co-culturing SAT and PVAT with SMC without known pathology, the cells were microscopically checked for vitality and in all wells, a solid group of live SMC and live adipose tissues were observed. **Figure 4A-D** shows a schematic overview and photographs of the incubation process. qPCR was used to detect the levels of genes. Not all tested genes resulted in quantifiable outcomes. For the following genes, results were retrieved: *IL6*, *CXCL8*, *TIMP1*, *TIMP2*, *MMP9*, *MMP2*, *MKI67*, *CNN1*, *ACTA2*, *SMTN*, *CXCR2*, *CD44*, *MCP1*, *PTAFR*, *PPARG*, *JUN*, *CCL5*, *TNF*. Analysis showed an increase of PVAT-co-cultured SMC ratio for MMP9 (2.15, p= .008) that encodes the matrix metallopeptidase 9 enzyme. Furthermore, analysis showed an increase of PVAT-co-cultured SMC ratio for *SMTN* (1.06, p =.008) that encodes the protein smoothelin. Furthermore, an increase of SAT-co-cultured SMC ratio was found for *SMTN* (1.18, p=.016). A complete overview of significant and non-significant outcome ratios is presented as box plots in Figure 4E.

Discussion

The purpose of this study was to elucidate if there was a role for PVAT in inflammation within AAA patients. An overview and interpretation of the results can be found in Figure 5.

Histology results showed that PVAT from patients with AAA has smaller adipocytes and more interstitial fibrosis. Although unconventional in basic research, we have composed a linear regression model for gender, age, AAA diameter, BMI, diabetes mellitus, statin use and smoking habits. After adjustment for these variables, the difference in PVAT area persisted between the groups, however, the difference in fibrosis did not. Current thinking on adipose tissue changes in obesity is that increased adipose tissue inflammation stimulates adipocyte lipolysis and tissue fibrosis, enhancing the release of free fatty acids from the adipose tissue. These excess fatty acids subsequently accumulate non-adipose tissues such as liver and skeletal muscle as ectopic fat, inducing lipotoxicity [31,32]. In human samples, omental fibrosis is also negatively correlated with adipocyte size in the same depot [33]. It is possible that increased inflammation in the adventitia of the aneurysmal wall is extends into the surrounding periaortic adipose tissue, leading to similar adipocyte morphology in the fat that surrounds the aneurysm. Our results, however, merely support a reversed route in which PVAT inflammation leads to aortic wall inflammation via adipokines.

In PVAT of AAA patients, we found increased expression of *PTPRC*, *CXCL8*, *MMP9*, *LCK* and *CCL5*, and a decreased expression of *PPARG* in PVAT of AAA patients compared to PVAT of controls. In patients suffering from PAOD, an increased expression of *MMP9* was found compared to both patients suffering from AAA and controls.

Taken together, an increased inflammatory gene expression was observed in PVAT of AAA patients. The enzyme PTPRC (also CD45 or leukocyte marker) is a member of the protein tyrosine phosphatase family and is involved cell growth, mitosis and differentiation. *PTPRC* was already shown to be upregulated in the aortic wall of AAA patients as an unspecific marker of inflammatory processes [34], and this also accounts for the enzyme [35,36]. *LCK* (Lymphocyte Cell-Specific Protein-Tyrosine Kinase) is a protein found inside lymphocytes, known for specific T-cell type selection and maturation of developing T-cells, which are elevated in AAA [37,38]. Although *LCK* was not broadly mentioned in literature considering AAA, our results show that this protein (and its gene expression) plays a role in an inflammatory response to antigens, potentially leading to a yet to be discovered activated T-cell cascade in the pathology of AAA. Future studies on the current topic might reveal the role of *LCK* in the differentiation of specific T-cells in AAA.

The proinflammatory role of PVAT is further underlined by the increased expression of *CXCL8* (IL8). *CXCL8* encodes for the eponymous chemokine and promotes inflammation, stimulates expression of proteases and it has pro-angiogenic effects via neutrophils and leukocytes, which are known for many years already to play a role in AAA pathology [39–42]. *CCL5* (Chemokine C-C motif ligand 5) is classified as a chemotactic chemokine, which

functions as chemoattractant for blood monocytes, memory T-helper cells and eosinophils, and recruits leukocytes into inflammatory sites. Therefore, the strong interaction level with *CXCL8* is not surprising. Recently, *CCL5* was found to be overexpressed in aortic wall tissue and mentioned as potential therapeutic target [43], since inhibition of *CCL5* in mice could limit AAA progression [44]. A potential therapeutic value might become clear after performing additional clinical studies.

In parallel and related to inflammation, PVAT may also play a role in the weakening of the aortic wall via increased expression of *MMP9*. Matrix Metallopeptidase 9 is a matrixin (enzyme) involved in the degradation of the extracellular matrix and is known to play a role in both progression and rupture of AAA, likely again as a consequence of inflammation as illustrated by the high interaction with *CXCL8* [45,46].
More recently, Kugo et al. showed greater *MMP9* positive areas around adipocytes than in the areas without adipocytes in the ruptured vascular wall [47], suggesting a link between the adipocytes in the vascular wall and the mechanisms leading to rupture. The putative contribution of PVAT to the appearance of adipocytes in the adventitia of the AAA wall remains unclear. Potentially, PVAT might lead to degradation of the aortic wall through *MMP9* via equal pathways.

Another interesting finding was the decreased expression of the seemingly protective gene *PPARG* (Peroxisome Proliferator-Activated Receptor Gamma), also known as glitazone receptor. This is a nuclear receptor mainly present in adipose tissue, colon and macrophages and regulates fatty acid storage, glucose metabolism and adipocyte differentiation. Strikingly, *PPARG* also decreases the inflammatory response of many cardiovascular cells [48–50]. One characteristic feature of the epidemiology of AAA is that diabetic patients are less prone to AAA presence, progression and rupture [51–56]. A underlying hypothesis is that common antidiabetic medications on their own inhibit growth of aortic aneurysms, potentially via *PPARG* ligation [57–60]. *PPARG* ligation was shown to significantly decrease suprarenal aortic expansion in the AAA animal model (apolipoprotein E deficient (ApoE[-/-]) mice treated with angiotensine II) [61]. *PPARG* increased the number of small adipocytes, which is in line with our current histological findings, and raises *IL10* [62,63]. The increase of *IL10* content in adipose tissue leads to macrophage (type 2) polarization which is important for remodeling and tissue repair. *PPARG* is likely to continue to upregulate the expression of anti-inflammatory cytokines, extend the 'compensatory stage', and decelerate the process of AAA development and rupture [64]. Taken together, a decrease in *PPARG* expression might play a key role in the increase of inflammation and the onset and/or progression of AAA.

The effect of live PVAT and SAT on SMC of 5 patients showed an increased expression of *MMP9* in healthy SMC co-culture with PVAT of AAA patients. This implicates that PVAT could play an important role in the breakdown of the extracellular matrix via SMC in the medial layer and consequently in progression and rupture of AAA [45,46]. In both SAT and PVAT co-cultured SMC, we found a mildly increased expression of smoothelin (*SMTN*). Smoothelin is a structural protein that is found exclusively in contractile SMC and is essential for contractility of SMC [65]. Potentially, as a response to early induced changes in healthy SMC by adipose tissue, these express more smoothelin. Interestingly, our research group previously found *decreased PTPRC* and *decreased SMTN* expression after stimulation of AAA SMC with TGF-β [30], while TGF-β is suggested to protect against AAA formation. [66–71] These findings seem to be in line with our current findings of *increased PTPRC* and *increased SMTN* expression in the pathologic *ex vivo* situation.

Most gene expression outcomes showed comparable results of PVAT and SAT, which might indicate that there is a systemic effect of adipose tissue in AAA patients. This finding could also be explained by interaction between SMC and adipose tissue. Additional research, studying also the effect of adipose tissue of healthy patients should be undertaken to further clarify the role of adipose tissue in gene expression of SMC.

Strikingly, Kugo et al (2019) [72] recently showed in a rat model that removal of PVAT led to a significant decrease in AAA diameter. This was along with a reduction in the adipocytes and $CD44^{+}CD90^{+}$ mesenchymal stem cells that express adipogenic transcription factors in the AAA wall of a hypoperfusion-induced AAA model. The role of PVAT in aneurysm progression was further emphasized during the study of Interleukin-18 in the AAA development [73]. Lesion adipocytes and PVAT were shown to contribute to AAA pathogenesis by releasing leptin and fatty acid binding protein 4 (FABP4) that induce the expression of IL18 and its receptors and promote IL18 action.

Surprisingly, it seems like there is an increased inflammatory PVAT activity in AAA patients, despite the higher use of statins in the AAA group (72.7%) versus the control group (0%). It is widely accepted that statins lower inflammation in vascular diseases [74–76] and van der Meij et al. specifically showed this for AAA patients [77]. Although a clinical relevance of statin use preventing AAA growth and or rupture was not found in that study, selective anti-inflammatory therapy focusing on PVAT might provide a new strategy to prevent progression of AAA. Moreover, this might explain the recent interest in the role of, not diabetes, but metformin use as protective against AAA progression, [78–81] since metformin proved to have widespread anti-inflammatory characteristics as well [82]. Further work is required to establish the role of metformin on PVAT.

A note of caution is due here since this study was limited by the relatively small number of cases that were included. Furthermore, the included patients had a Western European background. One could also argue that we should have included waist-to-hip ratio, especially since ethnic differences seems to exist. Therefore, these findings cannot be globally extrapolated to patients from varying geographical backgrounds, since incidence, pathophysiology and outcomes after intervention differ in at least Asian, Hispanic an Afro-American population [83–87].

Another weakness of the study was the collection of control tissue. Due to the clinical handling process, procurement times and protocol differed between PAOD and control tissues, since PAOD tissue was harvested directly in the OR and handled like AAA tissue, while control tissue was dissected for renal transplantation and transported in Custodiol®. The control tissue was only harvested for research after the surgery of the kidney receiving patient had started. Interestingly, though, we concluded that the results of the PAOD and control tissue are roughly comparable. This finding strengthens the differences found between the AAA and control group.

Furthermore, we were not able to perform all the different experiments within the same patient cohort. This was a direct consequence of the development of new research techniques which were unavailable at the initiation of the current study. Small quantities of valuable patient material were available since this originated from rest tissue of living patients. Furthermore, as shown in Table 1, there were statistically significant differences between the groups in age, diabetes and statin use. Therefore, we need to interpret and discuss the current findings under these limitations.

The model that we use has some limitations because stimulation by sections of adipose tissue were tested in SMC culture only. SMC dysregulation is central to the AAA pathophysiology as explained before, but it represents a fraction of the resident vascular cells in the aortic wall. Using this model, we were not able to address the effect of PVAT in the mesenchymal cells and adipose deposits of the adventitia itself, cellular elements that have recently been linked to AAA progression and rupture [88].

It is important to bear in mind that our results could possibly indicate an inflammatory cause or consequence of PVAT in the AAA pathophysiology. This question remains for each individual finding. Also, bidirectional communication between PVAT and aortic wall could lead to alterations in both PVAT and wall [89–91]. Further stimulation experiments might clarify the exact role of individual PVAT genes in the pathology of AAA.

In conclusion, our results show that PVAT of AAA patients have smaller adipocytes with higher interstitial fibrosis, present with higher inflammation and increased extracellular matrix degradation. Adipose tissue of patients suffering from AAA can induce inflammation in healthy SMC. We propose that PVAT plays an important role in the onset and progression of AAA and underlying pathways through PVAT might be a novel promising therapeutic target in both prevention and conservative treatment of AAA.

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References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study2010. *Lancet* 2012; **380**: 2095–128.
2. Assar AN, Zarins CK. Ruptured abdominal aortic aneurysm: A surgical emergency with many clinical presentations. Postgrad. Med. J. 2009; **85**: 268–73.
3. Toghill BJ, Saratzis A, Bown MJ. Abdominal aortic aneurysm—an independent disease to atherosclerosis? Cardiovasc. Pathol. 2017; **27**: 71–5.
4. Galkina E, Ley K. Immune and Inflammatory Mechanisms of Atherosclerosis. *Annu Rev Immunol* 2009; **27**: 165–97.
5. Dale MA, Ruhlman MK, Baxter BT. Inflammatory cell phenotypes in AAAs: Their role and potential as targets for therapy. *Arterioscler Thromb Vasc Biol* 2015; **35**: 1746–55.
6. Lindeman JHN, Abdul-Hussien H, Van Bockel JH, Wolterbeek R, Kleemann R. Clinical trial of doxycycline for matrix metalloproteinase-9 inhibition in patients with an abdominal aneurysm doxycycline selectively depletes aortic wall neutrophils and cytotoxic t cells. *Circulation* 2009; **119**: 2209–16.
7. Arnoud Meijer C, Stijnen T, Wasser MNJM, Hamming JF, Van Bockel JH, Lindeman JHN. Doxycycline for stabilization of abdominal aortic aneurysms: A randomized trial. *Ann Intern Med* 2013; **159**: 815–23.
8. Broz P, Ben-Haim N, Grzelakowski M, Marsch S, Meier W, Hunziker P. Inhibition of macrophage phagocytotic activity by a receptor-targeted polymer vesicle-based drug delivery formulation of pravastatin. *J Cardiovasc Pharmacol* 2008; **51**: 246–52.
9. Emeto T, Seto S-W, Golledge J. Targets for Medical Therapy to Limit Abdominal Aortic Aneurysm Progression. *Curr Drug Targets* 2014; **15**: 860–73.
10. Fuster JJ, Ouchi N, Gokce N, Walsh K. Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease. *Circ Res* 2016; **118**: 1786–807.
11. Koliaki C, Liatis S, Kokkinos A. Obesity and cardiovascular disease: revisiting an old relationship. *Metabolism* 2019; **92**: 98–107.
12. Ha EE, Bauer RC. Emerging Roles for Adipose Tissue in Cardiovascular Disease. *Arterioscler Thromb Vasc Biol* 2018; **38**. DOI:10.1161/ATVBAHA.118.311421.
13. Thanassoulis G, Massaro JM, Corsini E, *et al.* Periaortic adipose tissue and aortic dimensions in the Framingham Heart Study. *J Am Heart Assoc* 2012; **1**. DOI:10.1161/JAHA.112.000885.
14. Dias-Neto M, Meekel JP, van Schaik TG, *et al.* High Density of Periaortic Adipose Tissue in Abdominal Aortic Aneurysm. *Eur J Vasc Endovasc Surg* 2018; **56**: 663–71.
15. Greenstein AS, Khavandi K, Withers SB, *et al.* Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. *Circulation* 2009; **119**: 1661–70.
16. Spiroglou SG, Kostopoulos CG, Varakis JN, Papadaki HH. Adipokines in periaortic and epicardial adipose tissue: differential expression and relation to atherosclerosis. *J Atheroscler Thromb* 2010; **17**: 115–30.
17. Chatterjee TK, Aronow BJ, Tong WS, *et al.* Human coronary artery perivascular adipocytes overexpress genes responsible for regulating vascular morphology, inflammation, and hemostasis. *Physiol Genomics* 2013; **45**: 697–709.
18. van Hinsbergh VWM, Eringa EC, Daemen MJAP. Neovascularization of the atherosclerotic plaque: interplay between atherosclerotic lesion, adventitia-derived microvessels and perivascular fat. *Curr Opin Lipidol* 2015; **26**: 405–11.
19. Skiba DS, Nosalski R, Mikolajczyk TP, *et al.* Anti-atherosclerotic effect of the angiotensin 1-7 mimetic AVE0991 is mediated by inhibition of perivascular and plaque inflammation in early atherosclerosis. Br. J. Pharmacol. 2017. DOI:10.1111/bph.13685.
20. Lu D, Wang W, Xia L, Xia P, Yan Y. Gene expression profiling reveals heterogeneity of perivascular adipose tissues surrounding coronary and internal thoracic arteries. *Acta Biochim Biophys Sin (Shanghai)* 2017; **49**: 1075–82.
21. Folkesson M, Vorkapic E, Gulbins E, *et al.* Inflammatory cells, ceramides, and expression of proteases in perivascular adipose tissue adjacent to human abdominal aortic aneurysms. In: Journal of Vascular Surgery. 2017: 1171–9.
22. Blomkalns AL, Gavrila D, Thomas M, *et al.* CD14 directs adventitial macrophage precursor recruitment: role in early abdominal aortic aneurysm formation. *J Am Heart Assoc* 2013; **2**. DOI:10.1161/JAHA.112.000065.
23. Zhang Z-B, Ruan C-C, Lin J-R, *et al.* Perivascular Adipose Tissue-Derived PDGF-D Contributes to Aortic Aneurysm Formation During Obesity. *Diabetes* 2018; **67**: 1549–60.

24. Kakisis JD. Peria-ortic Adipose Tissue is More than Plain Fat. *Eur J Vasc Endovasc Surg* 2018; **56**: 672.
25. Parlee SD, Lentz SI, Mori H, MacDougald OA. Quantifying size and number of adipocytes in adipose tissue. *Methods Enzymol* 2014; **537**: 93–122.
26. Muir LA, Neeley CK, Meyer KA, *et al.* Adipose tissue fibrosis, hypertrophy, and hyperplasia: Correlations with diabetes in human obesity. *Obesity (Silver Spring)* 2016; **24**: 597–605.
27. Yeung KK, Bogunovic N, Keekstra N, *et al.* Transdifferentiation of Human Dermal Fibroblasts to Smooth Muscle-Like Cells to Study the Effect of MYH11 and ACTA2 Mutations in Aortic Aneurysms. *Hum Mutat* 2017; **38**: 439–50.
28. Bogunovic N, Meekel JP, Micha D, Blankensteijn JD, Hordijk PL, Yeung KK. Impaired smooth muscle cell contractility as a novel concept of abdominal aortic aneurysm pathophysiology. *Sci Rep* 2019; **9**: 6837.
29. Szklarczyk D, Franceschini A, Kuhn M, *et al.* The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res* 2011; **39**: D561–8.
30. Meekel JP, Groeneveld ME, Bogunovic N, *et al.* An in vitro method to keep human aortic tissue sections functionally and structurally intact. *Sci Rep* 2018; **8**. DOI:10.1038/s41598-018-26549-4.
31. Suganami T, Tanaka M, Ogawa Y. Adipose tissue inflammation and ectopic lipid accumulation. *Endocr J* 2012; **59**: 849–57.
32. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest* 2011; **121**: 2094–101.
33. Divoux A, Tordjman J, Lacasa D, *et al.* Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss. *Diabetes* 2010; **59**: 2817–25.
34. Hinterseher I, Erdman R, Elmore JR, *et al.* Novel Pathways in the Pathobiology of Human Abdominal Aortic Aneurysms. *Pathobiology* 2013; **80**: 1–10.
35. Schubl S, Tsai S, Ryer EJ, *et al.* Upregulation of Protein Kinase Cδ in Vascular Smooth Muscle Cells Promotes Inflammation in Abdominal Aortic Aneurysm. *J Surg Res* 2009; **153**: 181–7.
36. Treska V, Kocova J, Boudova L, *et al.* Inflammation in the wall of abdominal aortic aneurysm and its role in the symptomatology of aneurysm. *Cytokines Cell Mol Ther* 2002; **7**: 91–7.
37. Hauck F, Randriamampita C, Martin E, *et al.* Primary T-cell immunodeficiency with immunodysregulation caused by autosomal recessive LCK deficiency. *J Allergy Clin Immunol* 2012; **130**: 1144-1152.e11.
38. Téo FH, de Oliveira RTD, Villarejos L, *et al.* Characterization of CD4 + T Cell Subsets in Patients with Abdominal Aortic Aneurysms. *Mediators Inflamm* 2018; **2018**: 1–12.
39. Eliason JL, Hannawa KK, Ailawadi G, *et al.* Neutrophil Depletion Inhibits Experimental Abdominal Aortic Aneurysm Formation. *Circulation* 2005; **112**: 232–40.
40. Campa JS, Greenhalgh RM, Powell JT. Elastin degradation in abdominal aortic aneurysms. *Atherosclerosis* 1987; **65**: 13–21.
41. Middleton RK, Bown MJ, Lloyd GM, Jones JL, London NJ, Sayers RD. Characterisation of Interleukin-8 and Monocyte Chemoattractant Protein-1 Expression within the Abdominal Aortic Aneurysm and their Association with Mural Inflammation. *Eur J Vasc Endovasc Surg* 2009; **37**: 46–55.
42. Kokje VBC, Gäbel G, Dalman RL, *et al.* CXCL8 hyper-signaling in the aortic abdominal aneurysm. *Cytokine* 2018; **108**: 96–104.
43. Raffort J, Lareyre F, Clément M, Hassen-Khodja R, Chinetti G, Mallat Z. Monocytes and macrophages in abdominal aortic aneurysm. *Nat Rev Cardiol* 2017; **14**: 457–71.
44. Iida Y, Xu B, Xuan H, *et al.* Peptide Inhibitor of CXCL4–CCL5 Heterodimer Formation, MKEY, Inhibits Experimental Aortic Aneurysm Initiation and Progression. *Arterioscler Thromb Vasc Biol* 2013; **33**: 718–26.
45. Sakalihasan N, Heyeres A, Nusgens B V., Limet R, Lapiére CM. Modifications of the extracellular matrix of aneurysmal abdominal aortas as a function of their size. *Eur J Vasc Surg* 1993; **7**: 633–7.
46. Wilson WRW, Anderton M, Schwalbe EC. Matrix metalloproteinase-8 and -9 are increased at the site of abdominal aortic aneurysm rupture. *J Vasc Surg* 2006; **44**: 683–4.
47. Kugo H, Zaima N, Tanaka H, *et al.* Adipocyte in vascular wall can induce the rupture of abdominal aortic aneurysm. *Sci Rep* 2016; **6**: 31268.
48. Chinetti G, Fruchart J-C, Staels B. Peroxisome proliferator-activated receptors and inflammation: from basic science to clinical applications. *Int J Obes* 2003; **27**: S41–5.
49. Calder PC. Long-chain fatty acids and inflammation. *Proc Nutr Soc* 2012; **71**: 284–9.
50. Szanto A, Nagy L. The many faces of PPARγ: Anti-inflammatory by any means? *Immunobiology* 2008; **213**: 789–803.
51. De Rango P, Farchioni L, Fiorucci B, Lenti M. Diabetes and Abdominal Aortic Aneurysms. *Eur J Vasc Endovasc Surg* 2014; **47**: 243–61.
52. Lederle FA, Johnson GR, Wilson SE, *et al.* The aneurysm detection and management study screening

program: validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. *Arch Intern Med* 2000; **160**: 1425–30.
53. Brady AR, Thompson SG, Fowkes FGR, Greenhalgh RM, Powell JT, UK Small Aneurysm Trial Participants. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. *Circulation* 2004; **110**: 16–21.
54. Vega de Céniga M, Gómez R, Estallo L, Rodríguez L, Baquer M, Barba A. Growth rate and associated factors in small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2006; **31**: 231–6.
55. Golledge J, Karan M, Moran CS, *et al.* Reduced expansion rate of abdominal aortic aneurysms in patients with diabetes may be related to aberrant monocyte-matrix interactions. *Eur Heart J* 2008; **29**: 665–72.
56. Le MTQ, Jamrozik K, Davis TME, Norman PE. Negative Association between Infra-renal Aortic Diameter and Glycaemia: The Health In Men Study. *Eur J Vasc Endovasc Surg* 2007; **33**: 599–604.
57. Patel K, Zafar MA, Ziganshin BA, Elefteriades JA. Diabetes Mellitus: Is It Protective against Aneurysm? A Narrative Review. *Cardiology* 2018; **141**: 107–22.
58. Brunmair B, Staniek K, Gras F, *et al.* Thiazolidinediones, Like Metformin, Inhibit Respiratory Complex I: A Common Mechanism Contributing to Their Antidiabetic Actions? *Diabetes* 2004; **53**: 1052–9.
59. Takemura Y, Osuga Y, Yoshino O, *et al.* Metformin Suppresses Interleukin (IL)-1β-Induced IL-8 Production, Aromatase Activation, and Proliferation of Endometriotic Stromal Cells. *J Clin Endocrinol Metab* 2007; **92**: 3213–8.
60. Sundriyal S, Khanna S, Saha R, Bharatam P V. Metformin and glitazones: does similarity in biomolecular mechanism originate from tautomerism in these drugs? *J Phys Org Chem* 2008; **21**: 30–3.
61. Golledge J, Cullen B, Rush C, *et al.* Peroxisome proliferator-activated receptor ligands reduce aortic dilatation in a mouse model of aortic aneurysm. *Atherosclerosis* 2010; **210**: 51–6.
62. Okuno A, Tamemoto H, Tobe K, *et al.* Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J Clin Invest* 1998; **101**: 1354–61.
63. Rigamonti E, Chinetti-Gbaguidi G, Staels B. Regulation of macrophage functions by PPAR-alpha, PPAR-gamma, and LXRs in mice and men. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1050–9.
64. Wang W-D, Sun R, Chen Y-X. PPARγ agonist rosiglitazone alters the temporal and spatial distribution of inflammation during abdominal aortic aneurysm formation. *Mol Med Rep* 2018; **18**: 3421–8.
65. Kadirvel R, Ding Y-H, Dai D, Lewis DA, Kallmes DF. Gene Expression Changes: Five Years after Creation of Elastase-induced Aneurysms. *J Vasc Interv Radiol* 2011; **22**: 1447-1451.e2.
66. Wang Y, Krishna S, Walker PJ, Norman P, Golledge J. Transforming growth factor-β and abdominal aortic aneurysms. *Cardiovasc Pathol* 2013; **22**: 126–32.
67. Lareyre F, Clément M, Raffort J, *et al.* TGFβ (Transforming Growth Factor-β) Blockade Induces a Human-Like Disease in a Nondissecting Mouse Model of Abdominal Aortic Aneurysm. *Arterioscler Thromb Vasc Biol* 2017; **37**: 2171–81.
68. Wang Y, Ait-Oufella H, Herbin O, *et al.* TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. *J Clin Invest* 2010; **120**: 422–32.
69. Doyle AJ, Redmond EM, Gillespie DL, *et al.* Differential expression of Hedgehog/Notch and transforming growth factor-β in human abdominal aortic aneurysms. *J Vasc Surg* 2015; **62**: 464–70.
70. Bertoli-Avella AM, Gillis E, Morisaki H, *et al.* Mutations in a TGF-β Ligand, TGFB3, Cause Syndromic Aortic Aneurysms and Dissections. *J Am Coll Cardiol* 2015; **65**: 1324–36.
71. Angelov SN, Hu JH, Wei H, Airhart N, Shi M, Dichek DA. TGF-β (Transforming Growth Factor-β) Signaling Protects the Thoracic and Abdominal Aorta From Angiotensin II-Induced Pathology by Distinct Mechanisms. *Arterioscler Thromb Vasc Biol* 2017; **37**: 2102–13.
72. Kugo H, Moriyama T, Zaima N. The role of perivascular adipose tissue in the appearance of ectopic adipocytes in the abdominal aortic aneurysmal wall. *Adipocyte* 2019; **8**: 229–39.
73. Liu C-L, Ren J, Wang Y, *et al.* Adipocytes promote interleukin-18 binding to its receptors during abdominal aortic aneurysm formation in mice. *Eur Heart J* 2019; published online Dec 10. DOI:10.1093/eurheartj/ehz856.
74. Blum A, Shamburek R. The pleiotropic effects of statins on endothelial function, vascular inflammation, immunomodulation and thrombogenesis. *Atherosclerosis* 2009; **203**: 325–30.
75. Jain MK, Ridker PM. Anti-Inflammatory Effects of Statins: Clinical Evidence and Basic Mechanisms. *Nat Rev Drug Discov* 2005; **4**: 977–87.
76. Bäck M, Hansson GK. Anti-inflammatory therapies for atherosclerosis. *Nat Rev Cardiol* 2015; **12**: 199–211.
77. van der Meij E, Koning GG, Vriens PW, *et al.* A Clinical Evaluation of Statin Pleiotropy: Statins Selectively and Dose-Dependently Reduce Vascular Inflammation. *PLoS One* 2013; **8**: e53882.
78. Hsu C-Y, Su Y-W, Chen Y-T, *et al.* Association between use of oral-antidiabetic drugs and the risk of aortic

aneurysm: a nested case–control analysis. *Cardiovasc Diabetol* 2016; **15**: 125.
79. Hinchliffe RJ. Metformin and Abdominal Aortic Aneurysm. *Eur J Vasc Endovasc Surg* 2017; **54**: 679–80.
80. Golledge J, Morris DR, Pinchbeck J, *et al.* Editor's Choice – Metformin Prescription is Associated with a Reduction in the Combined Incidence of Surgical Repair and Rupture Related Mortality in Patients with Abdominal Aortic Aneurysm. *Eur J Vasc Endovasc Surg* 2019; **57**: 94–101.
81. Itoga NK, Rothenberg KA, Suarez P, *et al.* Metformin prescription status and abdominal aortic aneurysm disease progression in the U.S. veteran population. *J Vasc Surg* 2019; **69**: 710-716.e3.
82. Cameron AR, Morrison VL, Levin D, *et al.* Anti-Inflammatory Effects of Metformin Irrespective of Diabetes Status. *Circ Res* 2016; **119**: 652–65.
83. Kent KC, Zwolak RM, Egorova NN, *et al.* Analysis of risk factors for abdominal aortic aneurysm in a cohort of more than 3 million individuals. *J Vasc Surg* 2010; **52**: 539–48.
84. Laughlin GA, Allison MA, Jensky NE, *et al.* Abdominal Aortic Diameter and Vascular Atherosclerosis: The Multi-Ethnic Study of Atherosclerosis. *Eur J Vasc Endovasc Surg* 2011; **41**: 481–7.
85. Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nat Rev Cardiol* 2011; **8**: 92–102.
86. Arnaoutakis DJ, Propper BW, Black JH, *et al.* Racial and ethnic disparities in the treatment of unruptured thoracoabdominal aortic aneurysms in the United States. *J Surg Res* 2013; **184**: 651–7.
87. Wang XL, Thompson MM, Dole WP, Dalman RL, Zalewski A. Standardization of outcome measures in clinical trials of pharmacological treatment for abdominal aortic aneurysm. *Expert Rev Cardiovasc Ther* 2012; **10**: 1251–60.
88. Kugo H, Zaima N, Tanaka H, *et al.* Adipocyte in vascular wall can induce the rupture of abdominal aortic aneurysm. *Sci Rep* 2016; **6**: 31268.
89. Antonopoulos AS, Margaritis M, Coutinho P, *et al.* Adiponectin as a link between type 2 diabetes and vascular NADPH oxidase activity in the human arterial wall: the regulatory role of perivascular adipose tissue. *Diabetes* 2015; **64**: 2207–19.
90. Fernández-Alfonso MS, Gil-Ortega M, Aranguez I, *et al.* Role of PVAT in coronary atherosclerosis and vein graft patency: friend or foe? *Br J Pharmacol* 2017; **174**: 3561–72.
91. Quesada I, Cejas J, García R, Cannizzo B, Redondo A, Castro C. Vascular dysfunction elicited by a cross talk between periaortic adipose tissue and the vascular wall is reversed by pioglitazone. *Cardiovasc Ther* 2018; **36**: e12322.

Table 1. Description of baseline characteristics for patients included control, PAOD and AAA groups.

	Control (n = 8)	PAOD (n = 9)	AAA (n = 36) *Elective AAA (n = 9)* *Acute AAA (n = 27)*	p-value
Male gender (%)	100%	100%	74.3% *73.1%* *77.8%*	.133
Age in years (median)	65.0 (45.0 - 69.0)	61.0 (44.0 - 72.0)	70.0 (47.0 - 88.0) *73.0 (58.0 - 82.0)* *70.0 (47.0 - 88.0)*	**.009**
AAA diameter in mm (median)	*n/a*	*n/a*	64.0 (48.0 - 114.0) *62.0 (48.0 - 94.0)* *83.0 (60.0 - 114.0)*	*n/a*
BMI (median)	23.9 (16.7 - 32.6)	22.8 (21.4 - 32.0)	27.0 (20.1 - 37.0) *23.7 (20.1 - 37.0)* *28.3 (23.9 - 35.3)*	.386
Diabetes mellitus (%)	0.0%	44.4%	5.9% *4.0%* *11.1%*	**.015**
Statin use (%)	0.0%	100%	79.4% *80.0%* *77.8%*	**.007**
Smoking: non-smoking, formerly smoking, currently smoking (%)	8.3%, 75.0%, 16.7%	55.6%, 33.3%, 11,1%	21.9%, 31.3%, 46.9 *23.8%, 38.1%. 38.1%* *83.3%, 0.0%, 16.7%*	.058

Figure 1. A simplified flow chart describing the different steps performed in the current study. Perivascular adipose tissue, subcutaneous adipose tissue and demographic and clinical data were collected for analysis. Respectively, Hounsfield Units, histology analysis, RNA expression and data analysis were performed. PVAT indicates perivascular adipose tissue; AAA, abdominal aortic aneurysm; SAT, subcutaneous adipose tissue; PAOD, peripheral arterial occlusive disease and SMC, smooth muscle cells.

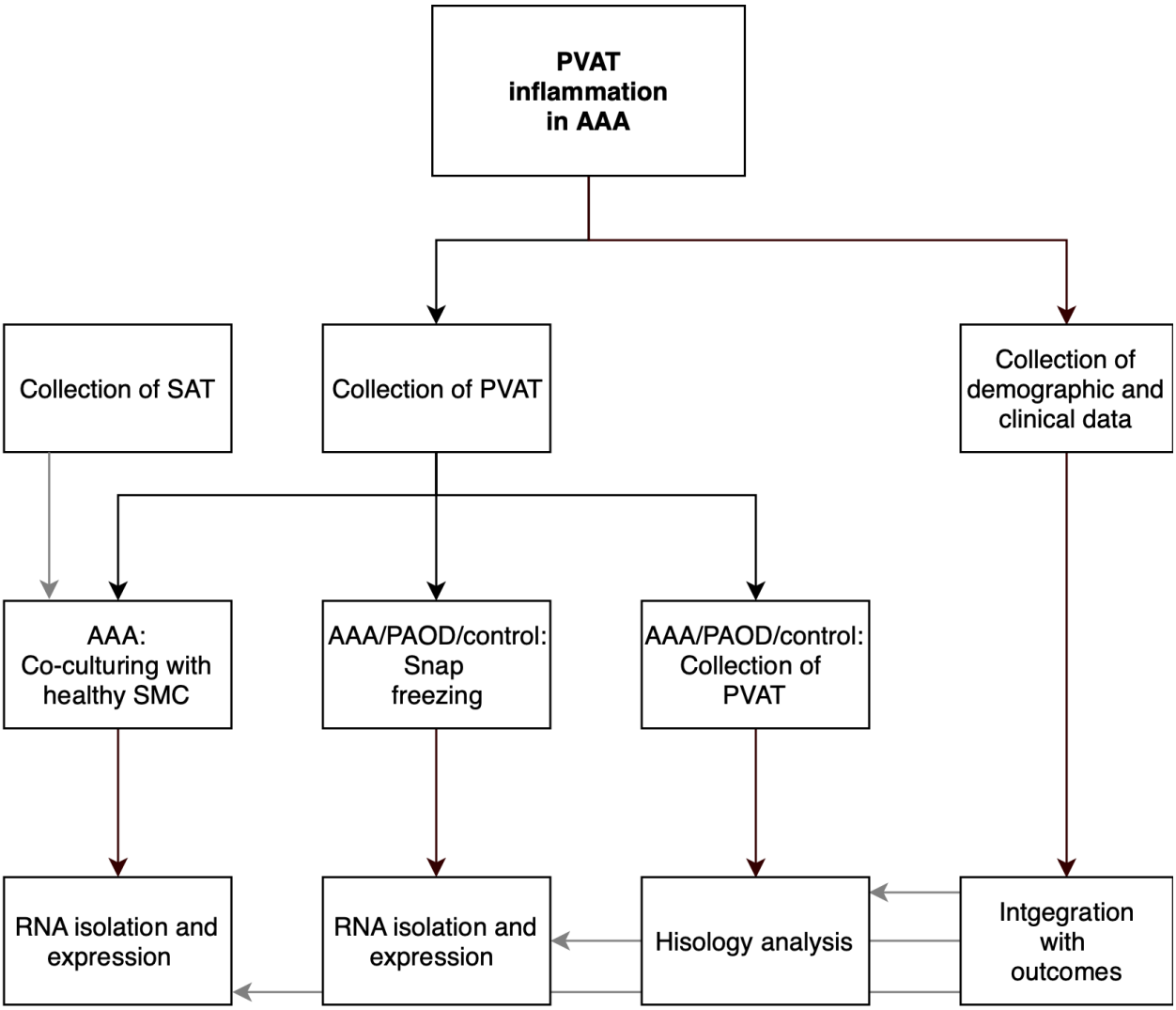
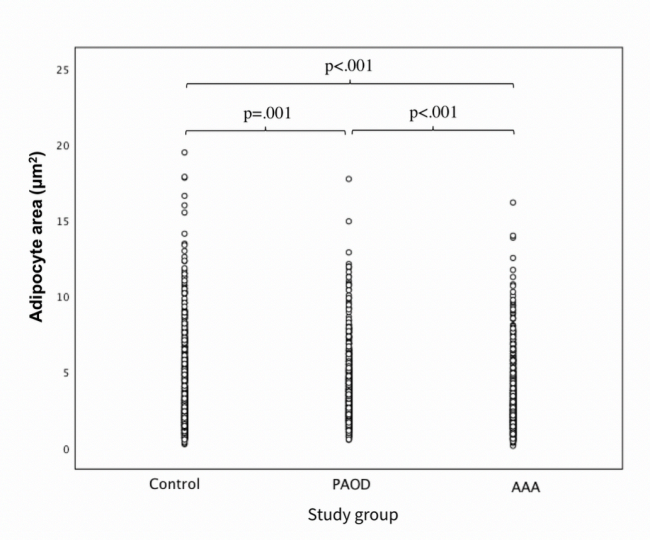


Figure 2. A) Adipocyte area of PVAT from Control, PAOD and AAA patients (p<.001 in Kruskal-Wallis test; p values represent all pairwise comparison using Bonferroni correction). **B)** Hematoxylin and eosin staining for quantification of the area of the PVAT adipocytes (10x magnification). **C)** Percentage of fibrosis in PVAT from Control, PAOD and AAA patients (p=.006 in ANOVA test using the log-transformation of the variable; p values represent all pairwise comparison using Bonferroni correction). NS – nonsignificant. **D)** Massons Trichrome staining for quantification of the fibrosis of the PVAT adipocytes (20x magnification), where blue indicates fibrosis.

A



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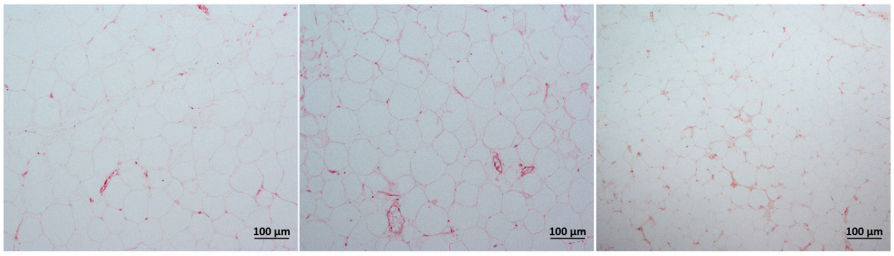

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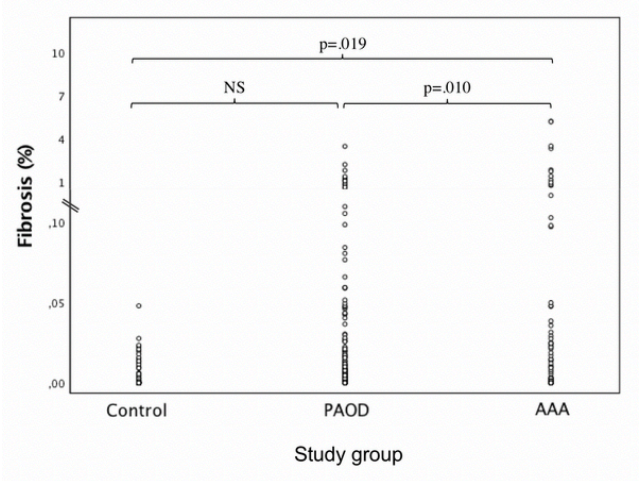

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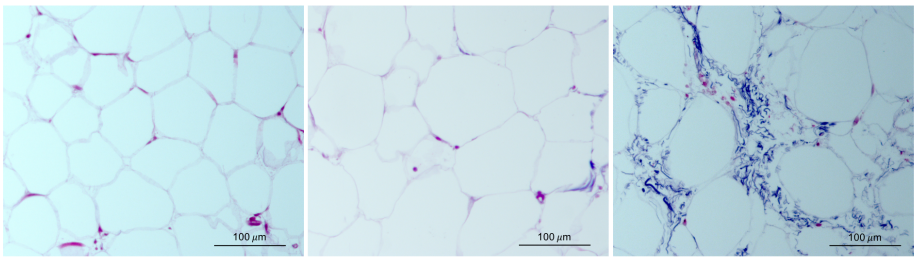
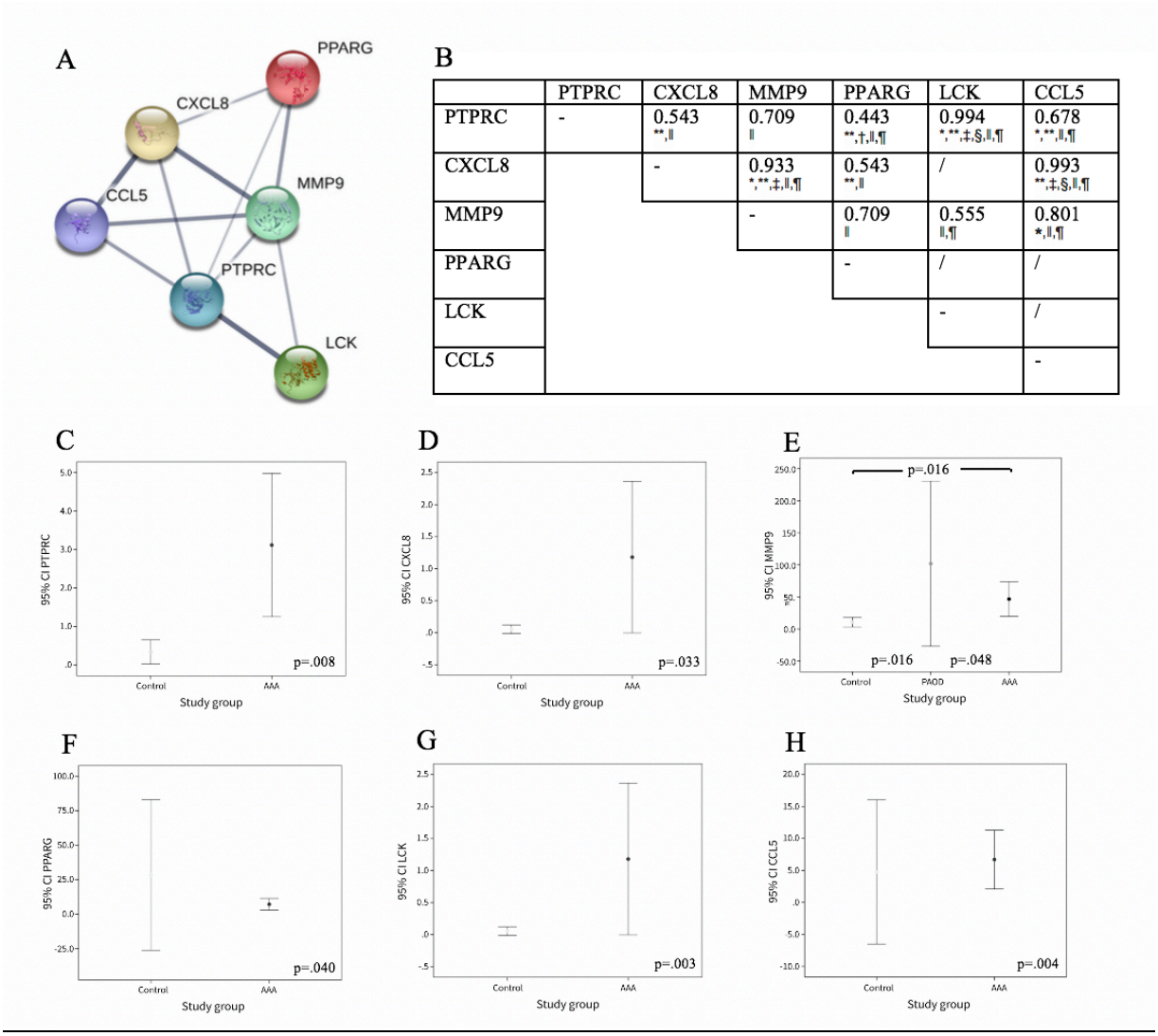


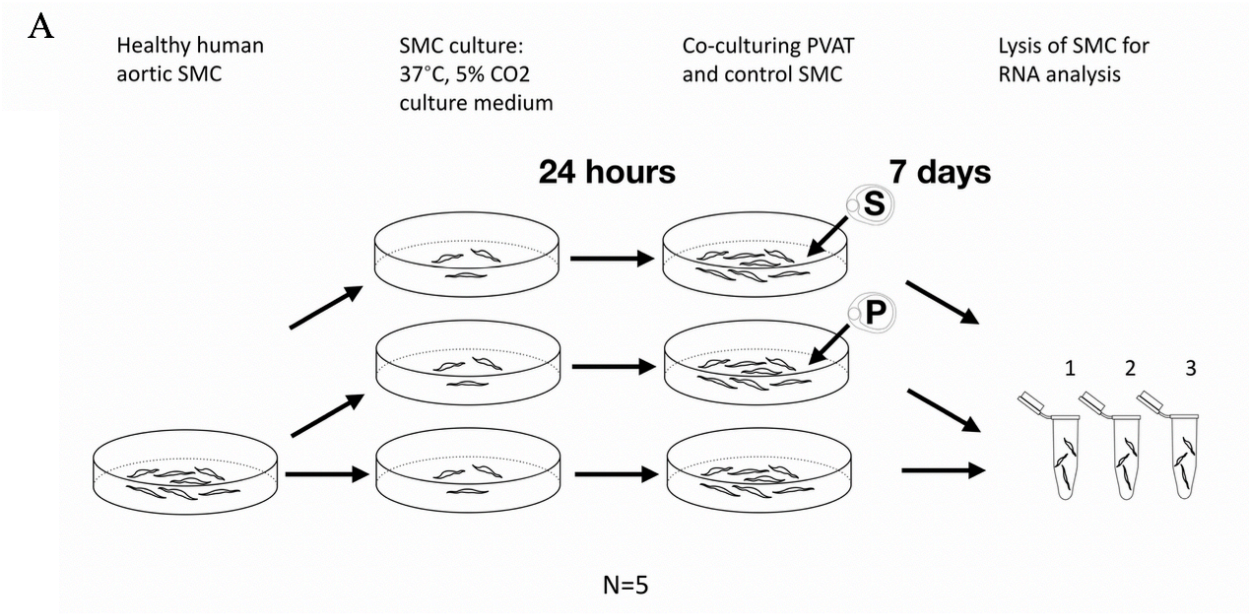
Control PAOD AAA

Figure 3. String is a biological database of known and predicted protein-protein interactions with a confidence level varying from 0 to 1. **A** Interaction of the significantly altered genes found in PVAT of AAA patients. The figure was licensed by STRING, ELIXIR, Cambridgeshire, United Kingdom under Creative Commons Attribution (CC BY) 4.0 license. **B)** The table presents the confidence level of interaction between significantly altered genes. Functional links are also provided: * Co-expressed, ** Putative homologs are coexpressed in other species, † Putative homologs were found interacting in other species, ‡ Experimental/biochemic data, § Association in Curated Databases, ‖ Co-mentioned in PubMed abstracts, ¶ Putative homologs are co-mentioned in PubMed abstracts in other species, / No known interaction. **C-H)** Error bars for the 95% Confidence Interval (CI) of the outcomes of the statistically significant different genes. Non-significant comparisons are not shown.



	PTPRC	CXCL8	MMP9	PPARG	LCK	CCL5
PTPRC	-	0.543 **,‖	0.709 ‖	0.443 **,†,‖,¶	0.994 *,**,‡,§,‖,¶	0.678 *,**,‖,¶
CXCL8		-	0.933 *,**,‡,‖,¶	0.543 **,‖	/	0.993 **,‡,§,‖,¶
MMP9			-	0.709 ‖	0.555 ‖,¶	0.801 *,‖,¶
PPARG				-	/	/
LCK					-	/
CCL5						-

Figure 4. A) Schematic overview of the incubation of live healthy SMC with PVAT and SAT. After seven days of co-culturing, the SMC were harvested for RNA expression analysis. S indicates subcutaneous adipose tissue and P, perivascular adipose tissue. **B)** SMC in culture wells co-incubated with adipose tissue after seven days of co-culturing. **C)** SMC focused in the upper part of the ocular and adipocytes not in focus in the lower part tissue after seven days of co-culturing and **D)** adipocytes focused in the lower part of the ocular and SMC not in focus in the upper part tissue after seven days of co-culturing. **E)** Box plots of gene expression results of healthy SMC, either as control or co-cultured with PVAT and SAT of AAA patients. As internal control, ratios are calculated by dividing either the outcomes of co-cultured SMC with PVAT or SAT by the outcome of not-co-cultured SMC, which are the controls. Therefore, the controls show an expression ratio of 1 and the PVAT and SAT outcomes present the alterations after co-culturing. * indicates statistical significance.



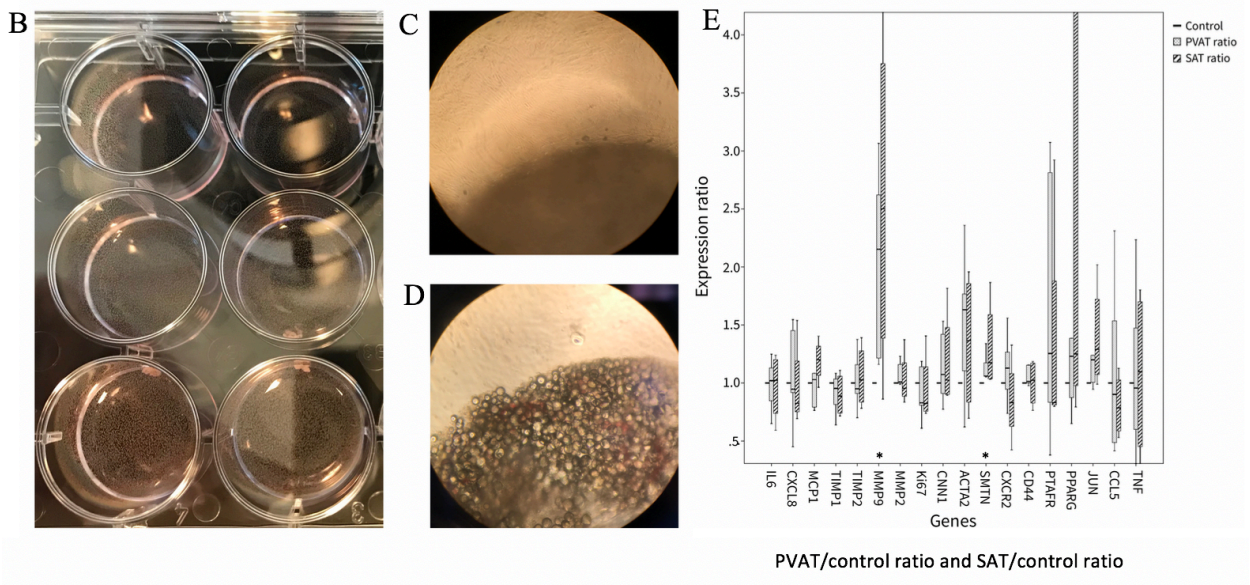
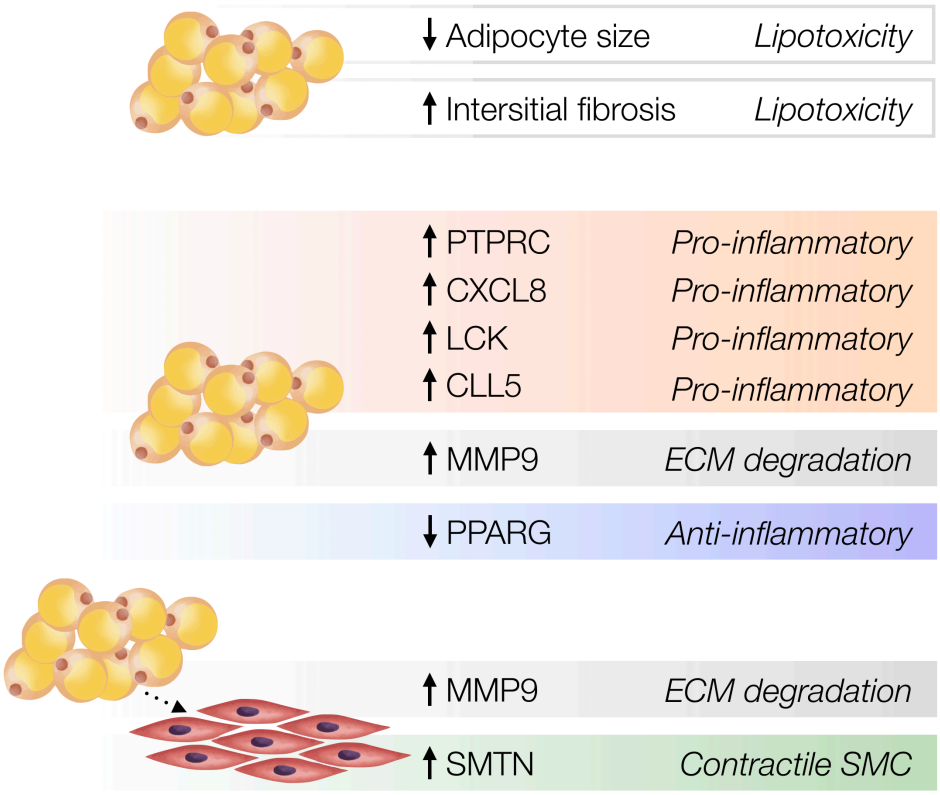


Figure 5. Schematic overview of the results of the current study and interpretation of the results. Histology showed decreased adipocyte size and increased interstitial fibrosis in AAA patients. qPCR of snapfrozen PVAT of AAA patients presented increased gene expression of pro-inflammatory PTPRC, CXCL8, LCK and CLL5. Furthermore, increased MMP9 expression which leads to ECM degradation and decreased anti-inflammatory

PPARG were observed. qPCR of co-cultured live non-pathologic SMC with live PVAT of AAA patients showed increased MMP9 and increased SMTN which is found in SMC that undergo contractile phenotype switch.



DISCUSSION AND FUTURE PERSPECTIVES

The **first part of the thesis** was dedicated to the evolving epidemiology of the AAA in Portugal.

1. iAAA admission

We were able to show that, in Portugal, the repair rate of iAAA continues to grow, especially in patients ≥75 years old, and did not reach a point of inversion yet. The absolute numbers, however, still lag behind those described in other European countries, even if a catching up effect is noticeable.

The increase in the rate of admission in the era where the incidence of the disease is supposed to be decreasing in Western countries might be paradoxical. The underlying causes of the changing epidemiology of AAAs are complex, and there are factors that may mask a decline in the incidence of nonruptured aneurysms. For that reason, a deeper look into the determinants of admission rate for repair is warranted.

a. Incidence of the disease

The admission rate reflects the incidence of the disease, that correlates with its known risk factors (such as smoking), something that was not directly addressed in this thesis. Data from Censos (INE), shows that in the population ≥ 15 years-old, 19.58% were smokers in 2005 and 19.85% were smokers in 2014. This lack of a significative variation does not exclude a role of this important risk factor in the incidence of AAA, as a longer lag between the risk factor modification and the change in the disease would be expected.

Although increasing availability of imaging techniques may contribute towards an increased diagnosis of incidental iAAA, an accurate measure of the iAAA incidence relays on screening. The most recent publication about a regional screening initiative (men with age ≥ 65 years old with an eligible population of about 900 males) yielded a prevalence of 2.1% (141), which is below of that described in the clinical trials supporting screening programs (prevalence of 4-7%) (142, 143).

b. AAA growth rate

There may be a long delay before the aneurysms expand to AAAs large enough to require repair, as suggested (44). In theory, it means that for the same incidence rate, the rate of repair would vary according to the speed of AAA growth until it reaches a threshold for repair.

c. Threshold for AAA repair

The workload may be maintained or increased by increased repair of smaller AAAs. A report from VASCUNET indicates that the threshold level for repair of small aneurysms varies between countries (144). The variation in the rate of small AAA repair may depend on differences in management of rapid growth and symptomatic aneurysms between regions, as well as differences in attitude towards treatment of small AAA with EVAR. Two groups assessed surveillance versus EVAR of small AAA (145, 146) and have reported no difference in mortality between surveillance and EVAR for small AAA. Recommendation for repair accordingly to the guidelines (1), remain ≥ 5cm in women or ≥ 5.5cm in men.

In our single centre-study the mean diameter of treated patients was 6.2±1.5cm, indicating that this might not be the main reason, unless other Portuguese centres do not follow the same trend.

d. Turn down for repair due to risk profile of the patient

The widespread use of EVAR brought another option to patients that would be otherwise denied for OSR. Although EVAR-2 randomized trial did not show an increase in overall life expectancy in patients ineligible for OSR, EVAR could reduce aneurysm-related mortality (147) and better results for the same unfit for OSR-patients were later described after EVAR (148). EVAR treatment might have led to a reduction in the turn down for repair in patients with the higher risk profiles such as older patients. Accordingly, in our results, the increase in the rate of admission was more marked in patients ≥ 75 years old, with probable age-related comorbidities.

Taken all together, while a direct assessment of the incidence was not possible in this thesis, nor an assessment of AAA growth rate, the increased incidence of repair was, at least, related to the extension of the treatment to older patients. Concomitantly, the rise in admission in older age-bands might mean a more benign presentation of the disease, if patients achieved larger AAAs only later in life (40). This would imply a decrease in the admission of younger patients, something that was also noticed. Finally, our study shows that this is happening beyond the aging of the population that occurred during that 16-year period, since incidences were adjusted for this confounder through age-standardization.

2. Mortality after iAAA repair

Operative mortality has been consistently decreasing in Portugal. In the recent years, while the operative mortality after EVAR continued to decline, an increased operative mortality after OSR was identified. Accordingly, all repair mortality

and EVAR mortality fairly fall into the range that was reported in the international VASCUNET Database (5.1±1.9% and 2.8±0.9% vs 2.0-5.0% and 0.3-3.0% in VASCUNET report, respectively) (144), whereas a greater concern is open surgery, where a high operative mortality was verified in the last period. Our study provided some explanations for this excessive mortality. While the center caseload was not associated with OSR mortality (but was with EVAR mortality), older patients presented higher mortality associated with both OSR and EVAR, but, for EVAR, differences between older and younger patients were minimal in the last period, meaning that older patients submitted to OSR are at higher risk. Female gender also performed worse regardless the type of repair, but even worse after OSR, where operative mortality reached about 10% in the third period under analysis.

3. rAAA incidence

Differently from iAAA, admission due to rAAA seems to have reached a peak and have been recently decreasing (2010-2015). Nonetheless, a deeper look into the assessment of the incidence requires taking into consideration the cases that presented to the hospital plus those that die before that. Combining both, the DRGs hospital database and the cause of death database, we showed that the incidence has recently stabilized after a previous increase. These findings are in accordance to the expected epidemiologic transition.

The determinants of rAAA incidence include:

a. The incidence of nonruptured AAA

This topic was discussed above.

b. AAA growth rate and other risk factors for rupture

Smoking is a known risk factor for rupture (22, 149, 150). Again, the lack of a significant variation in smoking habits described in the INE report cannot exclude a role of this important risk factor underlying the stabilization of rAAA.

The incidence of AAA increases with age, peaking later in females (4). We verified that the incidence of rAAA decreased in younger patients (<75 years-old) and increased in the older ones (≥75 years-old) during the last period. This points toward a later presentation of the disease or a lower growth speed, both in favor of a more benign disease. The hypothesis behind this is that medical management such as increased use of statin therapy and decline in smoking may have successfully reduced the risk of AAA rupture. Patients who would otherwise have presented with ruptured aneurysms may now present with intact aneurysms, and this effect may also obscure a fall in the incidence of nonruptured AAAs as explained before.

c. Success in diagnosis, case selection and repair of iAAA

In this thesis we could not describe the long-term outcomes after iAAA repair to ascertain in what extent the treatment of iAAA effectively prevented rupture.

d. Ability to arrive to the hospital in case of a rAAA

We noted a recent increase in incidence/mortality outside the hospital that deserves a special consideration. Possible causes include patients' profile-risk, emergent patients transportation logistics, medical teams' skills in the pre-hospital setting, number and distribution of the hospitals that receive these emergent patients, something that deserves further research. Most of these factors were not addressed in this thesis. However, we gave some insight into patients' profile-risk of, since the increase in age-standardized mortality in the third period occurred mostly among older cases. Again, this cannot be attributed to population aging per se, since incidences were adjusted for this confounder through age-standardization, but to an increased occurrence of the disease at an older age.

4. rAAA mortality

The mortality due to rAAA depends both on pre-hospital mortality and hospital mortality. Overall, 75% of patients with a rAAA died before reaching the hospital or during the hospitalization. The rAAA age-standardized mortality was stable between the first and second periods and increased slightly in the third period due to pre-hospital mortality, since hospital mortality actually decreased. Considerations on the pre-hospital mortality were described above. Hospital mortality for rAAA account for turn-down for repair rates and operative mortality.

The age-standardized rate of non-intervention is 22% on average and also followed a rise-fall pattern. These patients are more often women and old. We verified a trend that points to an expected higher proportion of these type of patients presenting to our hospitals because, despite the reduction in admission rates for all type of patients (those undergoing and not undergoing repair), the decrease in admission was higher in those undergoing repairs.

Finally, following the trend observed for iAAA, the operative mortality after rAAA repair decreased from 55% to 49% and then remained stable. Like in iAAA, the stabilization corresponded to an increase in operative mortality due to OSR compensated by a decrease in operative mortality after EVAR. Again, the subgroup of females and older patients performed worse. And although mortality after EVAR is decreasing, only 1 EVAR to 7.5 OSR are performed in emergent setting versus 1 EVAR to 1.7 OSR in elective setting.

Taking all these factors into account, a pattern where rAAA incidence is stabilizing but the rate of iAAA is still going up denote a middle stage in the epidemiology transition as suggested before (40). It is likely that the trends verified in Portugal are lagging behind those of Australia (39), New Zealand (39), and Sweden (44). If the assumptions remain constant, after this middle stage, it is anticipated that there will be a decline in overall iAAA admissions and iAAA repairs in the coming years.

Limitations

The databases used in this thesis to study the nationwide evolution of AAA provides sensitive clustered data coded with ICD-9 classification. Its features were presented in the Introduction. Despite being the unique readily available databases in Portugal, they have some limitations.

First, patients may have their AAA treated in private hospitals. The number of patients referred and treated in the private sector is a potential source for selection bias, if we are analyzing data that is exclusive from the public sector. Codification is mandatory for public hospitals since its reimbursement depends on that. However, for private hospitals, codification is not legislated, and institutions might or might not code their episodes of care. Even for those that perform codification, data is not publicly available.

For AAA, private health care providers mainly fulfil a supplementary role to the NHS rather than providing a global alternative to it. While, some patients have their iAAA repaired in private hospitals, because of the previous-mentioned features of the Portuguese health system, the amount of rAAA referred and treated in the private sector is null or, at least, neglectable in Portugal and not a realistic source of relevant selection bias.

Second, databases present episode-related data retrievable through ICD codes. In INE data, the episode is death and includes deaths outside the hospital as well as those certified at the hospitals. In NHS administrative database, each episode refers to a hospitalization. Furthermore, only data associated with a specific hospital episode is available and so the follow-up is limited. We believe that bias created by the use of hospitalization-related data instead of patient-related data is minimal, especially for rAAA, as being admitted twice in life with a rAAA is possible but rare. We also focus the analysis on the longitudinal time trend rather than absolute results, meaning that if the bias is relatively systematic, conclusions on variations along time are valid.

Third, reinterventions were not studied as they require linking of data and/or an available longitudinal patient history.

Four, errors during codification might be a source of bias. The correction of codification is ensured by internal institutional audits and external ACSS audits. The ICD-10 codification was introduced in Portugal in 2017, but our analysis ended in 2015. During the period from 2000-2015, only ICD-9 codification was used. The specific EVAR code was introduced only in October 2000. Due to lack of the specific ICD9 code for EVAR before 2000, the first period of the analysis was set to null to avoid the early years of the EVAR code use. Since 1 January 2015, the DRGs grouper "All Patient Refined DRG 31" started to be used instead of the previous grouper "All Patient DRG 27". The introduction of a new version of the DRG grouper entails the determination of new relative weights for each DRG (relative weights that will be used in the calculation of the Case Mix index of each institution). Our analyses did not use DRG but ICD-9 codes directly. This grouper still relies on ICD-9 codes that kept the same, so we believe this group change did not affect our analysis significantly.

Fifth, data from DRGs database cannot be paired with other systems of information such as social security number. As such, estimations of absence from work and/or assessment of incapacity could not be provided.

Sixth, the validity of the "outside the hospital mortality for rAAA" depends on the quality of both the cause of death and the hospital administrative databases. The cause of death database captures the deaths of all patients dying with rAAA at hospital or outside the hospital. While at the hospital, the cause of death is usually easy to address, in patients dying outside a hospital setting, the cause of death is prone to error owing to misdiagnosis of the cause of death (if no autopsy is performed and no previous diagnosis of AAA exists). In Portugal like in other countries, autopsy rates are declining, from 6.7±0.3% of all deaths in 2000-2005 to 6.4±0.2% in 2005-2009 and to 5.5±0.8% in 2010-2015. This is the main limitation

to the use of cause of death databases in general.

Future perspectives

Future perspectives for this project involve exploring in which extent the implementation of a national screening programs would improve the national AAA outcomes.

The global incidence of rAAA and the rates of admission due to iAAA and rAAA obtained in this paper compare, in general, inferiorly to other European countries.

Four randomized trials (142, 143, 151, 152) showed the benefit of screening a male population in 1980–1999, when the AAA prevalence rates detected were as high as 4–7.5%. These studies led to the implementation of national screening--programs. Screening for AAA has been shown to be effective at reducing AAA-related mortality and is cost-effective in many analysis (153). However, screening programs that fail to target high-risk populations are likely to see very low detection rates that could call the value of the program into question. Cost-effectiveness has been demonstrated at prevalences as low as 1.6% (154) or 0.5% (155).

Studies, including ours, have also indicated a shift in AAA disease to affect older patients (40, 155), meaning that 65 years-old patients with normal aortic diameter at screening might still be at risk of developing AAA. And on the other side, 18% patients with AAA were bellow 65 years old. Furthermore, there are significant variations in AAA screening protocols between healthcare systems (156). Further evidence of cost and clinical effectiveness is likely to be required before there is widespread international adoption of AAA screening. These concerns are of particular relevance in Portugal, where figures of intact and ruptured AAA lag behind those found in other European countries.

Therefore, it is crucial that these recent trends be monitored, and screening strategies evaluated accordingly on the basis of these trends in order to be effective.

Economic evaluations aim to inform the cost-effective use of scarce health care resources, ultimately to ensure that interventions or health care programs result in positive health outcomes at a reasonable cost (157).

The economic analysis must take into account several parameters (158):

a. Definition of the target population (gender, age, smokers)
b. Prevalence of the disease, that is, AAA ≥ 3.0cm
c. Management of sub-aneurysmal aortic dilatation, that is, aortic diameter 2.5-2.9cm
d. Yearly probability of AAA growing according to AAA diameter
e. Yearly probability of AAA rupture according to AAA diameter
f. Preferred method for ultrasound measurement of the aortic diameter (inner-to-inner, outer-to-outer and leading-edge-to-leading-edge (outer anterior wall to inner posterior wall)
g. Proportion of individuals complying with invitation
h. Rate of opportunistic diagnosis if not under a screening program
i. Threshold for AAA repair
j. Mortality associated with AAA elective repair (EVAR, OS and probability of undergoing each of them)
k. Mortality associated with AAA elective repair (EVAR, OS and probability of undergoing each of them)
l. Probability of reaching the surgery in case of rAAA
m. Degree to which lives are lengthened by detecting an AAA
n. Costs of ultrasound assessment
o. Costs of elective and emergent AAA repair

Most relevant cost-effectiveness analyses perform *incremental cost-effectiveness ratio* estimates based on either Markov Chain Monte Carlo analysis or actual retrospective data (159). Other aspects such as quality of the improved lifespan and the psychological effect of screening must also be considered.

The **second part of the thesis** was dedicated to pathophysiological features of AAA, both AAC and adipose tissue involvement.

1. Image assessment of AAC in AAA

Vascular calcification corresponds to the deposition of ectopic minerals in the vascular wall. This phenomenon is now recognized to recapitulate skeletal bone formation, involving competition between inhibition of mineralization and promotion of calcification (160-162). It is typically localized into the luminal neointimal plaques in atherosclerotic vessels, where it is present in a punctate or patchy pattern (163). Vascular calcification also occurs in the medial layer of the vessel, which is known as Monckeberg's medial sclerosis (164). One of the processes of vascular calcification involves the reprogramming and transdifferentiation of SMC to osteoblast-like cells (165). These cells lose their contractile properties but are able to generate and release of calcifying matrix vesicles, a collagen matrix and form calcium- and phosphorus-rich matrix vesicles, involved in vascular calcification (166). The mechanisms underlying vascular calcification remain under investigation.

In population studies, the prevalence of vascular calcification increased significantly with age, reaching 93% and 75% in males and females, respectively, who are age 70 years or older (167). The presence of calcification in any arterial wall was associated with a 3-4-fold higher risk for mortality and cardiovascular events (168).

In patients with iAAA, we found that the amount of AAC measured by the ACI method had a high correlation to that measured using the AS. From the three semiquantitative methods used, ACI was the most reproducible and was associated with a higher number of demographic and clinical variables that concomitantly associated with the AS.

In line with our hypothesis, different methods led to identification and attribution of statistical significance to different clinical variables. This finding supports the impression that the use of different methods to assess AAA AAC might contribute to conflicting reports noticed in the literature.

The clinical variables that exhibited a positive association with AAC in AAA include: older age, female gender, carotid artery disease and PAD. Taken together, the variables identified AAC as a marker of atherosclerotic burden in AAA (169).

The most common form of AAA is typically associated with the usual risk factors with occlusive atherothrombosis, except for diabetes mellitus. However, approximately 20% of the AAA patients have a positive family history for aneurysms (170). These patients tend to be younger, and less exposed to smoking. They were also shown to present higher aneurysm-related complications after endovascular repair (170), that were not due to baseline morphologic features, even if only the neck calcification was assessed.

Therefore, AAA is a complex disorder that integrates the influence of predisposing genes with lifestyle-associated risk factors. It is possible that the presence of AAC in the diseased aortic segments in AAA reflects a higher importance of lifestyle-associated risk factors than of predisposing genes, something that deserves further investigation.

2. The role of PVAT in AAA

Despite the amount of evidence from animal and clinical studies pointing towards a role of adipose tissue in AAA progression, the role of PVAT is still under discussion.

One of the main difficulties in the image study of PVAT in humans is the inability to resolve the concerns related to the retroperitoneal lining and the inherent correlations between abdominal aortic diameter and abdominal PVAT volume (171).

Thoracic PVAT can be distinguished from the remaining tissue using physiological landmarks (172). The posterior border is the anterior portion of the vertebral foramen. The anterior border and left/right lateral borders change as the images progress distally from the carina and include the left bronchus, esophagus, and crus of diaphragm. The first proximal image analyzed includes the carina, and then down through the vertebral body T12, and stopping at the pedicles of L1.

To achieve that in the abdomen, we developed a new method where a density approach was used instead of the absolute PVAT quantity assessment. Using the measure consisting of a ratio between the number of fat pixels in a certain area of interest and that area, the evaluation of the PVAT becomes independent of changes in the aortic diameter. Second, a systematic definition of sections around the aorta provides regions of interest enriched with PVAT that are being sampled in each section. Furthermore, PVAT densities were assessed in two separate regions of the aorta in the same patient, allowing the comparison of healthy and aneurysmal segments individually within the same patient.

Antonopoulos AS et al (173) hypothesized that phenotypic changes in coronary PVAT induced by vascular inflammation could be quantified using a CT approach. Because there is no clear biological definition of coronary PVAT, the region of interest was defined as the adipose tissue in a layer of tissue within a radial distance from outer coronary artery wall equal to

the average diameter of the tracked coronary artery segment, a process that follows the principals of our method. Instead of *density* of adipose tissue, where the *sum of all fat pixels* were count in a certain region of interest, they defined a fat attenuation index (FAI) consisting in the *average* HU of the fat pixels in the volume of interest (within a pre-specified window of HU that fit the adipose tissue, that is -190 to -30 HU). The working hypothesis was that adipocyte lipid content is the main driver of FAI; since larger adipocytes have a higher proportion of lipid phase (adipocytes) compared to aqueous phase (extracellular space), this would lead to more negative FAI. Interestingly, their FAI had excellent sensitivity and specificity for detecting tissue inflammation as assessed by tissue uptake of 18F-fluorodeoxyglucose in positron emission tomography.

The main finding of our multicenter case-control study is that AAA patients had higher intra-individual differences in PVAT density, with a higher PVAT density around the aneurysm sac than the healthy neck. Intra-individual PVAT differences also presented the highest positive correlation with aortic dimensions, after adjustment for other fat compartments, BMI, sex, and age. This is in accordance with the animal studies where the AAA diameter significantly decreased in the PVAT-removed vascular wall (131). One might hypothesize that the increased dilation in the region of higher PVAT density might be because of an increased number of adipocytes in the aortic wall/PVAT complex. Differently, we may input these changes to alterations in the inflammatory content, as variations under HU range of fat tissue correlated with tissue inflammation before (173).

However, a causal inference cannot be extrapolated from our paper due to its observational nature. In order to further investigate the mechanisms underlying the role of PVAT in the occurrence of AAA, we design a laboratorial study, using PVAT samples form patient with AAA and PAOD and controls.

In this laboratorial study, examined histological changes in PVAT and studied RNA expression of healthy and pathologic PVAT tissue. PVAT from patients with AAA display smaller adipocyte size and more interstitial fibrosis in univariate analysis, although only adipocyte size remained significant after adjusting for potential confounders. We hypothesize that these histological features might be due to tissue inflammation since, in obesity increased adipose tissue inflammation stimulates adipocyte lipolysis and tissue fibrosis (174, 175), features that resemble our findings. Moreover, inflamed human coronary vessels release cytokines that prevent lipid accumulation in PVAT-derived preadipocytes in vitro, ex vivo, and in vivo (173).

Gene expression was in accordance to our hypothesis since PVAT from AAA presented increased expression of inflammation-related genes (PTPRC, CXCL8, MMP9, LCK and CCL5) and decreased in PPARg that is linked to antiinflammatory cytokines.

To test if this inflammatory state could have impact in the adjacent aortic wall, we performed an *ex vivo* stimulation experiments using control aortic SMC without known aortic pathology. These cells were incubated with PVAT or SAT sections from AAA patients or were negative controls. Both adipose tissues increased the expression of smoothelin. Despite being a structural protein that is found exclusively in contractile SMC, reductions in smoothelin expression were noted after stimulation of AAA SMC with TGF-β, which is suggested to protect against AAA (176). This was a very interesting finding as it points into a systemic effect of adipose tissue in AAA patients, resembling an aneurysmatic signature. Furthermore, SAT sections are fairly easier to obtain from patients. Further research in this field is warranted.

PVAT was distinct from SAT because increased expression of MMP9 was observed in healthy SMC only when stimulated with PVAT but not with SAT of AAA patients. This supports the harmful contribution of PVAT to the AAA progression suggested before in animal studies, where the removal of PVAT led to a significant decrease in AAA diameter.

The model that we use has some limitations because stimulation by sections of adipose tissue were tested in SMC culture only. SMC dysregulation is central to the AAA pathophysiology as explained before, but they represent a fraction of the resident vascular cells in the aortic wall. Using this model, we were not able to address the effect of PVAT in the mesenchymal cells and adipose deposits of the adventitia itself, cellular elements that have recently been linked to AAA progression and rupture.

Taking all together, using an *ex vivo* stimulation study of human aortic and periaortic cells/tissues, our results corroborate what has been suggested in animal studies for the active PVAT role in the AAA progression. They suggest that PVAT is able to increase SMC expression directly or through paracrine processes. It is yet to be unrevealed the possible impact of the PVAT in the other aortic wall components. To accomplish that, a model of the aortic wall in which cell-to-cell and cell-to-matrix communications are not missed would be ideal, instead of constrained fixated cells or isolated cell cultures. Although animal models are of great utility and have been used before in this regard, the usage of animal models is costly, ethically and technically challenging and animal models do not completely resemble the human conditions, as the aortic aneurysms need to be created in the animal mode.

A recent paper presented a new in vitro method for extended preservation of aortic wall sections to study pathophysiological processes, where intraoperatively harvested, live aortic specimens were sectioned with a vibratome, and cultured (176). Aortic tissue viability could be preserved until at least 62 days after harvesting. Cultured tissues can be digested into viable single cells for additional techniques. Digested tissues showed different cell types and a viability up to 75% at day 14.

Future perspectives

Future perspectives for this project evolve two different and complementary tactics.

a. *Ex vivo* aortic wall stimulation by PVAT-conditioned medium

Live aortic tissues of AAA patients can be sectioned and cultured according to the recently described protocol (176). The culture medium would be supplemented with adipose tissue conditioned medium. This method was used before to address the importance of the substances produced by adipose tissue depots (177). After a period corresponding to the best viability outcomes of the aortic wall preparations (day 5 and 7), full sections and homogenates from conditioned and non-conditioned tissue sections would be generated. Immunofluorescence images showing aneurysmal aortic wall sections would permit to track mesenchymal and adipocyte cells after stimulation, something that could never be addressed before in human samples.

b. Systematic analysis of the lipid content of PVAT

Recent studies on large-scale lipidomic in large cohort CVD studies have been evolving as an alternative field of research able to provide insights into the lipid signature that precedes CVD events and the molecular mechanisms underlying its onset and progression. Taking plasma as an example, even though plasma lipids comprise more than half of the circulating metabolites, only recent attention has been paid, in part because of the challenges associated with sample collection, lipid extraction, analysis, quantification and data analysis of large datasets.

The two leading analytical approaches to metabolomics are MS and nuclear magnetic resonance spectroscopy. Recent technological advances in the development of user-friendly mass spectrometers equipped with fast scan rates, high sensitivity and high resolution, and polarity switching modes ideally suited for the high-throughput of complex biological samples, has led to the increasing popularity of MS-based approaches (178-192) over nuclear magnetic resonance -based approaches (193) in search for CVD lipid signature.

Assessment of the aortic wall resorting to these techniques was performed in a few studies. Through a MS-approach, Tanaka et all (70) revealed proinflammatory lipid molecules such as lyso-phosphatidylcholine (1-acyl 16:0) and phosphatidylcholine (16:0/20:4) distributed in all the layers of the AAA sac. Significantly greater accumulation of these molecules was observed in the adventitial side. In contrast, the distribution of cholesterol ester was not significantly different between the AAA neck and sac arterial walls (70). A characteristic distribution of triglycerides specifically in the aneurismal adventitia of the sac was also demonstrated by this group (194). The amount of triglycerides in the adventitia was correlated with AAA diameter while, on the contrary, the amount of triglycerides in the intima and media was not correlated with AAA diameter. Interestingly, the quantity of triglycerides in the vascular wall was not correlated with serum triglycerides levels, serum total cholesterol levels or BMI. The relevance of the triglycerides was underlined recently (195), particularly in patients with degenerative aortic aneurysms. Increased triacylglycerols and decreased ether-type phosphatidylethanolamines were observed throughout the normal, border, and aneurysm areas of thoracic and abdominal atherosclerotic aortic aneurysms, whereas phosphatidylethanolamines and triglycerides decreased in normal areas of thoracic atherosclerotic aortic aneurysms and thoracic nonatherosclerotic aortic aneurysms compared with the control tissues.

The lipidomic approach to study the features of the PVAT seems to be next step. Claria J et al addresses the identification and distribution of local chemical lipid mediators of inflammation resolution in different anatomically localized fat depots collected from patients with PAD undergoing major lower extremity amputation and a control group of patients that underwent total hip or knee replacement (196). Specifically, using MS-based metabolo-lipidomic approach, they revealed the signature profile of established specialized proresolving mediators derived from polyunsaturated fatty acids of the omega-6 and omega-3 series, namely lipoxins, resolvins, protectins, and maresins (e.g., LXA4, RvD1, RvD2, PD1, and RvE1) as well as their intermediate and pathway markers 17-HDHA and 18-HEPE. A key feature of the present study was that lipid mediators were determined in parallel with adipokine concentrations in SAT from patients with PAD and controls. PAD patients showed increased inflammatory adipokine (i.e., MCP-1, resistin, and PAI-1) in parallel with reduced anti-inflammatory SPM levels, which further support the determining role of this mediators in the vascular disease and their putative interest as therapeutic targets.

To our knowledge, only one group performed so far PVAT MS-based lipidomic analysis in AAA disease (197), establishing a ceramide profile of PVAT in AAA in humans. Confirmation and extension of these findings may disclosure the ultimate role for PVAT in ongoing damage to the adjacent aneurysmal aortic wall, as well as the pathways that can be tackle for innovative outer in inner therapeutic approaches.

REFERENCES

1. Wanhainen A, Verzini F, Van Herzeele I, Allaire E, Bown M, Cohnert T, et al. Editor's Choice - European Society for Vascular Surgery (ESVS) 2019 Clinical Practice Guidelines on the Management of Abdominal Aorto-iliac Artery Aneurysms. Eur J Vasc Endovasc Surg. 2019;57(1):8-93.
2. Erbel R, Aboyans V, Boileau C, Bossone E, Di Bartolomeo R, Eggebrecht H, et al. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases. Eur Heart J. 2014;35(41):2873-U93.
3. Gloviczki P, Pairolero P, Welch T, Cherry K, Hallett J, Toomey B, et al. Multiple aortic aneurysms: the results of surgical management. J Vasc Surg. 1990;11(1):19-27; discussion -8.
4. Lederle FA, Johnson GR, Wilson SE, Aneurysm D, Management Veterans Affairs Cooperative S. Abdominal aortic aneurysm in women. J Vasc Surg. 2001;34(1):122-6.
5. Lederle FA, Johnson GR, Wilson SE, Chute EP, Littooy FN, Bandyk D, et al. Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. Ann Intern Med. 1997;126(6):441-9.
6. Lederle FA, Johnson GR, Wilson SE, Chute EP, Hye RJ, Makaroun MS, et al. The aneurysm detection and management study screening program: validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. Arch Intern Med. 2000;160(10):1425-30.
7. Dattani N, Sayers RD, Bown MJ. Diabetes mellitus and abdominal aortic aneurysms: A review of the mechanisms underlying the negative relationship. Diab Vasc Dis Res. 2018;15(5):367-74.
8. Lederle FA, Wilson SE, Johnson GR, Reinke DB, Littooy FN, Acher CW, et al. Immediate repair compared with surveillance of small abdominal aortic aneurysms. N Engl J Med. 2002;346(19):1437-44.
9. Forsdahl SH, Solberg S, Singh K, Jacobsen BK. Abdominal aortic aneurysms, or a relatively large diameter of non-aneurysmal aortas, increase total and cardiovascular mortality: the Tromso study. Int J Epidemiol. 2010;39(1):225-32.
10. Singh K, Bonaa KH, Jacobsen BK, Bjork L, Solberg S. Prevalence of and risk factors for abdominal aortic aneurysms in a population-based study : The Tromso Study. Am J Epidemiol. 2001;154(3):236-44.
11. Powell JT, Greenhalgh RM. Clinical practice. Small abdominal aortic aneurysms. N Engl J Med. 2003;348(19):1895-901.
12. Vardulaki KA, Walker NM, Day NE, Duffy SW, Ashton HA, Scott RA. Quantifying the risks of hypertension, age, sex and smoking in patients with abdominal aortic aneurysm. Br J Surg. 2000;87(2):195-200.
13. Lindholt JS, Heegaard NH, Vammen S, Fasting H, Henneberg EW, Heickendorff L. Smoking, but not lipids, lipoprotein(a) and antibodies against oxidised LDL, is correlated to the expansion of abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2001;21(1):51-6.
14. Blanchard JF, Armenian HK, Friesen PP. Risk factors for abdominal aortic aneurysm: results of a case-control study. Am J Epidemiol. 2000;151(6):575-83.
15. Lederle FA, Nelson DB, Joseph AM. Smokers' relative risk for aortic aneurysm compared with other smoking-related diseases: a systematic review. J Vasc Surg. 2003;38(2):329-34.
16. Golledge J, van Bockxmeer F, Jamrozik K, McCann M, Norman PE. Association between serum lipoproteins and abdominal aortic aneurysm. Am J Cardiol. 2010;105(10):1480-4.
17. Ortiz-Munoz G, Houard X, Martin-Ventura JL, Ishida BY, Loyau S, Rossignol P, et al. HDL antielastase activity prevents smooth muscle cell anoikis, a potential new antiatherogenic property. FASEB J. 2009;23(9):3129-39.
18. Wahlgren CM, Larsson E, Magnusson PK, Hultgren R, Swedenborg J. Genetic and environmental contributions to abdominal aortic aneurysm development in a twin population. J Vasc Surg. 2010;51(1):3-7; discussion
19. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008;40(2):217-24.
20. Gretarsdottir S, Baas AF, Thorleifsson G, Holm H, den Heijer M, de Vries JP, et al. Genome-wide association study identifies a sequence variant within the DAB2IP gene conferring susceptibility to abdominal aortic aneurysm. Nat Genet. 2010;42(8):692-7.
21. Jones GT, Tromp G, Kuivaniemi H, Gretarsdottir S, Baas AF, Giusti B, et al. Meta-Analysis of Genome-Wide Association Studies for Abdominal Aortic Aneurysm Identifies Four New Disease-Specific Risk Loci. Circ Res. 2017;120(2):341-53.
22. Sweeting MJ, Thompson SG, Brown LC, Powell JT, collaborators R. Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. Br J Surg. 2012;99(5):655-65.

23. Baba T, Ohki T, Kanaoka Y, Maeda K, Ito E, Shukuzawa K, et al. Risk Factor Analyses of Abdominal Aortic Aneurysms Growth in Japanese Patients. Ann Vasc Surg. 2019;55:196-202.

24. Takagi H, Umemoto T, Group A. Association of Hypertension with Abdominal Aortic Aneurysm Expansion. Ann Vasc Surg. 2017;39:74-89.

25. Takagi H, Umemoto T. No association of chronic obstructive pulmonary disease with abdominal aortic aneurysm growth. Heart Vessels. 2016;31(11):1806-16.

26. Brady AR, Thompson SG, Fowkes FG, Greenhalgh RM, Powell JT, Participants UKSAT. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. Circulation. 2004;110(1):16-21.

27. Lederle FA, Johnson GR, Wilson SE, Littooy FN, Krupski WC, Bandyk D, et al. Yield of repeated screening for abdominal aortic aneurysm after a 4-year interval. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. Arch Intern Med. 2000;160(8):1117-21.

28. Vega de Ceniga M, Gomez R, Estallo L, Rodriguez L, Baquer M, Barba A. Growth rate and associated factors in small abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2006;31(3):231-6.

29. Matthews EO, Rowbotham SE, Moxon JV, Jones RE, Vega de Ceniga M, Golledge J. Meta-analysis of the association between peripheral artery disease and growth of abdominal aortic aneurysms. Br J Surg. 2017;104(13):1765-74.

30. Takagi H, Umemoto T, Group A. Association of peripheral artery disease with abdominal aortic aneurysm growth. J Vasc Surg. 2016;64(2):506-13.

31. Heilmaier C, Koester A, Moysidis T, Weishaupt D, Kroger K. Abdominal aortic calcification and its distribution in normal-sized and aneurysmatic abdominal aortas. Vasa. 2014;43(2):132-40.

32. Lindholt JS. Aneurysmal wall calcification predicts natural history of small abdominal aortic aneurysms. Atherosclerosis. 2008;197(2):673-8.

33. Takagi H, Umemoto T, Group A. Coronary artery disease and abdominal aortic aneurysm growth. Vasc Med. 2016;21(3):199-208.

34. Choke E, Cockerill G, Wilson WR, Sayed S, Dawson J, Loftus I, et al. A review of biological factors implicated in abdominal aortic aneurysm rupture. Eur J Vasc Endovasc Surg. 2005;30(3):227-44.

35. Mortality results for randomised controlled trial of early elective surgery or ultrasonographic surveillance for small abdominal aortic aneurysms. The UK Small Aneurysm Trial Participants. Lancet. 1998;352(9141):1649-55.

36. Anjum A, Powell JT. Is the incidence of abdominal aortic aneurysm declining in the 21st century? Mortality and hospital admissions for England & Wales and Scotland. Eur J Vasc Endovasc Surg. 2012;43(2):161-6.

37. Norman PE, Spilsbury K, Semmens JB. Falling rates of hospitalization and mortality from abdominal aortic aneurysms in Australia. J Vasc Surg. 2011;53(2):274-7.

38. Semmens JB, Norman PE, Lawrence-Brown MM, Bass AJ, Holman CD. Population-based record linkage study of the incidence of abdominal aortic aneurysm in Western Australia in 1985-1994. Br J Surg. 1998;85(5):648-52.

39. Sandiford P, Mosquera D, Bramley D. Trends in incidence and mortality from abdominal aortic aneurysm in New Zealand. Br J Surg. 2011;98(5):645-51.

40. Choke E, Vijaynagar B, Thompson J, Nasim A, Bown MJ, Sayers RD. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? Circulation. 2012;125(13):1617-25.

41. Nelissen BG, Herwaarden JA, Pasterkamp G, Moll FL, Vaartjes I. Shifting abdominal aortic aneurysm mortality trends in The Netherlands. J Vasc Surg. 2015;61(3):642-7 e2.

42. Sensi L, Tedesco D, Mimmi S, Rucci P, Pisano E, Pedrini L, et al. Hospitalization rates and post-operative mortality for abdominal aortic aneurysm in Italy over the period 2000-2011. PLoS One. 2013;8(12):e83855.

43. Stather PW, Sidloff DA, Rhema IA, Choke E, Bown MJ, Sayers RD. A review of current reporting of abdominal aortic aneurysm mortality and prevalence in the literature. Eur J Vasc Endovasc Surg. 2014;47(3):240-2.

44. Svensjo S, Bjorck M, Gurtelschmid M, Djavani Gidlund K, Hellberg A, Wanhainen A. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. Circulation. 2011;124(10):1118-23.

45. Reimerink JJ, van der Laan MJ, Koelemay MJ, Balm R, Legemate DA. Systematic review and meta-analysis of population-based mortality from ruptured abdominal aortic aneurysm. Br J Surg. 2013;100(11):1405-13.

46. Budtz-Lilly J, Bjorck M, Venermo M, Debus S, Behrendt CA, Altreuther M, et al. Editor's Choice - The Impact of Centralisation and Endovascular Aneurysm Repair on Treatment of Ruptured Abdominal Aortic Aneurysms Based

on International Registries. Eur J Vasc Endovasc Surg. 2018;56(2):181-8.

47. Budtz-Lilly J, Venermo M, Debus S, Behrendt CA, Altreuther M, Beiles B, et al. Editor's Choice - Assessment of International Outcomes of Intact Abdominal Aortic Aneurysm Repair over 9 Years. Eur J Vasc Endovasc Surg. 2017;54(1):13-20.
48. Simões J AG, Fronteira I, Hernández-Quevedo C. Portugal: Health system review. Health Systems in Transition. 2017;19(2):(2):1–184.
49. ACSS. Relatório anual de auditoria externa à codificação clínica - Ano 2010. Administração Central do Sistema de Saúde (ACSS). 2011.
50. Óbitos por causas de morte. Documento metodológico. Instituto Nacional de Estatística. 2009:1-19.
51. Mahapatra P, Shibuya K, Lopez AD, Coullare F, Notzon FC, Rao C, et al. Civil registration systems and vital statistics: successes and missed opportunities. Lancet. 2007;370(9599):1653-63.
52. Kristmundsson T, Dias N, Resch T, Sonesson B. Morphology of Small Abdominal Aortic Aneurysms Should be Considered before Continued Ultrasound Surveillance. Ann Vasc Surg. 2016;31:18-22.
53. Erentug V, Bozbuga N, Omeroglu SN, Ardal H, Eren E, Guclu M, et al. Rupture of abdominal aortic aneurysms in Behcet's disease. Ann Vasc Surg. 2003;17(6):682-5.
54. Matsumura K, Hirano T, Takeda K, Matsuda A, Nakagawa T, Yamaguchi N, et al. Incidence of aneurysms in Takayasu's arteritis. Angiology. 1991;42(4):308-15.
55. Towbin JA, Casey B, Belmont J. The molecular basis of vascular disorders. Am J Hum Genet. 1999;64(3):678-84.
56. Xu C, Zarins CK, Glagov S. Aneurysmal and occlusive atherosclerosis of the human abdominal aorta. J Vasc Surg. 2001;33(1):91-6.
57. Sakalihasan N, Limet R, Defawe OD. Abdominal aortic aneurysm. Lancet. 2005;365(9470):1577-89.
58. Melrose J, Whitelock J, Xu Q, Ghosh P. Pathogenesis of abdominal aortic aneurysms: possible role of differential production of proteoglycans by smooth muscle cells. J Vasc Surg. 1998;28(4):676-86.
59. Baxter BT, McGee GS, Shively VP, Drummond IA, Dixit SN, Yamauchi M, et al. Elastin content, cross-links, and mRNA in normal and aneurysmal human aorta. J Vasc Surg. 1992;16(2):192-200.
60. Sakalihasan N, Heyeres A, Nusgens BV, Limet R, Lapiere CM. Modifications of the extracellular matrix of aneurysmal abdominal aortas as a function of their size. Eur J Vasc Surg. 1993;7(6):633-7.
61. Dobrin PB, Mrkvicka R. Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation. Cardiovasc Surg. 1994;2(4):484-8.
62. Satta J, Juvonen T, Haukipuro K, Juvonen M, Kairaluoma MI. Increased turnover of collagen in abdominal aortic aneurysms, demonstrated by measuring the concentration of the aminoterminal propeptide of type III procollagen in peripheral and aortal blood samples. J Vasc Surg. 1995;22(2):155-60.
63. Huffman MD, Curci JA, Moore G, Kerns DB, Starcher BC, Thompson RW. Functional importance of connective tissue repair during the development of experimental abdominal aortic aneurysms. Surgery. 2000;128(3):429-38.
64. Herron GS, Unemori E, Wong M, Rapp JH, Hibbs MH, Stoney RJ. Connective tissue proteinases and inhibitors in abdominal aortic aneurysms. Involvement of the *vasa vasorum* in the pathogenesis of aortic aneurysms. Arterioscler Thromb. 1991;11(6):1667-77.
65. Holmes DR, Liao S, Parks WC, Thompson RW. Medial neovascularization in abdominal aortic aneurysms: a histopathologic marker of aneurysmal degeneration with pathophysiologic implications. J Vasc Surg. 1995;21(5):761-71; discussion 71-2.
66. Satta J, Soini Y, Mosorin M, Juvonen T. Angiogenesis is associated with mononuclear inflammatory cells in abdominal aortic aneurysms. Ann Chir Gynaecol. 1998;87(1):40-2.
67. Rodella LF, Rezzani R, Bonomini F, Peroni M, Cocchi MA, Hirtler L, et al. Abdominal aortic aneurysm and histological, clinical, radiological correlation. Acta Histochem. 2016;118(3):256-62.
68. Sano M, Sasaki T, Hirakawa S, Sakabe J, Ogawa M, Baba S, et al. Lymphangiogenesis and angiogenesis in abdominal aortic aneurysm. PLoS One. 2014;9(3):e89830.
69. Kessler K, Borges LF, Ho-Tin-Noe B, Jondeau G, Michel JB, Vranckx R. Angiogenesis and remodelling in human thoracic aortic aneurysms. Cardiovasc Res. 2014;104(1):147-59.
70. Tanaka H, Zaima N, Sasaki T, Hayasaka T, Goto-Inoue N, Onoue K, et al. Adventitial *vasa vasorum* arteriosclerosis in

abdominal aortic aneurysm. PLoS One. 2013;8(2):e57398.

71. Carrell TW, Burnand KG, Wells GM, Clements JM, Smith A. Stromelysin-1 (matrix metalloproteinase-3) and tissue inhibitor of metalloproteinase-3 are overexpressed in the wall of abdominal aortic aneurysms. Circulation. 2002;105(4):477-82.

72. Defawe OD, Colige A, Lambert CA, Munaut C, Delvenne P, Lapiere CM, et al. TIMP-2 and PAI-1 mRNA levels are lower in aneurysmal as compared to athero-occlusive abdominal aortas. Cardiovasc Res. 2003;60(1):205-13.

73. Sakalihasan N, Delvenne P, Nusgens BV, Limet R, Lapiere CM. Activated forms of MMP2 and MMP9 in abdominal aortic aneurysms. J Vasc Surg. 1996;24(1):127-33.

74. Carmeliet P, Moons L, Lijnen R, Baes M, Lemaitre V, Tipping P, et al. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat Genet. 1997;17(4):439-44.

75. Anidjar S, Salzmann JL, Gentric D, Lagneau P, Camilleri JP, Michel JB. Elastase-induced experimental aneurysms in rats. Circulation. 1990;82(3):973-81.

76. Deng GG, Martin-McNulty B, Sukovich DA, Freay A, Halks-Miller M, Thinnes T, et al. Urokinase-type plasminogen activator plays a critical role in angiotensin II-induced abdominal aortic aneurysm. Circ Res. 2003;92(5):510-7.

77. Newman KM, Jean-Claude J, Li H, Ramey WG, Tilson MD. Cytokines that activate proteolysis are increased in abdominal aortic aneurysms. Circulation. 1994;90(5 Pt 2):II224-7.

78. Walton LJ, Franklin IJ, Bayston T, Brown LC, Greenhalgh RM, Taylor GW, et al. Inhibition of prostaglandin E2 synthesis in abdominal aortic aneurysms: implications for smooth muscle cell viability, inflammatory processes, and the expansion of abdominal aortic aneurysms. Circulation. 1999;100(1):48-54.

79. Hinterseher I, Erdman R, Donoso LA, Vrabec TR, Schworer CM, Lillvis JH, et al. Role of complement cascade in abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol. 2011;31(7):1653-60.

80. Lopez-Candales A, Holmes DR, Liao S, Scott MJ, Wickline SA, Thompson RW. Decreased vascular smooth muscle cell density in medial degeneration of human abdominal aortic aneurysms. Am J Pathol. 1997;150(3):993-1007.

81. Riches K, Angelini TG, Mudhar GS, Kaye J, Clark E, Bailey MA, et al. Exploring smooth muscle phenotype and function in a bioreactor model of abdominal aortic aneurysm. J Transl Med. 2013;11:208.

82. Li G, Qin L, Wang L, Li X, Caulk AW, Zhang J, et al. Inhibition of the mTOR pathway in abdominal aortic aneurysm: implications of smooth muscle cell contractile phenotype, inflammation, and aneurysm expansion. Am J Physiol Heart Circ Physiol. 2017;312(6):H1110-H9.

83. Allaire E, Muscatelli-Groux B, Guinault AM, Pages C, Goussard A, Mandet C, et al. Vascular smooth muscle cell endovascular therapy stabilizes already developed aneurysms in a model of aortic injury elicited by inflammation and proteolysis. Ann Surg. 2004;239(3):417-27.

84. Bogunovic N, Meekel JP, Micha D, Blankensteijn JD, Hordijk PL, Yeung KK. Impaired smooth muscle cell contractility as a novel concept of abdominal aortic aneurysm pathophysiology. Sci Rep. 2019;9(1):6837.

85. Yoshimura K, Morikage N, Nishino-Fujimoto S, Furutani A, Shirasawa B, Hamano K. Current Status and Perspectives on Pharmacologic Therapy for Abdominal Aortic Aneurysm. Curr Drug Targets. 2018;19(11):1265-75.

86. Folsom AR, Yao L, Alonso A, Lutsey PL, Missov E, Lederle FA, et al. Circulating Biomarkers and Abdominal Aortic Aneurysm Incidence: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2015;132(7):578-85.

87. Franck G, Dai J, Fifre A, Ngo S, Justine C, Michineau S, et al. Reestablishment of the endothelial lining by endothelial cell therapy stabilizes experimental abdominal aortic aneurysms. Circulation. 2013;127(18):1877-87.

88. Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R, et al. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation. 2001;104(4):448-54.

89. Gao L, Siu KL, Chalupsky K, Nguyen A, Chen P, Weintraub NL, et al. Role of uncoupled endothelial nitric oxide synthase in abdominal aortic aneurysm formation: treatment with folic acid. Hypertension. 2012;59(1):158-66.

90. Sho E, Sho M, Hoshina K, Kimura H, Nakahashi TK, Dalman RL. Hemodynamic forces regulate mural macrophage infiltration in experimental aortic aneurysms. Exp Mol Pathol. 2004;76(2):108-16.

91. Heath JM, Fernandez Esmerats J, Khambouneheuang L, Kumar S, Simmons R, Jo H. Mechanosensitive microRNA-181b Regulates Aortic Valve Endothelial Matrix Degradation by Targeting TIMP3. Cardiovasc Eng Technol. 2018;9(2):141-50.

92. Platt MO, Ankeny RF, Shi GP, Weiss D, Vega JD, Taylor WR, et al. Expression of cathepsin K is regulated by shear

stress in cultured endothelial cells and is increased in endothelium in human atherosclerosis. Am J Physiol Heart Circ Physiol. 2007;292(3):H1479-86.

93. Liu J, Sukhova GK, Yang JT, Sun J, Ma L, Ren A, et al. Cathepsin L expression and regulation in human abdominal aortic aneurysm, atherosclerosis, and vascular cells. Atherosclerosis. 2006;184(2):302-11.

94. Keegan PM, Wilder CL, Platt MO. Tumor necrosis factor alpha stimulates cathepsin K and V activity via juxtacrine monocyte-endothelial cell signaling and JNK activation. Mol Cell Biochem. 2012;367(1-2):65-72.

95. Saito T, Hasegawa Y, Ishigaki Y, Yamada T, Gao J, Imai J, et al. Importance of endothelial NF-kappaB signalling in vascular remodelling and aortic aneurysm formation. Cardiovasc Res. 2013;97(1):106-14.

96. Hannawa KK, Cho BS, Sinha I, Roelofs KJ, Myers DD, Wakefield TJ, et al. Attenuation of experimental aortic aneurysm formation in P-selectin knockout mice. Ann N Y Acad Sci. 2006;1085:353-9.

97. Xiong W, Mactaggart J, Knispel R, Worth J, Zhu Z, Li Y, et al. Inhibition of reactive oxygen species attenuates aneurysm formation in a murine model. Atherosclerosis. 2009;202(1):128-34.

98. Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, et al. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nat Med. 2009;15(6):649-56.

99. Vega de Ceniga M, Gomez R, Estallo L, de la Fuente N, Viviens B, Barba A. Analysis of expansion patterns in 4-4.9 cm abdominal aortic aneurysms. Ann Vasc Surg. 2008;22(1):37-44.

100. Wolf YG, Thomas WS, Brennan FJ, Goff WG, Sise MJ, Bernstein EF. Computed tomography scanning findings associated with rapid expansion of abdominal aortic aneurysms. J Vasc Surg. 1994;20(4):529-35; discussion 35-8.

101. Speelman L, Schurink GW, Bosboom EM, Buth J, Breeuwer M, van de Vosse FN, et al. The mechanical role of thrombus on the growth rate of an abdominal aortic aneurysm. J Vasc Surg. 2010;51(1):19-26.

102. Parr A, McCann M, Bradshaw B, Shahzad A, Buttner P, Golledge J. Thrombus volume is associated with cardiovascular events and aneurysm growth in patients who have abdominal aortic aneurysms. J Vasc Surg. 2011;53(1):28-35.

103. Vorp DA, Lee PC, Wang DH, Makaroun MS, Nemoto EM, Ogawa S, et al. Association of intraluminal thrombus in abdominal aortic aneurysm with local hypoxia and wall weakening. J Vasc Surg. 2001;34(2):291-9.

104. Khan JA, Abdul Rahman MN, Mazari FA, Shahin Y, Smith G, Madden L, et al. Intraluminal thrombus has a selective influence on matrix metalloproteinases and their inhibitors (tissue inhibitors of matrix metalloproteinases) in the wall of abdominal aortic aneurysms. Ann Vasc Surg. 2012;26(3):322-9.

105. Fontaine V, Jacob MP, Houard X, Rossignol P, Plissonnier D, Angles-Cano E, et al. Involvement of the mural thrombus as a site of protease release and activation in human aortic aneurysms. Am J Pathol. 2002;161(5):1701-10.

106. Houard X, Rouzet F, Touat Z, Philippe M, Dominguez M, Fontaine V, et al. Topology of the fibrinolytic system within the mural thrombus of human abdominal aortic aneurysms. J Pathol. 2007;212(1):20-8.

107. Houard X, Ollivier V, Louedec L, Michel JB, Back M. Differential inflammatory activity across human abdominal aortic aneurysms reveals neutrophil-derived leukotriene B4 as a major chemotactic factor released from the intraluminal thrombus. FASEB J. 2009;23(5):1376-83.

108. Delbosc S, Diallo D, Dejouvencel T, Lamiral Z, Louedec L, Martin-Ventura JL, et al. Impaired high-density lipoprotein anti-oxidant capacity in human abdominal aortic aneurysm. Cardiovasc Res. 2013;100(2):307-15.

109. Moxon JV, Padula MP, Clancy P, Emeto TI, Herbert BR, Norman PE, et al. Proteomic analysis of intra-arterial thrombus secretions reveals a negative association of clusterin and thrombospondin-1 with abdominal aortic aneurysm. Atherosclerosis. 2011;219(2):432-9.

110. Martinez-Pinna R, Madrigal-Matute J, Tarin C, Burillo E, Esteban-Salan M, Pastor-Vargas C, et al. Proteomic analysis of intraluminal thrombus highlights complement activation in human abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol. 2013;33(8):2013-20.

111. Yamazumi K, Ojiro M, Okumura H, Aikou T. An activated state of blood coagulation and fibrinolysis in patients with abdominal aortic aneurysm. Am J Surg. 1998;175(4):297-301.

112. Lindholt JS, Jorgensen B, Fasting H, Henneberg EW. Plasma levels of plasmin-antiplasmin-complexes are predictive for small abdominal aortic aneurysms expanding to operation-recommendable sizes. J Vasc Surg. 2001;34(4):611-5.

113. Martinez-Pinna R, Lindholt JS, Blanco-Colio LM, Dejouvencel T, Madrigal-Matute J, Ramos-Mozo P, et al. Increased levels of thioredoxin in patients with abdominal aortic aneurysms (AAAs). A potential link of oxidative stress with AAA evolution. Atherosclerosis. 2010;212(1):333-8.

114. Martinez-Pinna R, Ramos-Mozo P, Madrigal-Matute J, Blanco-Colio LM, Lopez JA, Calvo E, et al. Identification of pero-

xiredoxin-1 as a novel biomarker of abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 2011;31(4):935-43.

115. Dejouvencel T, Feron D, Rossignol P, Sapoval M, Kauffmann C, Piot JM, et al. Hemorphin 7 reflects hemoglobin proteolysis in abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 2010;30(2):269-75.

116. Vaglio A, Greco P, Corradi D, Palmisano A, Martorana D, Ronda N, et al. Autoimmune aspects of chronic periaortitis. Autoimmun Rev. 2006;5(7):458-64.

117. Wilcox JN, Waksman R, King SB, Scott NA. The role of the adventitia in the arterial response to angioplasty: the effect of intravascular radiation. Int J Radiat Oncol Biol Phys. 1996;36(4):789-96.

118. Hurks R, Vink A, Hoefer IE, de Vries JP, Schoneveld AH, Schermerhorn ML, et al. Atherosclerotic risk factors and atherosclerotic postoperative events are associated with low inflammation in abdominal aortic aneurysms. Atherosclerosis. 2014;235(2):632-41.

119. Tieu BC, Ju X, Lee C, Sun H, Lejeune W, Recinos A, 3rd, et al. Aortic adventitial fibroblasts participate in angiotensin-induced vascular wall inflammation and remodeling. J Vasc Res. 2011;48(3):261-72.

120. Biros E, Walker PJ, Nataatmadja M, West M, Golledge J. Downregulation of transforming growth factor, beta receptor 2 and Notch signaling pathway in human abdominal aortic aneurysm. Atherosclerosis. 2012;221(2):383-6.

121. Hellmann DB, Grand DJ, Freischlag JA. Inflammatory abdominal aortic aneurysm. JAMA. 2007;297(4):395-400.

122. Doderer SA, Gabel G, Kokje VBC, Northoff BH, Holdt LM, Hamming JF, et al. Adventitial adipogenic degeneration is an unidentified contributor to aortic wall weakening in the abdominal aortic aneurysm. J Vasc Surg. 2018;67(6):1891-900 e4.

123. Krueger F, Kappert K, Foryst-Ludwig A, Kramer F, Clemenz M, Grzesiak A, et al. AT1-receptor blockade attenuates outward aortic remodeling associated with diet-induced obesity in mice. Clin Sci (Lond). 2017;131(15):1989-2005.

124. Gabel G, Northoff BH, Weinzierl I, Ludwig S, Hinterseher I, Wilfert W, et al. Molecular Fingerprint for Terminal Abdominal Aortic Aneurysm Disease. J Am Heart Assoc. 2017;6(12).

125. Niestrawska JA, Regitnig P, Viertler C, Cohnert TU, Babu AR, Holzapfel GA. The role of tissue remodeling in mechanics and pathogenesis of abdominal aortic aneurysms. Acta Biomater. 2019;88:149-61.

126. Kugo H, Zaima N, Tanaka H, Mouri Y, Yanagimoto K, Hayamizu K, et al. Adipocyte in vascular wall can induce the rupture of abdominal aortic aneurysm. Sci Rep. 2016;6:31268.

127. Kang JR, Gupta R. Mechanisms of fatty degeneration in massive rotator cuff tears. J Shoulder Elbow Surg. 2012;21(2):175-80.

128. Rodeheffer MS. Tipping the scale: muscle versus fat. Nat Cell Biol. 2010;12(2):102-4.

129. Weiss DJ, Casale GP, Koutakis P, Nella AA, Swanson SA, Zhu Z, et al. Oxidative damage and myofiber degeneration in the gastrocnemius of patients with peripheral arterial disease. J Transl Med. 2013;11:230.

130. Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol. 2010;12(2):153-63.

131. Kugo H, Moriyama T, Zaima N. The role of perivascular adipose tissue in the appearance of ectopic adipocytes in the abdominal aortic aneurysmal wall. Adipocyte. 2019;8(1):229-39.

132. Lim S, Meigs JB. Links between ectopic fat and vascular disease in humans. Arterioscler Thromb Vasc Biol. 2014;34(9):1820-6.

133. Brown NK, Zhou Z, Zhang J, Zeng R, Wu J, Eitzman DT, et al. Perivascular adipose tissue in vascular function and disease: a review of current research and animal models. Arterioscler Thromb Vasc Biol. 2014;34(8):1621-30.

134. Omar A, Chatterjee TK, Tang Y, Hui DY, Weintraub NL. Proinflammatory phenotype of perivascular adipocytes. Arterioscler Thromb Vasc Biol. 2014;34(8):1631-6.

135. Owen MK, Noblet JN, Sassoon DJ, Conteh AM, Goodwill AG, Tune JD. Perivascular adipose tissue and coronary vascular disease. Arterioscler Thromb Vasc Biol. 2014;34(8):1643-9.

136. Chang L, Milton H, Eitzman DT, Chen YE. Paradoxical roles of perivascular adipose tissue in atherosclerosis and hypertension. Circ J. 2013;77(1):11-8.

137. Fitzgibbons TP, Kogan S, Aouadi M, Hendricks GM, Straubhaar J, Czech MP. Similarity of mouse perivascular and brown adipose tissues and their resistance to diet-induced inflammation. Am J Physiol Heart Circ Physiol. 2011;301(4):H1425-37.

138. Padilla J, Jenkins NT, Vieira-Potter VJ, Laughlin MH. Divergent phenotype of rat thoracic and abdominal perivascular

adipose tissues. Am J Physiol Regul Integr Comp Physiol. 2013;304(7):R543-52.

139. Liu CL, Ren J, Wang Y, Zhang X, Sukhova GK, Liao M, et al. Adipocytes promote interleukin-18 binding to its receptors during abdominal aortic aneurysm formation in mice. Eur Heart J. 2019.

140. Parvizi M, Petersen AH, van Spreuwel-Goossens C, Kluijtmans S, Harmsen MC. Perivascular scaffolds loaded with adipose tissue-derived stromal cells attenuate development and progression of abdominal aortic aneurysm in rats. J Biomed Mater Res A. 2018;106(9):2494-506.

141. Castro-Ferreira R, Barreira R, Mendes P, Couto P, Peixoto F, Aguiar M, et al. First Population-Based Screening of Abdominal Aortic Aneurysm in Portugal. Ann Vasc Surg. 2019.

142. Ashton HA, Buxton MJ, Day NE, Kim LG, Marteau TM, Scott RA, et al. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. Lancet. 2002;360(9345):1531-9.

143. Norman PE, Jamrozik K, Lawrence-Brown MM, Le MT, Spencer CA, Tuohy RJ, et al. Population based randomised controlled trial on impact of screening on mortality from abdominal aortic aneurysm. BMJ. 2004;329(7477):1259.

144. Mani K, Venermo M, Beiles B, Menyhei G, Altreuther M, Loftus I, et al. Regional Differences in Case Mix and Peri-operative Outcome After Elective Abdominal Aortic Aneurysm Repair in the VASCUNET Database. Eur J Vasc Endovasc Surg. 2015;49(6):646-52.

145. Cao P, De Rango P, Verzini F, Parlani G, Romano L, Cieri E, et al. Comparison of surveillance versus aortic endografting for small aneurysm repair (CAESAR): results from a randomised trial. Eur J Vasc Endovasc Surg. 2011;41(1):13-25.

146. Ouriel K, Clair DG, Kent KC, Zarins CK, Positive Impact of Endovascular Options for treating Aneurysms Early I. Endovascular repair compared with surveillance for patients with small abdominal aortic aneurysms. J Vasc Surg. 2010;51(5):1081-7.

147. Sweeting MJ, Patel R, Powell JT, Greenhalgh RM, Investigators ET. Endovascular Repair of Abdominal Aortic Aneurysm in Patients Physically Ineligible for Open Repair: Very Long-term Follow-up in the EVAR-2 Randomized Controlled Trial. Ann Surg. 2017;266(5):713-9.

148. Sobocinski J, Maurel B, Delsart P, d'Elia P, Guillou M, Maioli F, et al. Should we modify our indications after the EVAR-2 trial conclusions? Ann Vasc Surg. 2011;25(5):590-7.

149. Brown LC, Powell JT. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance. UK Small Aneurysm Trial Participants. Ann Surg. 1999;230(3):289-96; discussion 96-7.

150. Jahangir E, Lipworth L, Edwards TL, Kabagambe EK, Mumma MT, Mensah GA, et al. Smoking, sex, risk factors and abdominal aortic aneurysms: a prospective study of 18 782 persons aged above 65 years in the Southern Community Cohort Study. J Epidemiol Community Health. 2015;69(5):481-8.

151. Scott RA, Wilson NM, Ashton HA, Kay DN. Influence of screening on the incidence of ruptured abdominal aortic aneurysm: 5-year results of a randomized controlled study. Br J Surg. 1995;82(8):1066-70.

152. Lindholt JS, Juul S, Fasting H, Henneberg EW. Hospital costs and benefits of screening for abdominal aortic aneurysms. Results from a randomised population screening trial. Eur J Vasc Endovasc Surg. 2002;23(1):55-60.

153. Thompson SG, Ashton HA, Gao L, Buxton MJ, Scott RA, Multicentre Aneurysm Screening Study G. Final follow-up of the Multicentre Aneurysm Screening Study (MASS) randomized trial of abdominal aortic aneurysm screening. Br J Surg. 2012;99(12):1649-56.

154. Lindholt JS, Juul S, Fasting H, Henneberg EW. Preliminary ten year results from a randomised single centre mass screening trial for abdominal aortic aneurysm. Eur J Vasc Endovasc Surg. 2006;32(6):608-14.

155. Svensjo S, Mani K, Bjorck M, Lundkvist J, Wanhainen A. Screening for abdominal aortic aneurysm in 65-year-old men remains cost-effective with contemporary epidemiology and management. Eur J Vasc Endovasc Surg. 2014;47(4):357-65.

156. Stather PW, Dattani N, Bown MJ, Earnshaw JJ, Lees TA. International variations in AAA screening. Eur J Vasc Endovasc Surg. 2013;45(3):231-4.

157. Hjelmgren J, Berggren F, Andersson F. Health economic guidelines--similarities, differences and some implications. Value Health. 2001;4(3):225-50.

158. Henriksson M, Lundgren F, Carlsson P. Informing the efficient use of health care and health care research resources - the case of screening for abdominal aortic aneurysm in Sweden. Health Econ. 2006;15(12):1311-22.

159. Ying AJ, Affan ET. Abdominal Aortic Aneurysm Screening: A Systematic Review and Meta-analysis of Efficacy and Cost. Ann Vasc Surg. 2019;54:298-303 e3.

160. Demer LL, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. Arterioscler Thromb Vasc Biol. 2014;34(4):715-23.

161. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. Circ Res. 2006;99(10):1044-59.

162. Bostrom KI, Rajamannan NM, Towler DA. The regulation of valvular and vascular sclerosis by osteogenic morphogens. Circ Res. 2011;109(5):564-77.

163. Fitzpatrick LA, Severson A, Edwards WD, Ingram RT. Diffuse calcification in human coronary arteries. Association of osteopontin with atherosclerosis. J Clin Invest. 1994;94(4):1597-604.

164. Shanahan CM, Cary NR, Salisbury JR, Proudfoot D, Weissberg PL, Edmonds ME. Medial localization of mineralization-regulating proteins in association with Monckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. Circulation. 1999;100(21):2168-76.

165. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, et al. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. J Am Soc Nephrol. 2004;15(11):2857-67.

166. Lau WL, Pai A, Moe SM, Giachelli CM. Direct effects of phosphate on vascular cell function. Adv Chronic Kidney Dis. 2011;18(2):105-12.

167. 1Wong ND, Kouwabunpat D, Vo AN, Detrano RC, Eisenberg H, Goel M, et al. Coronary calcium and atherosclerosis by ultrafast computed tomography in asymptomatic men and women: relation to age and risk factors. Am Heart J. 1994;127(2):422-30.

168. Rennenberg RJ, Kessels AG, Schurgers LJ, van Engelshoven JM, de Leeuw PW, Kroon AA. Vascular calcifications as a marker of increased cardiovascular risk: a meta-analysis. Vasc Health Risk Manag. 2009;5(1):185-97.

169. Jayalath RW, Mangan SH, Golledge J. Aortic calcification. Eur J Vasc Endovasc Surg. 2005;30(5):476-88.

170. Van de Luijtgaarden KM, Bastos Goncalves F, Hoeks SE, Majoor-Krakauer D, Rouwet EV, Stolker RJ, et al. Familial abdominal aortic aneurysm is associated with more complications after endovascular aneurysm repair. J Vasc Surg. 2014;59(2):275-82.

171. Thanassoulis G, Massaro JM, Corsini E, Rogers I, Schlett CL, Meigs JB, et al. Periaortic adipose tissue and aortic dimensions in the Framingham Heart Study. J Am Heart Assoc. 2012;1(6):e000885.

172. Shields KJ, Barinas-Mitchell E, Gingo MR, Tepper P, Goodpaster BH, Kao AH, et al. Perivascular adipose tissue of the descending thoracic aorta is associated with systemic lupus erythematosus and vascular calcification in women. Atherosclerosis. 2013;231(1):129-35.

173. Antonopoulos AS, Sanna F, Sabharwal N, Thomas S, Oikonomou EK, Herdman L, et al. Detecting human coronary inflammation by imaging perivascular fat. Sci Transl Med. 2017;9(398).

174. Suganami T, Tanaka M, Ogawa Y. Adipose tissue inflammation and ectopic lipid accumulation. Endocr J. 2012;59(10):849-57.

175. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest. 2011;121(6):2094-101.

176. Meekel JP, Groeneveld ME, Bogunovic N, Keekstra N, Musters RJP, Zandieh-Doulabi B, et al. An in vitro method to keep human aortic tissue sections functionally and structurally intact. Sci Rep. 2018;8(1):8094.

177. !!! INVALID CITATION !!! [142].

178. Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, et al. Plasma lipidomic analysis of stable and unstable coronary artery disease. Arterioscler Thromb Vasc Biol. 2011;31(11):2723-32.

179. Stegemann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A, et al. Comparative lipidomics profiling of human atherosclerotic plaques. Circ Cardiovasc Genet. 2011;4(3):232-42.

180. Fernandez C, Sandin M, Sampaio JL, Almgren P, Narkiewicz K, Hoffmann M, et al. Plasma lipid composition and risk of developing cardiovascular disease. PLoS One. 2013;8(8):e71846.

181. Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, et al. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. Circulation. 2014;129(18):1821-31.

182. Sigruener A, Kleber ME, Heimerl S, Liebisch G, Schmitz G, Maerz W. Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. PLoS One. 2014;9(1):e85724.

183. Ellims AH, Wong G, Weir JM, Lew P, Meikle PJ, Taylor AJ. Plasma lipidomic analysis predicts non-calcified coronary artery plaque in asymptomatic patients at intermediate risk of coronary artery disease. European Heart Journal -

Cardiovascular Imaging. 2014;15(8):908-16.

184. Sutter I, Velagapudi S, Othman A, Riwanto M, Manz J, Rohrer L, et al. Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL. Atherosclerosis. 2015;241(2):539-46.

185. Alshehry ZH, Mundra PA, Barlow CK, Mellett NA, Wong G, McConville MJ, et al. Plasma Lipidomic Profiles Improve on Traditional Risk Factors for the Prediction of Cardiovascular Events in Type 2 Diabetes Mellitus. Circulation. 2016;134(21):1637-50.

186. Croyal M, Kaabia Z, Leon L, Ramin-Mangata S, Baty T, Fall F, et al. Fenofibrate decreases plasma ceramide in type 2 diabetes patients: A novel marker of CVD? Diabetes Metab. 2018;44(2):143-9.

187. Razquin C, Liang L, Toledo E, Clish CB, Ruiz-Canela M, Zheng Y, et al. Plasma lipidome patterns associated with cardiovascular risk in the PREDIMED trial: A case-cohort study. Int J Cardiol. 2018;253:126-32.

188. Mundra PA, Barlow CK, Nestel PJ, Barnes EH, Kirby A, Thompson P, et al. Large-scale plasma lipidomic profiling identifies lipids that predict cardiovascular events in secondary prevention. JCI Insight. 2018;3(17).

189. Gerl MJ, Vaz WLC, Domingues N, Klose C, Surma MA, Sampaio JL, et al. Cholesterol is Inefficiently Converted to Cholesteryl Esters in the Blood of Cardiovascular Disease Patients. Sci Rep. 2018;8(1):14764-.

190. Harshfield EL, Koulman A, Ziemek D, Marney L, Fauman EB, Paul DS, et al. An Unbiased Lipid Phenotyping Approach To Study the Genetic Determinants of Lipids and Their Association with Coronary Heart Disease Risk Factors. J Proteome Res. 2019;18(6):2397-410.

191. Schlotterbeck J, Chatterjee M, Gawaz M, Lammerhofer M. Comprehensive MS/MS profiling by UHPLC-ESI-QTOF-MS/MS using SWATH data-independent acquisition for the study of platelet lipidomes in coronary artery disease. Anal Chim Acta. 2019;1046:1-15.

192. Ramo JT, Ripatti P, Tabassum R, Soderlund S, Matikainen N, Gerl MJ, et al. Coronary Artery Disease Risk and Lipidomic Profiles Are Similar in Hyperlipidemias With Family History and Population-Ascertained Hyperlipidemias. J Am Heart Assoc. 2019;8(13):e012415.

193. Kostara CE, Papathanasiou A, Psychogios N, Cung MT, Elisaf MS, Goudevenos J, et al. NMR-based lipidomic analysis of blood lipoproteins differentiates the progression of coronary heart disease. J Proteome Res. 2014;13(5):2585-98.

194. Tanaka H, Zaima N, Sasaki T, Yamamoto N, Inuzuka K, Sano M, et al. Imaging Mass Spectrometry Reveals a Unique Distribution of Triglycerides in the Abdominal Aortic Aneurysmal Wall. J Vasc Res. 2015;52(2):127-35.

195. Saito K, Yagi H, Maekawa K, Nishigori M, Ishikawa M, Muto S, et al. Lipidomic signatures of aortic media from patients with atherosclerotic and nonatherosclerotic aneurysms. Sci Rep. 2019;9(1):15472.

196. Claria J, Nguyen BT, Madenci AL, Ozaki CK, Serhan CN. Diversity of lipid mediators in human adipose tissue depots. Am J Physiol Cell Physiol. 2013;304(12):C1141-9.

197. Folkesson M, Vorkapic E, Gulbins E, Japtok L, Kleuser B, Welander M, et al. Inflammatory cells, ceramides, and expression of proteases in perivascular adipose tissue adjacent to human abdominal aortic aneurysms. J Vasc Surg. 2017;65(4):1171-9 e1.

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LIST OF PUBLICATIONS

1. Dias-Neto M, Pintalhao M, Ferreira M, Lunet N. Salt intake and risk of gastric intestinal metaplasia: systematic review and meta-analysis. Nutr Cancer. 2010;62(2):133-47. doi: 10.1080/01635580903305391. Review. PubMed PMID: 20099187.

2. Pintalhao M, Dias-Neto M, Peleteiro B, Lopes C, Figueiredo C, David L, Lunet N. Salt intake and type of intestinal metaplasia in Helicobacter pylori-infected Portuguese men. Nutr Cancer. 2010;62(8):1153-60. doi: 10.1080/01635581.2010.513799. PubMed PMID: 21058204.

3. Lopes-Conceição L, Dias-Neto M, Fontes-Sousa AP, Mendes-Ferreira P, Maia-Rocha C, Henriques-Coelho T, de Keulenaer G, Leite Moreira AF, Brás-Silva C. [Neuregulin1/ErbB system: importance in the control of cardiovascular function]. Acta Med Port. 2011 Dec;24 Suppl 4:1009-20. Epub 2011 Dec 31. Review. Portuguese. PubMed PMID: 22863512.

4. Dias-Neto M, Ramos JF, Dias PG, Sampaio S, Rocha E Silva A, Freitas A, Teixeira JF. [TEVAR: a strategy for the diversity of thoracic aorta disease. Series of cases and national prospect]. Rev Port Cir Cardiotorac Vasc. 2014 Jan-Mar;21(1):43-54. Portuguese. PubMed PMID: 25596395.

5. Dias-Neto M, Luísa-Neves A, Pinho S, Gonçalves N, Mendes M, Eloy C, Lopes JM, Gonçalves D, Ferreira-Pinto M, Leite-Moreira AF, Henriques-Coelho T. Pathophysiology of infantile pulmonary arterial hypertension induced by monocrotaline. Pediatr Cardiol. 2015 Jun;36(5):1000-13. doi: 10.1007/s00246-015-1111-y. Epub 2015 Jan 22. PubMed PMID: 25608696.

6. Vieira, Mário et al. Recanalização subintimal total femoropoplítea após falência de bypass infrapoplíteo: redefinindo estratégias. *Angiol Cir Vasc*, Dez 2015, vol.11, no.4, p.230-234. ISSN 1646-706X

7. Dias-Neto M, Ramos JF. Before and After: Superior Vena Cava Syndrome Treated by Fibrinolysis. Eur J Vasc Endovasc Surg. 2016 Apr;51(4):564. doi: 10.1016/j.ejvs.2016.01.012. Epub 2016 Feb 23. PubMed PMID: 26919934.

8. Reis P, Mendes L, Rodrigues M, Dias-Neto M, Oliveira R, Araújo R, Amaral C, Teixeira JF, Afonso G. Perioperative Drainage of Cerebrospinal Fluid for Endovascular Aortic Surgery. Rev Port Cir Cardiotorac Vasc. 2016 Jul-Dec;23(3-4):157-163. PubMed PMID: 29103224.

9. Dias-Neto, Marina et al. A transição epidemiológica no aneurisma da aorta abdominal. *Angiol Cir Vasc*, Mar 2017, vol.13, no.1, p.08-13. ISSN 1646-706X

10. Dias-Neto M, Reis PV, Rolim D, Ramos JF, Teixeira JF, Sampaio S. Strategies to prevent TEVAR-related spinal cord ischemia. Vascular. 2017 Jun;25(3):307-315. doi: 10.1177/1708538116671235. Epub 2016 Sep 30. Review. PubMed PMID: 27694555.

11. Dias-Neto M, Reis P, Mendes L, Rodrigues M, Amaral C, Afonso G, Fernando Teixeira J. Institutional Protocol for Prevention of TEVAR-related Spinal Cord Ischemia - The First 9 Cases. Rev Port Cir Cardiotorac Vasc. 2017 Jul-Dec;24(3-4):151. PubMed PMID: 29701382.

12. Dias-Neto M, Neves E, Sousa-Nunes F, Henriques-Coelho T, Sampaio S. Abdominal aortic aneurysm calcification: trying to identify a reliable semiquantitative method. J Cardiovasc Surg (Torino). 2018 Feb 8. doi: 10.23736/S0021-9509.18.10132-7. [Epub ahead of print] PubMed PMID: 29430885.

13. Dias-Neto, Marina, Ramos, José Fernando and Teixeira, José Fernando Tratamento endovascular de lesões femoro-poplíteas calcificadas. *Angiol Cir Vasc*, Mar 2018, vol.14, no.1, p.56-63. ISSN 1646-706X

14. Dias-Neto M, Ramos JF, Teixeira JF. Blunt Injury of the Innominate Artery: What Surprises to Expect? A Case Report. Vasc Endovascular Surg. 2018 Apr;52(3):226-232. doi: 10.1177/1538574418758230. Epub 2018 Feb 12. PubMed PMID: 29433408.

15. Dias-Neto M, Matschuck M, Bausback Y, Banning-Eichenseher U, Steiner S, Branzan D, Staab H, Varcoe RL, Scheinert D, Schmidt A. Endovascular Treatment of Severely Calcified Femoropopliteal Lesions Using the "Pave-and-Crack" Technique: Technical Description and 12-Month Results. J Endovasc Ther. 2018 Jun;25(3):334-342. doi: 10.1177/1526602818763352. Epub 2018 Mar 20. PubMed PMID: 29557221.

16. Dias-Neto, Marina et al. Novos conceitos sobre adiposidade na patologia da aorta abdominal. *Angiol Cir Vasc*, Jun 2018, vol.14, no.2, p.48-53. ISSN 1646-706X

17. Reis PV, Morgado M, Valdoleiros I, Neto MD, Mourão J. Complications of Endovascular Aneurysm Repair: Mortality, Myocardial Infarction and Acute Kidney Injury. Turk J Anaesthesiol Reanim. 2018 Jun;46(3):222-228. doi: 10.5152/TJAR.2018.37929. Epub 2017 Jun 1. PubMed PMID: 30140519; PubMed Central PMCID: PMC6097855

18. Duarte Silva J, Ferreira M, Neto M, Teixeira J, Duarte F. [Pseudoaneurysm of the popliteal artery associated with tibial osteochondroma]. Rev Port Cir Cardiotorac Vasc. 2018 Jan-Jun;25(1-2):103-104. Portuguese. PubMed PMID: 30317723.

19. Dias-Neto M, Meekel JP, van Schaik TG, Hoozemans J, Sousa-Nunes F, Henriques-Coelho T, Lely RJ, Wisselink W, Blankensteijn JD, Yeung KK. High Density of Periaortic Adipose Tissue in Abdominal Aortic Aneurysm. Eur J Vasc Endovasc Surg. 2018 Nov;56(5):663-671. doi: 10.1016/j.ejvs.2018.07.008. Epub 2018 Aug 14. PubMed PMID: 30115505.

20. Dias-Neto M, Neves E, Sousa-Nunes F, Leite-Moreira A, Henriques-Coelho T, Sampaio S. [Perspectives and research challenges in abdominal aortic aneurysm calcification]. Rev Port Cir Cardiotorac Vasc. 2018 Jan-Jun;25(1-2):55-60. Review. Portuguese. PubMed PMID: 30317711.

21. Marques C, Dias-Neto M, Sampaio S. Clinical outcomes after digital subtraction angiography versus computed tomography angiography in the preoperative evaluation of lower limb peripheral artery disease. Rev Port Cir Cardiotorac Vasc. 2018 Jul-Dec;25(3-4):133-140. PubMed PMID: 30599470.

22. Dias-Neto M. Vascular access complications in Extracorporeal Membrane Oxygenation: a joint effort of intensivists and vascular surgeons. Rev Port Cir Cardiotorac Vasc. 2019 Jan-Mar;26(1):17-18. PubMed PMID: 31104371.

23. Castro-Ferreira R, Barreira R, Mendes P, Couto P, Peixoto F, Aguiar M, Neto M, Rolim D, Pinto J, Freitas A, Dias PG, Mansilha A, Teixeira JF, Sampaio SM, Leite-Moreira A. First Population-Based Screening of Abdominal Aortic Aneurysm in Portugal. Ann Vasc Surg. 2019 Aug;59:48-53. doi: 10.1016/j.avsg.2018.12.091. Epub 2019 Feb 22. PubMed PMID: 30802591.

24. Alves-Ferreira J, Rocha-Neves J, Dias-Neto M, Braga SF. Poor long-term outcomes after carotid endarterectomy: a retrospective analysis of two Portuguese centers. Scand Cardiovasc J. 2019 Oct;53(5):266-273. doi: 10.1080/14017431.2019.1638518. Epub 2019 Jul 8. PubMed PMID: 31251084.

25. Vieira-Andrade JD, Rocha-Neves JP, Macedo JP, Dias-Neto MF. Onset of Neurological Deficit During Carotid Clamping With Carotid Endarterectomy Under Regional Anesthesia Is Not a Predictor of Carotid Restenosis. Ann Vasc Surg. 2019 Aug 2. pii: S0890-5096(19)30520-5. doi: 10.1016/j.avsg.2019.05.025. [Epub ahead of print] PubMed PMID: 31382007.

26. Poleri I, Dias-Neto M, Rocha-Neves J, Sampaio S. Type B Aortic Dissection - A Single Center Series. Rev Port Cir Cardiotorac Vasc. 2019 Apr-Jun;26(2):131-137. PubMed PMID: 31476814.

27. Pereira-Macedo J, Rocha-Neves JP, Dias-Neto MF, Andrade JPV. Prognostic effect of troponin elevation in patients undergoing carotid endarterectomy with regional anesthesia - A prospective study. Int J Surg. 2019 Sep 19;71:66-71. doi: 10.1016/j.ijsu.2019.09.015. [Epub ahead of print] PubMed PMID: 31542388.

28. Duarte-Gamas L, Rocha-Neves JP, Pereira-Neves A, Dias-Neto M, Baekgaard N. Contralateral deep vein thrombosis after stenting across the iliocaval confluence in chronic venous disease - A systematic review. Phlebology. 2019 Dec 2:268355519889873. doi: 10.1177/0268355519889873. [Epub ahead of print] PubMed PMID: 31793374.

29. Dias-Neto M, Mani K, Leite-Moreira A, Freitas A, Sampaio S. Nationwide analysis of intact abdominal aortic aneurysm repair in Portugal from 2000-2015. Ann Vasc Surg. 2020 Jan 6. pii: S0890-5096(19)31056-8. doi:10.1016/j.avsg.2019.12.013. [Epub ahead of print] PubMed PMID: 31917222.

CURRICULUM VITAE

Marina Felicidade Dias Neto was born December 3rd, 1986 in Santo Tirso, Portugal. She studied in Santo Tirso until attending FMUP from 2004 to 2010. During the course, she was invited by Professor Adelino Leite-Moreira to collaborate with the Department of Physiology and Cardiothoracic Surgery (later called Surgery and Physiology), where she worked with Prof. Doutor Tiago Henriques Coelho in the field of pulmonary hypertension. The interest in cardiovascular diseases became obvious by the end of the integrated master's degree in medicine. The filiation to UnIC sharped even more the scientific interest in the field. After a 1-year general training at CHUSJ, she started her specialized training in Angiology and Vascular Surgery at the same hospital in 2012. During that time, she visited University Hospital of Leipzig (Dr. Andreas Schmidt), the Clinic Hospital of Barcelona (Dr. Vincent Riambau) and Lille Hospital (Dr. Stephan Haulon). In 2015 she joined the PhD in Cardiovascular Sciencies, under the supervision of Professor Adelino Leite-Moreira, Prof. Doutor Tiago Henriques Coelho and Prof. Doutor Sérgio Sampaio. In 2016-2017 she attended and passed the Portugal Clinical Scholars Research Training Certificate Program, a collaboration between the Harvard Medical School and the *Fundação para a Ciência e Tecnologia.*

In April 2018 she passed the national exam for the degree of Specialist in Angiology and Vascular Surgery. In March 2019 she passed the European Board of Vascular Surgery Exam and obtained the degree of Fellow of the European Board of Vascular Surgery.

Aside from UnIC, the collaboration with the Department of Vascular Surgery, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centers location VU medical center (Amsterdam, the Netherlands), where she worked with Prof. Dr. Kak Khee Yeung, later becoming promoter of her PhD thesis, and the Uppsala Academic Medical Centre (Sweden), where she worked with Prof. Dr. Kevin Mani, contributed strongly to the accomplishment of her research.

She maintains the activity of Assistant Professor of Physiology and of Angiology and Vascular Surgery at the FMUP and is Promoter/Copromoter of several thesis of Integrated Master Degree in Medicine.

She is the associate editor of the Portuguese Journal of Cardiothoracic and Vascular Surgery (Rev Port Cir Cardiotorac Vasc) for Vascular Surgery since 2019.

She is also a reviewer for Angiologia e Cirurgia Vascular, the International Angiology and Journal of Endovascular Therapy.

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