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Biomechanical and histological analyses of a multilayer stent in a swine model of suprarenal aortic aneurysm

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Summary. Objectives. To analyze and compare, in an animal model, the treatment of thoracoabdominal aneurysms with multilayer stents and its hemodynamic effects through the biomechanical and histological analysis of the aortic wall in contact with the stent.

Methods. Large White pigs were randomized into two groups: Stent (n=6) and Control (n=5, non-stent). All animals were subjected to the creation of a suprarenal aneurysm with a bovine pericardial patch. In the Stent group, a multilayer stent was implanted immediately after aneurysm formation. After four weeks, all animals were subjected to angiographic assessment and intravascular ultrasound, and the stent was explanted before euthanasia for histological and biomechanical analyses.

Results. At histological analysis, the groups did not differ significantly in maximum thickness of the intima (p=0.526), media (p=0.129), or adventitia (p=0.662). Thrombus formation was observed in 100% of the animals on the intima and media layers of the stented aorta vs. none in the Control group (p=0.048). At biomechanical analysis, no statistical differences were observed in aortic wall elasticity (p=0.158), strength (p=0.360), or thickness (p=0.323).

Conclusion. We identified thrombosis of the aneurysmal sac through the presence of thrombi on the intima of the aorta in 100% of the animals in the Stent group; as for the biomechanical analysis, this study showed no statistical differences in vessel wall thickness, strength, and elasticity between groups.

Key words: Animal experimentation, Aortic aneurysm, Endovascular procedures, Animal models

Introduction

Aortic aneurysms are the 13th main cause of death in the United States of America, being the third main cause of sudden death (Reilly and Tilson, 1989; Gillum, 1995); their prevalence has increased with human longevity and the improvement of diagnostic imaging techniques. Studies have shown that, in 25% of patients, aortic aneurysms have thoracoabdominal involvement (more common in the descending aorta) and are associated with an infrarenal abdominal aortic aneurysm (Crawford and DeNatale, 1986; Clouse et al., 1998; Sakalihasan et al., 2005).

Endovascular procedures have greatly progressed during the 2000s and are currently widely used at specialized centers. Implantation of fenestrated or branched endografts is the procedure of choice in the treatment of thoracoabdominal aneurysms (Patel et al., 2009). However, inadequate sealing between stents and the aortic stent graft is not rare, leading to endoleaks, a complication with limited treatment options (O'Neill et al., 2006; Lee et al., 2007; Roselli et al., 2007; Hiramoto et al., 2009).

The multilayer stent was developed in the last decade, with a tridimensional geometry of overlapping layers and the purpose of modulating blood flow for use in the peripheral vascular system. Unlike conventional covered stent grafts, the multilayer stent is not covered and can be positioned over the origin of visceral branches without occluding them. Surgical planning is thus facilitated, and no specific techniques are required for the revascularization of visceral branches. For this reason, the multilayer stent was initially indicated in the treatment of peripheral aneurysms affecting collateral

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Abbreviations. AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; CETEC, Experimental and Surgical Training Center; CEUA, Ethics Committee on Animal Use; FAPESP, São Paulo Research Foundation; HIAE, Hospital Israelita Albert Einstein; SBCAL, Brazilian Animal Science Society; USP, Universidade de São Paulo; SBIBAE, Sociedade Beneficente Israelita Brasileira Albert Einstein.

vessels and is currently also indicated for complex abdominal and thoracoabdominal aortic aneurysms (Maynar et al., 2003). Its role is to create laminar blood flow within the stent and reduce pressure and velocity in the aneurysmal sac without excluding it from the circulation. Despite maintaining patency, the reduction in pressure and velocity inside the aneurysmal sac theoretically prevents its expansion, leading to a gradual reduction in its diameter with eventual thrombosis (Sincos et al., 2011). The multilayer stent theory suggests that eddies persist with the use of a monolayer stent; however, with a multilayer stent blood flow becomes laminar and eddies are eliminated (Monteiro et al., 2014).

As of 2009, the multilayer stent has been approved for use in the treatment of peripheral aneurysms and is currently available in Europe, Asia, and Latin America as an alternative for the treatment of complex aortic aneurysms (Chocron et al., 2011; Benjelloun et al., 2012; Natrella et al., 2012). Reports of its use in the treatment of thoracoabdominal aneurysms show a reduction, but not cessation, of blood flow in the aneurysmal sac. There are signs of a gradual reduction in aneurysm size. However, Lazaris et al. (2012) reported a case of a thoracoabdominal aneurysm treated with the multilayer stent that progressed with an increase in aortic diameter and rupture. Although this is a promising technique in the treatment of complex aortic aneurysms, the persistence of blood flow inside the aneurysmal sac may lead to its growth and rupture.

Therefore, this work aimed to analyze and compare, in a porcine model, the treatment of thoracoabdominal aneurysms with multilayer stents and its hemodynamic effects through the biomechanical and histological analysis of the aortic wall in contact with the stent.

Objectives

1 - To histologically assess the effects of myointimal hyperplasia and structural aspects of the aortic wall with and without a stent, such as the presence of thrombi on the intima.

2 - To analyze aortic wall biomechanics through vessel wall elasticity, strength, and thickness variables.

Materials and methods

Ethical aspects

The results of this research consist of an independent and strictly scientific analysis unassociated with the industry, which only donated the material for this study. Detailed development of the experimental swine model and the results obtained through angiography, intravascular ultrasound, and morphological analysis have been reported in a previously published work (Baptista-Strazzi et al., 2021).

It is registered at the Research Project Management System (SGPP) with No. 3312-18, CEUA No. 3312/2018, and was approved by the Ethics Committee on Animal Use of Sociedade Beneficente Israelita Brasileira Albert Einstein (CEUA/SBIBAE) on August 31, 2018.

The grant proposal was funded by the São Paulo Research Foundation (FAPESP), protocol No. 2016/03851-0. The Tecmedic company donated 10 multilayer stents for this study. The Vitoria Hospitalar company provided the intravascular ultrasound (IVUS) for this study.

This whole experiment was based on international ethical principles as stated by the Brazilian Animal Science Society (SBCAL), accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and with a standard anesthetic protocol as established by the Experimental and Surgical Training Center (CETEC) of Hospital Israelita Albert Einstein (HIAE).

The anesthetic protocol, analgesia, and postoperative protocol, along with the technique and fabrication of the aneurysm patch, were described by Baptista-Strazzi et al. (2021).

Experimental design

We used 16 Large White female pigs, aged 4 to 10 months, weighing between 37 kg and 75 kg, and obtained from Granja, São Paulo (SP). All animals underwent the creation of a suprarenal aneurysm with a bovine pericardial patch, similar to the technique published in 2003 by Maynar et al. (2003) and modified by Sincos et al. (2011), and were then divided into two groups with eight animals each. Animals in the Control group had a median age of 4 months (Q1-Q3, 4-4; min-max, 4-10) and a mean weight of 53.8 kg (SD, 15.5; min-max, 37-74). Animals in the Stent group had a median age of 4 months (Q1-Q3, 4-4; min-max, 4-5) and a mean weight of 47.5 kg (SD, 8.5; min-max, 40-63). The Control group was subjected only to the creation of a suprarenal aortic aneurysm but not to stent implantation. This group served as a baseline for morphological comparison considering animal growth and the hemodynamics of blood flow within the aneurysmal sac. In the Stent group, the suprarenal aortic aneurysm was created and the multilayer stent (Cardiatis, Isnes, Belgium, donated by Tecmedic) was implanted. For stent implantation, an ultrasound-guided puncture was performed in the right femoral artery, with insertion of a 5F sheath, followed by insertion of a standard 0.035'' hydrophilic guidewire, catheterizing to the suprarenal aorta, placement of a pigtail catheter for angiographic visualization, and then implantation of a multilayer stent 3 cm above the renal arteries. Not all stent lengths and diameters were available as they were donations, therefore, we chose to use stents covering the renal arteries and, in three animals, covering up to the superior mesenteric artery, along the inferior border to the infrarenal segment of the aorta. The stent was sized according to the proximal and distal diameters of the

healthy aorta.

The study was conducted between September 2017 and April 2019 at the CETEC of HIAE. Procedures were overseen by a team comprising a veterinarian, veterinary assistants, a medical team of surgeons with experience in animal surgery, and a surgical instrument technician.

All animals were followed up for four weeks and then subjected to control angiography with IVUS for the following assessments:

1) At arteriography: stent migration and renal artery patency, as well as the presence of endoleak. In the Stent group, there were no cases of migration or endoleak, and all animals had patent renal arteries. These results were presented in full in our previously published work (Baptista-Strazzi et al., 2021).

2) At IVUS: interaction between the stent and aortic wall, formation of mural thrombi, and aortic diameter measurements.

After endovascular analysis, four weeks later, animals were subjected to a xiphopubic transperitoneal laparotomy to explant the aorta-stent graft combination (or aorta with aneurysm only, in the Control group) and verify the morphological status and aortic wall biomechanics, along with histological analysis of thrombi and vessel walls, followed by euthanasia during the same intraoperative session.

Animal randomization, considering which would undergo multilayer stent implantation, was performed with a random draw (Control and Stent groups).

The animals underwent reintervention through angiography, control IVUS, and laparotomy with explant of the stent and aortic wall after four weeks. We assessed branch vessel patency, patch infection, or the presence of rupture or extravasation of the aneurysmal sac. The same anesthesia, medication, and examination protocols were employed during this procedure.

During the same intraoperative session, all animals were subjected to general anesthesia and euthanasia with potassium chloride. The material was then explanted and sent for histological and biomechanical analyses (Fig. 1).

Biomechanical analysis

Biomechanical analysis was performed on the same day of euthanasia, with a post-mortem interval of 1-3 hours accounting for the time to complete the procedure and to travel to the university's vascular laboratory. The method used for biomechanical analysis described below was developed jointly by researchers of the Vascular Biomechanics Laboratory of the Department of Surgery at Universidade de São Paulo (USP) Medical School and the Department of Bioengineering at the University of Iowa, USA. In 2001, the initial grant supported and funded by the National Science Foundation (USA) and FAPESP enabled a visit by the engineer Prof. Dr. Madhavan Lakshmi Raghavan to the Vascular Biomechanics Laboratory. In 2003, grant No. 0365408Z, funded by the American Heart Association, enabled the

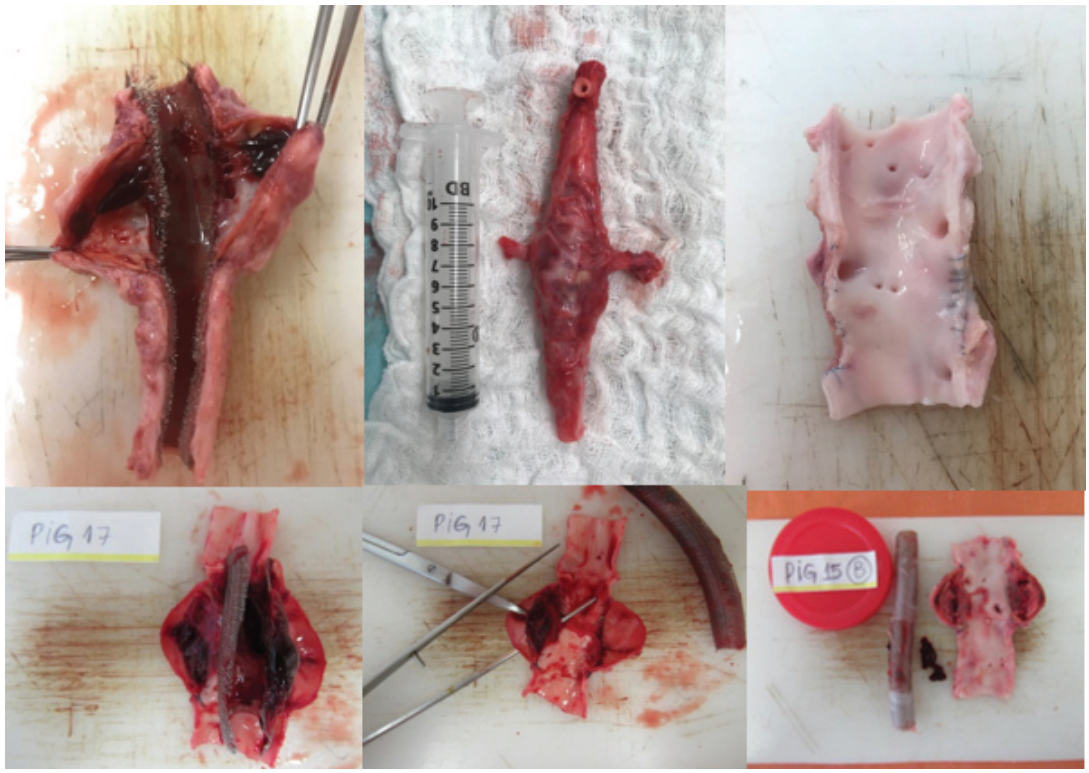

Fig. 1. Images of the explanted aorta with the stent. The size of the aortic segments taken for histology ranged (from 1.4 to 2.4 cm, 1.9 cm).

acquisition and installation of equipment required for destructive uniaxial tests using biological material.

In this experiment, each aorta was removed from the animal along with the stent and was taken to the Vascular Biomechanics Laboratory of the Department of Surgery at the USP Medical School. Careful bench dissection of the aorta was immediately performed, removing adipose and retroperitoneal tissue that was adhered to the aorta by the postoperative inflammatory reaction itself, without removing the adventitia. The specimen was then submerged in 0.9% sodium chloride at room temperature.

For sectioning the material, we idealized and constructed a cutting device with three parallel blades that, when applied to the aortic segment, produced two similar sectioned specimens that were 4 mm wide and had variable lengths according to the aortic diameters, ranging from 1.4 to 2.4 cm (mean, 2.4 cm). One specimen was referred to the biomechanical test and the other was submerged in 10% formaldehyde and sent for histological preparation.

Each specimen was then fixed in a grip system and placed on the In-Spec 2200 testing equipment (Instron Corporation, Norwood, USA), which was connected to a personal computer (PC, Compaq Presario 2500, Hewlett-Packard, Palo Alto, USA) and a palmtop (Clié, Sony, Japan). This universal equipment was constructed for performing destructive uniaxial tensile tests using the specimen as the workpiece. The whole operation involving the biomechanical test was computerized, being controlled by software on the PC and palmtop. The In-Spec PDA managing software on the palmtop received information on the length, width, and thickness of the test specimen. The PC, 21 through the SERIES IX data managing program (Instron Corporation, Norwood, USA), captured information provided by an electronic load cell at the uniaxial tensile testing machine, that is, the force applied to the specimen and the elongation to which it was subjected.

In the first phase of the biomechanical analysis, each workpiece was subjected to a pre-test to stabilize the material's mechanical behavior and reduce hysteresis, thus starting the test in similar conditions for all specimens. This pre-conditioning was performed through 10 cycles of elongation and relaxation, corresponding to an elongation of 5% of the length of the reduced section at a speed of 20% of the length of the reduced section per minute. This standardization was first described by Raghavan in 1996 (Monteiro et al., 2014). After the pre-test, the destructive biomechanical test was performed to failure, with a speed corresponding to 20% of the length of the reduced section per minute. Tests were not considered valid when the specimen slipped out of the grip or was ruptured less than 2 mm away from the grips. Length, width, and thickness were measured for each specimen. The maximum force applied before failure was measured to assess strength, while the maximum strain before failure was measured to assess elasticity.

Histological analysis

We collected three cross-sections of the following areas: the aorta proximal to the stent, the mid-portion in contact with the stent, and the distal aorta. All fragments underwent fixation with 10% buffered formaldehyde for 24 hours and were then processed and embedded in paraffin. Histological slides were stained with hematoxylin/eosin, Verhoeff's, Picrosirius, and Masson's trichrome stains for analyzing elastic and muscle fibers, collagen and fibrosis, inflammatory infiltrate, and neovascularization, respectively, with optical and polarized light microscopy (Monteiro et al., 2014).

Morphometric analyses were performed using a Quantimet 500 image analysis system (Leica Cambridge Ltd.) attached to an optical microscope at 20X magnification, along with a video camera (DONPISHA 3CCD). The main regions of interest were the media and intima layers, with emphasis on thicker areas and areas of alteration of normal histology. There were no artifacts that interfered with the measurements. The maximum intimal and medial thicknesses were measured in micrometers (μm). For the intimal layer, the mean thickness was obtained by measuring the average thickness at three different points of each segment of the aorta. The following special stains were performed for further qualitative analyses: Masson's trichrome, Verhoeff's, and Picrosirius. Masson's trichrome stain was used to detect areas of disruption of muscle fibers and collagen deposits. Picrosirius stain was used for the diagnosis of hyperplasia or intimal fibrosis. Verhoeff's stain was used to detect disruption of elastic fibers. Histological changes also included areas of accumulation of histiocytes, fibrin deposits, and hemorrhage.

The primary morphometric data of animal 16 (Control group) and animal 17 (Stent group) are provided as supplementary material for reference (Supplementary Tables S1 and S2, respectively).

Data analysis

Data are described as absolute frequencies and percentages for categorical variables and as means and SD or median, quartile, minimum, and maximum values for numerical values. The distributions of frequencies of numerical variables were verified using histograms, boxplots, and quartile distribution plots, in addition to Shapiro-Wilk tests.

We adjusted linear mixed models to assess the effects on measurements of the thickness of the aorta and intima, media, and adventitia layers. The models were adjusted considering the relationship between measurements obtained using different sections of the aorta of the same animal, and results were presented as mean estimated values and 95% confidence intervals.

For verifying associations between the study group and findings from histological assessments, we used Fisher's exact tests.

Analyses were performed using SPSS software and considering a 5% significance level.

Results

Histological analysis

Of 16 animals, five died before the four-week follow-up and were excluded from the analysis. The causes of death were acute abdominal obstruction on postoperative day 7 (n=1, Stent group), paraplegia in the immediate postoperative period (n=1, Control group), and refractory hypotension (n=3, Control group). Therefore, the histological analysis database had records of material belonging to 11 pigs: five from the Control group and six from the Stent group.

When comparing groups regarding aortic thickness measurements (Table 1), no differences were observed for point 1 (p=0.643), point 2 (p=0.658), point 3 (p=0.635), mean (p=0.753), or maximum values (p=0.722).

We also obtained the maximum thickness values for the intima, media, and adventitia layers in one or more sections of the animals' aortas. When comparing groups considering thickness, no differences were observed for the intima (p=0.526), media (p=0.129), or adventitia (p=0.662).

The characteristics of the aortas were analyzed on one or more sections of the same animal at the intima and media layers (Table 2).

We noticed evidence of differences between the Control and Stent groups regarding the presence of thrombi on the intima (p=0.048), where five (83.3%) animals in the Stent group presented thrombi in at least one section, including the intimal aortic wall in contact with the stent, whereas no animals presented thrombi in the Control group (Fig. 2). No evidence of differences was observed between groups regarding areas of intimal thickening (p>0.999).

When assessing the media layer, no evidence of differences was observed between the groups as to the presence of thrombi (p=0.076), injury (p=0.524), or calcification (p=0.400).

No evidence of significant associations was observed between the studied groups and findings regarding the disruption of collagen fibers according to the Picrosirius stain (p>0.999), disruption of elastic fibers according to Verhoeff's stain (p>0.999), and disruption of muscle fibers according to Masson's stain (p>0.999).

Biomechanical analysis

We found no differences between the Control and Stent groups regarding mean elasticity (p=0.158), strength (p=0.360), and thickness (p=0.323). In the Control group, animals had a mean elasticity of 0.86 (SD=0.22), whereas in the Stent group, it was 0.71 (SD=0.11). Mean strength was 480.00 N/cm^3 (SD=314.68 N/cm^3) in the Control group and 631.08 N/cm^3 (SD=131.07 N/cm^3) in the Stent group. Mean thickness values were 1.84 μm (SD=0.18 μm) and 2.02 μm (SD=0.35 μm) in the Control and Stent groups, respectively (Table 3).

Discussion

We designed a controlled experimental model with swine as they are excellent biomedical models owing to their anatomical, physiological, and immunological similarities to humans (Gertz et al., 1988; Hynecek et al., 2007; Czerski et al., 2013). Our database contained 16 animals, of which five died before the four-week follow-up and were excluded from the analysis. None of the deaths in either group was attributed to aneurysm rupture.

Even though the multilayer stent appears to be a promising alternative for the treatment of complex thoracoabdominal aneurysms, the high and notable morbidity of suprarenal aortic clamping along with the unprecedented nature of this experimental study led to an initial difficulty in maintaining the animals alive for four weeks. Therefore, we had to intensify monitoring and postoperative care, such as arterial blood gas tests before and after clamping and monitoring of mean arterial pressure in the carotid artery, with more

Table 1. Aortic thickness measurements of animals in each group.

Thickness, μm	Group		*p*-value
	Control (n=5)	Stent (n=6)	
Point 1 of the aorta	2175.2 (1570.1; 2780.3)	2344.6 (1873.8; 2815.4)	0.643
Point 2 of the aorta	2095.9 (1425.9; 2765.8)	1915.7 (1386.8; 2444.6)	0.658
Point 3 of the aorta	2111.3 (1313.7; 2908.9)	1879.4 (1235.5; 2523.2)	0.635
Mean value for the aorta	2149.1 (1653.2; 2645.1)	2054.0 (1654.6; 2453.4)	0.753
Maximum for the aorta	2660.1 (2080.5; 3401.2)	2804.2 (2315.6; 3395.9)	0.722
Maximum for the intima	113.3 (53.2; 241.4)	154.2 (77.8; 305.6)	0.526
Maximum for the media	1032.5 (760.6; 1304.4)	768.1 (545.7; 990.6)	0.129
Maximum for the adventitia	1874.6 (1251.5; 2497.6)	2041.5 (1538.4; 2544.5)	0.662

Estimated mean (95% confidence interval).

Table 2. Histological findings in the aortas of animals in each group.

Histological findings	Group, n (%)		*p*-value
	Control (n=5)	Stent (n=6)	
Thrombus on the intima			0.048
No	5 (100.0)	1 (16.7)	
Yes	0 (0.0)	5 (83.3)	
Area of intimal thickening			>0.999
No	3 (60.0)	3 (50.0)	
Yes	2 (40.0)	3 (50.0)	
Thrombus on the media			0.076
No	5 (100.0)	2 (33.3)	
Yes	0 (0.0)	4 (66.7)	
Injury on the media			0.524
No	4 (80.0)	2 (33.3)	
Yes	1 (20.0)	4 (66.7)	
Calcification on the media			0.400
No	4 (80.0)	6 (100.0)	
Yes	1 (20.0)	0 (0.00)	
PICRO – disruption of collagen fibers			>0.999
No	3 (60.0)	4 (66.7)	
Yes	2 (40.0)	2 (33.3)	
VERHOEFF – disruption of elastic fibers			>0.999
No	3 (60.0)	5 (83.3)	
Yes	2 (40.0)	1 (16.7)	
MASSON – disruption of muscle fibers			>0.999
No	3 (60.0)	4 (66.7)	
Yes	2 (40.0)	2 (33.3)	

p-value: Fisher's exact test. MASSON: Masson's stain; PICRO: Picrosirius stain; VERHOEFF: Verhoeff's stain.

hemodynamic control and better outcomes in the subsequent animals.

The duration of this study (four weeks) was chosen because it is sufficient to observe some degree of intimal hyperplasia and stent incorporation without the animal gaining too much weight and the aortic diameter significantly changing (Sincos et al., 2011; Ferrero et al., 2014). The Control group provided the baseline parameters for analyzing increases in the size of animals' aortas and aneurysms during the study and the impact of the stent on these parameters.

Table 3. Measurements obtained after four weeks at the euthanasia of animals subjected to aneurysm creation.

Measurements	Group		*p*-value
	Control (n=5)	Stent (n=6)	
Elasticity	No	Yes	0.158*
Mean (SD)	0.86 (0.22)	0.71 (0.11)	
Minimum; maximum	0.54 (1.15)	0.59 (0.87)	
Strength (N/cm3)			0.360*
Mean (SD)	480.00 (314.68)	631.08 (131.07)	
Minimum; maximum	178.80 (823.70)	451.90 (740.10)	
Thickness (μm)			0.323*
Mean (SD)	1.84 (0.18)	2.02 (0.35)	
Minimum; maximum	1.63 (2.07)	1.43 (2.49)	

*Student's t-test. SD: standard deviation.

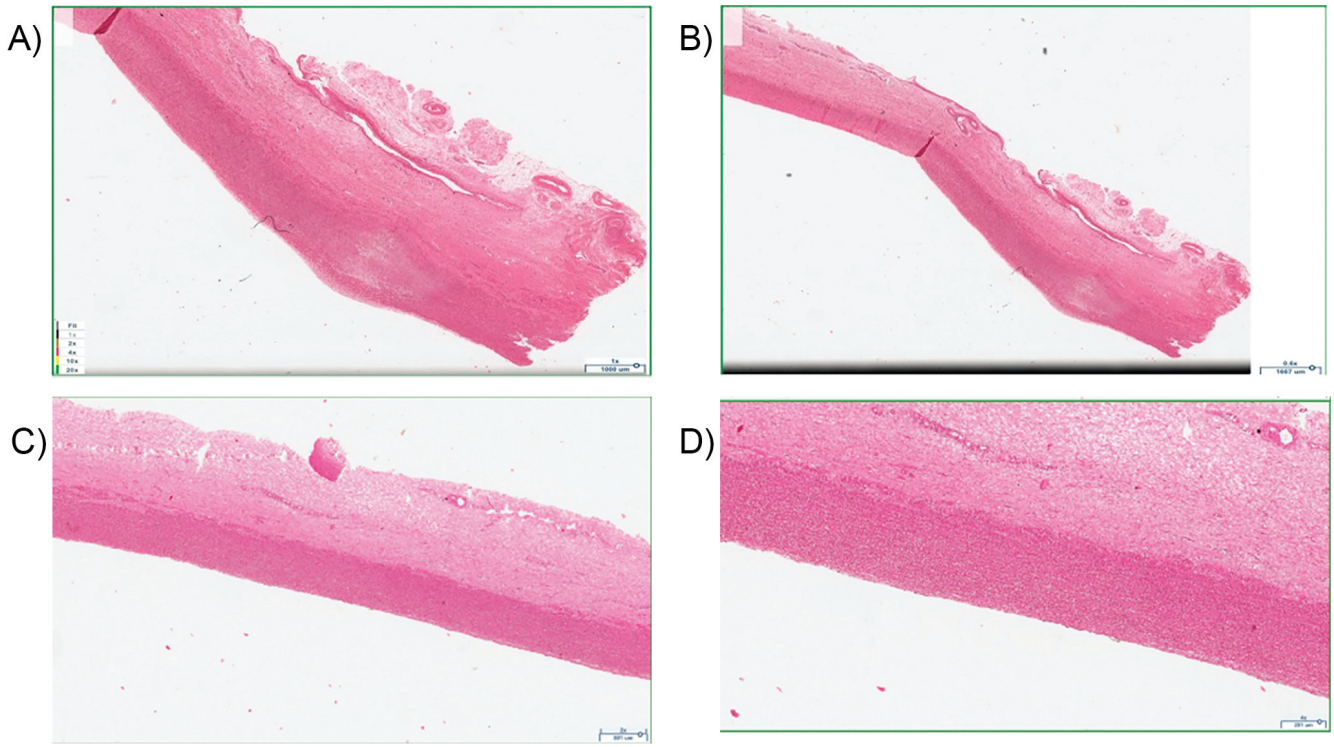

Fig. 2. A, B. Stent group. Micrographs of the intimal aortic wall showing the innermost layer of the wall, with the presence of thickening in the wall that was in contact with the stent (200x magnification). **C, D.** Control group. Micrographs of the intimal aortic wall showing the absence of thrombi. x 200.

The biomechanical analysis showed no statistically significant differences in the categories of elasticity, strength, and thickness. Regarding histological analysis, aortas in the Stent group presented more thrombi on the intima than those in the Control group, and there was no statistically significant difference between groups regarding intimal thickening, suggesting the same impact of the aneurysmal sac and stent on smooth muscle cells. We can speculate that the biomechanical alterations that could be related to the stability of the expansion of abdominal aortic aneurysms are thickness and stiffness, which are based on the remodeling of the wall of the aneurysmal sac at the expense of collagen deposition. The elastin/collagen ratio is relatively constant from the origin of the aorta to the renal arteries; however, in the infrarenal region, there is a drop in elastin content without a proportional decrease in collagen, leading to reduced compliance and increased stiffness in this region (Halloran et al., 1995; Cheuk and Cheng, 2005). This imbalance in the elastin/collagen ratio is a unique characteristic found in the infrarenal aorta and certainly plays a role in the genesis of atherosclerosis and aneurysms that typically manifest at this location. Elasticity would be an important factor, as a less elastic vessel wall would reduce the risk of aneurysm wall dilation and, consequently, the risk of rupture. However, because it is not possible to recover or replace lost elastic fibers, this variable is considered to be of no practical interest.

It is known that atherosclerotic lesions first develop in the abdominal aorta, with their progression producing atherosclerotic plaques mainly in the abdominal aorta. Extensive atherosclerotic lesions lead to dimensional changes in aortic diameter and length, which are not uniform throughout the aorta, resulting in an irregular, ectatic aorta with localized areas of aneurysmal dilatation, especially in its abdominal portion. In contrast, aortic medial degeneration can lead to dilatation, which is often most pronounced in the ascending aorta. Hypertension has also been associated with dilatation of the ascending aorta but not of other aortic segments, leading to accelerated atherosclerosis that results in dilatation and ectasia mainly of the abdominal segment. Compared with the remaining aortic segments, the specialized structural properties and architectural attributes of the lower abdominal aorta may set maladaptive restrictions to its function, considering that this aortic segment is very nearly avascular and has a thickness exceeding that allowed, beyond which it should have *vasa vasorum*. This leads to an improperly nourished lower abdominal aorta due to inadequate diffusion and plasma fluid flow from the lumen to supply nutrients to the entire wall thickness (Sokolis et al., 2002).

In our study, the biomechanical test was probably not significant for two reasons: (1) the short period of time of the stent in the aorta did not produce the chronic changes that would lead to changes in the tissue, with loss of elasticity and greater (or lesser) strength; and (2) the uniaxial tensile test may not be sensitive enough to detect subtle changes in tissue elasticity and strength. Although there was no statistically significant difference between the biomechanical parameters or in the area of intimal thickening, disruption of muscle fibers, collagen fibers, and elastic fibers, or injury to the media layer, we observed a tendency for porcine aortic tissue to have a more rigid wall, with greater strength and less elasticity in the Stent group vs. more elasticity and lower strength, and hence a less thick wall, in the Control group. This variation in structures of the inner and outer layers of the aortic wall, whether thoracic or abdominal, has been previously discussed in the study by Sokolis et al. (2002), in which morphometric analysis data showed a uniform peripheral reduction in the aortic diameter and medial thickness, with thickness changing proportionally to changes in diameter, leading to a very nearly constant ratio of thickness to diameter throughout the aorta's length. During growth, aging, or in response to increased wall stress, the tunica media would become nutritionally disadvantaged due to aortic wall thickening by further separating the smooth muscle cells from the lumen and exerting a major influence on the metabolism of the outer zone of the aortic wall (Sokolis et al., 2002). As suggested by the authors, similar studies could be performed with aortic specimens in pathological processes, such as aneurysms, to define the respective properties in these cases, as conducted in the present study.

As for limitations, our experimental study model was based on swine, which has physiological and anatomical similarities with humans and allows the use of endovascular devices with compatible diameters with the human aorta. However, the biological behavior of pig aortas is different from that of the human aorta, with greater intimal response in the presence of an aneurysmal sac with a bovine pericardial patch and stent, in addition to higher hypercoagulability, which makes intraoperative anticoagulation an essential aspect of the study. There are also anatomical variations such as the aortic trifurcation, producing right and left external iliac arteries as well as an internal iliac trunk, in addition to a difference in the number of renal arteries (Sincos et al., 2011). Another important context was the major initial procedure, with laparotomy and a xiphopubic incision exposing intestinal loops, post-clamping hypotension, and the absence of intensive postoperative support resources and infrastructure, which was one of the main limitations to guaranteeing animal survival for four weeks.

In conclusion, the histological study confirmed thrombosis of the aneurysmal sac through the presence of thrombi on the intima of the aorta in 100% of the animals in the Stent group. As for the biomechanical analysis, the study showed no statistically significant differences in vessel wall thickness, strength, and elasticity between groups.

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Conflict of Interest. The authors have no conflicts of interest to disclose.

References

Baptista-Strazzi A.P.W., Aun R., Sincos I.R., Tobita A.M., Portugal M.F.C., Paula V.P., Kaufmann O. and Wolosker N. (2021). Multilayer stents affect the final diameter of aortic aneurysms and maintain renal artery patency for a short time in a swine experimental model. Clinics 76, e2812.

Benjelloun A., Henry M., Ghannam A., Vaislic C., Azzouzi A., Maazouzi W., Khlifi A. and Benamor J. (2012). Endovascular treatment of a tuberculous thoracoabdominal aneurysm with the Multilayer stent. J. Endovasc. Ther. 19, 115-120.

Cheuk B.L. and Cheng S.W. (2005). Expression of integrin α5β1 and the relationship to collagen and elastin content in human suprarenal and infrarenal aortas. Vas. Endovascular Surg. 39, 245-251.

Chocron S., Vaislic C., Kaili D. and Bonneville J.F. (2011). Multilayer stents in the treatment of thoraco-abdominal residual type B dissection. Interact. Cardiovasc. Thorac. Surg. 12, 1057-1059.

Clouse W.D., Hallett Jr J.W., Schaff H.V., Gayari M.M., Ilstrup D.M. and Melton 3rd L.J. (1998). Improved prognosis of thoracic aortic aneurysms: a population-based study. JAMA 280, 1926-1929.

Crawford E.S. and DeNatale R.W. (1986). Thoracoabdominal aortic aneurysm: Observations regarding the natural course of the disease. J. Vasc. Surg. 3, 578-582.

Czerski A., Bujok J., Gnus J., Hauzer W., Ratajczak K., Nowak M., Janeczek M., Zawadzki W., Witkiewicz W. and Rusiecka A. (2013). Experimental methods of abdominal aortic aneurysm creation in swine as a large animal model. J. Physiol. Pharmacol. 64, 185-192.

Ferrero E., Gibello L., Ferri M., Viazzo A. and Nessi F. (2014). Aortic arch rupture after multiple multilayer stent treatment of a thoracoabdominal aneurysm. J. Vasc. Surg. 60, 1348-1352.

Gertz S.D., Kurgan A. and Eisenberg D. (1988). Aneurysm of the rabbit common carotid artery induced by periarterial application of calcium chloride *in vivo*. J. Clin. Invest. 81, 649-656.

Gillum R.F. (1995). Epidemiology of aortic aneurysm in the United States. J. Clin. Epidemiol. 48, 1289-1298.

Halloran B.G., Davis V.A., McManus B.M., Lynch T.G. and Baxter B.T. (1995). Localization of aortic disease is associated with intrinsic differences in aortic structure. J. Surg. Res. 59, 17-22.

Hiramoto J.S., Chang C.K., Reilly L.M., Schneider D.B., Rapp J.H. and Chuter T.A. (2009). Outcome of renal stenting for renal artery coverage during endovascular aortic aneurysm repair. J. Vasc. Surg. 49, 1100-1106.

Hynecek R.L., DeRubertis B.G., Trocciola S.M., Zhang H., Prince M.R., Ennis T.L., Kent K.C. and Faries P.L. (2007). The creation of an infrarenal aneurysm within the native abdominal aorta of swine. Surgery 142, 143-149.

Lazaris A.M., Maheras A.N. and Vasdekis S.N. (2012). A multilayer stent in the aorta may not seal the aneurysm, thereby leading to rupture. J. Vasc. Surg. 56, 829-831.

Lee W.A., Brown M.P., Martin T.D., Seeger J.M. and Huber T.S. (2007). Early results after staged hybrid repair of thoracoabdominal aortic aneurysms. J. Am. Coll. Surg. 205, 420-431.

Maynar M., Qian Z., Hernandez J., Sun F., DeMiguel C., Crisostomo V., Usón J., Pineda L.F., Espinoza C.G. and Castañeda W.R. (2003). An animal model of abdominal aortic aneurysm created with peritoneal patch: technique and initial results. Cardiovasc. Intervent. Radiol. 26, 168-176.

Monteiro J.A.T., da Silva E.S., Raghavan M.L., Puech-Leão P., Higuchi M.L. and Otoch J.P. (2014). Histologic, histochemical, and biomechanical properties of fragments isolated from the anterior wall of abdominal aortic aneurysms. J. Vasc. Surg. 59, 1393-401.e2.

Natrella M., Castagnola M., Navarretta F., Cristoferi M., Fanelli G., Meloni T. and Peinetti F. (2012). Treatment of juxtarenal aortic aneurysm with the multilayer stent. J. Endovasc. Ther. 19, 121-124.

O'Neill S., Greenberg R.K., Haddad F., Resch T., Sereika J. and Katz E. (2006). A prospective analysis of fenestrated endovascular grafting: intermediate-term outcomes. Eur. J. Vasc. Endovasc. Surg. 32, 115-123.

Patel R., Conrad M.F., Paruchuri V., Kwolek C.J., Chung T.K. and Cambria R.P. (2009). Thoracoabdominal aneurysm repair: hybrid *versus* open repair. J. Vasc. Surg. 50, 15-22.

Reilly J.M. and Tilson M.D. (1989). Incidence and etiology of abdominal aortic aneurysms. Surg. Clin. North. Am. 69, 705-711.

Roselli E.E., Greenberg R.K., Pfaff K., Francis C., Svensson L.G. and Lytle B.W. (2007). Endovascular treatment of thoracoabdominal aortic aneurysms. J. Thorac. Cardiovasc. Surg. 133, 1474-1482.

Sakalihasan N., Limet R. and Defawe O.D. (2005). Abdominal aortic aneurysm. Lancet. 365, 1577-1589.

Sincos I.R., Aun R., da Silva E.S., Belczak S., Higuchi M.L., Gornati V.C., Gigglio P.N., Baptista A.P. and de Figueiredo L.F. (2011). Impact of stent-graft oversizing on the thoracic aorta: experimental study in a porcine model. J. Endovasc. Ther. 18, 576-584.

Sokolis D.P., Boudoulas H., Kavantzas N.G., Kostomitsopoulos N., Agapitos E.V. and Karayannacos P.E. (2002). A morphometric study of the structural characteristics of the aorta in pigs using an image analysis method. Anat. Histol. Embryol. 31, 21-30.

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