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Histopathological reaction of the abdominal aorta wall to non-covered stents

Reação histopatológica da parede da aorta abdominal ao stent não recoberto

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Abstract

Objective: **To evaluate the histopathological reaction of the abdominal aorta wall in pigs' renal arteries to the presence of non-covered stainless steel stents.**

Methods: **The abdominal aorta of ten pigs (6 months old and weighing 86.6 kg on average) was histopathologically studied 100 days after the implant of stainless steel stents in the abdominal aorta, with one segment of the stent implanted in the renal artery. Self-expanding non-covered stents were released by laparotomy. The histological slices were made at the transition from the normal aorta and the aorta containing the stent; the aorta portion containing the stent; the portion with the ostia of renal arteries; periaortic lymph nodes and renal parenchyma. The samples were stained by the hematoxylin and eosin technique.**

Results: **Macroscopic findings showed periaortic lymphadenopathy, thickened aortic wall, patency of lumbar and renal arteries and normal renal anatomical structure. Microscopic analyses near the stents revealed thickening of vessel wall secondary to intima fibrosis, and media layer affected by interstitial fibrosis. Micrometric measurements of aorta wall with the stent, compared to the aortic portion without it, presented a 75.90% increase in the total thickness of the wall by thickening of the intima layer secondary to fibroblast proliferation, collagen deposits with lymphocytary inflammatory infiltrate and foreign body-type granulomas.**

Conclusion: **The non-covered stainless steel stents in pigs' aortas produced a significant inflammatory reaction with fibrosis in the media and intima layers evidenced by histopathological analyses; their presence did not interfere in the patency of the abdominal aorta or the renal and lumbar arteries.**

Descriptors: **Aorta, abdominal, surgery. Stents. Foreign-body reaction.**

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Resumo

Objetivo: **Avaliar a reação histopatológica da parede aorta abdominal, em suínos, no nível das artérias renais, na presença de *stent* metálico não recoberto.**

Método: **Foi estudada histopatologicamente a aorta abdominal de 10 suínos, com peso médio de 86,6 quilos e idade média de 6 meses, submetidos a implante de *stent* metálico posicionado na aorta, no nível das artérias renais, após 100 dias do implante. Os *stent*s foram liberados por auto-expansão com laparotomia. Os cortes histológicos foram realizados nos seguintes locais: 1) transição entre a aorta normal e aorta contendo *stent*; 2) aorta contendo o *stent*; 3) porção contendo os óstios das artérias renais, 4) linfonodos periaórticos e, 5) parênquima renal. As lâminas foram coradas pela técnica da hematoxilina e eosina.**

Resultados: **Os achados macroscópicos revelaram: linfonodomegalia periaórtica; espessamento da parede aórtica; artérias lombares e renais pérvias; estrutura anatômica renal normal. Análises microscópicas, próximas aos *stent*s, evidenciaram espessamento da parede vascular, secundário à fibrose intimal e camada média comprometida com fibrose intersticial. Medidas micrométricas da parede aórtica com o *stent*, comparada à aorta sem o *stent*, apresentaram aumento da espessura da parede (75,9%) por hiperplasia da camada íntima secundária à proliferação de fibroblastos; depósitos de colágeno com infiltrado inflamatório e granulomas do tipo corpo estranho.**

Conclusão: **O *stent* de aço inoxidável descoberto, implantado na aorta de suínos, produziu importante reação inflamatória, com fibrose nas camadas média e íntima, evidenciada pelas análises histopatológicas e a sua presença não comprometeu o estado pérvio da aorta e dos ramos lombares e renais.**

Descritores: **Aorta abdominal, cirurgia. Contenedores. Reação a corpo estranho.**

INTRODUCTION

An introduction to stents in the clinical practice by Parodi in 1995 [1] as an alternative treatment for abdominal aorta aneurysms, created new perspectives for the treatment of aneurysms and dissections in varying sections of aortas. Dake et al. [2] were the pioneers to use the stent in the thoracic aorta. Since then, several other groups have reported success in the treatment of different pathological conditions of the aorta with different types of endoprostheses, thereby reducing morbidity and mortality rates in specific groups of patients [3-7].

Stainless steel stents covered with polyester to treat type-B aortic dissections were first used by the cardiovascular surgery group of the Federal University of São Paulo (UNIFESP) as an alternative to elephant-trunk endoprostheses [3,4,8,9]. Recently, this group reported the success and complications with this type of prosthesis. Among the most common complications are occlusion of the left subclavian artery, which can be solved by a simple surgery utilizing tubes to connect the left carotid artery to the left subclavian artery and fever not related to an infectious process but probably due to an inflammatory reaction produced by the stent [9].

In a very interesting experimental and original study by Paula et al. [10], the authors verified the perfect adherence of the dacron prostheses mounted using metallic wire, with concomitant neointimal proliferation in the aorta of dogs demonstrating that the prosthesis implantation procedure in the thoracic aorta was feasible and that its displacement is very unlikely although possible if the prosthesis were not the perfect size for the diameter of the aorta.

Although the majority of available publications on intravascular procedures include studies on the coronary arteries, the mechanisms of neointimal formation in other human arteries such as the iliac arteries and the aorta, are considered to be similar [1]. Studies have shown, in the aorta and in other arteries, signs of inflammatory reaction related to neointimal hyperplasia with complications, specifically restenosis after the placement of the stent, in both humans and animal models.

The aim of the current study was to evaluate the histological reactions of the aorta caused by non-covered steel stents placed in the abdominal artery of pigs at the junction with the renal arteries.

METHOD

The experiments were performed in the Veterinary Hospital of the Santa Catarina State University in the city of Lages. The project was approved by the Animal Experimentation Ethics Committee of the Paulista Medical School – UNIFESP.

Ten pigs (F1 crossbreeds between Landrace and Large White) from the pig breeding farm of the Veterinary Agro Center (CAV) with a mean weight of 86.6 ± 2.44 kg and a

mean age of 6.35 ± 0.15 months in good conditions of health were included in the study. The animals were submitted to 12 hours of fasting before the surgical procedures.

All the animals were pre-medicated with an intramuscular injection of a mixture of 0.025 mg/kg atropine and, at 10-minute intervals, 1 mg/kg xylazine. After sedation, using a catheter, venoclysis of the marginal artery of the ear was performed for the administration of anesthetic drugs and hydration using a glucose saline solution (5 mL/kg/h). After induction, 10% vaporized lidocaine was utilized to numb the vocal cords and the larynx, followed by intubation with an endotracheal tube using a low-pressure balloon depending on the diameter of the trachea. After appropriate intubation and inhaled anesthesia with halotan, a minimum alveolar concentration of 1.5 was maintained in a semi-closed circuit with an oxygen flow of 40 mL/kg. Following this mechanical ventilatory support was established using a volume of 15 milliliters per kilogram weight. The following parameters were evaluated during the anesthesia procedure: heartbeat, respiration rate, systolic and diastolic arterial pressures by non-evasive monitoring and esophageal temperature.

Using transmesenteric dissection, the abdominal aorta was identified at the junction with the renal arteries and a 0.032 or 0.035 thick 'very-hard' guide wire was introduced by puncturing the vessel. Self-expanding non-covered aortic stent systems were used with 16-, 18- or 20-millimeter polytetrafluoroethylene (PTFE) catheters (Figure 1) depending on the diameter of the aorta estimated by using a pachymeter. After the introduction of the guide wire, the aortic stent was released into the abdominal aorta, positioned at the renal arteries by indirect measurements and identified by touch (Figure 2). With a conventional ultrasound apparatus, the position of the aortic stent was confirmed with the transducer placed directly on the aorta where the stent had been released.

After stent placement, the perforation was closed using a previously prepared purse-string suture made of 4-0 propylene thread without the use of vascular clamps and or any type of systemic coagulation. The abdominal cavity was then closed by layers without the use of drains. In the postoperative period, an association of antibiotics based on penicillin and streptomycin were administered at 24-hour intervals together with analgesia based on cetoprofene at a dose of 0.1 mg/kg for three days. Using routine care related to feeding and hygiene, the animals were kept for 100 days when they were slaughtered in a commercial abattoir inspected by government health inspectors. After a macroscopic examination the kidneys and aorta were placed into a thermal bag with ice and sent for anatomopathological and histologic studies.

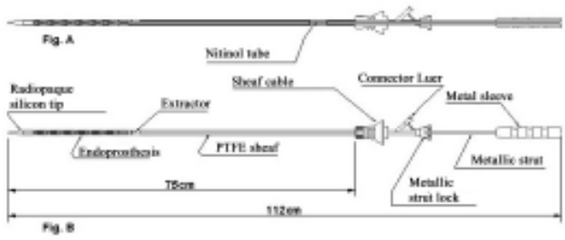

Fig. 1 – Diagram of the delivery device of the self-expanding aortic stent - A. Lengthwise section of the delivery device, showing the nitinol tube in which the device is mounted and through which a 0.035 mm or 0.032 mm extra-hard guide wire is passed. B. Diagram showing each component of the delivery device with a PTFE sheaf and a radiopaque silicone tip to facilitate the delivery and to protect the intima during the progression of the system within the aorta. The functional length of the sheaf is 75 cm.

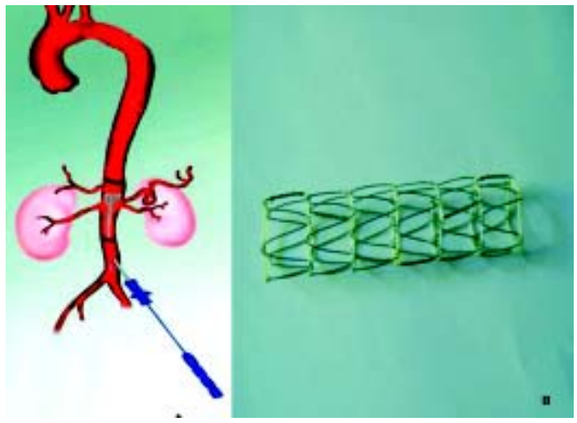

Fig. 2 – A. Diagram representative of the delivery and release of the stent - showing release of the stent close to the renal arteries B. expanded uncovered stent.

RESULTS

Macroscopy

The anatomopathological and histologic analyses of the aorta of the animals revealed significant periaortic lymphadenomegaly limited to the region of the stent (Figure 3).

In Figure 4, the segment of the aorta in which the stent was placed after being opened lengthwise is illustrated. In these specimens the transition from the normal aortic wall to the wall around the stent can be observed. The structure of the stent is completely covered by a thin layer of tissue with thickening in the regions close to the metallic supports of the stent.

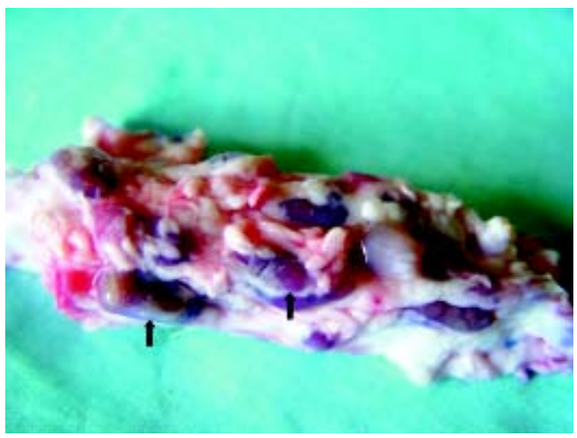

Fig. 3 – Periaortic lymphadenomegaly limited to the region of the stent – The arrows show the enlarged lymph nodes.

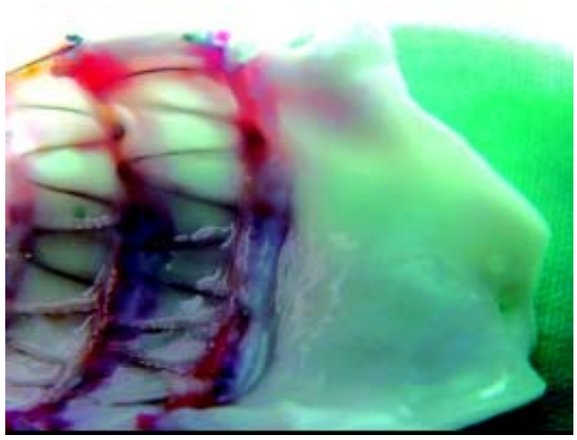

Fig. 4 – Lengthwise section of the aorta in which the transition between the normal aortic wall and the region thickened by the inflammatory reactions induced by the stent can be observed.

Note in Figure 5, the structure of the stent internally positioned close to the renal artery without signs of obstruction, both in the lumen of the renal artery or the aorta. Also note the hardened aspect of the aortic wall.

On macroscopic examination, the preserved renal structures can be observed without signs of embolism or ischemic damage in any of the pigs.

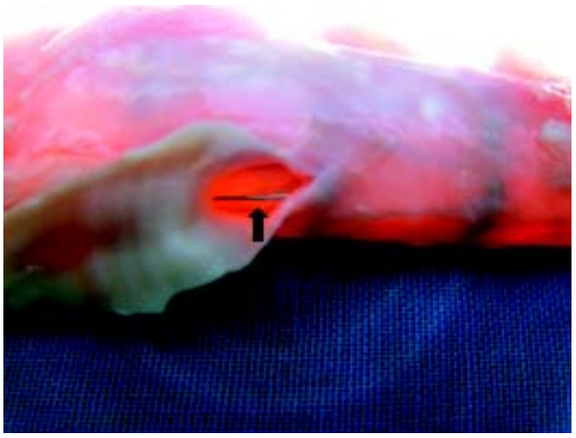

Fig. 5 – External view of the abdominal aorta with one metallic strut of the stent (arrows), over the ostium of the renal artery but without signs of obstruction and view of the hardened aortic wall.

Microscopy

Figure 6 shows the lengthwise sectioning of the aorta stained with hematoxylin and eosin at the transition between the normal distal segment and the gradual thickening of the intimal layer near to the stent showing intimal hyperplasia due to the inflammatory reaction induced by the stainless steel stent.

Additionally, the wall of the aorta with stent when compared to the aorta without stent presents a mean increase in thickness of 75.9%. The mean size of the aortic wall with stent was 3.87 mm and the mean size of the aortic wall without stent was 2.20 mm (Figure 7 and Table 1).

In Figure 8, the thickness of the aortic wall caused by the formation of the neointimal layer around the metallic supports and a polyester thread (utilized to unite the metallic struts) of the stent can be evidenced. Around the cavity created by the metallic wire, there is a pronounced thickening of the intimal layer secondary to the proliferation of fibroblasts and the deposit of collagen. It is also possible to identify accumulations of lymphocytic inflammatory infiltration and foreign body-type granulomas (Black arrow). Moreover, fibrotic proliferation with collagen deposits and moderate lymphocytic inflammatory infiltration were seen around the stent.

The aspect of the renal artery in the region near to the stent at the junction of the aorta revealed a histologically normal pattern, without hyperplasia. Finally, the renal parenchyma was histologically normal without signs of embolic or ischemic events.

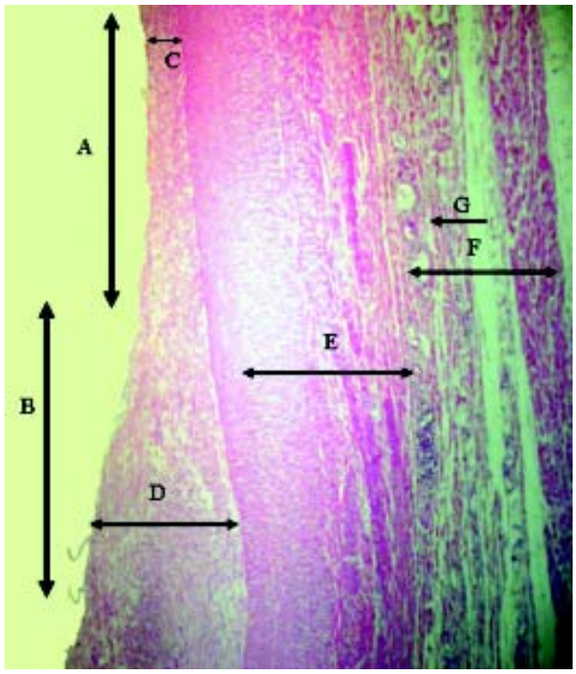

Fig. 6 – Lengthwise histologic section of the transition between the normal segment of the aorta (arrow A) and the intermediate segment before the start of the stent (arrow B) in the aorta of pigs: A and B correspond to the lumen of the artery; C – the intimal layer with normal thickness (endothelium, loose conjunctive tissue and internal elastic); D – thickened intimal layer (end closest to the stent) with accumulation of fibroblasts and collagen; E – Medial layer (muscle) containing the smooth muscle cells; F – adventitial layer containing the adipose cells, loose conjunctive tissue and vasa vasorum; G – vasa vasorum (100x HE).

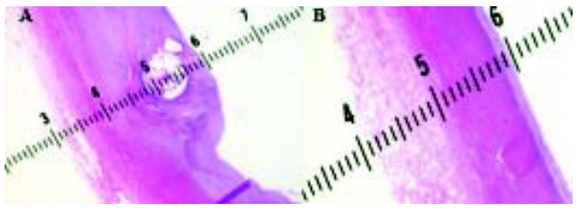

Fig. 7 – Lengthwise section of the aorta of pig Nº 7 at the site where the stent was placed showing the micrometric measurement of the thickness of the wall (3.0 mm) (10x HE) B. Histologic section of the aorta of pig Nº 7 at a location without the stent showing the micrometric measurement of the thickness of the wall (1.9 mm) (10x HE).

Table 1. Mean size of the aortic wall with and without stent

Nº	Pig	without Stent	with Stent	% increase
1	33 days	2.4 mm	2.9 mm	20.83
2	100 days	3.5 mm	6.0 mm	71.42
3	100 days	2.4 mm	4.2 mm	75.00
4	100 days	2.0 mm	3.6 mm	80.00
5	100 days	2.0 mm	4.8 mm	140.00
6	100 days	2.2 mm	4.7 mm	113.00
7	100 days	2.1 mm	3.0 mm	42.85
8	100 days	1.9 mm	3.0 mm	57.89
9	100 days	1.9 mm	3.4 mm	78.94
10	100 days	1.6 mm	3.1 mm	93.73
	Mean	2.20 mm	3.87 mm	75.90

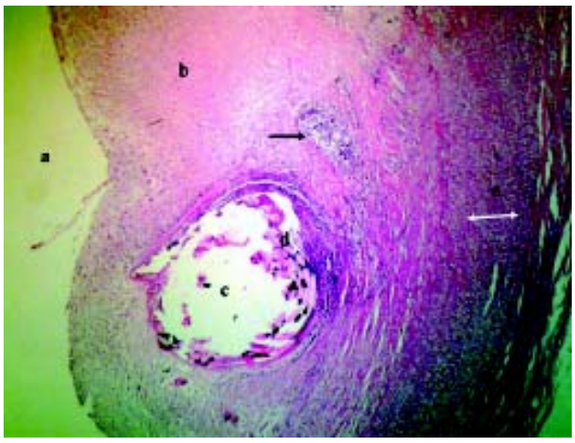

Fig. 8 - Lengthwise section of the aorta of a pig with concentration of inflammatory cells: a) aortic lumen; b) Thickened neointima with concentration of collagen and fibroblasts; c) cavity caused by the stent together with polyester fibers; d) polyester fibers; e) slightly compressed media muscle layer (white arrow); adventitial layer. A. Black arrow showing concentration of inflammatory cells (200x HE).

DISCUSSION

Experimental studies have demonstrated that the arterial wall presents a multifactorial response to mechanical lesions, denominated intimal hyperplasia. Topographically, this

response occurs mainly in the intima tunica and is characterized by cellular proliferation and intimal thickening that may result in a significant reduction in the lumen of the vessel [12-14]. Intimal hyperplasia is a relatively common complication after endovascular procedures and is considered the principal cause of restenosis after patients have been submitted to stent implantation as another important component, geometric remodeling, is minimized by the intravascular device [15-19].

Grüdtner et al. [20] evaluated morphological changes in the aortas of pigs submitted to the implantation of covered and non-covered stents compared to a Control Group. The authors did not find statistically significant differences among the groups in respect to the intimal thickness of the arteries between the proximal and distal segments of the stent. In another recent study using a pig model of atherosclerosis, Castaneda et al. [21] did not find significant differences in the thicknesses of the intima and media layers of the proximal and distal segments of the aorta and iliac arteries submitted to implantation of nitinol stents covered with polyester. Anyway, it seems, over the short term, the intimal reaction secondary to the implantation of stents in large caliber vessels and in high-flow situations does not extend much farther than the edges of the native artery.

In our study, we identified significant periaortic lymphadenomegaly limited to the region where the stent was placed (Figure 3), indicating a local inflammatory reaction. At histologic examination, these ganglions present with mixed reactive hyperplasia (follicular and sinusoidal) reinforcing the macroscopic findings of inflammatory reaction.

We made a detailed evaluation of the histological reaction produced by the stainless steel stent mounted using polyester thread in the aorta. We observed that a significant inflammatory reaction occurs in the region of the stent (Figure 8). Around the cavity created by the metallic wire, we observed a pronounced thickening of the intimal layer secondary to the proliferation of fibroblasts and deposits of collagen (Figures 6 and 8). Also, an accumulation of lymphocytic inflammatory infiltration is visible, as are foreign body-type granulomas (Figure 8). Fibrotic proliferation associated with the deposit of collagen and moderate lymphocytic inflammatory infiltration was also noted around the stent (Figure 8). With these findings, we proved there was significant neointimal hyperplasia secondary to an intense inflammatory response.

Grüdtner et al. [20], in their aforementioned work, demonstrated in a macroscopic analysis that the aortas were patent and the stents were firmly anchored to the artery wall. Moreover, a thin covering of translucent tissue was observed at the ends and the central region of the stents and on the PTFE which covered the stents. In the central region of the stents, the translucent lining of tissue did not appear to be continuous.

In this study, the aortic specimens analysed by lengthwise sectioning, showed that the stents were patent (Figure 5) and the stents were firmly adhered to the arterial wall (Figures 4 and 5). Additionally, a thin translucent lining was observed over the ends of the stents. In the transitional area between the metallic struts of the stents, the translucent lining was apparently not continuous. These findings are similar to those reported by Grüdtner et al [20]. We also clearly saw that the structural changes in the aortic wall were restricted to the region in which the stent was anchored (Figures 4 and 6). This evidence was confirmed by the histological analysis that plainly showed the transition between the normal endothelium and the intimal hyperplasia limited to the region of the stent (Figure 6).

Furthermore, we observed by microscopy the patency of the renal and lumbar arteries (Figure 5). Histological analysis of the renal arteries did not highlight any abnormality, proving that the histological alterations are really located only in the region of the stent. We also found, the renal structure without signs of embolic involvement at macroscopic analysis which was confirmed in detail by the microscopic assessment of the organ.

Our findings unmistakably showed that the uncovered stainless steel stent implanted in the aorta of pigs causes a significant inflammatory reaction evidenced by macroscopic analysis and confirmed by histological examination. This type of stent induces an intimal hyperplasia secondarily to the inflammatory response limited to the region of the stent, making the aortic wall thicker and more rigid due to the secondary fibrosis at this point. However, these changes do not interfere in the patency of the aorta, nor its lumbar and renal branches.

Considering among the etiologies of aortic aneurisms and dissections, Marfan's syndrome, which is a disease of the conjunctive tissue where the wall of the aorta suffers an important destructuring of the microfibrils [22-25] making it more prone to dilations and dissections, we can infer that these inflammatory reactions, the thickening of the aortic wall induced by non-covered metallic stents, may protect the aortic structure. Hence, we suggest that a line of new research using uncovered metallic stents in patients with Marfan's syndrome may change the tragic evolution of many of these patients.

The results of this study may also help to answer, at least in part, the questions raised in the literature, as well as other situations described by Albuquerque et al. [26] concerning the routine implantation of stents and the role of endoprosthesis implantation in the descending aorta during surgeries performed on the ascending aorta. According to our results, the implantation of the

'prophylactic stent' might increase the vascular rigidity and prevent catastrophic events over the medium and long terms.

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