6 – ORIGINAL ARTICLE

Digital morphometric analysis of the aortic wall in pigs following implantation of dacron-covered stents *versus* non-covered stents[1]

Análise morfométrica digital na parede aórtica de suínos após implante de s*tents* revestidos com dacron *versus stents* não revestidos

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ABSTRACT

PURPOSE: To evaluate, by digital morphometry, the intimal thickening after the placement of two different kinds of stents: polyester covered stainless steel stents (Dacron) and non-covered stents implanted in young pigs' infrarenal aortas.
METHODS: The experiment was carried out on two separate groups of pigs. Eight polyester-covered stainless steel stents (Dacron) and eight stainless steel stents (30-mm long, 8-mm diameter) were deployed through extraperitoneal approach in the normal infrarenal aorta of 16 normolipemic pigs. To allow the passage of the delivery system, a small arteriotomy was performed (phase I). After four weeks, the aorta with the *stent* was removed *en bloc*. (phase II). The values of the hematimetric and lipid analysis did not show any changes that could influence the study. Tissue samples of the fixing sites (proximal and distal) of the *stents* were taken. Microscope slices were prepared and submitted to Verhoeff's hematoxilin and eosin techniques and sent to morphometric analysis.
RESULTS: The intima immediately proximal to the device was thicker in the group of covered *stents* with marginal significance (p=0.054). The distal intimal layer (p=0.185), proximal medial layer of the proximal portion (p=0.141) and distal portion (p=0.375) did not present statistical difference between the two groups.
CONCLUSIONS: Patency was 100% in both groups of these normolipemic pigs. After four weeks the intimal layer immediately proximal to the covered stents was ticker when compared to uncovered stents, but this had a borderline significance. The intimal layer distal to covered stents and the media proximal or distal to the devices had similar morphometric features when covered and uncovered *stents* where compared.

Key words: Intimal thickness. Stainless steel stents. Dacron. Morphometric analysis.

RESUMO

OBJETIVO: Avaliar através de morfometria digital o espessamento intimal após o implante de dois tipos de *stents* de aço inoxidável, um recoberto com poliéster (dacron) e outro não recoberto, implantados na aorta infra-renal de suínos jovens.

MÉTODOS: O experimento foi realizado em dois grupos (*stents* não revestidos e revestidos com dacron) e duas fases (I e II). Oito *stents* recobertos com dacron e oito *stents* de aço inoxidável (30mm de extensão e 8 mm de diâmetro), não revestidos, foram implantados através de abordagem retroperitoneal na aorta infrarenal normal de 16 suínos normolipêmicos. Para a passagem do sistema de implante, foi necessário uma pequena arteriotomia na aorta distal (fase I). Após quatro semanas, a aorta com os *stents* foram removidas em monoblocos (fase II). Os valores de hematimetria e do lipidograma foram coletados nas duas fases e não apresentaram alterações que pudessem influenciar o estudo. Amostras de tecido dos sítios de fixação (proximal e distal) dos *stents* foram retiradas, confeccionadas lâminas, que foram coradas pelas técnicas de hematoxilina e eosina e de Verhoeff e enviadas para análise morfométrica digital.
RESULTADOS:A camada íntima proximal ao *stent* apresentou maior espessamento intimal no grupo de *stents* recobertos, mas com significância limítrofe(p=0.054). A camada íntima distal(p=0.185), a camada média das regiões proximal(p=0.141) e da porção distal (p=0.375) ao *stent* não apresentaram diferença entre os grupos.
CONCLUSÕES: Os dois tipos de *stents* apresentaram 100% de perviedade, boa biocompatibilidade e boa incorporação a parede aórtica de suínos normolipêmicos. A camada íntima proximal do grupo de stents revestidos com dacron apresentou maior espessamentodo que os *stents* não revestidos, mas com significância estatísitica limítrofe.

Descritores: Espessamento intimal. S*tents*. Dacron. Análise morfométrica digital.

Introduction

Regular *stents* can be constructed with an uncovered metal mesh, which can be steel, nitinol, tântalo or algiloy. They have been used to dilate arterial stenoses, or for fixation of atheroma plaques following angioplasty[1]. Stents can be covered with autogenous tissue, such as the saphenous vein, or synthetic material like expanded polytetrafluoroethylene (PTFEe) and polyethylene tereftelate (Dacron)[2,3,4]. Covered *stents* can be used in the treatment of traumatic vascular injuries, pseudoaneurysms, arteriovenous fistulas, aortic dissection or degenerative aneurysms [5,6,7].
An advantage of using a covered stent in occlusive arterial diseases is that the synthetic tissue can form a barrier excluding the ruptured wall from the vascular lumen. Another feature is that this barrier could prevent miointimal cell proliferation within the metallic mesh. In the other hand , some disadvantages can be named as the foreign body effect in the vascular lumen, higher cost, and the need of a delivery system of higher profile. [3,8,9].

Although stents seems to prevent the elastic retraction by means of positive geometric remodeling, they are not innocuous to the arterial system. Stents can be related to thrombi formation, higher inflammatory reaction and neointima tickening due to the fact that the endothelial lesion exposes the subintimal elements, causing a cicatricial answer to injury [10].

The most important difference between *stents* covered with polymeric materials and non-covered *stents* concerns their likehood of being incorporated by the vascular wall.

Naked *stents* are more likely to endotelize than when they are covered with polymers. These properties probably determine a stable binding to proteins when exposed to blood flow, followed by cells fixation onto the metallic mesh of the stents. The high energy found in the metal surface does not explain such interactions. The

electrical load on the surface, the chemical features, and the texture are most likely involved[11].

When compared to non-covered stents, covered stents are less biocompatible. There is some concordance between the behavior of Dacron and PTFE prostheses since they allow endothelization restricted to the perianastomotic region. In contrast, uncovered stents permit full endothelization, a feature that have been observed in several experimental studies carried out with baboons, dogs, pigs, sheep and veal[12].

The hypothesis in our study was that *stents* covered with extra-thin Dacron induces a reative intimal thickening greater than non-covered *stents.*

Methods

This study was approved by the Ethical Committee for the Post-Graduation Groups at the Clinical Hospital in Porto Alegre. The experiment was carried out at the facilities of the Animal Room in the University of Caxias do Sul and the digital morphometric analysis was performed at the Digital Medical Laboratory in Porto Alegre.

Stents were manufactured in 316L-series steel by the "Laboratório de Transformação Mecânica da Escola de Engenharia da Universidade Federal do Rio Grande do Sul, based in ELLA-CS® patterns. These stents were Z-shaped, self expandable, 3cm long and 8mm wide. Covered stents were manufactured by sewing an extra-fine Dacron covering with polypropylene thread to the internal surface of the metalic mesh (Figure 1).

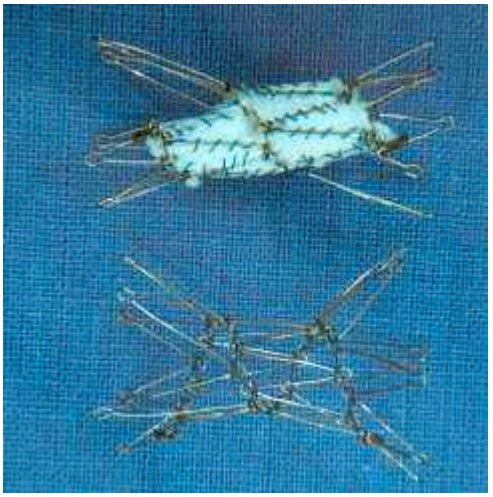

FIGURE 1 – Picture of the non-covered and covered stents.

The animals used in this study were male specimens of a double purpose breed from the Large White X Landrace. They were over eight weeks old and weighed between 16 and 25Kg [13,14.] The anesthetic technique employed was inhalatory general anesthesia, with a semi-closed reinhalation system. Pigs were sedated with tiletamine plus zolazepam (ZOLETIL®), at doses of 0.2ml/kg injected intramuscularly five minutes before the procedures. Anesthetic induction was carried out with intravenous thiopental sodium at 2.5%(10-12mg/kg) followed by tracheal intubation with a long laryngoscope [2,13,15.] To maintain the anesthesia, 0.5% to 1% halotane was used together with liquid replacement with physiological solution at 0.9%, 20ml/kg/hour.

An extraperitoneal approach through a left pararetal incision was used to expose the aorta. Following systemic heparinization (100UI/kg), a transversal arteriotomy was performed half-centimeter above the aortic trifurcation and the delivery system was introduced. The *stent* were then deployed. Arteriotomy synthesis was performed with a 5-0 polypropylene, and after the revision of the haemostasis, the abdominal wall was closed.

After 4 weeks, animals were subjected to the same anesthesia used for implantation. A supra and infra-umbilical laparotomy was performed to remove specimens (aorta including the stents) in one piece. After tissue collection, animals were submitted to euthanasia.

Preparing the tissue for histological analysis

Tissues removed were irrigated with sodium chloride solution at 0.9%, and vessel patency was macroscopically checked. Specimens were cut longitudinally and fixed with a solution of formaldehyde at 10% [16,17,18].
Tissue portions with 0.3-cm-long sections from the extension of the aorta artery from both ends (proximal and distal) were removed for histological analysis. Segments were processed and included in paraffin blocks and later submitted to histological cuts 4µm thick for preparation on the microscope slides. Slides were then prepared and blushed according to H-E(Hematoxilin-Eosin) and Verhoeff techniques.

Digital morphometry

Quantification methodology for intimal thickening of stents was performed with digital morphometric analysis according to an integrated morphometric program and image analysis - Media Cybernetics: Image Pro Plus. Images of histological sections were digitalized for morphometric analysis by conventional optical microscopy (Zeiss Microscope, Axiastar Model), by achromatic optical plane and photo-storage tube (Sony DXC 151 Video camera), generating image files in a PC. Images were digitalized with a 100-fold microscopic increase, and intimal and muscular areas were delimited, respectively, according to internal elastic membrane, and the endothelium. Dimensions of the areas were described in mm^2, using the average of eight microscopic fields.[19]
External and internal elastic membranes defined the area for the medial layers and the lines limited by the internal elastic membrane and the endothelium was considered as the intimal area. The ratio intimal area/medial layer was used as intimal index.

Statistical analysis

The data were analyzed according to SPSS v.6.0 Statistical Package for Social Sciences for Windows (Microsoft? - USA), by descriptive statistics. Student's *t*-test was used for matched samples between the groups and Student's *t*-test was used for independent samples in-between phases in order to analyze thickness of layers measured by digital morphometry.

Results

Morphometric analysis of the pig aorta

Morphometric analysis (proximal and distal to the stent) comparing the SNR and SRD groups were carried out with digital techniques including the intimal and medial layers. The intimal rate was established (ratio between the intimal layer and the medial layer) (Figure 2).

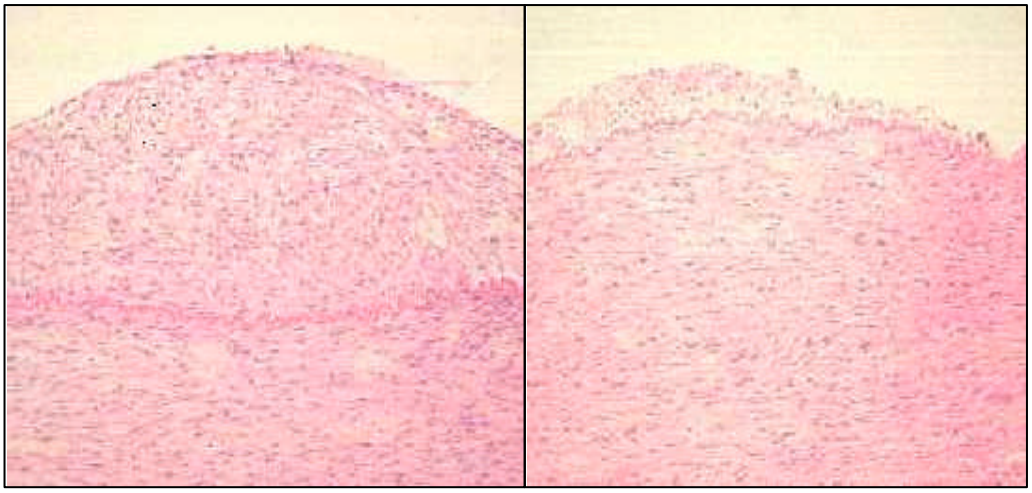

FIGURE 2 - Photomicrograph of one of the areas analyzed by digital morphometry at the proximal site adjacent to stent. **A -** Dacron-covered stent and **B** – non-covered stent. Intimal layer of covered stent features a larger thickening area than non-covered stents.

Non-covered stent group (SNR) *vs*. dacron-covered stent group (SRD) - proximal region.

Results obtained for proximal intimal area in the SNR group were 0.139 ± 0.069 mm², and 0.222 ± 0.089 mm² in the SRD group; the difference of 0.083mm² in the averages represented a borderline statistical difference (p=0.054). In the SNR group, proximal average was 5.158 ± 0.782mm², and in the SRD group it was 5.750 ± 0.733mm². The difference of 0.591mm² in the averages does not represent a significant statistical difference (p=0.141). The value of the proximal intimal index in the SNR group was 0,028 +- 0,017. Proximal intimal rate in the SNR group was 0.038 ± 0.013, whereas the difference of 0.009 in the averages does not represent a significant statistical difference (p=0.226) (Table 1).

TABLE 1 - Results of the morphometric analysis of the proximal area and its relationships with SNR and SRD Groups.

Variable	Group SNR (n=8)	Group SRD (n=8)	Difference in the Averages (IC 95%)	Statistical Significance	p*
Proximal Intimal Area(mm²)	0.139 ± 0.069	0.222 ± 0.089	0.083	NS	0.054
Proximal Media area (mm²)	5,158 ± 0.782	5,750 ± 0.733	0.591	NS	0.141
Proximal Intimal Rate	0.028 ± 0.017	0.038 ± 0.013	0.009	NS	0.226

Results: Average more or less standard deviation
Notation: Significance level = 0.05;
* Student *t* Test: for independent samples
n: number of animals per group

Non-covered stent group (SNR) ***vs.*** dacron-covered stent group (SRD) - distal region

The average results found for the distal intimal area in the SNR group were 0.252 ± 0.074mm², and, in the SRD group were 0.334 ± 0.149mm² (p=0.185). Results for distal average in the SNR group were 5.429 ± 0.715mm², and 5,089 ± 0.769mm² in the SNR group; difference of the averages was 0.340mm² (p=0.375). Results for distal intimal rate in the SNR group were 0.047 ± 0.017, and in the SRD group 0.068 ± 0.036, showing a difference of 0.020 in the averages (p=0.160).

When proximal and distal intimal layers of the same group of stents were compared, there were no statistical differences neither in the SNR group (p=0.608) nor in the SRD group (p=0.610) (Table 2).

TABLE 2 - Results of the morphometric analysis of the distal area and its relationships with the SNR and SRD groups.

Variable	Group SNR (n=8)	Group SRD (n=8)	Difference in the Averages (IC 95%)	Statistical Significance	p*
Area of Distal Intimal Layer(mm²)	0.252 ±0.074	0.334 ± 0.149	0.081	NS	0.185
Distal Media Area(mm²)	5,429 ± 0.715	5,089 ± 0.769	0.340	NS	0.375
Distal Intimal Rate	0.047 ± 0.017	0.068 ±0.036	0.020	NS	0.160

Results: Average more or less standard deviation
Notation: Significance level = 0.05;
* Student *t* Test: for independent samples
n: number of animals per group

Macroscopic and intraoperative analysis

During the surgical procedures (phases I and II) no vasospasms were observed in the aorta. We also carried out a macroscopic analysis of the specimens on phase II.

Discussion

Other authors [13, 20, 21] have also implanted stents in normal arteries and analyzed them through digital morphometry. Of course this king of study would be better performed in arteries with advanced atherosclerosis. However, animals models are difficult to obtain because they require long maintenance periods, hiperlipemic diets for long periods of time and specific genetic breeds [22].

The surgical or endovascular approach to the vessel being studied varies according to the personal preference of each author. Arteries that have a superficial

anatomical site, such as carotids and femoral arteries, are easily exposed to surgical procedures, while other deeper arteries like coronary arteries and the aorta are better approached by percutaneous catheters guided by fluoroscopy [22].

We approached the aorta through an extraperitoneal access as described by Weatherford et al [16], who carried out a similar research on dogs. This approach was chosen by us in this pig model because it is associated to lower rates of morbidity and mortality of the animals. It can be done expeditiously, the exposure of vessels is appropriate and blood loss is minimal.

In our study, 0.3cm-long sections were made in the aorta immediately proximal and distal to the stent edges. Based on digital morphometry techniques, areas from intimal and medial layers were measured in each artery and the average of the 8 microscopic fields with a 100-fold amplification was obtained. Schürmann et al[23] calculated also the average of the dimensions measured in eight fields with similar results.

All stents implanted in our study, whether they were covered or not, were patent after 4 weeks. The aorta of pigs used in the experiment had an average of 8-mm in diameter. There are some advantages in handling a vessel with such diameter when implanting tissue-covered stents, since there is a smaller probability for thrombosis in arteries with high flow. According to Cucina et al, [24] and Bassiouny et al, [25] grafts implanted in low flow arteries promote a more intense intimal hyperplasia.

Digital morphometric analyses showed that the intimal layer adjacent to the proximal margin of the covered stents (SRD) had greater intimal thickening than the intimal layer of the group with non-covered stents (SNR), though statistically this was of borderline significance (p=0,054). No differences were found in the groups when comparing areas of proximal media layer (p=0,141), distal intimal layer (p=0,185), distal media layer (p=0,375), and also when comparing the proximal intimal index (p=0,226) and distal index (0,160).

Hehrlein et al [26] found a larger intimal formation on the distal border of implanted stents than on the center portion. In our study, the proximal and the distal intimal layers were compared, in-groups, to check if there was larger intimal thickening in any of the analyzed implant sites. According to our analysis, there was no difference in intimal thickening between the proximal and the distal sites to the stents regardless they were covered or not.

Chalmers et al [27] evaluated, through morphometric analysis, intimal tickening in the anastomotic sites of uncovered stents implanted in dogs. A significant larger area of intimal hyperplasia was found adjacent to the borders of the stents. The intermediate region of the stent did not present any differences when compared to controls.

Karas et al [13] compared the proliferative response in pigs coronary arteries after implanting tantalum stents and after angioplasty with balloons in normolipemic pigs. After four weeks, arteries were processed for histopathological analysis. Data obtained indicated that the degree of intimal proliferation seems to be larger after stent implants than after balloon-injury. Intracoronary stents in pigs were associated to a strong inflammatory reaction around the stent mesh.

The clinical series with human beings that underwent angioplasty have shown conflicting results. Stenting is associated to lower patency rates than open surgery [28, 29, 30], but there has been considerable improvement in recent clinical series. The goal is reduction of intimal hyperplasia and restenosis rate. Some reports have already shown the results with covered stents impregnated with medicinal drugs and stents with radioactive substances that are capable of inhibiting intimal hyperplasia.

In our study, we could find an excellent patency, and a good incorporation for both kinds of stents (covered and non-covered with Dacron). Intimal thickening had similar characteristics in both groups of stents when comparing proximal and distal implant sites between and within the groups.

Even though the response to injury and biology of the vascular response after the implantation of these devices may be complex, we believe that clinical and experimental researches shall supplant the initial difficulties and will achieve the major goal, which is offering patients less invasive procedures with excellent results.

Conclusions

Neither of the two groups of stents (Dacron-covered and non-covered), when analyzed by digital morphometry for intimal thickening, presented any significant statistical difference when sites immediately distal to the stents were analyzed. There was only a borderline significant difference in the aorta proximal to the covered stents. When the analysis was carried out comparing samples within the same group, no statistical difference was observed either. All stents were patent after four weeks.

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