

EFFECT OF ANASTOMOTIC LENGTH ON THE DEVELOPMENT OF INTIMAL HYPERPLASIA IN THE DISTAL ANASTOMOSIS OF BYPASS GRAFT.

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ABSTRACT

Many hemodynamic factors have been shown to be associated with increased intimal hyperplasia at the distal anastomosis of arterial bypass graft. However, the relationship between the length of anastomosis and development of such complication has not been studied before. The aim of this study is to assess this relationship at the distal anastomosis with Dacron graft.

Material and Methods : Ileo-femoral bypasses were performed in 10 German shepherd dogs using 6-mm Dacron grafts. In accordance with preoperative randomization to individual animal legs, distal anasto-

moses were reconstructed applying four different groups (A, B, C and D) depending on the length of the arteriotomy: 3.0, 3.5, 4.0 and 4.5 times the internal diameter of the artery respectively. The vessels were harvested 6 months after the operation and specimens were processed for histological study. Quantitative analysis was performed to assess the extent of intimal hyperplasia at three zones (heel, toe and mid-zone of the arterial bed) of the distal anastomosis.

Results: Sixteen arterial bypasses were included in this study. Light microscopy revealed evidence of intimal hyperplasia in the four groups. Quantitative analysis showed significant

decrease in intimal hyperplasia with increasing the length of anastomosis at the heel, toe or mid-zone of the arterial bed. Mean ($\mu\text{m} \pm \text{SD}$) intimal hyperplasia of the three zones together was significantly higher in group A than B ($585\text{vs. } 423 \pm 8.6$ $p < 0.001$) and in B than C (423 ± 8.6 vs. 202 ± 15 $p < 0.001$). However, the difference between group C and group D (202 ± 15 vs. 162 ± 8.6 $p = 0.13$) was statistically insignificant.

Conclusion : The present study showed that the length of anastomosis is one of the hemodynamic factors involved in the development of intimal hyperplasia. Anastomotic techniques that resulted in the least intimal hyperplasia were end to side with length 4 or 4.5 times the internal diameter of the artery.

Key words : Intimal hyperplasia, arterial bypass, synthetic graft, transmission electron microscope

The paper was presented at 9th International Congress of the Asian Society for Vascular Surgery, Sept. 2008 Bangkok, Thailand

Vol. 40, No. 1 & 2 Jan., & April, 2009

INTRODUCTION

Prosthetic grafts are commonly used for lower-extremity arterial bypass grafting when no suitable autologous material is available. However, they accelerate the development of intimal hyperplasia, which remains the major cause of graft failure, especially in distal anastomosis⁽¹⁻³⁾. Compliance mismatch between the native vessel and the graft is considered one of the most important factors implicated in the development of intimal hyperplasia⁽⁴⁾. This factor can lead to adverse local hemodynamic effects at the anastomosis with consequent greater intimal thickening and eventual graft failure⁽⁵⁻⁷⁾.

Many hemodynamic factors^(4,8) have been shown to improve the compliance mismatch and hence associated with decreased intimal hyperplasia at the distal anastomosis of arterial bypass graft. However, the relationship between the length of arteriotomy and development of such complication has not been studied before. The aim of this study is to assess this relationship at the distal anastomosis using Dacron graft with different arteriotomy lengths.

MATERIAL AND METHODS

Ten apparently healthy adult German Shepherd dogs (3-5 years old) of both genders were used in this study. The study was carried out at Department of Surgery, Faculty of Medicine and the Surgical Department, Faculty of Veterinary Medicine, Suez Canal University, Egypt. The animal's weight ranged between 20-25 Kgms. The experimental study was performed in accordance with national laws on animal experiments, with the permission of the local university ethics commission.

About 10-15 minutes prior to the induction of general anesthesia, each dog was premedicated with intramuscular injection of chlorpromazine hydrochloride (1 mg / kg. Body Weight, Neurazine, Misr com. Pharm. Industries, Egypt)⁽⁹⁾. General anesthesia was then conducted by intravenous injection of thiopental sodium 2.5% (max dose is 20 mg/kg Bodyweight, Thiopental Sod., Egyptian International pharm. Industries co. Egypt)⁽¹⁰⁾.

Under sterile conditions, bilateral inguinal incisions were made. The terminal part of external iliac artery and

femoral arteries, on both sides, were dissected free. A segment approximately 3-4 cm in length was cut out from the femoral arteries after accomplishing proximal and distal control of the vessel. The segment (control) was divided in the mid sagittal plane and prepared for light microscopic study. Ileo-femoral bypass using 6-mm diameter, 10 cm length knitted Dacron grafts (Intergard Silver ultra-thin, Intervascular, France) were performed. In accordance with preoperative randomization to individual animal legs, distal anastomoses were reconstructed applying four different groups (A, B, C and D) depending on the length of the arteriotomy; 3.0, 3.5, 4.0, 4.5 times the diameter of the artery respectively. Standard end to side anastomosis were constructed. All anastomoses were constructed with continuous 6/0 polypropylene (EthiconTM; Johnson & Johnson Medical, Somerville, New Jersey, USA) with the aid of surgical magnifying loupes. Prophylactic antibiotic (3 doses of Cefotaxime 500 mg) were administered intravenously twice on the day of surgery and one on the following day. After surgery, the animals were kept under natural farming conditions

under the care of a veterinarian without any medication for 6 months. During the study period, follow up of the graft flow was checked with an ultrasound (Advanced Bi-directional Doppler Huntleigh UK) at one week post-operative and then every one month.

After 6 months, the dogs were brought back to the operating room for harvesting of the anastomosed vessels. Incisions were made on both legs under general anaesthesia. On table, angiography was performed to confirm patency of the distal anastomosis. The distal anastomotic regions were clamped and excised. The animals were euthanatized by intravenous administration of potassium solution (4.48 g potassium in 60 ml water). The excised segments were divided in the mid-sagittal plane and prepared for light microscopic study.

Light microscope :

Specimens for light microscope were fixed in 10% neutral buffered formalin solution. They were then processed to prepare 5 μm thick paraffin sections for histological stains (haematoxylin and eosin, and Elastic van Gieson's). The specimens were cut

transversely at three different locations: anastomotic heel, the mid-section at the floor of the artery and the anastomotic toe. Qualitative analysis of both control and distal anastomosis specimens was carried out. Quantitative assessments of intimal hyperplasia (distance; $\mu\text{m} \pm \text{SD}$, from lumen to internal elastic lamina) were measured in the different groups using the image analyzer (Super eye-Heidi software).

STATISTICAL ANALYSIS

Results have been summarized using descriptive statistics. These are presented as means ($\mu\text{m} \pm \text{SD}$) and compared using Student's t-test or ANOVA as appropriate. Significance was set at $P < 0.05$ for all comparisons. All statistical analyses were performed with the aid of SPSS-15 (Chicago, IL, USA) statistical analysis software.

RESULTS

Two dogs developed small hematomas in groins, which were treated conservatively. One groin was complicated with superficial inflammation, which settled down with antibiotics. One dog died without apparent rea-

son 4 months after the operation. Two grafts failed during the follow up period; one from group A and the other from group B. They were excluded from statistical analysis since pre-existing hyperplasia could not be distinguished from organized clot. Thus, sixteen arterial bypasses were included in this study. Each group comprised four bypass grafts. At 6 months, the distal anastomotic sites were patent in the 16 grafts. However, there was weak flow in all grafts of group A and 2 grafts in group B (50%). During the study period, there were no other morbidities.

Light microscopy revealed evidence of intimal thickening developed at the distal anastomosis of all grafts compared to normal artery. The intimal thickening was composed of a monolayer of endothelial cells (sometimes lost), proliferated smooth muscle cells and intercellular connective tissue formed of mainly collagen and

some elastic fibers (Fig1).

Quantitative analysis showed significant decrease in intimal hyperplasia at the heel with increasing the length of arteriotomy (595 ± 109 vs. 443 ± 129 vs. 185 ± 81 vs. 168 ± 94 μm , $p < 0.001$ at group A, B, C and D respectively) (Fig 2). The same was found at mid-zone of the arterial bed (566 ± 155 μm vs. 432 ± 87 μm vs. 192 ± 88 vs. 156 ± 46 $p < 0.001$ at group A, B, C and D respectively) (Fig 3) and at the toe (581 ± 131 vs. 394 ± 35 vs. 266 ± 162 vs. 176 ± 73 , $p < 0.001$ at group A, B, C and D respectively) (Fig 4).

Mean intimal hyperplasia of the 3 zones together was significantly higher in group A than B (585 ± 1.6 vs. 423 ± 8.6 $p < 0.001$) and in B than C (423 ± 8.6 vs. 202 ± 15 $p < 0.001$). However, the difference between group C and group D (202 ± 15 vs. 162 ± 8.6 $p = 0.13$) was statistically insignificant.

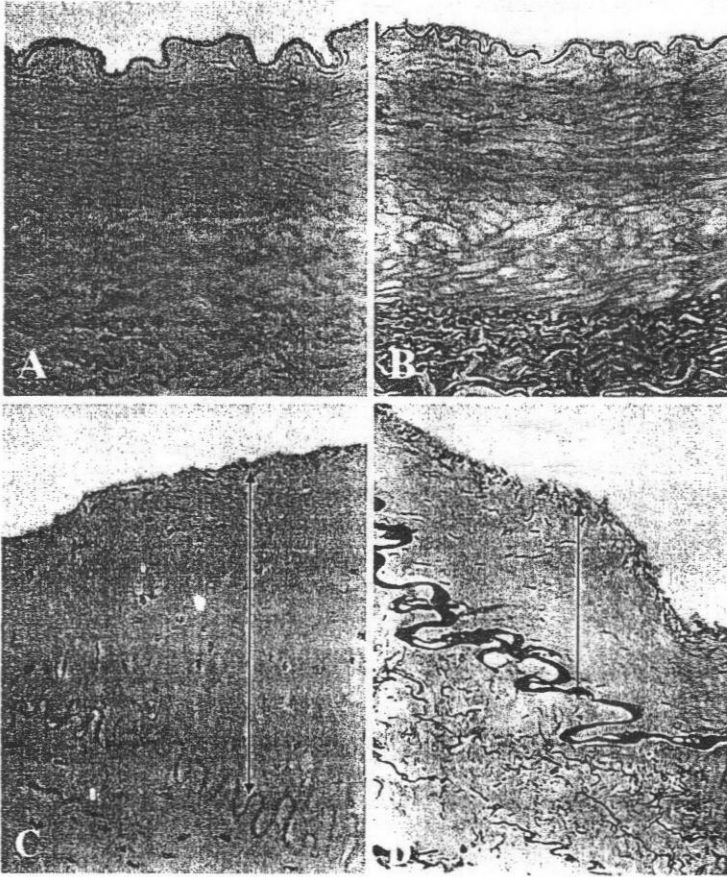


Fig1A &B: Sections in normal artery showing thin intima and black colored elastic fibers forming internal elastic lamina. (A:H&E=250, B:Elastic-van Gieson's =250) C D.. Sections at the distal anastomosis showing intimal thickening (arrow). It is composed of a monolayer of endothelial cells and proliferated smooth muscle cells. There is thickened black colored internal elastic lamina. (C:H&E=250, D:Elastic-van Gieson's =250)

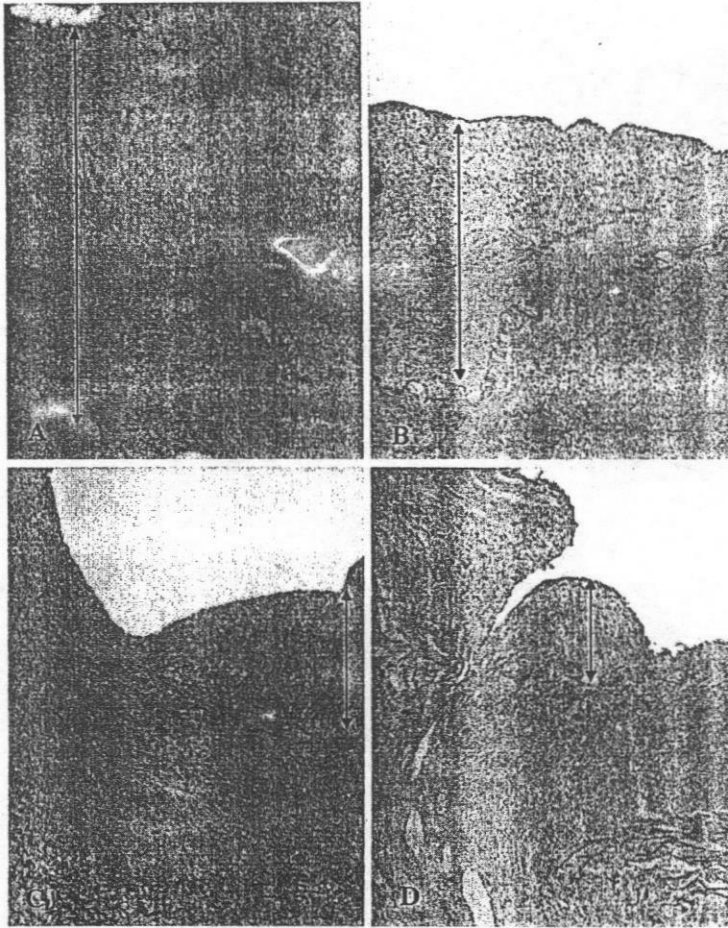


Fig2. Sections at the heel of distal anastomosis showing intimal hyperplasia (arrow) in different groups (A, B, C, D. Note that decrease in intimal hyperplasia with increasing the length of arteriotomy). H&E=100.

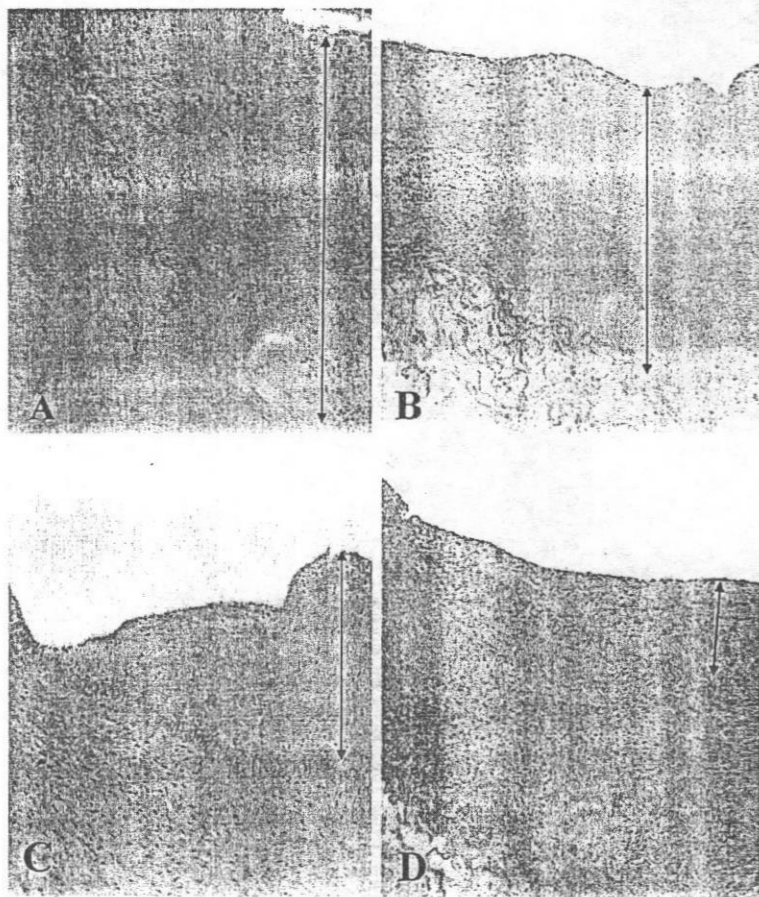


Fig3.Sections at the mid-zone of the arterial bed of distal anastomosis showing intimal hyperplasia (arrow) in different groups (A, B, C, D. Note that **decrease** in intimal hyperplasia with increasing the length of arteriotomy). H&E=100.

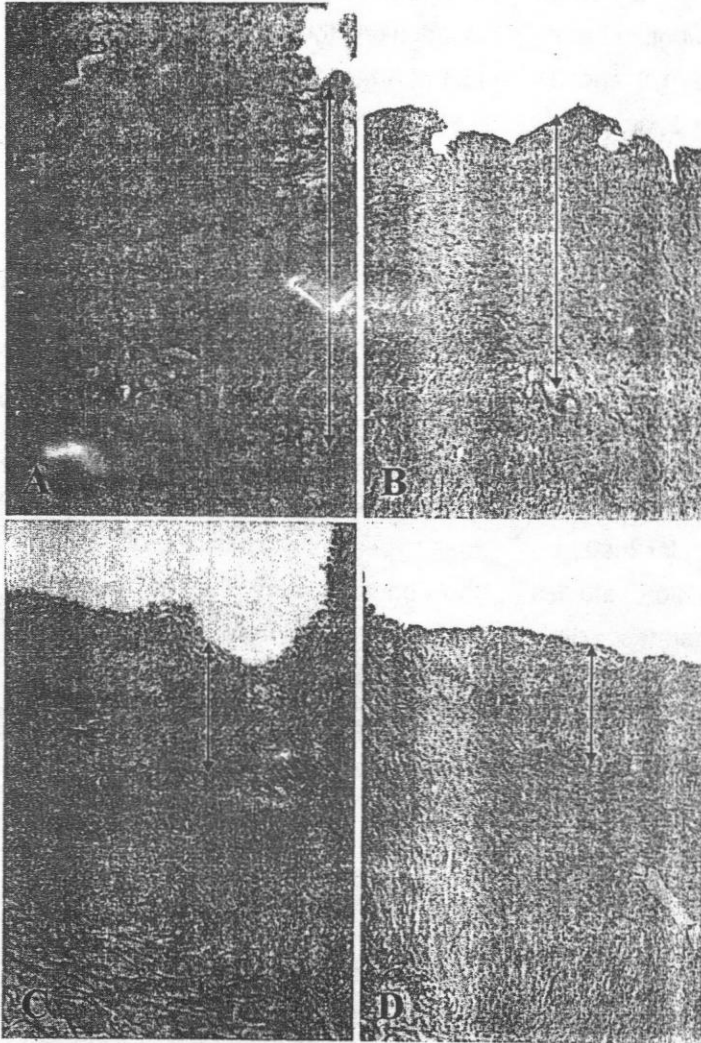


Fig4. Sections at the toe of distal anastomosis showing intimal hyperplasia (arrow) in different groups (A, B, C, D. Note that decrease in intimal hyperplasia with increasing the length of arteriotomy). H&E=100.

DISCUSSION

Although intimal hyperplasia was reported long time ago⁽¹¹⁾, its role as the main cause of thrombotic complications occurring between 2 and 24 months after a vascular intervention, has not been recognized until recently⁽¹⁾. Extensive researches have been performed to know more about the pathogenesis of this complication in order to reduce its development. Although some solutions have had promising results with respect to improved patency of peripheral bypass grafts, the impact of intimal hyperplasia on the field of vascular intervention remains high⁽¹²⁾. More studies are required to find pharmacological and hemodynamic interventions that can diminish the formation of intimal hyperplasia. The present study was made to assess the effect of one of these hemodynamic factors.

Intimal hyperplasia is commonly found at the toe, heel and the arterial floor near the distal anastomosis of arterial bypass graft⁽¹³⁾. At the floor of the arterial wall and at the heel of the anastomosis, where the blood flow divides proximally and distally, the wall shear stress will be low. This

affects expression of some vasoactive molecules, which are involved in the formation of intimal hyperplasia. In addition, the change in the direction of shear force that occurs during the systolic phase of the flow cycle, defined as the oscillatory shear index, is high at the anastomotic heel and the arterial floor⁽⁵⁾. This high oscillatory shear index may provide a stimulus for development of intimal hyperplasia⁽¹⁴⁾. At the toe of the anastomosis, the wall shear stress gradient is high^(15,16) which may be responsible for development of intimal hyperplasia at this site⁽¹⁷⁾. For the above reasons, the toe, the heel and the arterial floor near the distal anastomosis of arterial bypass graft were chosen for the measurement of intimal hyperplasia in the present study.

There are many factors which have been shown to be implicated in the development of intimal hyperplasia such as suture materials, surgical techniques^(4,8) and pharmacological intervention⁽¹²⁾. In the present study, there was no difference between groups as regards all the above mentioned factors. All bypasses were also

performed with the same material (Dacron), diameter and length. The previous measures minimized the differences between all operations apart from the length of arteriotomy.

The present study is the first, up to our knowledge, to assess the effect of length of arteriotomy on the degree of intimal hyperplasia at the distal anastomosis. It showed that increasing the arteriotomy length up to 4 times the internal diameter of the artery minimize the development of intimal hyperplasia. This is may be explained by Poiseuille's law which says that the pressure of flow for blood through a conduit is inversely proportional to the fourth power of its radius. This also explains how reduced flow velocity at the anastomotic floor of the Venafloe cuffed PTFE anastomosis prevents excessive shear stress, translating to reduced intimal hyperplasia.⁽¹⁸⁾ A vein cuff or the Distafloe PTFE graft widens the distal anastomosis and both are used clinically with better patency than PTFE alone ^(19,20). Conversely, grossly oversized distal conduits result in a sudden decrease in flow rate with relative stasis and flow separation. The resultant

low shear rate is associated with intimal hyperplasia as discussed earlier. Hence, the ideal diameter difference between graft and artery at the distal anastomosis is a balance to maintain the ideal flow velocity and resist turbulent flow⁽²¹⁾. This may explain that arteriomy of 4.5 times the internal diameter of the artery is not better than 4 times as regard the formation of intimal hyperplasia in the present study. It will be expected that intimal hyperplasia may increase if the arteriotomy length is more than 4.5 the internal diameter of the artery.

Intimal hyperplasia occurs as a result of abnormal proliferation and migration of cells to the tunica intima of a vessel wall⁽¹⁾. It is composed of about 20% of vascular smooth muscle cells that have migrated from the media to the intima, have proliferated, and deposited extracellular matrix, which comprises most of the intimal area. Other components of the intimal hyperplasia are macrophages and lymphocytes. The surface may or may not be endothelialised, depending on the extent of and time passed since the intimal damage⁽²²⁾. The same findings were found in the

present study as revealed by light microscopic study. Proliferation of smooth muscle cells results from endothelial cell damage as the normal endothelium produces factors, which inhibit vascular smooth muscle proliferation⁽²²⁾. This endothelial damage might result from surgical exposure and dissection damage to vasa vasorum and autonomic nerves⁽¹²⁾. The proliferated smooth muscle cells resemble the fetal type and can produce four to five times more extracellular matrix than the differentiated ones⁽²²⁾.

The present study has few limitations. It assessed the development of intimal hyperplasia in only medium sized vessels. It also investigates this complication using synthetic graft. Moreover, it is an experimental study and some experimental studies, which showed favorable outcome using certain technique, failed to prove this advantage in multicenter clinical trials⁽²³⁾.

In summery, the present study showed that the length of anastomosis is one of the hemodynamic factors involved in the development of intimal

hyperplasia. Anastomotic techniques that resulted in the least intimal hyperplasia were end to side with length 4 or 4.5 times the internal diameter of the artery. However, more extensive experimental and clinical studies are required on arteries of different sizes.

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