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# What is the major public health problem: the morbid obesity or the bariatric surgery in the unified health system? (Part II)

## *Qual o maior problema de saúde pública: a obesidade mórbida ou a cirurgia bariátrica no Sistema Único de Saúde (SUS)? (Parte II)*

Fernando de Barros, TCBC-RJ

There are scientific and political mobilizations worldwide that have the fight against morbid obesity (MO) as main objective. In February 2011, several ministers of health of the Americas participated in the High-Level Regional Consultation of the Americas Against Chronic Noncommunicable Diseases (ECNT), and signed a proposal with public health goals and guidelines against MO[1]. Europe already considered this epidemic one of the biggest challenges in public health[2].

In 2013, there were 72,000 operations to treat MO in Brazil, and in 2014, more than 80,000 operations, following the US, in which 140,000 operations were carried out[3]. Unfortunately, just over 10% of these operations in our country were performed in public hospitals. We are even more concerned when we see that much of obese patients (75%) who need this operation can only be treated by the Unified Health System (SUS) and not by the health insurance one[4]. We then have three quarters of a population "allocated" in a system which performs one tenth of bariatric operations in the country.

The cost of the problem, in 2011, for the SUS was R$ 487.98 million, representing 1.9% of spending on health care of medium and high complexity in the country[5]. Interestingly, spending on bariatric surgery itself were only R$ 31,5 million, which may reflect an insufficient number of operations to the size of the problem. According to Zilberstein *et al.*, the endless queues waiting for an operation on SUS reached 2.9 years in 2006, causing a mortality during the waiting period of 0.6[6]. Based on this work, and considering a linear growth of obesity of 0.024% a year[7], plus the finding in 2010 by IBGE, of the MO epidemic, we have enough reasons to understand why currently the waiting list in the SUS has a mortality rate equal to or greater than the operation itself, around 1% [8].

Bariatric surgery in SUS was regulated by Decree No 196 of 29 February 2000, and initially established 22 reference centers in the country[9]. However, this ordinance certainly did not contemplate the events of the years that followed and ended up scaling one reference service in bariatric surgery for every four million people. In 2007, a new ordinance was published[10], establishing guidelines for health care, with a view to preventing obesity and care to the obese, to be implemented in all federal units, respecting the competencies of the three levels of management. Six years later, through Ordinance on 425 of 19 March 2013, the Ministry of Health determined, among other things, the human resources and infrastructure for the adequacy and registration of the "High Complexity Center for Patients with Obesity"[11].

Brazil has now 78 units authorized by the Ministry of Health (MOH) in 20 different states[12]. Despite various guidelines and imposed bureaucratic regulations, the system offers little or no financial or training encouragement to the staff. The result of this mismatch is that 12 states in 2011, did not perform the 96 operations/year required as a prerequisite to remain qualified as reference centers. We have the impression, at this time, of a system biased towards a vicious cycle, where the productivity demand is made first and the initiative must be local; then the support resources arrive, which, in turn, does not cover 100% of the real needs. In 2011, 5,357 operations were performed in the SUS and, in 2012, slightly less than 6000. In the face of so many difficulties of everyday life of a Brazilian public hospital, plus the inefficient management model across the country to care for the morbidly obese, currently tertiary centers not registered by the MOH are implementing new bariatric surgery programs independently, which may indicate that the number of operations performed in the SUS may be underestimated. Again, the system turns against itself. If there is a highly complex program being carried out, but without proper recognition by managing entities, there is failure in statistics, there is no extra local funds reimbursement, there is no incentive, no staff training, programs are not sustainable, rendering losses to the local manager. This culminates in forming another "island", isolated in the system with their own and endless queues of patients waiting for an operation.

There is, no doubt, urgency in reviewing some guidelines. Even after almost 20 years of consecration of the method in Brazil, laparoscopic surgery is not covered by SUS for bariatric surgery. Several studies have demonstrated the highest efficiency and the lowest complication rate for this method[13,14]. In addition, it has been shown that the higher initial cost of laparoscopic surgery is offset by the savings in spending on length of stay, complications and readmissions[15]. Sussenbach *et al.* questioned 32 bariatric surgeons as to how would each of their services be should there be incorporation by SUS of the laparoscopic method for the surgical treatment of MO[12] and concluded that all respondents prefer the laparoscopic

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approach, believing that there would be more operations compared with laparotomy.

If there is a safer, less complication, preferably methods, preferred by the majority of surgeons and cheaper for public health, why not apply it? The cycle of a model of bureaucratic management not oriented to the up-to-date analysis of the population's needs repeats, confirming what for some is fact: SUS does not. But is it so? It is true that the SUS is currently undergoing a serious crisis. Since its creation in 1988, health in the country has become a universal right and duty of the state, becoming one of the largest public health systems in the world. Admittedly, who created the system did it with extreme perfection, but one detail was overlooked: exemplary management. After 27 years of its existence, the SUS is far from perfection. During this period we witnessed serious management failures and, according to press reports, misappropriation of health resources and hence scrapping of the services rendered to the population. If we make an analogy and observe the numerous decrees issued by the Ministry of Health for the care in the MO health-disease process, we can clearly see the gap between what is written and what is put into practice.

The approval of the Practice Area in Bariatric Surgery by the Federal Council of Medicine will expand the horizons of bariatric and metabolic surgery in Brazil, especially with regard to the formation of residents and to the continuing education of specialists in the field. In this moment of euphoria we have to think again about SUS. How will be the medical residency programs for surgeons interested in bariatric surgery? Will the SUS bariatric surgery reference centers be prepared to receive them? Will the residents be well-trained and ready to perform bariatric surgery in this current model? What will be the role of Surgical Societies in this training?

Certainly, major steps have been taken, but to consolidate them, we need to know how to advance in the political discussion, regulations, guidelines and undoubtedly in SUS management models. As we said, we live in a time of great changes in the world's epidemiological profile, and particularly in Brazil. Morbid obesity is among the serious health problems to be faced. Hopefully bariatric surgery in SUS – one of the important solutions to the problem – will not become a bigger problem than the disease itself due to its bureaucracy and inefficiency. Hopefully the first question answered here, "which is the largest public health problem: morbid obesity or bariatric surgery in SUS", will be quickly answered.

## REFERENCE

1. Castro C. Ministros da Saúde das Américas se comprometem a criar políticas públicas para reduzir a obesidade no continente. São Paulo: Associação Brasileira para o Estudo da Obesidade – ABESO; 2011. Acessado 06 mar 2011. Disponível em: http://www.abeso.org.br/.
2. Organização Pan-Americana da Saúde. Doenças crônico-degenerativas e obesidade: estratégia mundial sobre alimentação saudável, atividade física e saúde. Brasília: Organização Pan-Americana da Saúde; 2003.
3. Ramos AC. Brazil looking for completing his space in bariatric surgery. ABCD, arq bras cir dig. 2014;27(Suppl 1):1.
4. Fundação Instituto Brasileiro de Geografia e Estatística. Pesquisa Nacional por Amostra de Domicílios – PNAD/BrasiL. 1998. Análise de Resultados. Acessado em 23 jul 2010. Disponível em: http://www.ibge.gov.br/home/estatistica/populacao/trabalhoerendimento/pnad98/saude/analise.shtm
5. Oliveira ML. Estimativa dos custos da obesidade para o Sistema Único de Saúde do Brasil [tese]. Brasília, DF: Universidade de Brasília, Faculdade de Ciências da Saúde; 2013.
6. Zilberstein B, Halpern A, Monteiro SM, Lunardi A, Campoleone S, Souza AC, et al. Waiting time for bariatric surgery in a public hospital in Brazil: a problem to be solved. Obes Surg. 2006;16:1023.
7. Instituto Brasileiro de Geografia e Estatística. Diretoria de Pesquisas. Pesquisa de Orçamentos Familiares 2008-2009 – POF. Rio de Janeiro:IBGE; 2011.
8. Benotti P, Wood GC, Winegar DA, Petrick AT, Still CD, Argyropoulos G, et al. Risk factors associated with mortality after Roux-en-Y gastric bypass surgery. Ann Surg. 2014;259(1):123-30.
9. Ministério da Saúde. Portaria nº 196, de 29 de fevereiro de 2000, Institui os critérios clínicos para a indicação de realização de gastroplastia como tratamento cirúrgico da obesidade mórbida, no âmbito do Sistema Único de Saúde. Diário Oficial da União 01 mar 2000.
10. Ministério da Saúde. Portaria nº 1569, de 28 de junho de 2007, institui diretrizes para a atenção à saúde, com vistas à prevenção da obesidade e assistência ao portador de obesidade, a serem implantadas em todas as unidades federadas, respeitadas as competências das três esferas de gestão. Diário Oficial da União 02 jul 2007.
11. Ministério da Saúde. Portaria nº 425, de 19 de março de 2013, Estabelece regulamento técnico, normas e critérios para o Serviço de Assistência de Alta Complexidade ao Indivíduo com Obesidade. Diário Oficial da União 2013. Diário Oficial da União 20 mar 2013.
12. Sussenbach S, Silva EN, Pufal MA, Rossoni C, Casagrande DS, Padoin AV, et al. Implementing laparoscopy in Brazil's National Public Health System:the bariatric surgeons' point of view. ABCD, arq bras cir dig. 2014;27 (Suppl 1):39-42.
13. Tiwari MM, Reynoso JF, High R, Tsang AW, Oleynikov D. Safety, efficacy, and cost-effectiveness of commomlaparosocopic procedures. SurgEndosc. 2011;25(4):1127-35.
14. Reosch J, Mottillo S, Shimony A, Filion KB, Christou NV, Joseph L, et al. Safety of laparoscopic vs open bariatric surgery: a systematic review and meta-analysis. Arch Surg.2011;146(11):1314-22.
15. Nguyen NT, Goldman C, Rosenquist CJ, Arango A, Cole CJ, Lee SJ, et al. Laparoscopic versus open gastric bypass: a randomized study of outcomes, quality of life, and costs. Ann Surg. 2001;234(3):279-89; discussion 289-91.

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# Accuracy of doppler ultrasonography in the evaluation of hemodialysis arteriovenous fistula maturity

## *A acurácia da ultrassonografia com Doppler na avaliação da maturação da fístula arteriovenosa para hemodiálise*

João Humberto da Fonseca Junior[1]; Guilherme Benjamin Brandão Pitta[1]; Fausto Miranda Júnior[2]

A B S T R A C T

**Objective:** to determine the accuracy of Doppler ultrasonography (USD) for hemodialysis arteriovenous fistula (AVF) maturity. **Methods:** we included patients with no prior AVF. Each patient underwent two USD examinations. After initiation of hemodialysis, we followed the patients during the first month of the access use and verified its adequacy to hemodialysis sessions. At statistical analysis we measured specificity, sensitivity, accuracy, ROC curve (Receiver operator characteristic) curve, TG-ROC (Two graph - receiver operator characteristic) and logistic regression. **Results:** we included 76 patients, of which 51 completed the study. They formed two groups, those who have had good adequacy for hemodialysis (45) and those who had not (6). The average flow volume (FV) and the average draining vein diameter (DVD) of each group were, respectively: 940mL/min (95% CI: 829-1052) and 325mL/min (95% CI: 140-510); and 0.48cm (95% CI: 0.45-0.52) and 0.33cm (95% CI: 0.27-0.40). The area under the ROC curve of FV and DVD were 0.926 and 0.766, respectively. **Conclusion:** the accuracy of the measured volume flow measured at the draining vein to evaluate maturation of hemodialysis arteriovenous fistula was 85%.

**Key words:** Arteriovenous Fistula. Hemodialysis. Ultrasonography, Doppler, Color.

## INTRODUCTION

In Brazil, over 91,000 patients are undergoing dialysis, with 66.9% within the age group 19-64 years; the two main base diseases are hypertension and diabetes mellitus[1].

The vascular access for hemodialysis is critical to the proper treatment of patients with kidney failure, since through it patients' blood can be transferred to the dialysis filter and returned to the patient in a continuous process, which usually takes three to four hours, three times a week[2]. The monitoring and surveillance of these approaches provide better patency, reducing the complications inherent to their use[3].

The maturation of the arteriovenous fistula (AVF) has been studied by Doppler ultrasonography (DUS) and the results were related to the adequacy of hemodialysis sessions. Some studies have addressed this time of vascular access-related AVF, providing quantitative data on maturation of newly created accesses[4-7].

After installation of the AVF for hemodialysis, the patient may benefit from a quantitative criterion based on DUS to assess the maturity of this access and to allow the first puncture[4].

Quantitative and easily reproducible criteria for assessing fistula maturity and helping the clinical decision when conducting this access will be extremely useful in daily practice. The objective of this study was to determine DUS accuracy in assessing the maturity of hemodialysis AVF.

## METHODS

We studied patients with arteriovenous fistulas made in the upper limb. We conducted a prospective, diagnostic test cohort study at the Clínica de Nefrologia de Juazeiro, State of Bahia – BA, Brazil. The project was approved by the Ethics in Research Committee of UNCISAL (Universidade Estadual de Ciências da Saúde de Alagoas), Maceió, State of Alagoas – AL (29/05/2006/509). All patients who agreed to participate in the study signed a free and informed consent form. In this work, the prostheses will not be object of study.

Patients were referred by the nephrologist for their first surgical vascular access in the upper limb. Once we defined the best anatomical site for performing the access, the arteriovenous fistula (AVF) was made. We used a technique with a terminolateral anastomosis of the vein with the artery in all patients with 6-0 and 7-0 polypropylene sutures, according to the vessels diameter, with the aid of magnification loupes (Heine® 2.3x). Two groups were

1. Departamento de Cirurgia, UNIFESP – São Paulo – SP – Brasil; 2. Serviço de Cirurgia Vascular do Departamento de Cirurgia, UNIFESP – São Paulo – SP – Brasil.

formed, those who had good adaptation to hemodialysis and those who had not.

Each patient underwent two DUS exams, the first between the 10th and the 20th postoperative day and the second between the 30th and the 40th. After the second examination, we followed patients clinically as for the result of their hemodialysis sessions for a minimum period of one month after the start of AVF. The main investigator performed the DUS Exams (Figures 1 and 2). We used a Doppler ultrasound device (Hewlett-Packard®, model Image Point) with5-10 MHz variable frequency linear transducer.

The examination followed the evaluation protocol for the upper limb and was held in a room with the patient lying supine with the arm resting on the exam table[8].

The flow in a AVF draining vein typically has an increased systolic and diastolic velocity with a low resistance biphasic pattern (Figures 1 and 2), in a vessel with very superficial location. Adjustment is necessary regarding the depth, the pulse repetition frequency, the insonation angle, and the Doppler angle, which was set to 60 degrees, as well as the sample volume including the entire vessel lumen so that the spectral curve has the best graphical representation.

Once completed the fistula maturation period, which was 45 days, the patients were clinically examined for the presence of fremitus in the fistula drainage vein and were referred to the hemodialysis as any other patient from the nephrology clinic, using the AVF for hemodialysis sessions.

The adequacy of the AVF for hemodialysis sessions was set when the patient performed at least six hemodialysis sessions with four hours within one month, as prescribed by a nephrologist. Hemodialysis sessions were performed with devices of Baxter® brand, TINA 1200 model.

The accuracy of ultrasound was calculated based on the relationship between the DUS and the results of hemodialysis sessions[4]. With an estimated proportion of the population of 50%, with absolute precision of 15% and significance level of 5%, we calculated the sample size in 43 patients[9]. The alpha value was d"0.05%.

We used the programs "R"[10] and "SAS"[11] for the construction of the TG-ROC (Two Graph - Receiver operator characteristic) curve, the ROC curve (Receiver operator characteristic), the flow volume (FV) and the draining vein diameter (DVD), the area under the ROC curve, the confidence interval and the logistic regression.

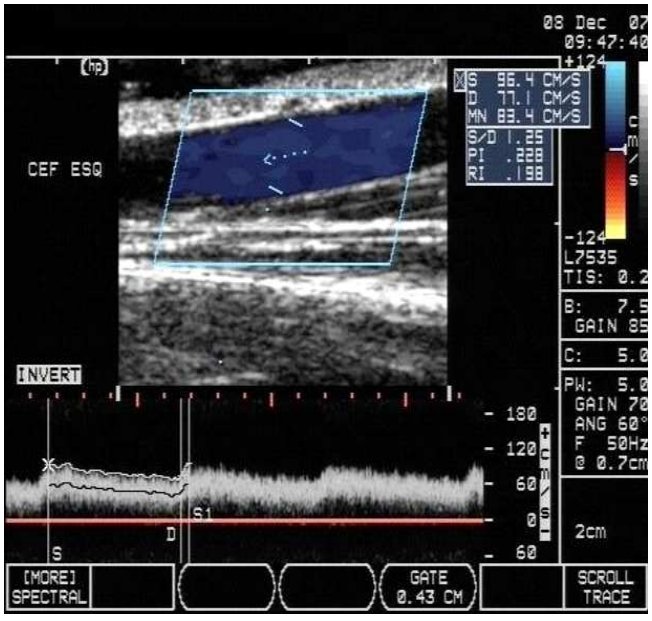


***Figure 1*** - *DUS of draining vein in the middle third of forearm, with the representation of image and spectral Doppler, and automatic measurement of average speed.*

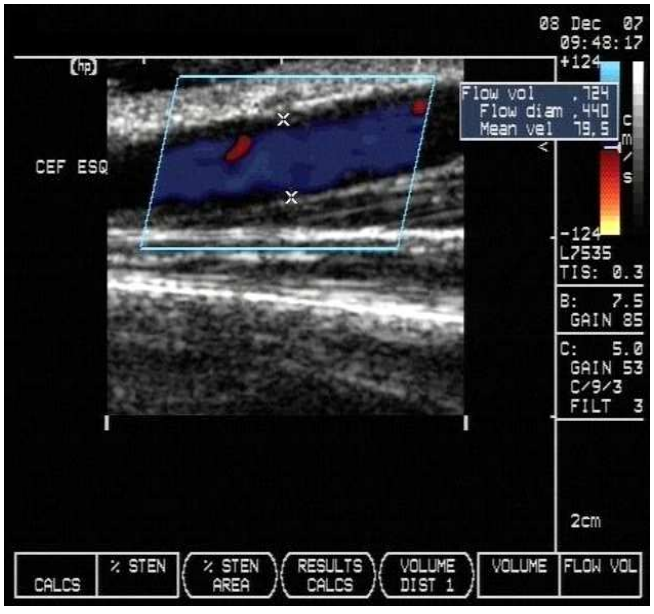


***Figure 2*** - *DUS of draining vein in the middle third of forearm, with the representation of the color Doppler image, ending with the manual measure of vessel diameter to calculate the flow volume.*

## RESULTS

We registered 76 patients, of which 25 did not complete the survey (Figure 3). We carried out 46 radiocephalic arteriovenous fistulae (AVF) and five brachiocephalic, in 30 men and 21 women. The performance of two Doppler ultrasound examinations (DUS) during the FAV maturation period allowed us to observe an increase in flow volume (FV) and average draining vein diameter (DVD) in 59% and 65% of patients, respectively.

The average fistula FV in 45 patients who had a good adaptation to hemodialysis sessions was 940ml/min (95% CI: 829-1052). The six patients who did adapt to hemodialysis presented an average flow of 325ml / min (95% CI: 140-510). The average flow adjusted by the nephrologist in the hemodialysis machine was 343ml / min (95% CI 341-345).

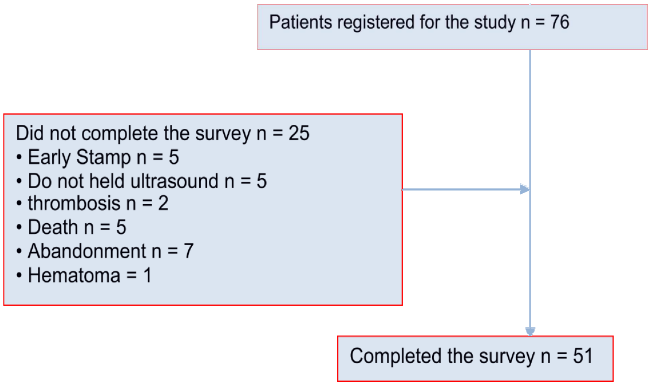


***Figure 3 -*** *Deviations in the research.*

The average DVD of the 45 patients who had a good adaptation to hemodialysis was 0.48cm (95% CI: 0.45-0.52). The six patients who did not meet the adequacy criteria had an average diameter of 0.33cm (95% CI: 0.27-0.40).

The accuracy, sensitivity and specificity of DUS were calculated based on the variation of the cut-off point (100ml / min) as for the FV and on the cutoff point variation (0.1cm) as for the DVD, with the three curves simultaneously demonstrating the measures for each cut-off point.

The FV cutoff that best represents DUS accuracy to assess AVF maturity for hemodialysis is located at the point of intersection of the accuracy, sensitivity and specificity curves (Figure 4), ie, between 500 and 600 ml/min. Using logistic regression, we estimated this point at 517ml/min, which corresponds to an accuracy of 85%.

The DVD cutoff point that best represents DUS accuracy to assess AVF maturity for hemodialysis is located at the point of intersection of the accuracy, sensitivity and specificity curves (Figure 5), ie, between 0.4 and 0.5 cm. Using logistic regression, we estimated this cutoff at 0.45 cm, which corresponds to an accuracy of 66%.

The DVD and FV areas under the ROC curve were, respectively, 0.766 and 0.926 (Figure 6), thereby showing that both have statistical significance, as they are higher than 0.5, but the FV proved to be the most important parameter when considering the relationship between curves.

## DISCUSSION

We conducted two Doppler ultrasonography (DUS) exams for the monitoring of the arteriovenous fistulas (AVF) maturation process. Results showed an increase in flow volume (FV) and the draining vein diameter (DVD), confirming the recommendation that the maturation process exists and needs to be respected for a better use of the vascular access.

An important contribution of our study is the finding of increased DVD and FV, by 50% and 52% of cases, respectively, between the completion of the first DUS exam and the second, suggesting that in half the cases there will be development and expansion of the circulatory system, confirming the need to wait for no less than a 40-day maturity period to start using this access.

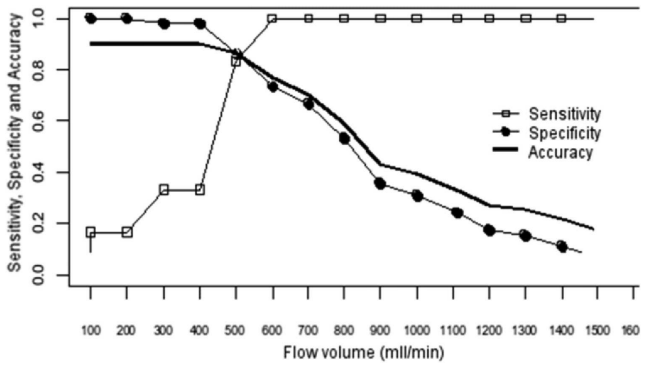


***Figure 4 -*** *TG-ROC Curve, sensitivity, specificity and accuracy percentage demonstration (ordinate) of cutoff points for the flow volume (abscissa). The curves intersect between 500 and 600 ml/min.*

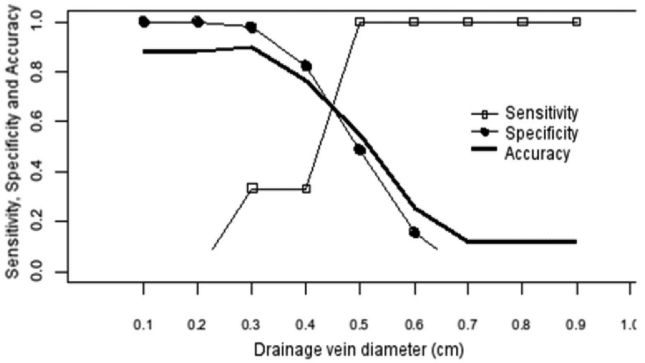


***Figure 5 -*** *TG-ROC Curve, sensitivity, specificity and accuracy percentage demonstration (ordinate) of cutoff points parts of the draining vein diameter (abscissa). The curves intersect between 0.4 and 0.5 cm.*

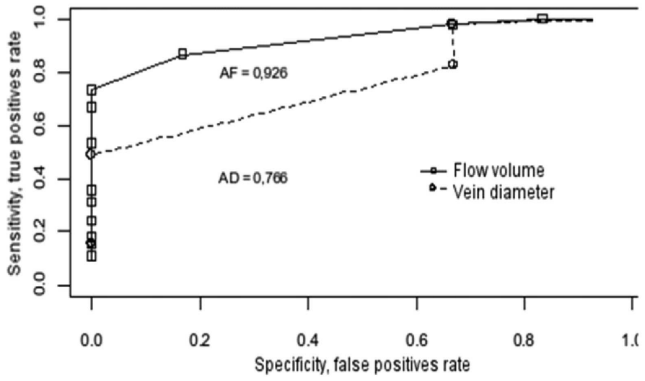


***Figure 6 -*** *ROC curve, sensitivity (ordinate), specificity (abscissa), squared ROC curve= flow, circle ROC curve = drainage vein diameter, AF = ROC curve area of the flow volume, AD = ROC curve area of the draining vein diameter.*

Our study found data similar to Toregeani *et al*. They evaluated the arteriovenous fistula maturation process by serial DUS monitoring as for the progression of the diameter of the draining vein and flow, especially for fistulas on the wrist[12].

Data from the NKF-K/DOQI[13], when compared to ours, differ in cutoff points, since the rule of three "6" could hardly be applied to our reality, especially when we would have to consider veins with a minimum of 0.6cm in diameter to achieve the optimum condition of maturation of said vein. This research found a flow volume and a drainage vein diameter cutoff point where we could suggest the creation of the rule of "5", with 500ml/min and 5mm, respectively, to the adequacy of hemodialysis sessions.

There have been attempts to establish quantitative parameters for AVF maturation[4,5,7,13]. Thus, this research is in accordance with these aspects and coincides with parameters used in different countries, especially concerning the FV with a cutoff of 500ml/min as a minimum suitable AVF value to perform hemodialysis sessions.

Robbin *et al*. did not calculate the area under the ROC curve[4], which undoubtedly is a failure in their article, since this parameter is of utmost importance to validate the diagnostic test used[14]. According to Zhu *et al.*, the area under the ROC curve can undergo stratification so as to indicate the diagnostic test's accuracy or overall precision, where close to 0.5 the diagnostic testing approaches randomness, and the ROC curve classified as insufficient, and when it approaches 1.0, it scores as excellent[14].

According to Martinez *et al.*, the TG-ROC curve proposes an alternative way of demonstrating the performance of a diagnostic test[15], with important additional information, the choice of the optimal cutoff point. In this same model it is possible to include the diagnostic test's accuracy curve. In our research, we found that, as for FV, the crossing point of sensitivity, specificity and accuracy was a point between 500 and 600 ml/min. This point best represented AVF maturation in 39/45 patients, with an accuracy of 86.3% (95% CI: 73-94) (Figure 4), and is in line with the literature[3,5]. The DVD TG-ROC, which shows the three curves simultaneously, revealed a crossing point between 4 and 5 mm, which is the cutoff point that best represented AVF maturation in 37/45 patients, with an accuracy of 66% (95% CI: 68-90) (Figure 5).

Biuckians *et al*.[6] make a reflection on the NKF-K/DOQI recommendations[13], so that the number of fistulas as the first patient access increases. However, at the end of the segment period, they found that only 48% of arteriovenous fistulas were being used, and 11% reached the maturation period without some kind of intervention. These data differ from our practice, since in our study 86% of patients achieved a good adaptation to hemodialysis in an average period of 45 days.

The problem of AVF maturation for hemodialysis has at least two facets with impact on clinical practice: the first would be the judgment about time of maturation, and the second is how this judgment would be best accomplished. This research contributed precisely with these two aspects, particularly with the form of judgment, because in our country, the average period of maturation is already well established (one to two months). But as for the form of judgment, where until recently the clinical examination was the only alternative, the DUS provides information that can help in the decision to consider that access mature.

DUS has contributed in this research, with the measure of FV and the DVD, establishing parameters that, once achieved, allow a more detailed access evaluation, thereby facilitating the doctor who is knowledgeable of these parameters to review the DUS report and, associated with a good clinical examination, conclude about the access evolution and about the maturation period. As a practical contribution, we can suggest that every DUS exam related to AVF assessment has the FV information measured in the draining vein. The FV should be quickly and easily available in DUS devices, reducing the bureaucracy of access to this information, which limits the execution of this test for many examiners.

The understanding of the AVF maturation process is constantly evolving. Being an affordable method, DUS allows the study of this process with minimal invasiveness and with parameters which can be added to better reflect AVF adequacy to hemodialysis.

Clinical studies have linked the accurate physical examination in association with the DUS as a way to substantiate the best clinical decision to be made in the AVF maturation period[4,16]. These studies, as well as the guidelines of the NKF-K/DOQI[14], allow to suggest that the investigation of the AVF maturation period can be completely unveiled, however, new study parameters provided by the DUS continue in development, leaving a door open for scientific research continue to work.

This study suggests a new approach to the evaluation of the AVF maturation process and correlates the DUS with the adequacy to the hemodialysis sessions. Deepening the study of this correlation is a new path that will continue bringing relevant information to the AVF management.

In conclusion, the accuracy of the flow volume measured in the draining vein to assess the fistula maturity for hemodialysis was 85%, which is the best available parameter alone. The combination of parameters can further improve these figures; there is need to deepen and develop new studies for the improvement of the technique.

## RESUMO

***Objetivo:*** *testar a acurácia da ultrassonografia com Doppler (USD) na avaliação da maturação do acesso vascular para hemodiálise.* ***Métodos:*** *foram incluídos pacientes que não haviam feito uma fistula arteriovenosa (FAV) anteriormente. Cada paciente foi submetido a dois exames de USD. Após o início da hemodiálise, foram acompanhados durante o primeiro mês utilizando o acesso e verificando sua adequação às sessões de hemodiálise. Foram aferidas: especificidade, sensibilidade, acurácia, curva ROC (Receiver operator characteristic), curva TG-ROC (Two graph – receiver operator characteristic) e regressão logística.* ***Resultados:*** *foram incluídos na pesquisa 76 pacientes, 51 concluíram o estudo. O volume de fluxo (VF) médio e o diâmetro médio da veia de drenagem (DVD) foram, respectivamente, para cada grupo: 940 ml/min (IC95%: 829-1052 ml/min); 325 ml/min (IC95%: 140-510 ml/min); e 0,48cm (IC95%: 0,45-0,52 cm); 0,33cm (IC95%: 0,27-0,40 cm). A área sob a curva ROC do VF e do DVD foram, respectivamente, 0,926 e 0,766.* ***Conclusão:*** *A acurácia da medida de volume de fluxo aferido na veia de drenagem para avaliar a maturação da fistula de hemodiálise foi 85%, o melhor parâmetro disponível isoladamente.*

***Descritores****: Fístula arteriovenosa. Hemodiálise. Ultrassonografia Doppler em Cores.*

## REFERENCES

1. Sociedade Brasileira de Nefrologia. Censo da Sociedade Brasileira de Nefrologia 2011 [online]. 2013 [capturado 10 de fev. 2011]. Disponível em: http://www.sbn.org.br/pdf/censo_2011_publico.pdf.
2. Lugon JR, Matos JPS, Warrak EA. Hemodiálise. In: Riella MC. Princípios de Nefrologia e Distúrbios Hidroeletrolíticos. 4ª ed. Rio de Janeiro: Guanabara Koogan; 2003. p. 869-907.
3. III. NKF-K/DOQI Clinical Practice Guidelines for Vascular Access: update 2000. Am J Kidney Dis. 2001;37(1 Suppl 1):S137-81.
4. Robbin ML, Chamberlain NE, Lockhart ME, Gallichio MH, Young CJ, Deierhoi MH, et al. Hemodialysis arteriovenous fistula maturity: US evaluation. Radiology. 2002;225(1):59-64.
5. Basile C, Casucci F, Lomonte C. Timing of first cannulation of arteriovenous fistula: time matters, but there is also something else. Nephrol Dial Transplant. 2005;20(7):1519-20.
6. Biuckians A, Scott EC, Meier GH, Panneton JM, Glickman MH. The natural history of autologous fistulas as first-time dialysis access in the KDOQI era. J Vasc Surg. 2008;47(2):415-21; discussion 420-1.
7. Brunori G, Ravani P, Mandolfo S, Imbasciati E, Malberti F, Cancarini G. Fistula maturation: doesn't time matter at all? Nephrol Dial Transplant. 2005;20(4):684-7.
8. Robbin ML, Lockhart ME. Avaliação ultra-sonográfica antes e depois de acesso para hemodiálise. In: Zwiebel WJ, Pellerito JS. Introdução à Ultra-Sonografia Vascular. 5ª ed. Rio de Janeiro: Elsevier; 2005. p. 325-40.
9. Lwanga SK. Sample size determination in health studies: a practical manual. Geneva: World Health Organization; 1991.
10. R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing [online]. Vienna, Austria; 2011. [capturado 10 fev 2011]. Disponível em: http://www.R-project.org/
11. The SAS Learning Edition 4.1; Copyright (c) 2002-2003 by SAS Institute Inc., Cary, NC, USA.SAS (r) 9.1 (TS1M3) [online]. 2013 [capturado 10 fev 2011]. Disponível em: http://www.sas.com/
12. Toregeani JF, Kimura CJ, Rocha AST, Volpiani GG, Bortoncello A, Shirasu K, et al. Avaliação da maturação das fistulas arteriovenosas para hemodiálise pelo eco-Doppler colorido. J vasc bras. 2008:7(3):203-13.
13. Vascular Access Work Group. Clinical practice guidelines for vascular access. Am J Kidney Dis. 2006;48 Suppl1:S248-73.
14. Zhu W, Zeng N, Wang N. Sensitivity, specificity, accuracy, associated confidence interval and ROC analysis with practical SAS Implementations. Baltimore: Health Careand Life Sciences; 2010.
15. Martinez EZ, Louzada Neto F, Pereira BB. A curva ROC para testes diagnósticos. Cad saúde colet. 2003;11(1):7-31.
16. Campos RP, Chula DC, Riella MC, Nascimento MM. O exame físico como método de detecção de estenose da fístula arteriovenosa. J Bras Nefrol. 2007;29(2):64-70.

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**Address for correspondence:**
Fausto Miranda Júnior
E-mail: fmiranda@apm.org.br

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Original Article

# Degloving injuries of trunk and limbs: comparison of outcomes of early versus delayed assessment by the plastic surgery team

## *Desenluvamentos de tronco e membros: comparação dos resultados da avaliação precoce ou tardia pela cirurgia plástica*

Daniel Francisco Mello[1]; José Cesar Assef, TCBC-SP[2]; Sílvia Cristine Soldá, TCBC-SP[2]; Américo Helene Jr[1]

A B S T R A C T

**Objective:** to analyze cases of degloving of the trunk and limbs, comparing outcomes of early versus delayed assessment by the plastic surgery team. **Methods:** we conducted a retrospective analysis of medical charts. Patients comprised two groups: Group I – early assessment, performed within 12 hours post trauma; and Group II – delayed assessment, performed more than 12 hours post trauma. We defined primary grafting as the use of skin from the traumatized skin flap. We excluded cases involving hands, feet or genitalia. **Results:** there were 47 patients treated with degloving injuries between 2002 and 2010. The mean body surface area affected was 8.2%. Lower limbs were the most frequently affected site (95.7%), whether alone or in association with lesions to other sites. Delayed assessment by the plastic surgery team occurred in 25 cases. Mean hospital stay was 36.1 days for Group I and 57.1 days for Group II (p=0.026). Regarding the number of surgical operations (skin grafts), Group I received a mean of 1.3, while Group II underwent 1.6 (p=0.034). **Conclusion**: based on length of hospital stay and number of operations in trauma patients with degloving of the trunk and limbs, plastic surgery assessment should be carried out early.

**Key words:** Skin Transplantation. Soft Tissue Injuries. Wound Closure Techniques. Dermatologic Surgical Procedures. Fascia/Surgery

## INTRODUCTION

Degloving results from the application of high intensity forces with tangential vectors that determine compression, stretch, twist and tissue friction, causing avulsion of skin and subcutaneous tissue from the fascia and muscle planes, with damage to the musculocutaneous and fasciocutaneous perforating vessels[1-4].

The first reports date back to the early twentieth century, in upper limb injuries caused by occupational accidents with drying machines in laundries, known in the literature as wringer arm (MacCollum, 1939). With the advent of the automobile industry, the most frequent mechanism became trampling[1-3,5].

The bearer of this type of injury is usually a multiple trauma patient, with high incidence of associated injuries, particularly fractures and vascular lesions[5-7]. The early and simultaneous participation of the plastic surgeon is essential in order to assess tissue viability and guide treatment[8]. The use of traumatized skin as partial or total thickness primary grafting, initially described by Farmer in 1939, is considered the ideal conduct[1-3,8,9].

This work aims to analyze patients suffering from degloving of trunk and limbs, comparing the results of early or late evaluation by the Plastic Surgery team.

## METHODS

We performed a retrospective analysis of medical records of patients suffering from degloving affecting the trunk and limbs treated between January 2002 and January 2010 at the Emergency units and evaluated by the Plastic Surgery team, Department of Surgery, Faculdade de Ciências Médicas, Santa Casa de Misericórdia de São Paulo. Cases involving hands, feet or genitals were excluded. This study was approved by the Comitê de Ética e Pesquisa (CEP) of the Irmandade da Santa Casa de São Paulo (in 161/10).

The evaluation by the Plastic Surgery team was requested after the initial care held by General Surgery, Pediatric Surgery and Orthopedics teams. It was considered an early assessment performed within 12 hours (Group I – 22 patients) and late, the ones performed after 12 hours of admission (Group II – 25 patients). To evaluate the

1. Serviço de Cirurgia Plástica, Departamento de Cirurgia, Faculdade de Ciências Médicas da Santa Casa de Misericórdia de São Paulo - SP - Brazil; 2. Serviço de Emergência, Departamento de Cirurgia, Faculdade de Ciências Médicas da Santa Casa de Misericórdia de São Paulo - SP - Brazil.

percentage of degloved body surface (DBS) we used Lund and Browder table[10].

We evaluated data regarding: gender, age, mechanism of injury, DBS, associated injuries (head, spinal cord, thoracic, abdominal and pelvic-perineal traumas), fractures, vascular injury, treatments performed, number of skin grafts, number of necessary interventions, graft integration rate, length of stay, complications and mortality.

We defined primary graft the one performed in the first 12 hours after admission, with the use of skin from the degloved area, in full or partial thickness, and late, the graft carried out after this period, with skin from non-traumatized donor sites.

## RESULTS

The study series comprised 47 patients, 22 (47%) evaluated early on and 25 (53%) late. Thirty-three (70%) patients were male and 14 (30%) female, with a mean age of 30.6 years (2-72, SD 18.8). There was no statistically significant difference in the comparison between gender (p=0.775) and age (p=0.091). The most common trauma mechanism was trampling, followed by motorcycle accidents in both groups (Figure 1a). There was also no statistically significant difference when comparing the trauma mechanisms (p=0.542). The average degloved body surface (DBS) was 8.2% (3-22%, SD = 4.5). There was no statistically significant difference when comparing the average DBS (Figure 1b) between groups (p=0.5). There were associated lesions (brain injury, spinal cord, thoracic, abdominal, pelvic-perineal) in 20 patients (42.5%); 33 (70%) presented associated fractures (Table 1). We observed a statistically significant difference when comparing the occurrence of fractures in Groups I and II, with no statistically significant difference as for the presence of vascular injuries and other associated injuries.

The mean hospital stay was 47.3 days (7-239, SD = 40). When comparing the length of stay and number of skin grafts, there were statistically significant differences (Table 2). Patients evaluated early underwent 14 primary graftings, two late graftings (15th and 17th day of hospitalization), two primary syntheses, two primary amputations with primary grafting and two primary amputations. The posteriorly evaluated ones underwent 18 late graftings (after the 14th up until the 30th day), three primary syntheses, three amputations and one debridement.

In the 16 patients undergoing primary grafting (Group I), DBS varied from four to 16%, there being

**Table 1 -** Comparative data on fractures, vascular injuries and associated injuries.

| Group | Fractures | | Vascular injuries | | Associated injuries | |
|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No |
| GI | 12 (54.5%) | 10 (45.5%) | 8 (36.4%) | 14 (63.6%) | 8 (36.4%) | 14 (63.6%) |
| GII | 21 (84%) | 4 (16%) | 5 (20%) | 20 (80%) | 12 (48%) | 13 (52%) |
| GI x GII (p) | 0.028 | | 0.211 | | 0.421 | |

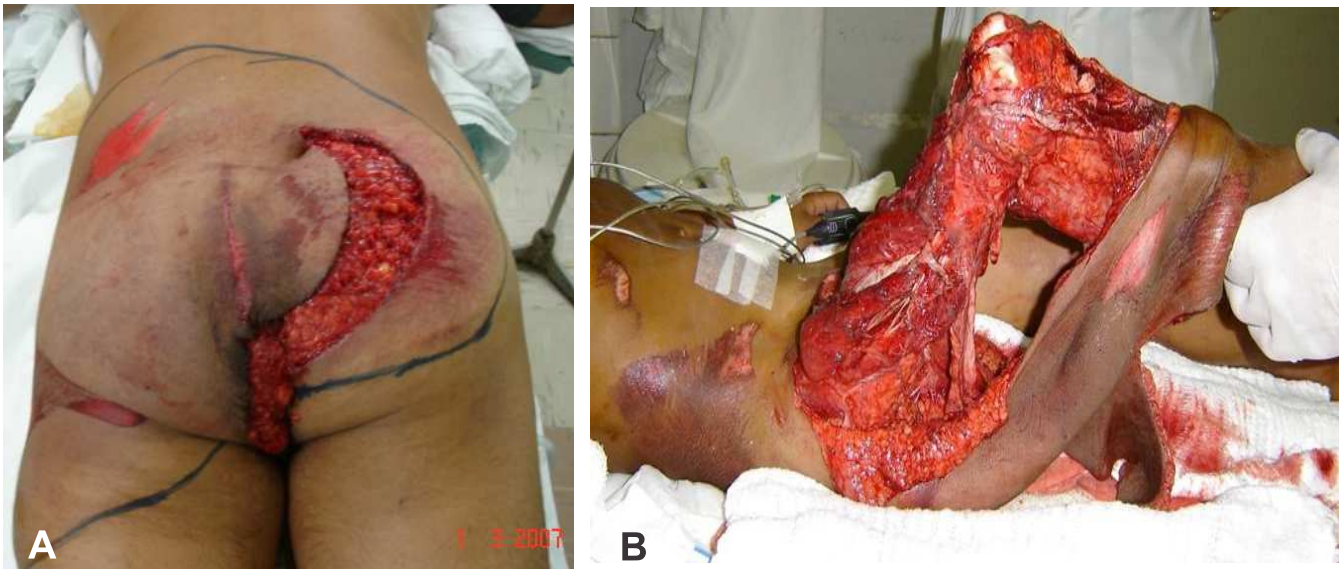


***Figure 1a*** – *Victim of motorcycle accident followed by trampling. Degloving Area Extension after demarcation. Figure 1b - Circumferential open degloving in lower limb - initial evaluation in the emergency room.*

integration of more than 95% in ten cases (Figures 2a and 2b), without the need for further skin-covering procedures. In the six other cases, there was integration of about 50% in three cases and subtotals losses in the other patients (who developed hemodynamic instability and need for abdominal and thoracic reoperations). In two cases we used skin from the areas of amputated limbs not affected by degloving.

In cases where primary grafting was not possible, it was necessary to wait a period of 14 to 30 days to begin the cover (late grafting). During this period we performed debridement and serial dressing exchanges until the healing wound presented an appropriate bed (granulation tissue). We used non-traumatized skin from donor sites mainly in the lower limbs and trunk. The evolution of these patients after grafting was satisfactory as for graft integration (above 95%).

We observed complications in 29 (62%) patients (Table 3). In 12 patients there was the development of infection in the degloved area, of which seven were evaluated late and five early. Among patients undergoing primary synthesis (5 cases), three developed infection (Figures 3a and 3b), which did not occur in any patient undergoing primary grafting. Two patients (4.2%) died after 15 and 25 days of hospitalization.

## DISCUSSION

Deglovings occur more frequently in males, since they are a condition consequent to trauma. The extent and the severity of injuries vary widely, making comparative analysis difficult. There may be extensive deglovings in patients without associated fractures or vascular injuries, as well as small extension injuries, injuries associated with fractures, vascular injuries and / or other associated injuries[11-13].

Fractures are present in 40-85% of cases in the degloving-affected areas, and the reduction and fixation should take place before the soft tissue approach, which may explain, though not delay, the Plastic Surgery assessment[2,7,8].

Associated injuries – head, spinal, abdominal, pelvic-perineal and chest traumas – are present in most patients, and there may be the need for operations to control

**Table 2** - Comparative data related to length of stay and number of skin grafts between groups.

| Group | Length of stay | | Number of grafting skin | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| GI | 36.1 | 29.2 | 1.3 | 1.1 |
| GII | 57.1 | 45.9 | 1.6 | 1.3 |
| GI x GII (p) | 0.026 | | 0.034 | |

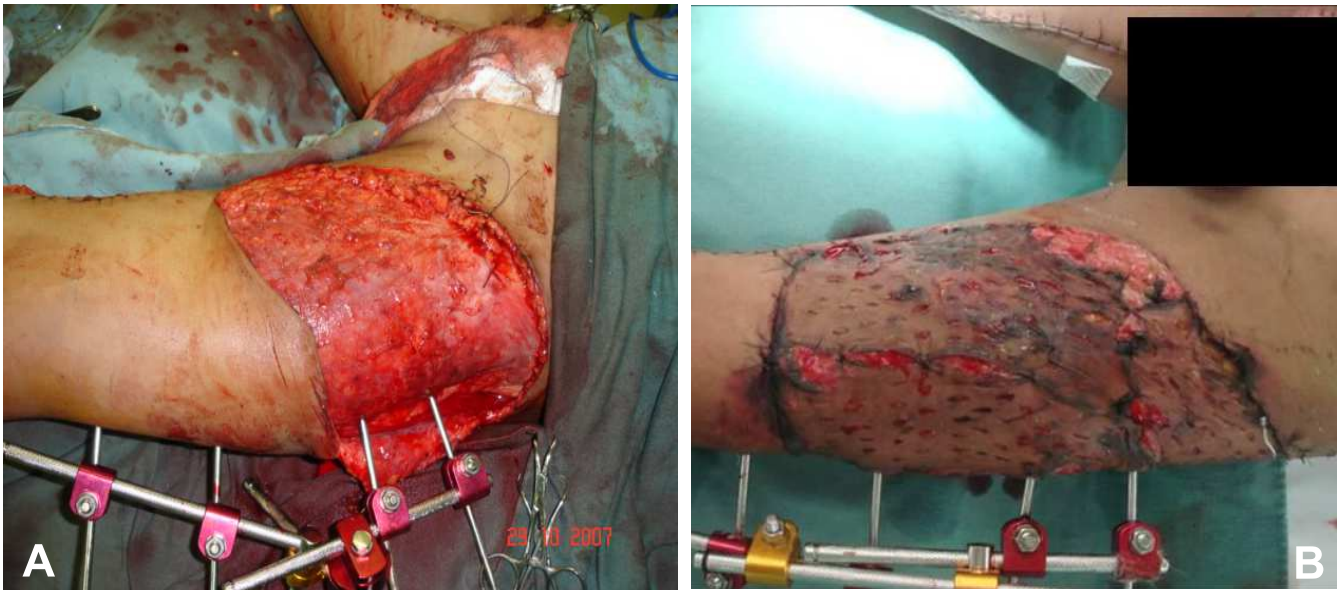


***Figure 2a*** – *Degloving in left lower limb. Intraoperative evaluation after external fixation for femur fracture. Figure 2b - Degloving in left lower limb. After treatment – primary grafting, partial thickness skin – fifth postoperative day – full integration.*

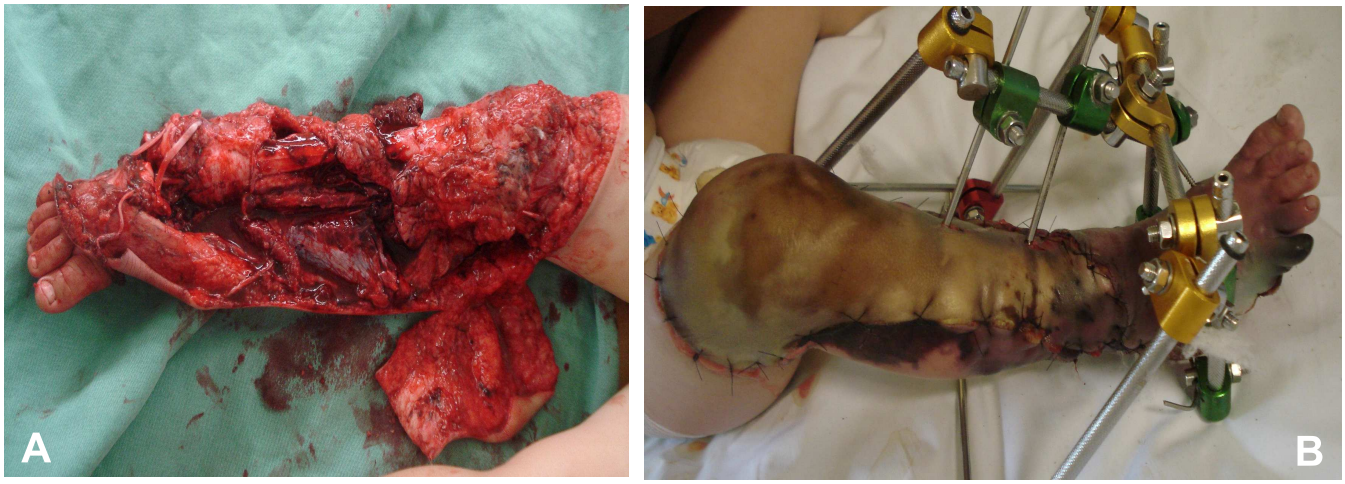


***Figure 3a*** – *Circumferential degloving of lower limb. Figure 3b - Circumferential degloving of lower limb - underwent primary synthesis. Fifth postoperative day – cutaneous ischemia and infection.*

**Table 3** - Number of complications (29 patients).

| Complications | GI (n=13) | GII (n=16) |
|---|---|---|
| Infection / necrosis of skin, soft tissue and amputation stump | 7 | 12 |
| Urinary infection | 4 | 6 |
| Problems related to synthesis and fixing materials | 4 | 5 |
| Pulmonary complications | 2 | 5 |
| Transfusion reaction / coagulopathy | 4 | 7 |
| Vascular complications | 2 | 2 |
| Pressure ulcer | 1 | 3 |
| Kidney failure | 1 | 1 |
| Other | 2 | 1 |

life-threatening injuries. In such critical situations, simultaneous and rapid participation of the plastic surgeon may be beneficial to the removal and preparation of the traumatic flap for later use, the skin being stores in a tissue bank[3,11,13].

We observed no statistical differences between groups regarding the following variables: gender, age, percentage of degloved body surface (% DBS), mechanism of trauma, presence of vascular injury and associated injuries. Thus, we could consider the groups comparable. On the other hand, there was statistical difference when analyzed the incidence of fractures, which was significantly higher in Group II. This may reflect the greater attention given to the fractured patient by the team performing the initial assessment and justify any delay of evaluation request for the Plastic Surgery team.

According Kudsk *et al.*, the principles of local treatment consists in evaluating the viability of the flaps, debridement of necrotic or mutilated tissues, use of non-viable flap areas as donor of skin grafts in partial or total thickness, fixation and immobilization of both grafts and fractures[2].

Controversy exists regarding the best option as to grafts taken from the traumatic flap, whether in partial or full thickness. Ideally, all available skin should be used, even when there is evidence of friction burns[1-3,5,9-12]. If there are no conditions for integration, this skin will work temporarily as a biological dressing.

Total skin grafts usually have better functional and aesthetic results due to lower secondary contracture. Grafting in partial thickness may be indicated for more critical situations, considering the greatest chance of integration, with lower aesthetic results, especially when subjected to prior expansion[14-17], although there seems to be significant difference in the rates of integration between the different graft thicknesses.

Primary synthesis should be avoided in areas with extensive deglovings, as noted by numerous authors[1-3,5,17]. We used it in five patients, with 80% complication rate (total necrosis of the repositioned traumatic flap), as well as local infection in three cases.

Hospital stay was prolonged in both groups (36 days in Group I and 57 in Group II), although significantly higher in Group II. Kudsk et al. reported an average hospi-

tal stay of 68 days[2]. Milcheski *et al*. described a mean hospital stay of 46.2 days for patients undergoing primary suture and 32.5 days for patients undergoing primary grafting ($p<0.001$)[18].

The variations in number, severity and heterogeneity of associated injuries, which occurred in 42.5% of patients, should be considered, since they may have impact on mortality and on the need for additional tests and procedures during the initial assessment. The presence of fractures and / or vascular lesions, even if isolated, could delay the perceived need for evaluation request by the plastic surgeon[17,19,20].

We observed that the determinant of hospital stay was the presence of extensive degloving area, especially in patients who did not receive the appropriate initial treatment. There was the need to wait for a delimitation of ischemic tissues and the subsequent healing (granulation) of the bed before grafting. Importantly, in these situations there is need of skin taken from non-traumatized donor areas, which may require multiple procedures[8,18,21].

Early coverage of the bloody areas decreases protein and electrolyte losses, as well as the basal energy expenditure, the need for dressing changes, the costs, the anesthetic risk, length of stay and functional sequelae[22,23].

Importantly, during the initial evaluation of the polytrauma patient, the plastic surgeon must be present to contribute to a better result in an attempt to decrease complications and mortality, as well as the length of stay and number of operations, as demonstrated in this study. For this, the awareness of multidisciplinary teams is fundamental[18,24,25].

The analysis of the data from this study shows that early assessment and the possibility of primary grafting have a positive impact on the evolution of patients victims of degloving, resulting in a shorter hospital stay and less operations.

## R E S U M O

***Objetivo***: *analisar os casos de desenluvamentos de tronco e membros, comparando os resultados da avaliação precoce ou tardia pela equipe de cirurgia plástica.* ***Métodos****: análise retrospectiva de prontuários. Os pacientes foram separados em dois grupos: Avaliação precoce – Grupo I (realizada no intervalo de até 12 horas após o trauma) e Avaliação tardia – Grupo II (realizada mais de 12 horas após o trauma). Definiu-se como enxertia primária aquela realizada com pele proveniente do retalho traumático. Foram excluídos os casos com acometimento de mãos, pés ou genitália.* ***Resultados:*** *foram atendidos 47 pacientes. A superfície corporal lesada média foi 8,2%. Os membros inferiores foram os locais mais acometidos, em 95,7%, isoladamente ou em associação com lesões em outros locais. A avaliação da Cirurgia Plástica foi solicitada tardiamente em 25 casos. Observou-se tempo médio de internação de 36,1 dias para o grupo I e de 57,1 para o grupo II (p=0,026). Em relação ao número de cirurgias (enxertias de pele), observou-se média de 1,3 no grupo I e 1,6 no grupo II (p=0,034).* ***Conclusão:*** *em doentes politraumatizados, vítimas de desenluvamento de tronco e membros, podemos concluir, no que se refere ao tempo de internação e número de operações, que a avaliação da Cirurgia Plástica deve ser precoce.*

***Descritores:*** *Transplante de pele. Lesões dos Tecidos Moles. Técnicas de Fechamento de Ferimentos. Procedimentos Cirúrgicos Dermatológicos. Fáscia/cirurgia.*

## REFERENCES

1. Mandel MA. The management of lower extremity degloving injuries. Ann Plast Surg. 1981;6(1):1-5.
2. Kudsk KA, Sheldon GF, Walton RL. Degloving injuries of the extremities and torso. J Trauma. 1981;21(10):835-9.
3. Hidalgo DA. Lower extremity avulsion injuries. Clin Plast Surg. 1986;13(4):701-10.
4. Askins G, Finley R, Parenti J, Bush D, Brotman S. High-energy roller injuries to the upper extremity. J Trauma. 1986;26(12):1127-31.
5. Slack CC. Friction injuries following road accidents. Br Med J. 1952;2(4778):262-4.
6. Tseng S, Tornetta P 3rd. Percutaneous management of Morel-Lavallee lesions. J Bone Joint Surg Am. 2006;88(1):92-6.
7. Phillips TJ, Jeffcote B, Collopy D. Bilateral Morel-Lavallée lesions after complex pelvic trauma: a case report. J Trauma. 2008;65:708-11.
8. Mello DF, Demario LA, Soldá SC, Helene Jr A. Desenluvamentos fechados: lesão de Morel-Lavallée. Rev Bras Cir Plást. 2010;25(2):355-60.
9. Farmer AW. Treatment of avulsed skin flaps. Ann Surg. 1939;110(5):951-9.
10. McManus WF, Pruit BA. Thermal injuries. In: Mattox RH, Moore EE, Feliciano DV, editors. Trauma. 2nd ed. Norwalk: Appleton and Lange; 1991. p. 751-64
11. MacKenzie EJ, Bosse MJ, Kellam JF, Burgess AR, Webb LX, Swiontkowski MF, et al. Factors influencing the decision to amputate or reconstruct after high-energy lower extremity trauma. J Trauma. 2002;52(4):641-9. Erratum in: J Trauma. 2002;53(1):48.
12. Southern SJ, Hart NB, Venkatramakishnan V, Nieuwoudt F, Villafane O. Lower limb salvage using parts of the contralateral amputated leg. Injury. 1997;28(7):477-9.
13. Steiner CL, Trentz O, Labler L. Management of Morel-Lavallee lesion associated with pelvic and/or acetabular fractures. Eur J Trauma Emerg Surg. 2008; 34(6):554-60.
14. Meara JG, Guo L, Smith JD, Pribaz JJ, Breuing KH, Orgill DP. Vacuum-assisted closure in the treatment of degloving injuries. Ann Plast Surg. 1999;42(6):589-94.
15. Josty IC, Ramaswamy R, Laing JH. Vacuum assisted closure: an alternative strategy in the management of degloving injuries of the foot. Br J Plast Surg. 2001;54(4):363-5.
16. Khan U, Ho K, Deva A. Exchanging split-skin grafts to reduce donor morbidity in limited pretibial degloving injuries. Plast Reconstr Surg. 2004;113(5):1523-5.

17. Arnez ZM, Khan U, Tyler MP. Classification of soft-tissue degloving in limb trauma. J Plast Reconstr Aesthet Surg. 2010;63(11):1865-9.
18. Milcheski DA, Ferreira MC, Nakamoto HA, Tuma Jr P, Gemperli R. Tratamento cirúrgico dos ferimentos descolantes nos membros inferiores: proposta de protocolo de atendimento. Rev Col Bras Cir. 2010;37(3):199-203.
19. Jeng SF, Wei FC. Technical refinement in the management of circumferentially avulsed skin of the leg. Plast Reconstr Surg. 1997;100(6):1434-41.
20. Ergün SS, Erözgen F, Akdemir OC, Ziyade S, Içten S, Egeli U. Missed closed degloving injury of the sacro-gluteal region. Eur J Plast Surg. 2010;33(1):41-44.
21. Hopgood B, Bessey PQ, Thorpe J, Blanton J, Yurt RW. Burn Center Management of Degloving Injuries: 177. In: Proceedings of the 38th Annual Meeting. April 4-7, 2006; Las Vegas, Nevada; 2006. 27(2) Suppl:S138 (J Burn Care Res. 2006;27(2):S138).
22. Sarlak AY, Buluç L, Alc T, Alponat A. Degloving injury of pelvis treated by internal fixation and omental flap reconstruction. J Trauma. 2006;61(3):749-51.
23. Schneider AM, Morykwas MJ, Argenta LC. A new and reliable method of securing skin grafts to the difficult recipient bed. Plast Reconstr Surg. 1998;102(4):1195-8.
24. Mellado JM, Pérez del Palomar L, Díaz L, Ramos A, Saurí A. Long-standing Morel- Lavallée lesions of the trochanteric region and proximal thigh: MRI features in five patients. AJR Am J Roentgenol. 2004;182(5):1289-94.
25. Morris M, Schreiber MA, Ham B. Novel management of closed degloving injuries. J Trauma. 2009;67(4):E121-3.

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**Address for correspondence:**
Daniel Francisco Mello
E-mail: mello.plastica@gmail.com

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# Inguinal hernia repair: can one identify the three main nerves of the region?

## *Herniorrafia inguinal: pode-se identificar os três principais nervos da região?*

João Vicente Machado Grossi, AsCBC-RS[1]; Leandro Totti Cavazzola, TCBC-RS[2]; Ricardo Breigeiron TCBC-RS[3]

A B S T R A C T

**Objective:** To identify the nerves in the groin during inguinal hernia repair by inguinotomy. **Methods**: We conducted a prospective, sequenced, non-randomized study comprising 38 patients undergoing inguinal hernia repair with placement of polypropylene mesh. **Results**: The male patients were 36 (94.7%), with a mean age and standard deviation of 43.1 ± 14.5, body mass index of 24.4 ± 2.8. Comorbidities were hypertension in two (5.2%), smoking in 12 (31.5%) and obesity in two (5.2%). The hernia was located only on the right in 21 (55.2%) patients, only on the left in 11 (28.9%), and was bilateral in six (15.7%) patients. Prior hernia repair was present in seven (18.4%) patients. The identification of the three nerves during operation was made in 20 (52.6%) patients, the ilioinguinal nerve and the iliohypogastric nerve were identified in 33 (86.8%), and the genital nerve branch of the genitofemoral nerve, in 20 (52.6%). Resection of at least one of the nerves was performed in seven (18.4%) cases, two iliohypogastric nerves and five ilioinguinal nerves. The average operating time was 70.8 ± 18.2 minutes. The hospital stay was 1.42 ± 1.18 days. Ten patients (26.3%) returned to physical activity around the first postoperative visit, and 37 (97.3%) in the last. The follow-up time was 95.6 ± 23.5 days. The inability to identify the ilioinguinal nerve was associated with previous repair (p = 0.035). **Conclusion**: The identification of the three nerves during inguinal hernia surgery has been described in more than half of the cases and prior repair interfered with the identification of ilioinguinal nerve.

**Key words:** Inguinal Hernia. Hernia repair. Peripheral Nerves. Chronic Pain / surgery. Neuralgia / surgery.

## INTRODUCTION

Hernias correspond to total or partial protrusion of an organ contained in a sac of peritoneal lining outside of the abdominal wall through a musculo-aponeurotic defect[1-3]. They may occur at various positions: umbilical (10%), epigastric (6%), incisional (10%), femoral (5%) or inguinal (69%)[1,4].

The only way to treat hernias is through hernioplasty[5]. There are numerous techniques for the surgical repair of abdominal wall defects and they have evolved in recent years. Basically, they are divided into techniques that use only primary aponeurotic suture – which must be free of tension – and techniques using synthetic prostheses (meshes)[6].

The repair of inguinal hernias carried out through with a mesh and tension-free surgical technique, introduced in 1989 by Lichtenstein, is widely used and presents growing popularity among surgeons[7]. Some studies showed that the techniques that use the patient's own tissue for hernial repair exhibit a relapse of 10 to 50%, while the use of prostheses reduces relapse to 3 to 17%[8,9]. In the United States, more than 90% of patients with abdominal wall hernias are subjected to correction with the use of prosthetic materials. Worldwide, it is estimated that one million such implants are used annually[6]. Despite the popularity and increased use of polypropylene mesh in hernioplasties, there are numerous complications arising from its use. Due to the mesh's large penetration capacity when in contact with intraperitoneal viscera, complications can occur such as adhesions, fibrosis, chronic pain, fistula and intestinal obstruction. Therefore, its contact with intra-abdominal organs is not recommended[4].

Chronic pain (inguinodinia) is an important postoperative complication and is associated with neural tissue damage during the operation, as well as with the healing process produced by the suture or the mesh itself. Such complications negatively impact the patient's quality of life. The identification and preservation of all three groin nerves – nerve ilioinguinal, iliohypogastric and the genital branch of the genitofemoral – during hernia correction by the open technique reduces the risk of chronic pain.

This study aims to identify the groin nerves during inguinal hernia repair by inguinotomy.

1. Disciplina de Clínica Cirúrgica do Adulto I, Faculdade de Medicina de Barbacena – MG - Brazil; 2. Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG) - MG - Brazil.

## METHODS

This was a prospective, sequenced, non-randomized study. The sample consisted of 38 patients who underwent hernia repair with polypropylene mesh placement and were evaluated during 12 months.

This study was approved by the Comitê de Ética da Prefeitura Municipal de Porto Alegre, under the registration number 001.031967.12.4. All patients were informed of the survey by informed consent. All procedures were supervised by a responsible medical preceptor.

The study examined the identification of nerves in the groin during inguinal hernia repair procedure, primary or recurrent, by the Lichtenstein technique. We used the Nyhus classification[10] to categorize the hernias.

We evaluated the following patients data: gender, age, body mass index (BMI) and associated comorbidities, including hypertension (SAH), smoking and obesity. We also considered in the analysis: the location of the hernia, the previous repair, the length of stay, type of daily physical activity, the return to work activities, the follow-up and surgical indication (emergency or elective). After the procedure, all patients were transferred to the recovery room with standard analgesia (dipyrone 1g EV 6/6h + morphine 4mg 4/4h, in case of strong pain). After the surgery, the description of the technique was filled according to the nerve identification protocol. In the immediate postoperative period, the patient was discharged the next day with recommendations established by the institution and outpatient return in seven days, accompanied by three more consultations. If patients did not refer pain during the postoperative evaluation in 90 days, they were released from monitoring. Should they have any symptoms, they remained in follow-up until the sixth postoperative month.

Statistical analysis was described by mean and standard deviation for quantitative variables. The qualitative variables were described by absolute and relative frequencies. Association between variables was evaluated by the Pearson's chi-square test or Fisher's exact test. The residues adjusted test was used to complement the associations with polytomic variables. The significance level was 5% ($p < 0.05$).

## RESULTS

All patients could be evaluated in the proposed period. Upon characterization of the sample there was a predominance of male patients, 36 (94.7%), with a mean age and standard deviation of 43.1 ± 14.5 years. The body mass index (BMI) was 24.4 ± 2.8kg/m$^2$, and obesity (BMI> 30 kg/m$^2$) was present in only two patients (5.2%).

The most prevalent type of daily physical activity was laborer, with 26 (68.4%), followed by administrative, with eight (21.1%). Two (5.2%) patients were athletes, one (2.6%) had physical disabilities and one (2.6%) was retired. The associated comorbidities identify were systemic arterial hypertension (SAH) in two (5.2%), alcoholism in one (2.6%), smoking in 12 (31.5%) and benign prostatic hyperplasia in one (2.6 %). Twenty-two (57.9%) patients were healthy.

The positioning of hernias was divided into right only, 21 (63.2%) patients, only on the left, 11 (36.8%) and bilateral, six (15.7%). Seven patients had previous repair, considered recurrent hernia, with the following techniques distribution: Bassini in one patient Shouldice in three, Lichtenstein in one and other techniques not identified during repair in two. Elective surgery was the most accomplished, with 31 (81.6%), and urgency, in seven (18.4%). The classification of the American Society of Anesthesiologists (ASA) was I in 29 (76.3%) and II in the remaining, not being performed surgeries in patients classified as ASA III and IV. Spinal anesthesia was performed in 36 (94.7%) and only two patients needed general anesthesia due to prolonged surgical time and intraoperative bleeding. The associated procedures were orchiectomy (n=1, 2.6%) and cord cyst excision (n=1, 2.6%).

Regarding the identification of nerves in the inguinal region during the procedure, the ilioinguinal nerve was identified in 20 (52.6%) patients, the iliohypogastric in 33 (86.8%) and the genital nerve branch of the genitofemoral in 20 (52.6%) patients (Figure 1).

Resection of at least one nerve was performed in seven (18.4%) patients due to technical difficulties, in two cases the iliohypogastric and in five, the ilioinguinal. The average operating time was 70.8 ± 18.2 minutes. The completion of the procedure was performed by a first-year resident in 21 (55.3%) cases and by a second-year one in 17 (44.7%). As a main auxiliary, 21 (55.3%) cases were made by a resident of the first year, 16 by a second-year and one by a medical school graduate.

As for the Nyhus classification, type 3a was predominant, with 17 (44.7%) patients, followed by type 2 in nine (23.6%), type 4 in seven (18.4%), type 3b in three

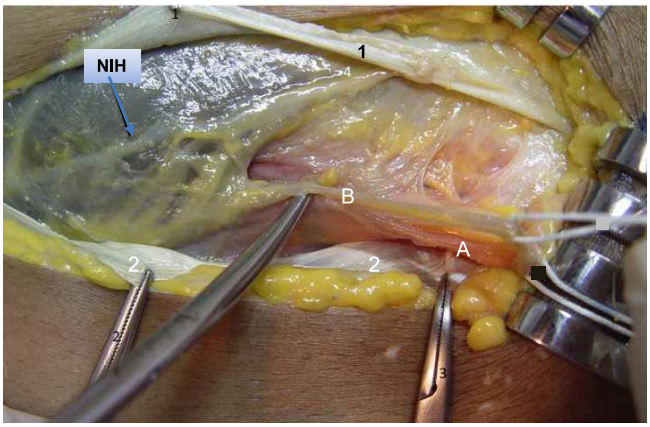


***Figure 1*** - *Identification of inguinal nerves.*

*A- inguinal cord; B- Iiioinguinal nerve; IHN- iliohypogastric nerve; 1) aponeurosis of the external oblique muscle; 2) Inguinal ligament.*

(7.8%) and the types 1 and 3c each with one patient (2.6%) (Table 1).

The most commonly used mesh was the polypropylene one, with medium weight (65mg/cm$^3$), in 36 (94.7%) patients. The remaining patients had the heavy weight polypropylene mesh (99mg/cm$^3$) implanted.

After the surgical procedure there were no complications in 32 (84.2%) patients. Hematoma occurred in five patients and seroma in one. The mean and standard deviation of hospital stay was 1.42 ± 1.18 in days, with 30 (79%) patients staying overnight, and more than 24 hours in only eight (21.1%) cases. As regards the personal response to surgery, in the first outpatient (seventh day after surgery) 24 (63.2%) patients answered that there was a significant improvement, 13 (34.2%) said that there was some improvement and one (2.6%), that there was no change.

Ten (26.3%) patients had returned to physical activity by their first visit, whereas 37 (97.4%) had returned by the last visit. The mean follow-up time was 95.6 ± 23.5 days.

When assessing the type of surgical indication, the identification of nerves in the inguinal region of the anterior wall did not show statistically significant differences between elective or emergency procedures. Although not statistically significant, the identification of the genital branch of genitofemoral nerve was lower in emergency operations, (p = 0.222,Table 2).

Prior repair – recurrent hernia – had an increased operative time and also showed statistically significant association in not identifying the ilioinguinal nerve (Table3).

## DISCUSSION

There was no need to exclude any patient. It was possible to monitor all patients for at least 90 days after surgery. The identification did not change operating time or required some special training. Thus, the study was feasible and reproducible, even in emergency procedures.

The sample's associated comorbidities found in this study were in only two patients with obesity criteria, which could contribute to greater technical difficulty and decreased nerves identification. Another comorbidity, such as smoking, prevalent in the sample, is a characteristic that can be modified. Cigarette smoking increases the risk of herniation development and, consequently, local symptoms increase, increasing the risk of turning an elective procedure into an emergency one[11].

The percentage of identification of the three nerves during the procedure was 52.6%, whereas the highest index described in publications is only 36%. The identification of the ilioinguinal and iliohypogastric nerves was 86.8% in our sample. The literature shows a tendency to identify the ilioinguinal nerve around 70%, and the iliohypogastric, of 59 %. This difference can be explained by the active search and identification of nerves during the procedure. Regarding the genital branch of the genitofemoral, we found in 56.2% of cases, corroborating the results from the literature, of 55.6% [12,13].

The identification of nerves following the anatomical description of the order of appearance on the

**Table 1** - Distribution of patients according to the Nyhus classification [10].

| NYHUS | Patients / Percentage |
|---|---|
| Type1 | 1 / 2.6% |
| Type 2 | 9 / 23.6% |
| Type 3a | 17 / 44.7% |
| Type 3b | 3 / 7.8% |
| Type 3c | 1 / 2.6% |
| Type 4 | 7 / 18.4% |
| Total | 38 / 100% |

**Table 2** - Identification of nerves in the inguinal region during hernia repair and its association with the type of surgical indication.

| Variables | Emergency surgery n (%) | Elective surgery n (%) | P |
|---|---|---|---|
| Iiioinguinal nerve | | | 1.000* |
| Yes | 6 (85.7) | 27 (87.1) | |
| No | 1 (14.3) | 4 (12.9) | |
| Iliohypogastric nerve | | | 1.000* |
| Yes | 6 (85.7) | 27 (87.1) | |
| No | 1 (14.3) | 4 (12.9) | |
| Genital branch of GF | | | 0.222* |
| Yes | 2 (28.6) | 18 (58.1) | |
| No | 5 (71.4) | 13 (41.9) | |

** Fisher's exact test.*
*GF= genitofemoral nerve.*

**Table 3** - Type of operation with identification of groin nerves during hernia repair.

| Variables | With prior repair n (%) | Without repair n (%) | p * |
|---|---|---|---|
| Iiioinguinal nerve | | | 0.035 |
| Yes | 4 (57.1) | 29 (93.5) | |
| No | 3 (42.9) | 2 (65) | |
| Iliohypogastric nerve | | | 1.000 |
| Yes | 6 (85.7) | 27 (87.1) | |
| No | 1 (14.3) | 4 (12.9) | |
| Genital branch of GF | | | 0.687 |
| Yes | 3 (42.9) | 17 (54.8) | |
| No | 4 (57.1) | 14 (45.2) | |

** Fisher's exact test.*
*GF= genitofemoral nerve.*

operation steps would have a higher rate of findings, with consequent correct identification of the groin nerves. However, when faced with a non-virgin area – recurrent hernia – or with a mesh already integrated to the tissue, one has a lower chance of isolating the nerves. In this sample, recurrent hernia showed a statistically significant difference in not identify the ilioinguinal nerve[13].

Even with training residents, it was possible to identify the three nerves in the inguinal region in more than half of cases. The study of the inguinal region and its anatomy should be part of the learning program and subsequent formation of new surgeons as a prevention of adverse effects of the procedure[14].

Even with the use of new techniques such as the use of PHS mesh, one can used the nerves identification to monitor cases of chronic pain or numbness associated with the procedure[15].

In conclusion, the identification of the three nerves during inguinal hernia surgery has been reported in over half of cases, and the prior repair interfered with the identification of ilioinguinal nerve.

## RESUMO

***Objetivo:*** *identificar os nervos da região inguinal durante hernioplastia inguinal por inguinotomia.* ***Métodos:*** *estudo prospectivo, sequenciado, não randomizado, composto por 38 pacientes submetidos à herniorrafia inguinal com colocação de tela de polipropileno.* ***Resultados:*** *Os pacientes masculinos eram 36 (94,7%), com média de idade e desvio-padrão de 43,1 ± 14,5, índice de massa corporal de 24,4 ± 2,8. As comorbidades eram HAS em dois (5,2%), tabagismo em 12 (31,5%) e obesidade em dois (5,2%). A hérnia localizava-se somente à direita em 21 (55,2%) pacientes, somente à esquerda em 11 (28,9%), e era bilateral em seis (15,7%) pacientes. O reparo prévio da hérnia foi feito em sete (18,4%) pacientes. A identificação dos três nervos durante a operação fez-se em 20 (52,6%) pacientes, o nervo ílio-inguinal e o nervo ílio-hipogástrico foram identificados em 33 (86,8%), e o ramo genital do nervo gênito-femoral em 20 (52,6%). A ressecção de ao menos um dos nervos foi realizada em sete (18,4%), sendo dois nervos ílio-hipogástricos e cinco nervos ílio-inguinais. O tempo médio de operação foi 70,8 ± 18,2 minutos. O tempo de internação hospitalar foi 1,42 ± 1,18 dias. Retornaram à atividade física no primeiro atendimento dez (26,3%) pacientes e, no último, 37 (97,3%). O tempo de acompanhamento foi 95,6 ± 23,5 dias. A impossibilidade de identificação do nervo ílio-inguinal associou-se ao reparo prévio (p=0,035).* ***Conclusão:*** *a identificação dos três nervos durante a hernioplastia inguinal foi descrito em mais da metade dos casos e o reparo prévio interferiu na identificação do nervo ílio inguinal.*

***Descritores:*** *Hérnia Inguinal. Herniorrafia. Nervos Periféricos. Dor Crônica/cirurgia. Neuralgia/cirurgia.*

## REFERENCES

1. Dabbas N, Adams K, Pearson K, Royle G. Frequency of abdominal wall hernias: is classical teaching out of date? JRSM Short Rep. 2011;2(1):5.
2. Lambert B, Manna BB, Montes JHM, Bigolin AV, Grossi JVM, Cavazzola LT. Does collagen-coated polyester mesh decrease teh rate of intraperitoneal adhesions in incisional hérnia repair? Bras J Video-Sur. 2012;5(1):37-43.
3. LeBlanc KA, Bellanger D, Rhynes KV 5th, Baker DG, Stout RW. Tissue attachment strength of prosthetic meshes used in ventral and incisional hernia repair. A study in the New Zeland White rabbit adhesion model. Surg Endosc. 2002;16(11):1542-6.
4. Puttini SMB. Avaliação da resposta inflamatória desencadeada pelas telas de polipropileno e politetrafluoretileno expandido im-

plantadas no espaço intraperitoneal. Estudo experimental em camundongos [dissertação]. Brasília, DF: Universidade de Brasília, Faculdade de Medicina; 2006.
5. Demir U, Mihmanli M, Coskun H, Dilege E, Kalyoncu A, Altinli E, et al. Comparison of prosthetic materials in incisional hernia repair. Surg Today. 2005;35(3):223-7.
6. N N, R S. A comparative study between modified Bassini's repair and Lichtenstein mesh repair (LMR) of inguinal hernias in rural population. J Clin Diagn Res. 2014;8(2):88-91.
7. Mizrahi H, Parker MC. Management of asymptomatic inguinal hernia: a systematic review of the evidence. Arch Surg. 2012;147(3):277-81.
8. Seiler C, Baumann P, Kienle P, Kuthe A, Kuhlgatz J, Engemann R, et al. A randomised, multi-centre, prospective, double blind pilot-study to evaluate safety and efficacy of the non-absorbable Optilene Mesh Elastic versus the partly absorbable Ultrapro Mesh for incisional hernia repair. BMC Surg. 2010;10:21.
9. Pundek MRZ, Czeczko NG, Yamamoto CT, Pizzatto RF, Czeczko LEA, Dietz UA, et al. Estudo das telas cirúrgicas de polipropileno/poliglecaprone e de polipropileno/polidioxanona/celulose oxidada regenerada na cicatrização de defeito produzido na parede abdominal em ratos. ABCD, arq bras cir dig. 2010:23(2) 94-9.
10. Nyhus LM, Klein MS, Rogers FB. Inguinal hernia, Curr Probl Surg. 1991;28(6):401-50.
11. Rosemar A, Angerås U, Rosengren A. Body mass index and groin hernia: a 34-year follow-up study in Swedish men. Ann Surg. 2008:247(6):1064-8.
12. Alfieri S, Rotondi F, Di Giorgio A, Fumagalli U, Salzano A, Di Miceli D, et al. Influence of preservation versus division of ilioinguinal, iliohypogastric, and genital nerves during open mesh herniorrhaphy: prospective multicentric study of chronic pain. Ann Surg. 2006:243(4):553-8.
13. Wijsmuller AR, Lange JF, Kleinrensink GJ, van Geldere D, Simons MP, Huygen FJ, et al. Nerve-identifying inguinal hernia repair: a surgical anatomical study. World J Surg. 2007;31(2):414-20; discussion 421-2.
14. Ergül Z, Kulaço□lu H, Sen T, Esmer AF, Güller M, Güneri G, et al. A short postgraduate anatomy course may improve the junior surgical residents' anatomy knowledge for the nerves of the inguinal region. Chirurgia. 2011;106(5):599-603.
15. Mottin CC, Ramos RJ, Ramos MJ. Utilização do sistema prolene de hérnia (SPH) para o reparo de hérnias inguinais. Rev Col Bras Cir. 2011,38(1):24-7.

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**Address for correspondence:**
João Vicente Machado Grossi
E-mail: jvicentegrossi@gmail.com

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# Predictive factors for short gastric vessels division during laparoscopic total fundoplication

## *Fatores preditivos da necessidade de secção dos vasos gástricos curtos nas fundoplicaturas totais videolaparoscópicas*

Alexandre Chartuni Pereira Teixeira[1]; Fernando Augusto Mardiros Herbella[1]; Adorísio Bonadiman[2]; José Francisco de Mattos Farah, ACBC-SP[2]; José Carlos Del Grande, TCBC-SP[1]

A B S T R A C T

**Objective:** to determine clinical variables that can predict the need for division of the short gastric vessels (SGV), based on the gastric fundus tension, assessing postoperative outcomes in patients submitted or not to section of these vessels. **Methods**: we analyzed data from 399 consecutive patients undergoing laparoscopic fundoplication for gastroesophageal reflux disease (GERD). The section of the SGV was performed according to the surgeon evaluation, based on the fundus tension. Patients were divided into two groups: not requiring SGV section (group A) or requiring SGV section (group B). **Results**: the section was not necessary in 364 (91%) patients (Group A) and required in 35 (9%) patients (Group B). Group B had proportionally more male patients and higher average height. The endoscopic parameters were worse for Group B, with larger hiatal hernias, greater hernias proportion with more than four centimeters, more intense esophagitis, higher proportion of Barrett's esophagus and long Barrett's esophagus. Male gender and grade IV-V esophagitis were considered independent predictors in the multivariate analysis. Transient dysphagia and GERD symptoms were more common in Group B. **Conclusion**: the division of the short gastric vessels is not required routinely, but male gender and grade IV-V esophagitis are independent predictors of the need for section of these vessels.

**Key words:** Fundoplication. Video-Assisted Surgery. Gastroesophageal Reflux. Gastric Fundus.

## INTRODUCTION

Laparoscopic total fundoplication is an effective procedure for the treatment of gastroesophageal reflux disease (GERD)[1]. However, some technical points are still controversial, especially the need for short gastric vessels (SGV) division[2]. While most authors believe this step brings better results[3-5], others showed similar outcomes whether SGV are divided or not or even complications attributed to SGV division[2,6-9].

This study aims to determine: (a) clinical variables that may predict the need of SGV division based on gastric fundus tension and (b) the outcomes in patients with or without SGV division.

## METHODS

We retrospectively studied 399 consecutive patients (50% male, mean age 49 years) recorded in a prospectively kept database that underwent laparoscopic total fundoplication for the surgical treatment of GERD. This study was approved by the local institutional review board. (CEP 0742/11).

Patients were questioned before the operation regarding the presence of symptoms. These were grouped into esophageal symptoms (heartburn and regurgitation), extra-esophageal symptoms (thoracic pain, respiratory symptoms, such as cough and asthma or ear, nose and throat symptoms) or dysphagia. Anthropometric variables were also recorded. Individuals with partial fundoplication, paraesophageal hernia, previous foregut operation or conversion to conventional laparotomy were excluded from the analysis.

All patients were submitted to an upper endoscopy to evaluate the presence of hiatal hernia (HH), esophagitis and Barrett´s esophagus. HH was classified according to size in <4cm or ≥4cm. Modified Savary-Miller endoscopic classification[10] was used for grading esophagitis. Barrett esophagus was defined by the presence of intestinal metaplasia and classified as short-segment (<3cm) or long-segment (≥3cm). Esophageal manometry was available to review in 283 (71%) patients. Ambulatory 24-hour esophageal pHmonitoring was only performed in

1. Departamento de Cirurgia, Escola Paulista de Medicina, Universidade Federal de São Paulo - SP - Brazil; 2. Departamento de Cirurgia Geral e Oncológica, Hospital do Servidor Público Estadual de São Paulo - SP - Brazil.

patients without esophagitis or with atypical symptoms. pHmonitoring results were available for review in 62 (15%).

Surgical technique has been previously described[2]. In summary, an extensive mobilization of the posterior wall of the gastric fundus followed the dissection of the distal esophagus and diaphragmatic crus in all patients. SGV division was done at the discretion of the surgeon based on tension of the gastric fundus after performing a specific maneuver ("drop-test" - Figure 1). A short-floppy total fundoplication was performed without the aid of a bougie. All procedures were performed by or under the supervision of a single experienced surgeon. Patients were grouped according to the necessity for SGV division (Group A – no division; Group B – SGV division).

Follow-up visits were scheduled for 15, 30, 90, 180 and 360 days after the surgery and then annually, irrespective of the presence of symptoms. Upper endoscopy was performed annually or earlier if the patient had any complaints related to the postoperative period. All selected patients had at least a 6-month postoperative follow-up period.

Chi-square, Student's t test and logistic regression were used when necessary. A value of *p* was considered significant at the 0.05 level. Variables are expressed as mean ± standard deviation.

## RESULTS

SGV division was deemed not necessary in 364 (91%) patients (Group A) but required in 35 (9%) patients (Group B). Demographic data, symptoms distribution, endoscopic and manometric data are depicted in table 1. Group B had more males and a higher height. Endoscopic parameters were worse for group B, with larger hiatal hernias, higher proportion of hiatal hernias >4cm, more severe esophagitis (Grade IV-V), higher proportion of Barrett esophagus, and higher rate of long-segment Barrett esophagus. Manometric parameters also disfavored group B with decreased lower esophageal sphincter basal pressure. Only male gender and grade IV-V esophagitis stood as independent predictive factors for the need of SGV division at the multivariate analysis (Table 2).

Average follow-up was 13.8 months. Outcomes at last follow-up are depicted in table 3. Transient dysphagia and GERD symptoms were more common in Group B.

If patients at higher risk for gastric fundus tension (namely males with severe esophagitis) that did not underwent SGV division are compared to the remaining patients no difference in symptoms were noticed.

## DISCUSSION

Our results show that: (a) male gender and severe esophagitis are independent predictors for the need to SGV division and (b) SGV division may lead to more transitory dysphagia and GERD symptoms.

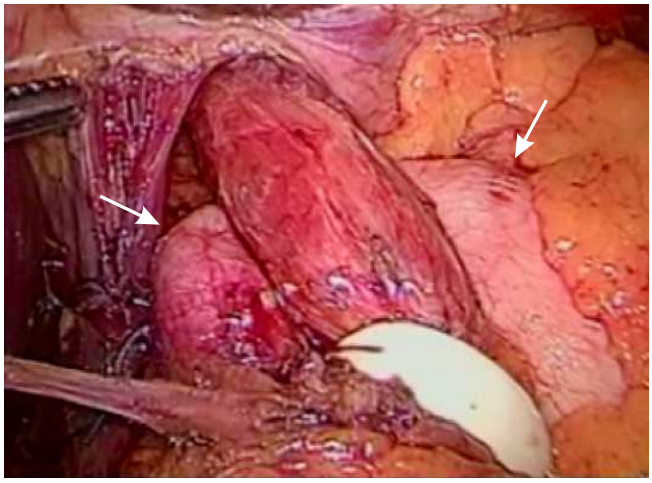

***Figure 1 -*** *Gastric fundus in place without the need of traction* - drop-test- *(Arrows).*

The effect of SGV division on the outcomes of laparoscopic fundoplication have been evaluated in five prospective randomized studies[2,6-9] and their meta-analysis[11-14]. These studies showed a longer operative time and intraoperative bleeding[2,6,8,9,11-14], higher incidence of transient dysphagia[2] and gas-bloating syndrome[7] when SGV are divided. Our results also showed more postoperative GERD symptoms. No benefit has been attributed to SGV division[15]. Very interestingly, however, authors that do not routinely divide SGV find this step necessary at the time of the fundoplication due to tension on the gastric fundus in up to 33% of patients[16] even after extensive gastric fundus mobilization by lysis of adhesions between the stomach and diaphragm[2,6].

In this study, 8.8% (35/399) of the patients needed SGVD for the completion of a short floppy fundoplication. The likely cause of this low need of SGVD is the extensive gastric fundus mobilization employed in all patients, as advocated by Farah *et al*[2] and Chrysos *et al*[6]. We believe that when this surgical step is used, one makes a larger length of the gastric fundus available for the construction of a floppy fundoplication around the esophagus. So, even in those cases in which there is an enlarged cardia, this maneuver lessens the likelihood of the need of SGVD.

Some previous studies attempted to identify anatomic parameters to predict gastric fundus tension and consequent need to SGV division. Szor *et al.*[16] found some sort of tension in half of the cases during fundoplications in cadavers, but no anatomic parameter predicted this tension. Huntington *et al.*[17] deemed necessary to divided SGV in some patients based on the gastric fundus length and the esophageal circumference. Severe esophagitis as a predictor for fundus tension in our study may be linked to esophageal circumference as progressive dilatation of the esophageal

**Table 1 -** Demographic data, symptoms distribution, endoscopic and manometric data.

| Variable | No SGVD(n = 364) | SGVD(n = 35) | *P* Value |
|---|---|---|---|
| Male | 47.3% | 80% | **<0.001$^{X2}$** |
| Male-female ratio | 0.89:1 | 4:1 | |
| Mean age (sd). years | 49.77 ± 13.05 | 51.15 ± 13.46 | 0.564$^{t-S}$ |
| Mean weight (sd). Kg | 74.56 ± 12.85 | 79.76 ± 16.86 | 0.084$^{t-S}$ |
| Mean height (sd). m | 1.67 ± 0.10 | 1.72 ± 0.08 | **<0.001$^{t-S}$** |
| Mean BMI (sd). Kg/m$^2$ | 26.9 ± 4.39 | 26.86 ± 5.02 | 0.969$^{t-S}$ |
| % Typical symptoms | 96.7% | 100% | 0.275$^{X2}$ |
| % Atypical symptoms | 29.7% | 14.3% | 0.054$^{X2}$ |
| % Dysphagia | 2.7% | 8.6% | 0.064$^{X2}$ |
| Mean duration of symptoms (sd). months | 66 ±51.17 | 65 ±41.63 | 0.921$^{t-S}$ |
| % Hiatal Hernia | 79.4% | 71.4% | 0.137$^{X2}$ |
| Mean hiatal hernia length (range). cm | 3 ± 2-9 | 3 ± 2-10 | **0.004$^{t-S}$** |
| % 2-4 cm | 68.7% | 48.6% | **0.016$^{X2}$** |
| % > 4 cm | 10.7% | 22.9% | **0.033$^{X2}$** |
| % Esophagitis | 85% | 94% | 0.129$^{X2}$ |
| I-II-III (Savary-Miller) | 58 | 37 | **0.019$^{X2}$** |
| IV-V (Savary-Miller) | 27 | 57 | **<0.001$^{X2}$** |
| % Barrett esophagus | 23.9% | 42.9% | **0.014$^{X2}$** |
| Mean size of Barrett (sd). cm | 2.17 ± 1.68 | 3.13 ± 2.29 | 0.056$^{t-S}$ |
| % Short-segment Barrett | 18.1% | 22.9% | 0.492$^{X2}$ |
| % Long-segment Barrett | 5.8% | 20% | **0.002$^{X2}$** |
| Mean LES pressure (sd). mmHg | 8.74 ± 4.98 | 6.01 ± 3.50 | **0.012$^{t-S}$** |

*SGVD: short gastric vessels division; n: number; sd: standard deviation; y: years; m: meters; mo: months; cm: centimeters; LES: lower esophageal sphincter; X2: chi-square; t-S: Student's t test.*

diameter is observed as esophagitis severity increases[18,19]. Male gender may bring a higher chance of fundus tension probably due to more exuberant visceral fat compared to females. To the best of our knowledge, no other series studied clinical parameters to predict gastric fundus tension.

The current study studied a large number of patients; however, it has the limitations of a retrospective case series. As such, some parameters that could help understand the results of the study were not evaluated, such as the amount of visceral fat. Also, the time of follow-up is short for a stronger conclusion that SGV division does not affect long-term outcomes. More importantly, even though a single surgeon operated all cases, gastric fundus tension was based on subjective parameters.

We conclude that SGV division is not necessary routinely but male sex and grade IV-V esophagitis are independent predictors of the need of SGV division. However, not all patients in these conditions need SGV division as a subanalysis of these population that did not underwent this step did not show worse outcomes compared to other patients. Gastric fundus tension must still be evaluated based on subjective parameters by experienced surgeons.

**Table 2 -** Multivariate analysis for the need to short gastric vessels division.

| Variable | *Odds Ratio*(95% CI) | *P* Value |
|---|---|---|
| Male gender | 28.3 (2.25-355.22) | **0.010** |
| Weight | 1.03 (0.98-1.09) | 0.216 |
| Hiatal hernia length | 1.64 (0.75-3.59) | 0.213 |
| Esophagitis grade IV-V | 19.5 (2.08-182.26) | **0.009** |
| Barrett esophagus and extension | 0.11 (0.01-1.06) | 0.056 |
| LES pressure | 0.79 (0.62-1.01) | 0.063 |

*LES, lower esophageal sphincter; CI, confidence interval.*

**Table 3 -** Clinical follow-up.

| Variable | No SGV division n=364 | SGV division n=35 | *P* |
|---|---|---|---|
| Transient dysphagia | 6 (1.6%) | 4 (11.4%) | **0.007** |
| Persistent dysphagia | 12 (3.3%) | 1 (2.8%) | 1 |
| Reflux symptoms | 6 (1.6%) | 3 (8.5%) | **0.04** |
| Subgroup analysis | | | |
| **Variable** | **Male sex. esophagitis IV-V, no SGV division n=58** | **Other patients n=341** | ***P*** |
| Transient dysphagia | 4 (6.9%) | 8 (2.3%) | 0.08 |
| Persistent dysphagia | 1 (1.7%) | 11 (3.2%) | 1 |
| Reflux symptoms | 1 (1.7%) | 8 (2.3%) | 1 |

*SGV: short gastric vessels.*

## R E S U M O

***Objetivo:*** *determinar variáveis clínicas que possam predizer a necessidade de secção dos vasos gástricos curtos (VGC), baseado na tensão do fundo gástrico, avaliando os resultados pós-operatórios em pacientes submetidos ou não à secção destes vasos.* ***Métodos:*** *foram analisados os dados de 399 pacientes consecutivos submetidos à fundoplicatura total laparoscópica para a doença do refluxo gastroesofágico (DRGE). A secção dos VGC foi realizada de acordo com a avaliação do cirurgião, baseado na tensão do fundo gástrico. Os pacientes foram distribuídos em dois grupos: sem necessidade de secção dos VGC (grupo A) ou com necessidade de secção dos VGC (grupo B).* ***Resultados:*** *A secção não foi necessária em 364 (91%) pacientes (Grupo A) e necessária em 35 (9%) pacientes (Grupo B). O Grupo B tinha proporcionalmente mais pacientes do sexo masculino e maior estatura média. Os parâmetros endoscópicos foram piores para o Grupo B, com maiores hérnias hiatais, maior proporção de hérnias com mais de quatro centímetros, esofagite mais intensa, maior proporção de esôfago de Barrett e esôfago de Barrett longo. O sexo masculino e as esofagites graus IV-V foram considerados fatores preditivos independentes na análise multivariada. A disfagia transitória e os sintomas de DRGE foram mais comuns no Grupo B.* ***Conclusão:*** *A secção dos vasos gástricos curtos não é necessária rotineiramente, porém o sexo masculino e as esofagites graus IV-V são fatores preditivos independentes da necessidade da secção destes vasos.*

***Descritores:*** *Fundoplicatura. Cirurgia Vídeoassistida. Refluxo Gastroesofágico. Fundo Gástrico.*

## REFERENCES

1. Dallemagne B, Weerts J, Markiewicz S, Dewandre JM, Wahlen C, Monami B, et al. Clinical results of laparoscopic fundoplication at ten years after surgery. SurgEndosc. 2006;20(1):159-65.
2. Farah JFM, Grande JCD, Goldenberg A, Martinez JC, Lupinacci RA, Matone J. Randomized trial of total fundoplication and fundal mobilization with or without division of short gastric vessels: a short-term clinical evaluation. Acta Cir Bras. 2007;22(6):422-9.
3. Soper NJ, Dunnegan D. Anatomic fundoplication failure after laparoscopic antireflux surgery. Ann Surg. 1999;229(5):669-76; discussion 676-7.
4. Wu JS, Dunnegan DL, Luttmann DR, Soper NJ. The influence of surgical technique on clinical outcome of laparoscopic Nissen fundoplication. Surg Endosc. 1996;10(12):1164-69; discussion 1169-70.
5. Hunter JG, Swanstrom L, Waring JP. Dysphagia after laparoscopic antireûux surgery. The impact of operative technique. Ann Surg. 1996;224(1):51-7.
6. Chrysos E, Tzortzinis A, Tsiaoussis J, Athanasakis H, Vasssilakis J, Xynos E. Prospective randomized trial comparing Nissen to Nissen-Rossetti technique for laparoscopic fundoplication. Am J Surg. 2001;182(3):215-21.
7. O'Boyle CJ, Watson DI, Jamieson GG, Myers JC, Game PA, Devitt PG. Division of short gastric vessels at laparoscopic Nissen fundoplication: a prospective double-blind randomized trial with 5-year follow-up. Ann Surg. 2002;235(2):165-70.
8. Blomqvist A, Dalenbäck J, Hagedorn C, Lönroth H, Hyltander A, Lundell L. Impact of complete gastric fundus mobilization on outcome after laparoscopic total fundoplication. J Gastrointest Surg. 2000;4(5):493-500.
9. Watson DI, Pike GK, Baigrie RJ, Mathew G, Devitt PG, Britten-Jones R, et al. Prospective double-blind randomized trial of laparoscopic Nissen fundoplication with division and without division of short gastric vessels. Ann Surg. 1997;226(5):642-52.
10. Savary M, Miller G. The esophagus. Handbook and atlas of endoscopy. Solothurn, Switzerland: Grassman; 1978. p.135-42
11. Engström C, Jamieson GG, Devitt PG, Watson DI. Meta-analysis of two randomized controlled trials to identify long-term symptoms after division of the short gastric vessels during Nissen fundoplication. Br J Surg. 2011;98(8):1063-7.

12. Markar SR, Karthikesalingam AP, Wagner OJ, Jackson D, Hewes JC, Vyas S, et al. Systematic review and meta-analysis of laparoscopic Nissen fundoplication with or without division of the short gastric vessels. Br J Surg. 2011;98(8):1056-62.
13. Khatri K, Sajid MS, Brodrick R, Baig MK, Sayegh M, Singh KK. Laparoscopic Nissen fundoplication with or without short gastric vessel division: a meta-analysis. Surg Endosc. 2012;26(4):970-8.
14. Kösek V, Wykypiel H, Weiss H, Höller E, Wetscher G, Margreiter R, et al. Division of the short gastric vessels during laparoscopic Nissen fundoplication: clinical and functional outcome during long-term follow-up in a prospectively randomized trial. SurgEndosc. 2009;23(10):2208-13.
15. Bonadiman A, Teixeira AC, Goldenberg A, Farah JF. Dysphagia after laparoscopic total fundoplication: anterior or posterior gastric wall fundoplication?.Arq Gastroenterol. 2014;51(2):113-7.
16. Szor DJ, Herbella FA, Bonini AL, Moreno DG, Del Grande JC. Gastric fundus tension before and after division of the short gastric vessels in a cadaveric model of fundoplication. Dis Esophagus. 2009;22(6):539-42.
17. Huntington TR, Danielson L. Variation in fundic dimensions with respect to short gastric vessel division in laparoscopic fundoplication. SurgEndosc. 2001;15(1):76-9.
18. Csendes A, Miranda M, Espinoza M, Velasco N, Henríquez A. Perimeter and location of the muscular gastroesophageal junction or 'cardia' in control subjects and in patients with reflux esophagitis or achalasia. Scand J Gastroenterol. 1981;16(7):951-6.
19. Korn O, Csendes A, Burdiles P, Braghetto I, Stein HJ. Anatomic dilatation of the cardia and competence of the lower esophageal sphincter: a clinical and experimental study. J Gastrointest Surg. 2000;4(4):398-406.

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**Address for correspondence:**
Alexandre Chartuni Pereira Teixeira
E-mail: alexandrechartuni@gmail.com

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# Endoscopic treatment of the fistulas after laparoscopic sleeve gastrectomy and Roux-en-Y gastric bypass

## *Tratamento endoscópico das fístulas após gastrectomia vertical e bypass gástrico em Y de Roux*

Luís Gustavo Santos Périssé[1]; Paulo Cezar Marques Périssé, ECBC/RJ[2]; Celso Bernardo Júnior[1]

A B S T R A C T

**Objective**: to evaluate the use of endoscopic self-expandable metallic prostheses in the treatment of fistulas from sleeve gastrectomy and Roux en y gastric bypass. **Methods:** all patients were treated with fully coated auto-expandable metallic prostheses and were submitted to laparoscopic or CT-guided drainage, except for those with intracavitary drains. After 6-8 weeks the prosthesis was removed and if the fistula was still open a new prostheses were positioned and kept for the same period. **Results:** the endoscopic treatment was successful in 25 (86.21%) patients. The main complication was the migration of the prosthesis in seven patients. Other complications included prosthesis intolerance, gastrointestinal bleeding and adhesions. The treatment failed in four patients (13.7%) one of which died (3.4%). **Conclusion**: endoscopic treatment with fully coated self-expandable prosthesis was effective in treating most patients with fistula after sleeve gastrectomy and roux en y gastric bypass.

**Key words:** Fistula. Postoperative Complications. Endoscopy, Gastrointestinal. Prosthesis.

## INTRODUCTION

Obesity is now a major worldwide public health problem. The World Health Organization estimates that one quarter of the world's population has overweight or obesity[1-3]. Obesity-related diseases such as type 2 diabetes, hypertension, dyslipidemia, myocardial infarction, stroke, sleep disorders, asthma, depression and degenerative diseases are responsible for about 2.5 million deaths a year in the world[2].

For patients diagnosed with morbid obesity, surgical treatment is considered the best treatment option. Currently, the two most used surgical techniques are the gastric sleeve (GS) and the Roux-en-Y gastric bypass (RYGB), both performed laparoscopically[4]. Major complications associated with these techniques are postoperative fistula and gastrointestinal bleeding (1.9%)[5-8].

Postoperative fistula is a major challenge for the medical staff, since it is difficult to diagnosis and its treatment is complex and multidisciplinary, involving the control of sepsis, multiple organ failure, nutritional deficit and the fistula itself[9,10]. The proposed therapeutic options range from conservative medical treatment to exploratory laparotomy with primary closure of the fistula and, in cases of abdominal contamination, which are the majority, radiological or laparoscopic drainage[8,10,11].

Endoscopic treatment is an alternative to surgery. Based on the use of self-expandable prosthesis, endoscopic treatment appeared initially to manage malignant fistulas and esophageal spontaneous perforation (Böerhaave syndrome), and subsequently has been proposed for postoperative leaks[12-14].

The aim of this study was to evaluate the result of using a self-expandable, completely coated, metal prosthesis in patients diagnosed with reducing post-gastric fistula.

## METHODS

In the Serviço de Endoscopia Digestiva of the Hospital Universitário Gaffrée e Guinle, we evaluated the medical records of patients who underwent surgical treatment for obesity between August 2011 and May 2014 who had postoperative fistula. All patients were from private institutions and operations were carried out by different teams.

Fistula diagnosis was made by clinical examination (tachycardia, fever, tachypnea), laboratory

1. Serviço de Endoscopia Digestiva, Hospital Universitário Gaffrée e Guinle, Universidade Federal do Estado do Rio de Janeiro (UNIRIO) - RJ - Brazil; 2. Curso de Pós-Graduação em Endoscopia Digestiva, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio) - RJ - Brazil.

tests (increased C reactive protein), computed tomography (identification of intra-abdominal collection) and the intake of methylene blue if the patient still had the abdominal drain.

After the surgical or radiological treatment of the collection, all patients were transferred to the operating room and underwent endoscopic procedure under general anesthesia in the supine position. After the passage of the endoscope and location of the fistula, contrast infusion was made for the fistula study. After this first step, the external marking was made with electrodes placed below the fistula and the fistula topography. In patients undergoing RYGB, the distal marking was located below the gastrojejunostomy. In patients undergoing GS, the distal marking was located below the surgical reinforcement near or just after the pylorus. In all patients, the proximal portion of the prosthesis was positioned in the distal esophagus. After labeling with electrodes, we positioned a Savary-Gilliard guide wire (Wilson Cook Medical Inc., Winston-Salem, NC) under endoscopic view and over which we introduced the 15cm self-expandable metal prosthesis (Boston Scientific of Brazil Ltda). With the prosthesis in its final position, we performed contrast infusion to confirm the path obliteration. In the absence of contrast material in the abdominal cavity, the procedure was considered satisfactory, and we then positioned the metal clips securing the proximal part of the prosthesis wall of the esophagus in order to reduce the possibility of migration. If there was prosthesis migration, we reassessed the patient before the end of the treatment and the prosthesis, repositioned, or replaced by a new one.

## RESULTS

We evaluated the records of 29 patients, 23 (79.3%) undergoing GS and six (20.6%), RYGB. Initial BMI ranged from 37.11 to 67.2. Eight patients had no risk factors, while 11 had more than one risk factor (Table 1). The mean age was 36.7 years and the average time between diagnosis of the fistula and the positioning of the prosthesis, six days. Twelve patients were male and 17 female, the average treatment time with the prosthesis being 63 days and the average number of endoscopies, three.

Endoscopic treatment closed the fistula in 25 patients (86.21%). There was recanalization of the fistula after six months of prosthesis placement in one patient. He underwent a new endoscopic treatment that permanently closed the fistula. The drainage of intra-abdominal abscess was required in 21 patients. In 19 (65.5%), surgical drainage was performed (laparotomy), in two (6.89%) CT-guided drainage was performed and seven patients (24.1%) had the cavity drain at diagnosis; in two of these patients the gastric banding had already been positioned. In one patient (3.4%) we observed a discrete amount of gas in the abdominal cavity without collection at CT, requiring no drainage.

In all patients undergoing GS, the fistula was located in the Hiss angle (Figures 1A, 1B and 1C). One patient, beyond this fistula, presented another one, located in the gastric antrum. In patients undergoing RYGB, two had total dehiscence of the gastric pouch suture (Figures 2A and 2B), two had fistula in the pouch suture line and two in the pouch anastomosis with the jejunum.

In the six patients undergoing RYGB, the fistula healed after placement of the prosthesis, with an average of 2.5 endoscopies per patient. Three patients required three endoscopies to rescue the prosthesis, which migrated to the jejunum. To such rescue we used the double balloon enteroscope in one patient and the colonoscope in two other patients; in three patients the fistulas were already healed.

For patients undergoing GS, the placement of the prosthesis was effective and healed the fistula in 19, with an average of 2.7 endoscopies per patient. In 11 patients (57.8%) two endoscopies were necessary. In two patients the fistula remained open on the date of prosthesis removal. New prostheses were positioned that lasted for over six weeks, when then were removed and there was complete healing of the fistula. In two other patients, at the time scheduled for withdrawal the prosthesis was adhered, requiring the placement of new fully coated prosthesis. In both cases, after 15 days, the two prostheses were removed and the fistula found to be closed. In four patients there was subsequent migration of the prosthesis, which led to an increase in treatment time and in the number of procedures. The main complications found were migration, adhesion, bleeding and intolerance (Table 2). In four patients (13.7%) endoscopic treatment was not effective, and one of these died after 22 days of treatment.

## DISCUSSION

Postoperative fistulas represent a serious complication of obesity surgical treatments. The increased intraluminal pressure caused by anastomotic stenosis, excessive tension on the suture line, tissue ischemia and hematoma are predisposing factors. In addition, there are preoperative factors that favor the emergence of complications in the postoperative period: BMI e" 40, hypertension, diabetes, infections, sleep apnea, age over 55 years, male gender, previous surgery and smoking[15]. In this study, 21 patients (72.4%) had at least one of these factors preoperatively.

The location of the fistula differs according to the technique proposed[2]. In this series all patients undergoing gastric sleeve (GS) had fistulas at the Hiss angle and one of them also had another leak in the gastric antrum. In patients undergoing Roux-en-Y gastric bypass (RYGB), fistulas were located in the gastrojejunostomy and at the suture line of the gastric pouch, location similar to that found by other authors[2].

**Table 1** - Epidemiological data.

| Patients | Gender | Age | BMI | Risk factors | Surgery |
|---|---|---|---|---|---|
| 1 | M | 27 | 41.02 | A | GB |
| 2 | M | 28 | 45.56 | | GS |
| 3 | M | 37 | 46.31 | A-H-D-B | GS |
| 4 | F | 35 | 40.22 | H-C | GS |
| 5 | F | 67 | 37.83 | H-D-B | GS |
| 6 | F | 34 | 37.26 | H-C | GS |
| 7 | M | 39 | 44.01 | | GS |
| 8 | F | 55 | 37.58 | | GS |
| 9 | M | 22 | 50 | A-H | GB |
| 10 | M | 35 | 46.61 | A-H | GB |
| 11 | M | 51 | 40.48 | A-C | GS |
| 12 | M | 40 | 46.08 | | GB |
| 13 | F | 18 | 45 | H | GS |
| 14 | F | 36 | 48.56 | | GS |
| 15 | F | 42 | 51.69 | C | GB |
| 16 | F | 34 | 67.2 | H | GB |
| 17 | M | 24 | 40.04 | A-H | GS |
| 18 | F | 40 | 40.65 | C | GS |
| 19 | F | 33 | 37.11 | H | GS |
| 20 | M | 25 | 40.07 | H | GS |
| 21 | F | 18 | 51.42 | | GS |
| 22 | F | 39 | 37.59 | A | GS |
| 23 | F | 53 | 44.66 | A-H-D | GS |
| 24 | M | 38 | 47.84 | A-H | GS |
| 25 | F | 36 | 44.41 | A-C | GS |
| 26 | F | 54 | 39,43 | A-H-D-C | GS |
| 27 | M | 38 | 38.89 | A | GS |
| 28 | F | 31 | 36 | | GS |
| 29 | F | 38 | 43.92 | | GS |

*Source: Digestive Endoscopy Service of the Gaffrée e Guinle University Hospital.*

*Legends: A - Obstructive sleep apnea; H - Systemic arterial hypertension; D - Diabetes; C - Previous laparotomy; B - gastric band; GB - gastric bypass; GS - sleeve gastrectomy.*

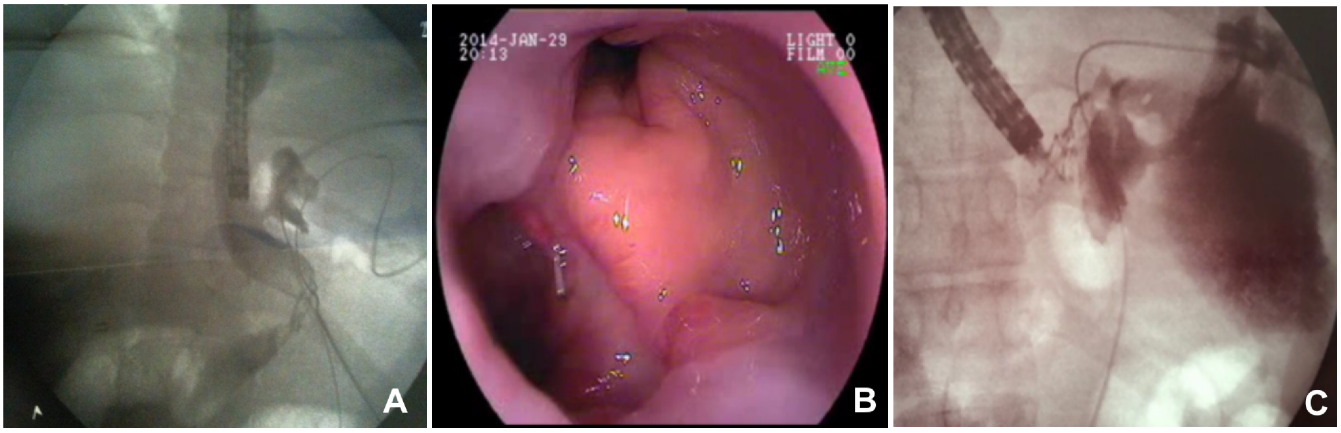


***Figure 1*** - *A) simple Hiss angle fistula; B) endoscopic appearance of a complex fistula at the Hiss angle; C) X ray image of the complex fistula at the Hiss angle with abdominal abscess.*

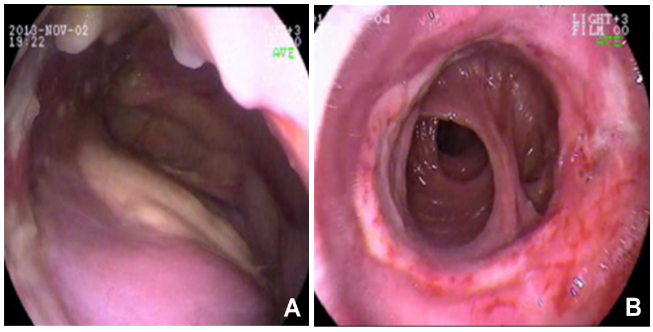


***Figure 2 -*** *A) total dehiscence of gastric pouch with visualization of the liver; B) gastric pouch after treatment.*

The male gender is an independent risk factor for fistula[6]. This can be explained by the higher central fat in relation to peripheral fat, leading to increased amount of intra-abdominal and mesenteric fat. In our study, 41.3% were men, 56.7% were women. This apparently contradictory results can be explained by the higher number of women undergoing surgical treatment.

Postoperative fistulas usually occur between the second and 12th day after the operation and are difficult to diagnose. The main symptom is tachycardia (72-92%), followed by nausea and vomiting (81%), fever (62%) and leukocytosis (48%)[1,16,17]. Computed tomography (CT) is the best imaging method for the diagnosis and assists the conduct as to whether or not to drain the abdomen, although its validity for the fistula diagnosis in patients with BMI e" 50 is questioned due to size of waist circumference[15]. In patients in whom the cavity drain remains positioned, methylene blue administered orally can be very useful for confirming the fistula. In 23 patients (79%) the diagnosis was made by the tenth day, in three (10.3%), until the 15th day, and in th remaining three (10.3%), after 30 days. In 21 patients the diagnosis was made by CT with contrast and in eight patients, with methylene blue. Once the fistula diagnosis is confirmed, operative treatment has been proposed by many centers of bariatric surgery in the world[18], but the technical difficulties associated with the high incidence of fistula recurrence, possibly caused by the intense inflammation in its surroundings, led this option to be underutilized. In order to reduce the complications inherent to operative rapprochement, it has been proposed that the operation be limited to drainage of possible abdominal abscesses and the fistula approach be made preferably endoscopically[9,15,19]. In our series, two patients submitted to RYGB underwent operative fistula rapprochement without success, increasing time between diagnosis and endoscopic treatment. In all other patients previously addressed by surgical teams the approach was the drainage of intra-abdominal collection.

The endoscopic technique is based on the use of a completely coated prosthesis associated, when required, the use of "Surgisis plugs" (Wilson Cook Medical, Inc., Winston-Salem, NC). After the positioning, the prosthesis leads to the formation of a mechanical barrier between the gastrointestinal tract and the fistula, allowing high protein oral nutritional support while healing occurs[8]. The use of oral feeding avoids complications related to parenteral nutrition. Moreover, premature discharge reduces the risk of infection[1,20]. After positioning, the prostheses are generally well accepted by patients. Usually found complications are migration, transient chest pain during expansion, nausea, gastrointestinal bleeding, adhesion and intolerance[4,15]. In this study there was prosthesis migration in three patients submitted to RYGB and in four submitted to GS. This complication is the biggest problem of the endoscopic treatment and is caused by the prostheses design, originally meant to be used in the esophagus[11]. One of the proposed ways to prevent prosthesis migration is the use of metal staples in the proximal part of the prosthesis in order to attach it to the esophagus[20,21]. We adopted this conduct for all patients in this series.

The literature describes one severe gastrointestinal bleeding case as a result of an aortic-esophagic fistula caused by the prosthesis[22]. Only one of our patients had bulky gastrointestinal bleeding, starting 24 hours before the expected prosthesis removal time. In this case, an approach to suppress the bleeding was not necessary, and during the removal of the prosthesis we observed a large stomach ulcer, probably caused by contact of the prosthesis with the gastric mucosa.

Although we have used only fully coated prostheses, in two patients there was silicone rupture, resulting in tissue growth and prosthesis adhesion. In these two patients we placed new fully coated prostheses of equal sizes inside the attached prostheses and patients were discharged. After 15 days, during a novel hospital admission, the two prostheses were withdrawn. This approach is used

**Table 2 -** Complications of endoscopic treatment.

| Complications | N (%) | Treatment |
|---|---|---|
| Migration | 7 (24.3) | 3 removed * |
| Adherence | 2 (6.8) | 4 replacements - prosthesis under prosthesis |
| Intolerance | 1 (3.4) | removed |
| Bleeding | 1 (3.4) | conservative treatment |

*Source: Digestive Endoscopy Service of the Gaffrée e Guinle University Hospital.*
** Fistulas already healed at the time of migration.*

in the removal of partially coated prostheses in which there is cell growth in the proximal and distal segments[21,23]. There is no consensus in the literature as to the time for prosthesis removal. Published papers report a period between four and eight weeks[20,24]. In our series, the average time of prosthesis stay was 9.4 weeks.

We removed the prostheses from four patients. One patient complained of severe heartburn and chest pain and requested the removal of the prosthesis before the time deemed necessary for fistula closure. In this patient there was a significant reduction in the size of the fistula and abdominal collection during the period in which the prosthesis remained positioned. After its withdrawal, we positioned "Surgisis plugs" (Wilson Cook Medical Inc., Winston-Salem, NC), with total occlusion of the path ten days after. In another patient there was a reduction of the fistula caliber after the proposed period and the patient was discharged on enteral feeding until the complete obliteration of the path. In the third patient there were numerous treatment attempts, endoscopic and clinical, all unsuccessful, and at the end of seven months the patient was referred for total gastrectomy. In this series, the one patient (3.4%) evolving to death presented one fistula located in the Hiss angle and another in the gastric antrum. Antrum fistulas are difficult to manage and often do not respond satisfactorily to treatment with the prosthesis; it is believed that antral postoperative anatomical changes hamper the obliteration of the fistula.

An important aspect to be considered in this work is that all patients were from different teams from various private institutions and that for the first patients there was no uniform acceptance of endoscopic fistulas treatment from all teams. Thus, there was a significant variation in the timing of indication of endoscopic treatment. This delay may have led to the formation of chronic fistula, reducing prosthesis effectiveness[18,20]. Since there are no randomized studies on the treatment of post-bariatric fistulas with the use of prostheses, the level of evidence is not strong. However, our work shows that treatment with prosthesis is safe and presents significant healing results. Other endoscopic techniques should always be complementary and one should not initially indicate surgical treatment for fistulas, which should be limited to collection drainage [9,15,19].

In conclusion, the endoscopic approach with the use of completely coated, self-expandable prosthesis was effective in treating most patients with fistula after gastric sleeve and Roux-en-Y gastric bypass.

## R E S U M O

***Objetivo:*** *avaliar a utilização das próteses metálicas autoexpansivas no tratamento das fístulas pós-gastroplastia redutora.* ***Métodos:*** *todos os pacientes foram tratados com próteses metálicas autoexpansivas totalmente recobertas e, exceto aqueles que apresentavam drenos intracavitários, foram submetidos à drenagem por via laparoscópica ou guiada por TC. Após seis a oito semanas, a prótese era retirada e, caso a fístula ainda estivesse aberta, novas próteses eram posicionadas e permaneciam por igual período.* ***Resultado:*** *o tratamento endoscópico obteve sucesso em 25 (86,21%) pacientes. A principal complicação foi a migração da prótese, ocorrida em sete pacientes. Outras complicações foram intolerância à prótese, hemorragia digestiva e aderência. O tratamento não teve êxito em quatro pacientes (13,7%), sendo que um (3,4%) faleceu.* ***Conclusão:*** *a abordagem endoscópica com a utilização de prótese autoexpansiva, totalmente recoberta, foi eficaz para tratar a maioria dos pacientes com fístula pós-gastroplastia redutora.*

***Descritores:*** *Fístula. Complicações Pós-Operatórias. Procedimentos Cirúrgicos Endoscópicos Gastrointestinais. Implante de Prótese.*

## REFERENCES

1. Kumar N, Thompson CC. Endoscopic management of complications after gastrointestinal weight loss surgery. Clin Gastroenterol Hepatol. 2013;11(4):343-53.
2. Yurcisin BM, Gaddor MM, DeMaria EJ. Obesity and bariatric surgery. Clin Chest Med. 2009;30(3):539-53.
3. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, et al. Bariatric surgery: a systematic review and meta-analysis. JAMA. 2004;292(14):1724-37. Erratum in: JAMA. 2005;293(14):1728.
4. Jurowich C, Thalheimer A, Seyfried F, Fein M, Bander G, Germer CT, et al. Gastric leakage after sleeve gastrectomy – clinical presentation and therapeutic options. Langenbecks Arch Surg. 2011;396(7):981-7.
5. El Mourad H, Himpens J, Verhofstadt J. Stent treatment for fístula after obesity surgery: results in 47 consecutive patients. Surg Endosc. 2013;27(3):808-16.
6. Simon F, Sicilliano I, Gillet A, Castel B, Coffin B, Msika S. Gastric leak after laparoscopic sleeve gastrectomy: early covered self-expandable stent reduces healing time. Obes Surg. 2013;23(5):687-92.
7. Babor R, Talbot M, Tyndal A. Treatment of upper gastrointestinal leaks with a removable, covered, self-expanding metallic stent. Surg Laparosc Endosc Percutan Tech. 2009;19(1):e1-4
8. de Aretxabala X, Leon J, Wiedmaier G, Turu I, Ovalle C, Maluenda F. Gastric leak after sleeve gastrectomy: analysis of its management. Obes Surg. 2011;21(8):1232-7.
9. Puli SR, Spofford IS, Thompson CC. Use of self-expansible stents in the treatment of bariatric surgery leaks: a sistematic review and meta-analysis. Gastrointest Endosc. 2012;75(2):287-93.
10. de Moura EG, Galvão-Neto MP, Ramos AC, de Moura ET, Glvão TD, de Moura DT, et al. Extreme bariatric endoscopy: stenting to reconnect the pouch to the gastrojejunostomy after a Roux-en-Y gastric bypass. Surg Endosc. 2012;26(5):1481-4.

11. Eubanks S, Edwards CA, Fearing NM, Ramaswamy A, de la Torre RA, Thaler KJ, et al. Use of endoscopic stents to treat anastomotic complications after bariatric surgery. J Am Coll Surg. 2008;206(5):935-8; discussion 938-9.
12. D'Cunha J, Rueth NM, Groth SS, Maddaus MA, Andrade RS. Esophageal stents for anastomotic leaks and perforations. J Thorac Cardiovasc Surg. 2011;142(1):39-46.e1.
13. Rajan PS, Bansal S, Balaji NS, Rajapandian S, Parthasarathi R, Senthilnathan P, et al. Role of endoscopic stents and selective minimal access drainage in oesophageal leaks: feasibility and outcome. Surg Endosc. 2014;28(8):2368-73.
14. Wie W, Ramaswamy A, de la Torre R, Miedema BW. Partially covered esophageal stents cause bowel injury when used to treat complications of bariatric surgery. Surg Endosc. 2013;27(1):56-60.
15. Márquez MF, Ayza MF, Lozano RB, Morales Mdel M, Díez JM, Poujoulet RB. Gastric leak after laparoscopic sleeve gastrectomy. Obes Surg. 2010;20(9):1306-11.
16. BhayaniNH, Swanström LL. Endoscopic therapies for leaks and fístulas after bariatric surgery. Surg Innov. 2014;21(1):90-7.
17. Ballesta C, Berindoague R, Cabrera M, Palau M, Gonzalez M. Management of anastomotic leaks after laparoscopic Roux-en-Y gastric bypass. Obes Surg. 2008;18(6):623-30.
18. Sakran N, Goitein D, Raziel A, Keidar A, Beglaibter N, Grinbaum R, et al. Gastric leaks after sleeve gastrectomy: a multicenter experience with 2,834 patients. Surg Endosc. 2013;27(1):240-5.
19. Donatelli G, Dhumane P, Perretta S, Dallemagne B, Vix M, Mutter D, et al. Endoscopic placement of fully covered self expanding metal stents for management of post-operative foregut leaks. J Minim Access Surg. 2012;8(4):118-24.
20. Nguyen NT, Nguyen XM, Dholakia C. The use of endoscopic stent in management of leaks after sleeve gastrectomy. Obes Surg. 2010;20(9):1289-92.
21. Aurora AR, Khaitan L, Saber AA. Sleeve gastrectomy and the risk of leak: a systematic analysis of 4,888 patients. Surg Endosc. 2012;26(6):1509-15.
22. Eisendrath P, Cremer M, Himpens J, Cadière GB, Le Moine O, Devière J. Endotherapy including temporary stenting of fístulas of the upper gastrointestinal tract after laparoscopic bariatric surgery. Endoscopy. 2007;39(7):625-30.
23. van Boeckel PG, Dua KS, Weusten BL, Schmits RJ, Surapaneni N, Timmer R, et al. Fully covered self-expansible metal stent (SEMS), partially covered SEMS and self-expansible plastic stents for the treatment of benign esophageal ruptures and anastomotic leaks. BMC Gastroenterol. 2012;12:19.
24. Almadi MA, Bamihriz F, Aljebreen AM. Fatal aortoesophageal fístula bleeding after stenting for a leak post sleeve gastrectomy. World J Gastrointest Surg. 2013;5(12):337-40.

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**Adress for correspondence:**
Luís Gustavo Santos Périssé
E-mail: luisperisse@yahoo.com.br

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# Holmium Laser enucleation of the prostate (HoLEP) versus Transurethral Resection of the Prostate (TURP)

## *Enucleação da próstata com Holmium Laser (HoLEP) versus Ressecção Transuretral Da Próstata (RTUP)*

Luís Eduardo Durães Barboza[1], Osvaldo Malafaia, ECBC-PR[2], Luiz Edison Slongo[3], Fernando Meyer[1], Paulo Afonso Nunes Nassif, TCBC-PR[1], Fernando Issamu Tabushi[1], Eduardo Wendler[1], Rafael Alexandre Beraldi[1]

A B S T R A C T

**Objective:** to evaluate the effectiveness and applicability of Holmium laser enucleation of the prostate (HoLEP) - in the treatment of benign prostatic hyperplasia (BPH) – in comparison to transurethral resection of the prostate (TURP). **Methods:** patients with symptomatic prostatic hyperplasia and candidates for surgical treatment were selected. Both procedures were explained and they had choosen HoLEP or TURP. At the hospital were collected: age, date of birth, international prostate symptom score, urinary peak flow rate, prostate volume, post-voiding residual urine, globular volume and serum PSA. At the procedure operating time, morcellating time (HoLEP), bladder mucosal injury and intercurrences were collected. At the first postoperative day, globular volume and sodium. Besides that were observed the catheter indwelling time and hospital stay and after 90 days, urinary peak flow rate and international prostate symptom score. Statistical analisys have been done partially by Sinpe® and also by a professional team. **Results:** twenty patients in HoLEP group and 21 at TURP were operated. Baseline urinary peak flow rate was 8 ml/s in both groups and preoperative international prostate symptom score was 22 in HoLEP and 20 in TURP, very similar. Operative time was 85 minutes in HoLEP and 60 in TURP, $p<0.05$. Hospital stay was 47 hours for HoLEP and 48 hours to TURP, $p<0.05$. At 90 day the urinary peak flow rate was raised to 21.5 ml/s in HoLEP group and to 20 ml/s in TURP and the median of international prostate symptom score had been reduced to score 3 in both groups. **Conclusion:** HoLEP is a feasible technique and is as effective as TURP on symptomatic prostatic hyperplasia surgical treatment.

**Key words:** Prostate. Prostatic hyperplasia. Transurethral resection of the prostate. Laser therapy.

## INTRODUCTION

Prostatic Benign Hyperplasia (BPH) is one of the most frequent diseases in men, and it has been considered part of the physiologic process of aging. The prevalence among 70 year-old men is around 40%[1]. The gold standard surgical treatment is Transurethral Resection of the Prostate (TURP). New surgical techniques using lasers, as vaporization and Holmium Laser Enucleation of the Prostate (HoLEP) or Thulium laser (ThuLEP), have been known through many studies[2]. Complications and morbidity related to TURP including blood loss, fluid balance disturbances, excessive fluid absorption, incontinence and erectile dysfunction, have been estimated to develop in about 15% of patients[3].

In this scenario the laser treatment for BPH has challenged TURP due to advances in laser technology, better understanding of tissue-laser interactions and growing clinical experience[4]. Holmium Laser Enucleation of the Prostate, introduced by Gilling *et al.*[5], seems to be an attractive alternative to standard TURP. The holmium: YAG laser (Lumenis®, Tel Aviv, Israel) is a pulse solid state laser with many characteristics that make it ideal for endo-urological surgery. It has a wavelength of 2.140nm that allows it to be strongly absorbed by tissue water, therefore, causing rapid vaporization of exposed tissues at a depth of approximately 0.4mm and producing coagulation 3 to 4 mm below the vaporization surface. This is useful and allows a precise, bloodless field, preventing systemic fluid absorption[3].

In recent studies, HoLEP was as effective as TURP in terms of improving subjective symptoms and urodynamic findings with a 12 month followup[3].

The purpose of this trial is to evaluate the effectiveness and applicability of Holmium Laser Enucleation of the Prostate (HoLEP) comparing it to TURP.

## METHODS

The work was realized in the Programa de Pós-Graduação em Princípios da Cirurgia of the Faculdade Evan-

1. Faculdade Evangélica do Paraná, Curitiba - PR - Brazil; 2. Serviço de Urologia do Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR) - PR - Brazil; 3. Pontifícia Universidade Católica do Paraná (PUC-PR) - PR - Brazil.

gélica do Paraná, Hospital Universitário Evangélico de Curitiba, Instituto de Pesquisas Médicas e Hospital Nossa Senhora das Graças, Curitiba, PR, Brazil.

Patients with symptomatic prostatic hyperplasia and candidates for surgical treatment were selected. Both procedures were explained and they had chosen HoLEP or TURP.

The including criteria were: age above 45 and under 80 years old, maximum urinary peak flow rate under 15ml/s and prostate sonography volume less than 100g. And the exclusion criteria were: neurogenic bladder, prostatic malignant disorder and previous surgery in the prostate, bladder neck or urethra.

We have created a protocol and these data were collected: age, date of birth, international prostate symptom score (IPSS), urinary peak flow rate, prostate volume, post-voiding residual urinary volume, globular volume and serum PSA. At the procedure: operating time, morcellating time (HoLEP), bladder mucosal injury and intercurrences (major and/or minor). At the first postoperative day, globular volume and sodium. After that catheter indwelling time and hospital stay. As major intercurrences/complications were considered: reoperation and blood transfusion; and as the minor ones: urinary retention and bladder mucosal injury during morcellation. After 90 days, new assessment of urinary peak flow rate and international prostate symptom score.

Patients have been operated on lithotomy position under regional anesthesia. Enucleation was performed at 2.0J and 50Hz, 100W potency. The surgical technique was the previously described by Gilling[5]. The device was a continuous flow 26FR resectoscope sheath (Storz®) and a working element prepared for laser with a 550 micra fiber stabilized inside a 4FR ureteral catheter.

Following the enucleation, the tissue was morcellated with Versacut Morcelator (Lumenis®). All fragmented tissue has been sent to histological evaluation.

At the end of the procedure, a triple lumen catheter was inserted into the bladder and continuous irrigation was started.

The transurethral resection of the prostate was performed with monopolar eletrocautery (Wem®) with a cutting current of 120W and coagulating current of 80W.

For the data analysis was made up a platform with the software Sinpe®[6,7]. We had chosen the Student t test. We also have performed statistical analysis by a Professional team to confirm the Sinpe outcomes inside each group and to evaluate between the groups. For HoLEP and TURP comparisons they had chosen the Student t test or the Mann-Whitney and also the Kolmogorov- Smirnov, to verify normal condition of the data. P values under 0.05 indicate statistical significance. These data were analyzed with the software Statistica v.8.0.

## RESULTS

Between June/2011 and May/2012, 20 patients in HoLEP group and 21 in TURP group were operated. The mean age was 68 (58–79) and 65 years (50-80) respectively. Prostate volume, total PSA, post-voiding residual urinary volume and pre-procedure urinary peak flow and IPSS are shown in table 1.

The difference in the post-voiding residual urinary volume was not statistically significant, neither the other data. Baseline urinary peak flow rate and IPSS were very similar, confirming the homogeneity between the groups. Operating time has considered all the time of the device in urethra, then including the morcellation in the Holmium technique. This mean time was 85 minutes in HoLEP and 60 in TURP, p=0.02. The mean morcellating time was 17 minutes. The mean hospital stay was 47 hours for HoLEP and 48 hours to TURP, p<0.05. The mean indwelling catheter time had no statistically significant difference, 48 hours for HoLEP and 45 hours for TURP. The baseline and post-procedure globular volume and also the post-procedure sodium were very similar in both groups. There were no major complications, i.e., blood transfusion or reoperation. As minor complications there were two patients in HoLEP group and one patient in TURP group who have had urinary

**Table 1** - Patients characteristics.

| | HoLEP | RTUp |
|---|---|---|
| Patients | 20 | 21 |
| Mean age | 68 (58-79) | 65 (50-80) |
| Prostate volume ($cm^3$) | 58 | 61 |
| PSA (ng/ml)* | 1.5 | 3 |
| Residual volume (ml) | 77.5 | 19 |
| Baseline Qmáx (ml/s) | 8 | 8 |
| Baseline IPSS** | 22.5 | 20 |

**PSA: prostatic specific antigen*
***IPSS: international prostate symptom score*

retention after catheter removal, and it was necessary to perform re-catheterization. Bladder mucosal injury, during morcellation, has occurred in 6 patients. Data shown in table 2.

One patient in HoLEP group was diagnosed with prostate cancer in the specimen analysis. He had been treated with radical prostatectomy.

On late assessment all patients were satisfied with both procedures and the mean IPSS was 3, for both groups. Urinary peak flow rate has risen to 21.5ml/s in HoLEP group and to 20ml/s in TURP group.

Comparing clinical outcomes before and after the procedures, there was remarkable improvement in both groups. Nineteen point IPSS reduction in HoLEP group and 17 in TURP. Also a peak urinary flow raising of 13.5ml/s in HoLEP group and 12ml/s in TURP. There was no statistically significant difference between the groups in these variables. Data shown in table 3.

On the other hand, when contrasting clinical outcomes before and after each intervention, we have seen a statistically significant difference (Figure 1 and 2).

## DISCUSSION

Holmium laser enucleation of the prostate has become an attractive technique due to low morbidity and high efficacy; this is testified by symptom relief and also by objective assessment of urinary peak flow rate and complete urodynamic findings[3].

In this study we have found a remarkable improvement on urinary symptoms in both groups, IPSS ranging from 22.5 to 3 points in HoLEP group and ranging from 20 to 3 in TURP group. Considering urinary peak flow rate we have seen the same improvement, 8ml/s to 21.5ml/s in HoLEP group and from 8 to 20 ml/s on the other group. Data on literature confirms that functional outcomes are at least the same of TURP technique[3,8].

Systematic review and meta-analysis have proved HoLEP to be the more testified technique with many reports confirming its safety and efficacy[4,9]. Ahyai *et al*.[8] report that HoLEP outcomes can be even better than TURP on IPSS reduction and also on raising urinary peak flow rate. We report a similar efficacy because there was no statistical significant difference on late IPSS and urinary peak flow rate between the groups, as well as other reports[3]. Although the time of hospital stay favors HoLEP in our report and in other ones[3,8,10,11] it was clinically irrelevant for the patient – just one hour. Many advantages of HoLEP technique are due to hemostatic abilities of Holmium laser[9]. Catheter indwelling time is systematically lower when evaluating HoLEP versus TURP[3,8,10,11]. In this report this time was longer on HoLEP group than in TURP, although there was no statistically significant difference. Operating time was longer in HoLEP than in TURP, but with no statistically significant difference. Literature data confirms the longer operating time in the HoLEP technique[3,8,10,11]. It seems reasonable due to the extra time of morcellation. Although the longer operating time blood loss is not higher. Blood transfusion was not necessary on any patient.

**Table 2** - Clinical data.

| | HoLEP | RTUp | p Value |
|---|---|---|---|
| Operating time(min) | 85 | 60 | 0.020 |
| Morcellating time (min) | 17 | NA* | |
| Hospital stay (h) | 47 | 48 | 0.002 |
| Catheter indwelling time (h) | 48 | 45 | 0.527 |
| Baseline GV** (%) | 44 | 45 | 0.927 |
| Post-Procedure GV** (%) | 41 | 42 | 0.281 |
| Post-Procedure Sodium | 140 | 140 | 0.306 |
| Bladder injury | 6 (30%) | NA* | |

**NA: not assessable*
***GV: globular volume*

**Table 3** - Comparing data between techniques.

| | HoLEP | RTUp | p Value |
|---|---|---|---|
| Baseline IPSS* | 22.5 | 20 | 0.603 |
| Baseline Qmáx(ml/s) | 8 | 8 | 0.533 |
| Post-procedure IPSS* | 3 | 3 | 0.533 |
| Post-procedure Qmáx(ml/s) | 21.5 | 20 | 0.329 |

**IPSS: international prostate symptom score*

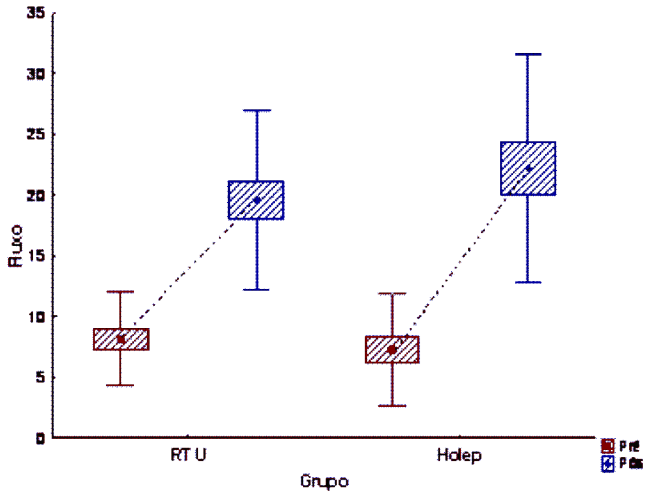


***Figure 1*** - *Varying urinary peak flow on each group (ml/s), p<0.001.*

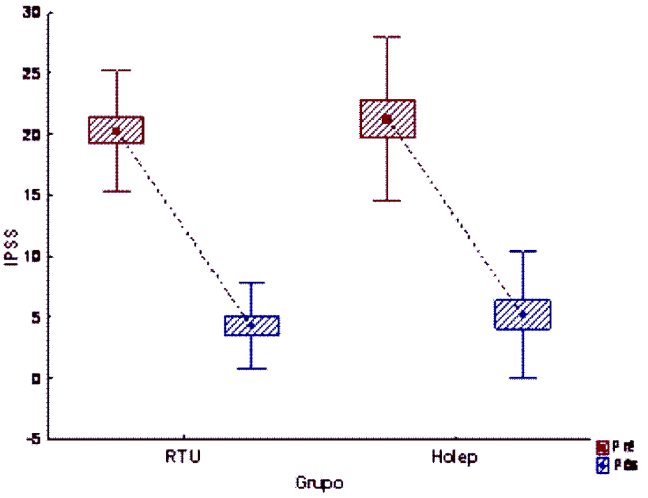


***Figure 2*** - *Varying IPSS on each group, p<0.001.*

Laser energy does not interfere on pathologist competence to prostate cancer detection. Placer *et al.*[9] reports 4.8% of incidental cancer. One patient in HoLEP group (5%) has been diagnosed with incidental adenocarcinoma. He had been treated with radical prostatectomy. This data ratifies that histological ability is preserved, as reported by other authors[3,9].

When analyzing intraoperative complications we have found a high percentage of bladder injury, six patients in HoLEP group, i.e. 30%. Placer *et al.*[9] reports 4%, Montorsi[3], 18% and Elzayat and Elhiali[10] less than 1% (1/118). The discrepancy between the values is marked and it happens because there is no uniformity on data classification. Some authors report all injuries and others report just that of clinical relevance. In the present analysis all injuries have occurred on morcellation, they were minimal mucosal lacerations that had not altered the surgery, nor catheter indwelling time and neither hospital stay.

Storage symptoms are frequent after HoLEP, 19.2%. Nevertheless they do not last more than 1-2 months and the patients have improved with anticholinergic therapy[9]. Urgency after the HoLEP procedure is more frequent than in TURP, 5.6% vs 2.2%[8]. It was not the objective of this study to evaluate storage symptoms.

On late complications, Montorsi reports that urethral stenosis was more frequent in TURP than in HoLEP[3]. Placer *at al.*[9] reports that five patients (4%) have developed bladder neck sclerosis. In our report there were no late complications, mainly by the short follow up period. One patient (5%) in HoLEP group has developed bladder neck sclerosis at the sixth month; therefore this data is not in our outcomes.

Summarizing early and late adverse events are similar in both techniques[8,10]. There are in literature studies even comparing HoLEP with the open prostatectomy to BPH treatment. Kuntz *at al.*[12] reported low per-operatory morbidity (15% vs. 26.7%), and reduction in hemoglobin values (1.9 vs. 2.8 g/dl), catheter indwelling time (30 vs. 194 h), and hospital stay (70 vs. 250 h) were significantly lower in HoLEP technique. HoLEP and the open procedure evidence the same early and late re-intervention rate.

Reviewing all laser treatment options for BPH, Gravas *et al.*[4] have evidenced that HoLEP represents the endoscopic alternative to open procedure on BPH treatment and it is the most advanced laser prostatic surgery. Ahyai *et al.*[8] report that prostatic cavity after enucleation is similar to that after the open procedure, and the enucleation technique has become candidate to replace TURP[8,10].

HoLEP has a remarkable outcome on immediate BPH symptoms relief but also on late assessment. Elzayat and Elhilali[10] has reported a five year follow up study demonstrating a 204% raise on urinary peak flow rate, 81% reduction in post-voiding residual volume and 67.6% IPSS reduction, with re-intervention rate of 4.2%.

The main disadvantage of Holmium laser enucleation technique is the steep learning curve[10,11], what keeps it in the large medical centers[8]. Placer *et al.*[9] reports that this steep learning curve has limited its spread. Elzayat e Elhilali[10] affirms that the curve is about 50 cases, but can be reduced to 27 under supervision of an experienced urologist.

HoLEP is internationally accepted, with evidence level 1, as an alternative for TURP and for open procedure[11]. Many clinical trials have proved its feasibility, efficacy, safety and cost-effectiveness[9]. Recent meta-analysis highlights HoLEP as a promising alternative[13]. In our study it was not different. We have got to perform all HoLEP procedures on adequate time. The patients are clinically well, both in symptomatic terms – assessed by IPSS – as in objective terms – assessed by urinary peak flow rate. These data were very similar to the TURP group, which is still the gold standard treatment for small and median volume prostates. Besides that, the technique has proved to be safe by the low blood loss and complication rate.

Holmium laser enucleation of the prostate (HoLEP) is an effective technique on BPH treatment and can be performed in our country, because it has outcomes – in terms of effectiveness and applicability – equal to TURP.

## R E S U M O

***Objetivo:*** *avaliar a eficácia e a aplicabilidade da enucleação prostática com Holmium Laser (HoLEP), no tratamento da hiperplasia prostática benigna (HPB), comparando-a à ressecção transuretral da próstata (RTUp).* ***Métodos:*** *ambos os procedimentos eram explicados aos pacientes com indicação de tratamento cirúrgico e eles escolhiam qual procedimento seria realizado, HoLEP ou RTUp. Eram coletados dados da internação, dados clínicos, escore de sintomas e pico de fluxo urinário. No ato operatório registravam-se tempo cirúrgico, tempo de morcelamento (nos casos de HoLEP), lesão vesical ou intercorrências. Noventa dias após a operação era feita uma nova avaliação do pico de fluxo urinário e escore de sintomas. A análise estatística foi realizada em parte pelo programa Sinpe® e também por uma equipe profissional.* ***Resultados:*** *foram operados 20 pacientes no grupo HoLEP e 21 no RTUp. O pico de fluxo urinário pré-operatório foi 8ml/s em ambos os grupos. O escore de sintomas pré-operatório foi 22 no grupo HoLEP e 20 no RTUp. O tempo operatório foi 85 minutos no grupo HoLEP e 60 minutos no RTUp, p<0,05. A internação hospitalar foi 47 horas para o grupo de HoLEP e 48 horas para RTUp, p<0,05. Na avaliação em 90 dias o fluxo urinário aumentou para 21,5ml/s no grupo HoLEP e para 20ml/s no RTUp e a mediana do escore de sintomas reduziu para 3 em ambos os grupos.* ***Conclusão:*** *o HoLEP é técnica tão eficaz quanto RTUp, no tratamento da HPB. A enucleação prostática com Holmium laser (HoLEP) é técnica eficaz no tratamento da HPB e pode ser aplicável, pois produz resultados, em termos de eficácia e aplicabilidade, comparáveis à RTUp.*

***Descritores:*** *Próstata. Hiperplasia Prostática. Ressecção Transuretral da Próstata. Terapia a Laser.*

## REFERENCES

1. BahiaLR, AraújoDV, PepeC, Trindade M, Camargo CM, JavaroniV. Cost-effectiveness analysis of medical treatment of benign prostatic hyperplasia in the Brazilian public health system. IntBraz J Urol. 2012;38(5):595-605.
2. JeongCW, Oh JK, ChoMC, Bae, JB, OhSJ. Enucleation ratio efficacy might be a better predictor to assess learning curve of holmium laser enucleation of the prostate. IntBraz J Urol.2012;38(3):362-71; discussion 372.
3. Montorsi F, Naspro R, Salonia A, Suardi N, Briganti A, Zanoni M, et al. Holmium laser enucleation versus transurethral ressection of the prostate: results of a 2-center, prospective, randomized trial in patients with obstructive prostatic hyperplasia. J Urol. 2004;172(5 Pt 1):1926-9.
4. Gravas S, BachmannA, Reich O, Roehrborn CG, GillingPJ,De La Rosette J. Critical review of lasers in benign prostatic hyperplasia (BPH). BJU Int. 2011;107(7):1030-43.
5. GillingPJ, KennettK, DasAK, ThompsonD, Fraundorfer MR. Holmium laser enucleation of the prostate (HoLEP) combined with transurethral tissue morcellation: an update on the early clinical experience. J Endourol. 1998;12(5):457-9.
6. Borsato EP. Modelo multicêntrico para elaboração, coleta e pesquisa de dados em protocolos eletrônicos [tese]. Curitiba: Universidade Federal do Paraná, Ciências da Saúde; 2005.
7. PintoJS. Interface de viabilização de informações para o Sistema Integrado de Protocolos Eletrônicos [tese]. 2005. Curitiba: Universidade Federal do Paraná, Ciências da Saúde; 2005.
8. Ahyai SA, Gilling P, Kaplan SA, Kuntz RM, Madersbacher S, Montorsi F, et al. Meta-analysis of functional outcomes and complications following transurethral procedures for lower urinary tract symptons resulting from benign prostatic enlargement. Eur Urol. 2010;58(3):384-97.
9. Placer J, Gelabert-Mas A, Vallmanya F, Manresa JM, Menéndez V, Cortadellas R, et al.Holmium laser enucleation of prostate: outcome and complications of self-taught learning curve. Urology.2009;73(5):1042-8.
10. Elzayat EA, Elhilali MM. Holmium laser enucleation of the prostate (HoLEP): long-term results, reoperation rate, and possible impact of the learning curve. Eur Urol. 2007;52(5):1465-71.
11. Ahyai SA, Chun FK, Lehrich K, Dahlen R, Zacharias MS, Fisch MM, et al. Transurethral holmium laser enucleation versus transurethral resection of the prostate and simple open prostatectomy—which procedure is faster? J Urol. 2012;187(5):1608-13.
12. Kuntz R, Lehrich K, AhyaiSA. Holmium laser enucleation of the prostate versus open prostatectomy for prostates greater than 100 grams: 5-year follow-up results of a randomised clinical trial. Eur Urol. 2008;53(1):160-6.
13. Yin L, Teng J, Huang CJ, Zhang X, Xu D. Holmium laser enucleation of the prostate versus transurethral resection of the prostate: a systematic review and meta-analysis of randomized controlled trials. J Endourol. 2013;27(5):604-11.

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**Address for correspondence:**
Luís Eduardo Durães Barboza
E-mail: luiseduardobarboza@hotmail.com

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# Effect of triamcinolone in keloids morphological changes and cell apoptosis

## *Efeito da triancinolona na apoptose celular e nas alterações morfológicas em queloides*

João Márcio Prazeres dos Santos[1]; Cláudio de Souza,ACBC-MG[2]; Anílton César de Vasconcelos[2]; Tarcizo Afonso Nunes[2]


## ABSTRACT

**Objective:** to assess the effects of injectable triamcinolone on keloid scars length, height and thickness, and on the number of cells undergoing apoptosis. **Methods:** This study consists in a prospective, controlled, randomized, single-blinded clinical trial, conducted with fifteen patients with ear keloids divided into two groups: group 1 - seven patients undergoing keloid excisions, and group 2 - eight patients undergoing keloid excisions after three sessions of infiltration with one ml of Triamcinolone hexacetonide (20mg/ml) with three week intervals between them and between the last session and surgery. The two groups were homogeneous regarding age, gender and evolution of the keloid scar. The keloid scars of patients in group 2 were measured for the length, height and thickness before triamcinolone injection and before surgery. A blinded observer performed morphological detailing and quantification of cells in hematoxylin-eosin-stained surgical specimens. An apoptotic index was created. **Results:** The apoptotic index in group 1 was 56.82, and in group 2, 68.55, showing no significant difference as for apoptosis (p=0.0971). The reduction in keloid dimensions in Group 2 was 10.12% in length (p=0.6598), 11.94% in height (p=0.4981) and 15.62% in thickness (p=0.4027). **Conclusion:** This study concluded that the infiltration of triamcinolone in keloid scars did not increase the number of apoptosit and did not reduce keloids' size, length, height or thickness.


**Key words:** Apoptosis. Keloid. Triamcinolone Acetonide. Wound Healing.

## INTRODUCTION

Keloids are fibroproliferative disorders[1] occurring in five to 15% of scars, regardless of gender[2]. They may be caused by inability of the body to interrupt the healing process, causing excess cell proliferation and increased collagen production[3]. Apoptosis is the most appropriate way to adjust the amount of fibroblasts and other cells during healing, as well as in other physiological events, by presenting less inflammation and being a regulated process[4].

Researches have not achieved complete success in elucidating keloids' causes and, as a consequences, treatments are varied and not always show good results[2,3]. The therapeutic approach currently adopted for keloids consists of several options and, among them, are surgical resection and intralesional corticosteroid infiltration[5-8]. Glucocorticoids are used for at least five decades[7] and act by decreasing the inflammatory process, which could be one of keloids' causes. However, this may be just one of the actions[9]. Investigations with *in vitro* fibroblast cultures have aimed at elucidating the effects of corticosteroids on apoptosis[10,11]. This fact motivated us to investigate the effects of triamcinolone in the *in vivo* keloid scar.

The objective of this study was to assess the effects of injectable triamcinolone in keloid scars on the number of cells undergoing apoptosis and changes in scars' length, height and thickness.

## METHODS

The research was conducted at the surgery clinic of the Hospital Escola da Faculdade de Medicina de Barbacena (Centro de Especialidades Médicas – CEMED) and at the Laboratório de Apoptose, Instituto de Ciências Biológicas (ICB) of UFMG, after approval by the Comitê de Ética em Pesquisa (CEP) of UFMG (# 0601.0.203.000-11), as well as by the Comitê de Ética em Pesquisa of the Universidade Presidente Antônio Carlos (UNIPAC) (# 911/2011).

This was a longitudinal, prospective, controlled, randomized, single-blinded clinical study[12], which included individuals with keloid scars located in the earlobe due to piercing and who agreed to participate by signing an informed consent form. We excluded individuals who had previous scar treatment, use of antimitotic and/or

1. Disciplina de Clínica Cirúrgica do Adulto I, Faculdade de Medicina de Barbacena – MG - Brazil; 2. Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG) - MG - Brazil.

immunomodulatory drugs or individuals not willing to participate in the survey.

The subjects were interviewed for anamnesis and the sample consisted of 15 individuals, seven (46.67%) male and eight (53.33%) females, three white, seven brown and five black (p=0.782).

We randomly created two groups of subjects: Group 1 - seven (46.67%) patients who underwent resection of keloids; and Group 2 - eight (53.33%) subjects who received intralesional corticosteroid infiltration prior to surgical resection.

All patients underwent extramarginal surgical resection of the keloids followed by suture with separate 6-0 Nylon monofilament stitches. The removal of the lesion was the only procedure in Group 1 individuals.

In Group 2, keloid scars were measured in centimeters in length, height and thickness, with the help of graph caliper (Rhosse®, São Paulo). Then we conducted three infiltration sessions of triamcinolone hexacetonide 1ml, at a concentration of 20mg/mL, in the central region of keloid scar; the sessions were three weeks apart[10,11]. Three weeks after the last infiltration session we repeated the keloid scars measurements, followed by their resection. Were removed the stitches ten days after surgery and individuals returned to monthly assessment for six months.

Surgical specimens were divided into three fragments of up to 0.5cm thick by longitudinal section, including the center and border of the lesion. All fragments were sent to the Laboratório de Apoptose at the UFMG, wherein one fragment was placed in a pH 7.4 buffered 10% formalin for analysis and the other two fragments were placed in vials with glutaraldehyde and liquid nitrogen to other research.

The specimens fixed in formalin were processed according to routine techniques, cut in 5ìm slices and stained with hematoxylin-eosin (HE) to morphological detailing.

We used the light microscope Olympus CH 300, with plan achromatic objective lenses with 40-times magnification; we also obtained photographs scanned through a JVC® TK 720V camera attached to the microscope to capture by Honestech TVR® images analyzer, version 2.5 for Windows®.

The quantification of the cells was performed manually in scanned images obtained from histological fields and analyzed with the software Image-Pro Plus®, version 4.5.0.29, with five fields obtained for each slide, and averaged between these measurements. This assessment was made by a single masked observer, which regarded as apoptotic the cells that had three or more of the following morphological characteristics: anoiquia, condensation of cytoplasm, nuclear condensation, nuclear fragmentation and cell fragmentation. The apoptotic index (AI) was determined by the following formula: AI = number of apoptotic cells X100 / number of total cells.

We carried out TUNEL (terminal deoxynucleotide transferase-mediated dUTP Nick-End Labelin) reactions to confirm the presence of apoptotic cells evidenced in the cytomorphological analysis (TdT-FragEL DNA Fragmentation Detection Kit ® TM, Calbiochem).

We employed nonparametric tests (Mann-Whitney), since groups showed asymmetric distribution and reduced number of individuals[13,14]. We adopted the significance level of 5% (p <0.05).

## RESULTS

The age ranged from 12 to 35 years; group 1 had a mean age of 19.29 years (±4.645) and group 2, 35.38 years (±30.336) (p=0.779). There was a predominance of browns and blacks in both groups, six individuals in each group, which corresponded to 85.8% in group 1 and 75.0% in group 2.

When comparing the initial with the final measurements of Group 2 individuals, after corticoid infiltration the keloid scars showed an average reduction in dimensions, 10.12% in length, 11.94% in height and 15.62% in thickness, with no significant difference (p> 0.05) (Table 1).

In Group 1 individuals, without the infiltration of triamcinolone in keloid scars, the apoptotic index was 56.82 (±15.82), while in Group 2, with triamcinolone infiltration, this index was 68.55 (±9,32), with no significant difference (p=0.0971). We observed no side effect of steroid use in Group 2 subjects.

## DISCUSSION

The differentiation between keloids and hypertrophic scars is authoritative, because, although they have the same probable etiology, they exhibit differences in clinical presentation and treatment. For example, hypertrophic scars tend to regression[15,16] and keloids present in several locations[2,9]. We then restricted the inclusion in the study to individuals with keloids on the ear lobe, so the sample would be homogeneous. Trauma is essential to trigger the keloids formation process, and can be a response to insect bites[9], acne[3], surgical incisions[17] and piercings, as in all this study's subjects.

In this series, 80% of subjects had pigmented skin, considering the members of both groups with black or brown skin, according to previous assessments[9,16,18]. As in the literature, we found a predominance of keloids between the second and third decades of life[9,16,18] and there was no difference in gender distribution between groups (p=0.782)[17]. There was also no difference (p=0.779) as to the keloids evolution time, what prevented the results to be influenced by more resistance of mature lesions[19,20].

Triamcinolone infiltrations were performed in the central region of keloids with the aim of causing more

**Table 1** - Initial and final dimensions of keloid scars of Group 2 individuals.

| Dimensions | Initial $\bar{\chi}$ ± sd | Final $\bar{\chi}$ ± sd | p * |
|---|---|---|---|
| Lenght[a] | 1.363 ± 0.6413 | 1.225 ± 0.5800 | 0.6598 |
| Height[a] | 0.8375 ± 0.3114 | 0.7375 ± 0.2615 | 0.4981 |
| Thickness[a] | 0.8000 ± 0.3071 | 0.6750 ± 0.2712 | 0.4027 |

*a: measure in centimeters; : Average; SD: standard deviation; *: Mann-Whitney test.*

effects, since this site's fibroblasts are more metabolically active[21]. There is no consensus on the dose of corticosteroids to be used[2,8,9,11] and the concentration of 20mg/mL is one commercially available presentation. The option of performing injections at three-week intervals was due to pharmacokinetics, as the drug form local depot and produces effect for 21 days, on average. The use of steroids may be accompanied by side effects, such as atrophy, depigmentation, telangiectasia, necrosis, ulceration and Cushing's syndrome[2,17,18,22]. The absence of side effects in this study could be due to treatment not being extended over three sessions and to the used concentration of 20mg/mL triamcinolone. This suggests the possibility of further investigation of such effects when using corticosteroids for more sessions or in higher concentrations. The clinical application of corticosteroids is one of the therapeutic resources to decrease keloid masses[7,8,18] and can be supported by the reduction in keloids size in this survey, which was 12.56% on average when jointly considering length, height and thickness.

The comparison of the apoptotic index between groups 1 and 2 showed no significant difference (p=0.0971), which can be explained by several reasons: small sample; speed with which apoptosis occurs, with the possibility of lasting only 24 hours, as described by Kerr, Wyllie and Currie, in 1972[23]; and the fact that keloids present heterogeneous tissues, despite all attempts by authors to homogenize the sample. Although research has not shown significant increase in apoptosis in infiltrated keloids, this increase is worthy of attention, since keloids' fibroblasts are more resistant to apoptosis and display apoptosis rates 22% lower when compared with fibroblasts from normal skin or from scars considered normal[24].

The TUNEL assay is an assay for DNA fragmentation and detection. Although not specific to apoptosis, since necrosis can also display this fragmentation, this reaction has been widely used to quantify apoptosis, both in cell cultures and preserved in frozen tissue, formalin or paraffin[25]. The TUNEL assay is a reliable method for apoptosis detection, especially when confirmed by another method such as cytomorphological analysis[25].

The need for the use of these tests has disadvantages, because for values to be significant, $p<0.05$, samples should be higher or the difference between them must be the greatest possible[14]. These two conditions were not met in this research.

Due to sample size and results obtained, other studies should be proposed in order to evaluate the role of triamcinolone in apoptosis, as well as investigations of other mechanisms of action of this drug on scars.

The infiltration of triamcinolone in keloid scars did not increase the number of apoptoses and caused no reduction in dimensions, length, height and thickness of infiltrated keloids.

## R E S U M O

***Objetivo:*** *comparar o efeito da triancinolona injetável em cicatrizes queloidianas quanto ao número de células em apoptose e avaliar o efeito da triancinolona quanto às alterações no comprimento, altura e espessura dessas cicatrizes.* ***Métodos:*** *estudo clínico longitudinal, prospectivo, controlado, aleatorizado, unicego, com 15 pacientes portadores de queloides de orelha distribuídos em dois grupos: grupo 1, com sete pacientes submetidos apenas às exéreses dos queloides; e grupo 2, com oito pacientes submetidos às exéreses das lesões após três sessões de infiltração de 1ml de hexacetonida de triancinolona (20mg/mL), com intervalos de três semanas entre elas, assim como entre a última sessão e a operação. Os dois grupos foram homogêneos quanto à: idade (p=0,867), sexo (p=0,782) e tempo de evolução da cicatriz queloidiana (p=0,779). As cicatrizes queloidianas dos pacientes do grupo 2 foram medidas quanto ao comprimento, altura e espessura antes da injeção da triancinolona e antes do procedimento cirúrgico. Um observador mascarado realizou detalhamento morfológico e quantificação das células nas peças cirúrgicas, coradas com HE. Foi criado um índice apoptótico.* ***Resultados****: os dois grupos foram homogêneos quanto à: idade (p=0,867), sexo (p=0,782) e tempo de evolução da cicatriz queloidiana (p=0,779). o índice apoptótico no grupo 1 foi 56,82 e no grupo 2, 68,55, sem diferença (p=0,0971). As reduções nas dimensões dos queloides dos grupos 2 foram 10,12% para o comprimento (p=0,6598), 11,94% para a altura (p=0,4981) e 15,62% para a espessura (p=0,4027).* ***Conclusão:*** *a infiltração de triancinolona nas cicatrizes queloidianas não aumentou o número de apoptoses e não houve redução das dimensões, comprimento, altura e espessura dos queloides.*

***Descritores****: Apoptose. Queloide. Triancinolona Acetonida. Cicatrização.*

## REFERENCES

1. Tredget EE, Nedelec B, Scott PG, Ghahary A. Hypertrophic scars, keloids, and contractures. The cellular and molecular basis for therapy. Surg Clin North Am. 1997;77(3):701-30.
2. Ceoviæ R, Lipozenciæ J, Bukviæ Mokos Z, Stulhofer Buzina D, Kostoviæ K. Why don't we have more effective treatment for keloids? Acta Dermatovenerol Croat. 2010;18(3):195-200.
3. Shih B, Garside E, McGrouther DA, Bayat A. Molecular dissection of abnormal wound healing processes resulting in keloid disease. Wound Repair Regen. 2010;18(2):139-53.
4. Ferrari CKB. Apoptose: a importância da maquinaria de morte celular no controle e na patogêneses das doenças. Rev ciênc méd. 2000;9(1):21-31.
5. de Oliveira GV, Nunes TA, Magna LA, Cintra ML, Kitten GT, Zarpellon S, et al. Silicone versus nosilicone gell dressings: a controled trial. Dermatol Surg. 2001;27(8):721-6.
6. Carroll LA, Hanasono MM, Mikulec AA, Kita M, Koch RJ. Triamcinolone stimulates bFGF production and inhibits TGF-beta1 production by human dermal fibroblasts. Dermatol Surg. 2002;28(8):704-9.
7. Muneuchi G, Suzuki S, Onodera M, Ito O, Hata Y, Igawa HH. Long-term outcome intralesional injection of traimcinolone acetonide for the treatment of keloids scars in Asian patients. Scand J Plast Recontr Surg Hand Surg. 2006;40(2):111-6.
8. Widgerow AD, Chait LA, Stals PJ, Stals R, Candy G. Multimodality scar management program. Aesthetic Plast Surg. 2009;33(4):533-43.
9. Juckett G, Hartman-Adams H. Management of keloids and hypertrophic scars. Am Fam Physician. 2009;80(3):253-60.
10. Darougheh A, Asilian A, Shariati F. Intralesional triamcinolone alone or in combination with 5-fluorouracil for the treatment of keloid and hupiertrophic scars. Clin Exp Dermatol. 2009;34(2):219-23.
11. Durani P, Bayat A. Levels of evidence for the treatment of keloid disease. J Plast Reconstr Aesthet Surg. 2008;61(1):4-17.
12. Hochman B, Nahas FX, Oliveira Filho RS, Ferreira LM. Desenhos de pesquisa. Acta Cir Bras. 2005;20(supl 2):2-9.
13. Siqueira AL, Tibúrcio JD. Estatística na área de saúde: conceitos, metodologia, aplicações e prática computacional. Belo Horizonte: Coopmed; 2011.
14. Triola MF. Introdução à estatística. Atualização da tecnologia. 11ª ed. Rio de Janeiro: Livros Técnicos e Científicos; 2013.
15. Gupta S, Sharma VK. Standard guidelines of care: Keloids and hypertrophic scars. Indian J Dermatol Venereol Leprol. 2011;77(1):94-100.
16. Wolfram D, Tzankov A, Pülzl P, Piza-Katzer H. Hypertrophic scars and keloids—a review of their pathophysiology, risk factors, and therapeutic management. Dermatol Surg. 2009;35(2):171-81.
17. Al-Attar A, Mess S, Thomassen JM, Kauffman CL, Davison SP. Keloid pathogenesis and treatment. Plast Reconstr Surg. 2006;117(1):286-300.
18. Mustoe TA, Cooter RD, Gold MH, Hobbs FD, Ramelet AA, Shakespeare PG, et al. International clinical recommendations on scar management. Plast Reconstr Surg. 2002;110(2):560-71.
19. Ueda K, Furuya E, Yasuda Y, Oba S, Tajima S. Keloids have continuous high metabolic activity. Plast Reconstr Surg. 1999;104(3):694-8.
20. Wang XQ, Liu YK, Qing C, Lu SL. A review of the effectiveness of antimitotic drug injections for hypertrophic scars and keloids. Ann Plast Surg. 2009;63(6):688-92.
21. Lu F, Gao J, Ogawa R, Hyakusoku H, Ou C. Biological differences between fibroblasts derived from peripheral and central areas of keloid tissues. Plast Reconstr Surg. 2007;120(3):625-30.
22. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. N Engl J Med. 2005;353(16):1711-23.
23. Kerr JF, Wyllie AH, Currier AR. Apoptosis: a basical biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972;26(4):239-57.
24. Ladin DA, Hou Z, Patel D, McPhail M, Olson JC, Saed GM, et al. p53 and apoptosis alterations in keloids and keloid fibroblasts. Wound Repair Regen. 1998;6(1):28-37.
25. Huerta S, Goulet EJ, Huerta-Yepez S, Livingston EH. Screening and detection of apoptosis. J Surg Res. 2007;139(1):143-56.

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**Address for correspondence:**
João Márcio Prazeres dos Santos
E-mail: jmprazsantos@hotmail.com

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Original Article

# Effect of cilostazol on neointimal hyperplasia in iliac arteries of pigs after transluminal angioplasty

## *Efeito do cilostazol na hiperplasia neointimal em artérias ilíacas de suínos submetidas à angioplastia transluminal*

Joel Alex Longhi[1]; Adamastor Humberto Pereira[1]

A B S T R A C T

**Objective**: to evaluate whether systemic administration of cilostazol reduces neointimal hyperplasia in iliac arteries of pigs submitted to balloon catheter angioplasty. **Methods**: twenty pigs underwent angioplasty with a 6x40 mm balloon catheter in the right common iliac artery, guided by Doppler ultrasound. The animals were randomized into two groups: group 1 (n=10), which received 50mg cilostazol twice a day, and group 2 (n=10), control. After 30 days, the animals were killed and the iliac arteries prepared for histological analysis. The histological sections were digitized and analyzed by digital morphometry. Statistical analysis was performed using the Student t and Mann-Whitney tests. **Results**: when comparing the iliac arteries submitted to angioplasty with those not subjected to angioplasty, there was significant neointimal hyperplasia (0.228 versus 0.119 $mm^2$; p=0.0001). In arteries undergoing angioplasty, there was no difference between group 1 (cilostazol) and group 2 (control) as for the lumen area (2.277 versus 2.575 $mm^2$; p=0.08), the tunica intima (0.219 versus 0.237 $mm^2$; p=0.64), the tunica media (2.262 vs. 2.393 $mm^2$; p=0.53) and the neointimal occlusion percentage (8.857 vs. 9.257 %; p=0.82). **Conclusion**: the use of cilostazol 50mg administered in two daily doses did not reduce neointimal hyperplasia in iliac arteries of pigs submitted to balloon angioplasty catheter.

**Key words:** Neointima. Hyperplasia. Phosphodiesterase Inhibitors. Angioplasty. Iliac Artery.

## INTRODUCTION

Peripheral arterial disease has a prevalence of up to 20% in patients over 70 years old and is an important cause of morbidity[1]. When surgical intervention is indicated, the endovascular technique is the initial treatment for most anatomical stages in different arterial sites[1,2] and in various clinical conditions. The endovascular treatment initial success rate is high, exceeding 90% in the aortoiliac segment, but the patency falls over time due to restenosis[3,4].

Drugs have been used in attempting to reduce the risk of restenosis after percutaneous vascular procedures. Cilostazol has antiplatelet, vasodilatory and antiproliferative effects[5-7]. It is a selective phosphodiesterase III inhibitor and promotes increase of adenosine 3',5'-cyclic monophosphate in platelets and smooth muscle cells. The sequence of events stimulates the rapid regeneration of endothelial cells, which inhibits neointimal formation by two mechanisms: blockage of the abnormal growth of vascular smooth muscle cells (VSMC) and endothelial function improvement[5,6,8-12]. These mechanisms may be responsible for reducing restenosis after coronary stent insertion observed in clinical trials and metanalyses[13,14].

There are studies with small numbers of patients analyzing the use of cilostazol after peripheral endovascular procedures, especially in the femoropopliteal segment, with favorable initial results[15,16]. However, it is not clear whether its application can be extended to other arterial sites.

The objective of this study is to evaluate whether the systemic administration of cilostazol reduces neointimal hyperplasia in iliac arteries of pigs submitted to balloon catheter angioplasty.

## METHODS

We performed the procedures in 20 Large White pigs, from different familial origins, with average eight weeks of age, from the Unidade de Experimentação Animal of Hospital de Clínicas of Porto Alegre (HCPA), accompanied by a veterinarian and a nurse. The study was approved by the Comitê de Ética of the Grupo de Pesquisa e Pós-Graduação of HCPA and conducted according to the protocol of the Unidade de Experimentação Animal of HCPA. Project number 09-150.

Exclusion criteria established previously to the experiment implementation were: thrombosis or rupture of the segment undergoing angioplasty; reoperation due to

1. Programa de Pós-Graduação em Medicina- Ciências Cirúrgicas, Universidade Federal do Rio Grande do Sul - RS - Brazil.

bleeding; death of the animal before the deadline of arterial segments withdrawal; technical glitches in tissues preparation or processing.

The pigs were pre-medicated with ketamine (15mg/kg) and midazolam (0.8mg/kg) intramuscularly. After ten minutes, we held an access in the cephalic vein for administration of 0.9% saline (5ml/kg/h). During the procedure, the animals received 100% oxygen through a face mask, which, coupled with the muzzle, allowed monitoring of respiratory rate. We also monitored heart rate and the anterior leg withdrawal reflex. We kept the animals in deep sedation by the administration of a propofol "bolus". Infiltration was performed with lidocaine (4mg/mg) in the site of the femoral artery access. Before the procedure, we administered the analgesic tramadol (2mg/kg) and the antimicrobial cefazolin (20mg/kg).

The right common femoral artery was surgically exposed. Through direct puncture of the common femoral artery with a 18G needle, we introduced the 0.035 inches hydrophilic guide wire and inserted the 6 Fr, 11cm sheath. Under ultrasound control, was directed the guide wire to the abdominal aorta. At this point, the animals received intravenous heparin (Heptar, Eurofarma) at a dose of 100 IU/kg. We performed doppler ultrasonography (TITAN equipment, SonoSite) during surgery to measure the diameters of the right common iliac artery and distal abdominal aorta. Then, we positioned the 6x40 mm balloon catheter (Passeo-35, Biotronik) in the right common iliac artery, from the trifurcation of the aorta, under ultrasound control. We carried out the angioplasty for one minute with an 8atm pressure. The diameter of the balloon catheter was selected to provide the sizing 10-20% larger than the common iliac artery lumen in this age group, similar to that used in interventions on human patients. At the end of angioplasty, we performed a Doppler ultrasound again to confirm the iliac segment arterial patency. After removal of sheath and guide wire, the common femoral artery was sutured, and the groin closed with nonabsorbable sutures. There were no intraoperative complications.

We divided the animals into two groups and randomized treatment through the site http://www.randomization.com. Group 1 – animals undergoing angioplasty and administration of cilostazol (Vasativ, Eurofarma) 50mg twice a day starting on the first day after surgery and administered until the 30th day; Group 2 (control) – animals submitted only to angioplasty. The animals were tagged with numbered earrings of different colors for identification.

In the postoperative period, animals were housed in specific bays, with running water and balanced chow for their age and no additional lipid supplementation. After 30 days, the animals were again anesthetized and underwent laparotomy with exposition of the abdominal aorta and iliac arteries. The surgical specimens, containing the distal abdominal aorta and iliac arteries, were removed *en bloc* after a lethal dose of 2.5% sodium thiopental.

For optical microscopy evaluation, surgical specimens were rinsed in a 0.9% NaCl solution and fixed in a neutral buffered 10% formalin solution. The common right iliac arteries (undergoing angioplasty) and left (not undergoing angioplasty) were transversally sectioned in three points measured from the aortic trifurcation: 10mm, 20mm and 30mm. These points correspond respectively to the locations 25, 50 and 75% of the used balloon length (40mm). The segments were embedded in paraffin blocks, sectioned into 4μm sections and stained with hematoxylin-eosin, Verhoeff and orcein techniques.

The images of histological sections were scanned for morphometric analysis through morphometry and image analysis with the Image-Pro Plus software version 6.0 (Media Cybernetics - Silver Spring, USA) and Image (Scion Corporation - USA). The images of histological sections were digitized from the conventional optical microscopy: microscope with plan-achromatic lenses (Zeiss Axiostar - Germany) with phototube adjusted to 0.25 magnification, color closed circuit camera (Sony DXC-151 - Japan) and digital analog conversion board (Image-Pro Plus Capture Kit, Media Cybernetics - Silver Spring, USA), generating image files with 2560x1920 pixels. Images were scanned with 40X microscopic magnification.

The planimetry of lumen area, the tunica intima and tunica media was performed with an automated technique, without interference from the observer. The lumen area was obtained by direct measurement of the area bounded by the endothelium; the intima, subtracting the area of the lumen from the area bounded by the internal elastic lamina; the media area by subtracting the area of the lumen and the intima delimited by the external elastic lamina. The results of the morphometric measurements of the lumen, intima and media areas are presented in absolute numbers (square millimeters), using the average of the obtained areas from the three segments of each artery. Additionally, we calculated the sum of the media and intima areas and the percentage of neointimal occlusion by dividing the intima area by the area delimited by the internal elastic lamina.

For descriptive statistics, we used the average and the standard deviation for parametric variables, and the median and interquartile ranges for non-parametric variables. When comparing groups, we used the Student t test and the Mann-Whitney test. We considered $p<0.05$ as statistically significant.

## RESULTS

All 20 animals undergoing angioplasty completed the study. We directly assessed the iliac arteries' patency during the removal of tissue for histological analysis. There was no difference animals weight between groups (24.83 versus 22.90kg; p=0.34). The diameter of the common iliac artery (mean of the

three measurements) was also not different (5.07 versus 4.87 mm, p=0.39).

By comparing the intima area in the right common iliac artery (submitted to angioplasty) with the intima area in the left common iliac artery (not submitted to angioplasty), we observed a significant neointimal hyperplasia (0.228 versus 0.119 $mm^2$; p=0.00001) (Figures 1 and 2). This difference persists even when stratified by Group 1 (cilostazol) and Group 2 (control) (Figure 3). Table 1 shows the data of the digital morphometry of the right common iliac arteries subjected to balloon catheter angioplasty. There was no difference in the lumen area, the intima area, the media area, the media + intima area and the neointimal obstruction percentage between groups (Table 1 and Figure 4).

## DISCUSSION

Restenosis after balloon catheter angioplasty is caused by the negative elastic remodeling and the proliferation and migration of vascular smooth muscle cells (VSMC)[17]. In this model, the response of the arterial wall to the damage caused by angioplasty is the release of growth factors and other biologically active factors, which change the composition of the extracellular matrix and promote VSMC phenotypic change, from contractile to synthetic (dedifferentiation), leading to cell proliferation in the tunica media and migration to the tunica intima, forming the neointima[18].

Animal models with pigs better reflect the pathophysiology of restenosis occurring in humans, with similar stages of neointimal formation[19-21]. In addition, pigs present similarities in the vascular anatomy, in the coagulation system and physiology; and in medium-sized animals, one can use the same materials used with humans without the need for adjustments[19].

The experimental porcine model of balloon catheter angioplasty in coronary arteries could cause significant neointimal hyperplasia only when there was rupture of the internal elastic lamina[22]. In rat carotid arteries, the results are similar[23]. Previous work comparing versus angioplasty versus angioplasty with stent in pigs' iliac arteries concluded that the use of the stent causes increased neointimal hyperplasia[23]. These previous studies have also applied the technique of multiple angioplasties aimed to cause greater damage to the arterial wall. In this study there was significant neointimal hyperplasia, even using the balloon catheter oversizing of up to 20% commonly used in clinical practice and one single inflation of the

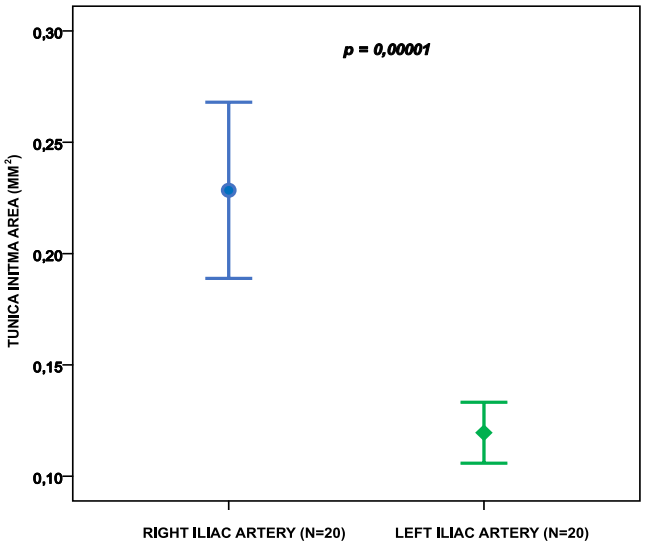


***Figure 2 -*** *Comparison between the intima area in the right iliac artery (subjected to angioplasty) and in the left iliac artery. The value of p shows a statistically significant neointimal hyperplasia in arteries undergoing angioplasty.*

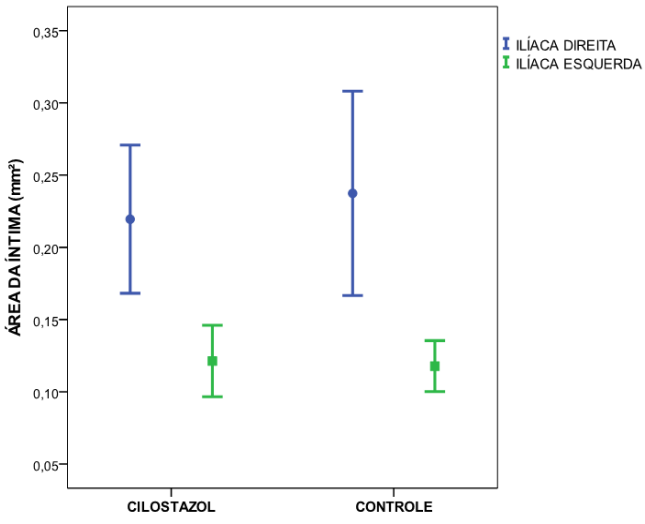


***Figure 3 -*** *Comparison between the intima area in the right iliac artery (submitted to angioplasty) and the left iliac artery. The value of p shows a statistically significant neointimal hyperplasia in arteries undergoing angioplasty, even when stratified by cilostazol and control groups.*

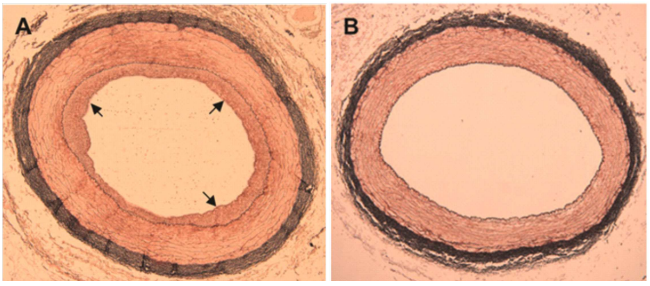


***Figure 1 -*** *A) Photomicrograph of the common iliac arteries of the same pig (orcein staining method, 40x original magnification); A) right common iliac artery submitted to angioplasty, with neointimal hyperplasia (arrow). B) left common iliac artery not subjected to angioplasty (normal artery).*

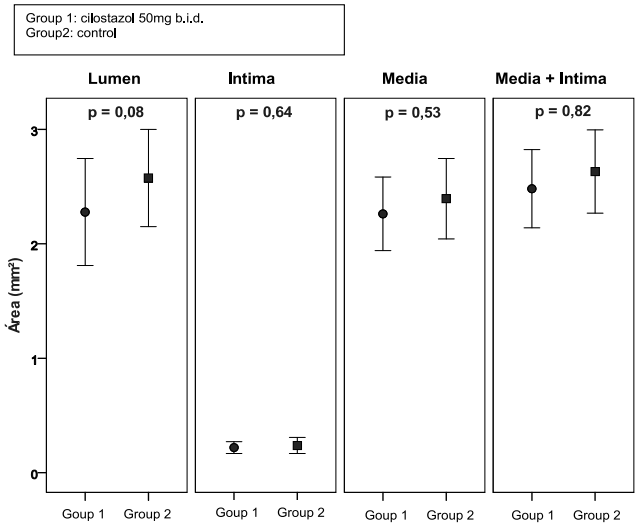


***Figure 4*** - *Comparison between the areas of the lumen, the intima, the media and media + intima between groups. The value of p shows that there was no difference between the cilostazol and control groups.*

balloon catheter, which might have caused minor injury to the arterial wall compared to studies using a larger oversize and multiple inflations. The option to apply the technique without a stent lies in the fact that although most studies evaluate the use of cilostazol in procedures with stent in coronary[13], carotid or peripheral[15,16] arteries, isolated balloon catheter angioplasty in iliac segment remains a significant procedure in the endovascular treatment.

Experimental studies have shown that around four weeks the neointimal hyperplasia is complete, similar to that identified in humans in six to 12 months[24,25]. A longer period of cilostazol administration probably would not add benefit. One limitation of this study is the assessment of the normal artery wall response. Extrapolation of the data is limited, since in the clinical setting the treated arteries present with atherosclerotic stenoses or occlusions. The adoption of an atherogenic diet does not appear to enhance neointimal hyperplasia in response to vascular wall injury in the porcine model[26].

The cilostazol dose of 50mg twice daily used in this study is similar to the maximum dose used in adults, and there were no adverse effects or change in behavioral pattern in treated pigs. Another limitation is the non-administration of other anti-platelet agents such as acetylsalicylic acid, ticlopidine and clopidogrel used in clinical trials[10]. However, the isolated use of cilostazol allows evaluating the effect of the drug, since the association with other drugs can have diverse biological effects. Previous studies with animal models demonstrated that the administration of cilostazol reduced neointimal hyperplasia in animal models for the study of the coronary artery [27], the carotid artery [28] and vein grafts [29,30]. Only one study in canine model evaluated the effect of cilostazol in the iliac artery. They performed angioplasty with stent associated with embolization with coils of the common femoral artery for reduced flow in the iliac axis. The results demonstrated reduced neointimal hyperplasia and absence of thrombotic occlusion in the cilostazol group compared with controls[31].

This is the first study in pigs that evaluated the use of cilostazol on neointimal hyperplasia of the iliac artery. Moreover, the work used the technique of angioplasty guided by Doppler ultrasonography, which allowed to accurately measuring arterial diameters and the consequent appropriate choice of the balloon catheter diameter.

Data from this study showed that the balloon catheter angioplasty caused significant neointimal hyperplasia in iliac arteries of pigs. The use of cilostazol 50mg twice daily for 30 days did not reduce neointimal hyperplasia in iliac arteries of pigs submitted to angioplasty. We observed no differences in the lumen area, tunica intima area and tunica media area.

## Acknowledgements

The authors thank the Animal Experimentation Unit and Experimental Pathology Unit of the HCPA for carrying out the experiment.

**Table 1** - Data from digital morphometry.

| | Cilostazol (n=10) | Controls (n=10) | p |
|---|---|---|---|
| Lumen area ($mm^2$) | 2.277 ± 0.654 | 2.575 ± 0.594 | 0.08 |
| Tunica Intima area ($mm^2$) | 0.219 ± 0.071 | 0.237 ± 0.098 | 0.64 |
| Tunica media area ($mm^2$) | 2.262 ± 0.449 | 2.393 ± 0.492 | 0.53 |
| Media + intima area ($mm^2$) | 2.481 ± 0.478 | 2.631 ± 0.508 | 0.50 |
| Neointimal obstruction (%) | 8.857 ± 3.464 | 9.257 ± 4.417 | 0.82 |

## R E S U M O

***Objetivo:*** *avaliar se a administração sistêmica de cilostazol reduz a hiperplasia neointimal nas artérias ilíacas de suínos submetidas à angioplastia com cateter balão.* ***Métodos:*** *vinte suínos foram submetidos à angioplastia com cateter balão 6x40 mm na artéria ilíaca comum direita, guiada por ultrassonografia com Doppler. Os animais foram randomizados em dois grupos: grupo 1 (n=10), no qual foi administrado cilostazol 50mg em duas doses diárias, e grupo 2 (n=10), considerado controle. Após 30 dias, os animais foram mortos, e as artérias ilíacas preparadas para análise histológica. Os cortes histológicos foram digitalizados e analisados por morfometria digital. A análise estatística foi realizada com o teste* t *de Student e o de Mann-Whitney.* ***Resultados:*** *comparando as artérias ilíacas submetidas à angioplastia com as artérias não submetidas à angioplastia, houve hiperplasia neointimal significativa (0,228* versus *0,119 mm²; p=0,0001). Nas artérias submetidas à angioplastia, não houve diferença entre o grupo 1 (cilostazol) e o grupo 2 (controle) na área do lúmen (2,277* versus *2,575 mm²; p=0,08), área da íntima (0,219* versus *0,237 mm²; p=0,64), área da média (2,262* versus *2,393 mm²; p=0,53) e no percentual de obstrução neointimal (8,857* versus *9,257 %; p=0,82).* ***Conclusão:*** *O uso de cilostazol 50mg administrado em duas doses diárias durante 30 dias não reduziu a hiperplasia neointimal em artérias ilíacas de suínos submetidas à angioplastia com cateter balão.*

***Descritores:*** *Neointima. Hiperplasia. Inibidores da Fosfodiesterase. Angioplastia Transluminal. Artéria Ilíaca.*

## REFERENCES

1. Kudo T, Chandra FA, Ahn SS. The effectiveness of percutaneous transluminal angioplasty for the treatment of critical limb ischemia: a 10-year experience. J Vasc Surg. 2005;41(3):423-35; discussion 35.
2. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Eur J Vasc Endovasc Surg. 2007;33 Suppl 1:S1-75.
3. Schwartz RS. Pathophysiology of restenosis: interaction of thrombosis, hyperplasia, and/or remodeling. Am J Cardiol. 1998;81(7A):14E-7E.
4. Ye W, Liu CW, Ricco JB, Mani K, Zeng R, Jiang J. Early and late outcomes of percutaneous treatment of TransAtlantic Inter-Society Consensus class C and D aorto-iliac lesions. J Vasc Surg. 2011;53(6):1728-37.
5. Rosa MP, Baroni GV, Portal VL. Cilostazol, um inibidor da fosfodiesterase III: perspectivas futuras na aterosclerose. Arq Bras Cardiol. 2006;87(5):e222-6.
6. Schror K. The pharmacology of cilostazol. Diabetes Obes Metab. 2002;4(Suppl 2):S14-9.
7. Weintraub WS. The vascular effects of cilostazol. Can J Cardiol. 2006;22(Suppl B):56B-60B.
8. Chen WJ, Chen YH, Lin KH, Ting CH, Yeh YH. Cilostazol promotes vascular smooth muscles cell differentiation through the cAMP response element-binding protein-dependent pathway. Arterioscler Thromb Vasc Biol. 2011;31(9):2106-13.
9. Jung WK, Lee DY, Park C, Choi YH, Choi I, Park SG, et al. Cilostazol is anti-inflammatory in BV2 microglial cells by inactivating nuclear factor-kappaB and inhibiting mitogen-activated protein kinases. Br J Pharmacol. 2010;159(6):1274-85.
10. Lee KM, Lee HJ, Kim MK, Kim HS, Jung GS, Hur SH, et al. Cilostazol inhibits high glucose- and angiotensin II-induced type 1 plasminogen activator inhibitor expression in artery wall and neointimal region after vascular injury. Atherosclerosis. 2009;207(2):391-8.
11. Liu Y, Shakur Y, Yoshitake M, Kambayashi Ji J. Cilostazol (pletal): a dual inhibitor of cyclic nucleotide phosphodiesterase type 3 and adenosine uptake. Cardiovasc Drug Rev. 2001;19(4):369-86.
12. Otsuki M, Saito H, Xu X, Sumitani S, Kouhara H, Kurabayashi M, et al. Cilostazol represses vascular cell adhesion molecule-1 gene transcription via inhibiting NF-kappaB binding to its recognition sequence. Atherosclerosis. 2001;158(1):121-8.
13. Geng DF, Liu M, Jin DM, Wu W, Deng J, Wang JF. Cilostazol-based triple antiplatelet therapy compared to dual antiplatelet therapy in patients with coronary stent implantation: a meta-analysis of 5,821 patients. Cardiology. 2012;122(3):148-57.
14. Sakurai R, Koo BK, Kaneda H, Bonneau HN, Nagai R. Cilostazol added to aspirin and clopidogrel reduces revascularization without increases in major adverse events in patients with drug-eluting stents: a meta-analysis of randomized controlled trials. Int J Cardiol. 2013;167(5):2250-8.
15. Iida O, Yokoi H, Soga Y, Inoue N, Suzuki K, Yokoi Y, et al Cilostazol reduces angiographic restenosis after endovascular therapy for femoropopliteal lesions in the Sufficient Treatment of Peripheral Intervention by Cilostazol study. Circulation. 2013;127(23):2307-15.
16. Soga Y, Iida O, Hirano K, Suzuki K, Kawasaki D, Miyashita Y, et al. Impact of cilostazol after endovascular treatment for infrainguinal disease in patients with critical limb ischemia. J Vasc Surg. 2011;54(6):1659-67.
17. Indolfi C, Torella D, Coppola C, Stabile E, Esposito G, Curcio A, et al. Rat carotid artery dilation by PTCA balloon catheter induces neointima formation in presence of IEL rupture. Am J Physiol Heart Circ Physiol. 2002;283(2):H760-7.
18. Mongiardo A, Curcio A, Spaccarotella C, Parise S, Indolfi C. Molecular mechanisms of restenosis after percutaneous peripheral angioplasty and approach to endovascular therapy. Curr Drug Targets Cardiovasc Haematol Disord. 2004;4(3):275-87.
19. Bayes-Genis A, Kantor B, Keelan PC, Altman JD, Lubbe DF, Kang JH, et al. Restenosis and Hyperplasia: Animal Models. Curr Interv Cardiol Rep. 2000;2(4):303-8.
20. Johnson GJ, Griggs TR, Badimon L. The utility of animal models in the preclinical study of interventions to prevent human coronary artery restenosis: analysis and recommendations. On behalf of the Subcommittee on Animal, Cellular and Molecular Models of Thrombosis and Haemostasis of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost. 1999;81(5):835-43.
21. Schwartz RS. Neointima and arterial injury: dogs, rats, pigs, and more. Lab Invest. 1994;71(6):789-91.
22. Humphrey WR, Simmons CA, Toombs CF, Shebuski RJ. Induction of neointimal hyperplasia by coronary angioplasty balloon overinflation: comparison of feeder pigs to Yucatan minipigs. Am Heart J. 1994;127(1):20-31.
23. Castro Júnior C, Pereira AH, Pasa MB. Morphometric analysis of the intimal reaction after stent implantation in iliac arteries submitted to angioplasty in pigs. Acta Cir Bras. 2006;21(3):139-43.
24. De Meyer GR, Bult H. Mechanisms of neointima formation—lessons from experimental models. Vasc Med. 1997;2(3):179-89.

25. Virmani R, Kolodgie FD, Farb A, Lafont A. Drug eluting stents: are human and animal studies comparable? Heart. 2003;89(2):133-8.
26. França LHG, Pereira AH, Perini SC. Self-expandable nitinol stent placement in homocysteinemic porcine aorta. Clinics. 2008;63(2):229-36.
27. Tsuchikane E, Suzuki T, Katoh O, Suzuki T. Examination of anti-intima hyperplastic effect on cilostazol-eluting stent in a porcine model. J Invasive Cardiol. 2007;19(3):109-12.
28. Ishizaka N, Taguchi J, Kimura Y, Ikari Y, Aizawa T, Togo M, et al. Effects of a single local administration of cilostazol on neointimal formation in balloon-injured rat carotid artery. Atherosclerosis. 1999;142(1):41-6.
29. Yamamoto K, Onoda K, Sawada Y, Fujinaga K, Imanaka-Yoshida K, Yoshida T, et al. Locally applied cilostazol suppresses neointimal hyperplasia and medial thickening in a vein graft model. Ann Thorac Cardiovasc Surg. 2007;13(5):322-30.
30. Malliaris SD, Munabi NC, Akelina Y, Ascherman JA. Topical cilostazol inhibits neointimal hyperplasia in a rat interposition vein graft model. Plast Reconstr Surg. 2014;134(6):895e-901e.
31. Kubota Y, Kichikawa K, Uchida H, Maeda M, Nishimine K, Makutani S, et al. Pharmacologic treatment of intimal hyperplasia after metallic stent placement in the peripheral arteries. An experimental study. Invest Radiol. 1995;30(9):532-7.

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**Address for correspondence:**
Joel Alex Longhi:
E-mail: joelalexlonghi@yahoo.com.br

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Original Article

# Cell therapy in the treatment of bronchiolitis obliterans in a murine model

## *Terapia celular no tratamento da bronquiolite obliterante em modelo murino*

Julio de Oliveira Espinel[1]; Carolina Uribe[1]; Fabíola Schons Meyer[1]; Rafael Bringheti[2]; Jane Ulbricht Kulczynski[1]; Maurício Guidi Saueressig[1]

A B S T R A C T

**Objective:** To evaluate the importance of stem cells derived from adipose tissue in reducing graft inflammation in a murine model of allogeneic heterotopic tracheal transplant. **Methods**: We performed a heterotopic tracheal allografting in dorsal subcutaneous pouch and systemically injected $5x10^5$ mesenchymal stem cells derived from adipose tissue. The animals were divided into two groups according to the time of sacrifice: T7 and T21. We also carried out histological analysis and digital morphometry. **Results**: The T7 animals treated with cell therapy had median obstructed graft area of 0 versus 0.54 of controls ($p = 0.635$). The treated T21 subjects had median obstructed graft area of 0.25 versus 0 in controls ($p = 0.041$). **Conclusion**: The systemically injected cell therapy in experimental murine model of bronchiolitis obliterans did not reduce the severity of the allograft inflammation in a statistically significant way in seven days; Conversely, in 21 days, it increased the allograft inflammatory process.

**Key words:** Transplantation, Heterotopic. Bronchiolitis Obliterans. Cell- and Tissue-Based Therapy. Stem Cells. Mesenchymal Stem Cell Transplantation.

## INTRODUCTION

Lung transplantation has become a viable alternative for many terminal pulmonary diseases. Bronchiolitis obliterans (BO) is the main constraint to long-term survival in lung transplantation. It displays inflammatory etiology. Multiple factors contribute to the development of this condition: repeated episodes of acute rejection, cytomegalovirus infections, gastroesophageal reflux disease, primary graft dysfunction, type of transplant performed, whether unilateral or bilateral, and also aspects related to autoimmunity.

The therapeutic alternatives for BO are limited and without a clearly established protocol. Possible treatments include changing the immunosuppressive medication, photopheresis, total lymphoid irradiation, azithromycin, plasmapheresis and inhaled cyclosporine[1,2]. Some national initiatives have tried to move forward on this issue and many alternatives keep arising[3,4].

The simplest experimental model to study BO (chronic rejection) is the allogeneic heterotopic tracheal transplantation with small rodents[5-7]. This model is shown to be robust to studies on immunogenesis, from the genetic and molecular points of view, and evaluation of new immunosuppressive therapies in the early stages.

Stem cells (SC) are those with self-renewal and differentiation capacity and there is a growing body of study of such cells[5,8-13]. Their role has been tested in different diseases, creating a new branch of knowledge - regenerative medicine.

It is known that mesenchymal stem cells (MSC) have immunomodulatory properties. It has been proposed that they are capable of modulating the response to ischemic injury and even attenuate the effects of immune-mediated diseases. MSC have been tested in clinical trials in humans graft versus host disease scenarios after transplantation of allogeneic hematopoietic stem cells and inflammatory bowel disease[8,9]. In an experimental model of heterotopic tracheal transplant, Grove *et al*. evaluated the role of MSC derived from bone marrow and detected a significant 60% reduction in BO occurrence[5].

Considering that BO is a disease of inflammatory nature, which limits the survival of lung transplantation and therapeutic options are limited, we prepared an experimental study to assess the importance of stem cells derived from adipose tissue in reducing the graft inflammatory process in a murine model of allogeneic heterotopic tracheal transplant.

1. Universidade Federal do Rio Grande do Sul - RS - Brazil; 2. Universidade Federal de Ciências da Saúde de Porto Alegre - RS - Brazil.

## METHODS

This randomized, blinded and controlled experimental study was developed at the Centro de Pesquisas of the Hospital Universitário de Porto Alegre, at the following laboratories: Unidade de Experimentação Animal, Centro de Terapia Gênica, Laboratório de Patologia Experimental e Laboratório de Vias Aéreas e Pulmão. The study was approved by the Comitê de Ética em Pesquisa of the Hospital de Clínicas de Porto Alegre under number 100 524.

The handling and accommodation of the mice were in accordance with Normative Resolution 196/96 of the National Health Council, with the Care and Use of Laboratory Animals Guide of the United State National Institutes of Health (NIH publication in 85-23, revised in 1996).

The mice were purchased from centers specialized in experimental animals. Altogether, we used 90 rats, 37 animals BalbC (receptors) and 37 C57BL6 animals (donors). We used 16 C57BL6 mice for extraction of mesenchymal stem cells. All animals were treated according to the current standard for experimental animals.

To obtain mesenchymal stem cells derived from adipose tissue, the C57BL6 mice were killed by cervical dislocation on the Animal Experimentation Unit (UEA). We removed the epididymal adipose tissue and placed it on a culture medium consisting of Dulbecco's Modified Eagle Medium, supplemented with 10% fetal bovine serum and 1% penicillin / streptomycin. This sample was transported to the Gene Therapy Center and placed in a sterile environment inside the exhaust hood. The tissue was subjected to mechanical digestion with scalpel and thereupon to enzymatic digestion with collagenase type I (1mg/ml) for 30 minutes at 37°C while stirring in ten minutes. The cell suspension was centrifuged and seeded into a six-well plate. The cell culture was maintained with culture medium. After two weeks of culture medium and subsequent exchanges, the cells obtained were called mesenchymal stem cells. When the culture reached confluence, they were treated with trypsin and transferred to culture bottles. The cells were grown till $5x10^5$ cells aliquots were obtained by animal being treated. Each aliquot was diluted in 100µl of phosphate buffered saline for injection in the mice.

Donor animals were anaesthetized with xylazine 10mg/kg and ketamine 100mg/kg. After the animal did not react topainful stimulation of the hind legs, we held a longitudinal incision from the xiphoid process to the cervical region of the animal. The anterior chest wall was excised to facilitate access and dissection of the trachea, from the larynx to the main carina. We dissected the trachea, freed it from the esophagus, and subsequently withdrew the larynx, thyroid remnants and adjacent lymphoid tissues by microdissection with appropriate instruments. After excision, the entire trachea was kept in cold buffered saline (between 4 and 10°C) until implantation in the receptor mouse.

The receptor was subjected to inhalation anesthesia with isoflurane in titrated dose to reach analgesia with spontaneous breathing. When the anesthesia plane was satisfactory, oxygen was offered through a hood. We preformed a longitudinal, 1cm incision in the animal's torso, 5mm caudal from the scapula tip at the dorsal midline. We made one subcutaneous pocket cranial to the incision by blunt dissection to place the graft (stretched entire trachea) to at least 1cm cranial to the incision. Surgical synthesis was performed with nylon 6.0 sutures, with separate stitches and buried knots. Tramadol was administered 10mg/kg in the immediate postoperative period and then the animals were sent back to cages and maintained with water and feed ad libitum.

The animals were sacrificed on the seventh and 21st post-transplant days in a $CO_2$ gas chamber. After sacrifice, we carried out a new incision in the recipient animal 3cm caudally from the previous one and inverted the skin of the back of the animal. After identification of the graft, we dissected it from the adjacent tissue and immersed it in formalin.

Each donor C57BL6 mouse had its trachea removed and implanted in a recipient, BalbC mouse. Upon implantation, the animals were given 100µl of cell therapy or of buffered saline through the tail vein.

The experimental groups received the following names according to treatment and the time for sacrifice: T7cel – ten animals sacrificed in seven days, treated with cell therapy; T7con – five animals sacrificed in seven days, treated with phosphate buffered saline; T21cel – 12 animals sacrificed at 21 days, treated with cell therapy; and T21con – ten animals sacrificed at 21 days, treated with normal saline (Figure 1).

Cell therapy was administered to the animals by injection of 100µl of buffered saline with $10^5$ mesenchymal stem cells into the tail vein with a 30G needle. The injection of cell therapy solution was administered slowly over one minute. If we observed leakage of the contents, assessed by visual inspection of the tail, we would administer a new standard aliquot of $5x10^5$ cells. Animals that died after injection of cell therapy were excluded from analysis.

For histological analysis, the tracheas, taken in specific times, were immersed in formaldehyde. Tracheas were cut in their middle portion in sections of 5µm thickness. After fixation, they were stained using hematoxylin and eosin (HE). Slides were evaluated semiquantitatively as to: 1) the intraluminal obliteration, classified as more or less than 50% (severe intraluminal obliteration if > 50%); 2) degree of preservation of the epithelium, classified as loss of more or less than 50% of the epithelium integrity (severe epithelial loss if > 50%); 3) presence of lymphocytic or plasmacytic inflammation, classified as mild (sparse infiltrate involving vessels or permeating adjacent connective tissue) or severe (diffuse infiltrate with transmural involvement); 4) circulatory disorders (vascular and extravascular), classified

as mild or severe according to the presence of swelling or polymorphonuclear infiltration involving vasculature, classification adapted from Boehler[14]. The slides were also stained with picrosirius, specific for distinguishing collagen fibrils, to identify whether the graft luminal obliteration was caused by a collagen-rich tissue or not.

Digital morphometric analysis (Image J - version 1.45s) was applied to histological sections of the grafts stained with picrosirius and HE and scanned for image capture software (Image-Pro Express). We manually set the luminal obstruction area (A2) and the original area of the graft lumen (A1). We calculated the A2/A1 ratio, which resulted in the graft obstructed area.

We conducted sample size calculation based on the primary outcome – evaluation of the inflammatory process by the luminal obstruction, based on a pilot study. We obtained a necessary sample of five animals per group.

We conducted a normality test for the continuous variable "graft obstructed area". We found that the standard deviation exceed half the average of the sample; I had a positively skewed distribution. Thus we presented the data as median and interquartile range. For the statistical analysis, we used the Fisher's test for categorical variables. The Mann-Whitney test was used for continuous variables, since they displayed a non-normal distribution and demanded a nonparametric treatment. We included in the statistical analysis the animals that survived until the scheduled day of death, excluding animals that died before this period.

## RESULTS

The analysis of the intraluminal obstruction of heterotopic grafts from sacrificed animals within seven days showed severe obliteration (for a cutoff of 50% obliteration) in 40% of animals treated with cell therapy and 50% in control animals (p=1) (Table 1). Analysis of the integrity of the grafts' heterotopic epithelium showed that 50% of animals of both T7cel and T7con groups showed severe loss of epithelial integrity (loss > 50% of epithelial integrity) (p=1). When analyzing lymphoplasmacytic inflammation, we found that sever inflammatory process occurred only in the T7con group (16.67% of animals) (p=0.38). Regarding circulatory disturbances (vascular and extravascular), we observed 60% of severe circulatory changes in T7cel and 100% in T7con (p=0.23) (Table 1, Figure 2).

At 21 days, severe intraluminal obliteration was 41.67% in T21cel group versus 20% in T21con (p=0.38). Analysis of the inflammatory response at 21 days revealed 75% severe loss of epithelial integrity in T21cel and 90% in T21con (p=0.59). When examining the lymphoplasmacytic inflammation, there was 75% severe inflammation in T21cel versus 50% in T21con (p=0.38). As for circulatory changes, there was 25% of severe circulatory changes in T21cel versus 40% in T21con. (p=0.65) (Table 2, Figure 3).

Digital morphometric evaluation applying Image J on scanned histological sections showed that in the seven-days grafts the median (25th percentile - 75th percentile)

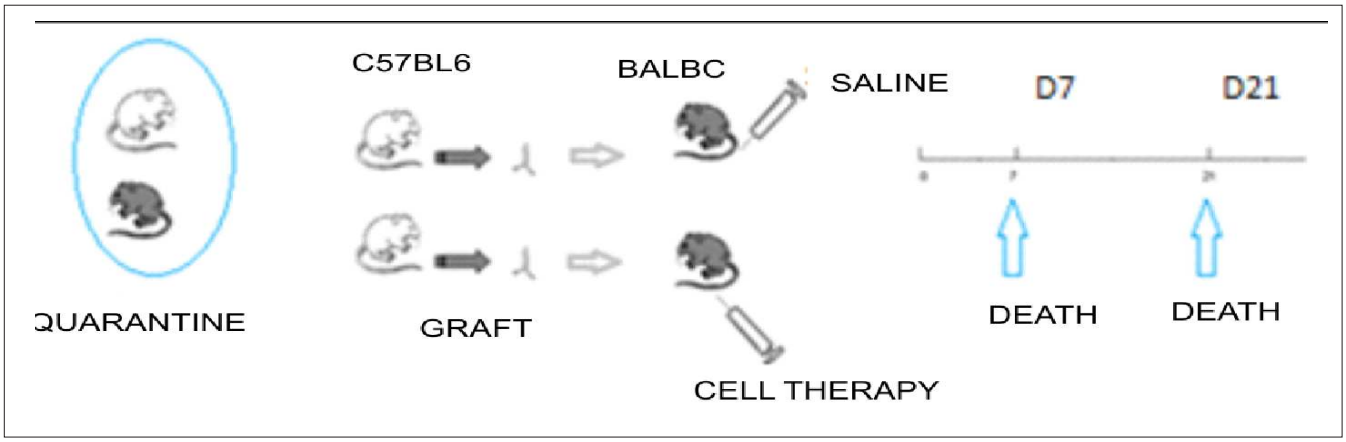


***Figure 1*** - *Experimental design.*

**Table 1 -** Histological analysis of animals sacrificed at seven days.

| | T7 com | T7cel | P |
|---|---|---|---|
| Severe intraluminal obliteration | 50% | 40% | 1 |
| Severe loss of epithelial integrity | 50% | 50% | 1 |
| Severe lymphoplasmacytic inflammation | 16,67% | 0% | 0.38 |
| Severe circulatory changes | 100% | 60% | 0.23 |

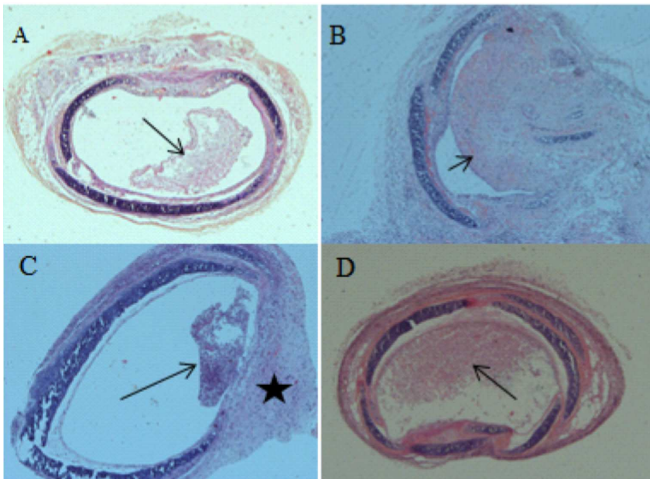


***Figure 2*** - ***A**: photomicrograph 40x, HE. Cross section of tracheal graft in T7cel group. Partially obstructed lumen (arrow); **B**: photomicrograph 100x, HE. Cross section of tracheal graft in T7con group. Grafting with severe luminal obliteration (> 50%) (arrow);**C**: photomicrograph 100x, HE. Cross section of tracheal graft in T21cel group. Graft without severe luminal obliteration (<50%) (arrow); lymphocytic infiltration (star). **D**: photomicrograph 40x, HE. Cross section of tracheal graft in T21con group. Grafting with severe luminal obliteration (<50%) (arrow).*

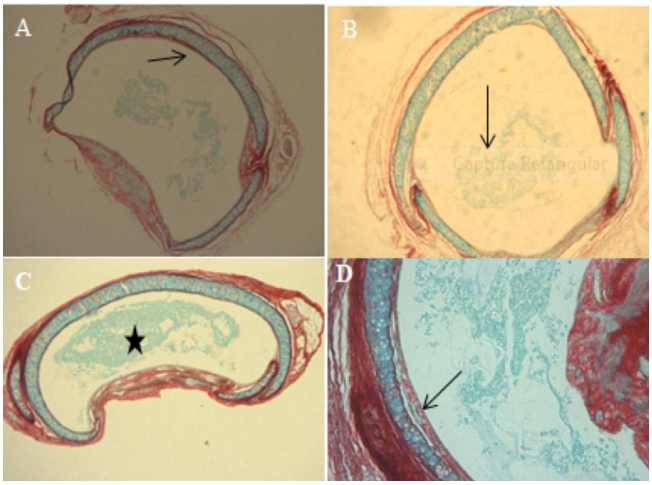


***Figure 3*** - ***A**: photomicrograph 100x, picrosirius. Cross section of tracheal graft in T7cel group. Graft without severe luminal obliteration (<50%). Arrow shows preserved epithelium. **B**: photomicrograph 100x, picrosirius. Cross section of tracheal graft in T7con group. Graft without severe luminal obliteration (<50%) (arrow). **C**: photomicrograph 100x, picrosirius. Cross section of tracheal graft in T21cel group. Graft without severe luminal obliteration (<50%) (star). **D**: photomicrograph 200x, picrosirius. Cross section of tracheal graft in T21con group. Intact epithelium (arrow).*

of the A2/A1 ratio (graft obstructed area) in T7con was 0.54 (0-0.71); in T7cel the median was 0 in (0-0.65) (p=0.635) (Figure 4).

In the animals sacrificed at 21 days, the median (25th percentile - 75th percentile) of obstructed areas in T21con was 0 (0-0.09) and in T21cel, 0.25 (0.05-1) (p=0.041) (Figure 5).

## DISCUSSÃO

Cell therapy with mesenchymal stem cells (MSC) shows immunomodulatory properties in several studies[5,6,10 12,15 -18]. Bronchiolitis obliterans (BO), which is the pathological expression of the lung transplantation chronic rejection, has treatments with limited efficacy. BO has inflammatory nature, and to test the effectiveness of MSC in this scenario, the murine experimental model of heterotopic tracheal transplant is simple and quick for the study new therapies.

Our study showed no decrease in heterotopic grafts inflammatory process in the animals treated with cell therapy and sacrificed at seven or at 21 days. We observed a slight decrease tendency in the inflammatory process at seven days, but not statistically significant.

In the digital morphometric analysis at 21 days, it is clear that the treated mice developed a larger luminal obliteration than the control group, in a statistically significant way. On the other hand, at 21 days the analysis has shown conflicting results regarding the semiquantitative evaluation of the inflammatory process. There was less severe loss of epithelial integrity and less vascular and extravascular changes in the treated group, but more severe luminal obliteration and increased lymphocytic infiltration. One must consider the limitations of a semi-quantitative analysis and the

**Table 2** - Histological analysis of animals sacrificed at 21 days.

| | T21 com | T21cel | P |
|---|---|---|---|
| Severe intraluminal obliteration | 20% | 41.67% | 0.38 |
| Severe loss of epithelial integrity | 90% | 75% | 0.59 |
| Severe lymphoplasmacytic inflammation | 50% | 75% | 0.38 |
| Severe circulatory changes | 40% | 25% | 0.65 |

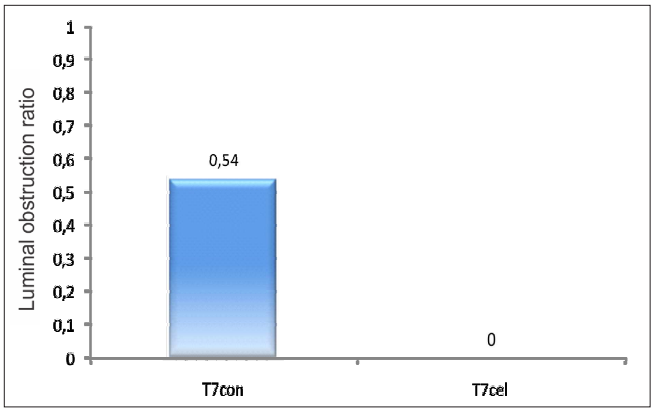


***Figure 4 -*** *Average of obstructed graft areas (A2/A1) at seven days; p = 0.635. A1: area of the original graft lumen; A2 graft luminal obstruction area. A2/A1: graft obstructed area.*

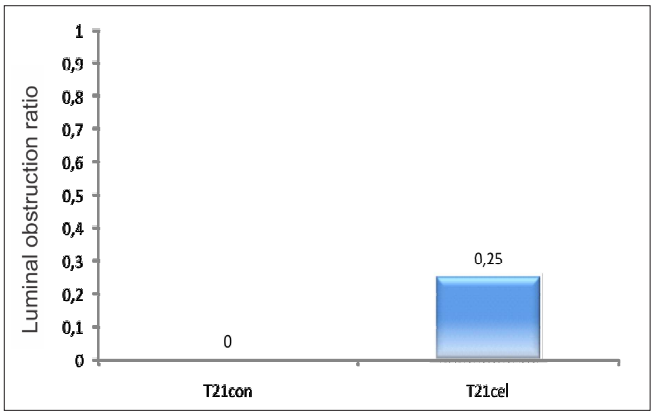


***Figure 5 -*** *Average of obstructed graft areas (A2/A1) at 21 days; p = 0.041. A1: area of the original graft lumen; A2 graft luminal obstruction area. A2/A1: graft obstructed area.*

fact that these differences did not reach statistical significance.

Importantly, there have been reduced inflammation rates of the tracheal wall in controls at 21 days. The luminal obliteration rate at 21 days in controls' allografts was low compared to the literature. There were 20% of severe obliteration (> 50% obstruction of the graft lumen) observed in our study against up to 75-90% reported in the literature[7,19]. Although we followed all surgical steps established in the literature[14] and increased the number of mice in the experiment (we doubled the initially calculated sample), the intensity of luminal obliteration was consistently lower than that described in the literature. It should be noted that the murine tracheal allograft model demonstrates variability in luminal obliteration between experiments, from 30% to 100%. This may account for the divergent data between different research groups[20], especially with regard to luminal obliteration at 21 days[7].

The explanation for the choice of times for allografts histological analysis derived from previous studies where it is clear that the seventh day after heterotopic transplantation is the culmination of lymphocytic infiltrate in the epithelial surface (intraepithelial and subepithelial), similar to bronchitis or lymphocytic bronchiolitis seen in transplanted humans. This time is one of the earliest stages of the lung rejection process, the lymphocytic phase[14]. On the seventh day, we also noted lymphocytic vasculitis, which is a manifestation of rejection targeted to the endothelium. The analysis in the 21st post-transplant day was chosen to represent the obliterative rejection phase, i.e., the terminal phase[5].

By 2011, to our knowledge there was no data in the literature on MSC applied in a BO murine model. A study published in 2011 by Grove *et al.* [5] tested a new form of immunomodulation with MSC derived from bone marrow. They carried out a retro-orbital injection of $5x10^5$ cells on allogeneic mice. There was a decrease of 60% in the intraluminal obstruction when treated animals were compared with controls, in groups sacrificed at seven and 14 days. This suggests that cell therapy can be effective in preventing heterotopic airway obstruction.

MSC showed significant immunomodulatory properties, including in lung diseases such as chronic obstructive pulmonary disease, pulmonary hypertension, asthma and pulmonary fibrosis[21]. The immunomodulatory mechanisms are still not fully understood but involve the inhibition of T cell proliferation and modulation of B cell function[22]. It has been shown, too, that there is no need of MSC integrate into the injured tissues to produce effect; paracrine effects explain this phenomenon[23]. Still on the mode of action of MSC, it has been shown that MSC injected systemically, besides migrating to the damaged tissue by an "homing" effect, remain viable and mixed with native cells for a few months[24] .

While most of the literature data point to an immunomodulatory effect of MSC, some authors have demonstrated a pro-fibrotic effect of cell therapy with MSC[25,26]. The reality is that MSC have a double role: they can exert an immunosuppressive function, protecting against BO through IL-8 and IL-10; or they may have a pro-fibrotic effect mediated by endothelin-1, TGF $\alpha$ and IL 13[27]. The local microenvironment to which MSC are exposed determines the predominant profile of their action. In our study, in animals of groups T21con and T21cel, there was a graft luminal obliteration lower than the literature; possibly, the dosage of the cytokines involved in the inflammatory process, which was not performed, could help in the understanding of our results. This can be exploited in our next work.

We should also consider that the way to obtain the MSC can modify their immunomodulatory properties. We obtained mesenchymal stem cells from epididymal adipose tissue, unlike Grove *et al.* [5], who obtained them from bone marrow. This, most likely, influenced our results. It is known that cells obtained from different sources have different immunomodulating potency, probably due to the

standard production of growth factors and interleukin. The profile of inflammatory and anti-inflammatory cytokines may help to unravel the effects observed in our study[28].

MSC can be obtained from several tissues: trabecular bone, periosteum, synovial membrane, skeletal muscle, skin, pericytes, peripheral blood, tooth, periodontal ligament, umbilical cord[8]. Adipose tissue-derived MSC have been much studied, since this tissue is abundant and capable of being accessed with low morbidity. There are reports that A-MSC have different characteristics depending on the location from which they were extracted[8]. Our study obtained MSC from epididymal adipose tissue. The choice was based on the experience of the gene therapy group and ease of tissue access. Another point relevant to MSC management is the source of obtaining the cells: autologous versus allogeneic; a single source or multiple sources. This seems to influence the effects of cell therapy[9]. Grove *et al.*[5] obtained MSC in an allogeneic form from three different animals breeds, which may have influenced the results. There is a clinical trial demonstrating that, in some scenarios, a smaller number of cells can produce more consistent effects in terms of damaged tissues recovery[29]. We chose to use $5x10^5$ cells because most of the studies in mice use this figure, although one can also use $10^6$ cells.

In order to analyze our results, we questioned whether a single injection at the selected time (at the time of transplantation) is the most suitable. The use of cell therapy involves test regarding both the infusion time and the number of injections. In this context, perhaps one could get different results by repeatedly applying the treatment – which has not been tested to date in this model which as we know. It is also known that delayed administration of cell therapy, when the fibrotic process is already established, does not bring benefits[9]. However, the reversal of the initial fibrotic process has been reported after cell therapy in a pulmonary fibrosis model[9].

The age of the MSC culture, as well as the number of cell expansions, determine changes in their morphology, differentiation capacity, viability, migration efficiency and competence to produce cytokines[30]. In our experiment, we took care to use cell cultures of the same age and the same number of expansions (four times). Hence, we do not believe that this has been the determining cause of the divergence between our results and the literature's ones.

The infusion route used in cellular therapy in murine models is variable. Injections may be intraperitoneal, locoregional and systemic via the tail vein, in the retro-orbital venous plexus and through dissection of the internal jugular vein[7]. These different routes may determine different results, as obtained in the present study. Some works show that the first-pass metabolism by the liver sinusoids can alter the morphology and physiology of the injected cells, which does not seem to have affected this study, since the tail vein drains to the caval system, not to the portal. In addition, some groups advocate the intra-arterial route as an option for cell therapy. Thus, a larger amount of cells can reach the target tissue, reducing systemic effects[13].

In a preliminary stage in our study, we tested different infusion routes: systemic injection through the tail vein, retro-ocular venous plexus and internal jugular vein. The results were not different in our pilot study. We opted for injection via the tail vein for convenience in performing a slow, technically easier injection. Still in the pilot phase, a limiting factor for the development of the experiment was the high mortality rate of animals during the cell therapy injection phase. We tested different dilutions of cell therapy (the same $5x10^5$ cells) diluted in 50μl, 200μl and 100μl of buffered saline. We found no differences in mortality between groups. We chose to use 100μl, this being the most used volume in the literature. On the other hand, when we rapidly injected the MSC, they would trigger a severe respiratory insufficiency in animals, which was followed in some cases by hemiplegia and even cardiac arrest – sometimes reversible. We noted that in our study, when, in some experiments with injection of MSC, mortality ranged from 50 to 100 % when we injected MSC in bolus (10 to 20 seconds). We considered that to be due to embolization of the injected cells in the right heart and brain arteries. This finding does not hold when the cell therapy is slowly injected – 100μl over one to two minutes –, a method developed by our group. Plock *et al.*[6] made a comment from their unpublished data, emphasizing the importance of slowly administering cell therapy.

The model applied in this experiment has many similarities to the human lung transplant. However, some aspects should always be considered: the allograft transplant is not functional, therefore not mimicking the actual environment of the transplanted tissue, sicni it is disconnected from the receiver airway; the allograft does not have its own primary vascular supply, which can impact on the dynamics of the immune response[5].

Associations between pathological outcomes and molecular biology ones can determine a better understanding of the dynamics of the inflammatory process and therapy impact. Consideration should be given to analysis of the cytokines already involved in immunogenesis of bronchiolitis obliterans, as well as to attempts in associating gene expression with histopathology[31].

There is a large number of publications involving murine model of heterotopic allogeneic tracheal transplantation[5,7,12,14,20,25,31]. Several immunogenesis steps were unraveled by this model. By advancing in immunogenesis, therapeutic alternatives were also tested over the past 20 years, some of them being clinically implemented, such as cyclosporin, tacrolimus, among others[32].

In this context this study increases the information about cell therapy with MSC in this model, and to date, to our knowledge, this is the first experiment using MSC derived from adipose tissue for this purpose.

As perspectives, one could test serial injections of cell therapy in animals in order to verify that a repetitive exposure could result in maximized effect in terms of reducing the inflammatory process.

Many therapeutic targets have been identified in the last decade, which increases the importance of testing immunomodulators associated with the measurement of the inflammatory process, dosing cytokines involved in the process, as well as using gene expression panels.

In conclusion, the systemically injected cell therapy in a experimental murine model of bronchiolitis obliterans did not reduce the severity of allograft inflammation in a statistically significant way at seven days; Conversely, in 21 days, it increased the allograft inflammatory process.

## R E S U M O

***Objetivo****: avaliar a importância das células-tronco derivadas de tecido adiposo na redução do processo inflamatório no enxerto em modelo murino de transplante traqueal heterotópico alogênico.* ***Métodos****: foi realizado alotransplante traqueal heterotópico em bolsa dorsal subcutânea e injetado 5x10$^5$ células-tronco mesenquimais, derivadas de tecido adiposo, sistemicamente. Os animais foram distribuídos em dois grupos, conforme o tempo de sacrifício: T7 e T21. Procedida a análise em HE e morfometria digital.* ***Resultados****: Os T7 tratados com terapia celular apresentaram mediana de área obstruída do enxerto de 0 contra 0,54 dos controles (p=0,635). Os T21 tratados apresentaram mediana de área obstruída da luz do enxerto de 0,25 nos tratados e 0 nos controles (p=0,041).* ***Conclusão: a*** *terapia celular injetada sistemicamente em modelo experimental murino de bronquiolite obliterante não reduziu a gravidade do processo inflamatório no aloenxerto de forma estatisticamente significativa em sete dias; de modo contrário, em 21 dias, aumentou o processo inflamatório no aloenxerto.*

***Descritores:*** *Transplante Heterotópico. Bronquiolite Obliterante. Terapia Baseada em Transplante de Células e Tecidos. Células-Tronco. Transplante de Células-Tronco Mesenquimais.*

## REFERENCES

1. Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR. The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant. 2010;29(4):424-31.
2. Fisher AJ, Rutherford RM, Bozzino J, Parry G, Dark JH, Corris PA. The safety and efficacy of total lymphoid irradiation in progressive bronchiolitis obliterans syndrome after lung transplantation. Am J Transplat. 2005;5(3):537-43.
3. Mayer E, Cardoso PF, Puskas JD, De Campos K, Oka T, Dardick I, et al. The effect of basic fibroblast growth fator and omentopexy on revascularisation and epitelial regeneration of heterotopic rat tracheal isograft. J Thorac Cardiovasc Surg. 1992;104(1):180-8.
4. Maksoud-Filho JG, Rodrigues CJ, Tannuri U, Maksoud JG. The effects of early and delayed immunosuppression in experimental tracheal transplantation with omentopexy. J Pediatr Surg. 1999;34(8):1223-8.
5. Grove DA, Xu J, Joodi R, Torres-Gonzales E, Neujahr D, Mora AL, et al. Attenuation of early airway obstruction by mesenchymal stem cells in a murine model of heterotopic tracheal transplantation. J Heart Lung Transplant. 2011;30(3):341-50.
6. Plock JA, Schnider JT, Schweizer R, Gorantla VS. Are cultured mesenchymal stromal cells an option for immunomodulation in transplantation? Front Immunol. 2013;4:41.
7. Hele DJ, Yacoub MH, Belvisi MG. The heterotopic tracheal allograft as an animal model of obliterative bronchiolitis. Respir Res. 2001;2(3):169-83.
8. Mizuno H, Tobita M, Uysal AC. Concise review: Adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells. 2012;30(5):804-10.
9. Weiss DJ, Ortiz LA. Cell therapy trials for lung diseases: progress and cautions. Am J Respir Crit Care Med. 2013;188(2):123-5.
10. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008;8(9):726-36.
11. Burt RK, Loh Y, Pearce W, Beohar N, Barr WG, Craig R, et al. Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. JAMA. 2008;299(8):925-36.
12. Guo Z, Zhou X, Li J, Meng Q, Cao H, Kang L, et al. Mesenchymal stem cells reprogram host macrophages to attenuate obliterative bronchiolitis in murine orthotopic tracheal transplantation. Int Immunopharmacol. 2013;15(4):726-34.
13. Zonta S, De Martino M, Bedino G, Piotti G, Rampino T, Gregorini M, et al. Which is the most suitable and effective route of administration for mesenchymal stem cell-based immunomodulation therapy in experimental kidney transplantation: endovenous or arterial? Transplant Proc. 2010;42(4):1336-40.
14. Boehler A, Chamberlain D, Kesten S, Slutsky A, Liu M, Keshavjee S. Lymphocytic airway infiltration as a precursor to fibrous obliteration in a rat model of bronchiolitis obliterans. Transplantation. 1997;64(2):311-7.
15. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotente stromal cells: the state of transdifferentiation and modes of tissue repair--current views. Stem Cells. 2007;25(11):2896-902.
16. English K. Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol. 2013;91(1):19-26.
17. English K, Wood KJ. Mesenchymal stromal cells in transplantation rejection and tolerance. Cold Spring Harb Perspect Med. 2013;3(5):a015560.
18. English K, Mahon BP, Wood KJ. Mesenchymal stromal cells; role in tissue repair, drug discovery and immune modulation. Curr Drug Deliv. 2014;11(5):561-71.
19. Lau CL, Zhao Y, Kron IL, Stoler MH, Laubach VE, Ailawadi G, et al. The role of adenosine A2A receptor signaling in bronchiolitis obliterans. Ann Thorac Surg. 2009;88(4):1071-8.
20. Neuringer IP, Aris RM, Burns KA, Bartolotta TL, Chalermskulrat W, Randell SH. Epithelial kinetics in mouse heterotopic tracheal allografts. Am J Transplant. 2002;2(5):410-9.

21. Iyer SS, Co C, Rojas M. Mesenchymal stem cells and inflammatory lung diseases. Panminerva Med. 2009;51(1):5-16.
22. Németh K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15(1):42-9.
23. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012;3:297.
24. Kuo YR, Goto S, Shih HS, Wang FS, Lin CC, Wang CT, et al. Mesenchymal stem cells prolong composite tissue allotransplant survival in a swine model. Transplantation. 2009;87(12):1769-77.
25. Salama M, Andrukhova O, Jaksch P, Taghavi S, Kelpetko W, Dekan G, et al. Endothelin-1 governs proliferation and migration of bronchoalveolar lavage-derived lung mesenchymal stem cells in bronchiolitis obliterans syndrome. Transplantation. 2011;92(2):155-62.
26. Walker N, Badri L, Wettlaufer S, Flint A, Sajjan U, Krebsbach PH, et al. Resident tissue-specific mesenchymal progenitor cells contribute to fibrogenesis in human lung allografts. Am J Pathol. 2011;178(6):2461-9.
27. Hardy JD, Webb WR, Dalton ML Jr, Walker GR Jr. Lung homotransplantation in man. JAMA. 1963;186:1065-74.
28. Dimarino AM, Caplan AI, Bonfield TL. Mesenchymal stem cells in tissue repair. Front Immunol. 2013;4:201.
29. Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA. 2012;308(22):2369-79.
30. Hematti P. Mesenchymal stromal cells and fibroblasts: a case of mistaken identity? Cytotherapy. 2012;14(5):516-21.
31. Lemaître PH, Vokaer B, Charbonnier LM, Iwakura Y, Field KA, Estenne M, et al. Cyclosporine A drives a Th17- and Th2-mediated posttransplant obliterative airway disease. Am J Transplant. 2013;13(3):611-20.
32. Baughman RP, Meyer KC, Nathanson I, Angel L, Bhorade SM, Chan KM, et al. Executive summary: monitoring of nonsteroidal immunosuppressive drugs in patients with lung disease and lung transplant recipients: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012;142(5):1284-8.

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**Address for correspondence:**
Julio de Oliveira Espinel
E-mail: julioespinel@gmail.com

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# Proximal endovascular blood flow shunt for thoracoabdominal aortic aneurism without total aortic clamping

## *Desvio de fluxo sanguineo endovascular proximal para derivaçao cirúrgica de aneurisma toracoabdominal sem clampeamento total da aorta*

Gaudencio Espinosa, TCBC-RJ[1]; Rivaldo Tavares[1]; Felippe Fonseca[1]; Alessandra Collares[1]; Marina Lopes[1]; Jose Luis Fonseca[1]; Rafael Steffan[1]

A B S T R A C T

The authors present a surgical approach to type III and IV Crawford aneurysms that does not need total aortic clamping, which allows the more objective prevention of direct ischemic damage, as well as its exclusion by the endoprosthesis implantation, shunting the flow to the synthetic graft.

**Key words:** Aortic Aneurysm, Blood Vessel Prosthesis. Endovascular Procedures. Mesenteric Ischemia.

## INTRODUCTION

Faced with the many advances in the surgical treatment of aortic aneurysms with involvement of visceral branches, there are still large morbidity and mortality related to the procedure. Among the most common complications, we highlight those inherent to the techniques employed, especially those related to total aortic occlusion (crossclamping). Approximately five decades ago, Etheredge *et al.*[1] described the first case of abdominal aortic aneurysms with involvement of visceral branches successfully surgically corrected. Since then, the surgical technique has undergone significant changes, especially those carried out by Crawford *et al.*[2], but the morbidity associated with crosscampling remained present. In this context, we emphasize the direct ischemic complications, largely dependent on the time of aortic clamping: injuries of the spinal cord, kidney and other abdominal viscera.

Over time methods appeared that sought to decrease ischemia caused by the crossclamping, including cardiopulmonary bypass and its variations (atrial-femoral bypass, hypothermic cardiac arrest, etc.), cerebrospinal fluid drainage, epidural cooling and induction of hypothermia. Although these approaches have proven some effectiveness in reducing complications, all refer to a way to minimize the insult caused rather than prevent it. Our aim is to describe a surgical technique without total aortic clamping, hence less likely to direct ischemic complications beyond endovascular bypass flow to the graft.

## TECHNIQUE

We recommended to perform the procedure with the patient under general anesthesia and selective intubation of each main bronchus. In addition to the basic monitoring parameters (bladder catheter, capnography, thermometer, oximeter) continuous assessments of blood pressure and central venous pressure are important. The use of direct cardiac output monitors and intraoperative echocardiography must be individualized for each case, being at the anesthesiology service discretion.

For better understanding, the procedure is described separately in four stage:

First stage – The proposed access is the left thoracophrenolaparotomy with internal paramedian incision. A thoracotomy is performed at the level of the sixth intercostal space. The abdominal aorta is approached by the retroperitoneal route with *en bloc* lateral retraction of the visceral structures and peritoneal sac integrity. The thoracotomy is communicated with the laparotomy through the diaphragm radial section. To perform this operative time it is essential that the patient is under exclusive ventilation of the right lung. By that time it is interesting to mark the diaphragm edges with identifiable sutures to facilitate its closure later. The diaphragmatic incision is extended to the diaphragmatic crus, completely exposing the thoracoabdominal aorta path till its bifurcation. The employment of retractors is practically mandatory to maintain an adequate surgical field. The combination of a chest retractor (Finochietto) with an abdominal retractor

1. Serviço de Cirurgia Vascular, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (HUCFF-UFRJ) - RJ - Brazil.

fixed to the table is satisfactory. After exposure of the aortic path, we must identify and repair the main visceral branches accessible to the left of the patient, including the celiac trunk, superior mesenteric artery and left renal artery. We also suggest that the Gerota's fascia under the left kidney is incised to release the kidney from the retroperitoneum, allowing proper kidney mobility, facilitating the manipulation of the aortic path and the reconstruction of renal flow. The maneuver also prevents the section of the left renal vein at the time of aneurysmectomy.

Second stage – With the aortic path and its main branches dissected and isolated, we proceed to the lateral clamping of the descending thoracic aorta with the Satinsky clamp (Figure 1). The partial isolation of the aorta allows the lateral aortotomy and proximal anastomosis with a bifurcated polyester prosthesis (eg: Dacron®). Next, the forked ends of the prosthesis can be termino-laterally anastomosed to the common iliac, external iliac or even common femoral arteries, completing the aortic bypass. The distal arteries can sustain both total and lateral clamping for the anastomosis, the former being easier and faster to perform. It is important to note that there was no need for total aortic clamping to date. However, we emphasize that, for the technique to be implemented successfully, there must be a free aortic aneurysm segment after the left subclavian artery, so that the proximal anastomosis is possible (Figure 2). With the flow set between the aorta and the lower limbs, we turn our attention to the revascularization of the visceral branches. Despite the possibility of making a polyester or polytetrafluoroethylene (PTFE) bridge for revascularization of the celiac trunk, in most cases, the celiac trunk simple ligation will not bring major complications due to the extensive network of anastomoses with collateral branches. The superior mesenteric artery is routinely ligated and sectioned, being anastomosed directly (termino-terminal) or indirectly (termino-lateral) to a polyester or PTFE bridge brought from the bypass (Figure 3). For the anastomosis, the bypass graft is clamped laterally. Next, the same technical principles are used in the approach of the left renal artery (Figure 3).

Third stage – We perform a suture in tobacco pouch on the anterior aspect of the aortic graft, followed by a puncture and catheterization with introduction of rigid guide wire (eg: Amplatz®) extra support 0.035mm – 260cm through which we introduce the 32 x 20 mm aorto-uni-iliac conical stent deployment system. The endoprosthesis is deployed under direct palpation, between the healthy thoracic aorta segment and the bypass graft, completely bypassing the aortic flow and excluding the aneurysm from the proximal flow (Figure 4).

Fourth stage – We held a wide longitudinal opening of the aneurysmal sac (Figure 5A). The origin of the iliac arteries is identified and ligated in both ostia. The right renal artery ostium receives a termino-terminal anastomosis with an interposed polyester or PTFE bridge directly from graft, ending all visceral bridges (Figure 5B). Finally, we suture up the remaining stump of the descending thoracic aorta. Note that the stump will not be subjected to aortic pressures, since the main flow has been diverted by the implantation of the endoprosthesis. The procedure ends with the synthesis of the thoracophrenolaparotomy and left water seal thoracostomy.

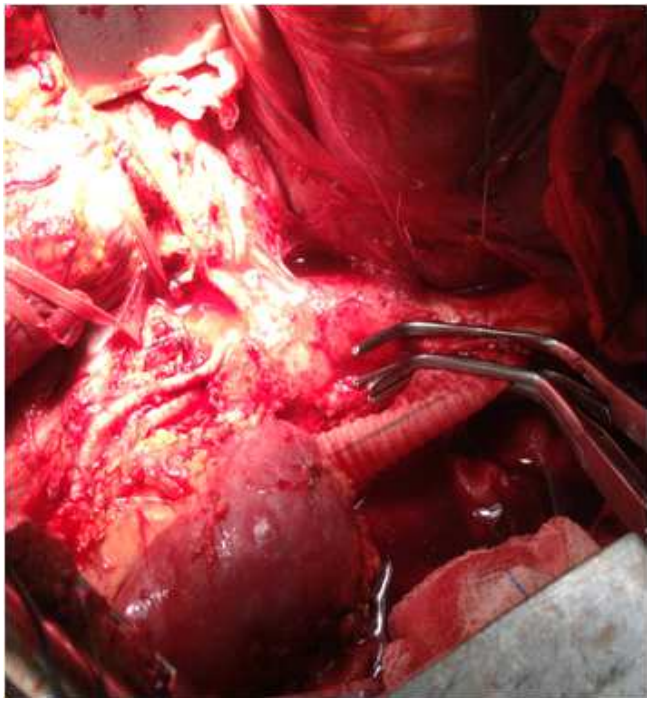

***Figure 1*** - *Lateral clamping of the descending thoracic aorta. Note the termino-lateral anastomosis with the Dacron graft.*

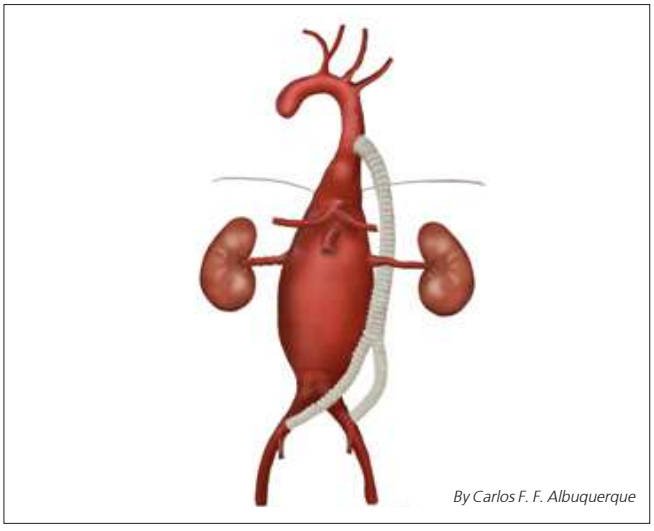


***Figure 2*** - *Aortoiliac bypass with synthetic graft.*

## DISCUSSION

The treatment of thoracoabdominal aneurysm still has high morbidity and mortality despite advances in

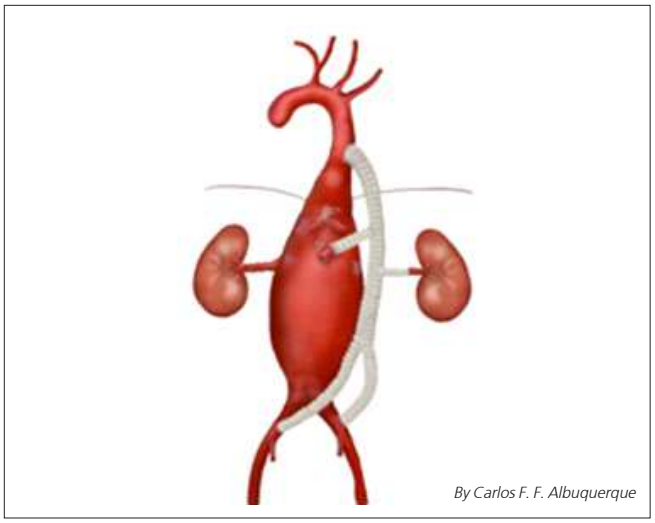


***Figure 3*** - *Bridges to the superior mesenteric and left renal arteries.*

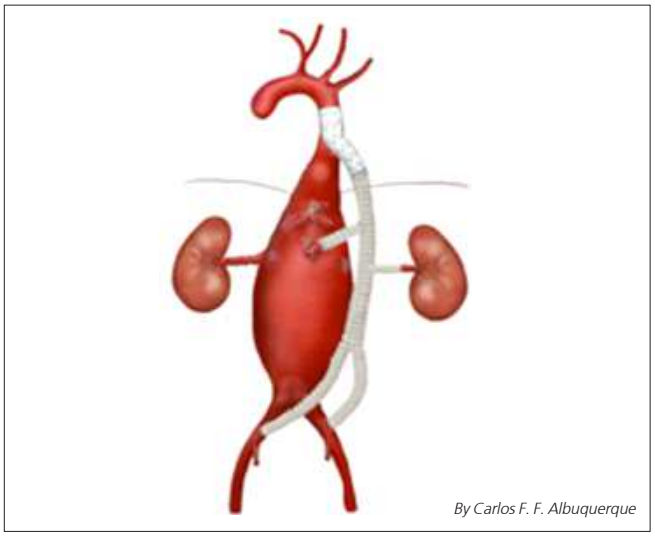


***Figure 4*** - *Blood flow shunt by implant of an endoprosthesis.*

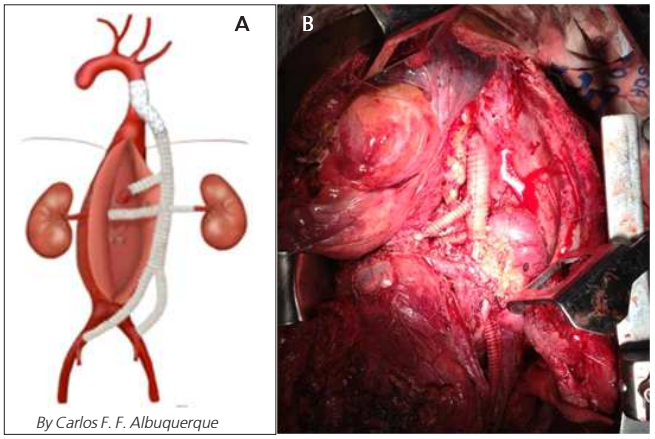


***Figure 5*** - *Opening the aneurysm sac (A) with manufacturing of bridges to the superior mesenteric and right renal arteries (B).*

techniques developed and discussed in the past 30 years in trying to minimize major complications: spinal cord ischemia, visceral ischemia, kidney failure, perioperative hypotension, need for multiple transfusions, among others. The technique described by us aims to minimize this damage in thoracoabdominal aneurysms of Crawford's[2] types III and IV, through the possibility of lateral aortic clamping, providing full flow, which is maintained throughout the procedure, avoiding greater hemodynamic instability, need of cardiopulmonary bypass (CPB), measures to prevent spinal cord ischemia and visceral hypoperfusion.

Preoperative evaluation is also important to estimate the risk of developing postoperative renal failure. According to Schepens *et al.*[3], preoperative age and serum creatinine are predictive variables of the postoperative dialysis need. Yang *et al.*[4] state that a time of renal ischemia greater than 25 minutes and the crossclamping are significant risk factors for the development of postoperative renal failure. In the lateral clamping technique described by us, renal ischemia time is restricted only to the time of manufacturing of the bridge to the renal artery.

The maintenance of perfusion distal to the total clamping has proved a necessity. Most authors cite the alternative use of CPB, celiac trunk and mesenteric catheterization with continuous infusion of nourishing solution and infusion of cold solution in the renal arteries is while the visceral branches anastomoses are performed[5]. Our strategy also renders CPB or solutions infusion unnecessary, the manufacturing time of the bridges to the visceral arteries being considered tolerable. The hybrid technique is also referred, mainly indicated in patients with high surgical risk, with direct bridges from the iliac vessels to the visceral vessels and subsequent endovascular aneurysm exclusion, thus avoiding the use of CPB and also the total clamping[6]. However, mores studies are needed to confirm the long-term benefits and safety of the hybrid procedures[6,7].

Conrad *et al.* showed that the mortality of patients who developed paraplegia or paresis was significantly higher than in those who did not develop them[8]. Thus, they recommend renal hypothermia via catheterization and cold solution infusion, epidural cooling and reconstruction of intercostal arteries, guided by continuous monitoring (motor-evoked potentials), and CPB.

The axilofemoral bypass is also a surgical option[9], which has the advantage of reducing afterload, lower incidence of spinal cord ischemia and preservation of visceral flow[10]. Although effective, this strategy significantly increases operating time. The aorto-bi-iliac bypass already performed in our technique allows for distal perfusion and reduces operative time.

Cambria *et al.* demonstrated a reduction in spinal cord ischemia in the open treatment of thoracoabdominal aneurysm (ATA) through epidural cooling and observed that mortality doubles in the postoperative period of non-elective

surgeries[11]. The demonstrated lateral clamping allows spinal cord perfusion during the entire procedure until the moment of flow bypass with the endoprosthesis implantation.

In summary, the conventional approach to ATA is still widely used, especially in non-elective conditions, for endovascular treatment failure and in complex[12] or infection cases. Moreover, the superiority of the hybrid technique in endovascular treatment over the conventional technique has not been proven[13,14]. Thus, we believe that the procedure has current utility and its main advantage is to maintain distal flow and shorter visceral ischemia, resulting in the reduction of postoperative morbidity and mortality. This fact stimulates new studies to evaluate the described benefits.

## RESUMO

*Os autores apresentam uma abordagem cirúrgica aos aneurismas do tipo III e IV de Crawford em que não é necessário o pinçamento total da aorta, o que permite a prevenção do dano isquêmico direto de forma mais objetiva, assim como sua exclusão por implante de endoprotese desviando o fluxo para o enxerto sintético.*

***Descritores:*** *Aneurisma Aórtico. Prótese Vascular. Procedimentos Endovasculares. Isquemia Mesentérica.*

## REFERENCES

1. Etheredge SN, Yee J, Smith JV, Schonberger S, Goldman MJ. Successful resection of large aneurysm of the upper abdominal aorta and replacement with homograft. Surgery. 1955;38(6):1071-81.
2. Crawford ES. Thoraco-abdominal and abdominal aortic aneurysms involving renal, superior mesenteric, celiac arteries. Ann Surg. 1974;179(5):763-72.
3. Schepens MA, Defauw JJ, Hamerlijnck RP, Vermeulen FE. Risk assessment of acute renal failure after thoracoabdominal aortic aneurysm surgery. Ann Surg. 1994;219(4):400-7.
4. Yang SS, Park KM, Roh YN, Park YJ, Kim DI, Kim YM. Renal and abdominal visceral complications after open aortic surgery requiring supra-renal aortic cross clamping. J Korean Surg Soc. 2012;83(3):162-70.
5. Hou Y, Zhao J, Guo W, Huang S, Wang C. Surgical repair of thoracoabdominal aortic aneurysms using the critical artery reattachment technique. J Biomed Res. 2011;25(3):220-3.
6. Chiesa R, Civilini E, Melissano G, Logaldo D, Calliari FM, Bertoglio L, et al. Management of thoracoabdominal aortic aneurysms. HSR Proc Intensive Care Cardiovasc Anesth. 2009;1(1):45-53.
7. Carrel TP, Signer C. Separate revascularization of the visceral arteries in thoracoabdominal aneurysm repair. Ann Thorac Surg. 1999;68(2):573-5.
8. Conrad MF, Ye JY, Chung TK, Davison JK, Cambria RP. Spinal cord complications after thoracic aortic surgery: long-term survival and functional status varies with deficit severity. J Vasc Surg. 2008;48(1):47-53.
9. Comerota AJ, White JV. Reducing morbidity of thoracoabdominal aneurysm repair by preliminary axilofemoral bypass. Am J Surg. 1995;170(2):218-22.
10. Conrad MF, Ergul EA, Cambria MR, Lamuraglia GM, Simon M, Cambria RP. Evolution of operative strategies in open thoracoabdominal aneurysm repair. J Vasc Surg. 2011;53(5):1195-201.
11. Cambria RP, Clouse WD, Davison JK, Dunn PF, Corey M, Dorer D. Thoracoabdominal aneurysm repair: results with 337 operations perfomed over a 15-year interval. Ann Surg. 2002; 236(4):4719; discussion 479.
12. LeMaire SA, Green SY, Kim JH, Sameri A, Parenti JL, Lin PH, et al. Thoracic or thoracoabdominal approaches to endovascular device removal and open aortic repair. Ann Thorac Surg. 2012;93(3):726-32; discussion 733.
13. Tshomba Y, Bertoglio L, Marone EM, Melissano G, Chiesa R. Visceral aortic patch aneurysm after thoracoabdominal aortic repair: conventional vs hybrid treatment. J Vasc Surg. 2008;48(5):1083-91.
14. Greenberg RK, Lu Q, Roselli EE, Svensson LG, Moon MC, Hernandez AV, et al. Contemporary analysis of descending thoracic and thoracoabdominal aneurysm repair: a comparison of endovascular and open techniques. Circulation. 2008;118(8):808-17.

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**Address for correspondence:**
Gaudencio Espinosa
E-mail: g.espinosa@uol.com.br

# A porcine model for teaching surgical cricothyridootomy

## *Modelo porcino no ensino da cricotiroidotomia cirúrgica*

Fernando Antonio Campelo Spencer Netto[1]; Patricia Zacharias[1]; Raphael Flavio Fachini Cipriani[1]; Michael de Mello Constantino[1]; Michel Cardoso[1]; Renan Augusto Pereira[1]

## A B S T R A C T

**Objective:** To evaluate the acceptability of an educational project using A porcine model of airway for teaching surgical cricothyroidotomy to medical students and medical residents at a university hospital in southern Brazil. **Methods**: we developed a teaching project using a porcine model for training in surgical cricothyroidotomy. Medical students and residents received lectures about this surgical technique and then held practical training with the model. After the procedure, all participants filled out a form about the importance of training in airway handling and the model used. **Results**: There were 63 participants. The overall quality of the porcine model was estimated at 8.8, while the anatomical correlation between the model and the human anatomy received a mean score of 8.5. The model was unanimously approved and considered useful in teaching the procedure. **Conclusion**: the training of surgical cricothyroidotomy with a porcine model showed good acceptance among medical students and residents of this institution.

**Key words:** Airway. Cricoid cartilage / surgery. Thyroid cartilage / surgery. Teaching / education.

## INTRODUCTION

Medical skills training, particularly in early phases, are increasingly based on simulation[1]. Simulation is an interactive and immersive method of teaching, recreating in whole or in part a clinical experience, without exposing patients to associated risks[1, 2]. Simulation modalities may vary according to the type of technology used. Among the low technology simulators, there are the models based on animals and on human or animal cadavers[3].

Airway management is key in emergency situations[4]. Patients requiring a surgical airway may represent 1% of procedures for obtaining a definitive airway in urgency settings. However, since this method is used when the other techniques are unsuccessful, failure in obtaining a surgical airway commonly will lead to death due to hypoxemia[5]. Among the surgical airways techniques, cricothyrotomy is preferred over tracheotomy in emergency situations due its simplicity and rapid execution[4].

This article presents and analyzes the acceptability of a teaching project using an airway porcine model for surgical cricothyrotomy to medical students and residents in a university hospital in the south of Brazil.

## METHODS

Since June 2013, in Universidade Estadual do Oeste do Paraná started a teaching project using porcine models in resuscitation procedures training for residents and last-year medical students. As part of this teaching project, trainees filled a feedback questionnaire about the used model. We analyzed the questionnaires used to assess the airways the porcine arway model filled by the participants from the start of the project until August 2014. Project approval: CR 40119/2013.

This teaching project comprised three steps: a) trainees attended a class about cervical anatomy and the surgical cricothyrotomy, according to the Advanced Trauma Life Support® (ATLS) principles; b) they performed the surgical cricothyrotomy in the porcine model, supervised by an ATLS instructor; c) they filled out an assessment questionnaire about the model (optional).

We obtained segments of porcine airways by donation from animals used for human consumption, according to regulatory sanitary rules.

We used a porcine arway segment from above the thyroid cartilage to about 10cm below the cricoid cartilage and a segment of pig skin (Figure 1). A plastic bag was adapted to the trachea end and the specimen was placed over a rigid wood surface (Figure 2). The specimen was covered with a segment of pork skin. In the internal segment of the pork skin, a rubber glove was attached and tensioned (Figure 3), to simulate a "new" cricothyroid membrane after the first procedure. The pork skin was then fixed to the rigid surface, allowing the airway segment to be moved below the skin in order to use the same model for several trainees (average of 10 trainees /

1. Faculdade de Medicina na Universidade Estadual do Oeste do Paraná.

model – Figure 4). A surgical cloth was placed over the plastic bag to simulate the chest movement seen during the bag ventilation. The step by step construction of the model and its operation can be observed on the site ( http://youtu.be/l8fDbost0O8 ).

The questionnaire used inquired about epidemiological aspects, previous training in airway management and adequacy of the used model for training medical student and residents. Some of the answers from the questionnaire were not object of this study, but used to improve graduation teaching opportunities.

Specifically, we requested assessments about the general quality of the model (robustness, ease in handling, similarity with biological tissues) and anatomic correlation (similarity with human anatomy). Both varied from 0 to 10.

The questionnaire was elaborated by the senior author and was not previously validated. The questionnaire data was grouped and presented in absolute numbers and percentages.

During the development phase, three independent senior surgeons with experience in this procedure tested this model. They unanimously approved it as an educational tool and gave the average score of 8.7 to its general quality and 7.6 to the anatomical correlation.

The teaching group was composed by: a) medical students from emergency room internship (last year of our medical school); b) medical residents (first and second year) from general surgery and internal medicine who requested this training.

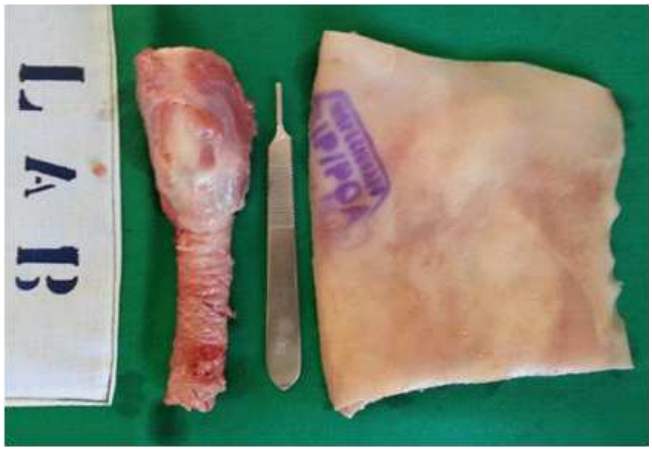

***Figure 1*** - *Porcine airway and skin specimens.*

***Figure 2*** - *Porcine airway on a rigid board with a plastic bag ("lung") adapted to its end.*

## RESULTS

This project had 63 participants, 54 medical students and nine medical residents. There was no refusal in participate in the training or in filling the questionnaire. The average age was 26 ± 3.1 years old (22-39). Thirty-two were males, 29 females and two did not disclose this information. Among the tested trainees, 60 (95.2%) informed no previous training in the procedure.

All participants considered the proposed model as an important tool in teaching surgical cricothyrotomy to medical students. The average overall model quality was 8.8 (scores varied from 6-10). The anatomical correlation of the model was scored in average as 8.5 (scores varied from 6-10). All trainees evaluated approved the use of this model as a teaching adjunct for medical graduation.

***Figure 3*** - *Porcine skin with glove fixed in the inner side. The glove simulates the cricothyroid membrane after the first procedure.*

## DISCUSSION

Since cricothyrotomy is relatively infrequently required and the patients who need the procedure are usually in physiologic extremes, the development of experimental animals models for professional training are important to provide medical training[6,7]. Using animal

***Figure 4*** - *Airway model ready to practice. The surgical cloth covers the "chest" and rises in each ventilation.*

models in airway procedures training is frequent, since biological tissues have higher similarity with human ones, when compared with synthetic materials[8, 9]. Also, animal models using porcine airways have low cost and are frequently easy to obtain[10].

Porcine models can present economical advantage when compared with mannequins[10]. In the current study, the porcine airway specimens were obtained as donation from trainees after these animals were abated for human consumption. Medical teaching based in passive techniques and knowledge acquisition verification based in written exams are not enough to assure competence, quality and safety when handling emergency situations[11]. Most of incidents and serious adverse events that happen in hospitals are related to human factors[12,13]. These figures are likely higher in emergency cases[12,13,14]. Simulation tries to reduce these undesired outcomes, providing education based on active learning in low-risk environment, improving knowledge, the technique, and non-technical skills[11]. Urgent surgical airway access is required when another technique is not feasible or failed[4], and frequently is performed by emergency physicians[15]. Performing this procedure can be difficult in the absence of adequate training[10]. Some suggest minimal training of 5 attempts or performing this procedure in 40 seconds in mannequins[9].

There are several teaching models used in airways management[8,15]. Traditionally, cricothyrotomy teaching is performed in mannequins and animals due to ethical and economical reasons[8,16]. Among the animal models, the use of porcine airways is well-established[17-19]. Porcine airways and skin are relatively similar to human tissues[8,18]. In a previous randomized study, the similarity of porcine models with human tissues and anatomy was considered greater than the one of mannequins[10].

Medical training in airways management was uniformly considered important by trainees. This study presented an experimental airway porcine model based on low technology and cost for teaching surgical cricothirotomy in medical graduation. The used model was approved for medical training among all study participants. The model used in this study was tested by three independent seasoned physicians, who corroborated the high correlation with human anatomy. Trainees also graded the model similarity with human anatomy to be medium to high. Other studies have shown similar results[20]. Each porcine airway model allowed 10 trainees in average to practice per specimen, due to the practice of sliding the model under the skin and glove. Alive models or cadavers do not allow repetition[6,8]. This possibility adds practicality and economical advantage to the model.

The model used was developed at our institution, using local conditions. However, there are other described porcine airway models[17-19]. Also, it does not recreate all anatomical characteristics present in real situations, such as protuberant jaw, cervical immobilization, hematomas, urgency, obesity, etc. However, performing this procedure in a real situation without previous training or simulation may incur in ethical inadequacy, and may increase failure rates.

There are few physicians seasoned in surgical cricothyrotomy in the region. In consequence, only three tested the model. Nevertheless, other studies used similar animal models for training[10,18,19,20]. The trainees opinion about anatomical correlation may not be as accurate, since they have relatively low knowledge of the anatomy and low experience in airway management. Also, trainees may tend to give higher scores to the models to please their teachers, even though they anonymously filled the questionnaires. Thus, due to such limitations, the presented model needs additional assessment and validation with specific methodology and/or by other institutions.

The proposed porcine airway model had good acceptability for surgical cricothyrotomy. Considering the low cost and easy preparation, it presents good potential for training medical professionals in developing countries. Further studies are necessary to validate this model as a teaching tool.

## RESUMO

***Objetivo:*** *avaliar a aceitabilidade de um projeto de ensino utilizando modelo porcino de vias aéreas no ensino da cricotiroidotomia cirúrgica para estudantes de Medicina e médicos residentes em um hospital universitário no sul do Brasil.* ***Métodos:*** *foi desenvolvido um projeto de ensino usando modelo porcino para treinamento em cricotiroidotomia cirúrgica. Estudantes de Medicina e residentes receberam aula teórica sobre esta técnica cirúrgica e, em seguida, realizaram no modelo o treinamento prático. Após o procedimento, todos os participantes preencheram um formulário acerca da importância do treinamento em manuseio de vias aéreas e do modelo utilizado.* ***Resultados:*** *houve 63 participantes. A qualidade geral do modelo porcino foi estimada em 8,8, enquanto a correlação anatômica entre o modelo e a anatomia humana recebeu o escore médio de 8,5 entre os treinandos. O modelo foi unanimemente aprovado e considerado útil no ensino do procedimento.* ***Conclusão:*** *o treinamento de cricotiroidotomia cirúrgica em modelo porcino apresentou boa aceitação entre os estudantes de Medicina e os residentes desta Instituição.*

***Descritores:*** *Manuseio das Vias Aéreas. Cartilagem Cricoide/cirurgia. Cartilagem Tireoidea/cirurgia. Ensino/educação.*

## REFERENCES

1. Maran NJ, Glavin RJ. Low-to high-fidelity simulation – a continuum of medical education? Med Educ. 2003;37 Suppl 1:22-8.
2. Beaubien JM, Baker DP. The use of simulation for training teamwork skills in health care: how low can you go? Qual Saf Health Care. 2004;13 Suppl 1:i51-6.
3. Dent JA, Harden RM, editors. A Practical Guide for Medical Teachers. 2nd ed. Edinburgh: Elsevier; 2005.
4. Comitê do Trauma do Colégio Americano de Cirurgiões. Suporte avançado de vida no trauma para médicos: manual do curso de alunos. 8ª Ed. Chicago: American College of Surgeons; 2008.
5. Bair AE, Panacek EA, Wisner DH, Bales R, Sakles JC. Cricothyrotomy: a 5-year experience at one institution. J Emerg Med. 2003;24(2):151-6.
6. Reeder TJ, Brown CK, Norris DL. Managing the difficult airway: a survey of residency directors and a call for change. J Emerg Med. 2005;28(4):473-8.
7. Wong DT, Prabhu AJ, Coloma M, Imasogie N, Chung FF. What is the minimum training required for successful cricothyrotomy?: a study in mannequins. Anesthesiology. 2003;98(2):349-53.
8. Stringer KR, Bajenov S, Yentis SM. Training in airway management. Anaesthesia. 2002;57(10):967-83. Erratum in: Anaesthesia. 2003;58(1):105.
9. Hedges J, Toomey TG. The 'Cambridge' surgical training device for cricothyrotomy and tracheostomy techniques. J Br Assoc Immed Care. 1989;12:11-2.
10. Cho J, Kang GH, Kim EC, Oh YM, Choi HJ, Im TH, et al. Comparison of manikin versus porcine models in cricothyrotomy procedure training. Emerg Med J. 2008;25(11):732-4.
11. Flato UAP, Guimarães HP. Educação baseada em simulação em medicina de urgência e emergência: a arte imita a vida. Rev Soc Bras Clin Méd. 2011;9(5), set-out.
12. Dellifraine J, Langabeer J, King B. Quality improvement practices in academic emergency medicine: perspectives from the chairs. West J Emerg Med. 2010;11(5):479-85.
13. Bion JF, Abrusci T, Hibbert P. Human factors in the management of the critically ill patient. Br J Anaesth. 2010;105(1):26-33.
14. Kothari D, Gupta S, Sharma C, Kothari S. Medication error in anaesthesia and critical care: A cause for concern. Indian J Anaesth. 2010;54(3):187-92.
15. Nelson MS. Models for teaching emergency medicine skills. Ann Emerg Med. 1990;19(3):333-5.
16. Cheong J. The use of animals in medical education: a question of necessity vs. desirability. Theor Med. 1989;10(1):53-7.
17. Gallagher EJ, Coffey J, Lombardi G, Saef S. Emergency procedures important to the training of emergency medicine residents: who performs them in the emergency department? Acad Emerg Med. 1995;2(7):630-3.
18. Fikkers BG, van Vugt S, van der Hoeven JG, van den Hoogen FJ, Marres HA. Emergency cricothyrotomy: a randomised crossover trial comparing the wire-guided and catheter-over-needle techniques. Anaesthesia. 2004;59(10):1008-11.
19. Wang EE, Vozenilek JA, Flaherty J, Kharasch M, Aitchison P, Berg A. An innovative and inexpensive model for teaching cricothyrotomy. Simul Healthc. 2007;2(1):25-9.
20. Kanji H, Thirsk W, Dong S, Szava-Kovats M, Villa-Roel C, Singh M, et al. Emergency cricothyroidotomy: a randomized crossover trial comparing percutaneous techniques: classic needle first versus ''incision first''. Acad Emerg Med. 2012;19(9):1061-7.

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**Mailing address:**
Fernando Antonio Campelo Spencer Netto
E-mail: fspencernetto@gmail.com

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# The writing of informed consent in accessible language: difficulties

## *A redação do termo de consentimento livre e esclarecido em linguagem acessível: dificuldades*

Nurimar C. Fernandes[1]

A B S T R A C T

In order to assess the adequacy of informed consent terminology of research projects developed at the Clementino Fraga Filho University Hospital (Federal University of Rio de Janeiro) , we conducted a review study on the terminology found in 55 projects (2008-20013) . Such projects belonged to different medical specialties and were all registered in the hospital's Ethics in Research Committee. Patients had difficulty in understanding the meanings of 76 medical terms and expressions; only 12 of them could be replaced. On the other hand, the present study reached the conclusion that, in most cases, the writing with scientific terms is essential in items such as justification/objectives and procedures, being insurmountable obstacles to the participants of this research and patients' understanding.

**Key words:** Informed consent. Biomedical research. Bioethics. Writing. Terminology. Comprehension.

## INTRODUCTION

The Declaration of Helsinki was broader than the Nuremberg Code, establishing the free and informed consent as a standard for accepted procedures in a ethical research. It is a very complex procurement document, since a lot of information must be provided to research participants in a simple and understandable way. To be truly free and informed, what is being consented should be clearly understood.

This study aims to assess the adequacy of terms from informed consent forms (ICF) of research projects developed at a university hospital.

## METHODS

This was a review study (2008-2013) of the ICFs from Project of the are of Health Sciences – Medicine, registered in the Comitê de Ética em Pesquisa of the Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (CEP-HUCFF/UFRJ).

The committee, whose members include one representative from the users, analyzed fifty-five projects of the specialties Anesthesiology, Cardiology, Surgery, Dermatology, Infectious and Parasitic Diseases, Endocrinology, Gastroenterology, Geriatrics, Gynecology, Hematology, Hepatology, Immunology, Nephrology, Neurology, Oncology, Ophthalmology, Orthopedics, Otorhinolaryngology, Pulmonology, Psychiatry, Radiology and Rheumatology.

## RESULTS

The following words/phrases (n=76) were considered difficult to understand and replacement by non-technical terms: 1) thiobarbituric acid, 2) endotracheal diffuse involvement , 3) retinal cells electrophysical findings, 4) low grade prostate adenocarcinoma, 5) antiendomysium antibody IgA, 6) non-steroidal anti-inflammatory agents, 7) non-probabilistic sample, 8) opioid analgesics, 9) anti-gliadin antibody, 10) social movement actors, 11) salivary scintigraphy, 12) understand perceptions about the disease. 13) demographic characteristics, 14) coagulopathy, 15) cohort, 16 liver cirrhosis, 17) coinfection, 18) choroidopathy, 19) corneal scarring, 20) dense cataract, 21) double-blind design, 22) breast density, 23) extraction / amplification / sequencing / quantification of DNA, 24) hepatic encephalopathy, 25) prospective / retrospective / Cross-sectional study, 26) risk stratification, 27) insulin-like growth factor, 28) neurocognitive function, 29) serum pharmacokinetics, 30) pituitary transcription factors, 31) genotype, 32) genome, 33) encoding genes, 34) portal

1. Serviço de Dermatologia, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro. Comitê de Ética em Pesquisa, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro - RJ - Brazil.

hypertension 35) contained distal herniation, 36) vitreous hemorrhage, 37) occult infection, 38) measurement of portal vein pressure gradient, 39) myelosuppression, 40) serum levels of hemoglobin and bilirubin, 41) serum levels of nitric oxide, 42) neurolysis, 43) peripheral neuropathy, 44) clinical research, performance status 45), 46) risk potential, 47) exploratory research, 48) pneumothorax, 49) prospects for the future, 50) peptides, 51) spontaneous bacterial peritonitis, 52) protein carbonylated, 53) analogue pictorial questionnaire, 54) psychosis, 55) cutaneous rash; 56) diabetic retinopathy, 57) randomized, 58) nonrandom selection, 59) genetic sequencing, 60) sialometry, 61) psychological symptoms, 62) , serology 63) histological subtype of Hodgkin's lymphoma, 64) viral susceptibility, 65) prevalence rate, 66) molecular biology technique, 67) biological therapy, 68) cognitive behavioral therapy, 69) optical coherence tomography, 70) toxicity, 71) pressure transmitter, 72) generalized anxiety disorder, 73) antiretroviral treatment, 74) nucleotide treatment, 75) non-infectious uveitis, 76) invasive mechanical ventilation.

For the following words (n = 12) replacements were possible: 1) aneurysm – dilation (increase) in a blood vessel, 2) disease course – what happens during an illness, 3) glucose curve – are the measures of blood sugar at a certain time, 4) adverse effects – are the negative effects that a medicine can cause, 5) exacerbation of the disease – the disease worsens, 6) noninvasive test – is the test that does not cut or pierces to skin, 7) nasal endoscopic exam – the exam is done inside the nose, 8) benign prostatic hyperplasia – is an increase in prostate size that is not malignant, 9) Post-inflammatory hyperpigmentation – is the darkening of the skin that can happen after a inflammation, 10) serum bilirubin levels – is the amount of a pigment produced in the liver that is found in the blood, 11) skin rash – red spots on the skin that can itch; they are caused by drugs or disease-causing microbes, 12) toxicity – bad for health[1].

## DISCUSSION

The epistemological question is obstacle to obtaining an informed consent; although the common sense and low-tech mechanism to spend more time talking to the participants of the study appears promising, the wording without scientific terms is, in most cases, an insurmountable obstacle in the sections of justification, objectives and procedures.

Confidence in the doctor affected the decision to participate in the clinical trial of the drug[2], there being also the influence of the patients' level of education. Other authors point out that even the text improvements through a syntactic lexical approach or workgroup did not impact final understanding[3]. The word choice is a particular challenge, as it requires a level of understanding that goes beyond the usually required in health assistance[1,4]. Biondo-Simões *et al*. argue that research participants should be those better-educated, with customary character for reading, with easy access to the internet and at a higher income group[5].

A recent review showed that understanding of the information about the study varies among participants, both in developing countries and in developed ones[6]. This fact highlights the complexity of the issue, since it was also shown that when it comes to randomized and controlled designs, understanding is compromised in both. Participants in developing countries are less likely to refuse to participate in research or to leave it, more concerned about the consequences of their decisions.

The readability of consent forms is a major problem for the ethics in research committees, researchers and research participants. The exclusion of technical terms is often the only way to reach it.

## R E S U M O

*Com o objetivo de avaliar a adequação dos textos do termo de consentimento livre e esclarecido de projetos de pesquisa desenvolvidos no Hospital Universitário Clementino Fraga Filho (Universidade Federal do Rio de Janeiro), foi realizado (2008-2013) um estudo de revisão da terminologia encontrada em 55 projetos. Tais projetos, todos registrados no Comitê de Ética em Pesquisa do hospital, pertenciam a diferentes especialidades médicas. Os participantes tiveram dificuldades em compreender os significados de 76 termos médicos e expressões; apenas 12 deles puderam ser substituídos. Por outro lado, o presente estudo chegou à conclusão de que, na maioria dos casos, a redação com termos científicos é essencial em itens como justificativa/objetivos e procedimentos, constituindo obstáculos intransponíveis para a compreensão dos participantes desta pesquisa e dos pacientes.*

***Descritores:*** *Consentimento livre e esclarecido. Pesquisa biomédica. Bioética. Redação. Terminologia. Compreensão.*

## REFERENCES

1. Rossi R, Goldim JR, Francisconi CF. Glossário de termos científicos para elaboração do consentimento informado. Rev Med ATM. 1999;19(1):304-9.
2. Meneguin S, Zoboli ELCP, Domingues RZL, Nobre MR, César LAM. Entendimento do termo de consentimento por pacientes partícipes em pesquisas com fármaco na cardiologia. Arq Bras Cardiol. 2010;94(1):4-9.

3. Paris A, Brandt C, Cornu C, Maison P, Thalamas C, Cracowski JL. Informed consent document improvement does not increase patients' comprehension in biomedical research. Br J Clin Pharmacol. 2010;69(3):231-7.
4. Goldim JR. Consentimento e informação: a importância da qualidade do texto utilizado. Rev HCPA Fac Med Univ Fed Rio Gd do Sul. 2006;26(3):117-22.
5. Biondo-Simões MLP, Martynetz J, Ueda FMK, Olandoski M. Compreensão do termo de consentimento informado. Rev Col Bras Cir. 2007;34(3):183-8.
6. Mandava A, Pace C, Campbell B, Emanuel E, Grady C. The quality of informed consent: mapping the landscape. A review of empirical data from developing and developed countries. J Med Ethics. 2012;38(6):356-65.

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**Adress for correspondence:**
Nurimar C. Fernandes
E-mail: nurimarfernandes@terra.com.br