

# CBC



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# The Journal of the Brazilian College of Surgeons

## *A Revista do Colégio Brasileiro de Cirurgiões*

GUILHERME PINTO BRAVO NETO, TCBC-RJ<sup>1</sup>

The Journal of The Brazilian College of Surgeons (CBC), published uninterruptedly since 1974, has been gaining greater visibility in recent years, since its indexing in the electronic library SciELO (Scientific Electronic Library Online) in 2005, and later in Medline/PubMed and other databases. Our articles have been read worldwide and quoted in several international publications of greatest impact, and our authors, contacted by editors of international journals to serve as ad hoc reviewers in several works, based on the expertise inferred through their publications on our journal. Our impact factor measured by the JCR (Journal Citation Reports) has been steadily increasing, and nationally, we reached the Qualis B1 level at CAPES (Higher Education Personnel Improvement Coordination), a degree now stamped on the cover of our journal. We are moving forward, but we still have much work ahead. More pressingly, we are striving to achieve more rapid assessments of the works sent and trying to substantially reduce the time for definition of approval, rejection or request of modifications suggested by our reviewers. For this, we rely on the promptness and seriousness of our editorial board, which will gain new signings in the coming months. Soon works submission will be done exclusively through our submission online platform, already in place and that can be accessed through the CBC website, which should further streamline the evaluation and review process and give greater transparency to the authors to monitor the progress of their work. We hope that in the first half of this year we managed to catch up on our publications and the journal can be accessed in full on the SciELO database and CBC site even during the last month of the bimonthly journal period. And that the printed journal reaches subscribers and CBC members already in the first days following the last month's cover. These perspectives are due to not only our respect and consideration to our authors and readers, but also to the guidelines set for maintenance of indexations already achieved and for approval in other search electronic platforms, such as PubMed Central, which has been in negotiation for a few months now. On the other hand, greater visibility and impact also require greater scientific quality of articles published and therefore higher rigor in assessments by the editorial board. We know of the

difficulties in conducting scientific work in our environment, particularly clinical trials. Inefficient patients referencing policy dilute the experience with certain diseases and reduce treatment effectiveness and degraded hospitals scrapped, especially university ones, are some of the many obstacles to be faced by Brazilian researchers, particularly by the surgeon ones. And once overcome the barriers to achieving the original work, one still need to deal with the difficulties in its publication, especially when opting for high-impact journals. Besides the indisputable quality of work, one needs an adequate translation into English, preferably by professionals experienced with the scientific language, and a renowned origin of institutions and authors. All these minutiae greatly hamper the publication possibilities for the most active surgeons, with all their professional activities, which often interfere even in their own family gatherings. On the other hand, in the academic world, the need to publish papers is increasing, which has generated serious distortions between professionals of the same university as it relates to clinical practice and scientific production, a ratio that is becoming contradictorily antagonistic. Unfortunately, we cannot change this reality, but we encourage our members to invest in quality clinical research, able to promote and extol their primary surgical activity. These difficulties have been chronically reflected in the types of articles we receive: largely case reports and review articles, to the detriment of quality original articles, the latter composed mostly by experimental work and master's and doctorate degree theses. For publication of these numerous Case Reports, the CBC has an online journal dedicated exclusively to these articles and videos, the Journal of Surgical Case Reports, which can be accessed through the CBC website and soon will also feature its own ISSN (International Standard Serial Number). We also have specialized translators who translate the work into English, as yet with no cost to the authors. We therefore believe that the CBC journal can not only further enhance and contribute to the dissemination of high quality scientific articles, but also stimulate our surgeons to develop their scientific and investigative potential, with the certainty of a good welcome in their home, the Brazilian College of Surgeons.

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# Comparison between open and laparoscopic elective cholecystectomy in elderly, in a teaching hospital

## *Comparação entre colecistectomia eletiva aberta e laparoscópica em idosos, em um hospital escola*

CÁSSIO PADILHA RUBERT<sup>1</sup>; ROBERTA ALVES HIGA, ACBC-MS<sup>1</sup>; FABIANO VILAS BOAS FARIAS<sup>1</sup>

### A B S T R A C T

**Objective:** to analyze the differences in mortality rates, length of hospital stay, time of surgery and the conversion rate between elective open cholecystectomies (OC) and laparoscopic ones (LC) in elderly patients. **Methods:** we evaluated medical records of patients 65 years of age or older undergoing open or laparoscopic cholecystectomy at the Hospital Regional de Mato Grosso do Sul between January 2008 and December 2011. We excluded individuals operated in non-elective scenarios or who underwent intraoperative cholangiography. **Results:** we studied 113 patients, of whom 38.1% were submitted to the OC and 61.9%, to LC. Women accounted for 69% of patients and men, for 31%. The conversion rate was 2.9%. The mean age and duration of the procedure was 70.1 and 84 minutes, respectively, with no significant difference between OC and LC. Patients undergoing LC had shorter hospital stays (2.01 versus 2.95 days,  $p=0.0001$ ). We identified operative complications in six patients (14%) after OC and in nine (12%) after LC, with no statistical difference. **Conclusion:** there was no difference in morbidity and mortality when comparing OC with LC. The laparoscopic approach led to shorter hospital stay. Operative time did not differ between the two access routes. The conversion rate was similar to other studies.

**Key words:** Cholecystectomy. Cholecystectomy, Laparoscopic. Aged. Postoperative Complications.

### INTRODUCTION

Life expectancy has increased over the past decades. Contributing factors for this change include improvements in primary prevention and advances in medical care, technology and pharmaceutical industries. The traditional definition of the World Health Organization (WHO) considers elderly people aged 60 or older if they live in developing countries, and 65 years of age and over in developed ones. However, in order to allow direct comparisons with other articles (which mostly are made in developing countries), we used as a cutoff age of 65 years.

In Brazil, approximately 7.3% of the population is over 65 years of age. About 50% of women and 16% men, 70 years of age, have biliary calculi<sup>1</sup>, and symptomatic or complicated cholelithiasis is the most common indication for abdominal surgery in the elderly<sup>2,3</sup>. Historically, associated diseases and the anesthetic risk have been an obstacle to perform interventions in the elderly. However, recent in anesthetic, surgical and postoperative care innovations made such patients candidates for surgery, even in the ninth and tenth decades of life<sup>4-7</sup>.

Laparoscopic cholecystectomy provides a safe alternative for symptomatic cholelithiasis or cholecystitis in elderly patients, and the benefits, lower morbidity and

reduced hospital stay, were demonstrated in prospective studies and meta-analyses<sup>8-11</sup>.

In the beginning of the minimally invasive surgery era, advanced age was a relative contraindication to the laparoscopic procedure. Despite recent evidence showing that the LC is feasible in elderly patients, including over 70 or 80 years of age, there are few studies in our country on the subject.

The aim of this study was to compare the conventional cholecystectomy with the laparoscopic, electively performed in elderly patients in a teaching hospital, a reference in the state, especially in relation to morbidity, length of stay, operative time and conversion rate to open surgery.

### METHODS

We searched The SAME database (Medical Records Section) of the Hospital Regional de Mato Grosso do Sul - Rosa Pedrossian (HRMS) for the medical records of patients aged over 65 years who underwent conventional and laparoscopic cholecystectomy between January 2008 and December 2011. We included all patients admitted for elective surgery, and excluded patients operated on a non-

1. Hospital Regional de Mato Grosso do Sul (HRMS), MS, Brasil.

elective setting. Patients who underwent intraoperative cholangiography were also excluded.

The variables studied were age, gender, cardiovascular surgical risk, duration of surgery, accidents and intraoperative complications, postoperative complications, length of stay and conversion to open surgery.

Nominal variables were compared using the chi-square test or Fisher's exact test, as appropriate. Ordinal variables were compared using the Mann-Whitney test, with a p-value <0.05 accepted as significant.

## RESULTS

Between January 1, 2008 and December 31, 2011 113 elective cholecystectomy without cholangiography were performed in patients aged over 65 years for symptomatic cholelithiasis, of which 43 (38.1%) corresponded to open cholecystectomy (OC) and 70 (61.9%), laparoscopic cholecystectomy (LC). Regarding gender, 78 (69%) patients were female and 35 (31%) were men.

The conversion from laparoscopic surgery to open one was necessary in two (2.9%) cases, one relating to the difficulty in identifying the Calot triangle structures, and the second converted at the end of the procedure, after removal of the gallbladder, to carry out an of an intestinal puncture lesion perceived at the end of the procedure.

Overall, the average age, length of stay and duration of surgery was 70.1 years (65-91), 2.3 days (1-9) and 84 minutes (30-180), respectively. When we stratified the groups by the procedure (open and laparoscopic), there was no difference in mean age (70.2 years OC versus 70 LC, p=0.873) or cardiovascular surgical risk (p=0.146). The duration of the procedure was also not different between procedures, with an average of 76±27 minutes (30-150) for OC and 88±31 minutes (40-180) for LC (p=0.582).

The length of stay was shorter in patients undergoing LC, averaging 2.01±0.9 days, while the OC group stayed for 2.95±1.5 days (p=0.0001). The distribution of hospital stay between the groups is shown in figure 1.

Postoperative complications were identified in six (14%) patients after OC and in nine (12%) after LC, with no statistical difference between the two groups (p=0.8675). In LC there was one (1.4%) case of desaturation, corrected with changes in the mechanical ventilator, and one (1.4%) case of accidental damage of the small intestine, corrected during the procedure. The frequency of complications is shown in table 1 (two patients in the OC group and one of the LC group had two complications simultaneously).

## DISCUSSION

Laparoscopic cholecystectomy (LC) causes less pain after surgery, shorter hospital stay, faster return to

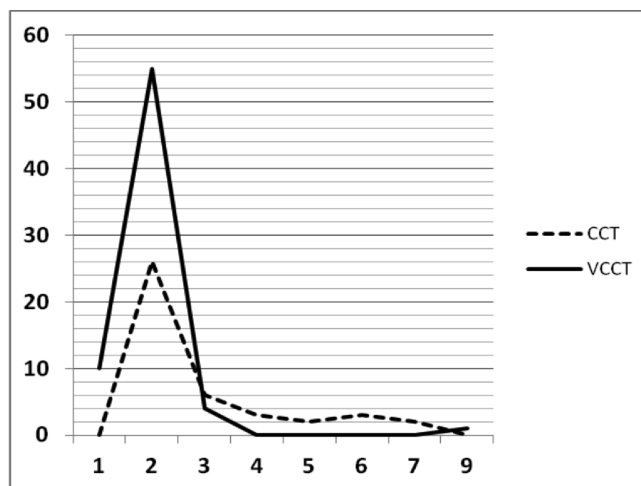


Figure 1 - Distribution of patients' hospital stay.

work activities and a lower metabolic-endocrine-immune response to trauma (REMIT)<sup>12-15</sup>. This procedure has been the gold standard for elective cholecystectomy for the general population in the last two decades<sup>16</sup>. Elderly patients with biliary tract disease have higher rates of complications, which explains their higher mortality.

LC could increase morbidity and mortality in the elderly, many of which have limited cardiopulmonary reserve. Although Behrman *et al.*<sup>17</sup> have not shown a higher incidence of hypotension and hypercarbia during the procedure in their series, they still recommend that LC be performed with caution in the elderly population, with a low threshold for conversion and considering open cholecystectomy (OC) as the initial indication.

However, LC has demonstrated results superior to OC in elderly patients with symptomatic cholelithiasis in terms of morbidity and hospital stay<sup>18</sup>. There is variability in global practices for the treatment of this disease in the elderly, and social, physiological and pathological

Table 1 - Postoperative complications.

Complications	OC (n=43)	LC (n=70)
Wound Seroma	3 (7%)	2 (2.9%)
Incisional hernia	3 (7%)	2 (2.9%)
Wound Hematoma	1 (2.3%)	1 (1.4%)
Surgical site infection	1 (2.3%)	1 (1.4%)
"Benign" biliary fistula	0 (0%)	1 (1.4%)
Cystic duct lesion	0 (0%)	1 (1.4%)
Desaturation	0 (0%)	1 (1.4%)
Small bowel lesion	0 (0%)	1 (1.4%)
Total	6 (14%)	9 (12.9%)

Source: SAME (Medical Records Section) database, Hospital Regional de Mato Grosso do Sul – Rosa Pedrossian (HRMS) – jan 2008-dec 2011.

characteristics of the elderly population also differ greatly between regions. In Brazil, there are few studies on the subject<sup>19-21</sup>. When one considers the population we studied (patients from SUS - National Health System) and procedures performed in the public system teaching hospitals, researches are even scarcer.

There were complications in 13.3% of patients, without differences in morbidity rates between groups, which differs from similar studies<sup>8,22</sup>, in which LC resulted in less morbidity. We believe that this divergence is due to under-reporting of minor complications. The conversion rate to open surgery in our series was 2.9%, compared with 2.5 to 14% in LC other series studying elective for symptomatic cholelithiasis in the elderly<sup>4,17-26</sup>, being similar to the conversion rate in young patients<sup>4,27,28</sup>, unlike Qasaimeh *et al.*<sup>29</sup>, who reported higher conversion rates in the elderly.

Many publications have reported that LC is associated with shorter hospital stay<sup>8,22,30</sup>. We also observed this result, with average length of stay of 2.01 days for LC, versus 2.95 days for OC.

As in other studies<sup>17,18</sup>, LC did not prolong surgical time, averaging 12 minutes greater than OC, with no statistical significance. We deem this result satisfactory, since the procedures are performed mostly by resident physicians, with less experience in laparoscopy.

There were no deaths in our study, as reported by Caglià<sup>31</sup> in his series of 50 patients.

We emphasize that possible biases related to the retrospective study and the small number of patients involved must be taken into account when interpreting the results.

In conclusion, elective laparoscopic cholecystectomy is a safe procedure in elderly patients, with no increased risk of complications compared with the open procedure. The recovery is faster and the hospital stay, shorter. It is important to correctly assess the cardiovascular surgical risk, since this group of patients have lower vital reserve, being more sensitive to surgical trauma. In the era of laparoscopic surgery, with increasing experience of surgeons and the advent of new technologies, old age is not a contraindication for LC, and there are no major complications of this surgery when electively performed.

## R E S U M O

**Objetivo:** analisar as diferenças nas taxas de morbimortalidade, o tempo de permanência hospitalar, o tempo de cirurgia e a taxa de conversão entre colecistectomia aberta (CA) e laparoscópica (CL) eletiva, em pacientes idosos. **Métodos:** pesquisa dos prontuários dos pacientes com 65 anos de idade ou mais, submetidos à colecistectomia aberta ou laparoscópica no Hospital Regional de Mato Grosso do Sul entre janeiro de 2008 e dezembro de 2011. Foram excluídos os operados em caráter não eletivo ou que realizaram colangiografia intraoperatória. **Resultados:** foram estudados 113 pacientes, 38,1% dos quais submetidos à CA e 61,9% à CL. Mulheres corresponderam a 69% dos pacientes e homens, 31%. A taxa de conversão foi 2,9%. A média de idade e duração da operação foram 70,1 anos e 84 minutos, respectivamente, sem diferença significativa entre CA e CL. Os pacientes submetidos à CL tiveram menor tempo de internação (2,01 x 2,95 dias,  $p=0,0001$ ). Complicações operatórias foram identificadas em seis (14%) pacientes após CA, e em nove (12%) pacientes após CL, sem diferença estatística. **Conclusão:** Não houve diferença de morbidade e mortalidade quando comparadas a CA e CL. A via laparoscópica propiciou menor tempo de hospitalização. O tempo de operação não diferiu entre as duas vias de acesso. A taxa de conversão foi semelhante a outros estudos.

**Descritores:** Colecistectomia. Colecistectomia Laparoscópica. Idoso. Complicações Pós-Operatórias.

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# Fifty consecutive pancreatectomies without mortality

## *Cinquenta pancreatectomias consecutivas sem mortalidade*

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### A B S T R A C T

**Objective:** to report the group's experience with a series of patients undergoing pancreatic resection presenting null mortality rates. **Methods:** we prospectively studied 50 consecutive patients undergoing pancreatic resections for peri-ampullary or pancreatic diseases. Main local complications were defined according to international criteria. In-hospital mortality was defined as death occurring in the first 90 postoperative days. **Results:** patients' age ranged between 16 and 90 years (average: 53.3). We found anemia (Hb < 12g/dl) and preoperative jaundice in 38% and 40% of cases, respectively. Most patients presented with peri-ampullary tumors (66%). The most common surgical procedure was the Kausch-Whipple operation (70%). Six patients (12%) needed to undergo resection of a segment of the mesenteric-portal axis. The mean operative time was 445.1 minutes. Twenty two patients (44%) showed no clinical complications and presented mean hospital stay of 10.3 days. The most frequent complications were pancreatic fistula (56%), delayed gastric emptying (17.1%) and bleeding (16%). **Conclusion:** within the last three decades, pancreatic resection is still considered a challenge, especially outside large specialized centers. Nevertheless, even in our country (Brazil), teams seasoned in such procedure can reach low mortality rates.

**Key words:** Pancreas. Surgical Procedures, Operative. Pancreatectomy. Pancreaticoduodenectomy. Mortality.

### INTRODUCTION

Pancreatic resection is still a complex procedure. Although described in the first half of the twentieth century, the cephalic resection of the pancreas was infrequently practiced because of poor early results<sup>1</sup>. Until the 70s, mortality rates were high, even in North America<sup>1</sup>. Mortality of around 25% with pancreaticoduodenectomy was frequent, which raised doubts about the implementation of this operation in the treatment of pancreas adenocarcinoma<sup>1</sup>. It was not until the 60s, with a pioneer creation of specialized services for the treatment of pancreatic diseases, that the John Hopkins Hospital in Baltimore, under the leadership of John Cameron, achieved better results<sup>2</sup>. Currently, in major centers the procedure is routinely performed; its indications for some pre-malignant diseases, or even benign ones, have become accepted; the length of stay has become smaller; and, above all, the mortality rate has become less than 3%<sup>3</sup>.

This study presents a series of consecutive cases of pancreatic resections with no mortality. We discuss aspects related to preoperative preparation, surgical technique and postoperative care.

### METHODS

This study was approved by the Ethics in Research Committee of the hospitals where surgical procedures were performed: Hospital Universitário Onofre Lopes, Liga Norte Riograndense Contra o Câncer and Casa de Saúde São Lucas, Natal, RN, Brasil; the number in Brazil Platform was 04198212.6.0000.5292.

We prospectively analyzed 50 consecutive patients undergoing pancreatic resections, from June 2010 to November 2013, for the treatment of peri-ampullary or pancreatic diseases.

Preoperatively, patients over 60 years of age or with cardiovascular risk factors regardless of age underwent cardiac tests to identify coronary artery disease and heart failure. Nutritional assessment was performed in all patients. For severe malnutrition, we indicated preoperative enteral nutritional support for a period not less than 15 days. We did not indicate endoscopic prosthesis preoperatively in patients with obstruction of the bile duct unless we foresaw a prolonged preoperative preparation or in the presence of cholangitis.

The technical steps of the two main types of operation carried out are described in a previous

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publication<sup>4</sup>. While in the first half of the series to a termino-terminal invaginating pancreato-jejunal anastomosis had been preferred, from the 25th case on we chose the termino-lateral invaginating pancreato-jejunal anastomosis in a single plan with separate stitches (5.0 prolene), for cases of pancreas with softened texture and/or main pancreatic duct with normal or decreased caliber. In operations of Kausch-Whipple (gastroduodenopancreatectomy without preservation of the pylorus) and central pancreatectomies, we used two silicone laminar drains externalized one on each side. In body and tail pacreatectomies, we used a silicon single laminar drain exteriorized in the left flank. A routine Witzel's jejunostomy was made independently of the type of procedure.

In the immediate postoperative period until the first 48 hours of operation, we used liberal fluid replacement, consisting of intravenous infusion of crystalloids, preferably Ringer Lactate solution (60ml/kg/day) combined with 30g/day of human albumin. A basic monitoring through continuous and serial measurement of central venous pressure, urine output, blood gases and serum lactate, served to guide hydration adjustments.

For prophylaxis of pancreatic fistula, in most patients we used octreotide subcutaneously, depending on the hospital availability, at a dose of 0.3mg/day, in eight-hour intervals for seven days. We daily recorded the drains debt and held dosages of in the fluid in the first, third, fifth and seventh days after surgery. In the last 15 cases, we also measured amylase of the fluid drains on the ninth day after surgery.

In most cases, on the seventh day after surgery, we carried out a control ultrasound or CT study; we then removed the drains on the eighth day after surgery for cases with low amylase on the fluid drains (less than three times the upper limit of normal serum amylase) and imaging exam without abdominal collections. In the last 15 patients we delayed in two days the imaging exam and drains removal. In the presence of pancreatic fistula, the patient was kept in zero oral intake, with enteral nutrition introduced via jejunostomy and the subcutaneous octreotide was maintained. In the presence of abdominal abscesses, we instituted antibiotic therapy and proceeded to percutaneous drainage of the collection. In case of persistent septic focus, we carried out a reoperation for drainage of the collections. In the absence of abdominal collections and high level of drain fluid amylase in the ninth day after surgery, the patient was discharged.

For the diagnosis of pancreatic fistula, we used the criteria of the International Study Group on the Definition of Pancreatic Fistula (GIEDFP)<sup>5</sup>, and for the diagnosis of bleeding and delayed postoperative gastric emptying, the criteria of the International Study Group of Pancreatic surgery (GIECP)<sup>6,7</sup>.

We defined in-hospital mortality as death occurred within the first 90 days postoperatively.

## RESULTS

Fifty patients consecutively underwent some type of pancreatic resection. Adenocarcinoma of the duodenal papilla was the most frequent disease (Table 1). The ages ranged between 16 and 90 years (mean=53.5) and the majority were male (42%). Anemia (Hb<12g/dl) and jaundice were part of the initial clinical presentation, respectively, in 38% and 40% of patients.

Two patients were preoperatively diagnosed with significant coronary disease. In one operation was postponed after implantation of coronary prosthesis and on the other, pancreatic surgery was contraindicated, since there was triple vessel coronary artery disease, with CABG indication. The recognition of severe malnutrition after nutritional assessment also delayed surgical treatment in at least seven patients in the series.

The Kausch-Whipple procedure was the most common (Table 2). There was need for red blood cell transfusion in 19 patients (39.5%).

In six patients (12%) there was need for resection if a mesenteric-portal axis segment. Mean operative time was 445.1 minutes (165-720).

When excluding grade A pancreatic fistulas, 22 patients (44%) had no clinical complications and had an average length of stay of 10.3 days. The remaining displayed one or more complications (Table 3).

Six patients had late pancreatic fistula (>8 days). In four of these patients, the drain was still present and the diagnosis was made by low amylase values until the seventh day after surgery and their increase after the ninth day. In the other two cases, the drains had been removed and the patients had infected abdominal collection with high amylase values. In one such case the collection was punctured, guided by CT, and in the other case where there was no window for percutaneous drainage, a chest

**Table 1 -** Distribution of diseases.

Disease	N
Adenocarcinoma of the duodenal papilla	19
Adenocarcinoma of the pancreas	12
Frantz tumor	6
Serous cystadenoma	3
Focal chronic pancreatitis	3
Neuroendocrine tumor	2
Cholangiocarcinoma	1
Adenocarcinoma of the duodenum	1
Adenoma of the duodenum	1
Mucinous cystadenoma	1
Insulinoma	1
TOTAL	50

Source: Medical records of patients undergoing pancreatic resections (June / 2010-November / 2013).

**Table 2 -** Distribution of types of pancreatic resections.

Procedures	Incidence (%)
Kausch-Whipple	35 (70%)
Body-tail pancreatectomy with splenectomy	5 (10%)
Central pancreatectomy	4 (8%)
Body-tail pancreatectomy with laparoscopic splenectomy	3 (6%)
Enucleation	2 (4%)
Uncinectomy	1 (2%)
Total	50 (100%)

Source: Medical records of patients undergoing pancreatic resections (June / 2010-November / 2013).

tube was inserted by the previous surgery drain orifice (Figure 1). These were the only two cases of readmission due to complications related to the surgical procedure (4%).

## DISCUSSION

In Brazil, few publications are devoted to the overall results of pancreatectomy performed for various diseases in the same service or the same group of surgeons.

In general, mortality rates found in national publications by the year 2012 ranged between 4.3% and 21.9%<sup>8-14</sup>, being superior to those described in the international literature<sup>3</sup>.

**Table 3 -** Distribution of identified postoperative complications.

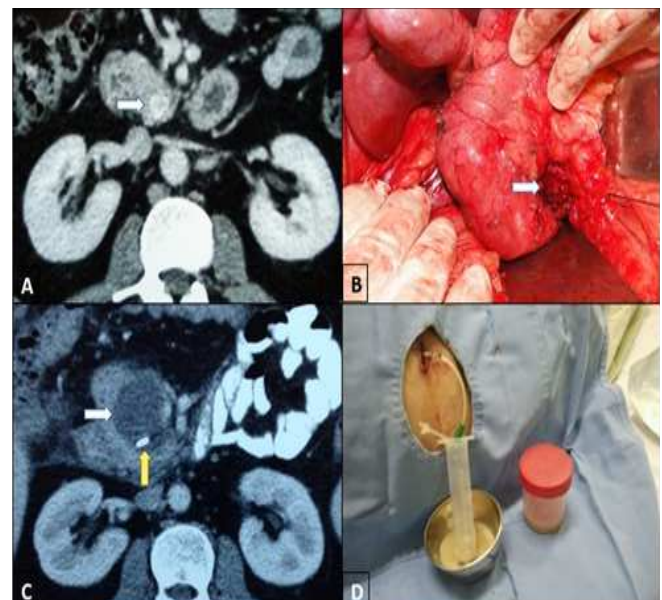
Complications	Incidence (%)
Pancreatic fistula	28 (56%)
Grade A	11 (22%)
Grade B	13 (26%)
Grade C	4 (8%)
Delayed Gastric Emptying (Just after the Kausch-Whipple operation)	6 (17.1%)
Grade A	2 (5.7%)
Grade B	3 (8.6%)
Grade C	1 (2.8%)
Bleeding	8 (16%)
Grade A	3 (6%)
Grade B	1 (2%)
Grade C	4 (8%)
Intra-abdominal collection	5 (10%)
Biliary fistula	3 (6%)
Renal insufficiency	2 (4%)
Wound infection	1 (2%)

Source: Medical records of patients undergoing pancreatic resections (June / 2010-November / 2013).

Note: When excluding the Grade A pancreatic fistulae, twenty eight patients (56%) developed one or more concurrent complications presented in this table.

Two recent national studies should be highlighted due to their excellent results.

In the first, Machado *et al.* reported a significant number of patients undergoing laparoscopic pancreatectomy, with zero mortality<sup>15</sup>. These authors described their personal experience in 11 years with the most diverse types of pancreatic resection, ranging from enucleation to pancreaticoduodenectomy. Most procedures (91.6%) were performed by completely laparoscopic technique, the conversion rate was only 3.1%, and the rate of pancreatic fistula, 28.1%<sup>15</sup>. Although one can notice a progressive increase in the complexity of procedures over time, most cases were operated on due to benign disease and subjected to resection of body and tail of pancreas, reflecting a



**Figure 1 -** A) Computerized tomography showing vascular lesion in the pancreas uncinata process. B) Intraoperative aspect: enucleation of insulinoma. C) postoperative collection of surgical site and surgical clip. D) Abscess drainage: chest tube inserted through the drain orifice.

selection of less complex cases justified for the application of the laparoscopic approach.

The other study<sup>16</sup>, although including only patients undergoing pancreaticoduodenectomy, put greater resemblance to the current series. In it, Fontes *et al.* performed a retrospective analysis of 97 patients over 12 years in the Hepato-bilio-pancreatic Surgery Service of the University of Medical Sciences of Porto Alegre<sup>16</sup>. In this study, the average hospital stay was 15 days, the fistula rate of pancreatic clinic, 10.3%, and the mortality rate, 2.1%<sup>16</sup>.

The number of cases presented in this study consisted essentially of unselected patients from the point of view of their disease, seen mostly in a tertiary public service reference for complex diseases of the pancreas. At least 1/3 of the patients found itself weakened, with jaundice and anemia. Malignancy was the main indication for operation in 41 patients (82%). The mostly performed procedure was the Kausch-Whipple surgery (70% of cases), and in 12% of cases there was the need to resect any portion of the mesenteric-portal axis. Although this series is small and we recognize that the death rate alone does not necessarily reflect overall treatment quality, especially in patients undergoing cancer surgery, our immediate results may be comparable to those of large referral services in pancreatic surgery<sup>2,17</sup>.

Luft *et al.*, in 1979, were the first to relate surgical procedures of high complexity and hospital volume with lower mortality rates<sup>18</sup>. In an important publication in the *New England Journal of Medicine*, in 2002, Birkmeyer *et al.* analyzed the results of 2.5 million patients undergoing cardiac procedures and cancer surgery between 1994 and 1999, and observed mortality rates after pancreatectomy in patients operated on at low volume hospitals compared with those treated in high volume ones, respectively, of 16.2% and 3.8%<sup>19</sup>. The next year, the same authors assessed the individual volume of each surgeon and found a mortality rate three times higher for surgeons who had performed less than two pancreatectomies a year<sup>20</sup>.

Even when different surgeons operate in the same service, one can find different results. Tseng *et al.* reported a "learning curve" for pancreatectomy from a sample with surgeons at MD Anderson Cancer Center<sup>21</sup>. The results relating to blood loss, operative time and length of stay were better for surgeons who had performed more than 60 procedures<sup>21</sup>. From these publications, regionalization to highly complex surgery has been recommended, although with conflicting results in the United States and Europe<sup>22</sup>.

Our experience with pancreatic resection began in 2002 and we have held 84 surgeries so far. In the first 34 cases, mortality was 11.7%, compared with zero mortality rate obtained in the last 50 cases. This allows us to recognize that we lived a real terms "learning curve". Based on this experience, we updated our routines protocol, which is now applied in all hospitals where we perform the procedure, which includes a thorough preoperative evaluation, with

particular emphasis on improvement of preoperative nutritional status, a standardized operative tactical technique and with little blood loss, and strict postoperative control, recognizing and treating early complications when present, especially pancreatic fistulas.

Recently there has been much emphasis in the literature the concern in establishing strategies to reduce the stress of surgical procedures and maintain body homeostasis. Widely described for colectomy, the fast-track programs have also been applied for pancreatic surgery. Thus, Berberat *et al.*<sup>8</sup>, Balzano *et al.*<sup>23</sup> and di Sebastiano *et al.*<sup>24</sup> found an average hospital stay of 10, 13 and 10 days, respectively, in series of patients undergoing pancreatectomy. Postoperative fluid restriction, early oral intake and mobilization, effective analgesia and early removal of drains and catheters form, in general, the main recommendations suggested by these and other authors<sup>8,23-25</sup>. While we agree with most of these measures, we do not practice fluid restriction, neither the early removal of abdominal drains.

The perioperative excess in fluids administration is implicated in the increased rate of complications and delay in return of intestinal transit in colorectal surgery<sup>26</sup>. For pancreatic surgery, however, there is insufficient data. In a recent publication, Grant *et al.* retrospectively studied 1,030 patients undergoing pancreatic resection and were unable to find any correlation between the amount of administered liquid and postoperative complications<sup>27</sup>. We do not deem reasonable to compare the third space volume formed in the operated area after pancreatic resection with the ones formed after other digestive procedures. The dissection area of the Kausch-Whipple operation is larger, and so is the third space, the restrictive fluid infusion being unable to maintain an adequate effective blood volume. We clearly recognized this fact in the first part of our series, when we had a more restrictive policy for the administration of crystalloid solutions in pancreatectomy intra- and postoperative periods, particularly in cephalic pancreatic resections. In such circumstances, oliguria was common, and we even had two cases of postoperative acute renal failure. From these cases, we initiated a liberal policy for volume replacement with crystalloid and colloid solutions. Postoperative acute renal failure requiring dialysis seems more deleterious than fluid overload that may subsequently develop due to more liberal fluid infusion in patients with previously appropriate renal or cardiac function.

Early removal of abdominal drains and even non-use in pancreatic resection surgery has been widely recommended<sup>28-30</sup>. Contrary to this trend, a recent multicenter study at nine US academic centers with a high volume of surgeries in which they randomized the use or not of abdominal drain after pancreaticoduodenectomy concluded for the lack of safety in the abolition of the drain after the procedure<sup>31</sup>. The mortality rate was four times higher (12% vs. 3%) in cases where the drain was not used, which led to discontinuation of the study<sup>31</sup>. We agree

with the practice of routinely using the abdominal drain in pancreatectomies. Our current policy is the routine use of two abdominal drains (in cephalic resections) and not remove them before the ninth day after surgery, at which time we make the last dosage of amylase and imaging exam. This approach is justified for the following reasons: 1) late pancreatic fistulas are not uncommon; 2) professional that percutaneously drain abdominal collections are not available at any time, especially in public hospitals; 3) once the collection is diagnosed, there is not always a window for percutaneous drainage and further surgery may be required; and 4) the presence of the drain does not prevent discharge, especially in pancreatic resections of body and tail of the pancreas.

Although the early removal of drains may be related to a reduction in length of stay, particularly in cephalic resections, we are convinced that the most important factor is, in fact, the development or absence of complications, particularly pancreatic fistula. This can be seen by the average length of stay of 10.3 days obtained with our patients without any clinical complications. The cost of maintaining the patient hospitalized for two days is not high because the patient without complication on the eighth day after surgery is already feeding, ambulating without intravenous access and with appropriate, oral drug pain control. Conversely, patients who develop pancreatic

fistula have longer hospital stay depending on the fistula characteristics. For this group we prefer that the drain placed during surgery is still in place, which guarantees, in most patients, the proper treatment of the fistula, combined with enteral nutrition by the jejunostomy.

For the accounting of our clinical complication rate, we excluded Grade A pancreatic fistulae, essentially because it is a laboratory diagnosis without any implication on the patient's clinical, which can be observed in our patients<sup>32</sup>. Although we measured the amylase value on the third day after surgery for the diagnosis of fistula in accordance with the GIEDFP criteria<sup>5</sup>, we consider of much greater practical importance the amylase measurements of the seventh and ninth postoperative days. Although not the subject of this study, apparently amylase levels increased in this period were much more correlated with the clinical development of fistula and prolonged hospitalization.

Despite major advances over the past three decades, pancreatic resection is still considered a challenge, especially outside the major specialized centers. Nevertheless, a low or nil mortality rate is possible. With practice, one accumulates knowledge about the disease and surgical treatment, allowing patients to be better prepared, the technical steps of the surgery to become standardized and easier, and postoperative complications to be more clearly recognized and properly treated.

## R E S U M O

**Objetivo:** apresentar uma série de casos consecutivos de ressecções pancreáticas com mortalidade nula, discutindo os aspectos relacionados ao preparo pré-operatório, técnica cirúrgica e cuidados pós-operatórios. **Métodos:** foram analisados prospectivamente 50 pacientes consecutivos submetidos à ressecções pancreáticas para o tratamento de doenças pancreáticas ou periampulares. As principais complicações locais foram definidas segundo critérios internacionais. A mortalidade intra-hospitalar foi considerada quando o óbito ocorreu nos primeiros 90 dias do pós-operatório. **Resultados:** a faixa etária variou entre 16 e 90 anos (média: 53,3 anos). Anemia (Hb<12g/dl) e icterícia pré-operatória estavam presentes, respectivamente, em 38% e 40% dos casos. A maior parte dos pacientes era portadora de tumor periampular (66%). O procedimento cirúrgico mais realizado foi a operação de Kausch-Whipple (70%). Em seis pacientes (12%) houve necessidade de ressecção de segmento do eixo mesentericoportal. O tempo cirúrgico médio foi 445,1 minutos. Vinte e dois pacientes (44%) evoluíram sem nenhuma complicação clínica e tiveram tempo médio de internação de 10,3 dias. As complicações mais frequentes foram: fistula pancreática (56%), retardo do esvaziamento gástrico (17,1%) e sangramento (16%). **Conclusão:** nas últimas três décadas a ressecção pancreática ainda é considerada um desafio, principalmente fora dos grandes centros especializados. Apesar disso, com uma equipe habituada com esse procedimento, um baixo índice de mortalidade é possível.

**Descritores:** Pâncreas. Procedimentos Cirúrgicos Operatórios. Pancreatectomia. Pancreaticoduodenectomia. Mortalidade.

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# Unintentionally retained foreign bodies after surgical procedures. Analysis of 4547 cases

## *Retenção inadvertida de corpos estranhos após intervenções cirúrgicas. Análise de 4547 casos*

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### A B S T R A C T

**Objective:** this study aims to explore the experience of Brazilian surgeons on Unintentionally Retained Foreign Bodies (RFB) after surgical procedures. **Methods:** A questionnaire was sent to surgeons by electronic mail, between March and July 2012. The questions analyzed their experience with foreign bodies (FB), foreign bodies' types, clinical manifestations, diagnoses, risk factors and legal implications. **Results:** in the 2872 eligible questionnaires, 43% of the surgeons asserted that they had already left FB and 73% had removed FB in one or more occasions, totalizing 4547. Of these foreign bodies, 90% were textiles, 78% were discovered in the first year and 14% remained asymptomatic. Among doctors with less than five years after graduation, 36% had already left a FB. The most frequently surgical procedures mentioned were the elective (57%) and routine (85%) ones. Emergency (26%), lack of counting (25%) and inadequate conditions of work contributed (12.5%) to the occurrence. In 46% of the cases patients were alerted about the FB, and 26% of them sued the doctors or the institution. **Conclusions:** challenging medical situations, omission of security protocols and inadequate work conditions contributed to RFB. However, RFB occurs mostly in routine procedures such as cesarean or cholecystectomy, and at the beginning of the professional career, highlighting, particularly in poorest countries, the need for primary prevention. Textiles predominated causing clinical repercussions and they were diagnosed in the first postoperative months. Surgeons were sued in 11.3% of the RFB cases.

**Key words:** Foreign Bodies. Postoperative Complications. Surgical Instruments.

### INTRODUCTION

Unintentionally Retained Foreign Bodies (RFB) causes 70% of reinterventions<sup>1</sup>, reaching 80% morbidity and 35% mortality<sup>2,3</sup>, in addition to significant medical and legal costs<sup>4,5</sup>. However, RFB still represents a problem without solution<sup>6</sup>.

The sporadic nature of this event (1:1000 to 1:2000)<sup>7-9</sup>, as well as the stigma of medical malpractice that follows it, still hinder its report and understanding, which is based on case-control studies and limited series. In order to make progress in understanding their occurrence, covering a greater number of cases, this study will examine the experience of surgeons with RFB, assessing some of their characteristics and consequences.

### METHODS

The project was approved by the Ethics in Research Committee of the University de São Paulo Medical School under number 493/11. It is a cross-sectional,

observational study carried out by means of a survey developed with the help of the Jotform tool ([www.jotform.com](http://www.jotform.com)) and sent over the Internet to the members of Brazilian societies related to the specialties of Urology, Gynecology, Obstetrics, General, Oncologic and Thoracic Surgery, Coloproctology, and Surgery of the Digestive Tract and Trauma. The responses were voluntary, confidential and anonymous.

In March 2012 those societies began to send e-mails to their members. The forms could be completed for a period of up to three months. Since there were overlapping email addresses between those doctors who participate in more than one society, the site blocked answers coming from the same e-mail and/or Internet Protocol (IP) number, in order to avoid duplicated answers.

Doctors without a specialist title, completed residency or without electronic addresses were excluded from the project. Members with specialist titles in other areas, such as mastology, vascular surgery, head and neck surgery and plastic surgery, were also excluded.

The questionnaire was divided into four parts. The first concerned the information about the doctor's

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experience, such as graduation time and if he/she had already removed, or unintentionally left, some FB. In the second part, in those cases in which RFB had occurred, we verified the information directly related to the FB, as type of surgical instrument, retention time and clinical manifestations. The third part assessed the triggering factors of RFB and analyzed the surgeries that had a higher prevalence. The last part focused on how doctors and patients dealt with ethical issues of RFB and if there were legal implications.

None of the answers was mandatory, although some questions depended on the previous one's affirmative answer. Thus, the percentage of each item was calculated based on the number of responses for each question, which were not always coincident.

## RESULTS

We received 2,885 answers. Thirteen forms were discarded since they were incomplete or duplicate, leaving 2,872 questionnaires for analysis.

The respondents comprised 1,021 general surgeons (36%), 1,613 gynecologists and obstetricians (56%) and 238 urologists (8%). Concerning graduation time, 20% of them had less than 10 years; 27% from 11 to 20 years; 28% from 21 to 30 years and 25% more than 30 years.

When asked if they had already removed a FB left by another colleague, 46% of the surgeons graduated for less than five years answered positively, as well as 69% of those graduated up to 10 years, 74% up to 30 years and 78% more than 30 years. On average, 73% of the participants had already removed some FB.

Among those surgeons with less than five years of practice, 36% had already left some FB. This index

amounted to 40% in the group with 11 to 20 years experience and reached 51% in the group with more than 30 years. On average, 43% had left some FB and, of these, 36% more than once.

Of the 4,547 foreign bodies reported, textiles accounted for 90% (Table 1). The retention was diagnosed in the first two months after the procedure in 42% of the cases, and within the 10 consecutive months in 36 % of them, totalizing 78% during the first year. The remaining 14% were diagnosed between one and five years, and 8%, after this period.

Regarding the clinical picture, 14% of the patients were asymptomatic, 61% reported mild symptoms such as unspecific abdominal discomfort or palpable mass, and 25% developed severe manifestations such as peritonitis, fistula or intestinal obstruction.

When we correlated the time for FB diagnosis to the clinical picture, we found that 96% of patients had some symptom two to six months after surgery, whereas 23% were asymptomatic in the period of more than five years after the procedure. Severe manifestations appeared in the first two months in 20% of the patients, decreasing to 11% up to sixty months, and to 23% after this period.

The relation of the clinical picture and the type of FB is shown in table 2. When we analyzed the severe manifestations in relation to the type textiles, we found 6.3% for gauze, 12.2% for small sponge and 29.9% for large sponge.

The majority of RFB occurred in open surgical procedures (94%) and elective surgeries (54%), which the surgeons classified as usual (85%) but complex (57%). Among the operations in which the surgeons left any FB, we identified 115 types of procedures, showing the seven most frequent in table 3.

**Table 1 -** Distribution of Foreign Bodies according to type.

Type of Retained Foreign Body	Percentage (%)
Large Sponge	42.01
Small Sponge	26.11
Gauze	22.10
Surgical Instrument	5.21
Needle	2.84
Others	1.74

**Table 2 -** Clinical manifestations according to Foreign Body type.

Type of Clinical Manifestation	Foreign Body		
	Textiles	Surgical Instrument	Needle
Asymptomatic	12%	67%	10%
Oligosymptomatic	71%	33%	90%
Severe manifestations	17%	-	-



When asked about which single factor has contributed more to RFB, respondents most frequently pointed emergency situations, not counting the sponges and inadequate work conditions. They highlighted difficulties related to incomplete and unprepared surgical teams, excess work, inappropriate operating rooms, and unprepared and undersized nursing staff table 4.

In the group of surgeons who had already left FB, 54% of their patients were not informed about the incident. Among those who were aware of the fact, 26% sued the doctor and/or the Institution. In the group of surgeons who had not left FB, only 26% would inform the patient if there had been a RFB.

## DISCUSSION

The incidence of unintentionally Retained Foreign Bodies (RFB) is underestimated<sup>10,11</sup>. The explanation for those occurrences are, in addition to difficulties in making the diagnosis<sup>11,12</sup>, potential legal repercussions<sup>1,5</sup> and difficulty in reporting the malpractice and dealing with its consequences<sup>13,14</sup>. This study decided to explore the issue from the point of view of the surgeons who have already left and/or removed FB. Therefore, we needed a project that would reduce the discomfort caused by the matter and that would reach a large number of doctors of the main specialties involved on it, preserving their anonymity.

**Table 3 -** Distribution of RFB reports among the procedures. Seven most frequent types.

Procedure	Percentage
Cesarean	17.96%
Abdominal Hysterectomy	16.33%
Exploratory Laparotomy for Acute Abdomen	13.54%
Exploratory Laparotomy for Trauma	7.26%
Cholecystectomy via subcostal incision	6.62%
Colectomy	4.12%
Appendectomy	3.60%

**Table 4 -** Factors that contributed to RFB.

Factors	Percentage (%)
Urgency / Emergency	26
Not counting the sponges	25
Inadequate work conditions	12
Patient's Obesity	11
Unexpected change of plans or surgical accident during surgery	11
Fatigue	9
Change of medical team during surgery	5
Teams of different specialties acting simultaneously	2

As, according to Scriven *et al.*, the internet provides distance and makes it easier to answer to delicate issues or to less socially desirable ones<sup>14</sup>, we asked to medical Brazilian societies to send the questionnaire to their members by e mail. In this way, were excluded surgeons who were in the basic years of residence or with an insufficient technical preparation.

As it was a voluntary research, we obtained a convenience sample. Some questions, such as the percentage of surgeons that have already left some FB or been sued, shall be analyzed taking this limitation into consideration. Nevertheless, the method allowed us to examine the experience of 2,872 professionals with 4,547 cases of RFB, representing approximately 7% of all general surgeons, urologists, gynecologists and obstetricians registered by the Federal Council of Medicine in Brazil (most of these doctors are not associated to the participating medical societies)<sup>15</sup>. We believe that it is an expressive number since Wan *et al.*, in the major review of cases published since 1963, collected 254 cases of retained sponges<sup>16</sup>.

When analyzing when RFB could have happened, we found that the peak incidence was at the beginning of the surgeons' career. Thereafter, the number slowly increased until it reached half of the interviewed among those with more than thirty years of practice. These data suggest that RFB happens more often than we think, and that doctors in training should receive more attention, so that they could learn techniques to prevent their own failures before they happen.

Of the 4,547 FB reported, 90% of them were textiles and, among these, large sponges were the most common. We had only 129 cases of needles, surpassed even by 237 surgical clamps, seldom mentioned in the medical literature. The most widespread RFB preventive measure is the instrumental counting, which, in many surgical centers, is not standardized or uses only textiles counting<sup>17-19</sup>. Even in the places that follow all recommendations of the Association of Perioperative Registered Nurses (AORN), needles represent the most discrepancies in counting<sup>20</sup>. So, considering these data, we asked ourselves why were the textiles, not the needles or clamps, the most retained items<sup>21</sup>.

These data support the explanation for the majority of RFB cases, that is, only FB intentionally inserted, released inside the cavity and then forgotten by the surgical team would be retained in surgeries. Since doctors, even temporarily, must not release a scalpel, anatomical tweezers or a needle and suture thread in the cavity, those instruments are hardly retained.

Taking into account the levels of diseases prevention that Leavall *et al.* popularized in 1965<sup>22</sup>, the same emphasis given to techniques for detecting something placed in the cavity should be given to disseminate methods to not release anything in the cavity. For example, in order to prevent a myocardial infarction, primary measures as avoiding sedentary lifestyle and overweight are more important than secondary measures, such as performing a periodic coronary tomography. In the same way, it might be more important to use sponge holding forceps than to count the sponges.

Other primary measures should also be used such as to always fix gauze to a clamp, or keeping the malleable valve extremity outside during the incision closure. The same concept can also be applied when, for example, a tired surgeon with an incomplete team chooses to postpone a complex surgery that is not urgent.

Regarding the time of FB detection, the peak incidence had usually occurred in the first two months, as was expected<sup>11,16,23</sup>. Those numbers can be explained by the fact that patients undergo more imaging examinations in the early post-operative period and by the greater tendency of the textiles to evolve to exudatives and symptomatic processes at this stage<sup>7</sup>. In late detection, the fibrotic reaction predominates, usually minimizing clinical manifestations<sup>24-26</sup>. Although 8% of the cases were detected after five years from the initial surgery, 23% of these patients developed serious complications, justifying the surgical removal though being asymptomatic<sup>24</sup>. On the other hand, the FB type should also be taken into consideration. After all, the rate of serious complications doubled according to the increase in the size of the textile, being 6% for gauze, 12% for small sponge and 24% for large sponge.

Brazilian surgeons highlighted the same risk factors emphasized in the studies of Gawande *et al.*<sup>1</sup>, Lincourt *et al.*<sup>10</sup> and Stawicki *et al.*<sup>6,27</sup>. Exploratory laparotomies usually include these risk factors, being urgent/emergency and complex surgeries performed in unstable patients, with unplanned changes in the surgical procedure, needing textiles to hemostasis and, often, performed by tired medical teams and in improper environments. For these reasons, it was not a surprise that exploratory laparotomies were in our list of surgeries with FB (20.8%). According to Cima *et al.*<sup>21</sup>, the majority of RFB occurred in routine and elective open surgeries. Although the 115 listed interventions may share these characteristics, there was a significant number of Cesarean sections, Hysterectomies and Cholecystectomies (40.91%). Perhaps, that may be explained by their high prevalence (350,000 Cesareans,

61,000 Cholecystectomies and almost 45,000 hysterectomies from October 2011 to March 2012) in Brazil<sup>28</sup>. However, the most important fact is that, in these interventions, sponges are routinely inserted in cavities to expose the operative field. Their removal depends on secondary prevention mechanisms, exposing their fallibility<sup>29-31</sup>. Eventually, in these cases, some RFB could be prevented by using sponge holding forceps. In addition to the previously mentioned factors, many interviewees reported structural and procedural failures, revealing worrisome workplaces frequented by many Brazilian and third world surgeons<sup>19</sup>.

Some important studies on RFB came from the registry of legal processes<sup>1,5</sup>. If this methodology were applied to that sample, we would be assessing only 11% of the cases and underestimating their occurrence. Furthermore, claiming to be an inherent risk in surgery, with possible legal and professional implications, 74% of surgeons stated that they would not tell the patient about the removal of a FB left by another colleague. Therefore, some paradigms need to be broken in order to better understand this phenomenon.

Unfortunately, despite all advances, the doctrines of "res ipsa loquitur" (the thing speaks for itself) and "captain-of-the-ship" (that blame mainly the surgeon), are still in force<sup>32</sup>. The focus on a forgotten FB would need to be changed to that on the safety in surgery. The RFB should be approached as a system failure and not as the product of negligence or incompetence of a specific professional<sup>33,34</sup>.

Despite not always having high technology, there are accessible and simple measures that must be disclosed and followed<sup>6,35-37</sup>. Among these, it is worth emphasizing the primary prevention and an appropriate work environment, so that professionals act in a dignified and safer manner. Although some of these results have not been based on a statistical risk analysis, they suggest some reflections.

In conclusion, the challenging medical situations, the omission of security protocols and inadequate working conditions contributed to RFB. However, inadvertent retentions occurred mainly in routine operations such as Caesarean sections and cholecystectomy, especially early in the medical career, highlighting, especially in poorer countries, the need for primary prevention. Textiles predominated, leading to clinical manifestations and being diagnosed in the first months after the postoperative. Doctors were sued in 11.3% of RFB cases.

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## RESUMO

**Objetivo:** avaliar a experiência de cirurgiões brasileiros com a retenção inadvertida de corpos estranhos (RICE) após procedimentos cirúrgicos. **Métodos:** foi enviado por correio eletrônico um questionário para cirurgiões, de março a julho de 2012. As questões avaliavam a sua experiência com RICE, os tipos de corpos estranhos, suas manifestações clínicas, diagnósticos, fatores de risco e implicações jurídicas. **Resultados:** 2872 questionários foram analisados. Destes, 43% dos cirurgiões já teriam deixado algum corpo estranho (CE) e 73% removido um CE em uma ou mais ocasiões. De um total de 4547 CE, 90% eram têxteis, 78% foram descobertos dentro do primeiro ano, e 14% assintomáticos. No grupo dos médicos graduados há menos de cinco anos, 36% já havia deixado um CE. Os procedimentos operatórios mais relacionados eram eletivos (54%) e rotineiros (85%). Emergência (26%), ausência de contagem (25%) e condições inadequadas de trabalho também contribuíram com a ocorrência (12,5%). Em 46% dos casos os pacientes tomaram ciência da retenção e 26% deles processaram os médicos ou as instituições. **Conclusão:** situações médicas desafiadoras, omissão de protocolos de segurança e condições inadequadas de trabalho contribuíram com a RICE. Entretanto, as RICE ocorreram principalmente em operações de rotina, como cesarianas e colecistectomias, principalmente no início da carreira profissional, ressaltando, principalmente em países mais pobres, a necessidade de prevenção primária. Os têxteis predominaram, acarretando repercussões clínicas e sendo diagnosticados nos primeiros meses de pós-operatório. Os médicos foram processados em 11,3% dos casos de RICE.

**Descritores:** Corpos Estranhos. Complicações Pós-Operatórias. Instrumentos Cirúrgicos.

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# Use of raw *Euphorbia tirucalli* extract for inhibition of ascitic Ehrlich tumor

## *Avaliação do uso do extrato bruto de Euphorbia tirucalli na inibição do tumor ascítico de ehrlich*

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### A B S T R A C T

**Objective:** to evaluate the effect of the *Euphorbia tirucalli* hydroalcoholic extract (ETHE) on the development of Ehrlich Tumor, in its ascitic form. **Methods:** we intraperitoneally inoculated 15 Swiss mice with  $10.44 \times 10^7$  cells of Ehrlich Tumor and divided them in two groups one day after: ETHE Group (eight mice), treated with a dosage of 125 mg/kg/day of ETHE for five days; and Control Group (seven mice), treated only with 0.9% isotonic saline solution over the same period. The treatment was done by gavage. Ten days after inoculation, four mice from each group were sacrificed for quantification of tumor cell number, ascitic fluid volume and bone marrow cell number. The remaining animals were maintained to evaluate survival. **Results:** The ascitic fluid volume and the tumor cell number were decreased in the ETHE group when compared with the control group, but with no statistical significance. On the other hand, survival was higher in the ETHE group, as well as the number of bone marrow cells. **Conclusion:** Treatment with ETHE after inoculation of Ehrlich Tumor decreases its development and increases survival and the bone marrow cellularity, thus reducing the myelosuppression present in the Ehrlich Tumor bearing mice.

**Key words:** Physical Therapy Specialty. Plant Extracts. Euphorbiaceae. Carcinoma, Ehrlich Tumor. Mice.

### INTRODUCTION

*Euphorbia tirucalli* L. [Euphorbiaceae] is known in Brazil as "aveloz", and its latex has been used as an anti-helminthic, antisiphilitic and anti-tumoral agent by native people as traditional medicine<sup>1-4</sup>. Some biological properties of *E. tirucalli* have been confirmed such as larvicidal, molluscicide, bactericidal and anti-herpes ones<sup>5-9</sup>. These activities are likely related to the presence of phytosterols and triterpenes<sup>2</sup>.

Ehrlich Tumor is a rapidly growing carcinoma with very aggressive behavior, which induces myelosuppression in mice and strongly affects the inflammatory response. It was shown that the prostaglandin E2 (PGE2) levels, which is dramatically increased in tumour bearing mice, was also abrogated by the treatment with *E. tirucalli* extract<sup>10-12</sup>.

Based on the popular use and on results about the effect of *E. tirucalli* on inflammatory response, our aim was to examine the *in vivo* antitumor activity of oral *Euphorbia tirucalli*, using the Ehrlich tumor in the ascitic form.

### METHODS

The project was evaluated and approved by the Ethics in Research Committee at the Universidade Federal do Maranhão (UFMA), protocol nº 23115 008767/2008-40. The study followed the guidelines for animal research as set out by the Brazilian College of Animal Experimentation (COBEA).

The sample consisted of 15 male Swiss mice (*Swiss webster*, Rodentia Mammalia), supplied by the UFMA vivarium. They were between 60 and 100 days old (mean 72) and weighted between 20g and 25g (mean 22.3g). They were kept in groups of five per standard cage for the species at the Research Laboratory of Immunophysiology, where light-dark cycle of 12 hours and humidity of 44-56%, the same as a normal environment without any artificial regulation. The temperature was constant at  $26 \pm 2$  °C. The animals were fed on standard commercial food and had free access to water.

The barks of *E. tirucalli* were collected and identified at the UFMA Ático Seabra Herbarium (São Luís, MA, Brazil) (specimen's voucher N° 1373). The barks were

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mechanically triturated, added to 1L of ethanol (70%) and mixed each 8h for 72h. After this period the hydroalcoholic extract was filtered using a cotton funnel. After this process the extract was concentrated using a rotatory evaporator and filtered again, when we obtained 190ml of hydroalcoholic extract. Finally, the extract was dried and the dry residue obtained was diluted in distilled water in a concentration of 15mg/ml.

To treat the mice, the *E. tirucalli* extract was diluted again in water and given by gavage with a daily single doses (125mg/kg body weight) during five days. The treatment was initiated 24 hours after the Ehrlich tumor implantation. The control group was treated only with isotonic saline solution (0,2ml).

The Ehrlich ascitic tumor, derived from a spontaneous murine mammary adenocarcinoma, was maintained in the ascitic form by passages in Swiss mice, by weekly transplantation at the UFMA Immunophysiology Laboratory. The ascitic fluid was removed by opening the abdomen and carefully collecting all the fluid with the help of a sterile 3ml syringe. Ascitic tumor cell counts were done in a Neubauer hemocytometer. The cells were found to be more than 99% viable by the Trypan blue dye exclusion method and amounted  $5.22 \times 10^8$  cells/ml of ascitic fluid.

A volume of 0,2ml of Ehrlich tumor cells was injected intraperitoneally for the development of the ascitic form. The final amount of tumor cell suspension used in each application was  $10.44 \times 10^7$  viable cells.

After inoculation, the animals were randomly distributed into two groups: ETHE Group (EG), with eight mice, and control group (CG), with seven animals. The animals with ascitic tumor were weighted each three days.

On the day following inoculation, EG animals received daily 125mg/kg of the hydroalcoholic extract by gavage and the CG, the same volume of 0.9% isotonic saline solution, all for five consecutive days.

On the 10<sup>th</sup> day after inoculation, four mice from each group were killed by a lethal dose of 2% xylazine (20mg/kg, IM) and 5% ketamine hydrochloride (30mg/kg, IM) for cell counting from the femoral bone marrow, as well as dosage of ascitic fluid volume and cellularity. The remained mice (four in EG and three in CG) were maintained to survival evaluation.

Results were expressed as the mean  $\pm$  standard deviation from four animals per group. Statistical evaluation was done by ANOVA test. The survival of mice was demonstrated using the Kaplan-Meier curve and the log-rank statistical test was applied to compare the curves. Differences were considered significant at  $p < 0.05$  and are represented by an asterisk. All experiments were repeated at least two times.

## RESULTS

The total ascitic volume and total tumor cell number were lower in mice treated with *E. tirucalli* (125mg/

kg), though with no statistical significance (Figure 1 A e B). The *E. tirucalli* treatments also significantly increased the life expectancy (Figure 1C).

There was also a significant increase in the femoral bone marrow cell number in the EG (Figure 2).

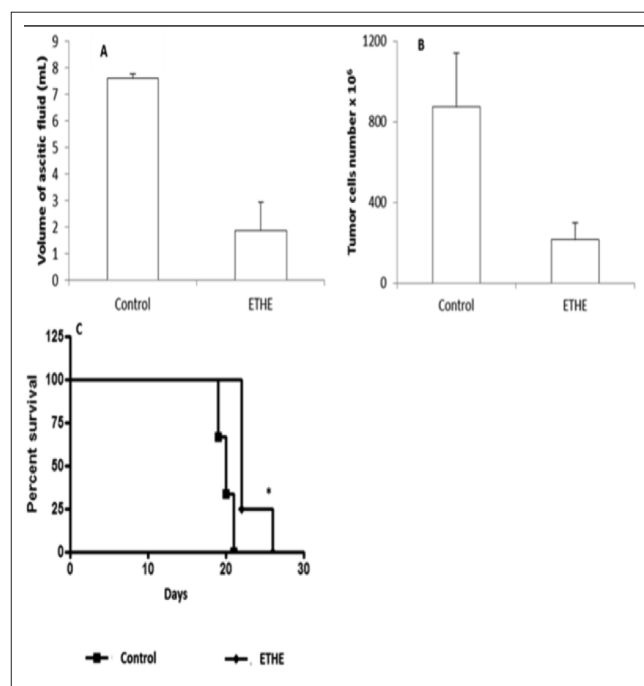
The average weights were significantly lower in EG (Figure 3).

## DISCUSSION

We observed that the ascitic fluid volume and the number of Ehrlich tumor cells in the peritoneum were lower in mice treated with *E. tirucalli*, but this difference was not statistically significant. Despite that, this decrease could mean the inhibition of ascitic growth in EG mice. This fact, combined with the significantly lower average weights in EG, suggests tumor growth slowing in animals treated with the extract.

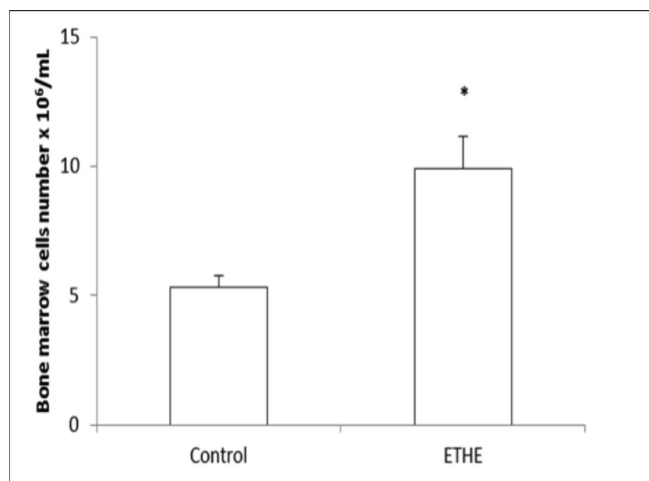
The exact mechanism by which *E. tirucalli* mediates its anti-tumor effect is unknown. However, some compounds present in *E. tirucalli*, such as terpenes, could explain these results<sup>13</sup>. These compounds have been mentioned as antioxidant and consequently can be involved in anti-tumor activities<sup>14</sup>. Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animals<sup>15-17</sup>.

The inflammatory response is, in fact, essential to modulate the development of tumors. It was



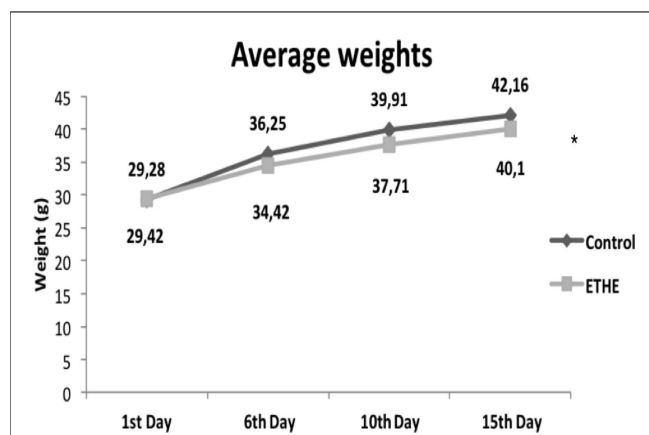
**Figure 1** - Effect of *E. tirucalli* on ascitic Ehrlich tumor on ascitic fluid volume(A), number of tumor in ascitic fluid (B) and mice survival (C).

\* $p < 0.05$  in comparison with the control group.



**Figure 2** - Effect of *E. tirucalli* on femoral bone marrow cell number in Ehrlich tumor bearing mice.

\* $p < 0.05$  when compared with the control group.



**Figure 3** - Evolution of the average weight of EG and CG mice on the 1<sup>st</sup>, 6<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> days of trial.

\* $p < 0.05$  when compared with the control group.

demonstrated that the neutrophilic inflammatory response is essential to the Ehrlich tumor's control. However, the high influx of these cells promotes tumor development<sup>18</sup>.

## R E S U M O

**Objetivo:** avaliar o efeito do extrato hidroalcoólico de *Euphorbia tirucalli* (ETHE) sobre o desenvolvimento do tumor de Ehrlich em sua forma ascítica. **Métodos:** quinze camundongos Swiss foram inoculados via intraperitoneal com  $10,44 \times 10^7$  células do tumor de Ehrlich e um dia depois foram divididos em dois grupos: Grupo ETHE (oito camundongos), tratados com a dose de 125mg/kg/dia de ETHE por cinco dias e Grupo Controle (sete camundongos), tratado apenas com 0,9% de solução salina isotônica em relação ao mesmo período. O tratamento foi realizado por gavagem. Dez dias após a inoculação, quatro animais de cada grupo foram sacrificados para a quantificação do número de células de tumor, do volume de fluido ascítico e do número de células da medula óssea. Os demais animais foram mantidos, para avaliar a sobrevivência. **Resultados:** o volume de líquido ascítico e do número de células tumorais foram menores no grupo ETHE quando comparado ao grupo controle, porém sem significância estatística. Por outro lado, a sobrevivência dos animais foi maior no grupo de ETHE, bem como, a quantidade de células de medula óssea. **Conclusão:** o tratamento com ETHE, após a inoculação do tumor, diminuiu o seu desenvolvimento e aumentou a sobrevivência, bem como, a celularidade da medula óssea, reduzindo assim, a mielossupressão presente nos animais portadores de tumor de Ehrlich.

**Descritores:** Fitoterapia. Extratos Vegetais. Euphorbiaceae. Carcinoma de Ehrlich. Camundongos.

This effect is probably related with the angiogenesis and growth factors induced by inflammation, which are necessary to tumor development. The implantation of the Ehrlich ascitic tumor itself is sufficient to induce a local inflammatory reaction, with increased vascular permeability, which results in intense edema formation, cellular migration and a progressive ascitic fluid formation<sup>19</sup>. The ascitic fluid is essential to the tumor growth, since it constitutes the direct nutritional source for the tumor cells<sup>20</sup>.

The Ehrlich tumor growth leads to inhibition of superoxide dismutase and catalase enzymes, which are fundamental in the elimination of free radicals such as superoxide and hydrogen peroxide<sup>21,22</sup>. In Ehrlich tumor-bearing mice the anti-oxidants act by a mechanism that involves modulating lipid peroxidation and augmenting the antioxidant defense system<sup>20</sup>.

We propose that the additive and synergistic antioxidant activity of phytochemicals such as terpenoids, present in *E. tirucalli*, are responsible for its potent antitumor activity, which can be inferred from the increased life span of tumor bearing mice and from the inhibition of ascitic growth.

As for the femoral bone marrow cellularity, there was a significant increase in the number of cells in the group treated with aveloz, a result that is consistent with what was exposed by Valadares *et al.*<sup>23</sup>. This author described the property of the extract of *Euphorbia tirucalli* to restore marrow myelopoiesis suppressed by Ehrlich tumor during its natural course, not finding, however, differences between the three doses (125, 250 and 500 mg/kg). Studies in mice inoculated with Ehrlich tumor showed a rapid decrease of granulocytic and macrophage colony forming units (CFU-GM) in the bone marrow, while observing a progressive increase in cell number and CFU-GM in the spleen, followed by splenomegaly<sup>24</sup>.

Regarding survival, we found a statistically significant increase in animals treated with aveloz. The same was reported by Valadares for all doses offered (125, 250, and 500 mg/kg/day for five days), suggesting a dose-dependent increase in survival (survival greater in animals treated with 500mg/kg of the extract)<sup>23</sup>.

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# Epidemiological evaluation of hepatic trauma victims undergoing surgery

## *Avaliação epidemiológica de vítimas de trauma hepático submetidas a tratamento cirúrgico*

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### A B S T R A C T

**Objective:** to evaluate the epidemiological variables and diagnostic and therapeutic modalities related to hepatic trauma patients undergoing laparotomy in a public referral hospital in the metropolitan region of Vitória-ES. **Methods:** we conducted a retrospective study, reviewing charts of trauma patients with liver injuries, whether isolated or in association with other organs, who underwent exploratory laparotomy, from January 2011 to December 2013. **Results:** We studied 392 patients, 107 of these with liver injury. The male: female ratio was 6.6 : 1 and the mean age was 30.12 years. Penetrating liver trauma occurred in 78.5% of patients, mostly with firearms. Associated injuries occurred in 86% of cases and intra-abdominal injuries were more common in penetrating trauma ( $p < 0.01$ ). The most commonly used operative technique was hepatorrhaphy and damage control surgery was applied in 6.5% of patients. The average amounts of blood products used were 6.07 units of packed red blood cells and 3.01 units of fresh frozen plasma. The incidence of postoperative complications was 29.9%, the most frequent being infectious, including pneumonia, peritonitis and intra-abdominal abscess. The survival rate of patients suffering from blunt trauma was 60%, and penetrating trauma, 87.5% ( $p < 0.05$ ). **Conclusion:** despite technological advances in diagnosis and treatment, mortality rates in liver trauma remain high, especially in patients suffering from blunt trauma in relation to penetrating one.

**Key words:** Liver. Abdominal Injuries. Wounds and Injuries. Firearms. Accidents, Traffic.

### INTRODUCTION

Trauma is a public health problem of great magnitude in Brazil. It is one of the leading causes of death today, due to the increase in urban violence and the technological advancement of the automotive industry, which enabled the production of increased power vehicles<sup>1,2</sup>.

Abdominal trauma can be classified into two distinct types: penetrating or blunt. Blunt trauma is usually due to accidents involving motor vehicles, falls, explosions and sports injuries. Penetrating trauma, on its turn, can be caused by stabbing or gunshot projectiles<sup>3</sup>.

The most commonly affected organs in the blunt abdominal trauma are the spleen (40 to 55%), liver (35 to 45%) and small intestine (5 to 10%). The injuries caused in penetrating abdominal trauma normally affect the liver (40%), small intestine (30%), the diaphragm (20%) and colon (15%). As for the injuries caused by firearm projectiles, they typically affect the small intestine (50%), colon (40%), liver (30%) and abdominal vessels (25%)<sup>1,4</sup>.

The high rates of liver injury are justified by its size and anatomical position<sup>5,6</sup>. The right lobe of the liver,

being the portion of the bulkier hepatic parenchyma, is the most affected region in abdominal injuries<sup>7</sup>.

This study aims to assess the epidemiological variables of, and the diagnostic and therapeutic modalities applied to, patients submitted to surgical treatment of liver trauma at a referral hospital for trauma.

### METHODS

We conducted a retrospective review of medical records of patients undergoing laparotomy in the period from January 2011 to December 2013, at the Hospital Estadual São Lucas, a referral center for trauma, located in the Metropolitan Region of Vitória-ES.

We included trauma patients with liver injury, isolated or in association with other intra or extra-abdominal organs. We excluded surgical re-approaches in patients operated on in other services and subsequently transferred to our hospital. The sample therefore comprised 107 patients undergoing surgery for hepatic injury during this period.

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The variables analyzed were: age, gender, date and time of admission, the first operation time, length of stay, mechanism of injury, the presence of associated intra or extra-abdominal lesions, surgical technique, the intra-operative need for blood products, need for peri-hepatic drainage, postoperative complications and mortality.

The collected data were tabulated in electronic spreadsheets and statistical analyzes were performed according to Levine *et al.*, at 2012<sup>8</sup>. We carried out a descriptive analysis of the categorical variables, expressed in absolute numbers and percentages, and the continuous ones as position measurements. To compare the trauma mechanisms in relation to the categorical variables, we used the chi-square test. We considered a p value <0.05 as statistically significant.

This study was approved by the Ethics in Research Committee of the Santa Casa de Misericórdia de Vitória – EMESCAM, on 30 of April 2014, under number 632.212.

## RESULTS

During the study period, from January 2011 to December 2013, 392 patients underwent laparotomy, 107 of them sustaining liver injury. Of these, 93 were male (86.9%) and 14 female (13.1%). The mean age of patients suffering liver trauma was 30.12 years, ranging from 14 to 72 (median 28), and 83.2% were in the first four decades of life.

As for the mechanism of injury, the most common was penetrating trauma, which occurred in 84 patients (78.5%). Of these, gunshot wounds (GW) accounted for 72 cases (85.7%) and stab wounds (SW), for 12 cases (14.3%). Blunt trauma occurred in 23 patients (21.5%), whose origins were 12 motorcycle accidents (52.2%), six car accidents (26.1%), two falls from a height (8.7%), running over in two (8.7%) and unidentified in one (4.3%).

Considering the anatomical site of liver injury, the right lobe was the most affected (46.73%), followed by the left one (25.23%) (Table 1). The caudate lobe was less affected, only in one case (0.93%). Simultaneous injury of right and left lobes or the right and caudate lobes occurred in 5.61% and 0.93% of patients, respectively.

We found associated intra (Table 2) and extra-abdominal (Table 3) lesions in 92 patients (86%). Associated Intra-abdominal lesions occurred in 67 patients (62.6%), the most damaged organs being the diaphragm, colon and stomach. Concomitant extra-abdominal lesions were found in 77 patients (72%), mostly in the chest.

As for the associated extra-abdominal lesions (Table 3), there was a higher incidence of head injury associated with blunt trauma (30% of cases) when compared with penetrating trauma (10.9% of cases).

The days of the week with more liver trauma victims were Sunday (25.2%), Wednesday (16.8%) and Saturday (15.9%) (Table 4). The days when with less victims

were Monday (14%), Friday (10.3%), Thursday (9.3%) and Tuesday (8.4%).

The time between hospital admission and the first surgery was less than two hours in 48.8%, between two and four hours in 17.9% patients and more than four hours in 33.3%. The average hospitalization time for patients with blunt trauma was 13.96 days, and for patients with penetrating trauma, 12.23 days.

Regarding the surgical technique used to control liver bleeding, the most used was hepatorrhaphy (80.37%) (Table 5). Cauterization of the liver injury has been reported in four patients (3.74%), being a single and sufficient measure to stop bleeding in two of these patients. It was also described the use of topical hemostatic agents in two cases (1.87%) and omentum transposition in only one case (0.93%). In 12 patients (11.21%) no measures of hemostasis were necessary, since the liver injury showed no active bleeding. Segmentectomy was performed in two patients (1.87%) and only one (0.93%) required left hepatectomy. Damage control surgery was performed in seven patients (6.54%). Eighteen patients (16.82%) needed further surgical intervention, mostly to withdraw textiles from the peritoneal cavity. Laparotomy was not therapeutic in four patients (3.74%).

**Table 1 -** Anatomical liver lobes affected in patients sustaining liver trauma.

Hepatic lobe	Frequency	%
Right	50	46.73
Left	27	25.23
Caudate	1	0.93
Right and left	6	5.61
Right and caudate	1	0.93
Not identified	22	20.56
Total	107	100.00

Source: Medical records of Hospital Estadual São Lucas (January 2011 to December 2013).

**Table 2 -** Associated intra-abdominal lesions in patients sustaining liver trauma.

Injured organ	Frequency	%
Diaphragm	30	20.27
Colon	26	17.57
Stomach	25	16.89
Kidney	17	11.49
Small intestine	17	11.49
Spleen	10	6.76
Extrahepatic biliary tree	9	6.08
Great vessels	8	5.4
Pancreas	6	4.05
TOTAL	148	100.00

Source: Medical records of Hospital Estadual São Lucas (January 2011 to December 2013).

**Table 3 -** Associated extra-abdominal lesions in patients sustaining liver trauma.

Injured region	Frequency	%
Chest	64	54.7
Limbs	37	31.62
Head	13	11.11
Neck	3	2.56
Total	117	100.00

Source: Medical records of Hospital Estadual São Lucas (January 2011 to December 2013).

**Table 4 -** Days of the week on which medical care was provided to patients sustaining liver trauma.

Weekday	Frequency	%
Sunday	27	25.2
Monday	15	14
Tuesday	9	8.4
Wednesday	18	16.8
Thursday	10	9.3
Friday	11	10.3
Saturday	17	15.9

Source: Medical records of Hospital Estadual São Lucas (January 2011 to December 2013).

Twenty-eight patients required blood products transfusion during surgery (26.2%) and the average amount of blood products used was 6.07 units of packed red blood cells and 3.01 fresh plasma units. Perihepatic drains were placed in 27 cases (25.2%), a Penrose drain being the most commonly used (n=15).

The incidence of postoperative complications was 29.9%, and the most frequent were infectious, including pneumonia, peritonitis and intra-abdominal abscess, which

represented 73.9% of complications. Hemobilia occurred in one patient, who was treated with hepatic artery embolization.

The survival rates were 60% in patients with blunt trauma, and in patients with penetrating trauma, 87.5% ( $p < 0.05$ ) (Table 6). The mortality rate was 17.8% (n=19), the most common causes of death being hemorrhagic shock, responsible for ten deaths (52.6%), and septic shock, amounting to four deaths (21%).

## DISCUSSION

Hepatic trauma occurred more frequently in males (86.9%) in the first four decades of life (83.2%). These results are similar to those reported by many researchers<sup>2,3,9,10</sup>. The highest incidence of trauma in young adult males is associated with increased risk behavior due to exposure to alcohol and illicit drugs<sup>10</sup>.

Due to the increase of traffic accidents and violence in Brazil, the proportion of trauma admissions has gradually increased and hence the proportion of government spending. Likewise, hospital costs are directly proportional to the length of hospital stay<sup>10</sup>. In this study, the average hospitalization time for patients with blunt trauma was 13.96 days, and for patients with penetrating trauma, 12.23 days, agreeing with the average found in the literature<sup>9</sup>.

Lima *et al.* studied the epidemiology of abdominal trauma undergoing laparotomy and, as well as in our work, observed that there was a higher prevalence of trauma on weekends<sup>10</sup>.

Mortality in hepatic trauma in most studies is close to 20%, considering all cases admitted to hospital<sup>9</sup>. The mortality rate in this study was 17.8%. Of the eleven deaths from penetrating trauma, ten were the result of gunshot wounds.

Other intra-abdominal injuries concomitant with liver lesions were more common in penetrating trauma ( $p < 0.01$ ), as shown in table 6. In penetrating abdo-

**Table 5 -** Surgical techniques applied in the treatment of patients sustaining liver trauma.

Technique*	Patients	%
Hepatorrhaphy	86	80.37
Segmentectomy	2	1.87
Left hepatectomy	1	0.93
Electrocauterization	4	3.74
Topical hemostatic agents	2	1.87
Epiploplasty	1	0.93
Damage Control	7	6.54
No action (liver damage with no active bleeding)	12	11.21
Nontherapeutic laparotomy	4	3.74

Source: Medical records of Hospital Estadual São Lucas (January 2011 to December 2013).

\* In some patients, more than one approach was performed

**Table 6 -** Associated intra-abdominal lesions and survival rate in blunt and penetrating trauma in patients sustaining liver trauma.

	Blunt trauma(n=20)	Penetrating trauma (n=64)
Associated intra-abdominal lesions*	6 (30%)	46 (71.9%)
Survival rate **	12 (60%)	56 (87.5%)

Source: Medical records of Hospital Estadual São Lucas (January 2011 to December 2013).

\* The result of the chi-square test indicated rejection of the null hypothesis at a significance level of <1% ( $p < 0.01$ ).

\*\* The result of the chi-square test indicated rejection of the null hypothesis at a significance level of <5% ( $p < 0.05$ ).

minal trauma, the injuries of large intra-abdominal vessels stand out<sup>11</sup>. In this study, all eight injuries of large intra-abdominal vessels were caused by penetrating trauma, seven of them by firearms. The vessels involved were: inferior vena cava, middle hepatic vein, splenic artery, right common iliac artery and vein, superior mesenteric vein and artery.

Hepatorrhaphy was the most used surgical technique to control liver bleeding. Other techniques include direct ligation of bleeding vessels, cauterization, the use of topical hemostatic agents, partial liver resection and hepatic artery ligation. In selected cases, refractory bleeding can be controlled with liver packing, the damage control surgery<sup>6</sup>.

The damage control surgery was performed in seven cases (6.54%) and patient survival was 100%, which confirms damage control as a measure which increases the survival rate of seriously injured patients who face the so-called triad of death – hypothermia, coagulopathy and metabolic acidosis<sup>6,11,12</sup>.

Research on the surgical treatment of hepatic injury began to grow in the early twentieth century. The maneuver of Pringle and liver packing technique were described in 1908, giving rise to the concept of damage control surgery<sup>11,13</sup>. Liver packing became a common practice a few years later, during the two Great World Wars. At that time, mortality related to hepatic trauma was 60%<sup>13,14</sup>.

After World War II, the mortality of liver trauma decreased due to increased experience in the repair of injuries. This led to the abandonment of surgery for damage control, which at the time was associated with a high incidence of late onset sepsis and rebleeding after packing removal. From the 70s on, surgery for damage control regained its importance in selected patients<sup>14</sup>.

Until the early 90s, surgical treatment was the standard treatment of liver injury<sup>15</sup>. Since then, the diagnostic and therapeutic approach to abdominal trauma victim suffered intense changes<sup>6</sup>. Initially, it was found that most liver injuries spontaneously stopped bleeding<sup>6,16</sup>. In 1908, Pringle had suggested that minor liver damage occasionally could heal without surgical intervention<sup>13</sup>. However, little had been published about non-operative treatment until the 80's and the surgeons were resistant to conservative treatment, especially in blunt trauma<sup>16,17</sup>.

This resistance resulted primarily from three factors: the belief that liver bleeding would not cease unless surgically controlled; the concern that the lack of bile drainage would result in biliary fistulas and infectious complications; and the concern about associated lesions in face of a positive peritoneal lavage<sup>17</sup>.

With technological advances in imaging and greater accessibility to computed tomography and FAST (Focused Assessment with Sonography for Trauma), conservative treatment has become possible for patients with hemodynamically stable hepatic trauma<sup>15,18</sup>, which contributed to reduce unnecessary laparotomy<sup>7,12,18-21</sup>. Thus, there is a tendency to avoid routine laparotomy, especially for patients with hepatic trauma who are hemodynamically stable and display signs of peritonitis<sup>19</sup>.

Besides the advantage of avoiding the morbidity of an unnecessary laparotomy<sup>15</sup>, conservative treatment has shown other advantages over the surgical one, such as lower complication rates, less need for blood products transfusions, shorter hospital stay, especially in Intensive Care Units, and lower mortality<sup>15,16,19,21</sup>.

FAST is an exam with high sensitivity for the diagnosis of hemoperitoneum in hemodynamically unstable patients, as well as to identify liver damage<sup>18,22</sup>. A major advantage of this test is that it can be done at the bedside, without the need to move the patient from the emergency room<sup>18</sup>.

On the other hand, when the patient is hemodynamically stable, triple contrast CT is the method of choice in patients with blunt abdominal trauma. Computed tomography allows to determine the extent of liver damage, document the presence of active bleeding and detect associated lesions<sup>22</sup>. It is very useful in defining the severity of liver injury and the decision of conservative treatment<sup>18</sup>.

The diagnostic peritoneal lavage (PL) is a useful test for the diagnosis of hemoperitoneum when the patient is hemodynamically unstable and has sensory changes, and when there's no ultrasonography and CT available in the institution. Therefore, the PL can be replaced by ultrasound and, in more stable patients, CT<sup>22</sup>.

According to Zago *et al.*, there has been a decrease in the incidence of penetrating trauma and increased incidence of blunt trauma in Brazil in recent years<sup>9</sup>.

However, this study found a higher prevalence of penetrating trauma (76.2%). This discrepancy can be explained by the fact that our study only examined the operated patients, and patients treated conservatively – most victims of blunt trauma – were not counted. It is noteworthy that the conservative treatment of hepatic trauma in patients with hemodynamic stability has become standard in most trauma centers<sup>16,22</sup>.

In this study, the right lobe of the liver was the most affected, which was also demonstrated by Talving *et al.*<sup>23</sup>. According to Romano *et al.*, the right lobe is the most affected because it is the most voluminous part of the hepatic parenchyma<sup>7</sup>. Associated lesions were found in 72 patients (85.7%). The high number of associated injuries is a major challenge for the management of patients because their presence hinders the decision of conservative treatment<sup>2,3</sup> and often determines the evolution of the patient<sup>12</sup>.

We observed a low incidence of postoperative complications (29.9%). This complication rate obtained is below the parameters found in some studies on the epidemiology of liver trauma, the rates varying from 36 to 38.9%<sup>9,24</sup>.

We found (Table 6) that the mortality rate for patients with hepatic injury was greater for blunt trauma (40%) than for penetrating one (12.5%), possibly due to the higher rates of cranial injuries associated with blunt trauma. Zago *et al.* found similar results, with a mortality rate of 26.5% for blunt trauma and 15.8% for the penetrating one<sup>9</sup>.

Despite technological advances in diagnosis and treatment, and the systematization of care for polytrauma proposed by the Advanced Trauma Life Support (ATLS®), morbidity and mortality rates in liver trauma remain high<sup>7,24,25</sup>. Therefore, liver trauma is a serious public health problem with significant social and economic costs, especially since it affects people of working age.

## R E S U M O

**Objetivo:** avaliar as variáveis epidemiológicas e as modalidades diagnósticas e terapêuticas relacionadas ao trauma hepático de pacientes submetidos à laparotomia exploradora em um hospital público de referência da Região Metropolitana de Vitória-ES.

**Métodos:** estudo retrospectivo de revisão de prontuários dos pacientes vítimas de trauma com lesão hepática isolada ou associada a outros órgãos, submetidos à laparotomia exploradora, no período de janeiro de 2011 a dezembro de 2013. **Resultados:** foram estudados 392 pacientes submetidos à laparotomia, dos quais 107 com lesões hepáticas. A relação masculino:feminino foi 6,6:1 e a média de idade dos pacientes foi 30,12 anos. O trauma hepático penetrante ocorreu em 78,5% dos pacientes, principalmente por arma de fogo. Lesões associadas ocorreram em 86% dos casos e as lesões intra-abdominais foram mais comuns no trauma penetrante ( $p<0,01$ ). A técnica operatória mais utilizada foi a hepatorrafia, e a cirurgia para controle de danos foi feita em 6,5% dos pacientes. A quantidade média de hemoderivados utilizados foi 6,07 unidades de hemoconcentrado e 3,01 unidades de plasma fresco. A incidência de complicações pós-operatórias foi 29,9%, e as mais frequentes foram as infecciosas, incluindo pneumonia, peritonite e abscesso intra-abdominal. A taxa de sobrevida dos pacientes acometidos de trauma contuso foi 60% e de trauma penetrante, 87,5% ( $p<0,05$ ). **Conclusão:** apesar dos avanços tecnológicos de diagnósticos e tratamentos, as taxas de morbimortalidade nos traumas hepáticos permanecem elevadas, especialmente nos pacientes acometidos de trauma hepático contuso em relação ao trauma penetrante.

**Descritores:** Fígado. Traumatismos Abdominais. Ferimentos e Lesões. Armas de Fogo. Acidentes de Trânsito.

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# Iliac artery myointimal hyperplasia in rabbits submitted to angioplasty and treated with *Moringa oleifera*

## *Hiperplasia miointimal na artéria ilíaca em coelhos submetidos à angioplastia e tratados com Moringa oleifera*

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### A B S T R A C T

**Objective:** to assess post-angioplasty myointimal hyperplasia in iliac artery of rabbits treated with extract of *Moringa oleifera* leaves. **Methods:** we conducted a randomized trial in laboratory animals for five weeks of follow-up, developed in the Vivarium of Pharmaceutical Technology Laboratory of the Universidade Federal da Paraíba. We used rabbits from the New Zealand breed, subjected to a hypercholesterolemic diet and angioplasty of the external iliac artery, randomized into two groups: M200 Group (n=10) – rabbits treated with 200mg/kg/day of *Moringa oleifera* leaves extract orally; SF group (n=10) – rabbits treated with 0.9% saline orally. After five weeks, the animals were euthanized and the iliac arteries prepared for histology. Histological sections were analyzed by digital morphometry. Statistical analysis was performed using the Student's t test. The significance level was 0.05. **Results:** there was no significant difference in myointimal hyperplasia between M200 and SF groups when comparing the iliac arteries submitted to angioplasty. **Conclusion:** there was no difference of myointimal hyperplasia between groups treated with saline and *Moringa oleifera* after angioplasty.

**Key words:** Hyperplasia. Iliac Artery. *Moringa oleifera*. Angioplasty. Rabbits.

### INTRODUCTION

In its history, Reconstructive Vascular Surgery has faced a complex biological phenomenon affecting results, which is the anastomosis myointimal hyperplasia and the fibroplasia, developed in vascular anastomosis or after balloon angioplasty, often leading to vascular occlusion and failed revascularization. Over the years, several researches have been dedicated to the study of the intima and the control of such phenomenon by pharmacotherapeutic agents, but the results are still far from satisfactory<sup>1</sup>.

Myointimal hyperplasia is the proliferation and migration of smooth muscle cells of the arterial medial layer and bone marrow cells to the intimal layer, thus decreasing the vascular lumen<sup>2</sup>.

*Moringa oleifera* (M. oleifera), also known as *Moringa pterygosperma Gaertn*, is a member of the plant family *Moringaceae*, perennial angiosperms, including 12 other species. Native of sub-Himalayan northern parts of India, it is cultivated in all tropical and subtropical areas of the world, where it is known by several common names:

drumstick tree, horseradish tree and malunggay, this most commonly found in the literature<sup>3</sup>.

*Moringa oleifera* is an edible plant. A wide variety of nutritional and medicinal properties have been attributed to its roots, bark, leaves, flowers, fruits and seeds<sup>4,5</sup>. Phytochemical analyses have shown that the leaves are particularly rich in potassium, calcium, porous iron phosphate, vitamins A and D, essential amino acids, and known antioxidants such as  $\beta$ -carotene, vitamin C and flavonoids<sup>6-10</sup>.

Moringa's flower has high medicinal value as a stimulant, aphrodisiac, abortifacient and anti-inflammatory. It acts in muscle diseases, has antitumor action, decreases triglycerides, as well as serum cholesterol and its fractions – Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), improving the atherogenic index. It decreases lipid profile in the heart, liver and aorta of hypercholesterolemic rabbits and increases fecal excretion of cholesterol. The leaves have purgative activities, applied as a poultice for wounds. It has been used in temples for headaches, as well for hemorrhoids, fever, sore throat,

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bronchitis, eye and ear infections, scurvy and phlegm. It is believed that the leaf juice is effective for controlling glucose levels<sup>4</sup>.

Experimental data<sup>11</sup> and the first clinical trials have shown that inhibition of myointimal hyperplasia can be achieved by local administration of antiproliferative drugs such as paclitaxel loaded on the surface of angioplasty balloons. Therefore, drug-eluting balloons are a promising tool to prevent restenosis and unwanted persistence of the polymers of drug-eluting stents in the vessel wall, thereby, potentially increasing the safety of the percutaneous coronary intervention<sup>12-14</sup>.

This research aimed to determine the difference in frequency of post-angioplasty myointimal hyperplasia in the iliac artery of rabbits with experimental atherosclerosis treated with extract from the leaves of *Moringa oleifera*.

## METHODS

The project was approved by the Ethics Committee on Animal Research of the Pharmacological Technology Laboratory (CEPA / LTF) of the Universidade Federal da Paraíba (UFPB), Campus I, Joao Pessoa, PB (protocol 602/2011), and the research developed in the Vivarium of the Laboratory of Pharmaceutical Technology.

### Study Type

Randomized clinical trial in experimental animals for five weeks follow-up.

### Sample

#### Inclusion criteria

We included 20 young, adult, female rabbits (12 months of age and weighing over 2kg) from *Oryctolagus cuniculus* species and New Zealand strain, undergoing an intimal hyperplasia process of the left common iliac artery after experimental atherosclerosis by egg yolk<sup>15</sup>.

#### Exclusion criteria

We excluded rabbits older than six months, body weight less than 2kg and more than 4kg, with preexisting disease or anatomical changes in studied structures.

### Sampling

We studied the selected rabbits, which formed a probabilistic sample.

### Randomization

The draw of the animals for each group was made randomly by block permutation<sup>16</sup> with the aid of the Research Randomizer software (available at: <http://www.randomizer.org/form.htm>), two blocks with ten numbers. Each block corresponded to a group, and the numbers generated, to animals. They formed two groups with ten rabbits each.

Animals received a letter corresponding to the group and a number (example: M1 – *Moringa* group, Animal 1; C2 – Control group, animal 2). This marking was performed by writing made by blue indelible ink (permanent mark) on the internal aspect of the base of the ear.

Atherosclerosis was induced by a hypercholesterolemic egg yolk-based diet, 20 ml / day divided into two oral doses for a period of 100 days.

We opted for closed animal management and the experiment was carried out on the same vivarium of origin, where there is an enabling environment for them, with forced ventilation, exhaust system, periods of natural luminescence, average temperature of 20°C, minimum noise and moisture around 50%. We kept the animals in individual cages with appropriate area 0.64m<sup>2</sup>, without contact with the natural secretions, since they are adapted in order to maintain proper hygiene. The diet consisted of water and granulated commercial chow *ad libitum* before and during the experiment, according to the literature<sup>17</sup>.

We induced the myointimal hyperplasia by the following technique: The rabbits were anesthetized with xylazine at a dose of 10mg/kg and ketamine at a dose of 40mg/kg intramuscularly in the thigh. Oxygenation was maintained by endotracheal intubation by direct visualization, coupled to the source of O<sub>2</sub> with the aid of anesthesia<sup>18</sup>. After anesthetic induction, we carried out trichotomy of the lower abdomen and groin followed by adequate local cleaning and antisepsis with iodized alcohol. The procedure was then the skin incision of 2 to 3 cm in length in the longitudinal direction in the inguinal fold with a 15 scalpel blade, for exposing the right femoral artery, which was repaired with simple interrupted sutures of nylon 3.0 at the end of the procedure. A blood sample was collected for Total Cholesterol, High Density Lipoprotein (HDL), LDL, VLDL and triglycerides. This blood was sent to the vivarium biochemistry laboratory for the respective dosage. We then performed a small cross arteriotomy with a 11 scalpel blade, by means of which we introduced a metal guide number 0.014, over which we introduced the balloon catheter of 3 mm diameter by 20mm length (balloon/artery ratio of 2.5-3.0: 1) which was inserted into the lumen of the right iliac artery (RIA). In each artery, the balloon catheter was inflated for a minute at rated pressure, leading to distension of the arterial wall. After deflation of the balloon we performed ligation of the arteries with 3.0 cotton sutures. Finally, the skin was sutured with 3.0 nylon sutures. We administered analgesics (Ibuprofen 10mg/kg orally for five days) and antibiotics (cefaclor 20mg/kg/day divided into two doses) for both groups postoperatively to prevent animal suffering. Twenty-four hours after the arterial injury, we began the administration of our research drugs in both groups.

Group M (*Moringa*) received 200mg/kg of ethanol extract of *Moringa oleifera* leaves by gavage for



five weeks and group C (negative control) received 0.9% saline, 10ml/day for five weeks by gavage.

By the end of the experiment, the animals were euthanized by a lethal dose of the anesthetic, and we harvested the common iliac arteries with the segment subjected to the lesion by the balloon. In addition, we collected new blood samples to measure total cholesterol, HDL, LDL, VLDL and triglycerides. Arterial specimens were fixed in 10% formalin for at least 24 hours and then brought to the routine preparation of histological sections for light microscopy: gradual and increasing dehydration in 70% alcohol to absolute alcohol – diaphanization in xylene and embedding in liquid paraffin at 60°C. Paraffin blocks thus prepared were cut with a microtome at a thickness of 3µm and cuts mounted on extra-thin glass slides (76x25 mm). They were stained with hematoxylin-eosin (HE), then mounted with coverslips and natural resin. The slides were prepared and examined by a pathologist duly accredited with increases of 10 and 40 times.

The blood was sent to the laboratory, where we held the standard dosage of the above parameters.

Pathologist blinding was performed by sequential numbering, the true correlation being known only by the one who had marked the ears (Principal Investigator). The new identification was kept in a sealed envelope, which was not opened until the time of data analysis, after the measurement of the primary variable.

After histological analysis, the slides were photographed with a digital camera (Canon PowerShot A640®) in 4x optical magnification associated with the optical microscope (Nikon®) increases of 2x and 10x.

The images of histological sections stained by HE were scanned for morphometric analysis from the conventional optical microscope with objective 2x with plan-achromatic lenses, colored closed circuit camera with 4x optical zoom, generating image files of 3,648 x 2,736 pixels. The images were scanned using 2x microscopic enhancement. The morphometric measurements were performed by the processing and digital analysis system "ImageJ64 NIH Image", without the interference of the observer, kept "blind" during the measurement. For each segment analyzed we measured the lumen area and the inner area of the internal and external elastic lamina. Based on these results, we calculated: 1) the area of the medium-intimal layer – subtracting the vessel wall region between the external elastic lamina and the lumen of the vessel; 2) the index of medium-intimal hyperplasia – dividing the average area of intimal layer by its sum with the lumen of the vessel.

The primary variable was the frequency difference of average myointimal hyperplasia.

For effective treatment, the myointimal hyperplasia was assessed by morphometry; values were in area and pixels, then we calculated the mean of each group. The difference of these mean area was the one used to tell which treatment was more efficient.

Secondary variables were total cholesterol and its fractions (HDL, LDL, VLDL) and triglycerides.

As additional data, we studied the animals body weight. The kilogram is the amount of mass, it is equal to the mass of the international kilogram prototype<sup>19</sup>; age (calculated in full months, on the last day of the reference month of the research, based on the day, month and year of the animal's birth).

## Statistical Method

### Calculating the sample size

The sample size was arbitrated in 20 rabbits, based on the literature, where there are works that demonstrate acceptable statistical results with fewer animals in the experiment with similar animal models<sup>20-22</sup>, and to respect the rules of the Brazilian Society of Science in Animal laboratories (SBCAL). Statistical analysis was performed with the Student's t test and calculating the 95% confidence interval (CI) for each point estimate.

### Statistical analysis

We collected data in a standardized form and stored them in a spreadsheet. We performed descriptive analysis by calculating the 95% confidence interval for each estimated point. The calculations were performed with the aid of statistical GraphPad InStat® Prism 5 (2012), Mac version.

Data are presented as mean and standard deviation. The comparison between groups was performed using the Mann-Whitney U test for morphometric data. The calculations were performed with the aid of statistical GraphPad Prism InStat® 5 (2012), Mac version.

## RESULTS

When comparing the average of the medium-intimal complex of groups (SF = 35.74% of the artery versus M200 = 38.66% of the artery), there was no statistical difference when we applied the Mann-Whitney U test ( $p=0.33$  – Table 1).

When comparing the values of total cholesterol, HDL, LDL and triglycerides, there was no statistically significant difference.

As for weight, there was difference in the means of both groups when comparing the periods before and after the experiment (Tables 2-5 and Figure 1).

## DISCUSSION

The study was performed in rabbits, as Gianni showed that New Zealand rabbits (*Oryctolagus cuniculus*) are very sensitive to induction of atherosclerotic lesions and considers this strain as one of the most important atherosclerosis study models<sup>20</sup>. The construction of models inducing atherosclerosis, pharmacologically or by

**Table 1 -** Medium-Intimal Complex of the iliac arteries.

Group	Mean (%)	Standard Deviation (+/-)	95% CI	Min	Max
SF 0.9%	35.74	5.56	31.76 a 39.71	29.23	45.46
M 200	38.66	7.39	33.37 a 43.94	24.1	47.02

*P* = 0.33

atherogenic diet, associated with angioplasty balloon injury, results in formation of plaques similar to those found in human coronary arteries<sup>20</sup>.

Brasselet *et al.* also used iliac arteries of rabbits subjected to experimental atherosclerosis diet with high cholesterol content<sup>21</sup>. However, the analysis of the iliac arteries of rabbits countered the prevalence of atherosclerotic lesions, which are more often in the aortic arch and thoracic aorta of rabbits submitted to experimental atherosclerosis, according to Taylor and Fan<sup>22</sup>.

The iliac area was chosen for the availability of data showing that this site is a territory in which

angioplasty may be performed, including selective implantation of stents<sup>23</sup>, which was not done in this study since we opted only by angioplasty balloon. The classic study from Indolfi *et al.*, who used a Fogarty catheter to cause endothelial injury, showed that restenosis after balloon catheter angioplasty was caused by the negative elastic remodeling and the proliferation and migration of vascular smooth muscle cells (VSMC)<sup>24</sup>. In said model, using carotid arteries of rats, the response of the arterial wall damage caused by angioplasty is the release of growth factors and other biologically active factors, which changing the composition of the extracellular matrix and

**Table 2 -** Premedication lipid profile - SF Group.

	0.9% Saline				
	Mean	Standard Deviation (+/-)	95% CI	Max	Min
Cholesterol Total (mg/dl)	53.1	50.11	17.26 - 88.94	194	25
HDL (mg/dl)	20.87	6.966	15.887 - 25.853	32.8	3.9
LDL (mg/dl)	14.8	37.37	11.93 - 41.53	120	-6
VLDL (mg/dl)	17.5	18.65	4.16 - 30.54	70	9
Triglycerides (mg/dl)	88.5	95.44	20.22 - 156.78	269	39

**Table 3 -** Post-medication lipid profile - SF Group.

	0.9% Saline				
	Mean	Standard Deviation (+/-)	95% CI	Max	Min
Cholesterol Total (mg/dl)	46.4	19.66	32.33 - 60.47	97	31
HDL (mg/dl)	19.08	16.189	7.499 - 30.661	53.8	7.8
LDL (mg/dl)	11.17	9.815	4.149 - 18.191	38	4.7
VLDL (mg/dl)	16.15	6.627	11.409 - 20.891	26.7	5.2
Triglycerides (mg/dl)	95.4	80.95	37.49 - 153.31	269	39

**Table 4 -** Premedication lipid profile – M 200 Group.

	M 200				
	Mean	Standard Deviation (+/-)	95% CI	Max	Min
Cholesterol Total (mg/dl)	44.75	11.23	35.36 - 54.14	61	29
HDL (mg/dl)	23.513	3.702	20.418 - 26.607	55	28
LDL (mg/dl)	5.13	12.02	4.92 - 15.17	25	-9
VLDL (mg/dl)	16.25	3.58	13.26 - 19.24	20	10
Triglycerides (mg/dl)	82.63	18.41	67.24 - 98.01	100	51

**Table 5 -** Post-medication lipid profile - M 200 Group.

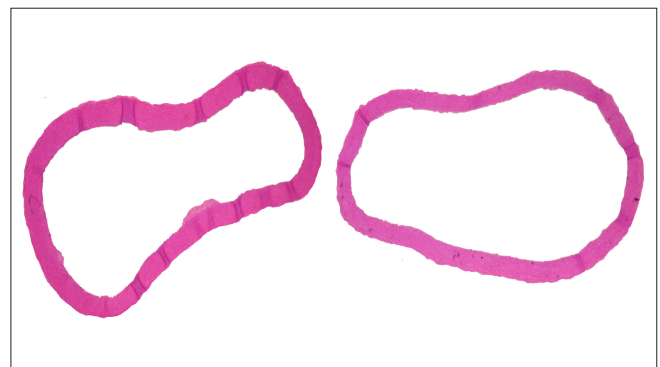
	M 200				
	Mean	Standard Deviation (+/-)	95% CI	Max	Min
Cholesterol Total (mg/dl)	42.56	11.2	33.94 - 51.17	55	28
HDL (mg/dl)	22.778	9.101	15.782 - 29.773	37.5	5.1
LDL (mg/dl)	3.889	8.525	2.664 - 10.442	22.7	-5.2
VLDL (mg/dl)	15.9	6.13	11.188 - 20.612	27.2	9.4
Triglycerides (mg/dl)	79.44	30.66	55.87 - 103.01	136	47

promote phenotypic change in VSMC, from the contractile to synthetic (dedifferentiation), leading to cell proliferation in the tunica media and migration into the intima, forming the neointima<sup>24</sup>. Likewise, Inouye *et al.* used balloon angioplasty to simulate, with greater reliability, the stress induced by angioplasty<sup>25</sup>. This research was carried out by inflating a 3.0 x 20 mm angioplasty balloon in the iliac artery of rabbits using the maximum balloon rated pressure (burst pressure) for a period of one minute and following a balloon:artery ratio of 2.5 3.0: 1, while in the study by Taylor<sup>22</sup> this ratio was 1.0-1.2: 1.

The survey results showed no significant effect in reducing weight, cholesterol or triglycerides, the secondary variables, unlike the work of Metha *et al.*, which showed a hypolipidemic effect and influence in the decrease in the rabbits' weight<sup>26</sup>. This research, as well as the one from Mehta *et al.*, used the dosage of 200mg/kg/day of an alcoholic extract of leaves, and that author used the fruits during the period of 120 days. In our study, the intervention was made in 35 days, as the main objective was to evaluate the effect of *Moringa* leaf extract in intimal hyperplasia, since the phases of intimal hyperplasia revolve around four weeks, according to the literature and to Indolfi *et al.*<sup>24</sup>.

Karas *et al.* defend the pig model as an ideal one for reproduction of hyperplasia<sup>27</sup>. However, a good restenosis model requires more affordable, accessible and easy to handle animals. As Le Tourneau *et al.*<sup>28</sup>, we chose to use the iliac arteries of hypercholesterolemic rabbits as the animal model for atherosclerosis and myointimal hyperplasia.

Jain *et al.* used up to 600mg/kg/day when evaluating hypolipidemic activity of the *Moringa oleifera* Lam<sup>29</sup>. Thus, other higher concentrations may be tested.



**Figure 1 -** Rabbits' arteries medium-intimal complex photomicrograph. Left – *Moringa oleifera* Group 200mg/kg/day. Right – Control Group; 8x magnification, HE.

Regarding lipid levels, we did not observe statistical difference either, perhaps because the atherogenic diet has been suspended in the period following angioplasty.

Finally, the investigation showed no significant difference from the myointimal area when comparing the control group, which used saline, with the group that was treated with the *Moringa* leaf extract at a dose of 200mg/kg/day, this dosage of *Moringa* extract possibly being too low.

Studies with higher doses of *Moringa* leaf extract should be performed, and with the testing of other parts of the plant, since there is a growing number of studies showing beneficial effects when it is used for medicinal purposes.

In conclusion, there was no difference in the frequency of myointimal hyperplasia between iliac arteries of rabbits treated with saline and with *Moringa oleifera* after angioplasty at the tested concentration.

## R E S U M O

**Objetivo:** determinar a diferença da média de hiperplasia miointimal pós-angioplastia na artéria íliaca de coelhos tratados e não tratados com extrato das folhas de *Moringa oleifera*. **Métodos:** ensaio aleatório em animais de laboratório por cinco semanas de seguimento, desenvolvido no Biotério do Laboratório de Tecnologia Farmacêutica da Universidade Federal da Paraíba. Foram utilizadas coelhas da raça Nova Zelândia, submetidas à dieta hipercolesterolêmica e angioplastia da artéria íliaca externa, randomizadas em dois grupos: Grupo M200 (n=10), coelhas tratadas com 200mg/kg/dia de extrato de folhas de *Moringa oleifera*, por via oral; Grupo SF (n=10), coelhas tratadas com soro fisiológico 0,9%, por via oral. Após cinco semanas, os animais foram eutanaziados e as artérias ílicas preparadas para histologia. Os cortes histológicos foram analisados por morfometria digital. A análise estatística foi realizada com o teste t de Student. O nível de significância foi 0,05. **Resultados:** comparando as artérias ílicas submetidas à angioplastia do grupo M200 com as do grupo SF, não houve diferença significativa da hiperplasia miointimal. **Conclusão:** não houve diferença da hiperplasia miointimal nos grupos tratados com soro fisiológico e *Moringa oleifera* após angioplastia.

**Descritores:** Hiperplasia. Artéria Íliaca. *Moringa oleifera*. Angioplastia. Coelhos.

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# The role of P16<sup>INK4a</sup> and P53 immunostaining in predicting recurrence of HG-CIN after conization treatment

## *O papel da expressão imunoistoquímica do P16<sup>INK4a</sup> e do P53 na predição da recorrência da nic-ag após tratamento por conização*

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### A B S T R A C T

**Objective:** To evaluate the expression of p16<sup>INK4a</sup> and p53 biomarkers in conization specimens from patients with high grade cervical intraepithelial neoplasia (HG-CIN), correlating them with the ability to predict the recurrence. **Methods:** we conducted a retrospective study of patients with HG-CIN in cervical biopsy treated with conization between January 1999 and January 2006 who had a minimum follow-up of 18 months. The expression of the p16 and p53 was assessed by tissue microarrays and correlated with disease recurrence. For analysis, we used the test of proportions (chi-square), considering value  $p \leq 0.05$ , 95% CI and calculations of sensitivity, specificity and accuracy of these immunomarkers in predicting recurrence. **Results:** the series comprised 83 patients aged between 16 and 86 years ( $35 \pm 11.7$ ), divided into two groups: 30 with HG-CIN recurrence (study group) and 53 without recurrence (control group). Mean age, parity, smoking and conization technique were similar in both groups. The p53 expression was present in 43% of the study group and 57% of the control group, and the p16 was present in 43% of the study group and in 57% of the control group ( $p > 0.05$ ). p53 had a positive predictive value (PPV) of 42% and negative predictive value (NPV) of 73%, sensitivity 70%, specificity of 47% and accuracy of 59%. The p16, PPV 42%, NPV 72%, sensitivity 66%, specificity of 49% and accuracy of 56%. **Conclusion:** immunohistochemistry expression of p53 and p16 showed low sensitivity and low specificity as predictors of HG-CIN recurrence after conization treatment.

**Key words:** Cervical Intraepithelial Neoplasia. Conization. Recurrence. Biological Markers.

### INTRODUCTION

Cervical cancer still presents itself as a public health problem in Brazil and worldwide, both for its high incidence and for its high morbidity and mortality<sup>1,2</sup>.

What differentiates this from other cancers is its development from well-defined, pre-invasive lesions, of known behavior and slow evolution, called "cervical intraepithelial neoplasia (CIN)"<sup>3</sup>.

Apart from the lesion recurrence in the first years of follow-up, women who were treated for CIN 2 or 3 remain at risk of developing carcinoma for a long period<sup>4</sup>.

The recurrence rates after conservative treatment vary between 13% and 26% in the most recent works<sup>5-7</sup>. It is still not possible to predict which cases of CIN will progress or regress. Regular follow-up by cytology can provide good efficacy by detecting cell changes and thus achieve significant reduction in cervical cancer morbidity and mortality through early diagnosis. However, the cost-effectiveness of this follow-up is still under debate<sup>8</sup>.

The detection of cell changes caused by the unregulated expression of viral oncoproteins appear promising as the characterization of tumor progression markers. Identifying and establishing the changing pattern of these proteins may define markers with high positive predictive power<sup>4</sup>.

The cellular tumor suppressor protein p16<sup>INK4a</sup> has been identified as a marker of infection by HPV (human papilloma virus). In a transformant HPV infection, E6 and E7 viral oncogenes substantially interfere with apoptosis and cell cycle regulation. Affected cells strongly express p16 to control the activation of the irregular cell cycle, and can be detected by immunohistochemistry<sup>9,10</sup>.

The protein 53 (p53) is a tumor suppressor protein which in humans is encoded by the TP53 gene<sup>11</sup>. As the "guardian of the genome", it may stop the cell cycle in response to DNA damage. The HPV E6 viral oncogene modifies the p53 and inactivates it, interfering with cell cycle control<sup>11</sup>.

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The importance of p53 inactivation in cervical carcinogenesis has been well documented. However, studies have failed to demonstrate different scores of p53 immunoreactivity in cervical intraepithelial neoplasia and cervical cancer. Conflicting results have also reported the relationship between p53 expression and progression of cervical intraepithelial neoplasia<sup>12</sup>.

Few studies have correlated the ability to predict HG-CIN recurrence with the immunohistochemistry expression of tumor proteins<sup>13,14</sup>.

Given the importance of assessing the role of biomarkers in predicting the progression of HG-CIN injuries, this study aims to evaluate the expression of p16<sup>INK4a</sup> and p53 in conization specimens from patients with cervical intraepithelial neoplasia using tissue microarrays immunohistochemistry, and correlate their expression with the ability to predict disease recurrence.

## METHODS

We evaluated 83 patients with histologically confirmed high grade cervical intraepithelial neoplasia (CIN 2 and 3), which were treated by conization at the Hospital Erasto Gaertner, Curitiba / PR from January 1999 to January 2006.

The patient sample was divided into two groups: Study group (patients with HG-CIN recurrence after conization within a follow-up of 18 months); and Control group (patients with no disease recurrence after conization treatment within the same period).

The study was duly approved by the Ethics in Research Committee of HEG, under Protocol number 1,947.

### Sample selection

**Inclusion criteria:** women followed in the HEG Cervical Pathology Service, between 16 and 86 years of age, who underwent cytology, colposcopy and biopsy of the cervix showing HG-CIN, and treated by conization, with identification of any CIN degree in the cone histological product, and with a minimum of 18-month follow-up. The follow-up was considered appropriate when comprised of cytology, colposcopy and, if necessary, biopsy in six-month intervals.

**Exclusion criteria:** insufficient data from medical records, post-conization clinical follow-up time less than 18 months, patients undergoing hysterectomy for benign disease, presence of invasive carcinoma in biopsy or conization specimen, absence of CIN evidence after microscopic evaluation of the conization specimen, and paraffin blocks in inadequate conditions for the realization of immunohistochemistry.

the presence of CIN 1, 2 or 3 in cytology, colposcopy and / or cervical biopsy during follow-up was determined as disease recurrence.

### Immunohistochemistry preparation technique and reading

The expression of the proteins was observed in tissue microarray slides prepared from the conization specimen paraffin block.

New sections were made from the original blocks of each patient by the tissue microarray technology, to be submitted to the application of p53 and p16<sup>INK4a</sup> immunomarkers, by hand, at the Experimental Pathology Laboratory PUCPR.

Each tissue slide was made of 20 samples, one patient each, totaling six slides per tissue immunomarker, without identifying the group of origin, so that the immunohistochemistry reading was performed avoiding contamination bias.

The immunohistochemical kits used were: prediluted p53 antibody (mouse monoclonal, clone DO-7, diluted 1:100, Biocare Medical®, Concord, USA); prediluted p16 Antibody (monoclonal mouse, clone 16p04-JC2, diluted 1:100, Bio Sb®, Santa Barbara, California, USA).

After preparation, the slides were subjected to the following processes: deparafinization; antigen retrieval with pH 6.0 citrate; endogenous peroxidase blocking; 1:100 dilution of each antibody and application of the primary antibody on each slide after washing with PBS specific to each immunomarker; application of the secondary antibody, and finally, application of specific DAB and counterstaining.

We determined using following immunohistochemical elements: reaction positivity and its intensity (qualitative analysis), reaction positivity standard (quantitative analysis) and immunolocalization (assessed only for p16 antibody).

Immunohistochemical elements were defined in similarly to the Jung *et al.* research, published in 2010, in their study of tumor progression markers<sup>15</sup>.

Qualitative analysis was divided into positive and negative reaction. The reaction was considered positive when impregnated, with brownish staining, the nuclei and/ or cytoplasm of at least 25% of the viable and evaluable tumor sample. A negative reaction was considered when there was no characteristic color of the immunohistochemical reaction.

The positive reaction was divided into two groups: strongly positive (when the staining intensity was similar to the used control) and weakly positive (when the intensity of staining was substantially lower than that of the positive control used, which can only be clearly observed with 100x magnification).

The quantitative analysis was classified as: diffuse positivity (the reaction was positive in an extension which included more than 50% of the viable and evaluable sample); severe multifocal positivity (the reaction was positive to an extent comprised between 25 and 50% of the viable and evaluable tumor sample); and mild multifocal positivity (the reaction was positive in an extension which included less than 25% of the viable and evaluable sample).

For p16 we also determined the immunolocalization, which we classified as follows: nuclear pattern of positivity, cytoplasmic pattern of positivity, simultaneous nuclear and cytoplasmic pattern of positivity.

The immunomarker showed brownish coloration at the nuclear level to the positive cuts for p53 and brownish at the nuclear and cytoplasmic level for p16 (Figure 1).

After applying these criteria, the study resulted in 83 patients, 30 in the study group and 53 patients in the control group (Figure 2).

### Statistical analysis

We statistically studied the variables with SPSS 12.0 software, seeking a confidence interval higher than 95% and a 5% significance level.

For pairing of the two groups we evaluated age, parity, type of treatment performed and time of post-treatment follow-up.

For the comparison of the age variable, we used the Student's t test. For comparison of the variable parity was used the nonparametric Mann-Whitney test.

In the analysis of immunomarkers, for the comparison of positivity immunoreaction between groups we used the chi-square and/or the Fisher's exact tests and identified the value of p. We also performed the calculation

of the positive predictive value (PPV) and negative predictive value (NPV), sensitivity, specificity and accuracy of each marker in predicting recurrence.

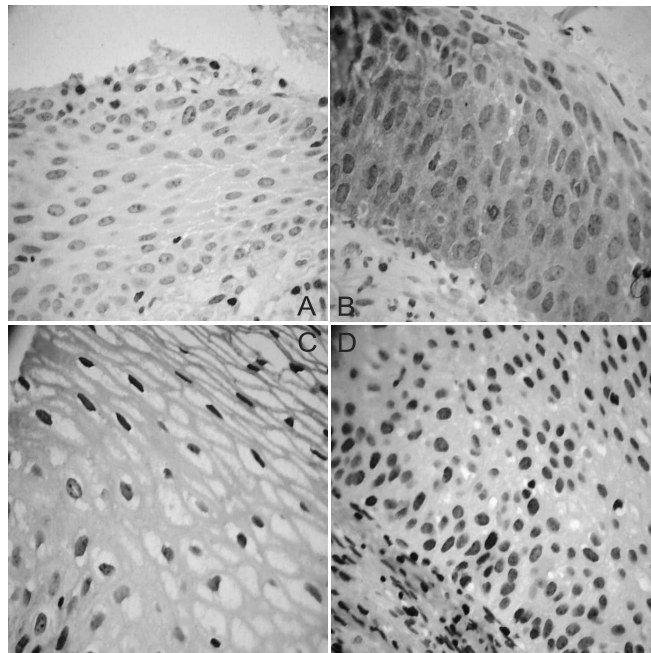
## RESULTS

The study group had a mean age of 36±12 years (95% CI: 33-40), average parity of 3±2 children (95% CI: 2-4), 42% were smokers and 95% were treated with conization by high frequency cautery (HFC) and 5% with the cold knife conization.

The control group had a mean age of 34±12 years (95% CI: 31-37), average parity of 3±2 children (95% CI: 2-3), 50% were smokers and 92% were treated by HFC and 8% with cold knife conization.

There was no statistically significant difference between groups when compared by age (p=0.2), parity (p=0.2), smoking (p=0.5) and conization technique (p=0.7).

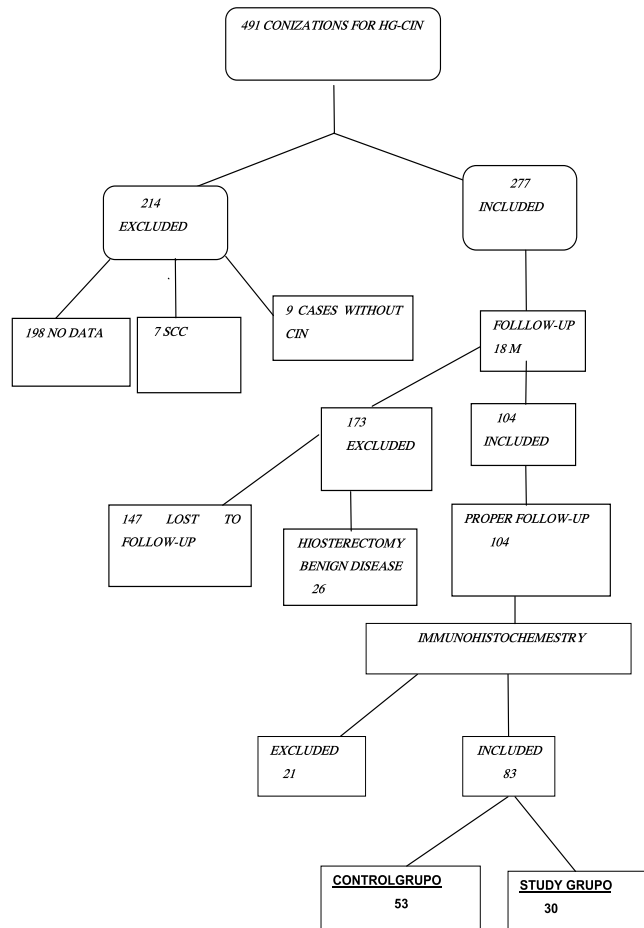
Of the 83 patients analyzed, all had CIN 2 or 3 in the biopsy prior to cervical conization and the cone specimen histology showed results between CIN 1 and 3. Conization specimens of the control group comprised three cases of CIN 1, 25 cases of CIN 2, 26 cases of CIN 3, while the ones



**Figure 1** - The contrast of the immunohistochemical expression of p53 and p16<sup>INK4</sup> biomarkers in histological specimens of cervical intraepithelial neoplasia.

Source: Anatomical pathology laboratory, HEG.

A- negative reaction for p16 (neither nucleus nor cytoplasm gets stained); B- positive reaction for p16 (brownish color in the nuclei and cytoplasm); C- negative reaction for p53 (no staining in the nuclei); D- positive reaction for p53 (brownish coloring of the nuclei).



**Figure 2** - Consort Diagram (Study Design).



from the study group consisted of three cases of CIN 1, nine cases of CIN 2 and 18 cases of CIN 3, showing no statistical difference of CIN severity degree between groups with respect to the recurrence risk ( $p = 0.1$ ).

As for immunomarkers, each group was individually evaluated for recurrence and/or clinical cure and we computed the positive predictive value (PPV), negative positive value (NPV), sensitivity, specificity and accuracy of each marker in predicting recurrence.

The p53 was present in 43% ( $n=21$ ) of the study group patients and in 57% ( $n=28$ ) of the control group, and was not identified in 73% ( $n=25$ ) of patients in control group and in 26% ( $n=9$ ) of the study one. It therefore exhibited no statistical significance to predict disease recurrence, with  $p$  value of 0.1 (Table 1).

The p16 was found in 43% ( $n=20$ ) of the study group patients and in 57% ( $n=27$ ) of the control group. It was not detected in 72% ( $n=26$ ) of the control group patients and in 28% ( $n=10$ ) of the study group. So, it also showed no statistical significance to predict recurrence, with  $p=0.1$  (Table 1).

Based on these data, the p53 PPV was 42%, and the NPV, 73%. It had a sensitivity of 70%, specificity of 47% and accuracy of 59% for predicting CIN recurrence.

For p16, the PPV was 42%, and NPV, 72%, with a sensitivity of 66%, specificity of 49% and accuracy of 56% of the test to predict disease recurrence.

There was no significant difference in the quantitative analysis of immunohistochemical reaction between the groups studied for p53 (Table 1).

For the p16 quantitative analysis, the only pattern clearly identified in the immunohistochemical reaction was

the one distinguishing between strong, weak and negative protein presence, as among those who exhibited strong reaction, 58% were from the study group, among the ones showing weak reaction, 74% were in the control group and for those who showed no reaction, 72% were in the control group, identifying a value of  $p=0.02$  (Table 1).

Also regarding p16, when the groups were compared in quantitative analysis, we identified that 70% of those who showed no reaction were in the control group, and among those with strong/diffuse pattern of reaction staining the nucleus and cytoplasm, 61% were in the study group (Table 1).

## DISCUSSION

Over the past decades, several epidemiological and laboratory studies have shown that invasive cervical cancer is a complex disease with many genetic and environmental determinants<sup>3</sup>. Despite adequate treatment of the precursor lesion, CIN recurrence occurs on average in 1-25% of cases, which increases the risk of invasive cancer<sup>16,17</sup>.

Determining the risk of development and prognosis, as well as the success of treatment in response to a particular medication and/or procedure, are the main reason for the identification of biomarkers<sup>8</sup>.

Many recent studies have found the importance of p16 and p53 in cervical neoplasia<sup>9,12-14,17-34</sup>. The vast majority rated the immunomarker positivity percentage for the presence of cervical intraepithelial neoplasia and correlated with the lesion severity<sup>9,12,13,17,18,21-29</sup>. However,

**Table 1 -** Qualitative and quantitative analysis of immunohistochemical p16 and p53 reactions as tests able to predict recurrence of HG-CIN treated by conization.

	Immuno reaction	P53		P16	
		Control	Study	Control	Study
General analysis	Positive	28 (57%)	21 (43%)	27 (57%)	20 (43%)
	Negative	25 (73%)	9 (27%)	26 (72%)	10 (28%)
	Significance	P= 0.1		P= 0.1	
Quantitative analysis	Difuse (+)	10 (63%)	6 (37%)	19 (56%)	15 (44%)
	Multifocal, intense (+)	7 (54%)	6 (46%)	2 (67%)	1 (33%)
	Multifocal, mild (+)	11 (55%)	9 (45%)	6 (60%)	4 (40%)
	Negative	25 (73%)	9 (27%)	26 (68%)	12 (32%)
Qualitative analysis	Strong (+)	15 (62%)	9 (38%)	10 (42%)	14 (58%)
	Weak (+)	13 (52%)	12 (48%)	17 (74%)	6 (26%)
	Negative	25 (73%)	9 (27%)	26 (73%)	10 (27%)
Positive predictive value		42%		42%	
Negative predictive value		73%		72%	
Sensitivity		70%		66%	
Specificity		47%		49%	
Accuracy		59%		56%	

few studies related immune reaction with disease recurrence and/or prognosis<sup>13,14,31</sup>.

A Korean study<sup>13</sup> tried to correlate the route related to pRb with the risk of recurrence. It analyzed 265 histological blocks of patients treated for CIN with immunohistochemistry for p16 and found a smaller percentage of such reaction in CIN 1, 2 and 3 patients who had recurrence than in the group without recurrence, similar to our findings.

A more recent study<sup>26</sup>, conducted in Greece, sought to identify the main changes that occurred in biomarkers related to HPV after six months of CIN treatment and tried to identify how their expression could predict treatment failure. While most of the evaluated markers, including p16, showed high rates of negative expression, they concluded that it is still necessary to examine more treatment failure cases to be able to identify a marker with high accuracy and ensure a quality follow-up.

Another recent study<sup>14</sup>, analyzing 55 cases of CIN 2 and 3 in cervical biopsies, studied how markers related to pRb and p53 pathways could be useful in identifying lesion regression. It concluded that the high percentage of pRb and p53 expressions were associated with increased odds of regression, which, in a way, contradicts our findings, which could not relate overexpression of p16 and p53 with disease recurrence.

Differently, some older studies have attempted to relate the expression of p53 and p16<sup>27,28</sup> with the prognosis of invasive carcinoma. The first<sup>27</sup> found a relation between p53 overexpression and poor prognosis of cervical invasive carcinoma, correlating such expression with shorter disease free-survival and increased recurrence risk. The second<sup>28</sup>, however, evaluating the expression of p53 and p16 in patients with clinical stage 1b and 2a of cervical cancer, could not identify this correlation with prognostic factors.

Since there is not in the literature an absolute standard reading of these immunomarkers, the criteria set out in this study were determined based on the manufacturer's instructions, the standards set by most studied and also in the authors' subjective evaluation, determining the following immunohistochemical elements: positivity of the reaction and its intensity (qualitative analysis), reaction positivity pattern (quantitative analysis) and immunolocalization (only evaluated for p16 antibody).

Most of the studies listed above<sup>9</sup> can identify a direct relationship between the percentage of positive expression of these markers with disease severity, but have difficulty finding an absolute standard and 100% reproducible that can identify the severity of injuries<sup>9</sup>. Maybe this subjectivity can be seen as a limiting factor of using this technology.

American authors<sup>17</sup> found positivity percentage of 100% in p16 expression in high-grade intraepithelial lesions, but correlated this positivity with the presence of high oncogenic risk HPV, showing a strong association

between diffuse and strong positivity and high-risk HPV lesions<sup>17</sup>.

In the same study<sup>17</sup>, p16 expression showed diffuse positivity in 70.2% of cases of high-grade lesions and 37.5% of low-grade lesions, and 84.8% of diffuse p16 positivity was related to the presence of high oncogenic risk HPV<sup>17</sup>.

In the present study, we could see a higher positivity of the p16 in patients whose evolution of CIN 3 was unfavorable, which can be correlated with HPV type, since different types of HPV may have different degrees of immunoreactivity, but this was a limitation of this study, since we did not genotype HPV.

When comparing the two groups, we did not observe significant difference in p16 positivity, but the comparative positivity in both groups at the same CIN degree was higher in the study group; and 61% of cases with strong and diffuse positivity were in the group with unfavorable evolution, suggesting a trend. A study with a larger number of cases might confirm this pattern of p16 marking as a feature of unfavorable evolution and/or high risk HPV transforming infection.

A study conducted in Costa Rica, generalizing their data for a cohort of 10,000 women, found a positive predictive value of 13.9% of p16 in CIN 3 and a negative predictive value of 100%, concluding that future studies are needed to assess when the clinical management should be modified based on the p16 positive results<sup>22</sup>.

A meta-analysis, in 2006, argued that in recent years, p16 has been extensively studied as a diagnostic aid in various scenarios of gynecological disease. So, like many markers, p16 is not 100% sensitive and specific for all injuries. However, there are many areas where there is no doubt about its value, often in combination with other markers, which includes identification of cervical high degree focal lesions and the separation of high grade lesions from benign ones mimicking high degree<sup>10</sup>.

Another more recent meta-analysis concluded that despite good evidence of correlation between the HPV infection severity with the positivity of p16, its reproducibility is still insufficient to standardize it in clinical practice<sup>9</sup>.

Evaluating the data found p53 positivity in this study, when considering its expression in the control and study groups, we could not find any more frequent pattern of positivity in the immunomarker quantitative analysis, reinforcing the idea of better negative than positive predictive value of such immunomarker, showing that when the marker is negative the chance of unfavorable evolution of post-conization CIN is very small, but when it is positive, one cannot predict its evolution, except by follow-up.

By studying the low-grade intraepithelial lesions and correlating them with HPV type, some<sup>11</sup> concluded that p53 expression in low-grade lesions increases progressively in low oncogenic risk HPV infections and is less proportionately expressed in intermediate and high oncogenic risk HPV infections, which may be related to the

different functions of the E6 protein on the HPV type and p53 degradation<sup>11</sup>.

Other investigators<sup>19,32</sup> failed to identify a gradually larger p53 expression pattern the higher the severity of the disease, concluding that alterations in p53 play a major role in the pathogenesis of cervical squamous cell carcinoma, but p53 expression is not sufficient to conclude on cervical carcinogenesis<sup>19</sup>.

Similar to our study, three other studies<sup>12,25,30</sup> simultaneously evaluated the positivity of p16 and p53 in

cervical HPV-induced lesions. However, they assessed the increased markers positivity regarding CIN severity, not recurrence.

Taking all these data into account, it seems the expression of both p16 as p53 in conization biopsies strongly emphasizes the relationship of the HPV infection severity with the development of cervical intraepithelial neoplasia, but cannot be considered markers capable to predict disease recurrence after treatment with conization.

## R E S U M O

**Objetivo:** avaliar a expressão dos biomarcadores p16<sup>INK4a</sup> e p53, nas peças de conização de pacientes com neoplasia intraepitelial cervical de alto grau (NIC-AG), correlacionando com a capacidade de prever o risco de recorrência. **Métodos:** estudo retrospectivo de pacientes com NIC-AG em biópsia de colo uterino, tratadas por conização, entre janeiro de 1999 e janeiro de 2006 e seguimento mínimo de 18 meses. A expressão dos biomarcadores p16 e p53 foi avaliada através de técnica de microarranjos teciduais e correlacionada com a recorrência da doença. Para análise utilizou-se o teste das proporções (qui-quadrado), considerando o valor  $p \leq 0,05$ , IC95% e cálculos de sensibilidade, especificidade e acurácia destes imunomarcadores na predição de recorrência.

**Resultados:** oitenta e três pacientes, idade entre 16 e 86 anos ( $35 \pm 11,7$ ), divididas em dois grupos: 30 com recorrência da NIC-AG (grupo estudo) e 53 sem recorrência (grupo controle). A média de idade, paridade, hábito de fumar e técnica de conização foram semelhantes nos dois grupos. A expressão do p53 esteve presente em 43% do grupo estudo e 57% do grupo controle e para o p16 esteve presente em 43% do grupo estudo e 57% do grupo controle ( $p > 0,05$ ). O p53 apresentou valor preditivo positivo (VPP) de 42% e valor preditivo negativo (VPN) de 73%, sensibilidade de 70%, especificidade de 47% e acurácia de 59%. O p16, VPP de 42% e VPN de 72%, sensibilidade de 66%, especificidade de 49% e acurácia de 56%. **Conclusão:** a expressão imunohistoquímica do p53 e do p16 apresentaram baixa sensibilidade e baixa especificidade como marcadores capazes de prever a recorrência da NIC-AG tratada por conização.

**Descritores:** Neoplasia Intraepitelial Cervical. Conização. Recidiva. Marcadores Biológicos.

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# Survival following orbital exenteration at a tertiary brazilian hospital

## *Sobrevida pós exenteração de órbita em hospital de referência*

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### A B S T R A C T

**Objective:** to analyze the epidemiology, clinical features and survival rate of patients undergoing orbital exenteration (OE) in a tertiary referral hospital. **Methods:** we conducted a retrospective study of all patients undergoing OE at the Hospital das Clínicas, FMUSP between January 2007 and December 2012. We collected data records related to gender, age, origin, length of stay, duration of the disease, other treatments related to the disease, number of procedures outside of the face related to the disease, follow-up and histological diagnosis. **Results:** we treated 37 patients in the study period. The average survival in one year was 70%, in two years, 66.1%, and 58.3% in three years. There was no significant difference in the one-year survival related to histological diagnosis ( $p=0.15$ ), days of hospitalization ( $p=0.17$ ), gender ( $p=0.43$ ), origin ( $p=0.78$ ), disease duration ( $p=0.27$ ) or the number of operations for the tumor ( $p=0.31$ ). Mortality was higher in elderly patients ( $p=0.02$ ). The average years of life lost was 33.9 in patients under 60 years, 14.7 in patients in the 61-80 years range and 11.3 in patients over 80 years. **Conclusion:** the present series of cases is significant in terms of prevalence of orbital exenteration; on the other hand, it shows one of the lowest survival rates in the literature. This suggests an urgent need for improved health care conditions to prevent deforming, radical resections.

**Key words:** Orbital Evisceration. Survival Rate. Carcinoma, Squamous Cell. Carcinoma, Basal Cell.

### INTRODUCTION

Orbital Exenteration (OE) is one of the most disfiguring procedures among ophthalmologic operations, and is characterized by the complete removal of the contents of the orbital cavity. According to the resection extent, it can be classified into: 1) total, if there is resection of the eyelids; 2) subtotal, when preserving the eyelids; or 3) extensive, when including removal of the bone surrounding walls<sup>1,3</sup>.

OE is the therapy of choice when other less radical methods do not result in better prognosis. It is usually indicated in oncologic resections for local control of malignant tumors. However, aggressive diseases or benign tumors that cause uncontrollable pain and structural and/or extensive lesions also require it. Among the malignant lesions, Basal cell carcinoma (BCC) is the most common skin cancer (80-90%), followed by squamous cell carcinoma (SCC). Examples of non-malignant diseases include: neurofibromatosis, fibrous dysplasia, mucormycosis, sharply contracted anophthalmic cavity, recurrent meningioma and orbital myiasis<sup>4,5</sup>.

The aesthetic consequences have a strong psychological impact on the patient and require a multidisciplinary approach. Many patients are referred to

psychological services after the operation or even refuse to undergo the surgical procedure. Constant vigilance, good doctor-patient relationship, early diagnosis and prompt treatment would provide better prognosis, especially in emerging countries<sup>6,7</sup>.

This retrospective study aims to analyze the epidemiology, clinical features and survival rate of patients undergoing orbital exenteration (OE) in a tertiary referral hospital.

### METHODS

The research project was approved by the of the Hospital das Clínicas, University of São Paulo and we carried out a retrospective study of medical records and pathology reports of all patients who underwent orbital exenteration at the facility between January 2007 and December 2012.

We identified cases by the International Classification of Diseases (ICD-10). We requested the medical records and analyzed them manually. the following data were collected: gender, age, origin, days of hospitalization, time of disease, other operations/treatments performed related to the disease, number of procedures

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performed outside the area of the face related to the disease, follow-up, histologic diagnosis and recurrence of lesions. To analyze the survival rate, we contacted the patient's family members by telephone with the help of Social Service for identification and active search for the occurrence of death.

We analyzed the variables by the Kaplan-Meier method, and compared survival curves using the log-rank test, with the R software, version 3.1.1. We calculated the Years of Potential Life Lost (YPLL) by the method proposed by Romeder<sup>8</sup>, adjusted to the life expectancy of Brazilians in 2013<sup>9</sup>. The age of reference used was 78.6 for patients under 60 years of age, 83.7 for patients between 61 and 81 years, and 96.7 for patients over 80.

## RESULTS

We identified 39 patients, of whom two were excluded due to incorrect coding of the disease.

### Demographic and clinical characteristics of patients

The study cohort included 17 men and 20 women, between 0 and 94 years of age (mean 62.2 years). São Paulo, capital, was the origin of 15 patients (40.5%), 13 (35.1%) were from towns in the interior of São Paulo and nine (24.4%) from other Brazilian regions. Thirty-three patients were white (89.2%), one was black (2.7%) and three brown (8.1%).

The average time of diagnosis was 43.4 months (range three months to 12 years), except for congenital cases. The days of hospitalization ranged from 0 to 62, average 14. Twelve patients (35.3%) were not subjected to any other surgical procedure related to the current injury, another 12 (35.3%) underwent one operation and 10 (29.4%) underwent more than one. Seventeen patients had additional treatment such as radiotherapy (ten patients – 27%), chemotherapy (two patients – 5.4%) and cryosurgery (three patients – 8.1%). Most were not submitted to any other operation outside the face area (81.8%) and eight (21.6%) were previously treated at least once.

### Histopathology

Histopathological findings included 16 cases of squamous cell carcinoma (43.2%) and ten of basal cell carcinoma (27.0%). Other diagnoses included adenoid cystic carcinoma, found in two patients, adenocarcinoma, sebaceous glands, cystic formation, inflammatory process, oncocytic schneiderian papilloma, esthesioneuroblastoma, capillary hemangioma, immature teratoma and malignant melanoma, each found in one patient (Table 1).

### Survival Rate

We excluded congenital cases from the survival analysis. Two patients died during hospitalization.

At the time of the study, 15 patients had died, 15 were alive and six could not be contacted. The average survival rate at one year was 70% and this figure decreased to 66.1% and 58.3% in two and three years, respectively. Mean survival was 47.3 months.

The mortality rate was higher in older patients ( $p=0.02$ ). There was no significant difference in one-year survival as for the histological diagnosis, if SCC (Figure 1), BCC or non-ECC/non-BCC ( $p=0.15$ ), days of hospitalization ( $p=0.17$ ), gender ( $p=0.43$ ), origin ( $p=0.78$ ), time of disease progression ( $p=0.27$ ) or number of operations related to the tumor ( $p=0.31$  – Table 2).

The average age of death in the age group under 60 was 44.7 years; between 61 and 80 years, 69, and in patients aged over 80 years, 85.4. Considering the life expectancy of Brazil in 2013, the average years of life lost were, respectively, 33.9 years, 14.7 years and 11.3 years. The total YPLL was 191 years (Figure 2).

## DISCUSSION

Orbital exenteration is not a common procedure and is usually done in tertiary referral centers. Our case series presented one of the largest series per year (37 patients in six years). Rahman *et al.* reported 64 cases in a period of 13 years<sup>10</sup>; Mohr and Esser had 77 in 20 years<sup>11</sup>; Bartley *et al.* described 102 in 20 years<sup>12</sup>; and Maheshwari *et al.* published 15 in 10 years<sup>13</sup>.

As the hospital where the study was conducted is a tertiary center, it is expected that 59.9% of patients originate from other cities as well as from São Paulo. The geographical distance from the origin to the hospital also explains the choice for OE, as the imprecise diagnosis of other health services and lagged time to admission to the



Figure 1 - Example of squamous cell carcinoma with orbital invasion.

**Table 1 -** Characteristics of patients undergoing orbital exenteration.

Patient	Gender	Age	Hospital stay (days)	Origim (State - City)	Time of disease till procedure (years)	Histopathology diagnosis	Other treatments	Number of procedures related to current lesion
1	M	74	49	SP – São Paulo	5	BCC	0	0
2	F	94	8	BA – Caculé	5	BCC	0	1
3	F	72	2	SP – São Paulo	5	CAC	RT	0
4	F	83	4	SP – Santos	7	CGS	0	2
5	M	81	22	CE – Cedro	7	SCC	0	1
6	F	65	1	MG	8	SCC	Cryosurgery	4
7	F	78	10	CE – Itapipoca	5	BCC	0	0
8	M	52	6	SP – Santos	1,7	SCC	QT	> 1
9	F	64	62	SP – São Lourenço da Serra	2	SCC	0	0
10	M	71	16	SP – São Paulo	0,4	SCC	RT + Cryosurgery	1
11	M	31	44	AM – Boca do Acre	0,3	SCC	0	0
12	F	49	16	SP – São Paulo	Unknown	SCC	Unknown	Unknown
13	F	63	2	SP – Mogi Guaçu	0,8	SCC	0	1
14	M	72	39	SP – Presidente Prudente	1	SCC	RT	1
15	F	66	2	SP – Uchôa	5	Cystic formation	0	7
16	M	49	9	SP – São Paulo	2	BCC	RT indicated	1
17	M	51	30	SP – São Paulo	2	SCC	RT	0
18	M	50	10	SP – São Paulo	5	Schneiderian papilloma	0	1
19	F	71	24	SP – Pompéia	0,5	Inflammatory process	0	2
20	M	58	9	SP – São Paulo	12	Esthesioneuroblastoma	RT	2
21	F	81	21	SP – São Paulo	2,3	SCC	Cryosurgery	2
22	M	82	4	SP – Santo Amaro	0,5	BCC	0	0
23	M	59	7	SP – Guarulhos	0,5	SCC	0	0
24	F	82	9	BA – Jequié	7	BCC	0	1
25	F	9	4	SP – Mogi Mirim	0	Capillary hemangioma	0	> 1
26	F	0	0	SP – São Paulo	0	Immature teratoma	QT	0
27	M	49	9	SP – Ibiúna	0,67	SCC	0	0
28	M	42	5	AM – Manaus	1	CAC	RT	0
29	M	69	7	SP – São Paulo	0,67	SCC	RT	0
30	F	82	7	BA – São Felix	5	BCC	0	1
31	F	42	8	SP – São Paulo	0,67	Adenocarci-noma	RT + QT	0
32	M	79	3	SP – Jandira	1	SCC	RT	1
33	M	51	7	AM – Manaus	2	SCC	0	4
34	F	82	27	SP – Mairipora	2	Malignant melanoma	0	1
35	M	60	21	SP – São Paulo	11	BCC	RT	6
36	M	51	11	SP – São Paulo	8	BCC	RT indicated	1
37	M	86	5	SP – São Paulo	6	BCC	0	3

Source: Medical records of the Hospital das Clínicas, Universidade de São Paulo (2007-2012).

BCC: basal cell carcinoma; CAC: cystic adenoid carcinoma; SGC: Sebaceous Glands Carcinoma; SCC: squamous cell carcinoma; RT: radiotherapy; QT: chemotherapy.

tertiary hospital may have made OE the only possible procedure for the control of local disease.

Among the patients cohort, three constituted non-malignant cases. SCC and BCC together accounted for 70.2% of the histological diagnosis, which is consistent with other studies. BCC is the most common skin cancer in the periorbital area, but SCC spreads more easily and requires a quick management to prevent disease

progression<sup>2,10,12,14,15</sup>. Our findings are similar to current literature, insofar as BCC represented 27% of the OE cases, while SCC accounted for 43.2%.

Although SCC is more aggressive than BCC, the difference in survival at one year was not statistically significant between histopathologic diagnoses ( $p=0.15$ ). The difference was evident only among the first 30 months or so. Some studies, however, had higher mortality after SCC

**Table 2 -** Comparison of age, gender, days of hospitalization, origin, time of disease, number of operations and histological diagnosis with survival rate.

Variable	Number of Cases	Number of Deaths	Mean Survival <sup>a</sup> (months)	One-year Survival
Age range				
< 60	11	4	47.1	72.7
61-80	10	3	48.4	70
> 80	9	8	23.2	66.7
Gender				
F	13	8	41.8	61.6
M	17	7	52.4	76.6
Hospital stay (days)				
< 15	18	7	54.7	83.3
15-30	8	6	28.7	60
> 30	4	2	43.8	50
Origin				
Other States	8	5	37.9	76
Capital	13	5	46.7	69.2
Towns from State interior	9	5	39	66.7
Time to diagnosis				
< 1 year	7	5	27.1	42.9
1-5 years	14	6	47	78.6
> 5 years	8	3	44.9	87.5
Number of procedures				
0	11	6	38.6	63.6
1	10	6	35.3	70
> 1	8	2	56.3	87.5
Histological diagnosis				
BCC	9	4	46.3	88.9
SCC	13	9	33.7	46.2
Non-BCC / non-SCC	8	2	62.8	87.5

Source: Medical records of the Hospital das Clínicas, Universidade de São Paulo (2007-2012).

\* LogRank Test

BCC: basal cell carcinoma; SCC: squamous cell carcinoma.

than BCC<sup>16-18</sup>. Additional treatments, such as Mohs micrographic surgery, may have been beneficial in the management of some SCC cases<sup>19,20</sup>.

The average mortality rate after OE also differs from the literature, since our series showed lower survival. Rahman et al. reported a survival rate of 93% in one year<sup>10</sup>;

Mohr and Esser had 89%<sup>11</sup> and Chih-Hung Kuo, 97%<sup>15</sup>. Karabekmez *et al.*, whose study also come from an emerging country, showed a low survival rate of 50.5%<sup>7</sup>. Bartley *et al.* reported a survival rate of 88.6%<sup>12</sup>.

Younger patients had on average 33.9 years of life lost as a result of diseases that lead to OE, and older

**Table 3 -** Years of life lost according to age group.

Age Group	N	Interval	Mean age at death time	Average years of life lost	YPLL
< 60	4	31-51	44.7	78.6	33.9
60-80	3	64-71	69	83.7	14.7
> 80	8	81-94	85.4	96.7	11.3

Source: Medical records of the Hospital das Clínicas, Universidade de São Paulo (2007-2012).

YPLL: Years of Potential Life Lost.

\* According IBGE (Instituto Brasileiro de Geografia e Estatística), 2013



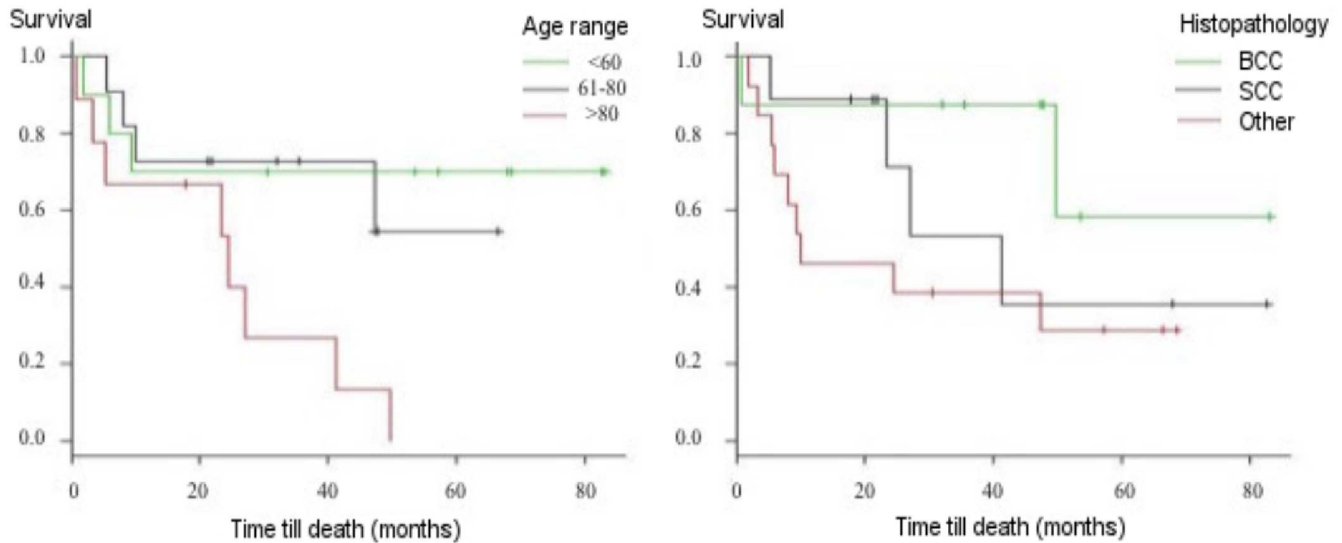


Figure 2 - Comparison of age and histological diagnosis with survival rate.

patients lost more than ten years. Not only the aggressiveness of the disease, but also the lack of information, difficulty in access to health care and delay in correct diagnosis justify the current low survival rate<sup>6,21</sup>. Studies suggest differences in post-SCC mortality between developed and developing countries<sup>22</sup>.

Advanced age may act as a confounding variable because, generally, it is related to comorbidities and other causes of death unrelated to the tumor. However, the

predominance of advanced malignant disease is already an indicator of difficulty in access to adequate medical services for immediate treatment, which could improve survival even in the older age group.

In conclusion, this case series is significant in terms of prevalence of Orbit Exenteration; On the other hand, it displayed one of the lowest survival rates in the literature. This suggests an urgent need for improved health care conditions to prevent deforming, radical resections.

## R E S U M O

**Objetivo:** analisar o perfil epidemiológico, as características clínicas e a taxa de sobrevivência dos pacientes submetidos à exenteração orbitária (EO) em um hospital de referência terciário. **Métodos:** estudo retrospectivo de todos os pacientes submetidos à EO no Hospital das Clínicas da FMUSP entre janeiro de 2007 e dezembro de 2012. Foram coletados em prontuários dados referentes ao sexo, idade, procedência, dias de internação, tempo de evolução da doença, outros tratamentos relacionados à doença, número de procedimentos fora da face relacionados à doença, tempo de seguimento e diagnóstico histológico. **Resultados:** trinta e sete pacientes foram identificados no período de estudo. A sobrevivência média em um ano foi 70%, em dois anos, 66,1% e em três anos 58,3%. Não houve diferença significativa na taxa de sobrevivência de um ano em relação ao diagnóstico histológico ( $p=0,15$ ), dias de hospitalização ( $p=0,17$ ), sexo ( $p=0,43$ ), procedência ( $p=0,78$ ), tempo de evolução da doença ( $p=0,27$ ) ou número de operações referentes ao tumor ( $p=0,31$ ). A mortalidade foi maior em pacientes idosos ( $p=0,02$ ). A média de anos de vida perdidos foi 33,9 em pacientes com menos de 60 anos, 14,7 em pacientes de 61-81 anos e 11,3 em pacientes com mais de 80 anos. **Conclusão:** a presente série de casos é significativa em termos de prevalência de exenteração orbitária; por outro lado, apresenta uma das menores sobrevivências da literatura. Isso sugere uma necessidade urgente de melhora das condições de assistência médica para a prevenção de ressecções radicais deformadoras.

**Descritores:** Exenteração Orbitária. Taxa de Sobrevivência. Carcinoma de Células Escamosas. Carcinoma Basocelular.

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# Effect of *Hevea brasiliensis* latex sap gel on healing of acute skin wounds induced on the back of rats

## *Efeito do gel da seiva do látex da Hevea brasiliensis na cicatrização de lesões cutâneas agudas induzidas no dorso de ratos*

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### A B S T R A C T

**Objective:** to evaluate the effect of topical delivery of latex cream-gel in acute cutaneous wounds induced on the back of rats. **Methods:** we subjected sixteen rats to dermo-epidermal excision of a round dorsal skin flap, with 2.5cm diameter. We divided the animals into two groups: Latex Group: application of cream-gel-based latex throughout the wound bed on postoperative days zero, three, six and nine; Control group: no treatment on the wound. Photographs of the lesions were taken on the procedure day and on the 6<sup>th</sup> and 14<sup>th</sup> postoperative days, for analyzing the area and the larger diameter of the wound. We carried out euthanasia of all animals on the 14<sup>th</sup> postoperative day, when we resected the dorsal skin and the underlying muscle layer supporting the wound for histopathological study. **Results:** there was no statistically significant difference in the percentage of wound closure, in the histopathological findings or in the reduction of the area and of the largest diameter of the wounds among the groups studied on the 14<sup>th</sup> postoperative day. **Conclusion:** according to the experimental conditions in which the study was conducted, latex cream-gel did not interfere in the healing of acute cutaneous wounds in rats.

**Key words:** Wound healing. Latex. Treatment. Skin. Rats.

### INTRODUCTION

Wound healing consists of a coordinated cascade of cellular and molecular events that interact to enable tissue reconstruction. Tissue loss is a triggering factor for wound healing and it initiates a series of steps that overlap from time to time. It includes inflammation, neoformation and tissue remodeling. Immediately after injury, the healing process begins through the action of a series of growth factors, cytokines and substances released from platelets and damaged blood vessels. After blood clots are formed, inflammatory cells invade the tissue and exert protecting functions against contaminating microorganisms. They are also major sources of growth factors and cytokines that trigger the wound healing proliferative phase. Such phase, in turn, begins with the migration and proliferation of keratinocytes at the wound edges, followed by the multiplication of dermal fibroblasts in the vicinities of the damaged tissue. Subsequently, the fibroblasts begin to produce large quantities of extracellular matrix. Still in the proliferative phase, there is formation of granulation tissue, thus named because of the granular characteristic due to the presence of newly formed capillaries that are essential to the healing process. Finally, there is the transition of the

granulation tissue into a mature scar, which is characterized by collagen continuous synthesis and degradation. The scar is a mechanically insufficient tissue, which lack epidermal appendages<sup>1,2</sup>.

Since ancient times, mankind tries to interfere in the tissue repair process. In recent decades, much effort has been made in identifying substances and techniques capable to promote healing to be used in wound management. The search for substances with angiogenic activity has also been intense due to its strong potential for clinical application. However, wound healing still remains a challenging clinical issue<sup>3</sup>.

The use of natural latex from *Hevea Brasiliensis* rubber tree for medicinal purposes is an alternative that links biocompatibility and low cost. Several studies have suggested that latex presents growth factors capable of acting in human tissues by stimulating neovascularization, cell adhesion and the formation of extracellular matrix, although such action mechanisms has not been fully elucidated so far<sup>4</sup>. Rubber tree latex biomembrane was developed by Coutinho-Netto for therapeutic purposes in the Laboratory of Biochemistry at the Medical School, USP, Ribeirão Preto / SP. In 1996, the first study used this material for the reconstruction of experimental defects in the

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esophagus of dogs. It demonstrated its influence on tissue neoformation, suggesting the possibility of its use as a substitute or a trigger to the formation of organs and tissues, although there has been elimination of the material<sup>5</sup>. Subsequent experimental studies have demonstrated the action of the biomembrane favoring the repair of abdominal wall defects in rats<sup>6</sup>, conjunctival reconstruction in rabbits<sup>7</sup>, as a prosthesis in videolaparoscopic inguinoplasty in dogs<sup>8</sup>, in the lining of the open cavity in tympanomastoidectomies<sup>9</sup> and pericardium replacement in dogs<sup>10</sup>. The biocompatibility of the biomembrane, necessary for its use as a biomaterial, has been proven by a number of experimental studies<sup>11,12,13</sup>. The biomembrane, in the form of dressing, is useful for treating pressure ulcers, promoting rapid debridement, *granulogenesis* and healing acceleration<sup>14</sup>. Similar effects were observed in chronic flebo-pathic ulcers<sup>15</sup>.

Subsequent studies showed that the angiogenic and healing acceleration properties are due to the action of a protein substance found in the latex serum fraction<sup>4,16</sup>. Such discovery stimulated the development of a cream-gel for topical use, which is indicated for the treatment and healing of wounds, produced from the base serum containing the bioactive protein fractions added to a cream-gel base.

This study aimed to evaluate the healing effect of latex cream-gel on induced skin wounds in rats.

## METHODS

This study was performed at the Laboratory of Experimental Surgery at the Medical School, University of Brasilia, Brazil. We adopted the Ethical Principles in Animal Experimentation recommended by the Brazilian College of Animal Experimentation (COBEA - Colégio Brasileiro de Experimentação Animal) affiliated to the International Council for Laboratory Animal Science, and the Brazilian Legislation on Animal Experimentation, Federal Law No. 6638 (1979). The research project was submitted to and approved by the Ethics in Research Committee on Animal Use of the Medical School, University of Brasília (protocol approval number 52439/2011).

We studied 16 adult, male Wistar rats (*Rattus norvegicus albinus*, *Rodentia mammalia*), with mean age of 60 days and weighing between 188 and 386 grams. We established an acclimatization period of seven days before initiating the experiment. The animals remained in their own accommodation, under room temperature and humidity, in circadian cycle (light / dark), with free access to water and specific diet (Purina® - Labina).

### Experimental design

We randomly distributed the animals into two groups, with eight animals each: Latex group – application of latex serum in cream-gel base on the wound bed on

postoperative days zero, three, six and nine; Control group: no treatment on the wound. We performed euthanasia of all animals on the 14<sup>th</sup> postoperative day.

### Surgical procedure

We anesthetized the animals with xylazine hydrochloride at a dose of 10 mg / kg of body weight, combined with ketamine hydrochloride at a dose of 75 mg / kg of body weight, intramuscularly delivered. Once anesthetized, each animal was placed on the surgical board in prone position. After trichotomy, the surgical technique started, with the same standardization for the animals in all groups, as we previously described in another publication<sup>17</sup>. The center of the epilated region was previously marked with a metallic, 2.5 cm diameter dermatological punch, and the excision of the skin was completed with a scalpel (figure 1). Hemostasis was performed by digital compression with gauze. Then, the animals from the latex group received manual application of latex in cream-gel base, in an amount enough to cover the surface of the wound. In the control group, the lesion was induced, followed only by hemostasis.

The latex cream-gel was reapplied on the third, sixth and ninth postoperative days. All animals, including the ones from the control group, were anesthetized on the mentioned days above. On the third and ninth days, the experimental animals were anesthetized for proper application of the gel-cream on the wound, and the control ones so that the animals were exposed to the same stressful situations, except the application of gel-cream.

Neither group received occlusive dressing after the treatments were applied. At the end of the procedures, the animals were put back in their respective cages, in the same preoperative conditions.

### Documentation of the wounds evolution

Once the animals were fixed on the operating table, the largest and smallest diameters of the wounds were measured with the help of a caliper in order to be



Figure 1 - Excision of the skin. Detail of the area marked by the punch and sectioned, deeply limited by the muscular plane.

compared with the standard initial measurement. At that time, we recorded the wounds with digital photography. This procedure was performed on the day the surgery was appointed to happen, and repeated on the 6<sup>th</sup> and on the 14<sup>th</sup> postoperative days. The image of the lesion was transferred to the Image J® software, and after establishing the periphery by means of the *polyline* method (demarcation of all points of the injury), the wound image was analyzed according to area and the largest diameter parameters.

### Material collection for the study

On the 14<sup>th</sup> postoperative day, the 16 animals were anesthetized with intramuscular ketamine and xylazine. Then, a dorsal pad containing the wound and the underlying muscle layer was excised. The animals were sacrificed with a lethal dose of thiopental intraperitoneally delivered at a dose of 25 mg / kg. The specimens were preserved in formaldehyde for histopathological study.

### Histopathology

Fragments embedded in paraffin were stained with hematoxylin and eosin and examined under an optical microscope. We analyzed the amount of collagen, fibroblasts and mononuclear and polymorphonuclear infiltrates. These parameters were graded on a 0-3 scale, indicating, respectively, samples with no, little, moderate or great amounts of the analyzed variable. Neovessels were quantified in five high magnification fields. The presence or absence of reepithelialization, foreign body, abscess and hair follicles in the scar were also documented.

### Statistical analysis

Data were analyzed using the Sigma Stat® 3.5 software. Comparisons of areas and the larger diameters of the wounds in latex and control groups in each of the study days were done by One Way Analysis of Variance (ANOVA). The Fisher's Exact and Chi-square tests were used for histological variables. The significance level ( $p$ ) used for rejecting the null hypothesis was 0.05.

## RESULTS

### Measurements of the wounds

when comparing the control and the latex groups on the day the surgery was performed, on the 6<sup>th</sup> and on

the 14<sup>th</sup> postoperative days, the wound area did not show a statistically significant difference (Table 1).

The percentage of wound closure from day zero to day six showed no statistically significant difference in the intergroup comparison ( $p=0.136$ ). There was, however, a higher wound closure percentage in the latex group compared with the control one, 63.1% and 59.5%, respectively.

### Microscopic evaluation

Tables 2, 3 and 4 show the histological intergroup comparison on the 14<sup>th</sup> postoperative day, with no statistically significant difference, though the number of neovessels in the latex group, observed in higher magnifications microscopic fields, was higher when compared with the control group (Figure 2).

## DISCUSSION

Several researches have shown that the biomembrane produced from natural latex of *Hevea brasiliensis* is biocompatible and has angiogenic, cell adhesion and extracellular matrix formation properties<sup>11</sup>. In pressure ulcers, the biomembrane facilitated the rapid debridement of wounds, granulogenesis and complete healing, producing flat and aesthetic scars<sup>14</sup>. Similar results were observed in diabetic patients with abnormal wound healing<sup>18</sup>. When used in patients with chronic venous ulcers, the biomembrane worked as a wound healing inducing factor, particularly in the inflammatory phase, confirmed by the intense exudation and debridement of the lesions, leading to changes in the chronic venous ulcer microenvironment<sup>19</sup>.

The preparation of a latex gel containing the protein fractions responsible for the induction of angiogenesis corresponds to the biotechnological enhancement of the research on *Hevea brasiliensis* natural latex. The product was obtained by a technique used to separate protein fractions through a high performance liquid chromatography, lyophilization and cream-gel formulation. According to studies conducted by the manufacturer, the protein fractions show biological activities that stimulate angiogenesis, fibroblasts cell proliferation, collagen synthesis and extracellular matrix strengthening and collagenase inhibition<sup>20</sup>. A study using latex gel in patients with chronic ulcers was also conducted by the same group with favorable results.

**Table 1** - Areas of the lesions (in cm<sup>2</sup>) in the latex and control groups.

Day of experiment	Control (n=8)	Mean ± SD	Latex (n=7)	Mean ± SD	p
0	4.17 ± 0.88		4.44 ± 0.88		0.544
6	1.69 ± 0.52		1.60 ± 0.44		0.711
14	0.04 ± 0.04		0.06 ± 0.03		0.083

**Table 2** - Histological comparison between the control and the latex groups on the 14<sup>th</sup> postoperative day.

	Control (n=8)		Latex (n=8)	
	Mean	Max/min	Mean	Max/min
Collagen	2.0	2/2	2.0	2/2
Fibroblasts	3.0	3/3	3.0	3/3
Mononuclear	2.0	2/2	2.0	2/2
Polymorphonuclear	2.0	2/2	2.0	2/2
Epithelial hyperplasia	1.4	2/1	1.8*	2/1

\* Latex x control  $p=0.234$

**Table 3** - Histological comparison between the control and the latex group in the 14<sup>th</sup> postoperative day (2).

	Control (n=8)		Latex (n=8)	
	Present	Absent	Present	Absent
Hair follicle	0	8	0	8
Abscess	0	8	0	8
Foreign body	8	0	8	0
Epithelialization	4	4	3	5

**Table 4** - Comparison between the control and the latex group – quantification of neovessels on the 14<sup>th</sup> postoperative day.

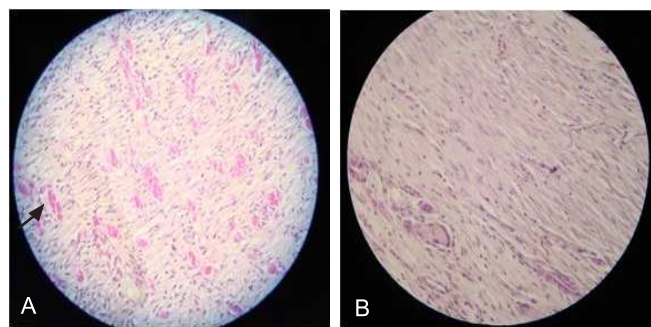
	Control (n=8) Mean $\pm$ SD	Latex (n=8) Mean $\pm$ SD	p
Vessels /field	24.50 $\pm$ 9.24	26.25 $\pm$ 12.41	0.561

In a study assessing the biocompatibility of the biomembrane, Mrué and colleagues assessed the biomaterial-induced healing by using a model of 0.5 cm circular acute skin ulcers induced in rabbits' ears. The group treated with biomembrane showed early epithelialization when compared with the control one, and in the histopathological samples, the presence of organized

collagen fibers was evident and presented no sign of fibrosis and neovessels<sup>11</sup>.

We could not prove the effectiveness of latex gel as for wound area reduction and histopathological findings, since these parameters were not statistically significant when compared with the control group. In the study by Mrué<sup>11</sup>, the biomembrane flexible conformation allowed direct and permanent contact of the ulcer by means of stitches. In a study evaluating angiogenesis, vascular permeability and healing, the latex serum added to a carboxymethylcellulose gel was applied on the day of surgery and on the 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> postoperative days, showing accelerated healing<sup>16</sup>. The current study used latex with the same application frequency. However, unlike the above mentioned study, the ulcers have not received occlusive dressing after each application, which may have caused the product to stay less time in contact with the wound. These facts may have interfered in the observation of any difference between this and the other groups. Another limitation of this study is the sample size, with only eight animals in each group. A larger sample could increase its statistical power.

For further studies, we suggest that different quantities of the product should be used, in order to



**Figure 2** - Photomicrograph of the repair tissue from the rats' skin – 14 days after surgery. A=Latex group rat; B=Control group. H&E stain, 40x.

Note: neovessels in greater quantities in A (arrow) than in B.

propose a dose, related to the size of the lesion, which is enough to promote a possible satisfactory effect. Serial biopsies of the lesions may also be useful to assess the influence of the latex cream-gel in specific healing evolutionary periods.

## R E S U M O

**Objetivo:** avaliar o efeito da administração tópica do gel-creme de látex em feridas cutâneas agudas induzidas no dorso de ratos. **Métodos:** dezesseis ratos foram submetidos à excisão dermoepidérmica de retalho cutâneo dorsal, circular com 2,5cm de diâmetro. Os animais foram distribuídos em dois grupos, um experimental e outro controle: Grupo Látex- aplicação em todo o leito da ferida do látex em base gel-creme no período zero, no terceiro, no sexto e no nono dias pós-operatórios; Grupo Controle- sem nenhum tratamento sobre a ferida. Foram feitas fotografias das lesões no dia da operação, no sexto e no 14º dia pós-operatório, para análise de área e do maior diâmetro da ferida. Realizou-se a eutanásia de todos os animais no 14º dia pós-operatório. Ressecou-se a pele dorsal e o plano muscular subjacente contendo a ferida para estudo histopatológico. **Resultados:** não houve diferença estatisticamente significativa no percentual de fechamento, nos achados histopatológicos ou na redução da área e do maior diâmetro das feridas, entre os grupos estudados no 14º dia pós-operatório. **Conclusão:** nas condições experimentais em que o estudo foi realizado, o gel-creme de látex não interferiu na cicatrização de feridas cutâneas agudas em ratos.

**Descritores:** Cicatrização. Látex. Terapêutica. Pele. Ratos.

## CONCLUSION

According to the experimental conditions in which the study was conducted, the latex cream-gel did not influence the healing of acute cutaneous wounds in rats.

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# Enhanced muscle strength with carbohydrate supplement two hours before open cholecystectomy: a randomized, double-blind study

## *Melhora da força muscular com suplemento contendo carboidratos duas horas antes de colecistectomia por laparotomia: estudo randomizado e duplo cego*

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### A B S T R A C T

**Objective:** to investigate the effects of preoperative fasting abbreviation with oral supplementation with carbohydrate in the evolution of grip strength in patients undergoing cholecystectomy by laparotomy. **Methods:** we conducted a clinical, randomized double blind study with adult female patients, aged 18-60 years. Patients were divided into two groups: Control Group, with fasting prescription 6-8h until the time of operation; and Intervention Group, which received prescription of fasting for solids 6-8h before surgery, but ingested an oral supplement containing 12.5% carbohydrate, six (400ml) and two (200ml) hours before the procedure. The handgrip strength was measured in both hands in both groups, at patient's admission (6h before surgery), the immediate pre-operative time (1h before surgery) and 12-18h postoperatively. **Results:** we analyzed 27 patients, 14 in the intervention group and 13 in the control group. There was no mortality. The handgrip strength (mean [standard deviation]) was significantly higher in the intervention group in the three periods studied, in at least one hand: preoperatively in the dominant hand (27.8 [2.6] vs 24.1 [3.7] kg;  $p=0.04$ ), in the immediate preoperative in both hands, and postoperatively in the non-dominant hand (28.5 [3.0] vs 21.3 [5.9] kg;  $p=0.01$ ). **Conclusion:** the abbreviation of preoperative fasting to two hours with drink containing carbohydrate improves muscle function in the perioperative period.

**Key words:** Fasting. Carbohydrates. Muscle Strength. Cholecystectomy. Preoperative Care.

### INTRODUCTION

Modern Anesthesiology guidelines support the reduction of preoperative fasting<sup>1-3</sup>. These guidelines recommend a shift from overnight fasting of six to eight hours to an approach in which solid foods are allowed up to 6-8 hours and clear fluids with carbohydrates (CHO) up to two hours before anesthesia induction<sup>4-7</sup>. Several studies show that intake of clear liquids with CHO up to two to three hours before the operation is safe and is not related to anesthetic complications or mortality risk<sup>8</sup>.

Prolonged preoperative fasting increases insulin resistance and induces gluconeogenesis<sup>9,10</sup>. Together with the metabolic response to trauma, prolonged fasting can in theory increase muscle proteolysis and impair muscle function in the early postoperative period<sup>11-13</sup>. Functional tests such as grip strength (dynamometry – DM) can detect these early changes in muscle function and thus demonstrate loss of functional capacity associated with prolonged fasting<sup>14-16</sup>. The objective of this study was to

investigate the effects of preoperative fasting abbreviation with oral supplementation containing CHO in Handgrip Strength (HGS) in patients undergoing cholecystectomy by laparotomy.

### METHODS

This is a prospective, randomized, double-blind clinical study, with therapeutic intervention, held at Hospital Beneficente Santa Helena (HBSH) in Cuiabá, State of Mato Grosso, Brazil. This study was approved by the Ethics in Research Committee of the Hospital Universitário Júlio Müller (Protocol 070/CEP-HUJM/2011) and all volunteers signed the informed consent form containing the description of the procedures involved in the project. The work was registered with the Brazilian Registry of Clinical Trials ([http://www.ensaiosclinicos.gov.br/](http://www ensaiosclinicos.gov.br/)) receiving the number RBR – 4rcsc6.

We included all adult, female patients aged between 18 and 60 years undergoing open

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cholecystectomy, from December 2011 to March 2012. They should not have diabetes mellitus, chronic renal failure, clinically significant liver disease (history of jaundice, ascites, chronic alcoholism or chronic hepatitis), gastroesophageal reflux disease, achalasia, lung disease or heart disease, and should be classified with the ASA score (American Society of Anesthesiologists) class I or II. Regarding nutritional status, they should be nourished (ASG – Subjective global assessment – A) and have body mass index (BMI) less than 35Kg/m<sup>2</sup>.

The exclusion criteria were: patient not adherent to any phase of the study protocol, laparoscopic operations, need for immediate surgical intervention, major intraoperative complications, such as severe hypotension, cardiac arrest and bowel perforation. We also decided to exclude prolonged operations, lasting more than three hours.

We randomized patients following the precepts of the CONSORT Group<sup>17</sup>, through numbers random generation by a computer program (available at: www.graphpad.com) that determined which patient would be part of the control group and of the intervention one. This randomization was kept in a sealed envelope until the time of patients allocation. To collect data, six professionals were initially trained by the researcher and subsequently selected for HGS measurement. None of the examiners knew which patients belonged to each group. Only one researcher knew the allocation of the groups, being the only one responsible for offering the carbohydrate-containing drink to selected patients and did not perform any measurements in the subjects. Patients who were in the intervention group also did not know the usefulness of the liquid ingested. Hospital discharge was determined by a doctor that was not informed of the ongoing study.

### Preoperative Protocol

All patients were hospitalized in the morning of the operation day, and the procedures performed in the afternoon. They followed the routine fasting of minimum six hours for solid foods. Those who belonged to the intervention group received 400ml of a drink containing carbohydrate about six hours before the surgical procedure and 200ml two hours before the operation. This drink was manipulated and supplied by the hospital Nutrition and Dietetic Service. The drink contained 12.5% carbohydrate (100% maltodextrin – Carboplex, Advanced Nutrition, Rio de Janeiro, Brazil). The conventional group, in turn, remained in complete fasting with a minimum requirement of 6-8 hours until the operation<sup>10</sup>.

### Postoperative protocol

With the exception of short preoperative fasting, all patients received some of the prescriptions and guidelines recommended by the ACERTO Project protocol, already a

routine in the service. Thus, all were instructed to early ambulation, intravenous fluids restriction (no volume or up to 20ml/kg/day in the immediate postoperative), early oral refeeding, up to six hours after the operation and no use or minimal use of opioids for analgesia<sup>18</sup>. All patients received epidural anesthesia.

The HGS was measured by a dynamometer (Baseline®, Pakistan) in both groups at admission (HGS-Pre), ie at 7am (six hours before surgery), in the immediate preoperative period (1h before surgery; HGS-Im) and after the end of the operation (HGS-Post – 12-18h postoperatively), in the dominant and nondominant hands. To this we adopted the technique described by Mathiowetz *et al.*<sup>19</sup> and validated by Bragagnolo<sup>20</sup>. We made three measurements and considered the highest obtained value to express each patient's HGS.

### Statistical method

We calculated a sample containing 12 patients in each group as being sufficient to obtain a beta error of 80% and alpha error of 5%, estimating that there would be a reduction of HGS by 50% in the control group. We used the Fisher's exact test or the chi-square test to compare categorical variables.

For continuous data, we used the ANOVA test for repeated measures. We analyzed the results with the Statistical Package for Social Sciences (SPSS) for Windows 9.0, with the significance level of 5% ( $p < 0.05$ ).

## RESULTS

Seventy-eight patients were eligible. Of these, 40 patients were randomized, with 21 and 19 for the intervention and control groups, respectively. Subsequently, 13 patients were excluded due to not following the perioperative protocol. Thus, 27 patients were analyzed, 14 in the intervention group and 13 in the control one (Figure 1). All patients were female and underwent cholecystectomy by laparotomy. Even presenting an age range 24 years (36 to 60), this did not affect the data obtained, since the force analysis was performed individually, comparing the individual's own strength, before and after surgery and/or after intake of the carbohydrate drink, there being no comparison between individuals. The distribution of cases according to the preoperatively demographic and clinical data in both groups is shown in table 1. There were no deaths or postoperative complications in both groups. Control group patients were on average fasting for 19 hours (3-19 hours). All patients were discharged early (within 24 hours postoperatively).

The mean values found for the HGS measures are in table 2 and figure 2. The group of patients who ingested the drink with CHO showed significantly higher HGS in at least one hand in the three periods.

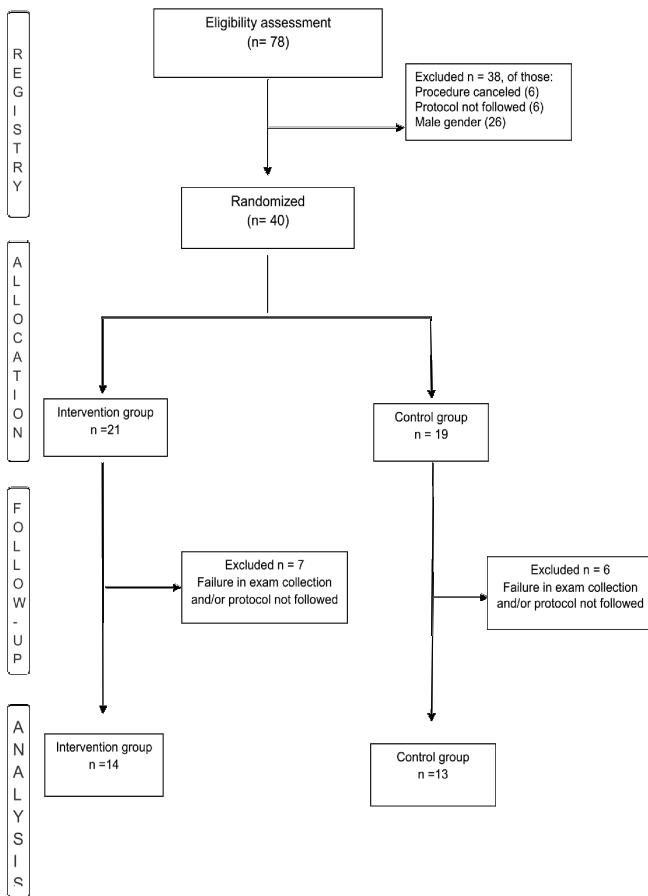


Figure 1 - Randomization Flowchart.

## DISCUSSION

The results of this study support other studies that showed that the abbreviation of preoperative fasting with carbohydrate-containing beverage is safe and does not cause damage to the patient. On the contrary, the current data show that fasting abbreviation with CHO was associated with a significant increase in HGS in all phases of the study, suggesting that the abbreviation of fasting exerts a beneficial effect on muscle strength in patients undergoing cholecystectomy by laparotomy.

Our results agree with several studies that suggested benefits associated with intake of carbohydrates drinks two hours before the surgical procedure<sup>9,10,21</sup>. We observed, in agreement with other ACERTO group studies, that traditional fasting is in fact more dilated and is on average greater than eight hours<sup>8,22,23</sup>. In a multicenter national study called BIGFAST<sup>23</sup>, also held by the ACERTO group, we found that patients remain on average 12 hours in preoperative fasting, regardless of the prescription of preoperative fasting being based on traditional protocols (6-8 hours absolute fasting) or on modern ones (6-8 hours of fasting to solids, and clear liquids allowed up to two hours before surgery). In this study, the conventional group got an average of 19 hours fasting, similar to the average of some BIGFAST study hospitals. Unlike the other cited multicenter study, ours observed that patients in the intervention group stayed on average for only three hours of preoperative fasting, really approaching the prescribed routine, versus eight hours of real preoperative fasting observed in patients throughout Brazil when oriented in modern fasting protocols. Possible causes for this spacing of preoperative fasting, not measured in this study but identified in other works, are the changes in the scheduling of operations, delay in timing of operations, and the higher patient compliance to fasting, believing it to be more beneficial to herself<sup>23,24</sup>.

Several studies have solidified the reliability of anthropometric measurements taken in the individual's non-dominant side, since it is the side that receives less external influences<sup>25</sup>. Corroborating this fact, the measurements taken in the non-dominant hand of the subjects studied in this work showed a significant difference between the control group and the intervention group immediately preoperatively and postoperatively, as a result of preoperative carbohydrate drink intake. At times, this influence was also found in the measurements of the dominant hand at the preoperative and immediate preoperative periods.

Noblett *et al.*<sup>13</sup>, when measuring the grip strength of their patients, also found a significant reduction of preoperative strength among patients who underwent

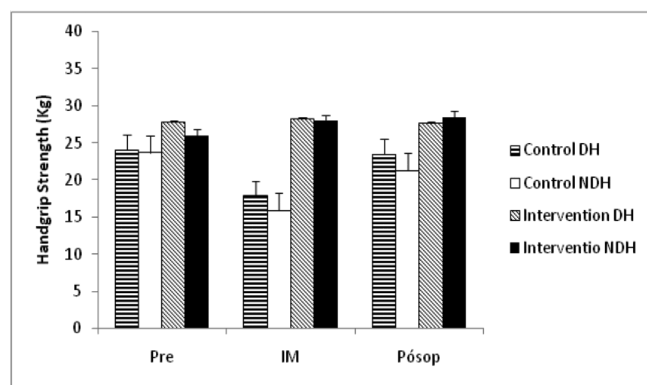
Table 1 - Demographic and clinical data of patients studied in both groups.

Variable	Control Group	Intervention Group	p (ANOVA test)
Age (years) (mean and SD)	48 ± 12	49 ± 10	0,89
Operative time (min) (mean and SD)	84 ± 31	75 ± 26	0,39
Type of anesthesia (N,%)Blockade	13 (100)	14 (100)	1,0
Preoperative fasting (hours) (mean and SD)	19 ± 3	3 ± 1	< 0,001
Nutritional status (N,%)Eutrophic	13 (100)	14 (100)	1,0
BMI (kg/m <sup>2</sup> )	24,4 ± 5,4	25,0 ± 3,9	0,79
ASA Score	13 (100)	14 (100)	1,0

SD: Standard deviation

BMI: Body mass index

ASA: American Society of Anesthesiologists - physical evaluation score.



**Figure 2** - Handgrip strength in the dominant and nondominant hands in the three observation periods in both groups.

\*,  $p < 0.05$  (ANOVA) versus the control group in the same reference hand and the same observation period. Data represent mean and standard error.

Pre= Preoperative; IM= immediate preoperative; Pós= postoperative period; DH= dominant hand; NDH= non-dominant hand.

conventional fasting, but this reduction was not found in patients receiving water or CHO.

Our results found a higher HGS in the intervention group. However, the lack of a group that received just water without CHO limits our conclusions. Therefore, we cannot clearly say whether this increase was due to the carbohydrate in the drink or simply because the patient is in an abbreviated fasting protocol. Indeed, Noblett *et al.*<sup>13</sup> found an improvement of HGS both in the group that ingested only flavored water and in the group that ingested the drink containing carbohydrate before the operation. Still, be it due to the hydration or to the glycidic load of the drink, there was improvement in the individual's functional status, and this suggests that preoperative fasting abbreviation benefits patients.

Hunt *et al.*<sup>26</sup> analyzed several nutritional status parameters, such as arm muscle circumference, forearm

muscle circumference, triceps fold, ideal body weight percentage, serum albumin and usual weight percentage, in addition to the DM in surgical patients. They showed that DM was the most sensitive indicator among those to assess risk of postoperative complications and hospital stay. Accordingly, Bragagnolo *et al.*<sup>20</sup> found that HGS is a good prognostic indicator in patients eligible for gastrointestinal surgery. In addition, Ali *et al.*<sup>27</sup> evaluated patients in the intensive care unit and found that muscle strength is an independent predictor of hospital mortality. In major surgery, early ambulation is very important to reduce complications and speed recovery. Thus, a better-preserved muscle function is beneficial to the patient and is associated with lower rates of postoperative complications<sup>26</sup>.

In summary, our findings showed that candidates for cholecystectomy by laparotomy may benefit from fasting abbreviation two hours before the operation. These patients had greater muscle strength throughout the perioperative period. These data suggest that the nutritional and metabolic state was better preserved in this treatment group. Indeed, in the literature, there is evidence that the abbreviation of fasting reduces hospital stay, thus there may be a higher turnover of hospitalizations<sup>24</sup>.

Although with a small number of cases, these results are significant. We evaluated a sample of patients with good general condition, relatively young and without chronic diseases. In addition, the operation was appraised as medium-sized, and associated with a low risk of complications and mortality. This was a double blind, randomized study, which is an ideal study type to evaluate an intervention. Either way, the results are consistent with most of the literature and suggest the benefits of a beverage containing maltodextrin two hours before the operation<sup>5,9,10,21,28</sup>.

According to the results obtained in our study, we conclude that the abbreviation of preoperative fasting to two hours with a carbohydrate containing drink improves muscle strength in the perioperative period.

**Table 2** - Results of measurements of Handgrip Strength pre and postoperatively in both groups. Data is expressed in mean and standard deviation.

Hand Grip Strength (kg)	Control	Intervention	p (ANOVA test)
Preoperative, dominant hand	24.1 (3.7)	27.8 (2.6)	0.04
Preoperative, non-dominant hand	23.7 (2.1)	26.0 (4.0)	0.23
Immediate preoperative, dominant hand	17.9 (12.8)	28.3 (2.7)	0.03
Immediate preoperative, non-dominant hand	15.9 (11.4)	28.0 (2.0)	0.01
Postoperative, dominant hand	23.5 (6.6)	27.7 (4.0)	0.19
Postoperative, non-dominant hand	21.3 (5.9)	28.5 (3.0)	0.01

## R E S U M O

**Objetivo:** investigar os efeitos da abreviação do jejum pré-operatório com suplementação oral contendo carboidrato na evolução da força de preensão palmar em indivíduos submetidos à colecistectomia por laparotomia. **Métodos:** estudo clínico randomizado e duplo cego em pacientes adultos do sexo feminino, com idade entre 18 a 60 anos. Os pacientes foram divididos em dois grupos: grupo controle, com prescrição de jejum de 6-8h até o momento da operação e o grupo intervenção, que recebeu prescrição de jejum para sólidos de 6-8h, porém ingeriu um suplemento oral contendo 12,5% de carboidrato, seis (400ml) e duas (200ml) horas antes do procedimento operatório. A força de preensão palmar foi aferida em ambas as mãos nos dois grupos, no momento de internação do paciente (6h antes da operação), no pré-operatório imediato (1h antes da operação) e com 12-18h de pós-operatório. **Resultados:** foram analisados 27 pacientes, 14 do grupo intervenção e 13 do grupo controle. Não houve mortalidade. A força de preensão palmar (média [desvio padrão]) foi significativamente maior no grupo intervenção nos três períodos estudados, em ao menos uma das mãos: no pré-operatório na mão dominante (27,8 [2,6] vs. 24,1 [3,7] kg;  $p=0,04$ ), no pré-operatório imediato nas duas mãos, e no pós-operatório na mão não dominante (28,5 [3,0] vs. 21,3 [5,9] kg;  $p=0,01$ ). **Conclusão:** a abreviação do jejum pré-operatório para duas horas com bebida contendo carboidrato melhora a função muscular no período perioperatório.

**Descritores:** Jejum. Carboidratos. Força Muscular. Colecistectomia. Cuidados Pré-Operatórios.

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# Teaching project: a low-cost swine model for chest tube insertion training

## *Projeto de ensino: modelo suíno de baixo custo para treinamento de drenagem torácica*

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### A B S T R A C T

**Objective:** to describe and evaluate the acceptance of a low-cost chest tube insertion porcine model in a medical education project in the southwest of Paraná, Brazil. **Methods:** we developed a low-cost and low technology porcine model for teaching chest tube insertion and used it in a teaching project. Medical trainees – students and residents – received theoretical instructions about the procedure and performed thoracic drainage in this porcine model. After performing the procedure, the participants filled a feedback questionnaire about the proposed experimental model. This study presents the model and analyzes the questionnaire responses. **Results:** seventy-nine medical trainees used and evaluated the model. The anatomical correlation between the porcine model and human anatomy was considered high and averaged  $8.1 \pm 1.0$  among trainees. All study participants approved the low-cost porcine model for chest tube insertion. **Conclusion:** the presented low-cost porcine model for chest tube insertion training was feasible and had good acceptability among trainees. This model has potential use as a teaching tool in medical education.

**Key words:** Training Programs. Education, Medical. Thoracic Wall. Models, Animal. Swine.

### INTRODUCTION

Simulators use represents a significant evolution in medical teaching<sup>1</sup>. They are a useful tool in training of students and professionals, decreasing patients' risks<sup>2-4</sup>.

Chest tube insertion is a medical skill used frequently in urgent and life-threatening situations, particularly in trauma<sup>5</sup>. It is generally accepted that every physician should master this life-saving skill<sup>1,5,6</sup>.

The present article demonstrates a simple, low-cost and low technology porcine model, used in a teaching project for chest tube insertion training. Also, it analyzes its acceptance by medical students and residents from a medical university in southwest of Paraná State, Brazil.

### METHODS

We carried on a teaching project using porcine models in resuscitation procedures training for last-year medical students and residents, from June 2013 to June 2014, at Universidade Estadual do Oeste do Paraná, Cascavel. As a part of this project, trainees filled a feedback

questionnaire about the used model. The chest tube insertion porcine model is presented and the questionnaires used for its assessment are currently analyzed. Before starting the project, it was submitted and analyzed, obtaining institutional approval (Prograd CR 40119/2014).

#### Teaching Project Steps

Each session of this educational project included groups of about ten medical students or residents, and consisted of three steps: a) trainees attended a class on thoracic anatomy, thoracentesis procedures and closed thoracic drainage<sup>5</sup>; b) they then practiced thoracic drainage in the porcine model under supervision; and c) trainees were asked to fill out a model evaluation questionnaire (optional).

#### Chest tube insertion porcine model

We used a rack of porcine ribs, from skin to pleural space. Each porcine hemithorax was divided and resulted in two models. Each model trained approximately five students. The ribs racks were purchased in local food markets, appropriate for human consumption, according to sanitary rules.

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Each chest specimen was fixed to a fenestrated basin, placed upside down (Figure 1). The remaining materials used were from the Medical Skills Lab, obtained by donation, without cost.

Details about the model confection can be found at the link: <https://www.youtube.com/watch?v=czKJEDP3gig>. During the study, different trauma situations were simulated, such as traumatopnea and penetrating chest trauma.

### Questionnaire

The evaluation questionnaire asked about the epidemiological aspects, previous training in the management of chest trauma, resuscitation procedures on the chest and adequacy of the model for training medical students and residents. Some of the answers were not objects of this study, but used in order to improve graduation educational opportunities.

Specifically, we requested evaluations of the overall quality of the model (robustness, ease of handling and tissue similarity criteria) and anatomical correlation (similarity to the expected anatomy in humans), both with scores ranging from 0 to 10.

The questionnaire was prepared by the main author and was not previously validated.

All information obtained by the questionnaire were grouped into tables using a spreadsheet, and analyzed with averages and percentages. When comparing the subgroups of students and residents, the values were expressed as mean and standard deviation, and we performed unpaired Student's t and chi-square tests, as appropriate.

### Teaching Group

Seventy medical students in their Emergency Department internship rotation and nine residents from Internal Medicine took part in this study after undergoing chest tube insertion training, from June 2013 to June 2014.

## RESULTS

The study group characteristics are described in Table 1. There was no refusal from students or residents to take part in the training or filling up the feedback questionnaire. Regarding the general quality of the used model, the mean score given by medical students was  $8.7 \pm 0.9$  and the mean score given by medical residents was  $9.5 \pm 0.7$  ( $p = 0.01$ ). The final general quality score was  $8.8 \pm 0.9$ . When comparing anatomic correlation with humans, the given general average score was  $8.1 \pm 1.0$ . The scores for anatomical correlation given by residents were higher than the ones by medical students ( $9.0 \pm 0.8$  vs.  $8.0 \pm 1.0$  respectively;  $p=0.005$ ). All trainees approved the porcine model as an adjunct for training chest tube insertion.

## DISCUSSION

Simulation is increasingly used in medical education<sup>1</sup>. Among the several available models, artificial simulation models have a relatively high initial cost and a low maintenance cost. In Brazil, the average cost of an artificial model for training of resuscitation procedures is between 10,000 and 12,000 American dollars. For comparison, in Brazilian ATLS centers using only artificial models in their courses, the cost for substitution of simulated skins and material reach in average US\$ 200 for 16 participants. In spite of the recent increase in artificial models for procedure training, the use of animal models is frequent, due to costs and higher handling similarity with human tissues when compared with synthetic materials<sup>7</sup>.

Live animal models have been used for a long time in medical education<sup>8,9</sup>. They require a series of maintenance costs related with animal care and ethical issues and may not be advantageous for basic procedures. Use of human cadavers requires ethical considerations and

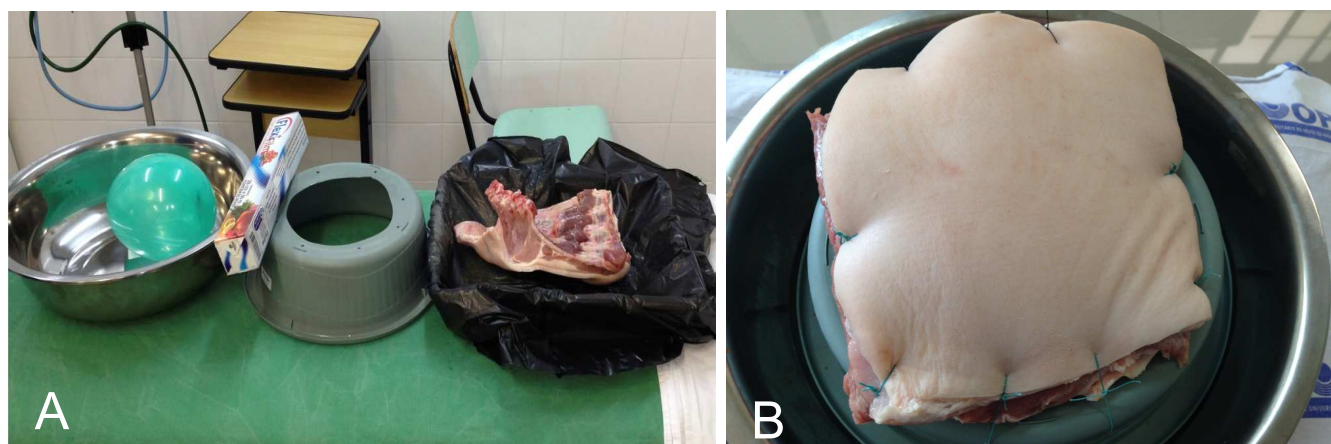


Figure 1 - Materials applied and porcine model ready for use.



**Table 1** - Demographics of the study group.

Characteristics	Medical Students	Residents	All Trainees
Age			
Mean (min-max)	25.7 (22-39)	26.6 (24-31)	25.8 (22-39)
Gender n (%)			
Female	35 (50%)	8 (88.9%)	43 (54.4%)
Male	35 (50%)	1 (11.1%)	36 (45.6%)
Previous experience in CTIP n (%)			
None	9 (12.8%)	1 (11.1%)	10 (12.6%)
1-5 CTIPs	51 (73%)	3 (33.4%)	54 (68.5%)
6-10 CTIPs	10 (14.2%)	5 (55.5%)	15 (18.9%)

CTIP: chest tube insertion procedure.

previous agreements among institutions<sup>10</sup>. Animal segments are cheaper and easier to obtain when compared to whole animal cadavers<sup>7</sup>.

As expected, the analysis of the anatomical correlation of this porcine chest model with human tissues was considered high<sup>11</sup>. Interestingly, the smaller but slightly more experienced group of medical residents attributed higher scores at this criterion when compared with medical students. Even though most of the trainees (87%) had previously participated in chest tube insertion procedures in human patients, they unanimously approved training in the presented model. It may have given the opportunity to review the procedure in a controlled environment.

There are several models using animal segments in training resuscitation skills and chest tube insertion<sup>11,12</sup>. The presented model is simple, reproducible and cheap.

Each training session (average of ten trainees divided in two models) had an average cost of US\$ 15, resulting in an average cost of less than two American dollars per trainee. Due to its simplicity and low cost, this model is attractive in the initial phases of medical training, particularly in centers where resources are limited.

### Limitations

This teaching project was based in a porcine hemithorax, not bringing real anatomic difficulties as hematomas, rib fractures, obesity, pain when manipulating the injured chest, urgency scenario and so on.

The presented porcine model can be improved. Also, it can be tested and validated by physicians with expertise in chest tube insertion. This study was an early step in developing a simple and cheap instrument to be used in medical education.

## R E S U M O

**Objetivo:** descrever e avaliar a aceitação do modelo porcino de baixo custo para drenagem torácica em projeto de ensino médico no oeste do Paraná, Brasil. **Métodos:** um modelo suíno de baixa tecnologia e baixo custo foi desenvolvido em projeto de ensino de drenagem torácica. Alunos de Medicina e médicos residentes receberam instruções teóricas sobre o procedimento e realizaram a drenagem torácica no modelo porcino. Após realizarem o procedimento, os participantes responderam a um questionário sobre o modelo experimental proposto. Esse estudo apresenta o modelo e analisa as respostas ao questionário. **Resultados:** setenta e nove participantes usaram e avaliaram o modelo. A correlação anatômica entre o modelo porcino e a anatomia humana foi considerada alta com média de 8,1+1,0. Todos os participantes aprovaram o modelo porcino de baixo custo para o ensino de drenagem torácica. **Conclusão:** o modelo porcino de baixo custo para drenagem torácica apresentado neste projeto de ensino foi facilmente montado e teve boa aceitação local entre os participantes. Esse modelo tem potencial para ser usado como ferramenta de ensino na educação médica.

**Descritores:** Programas de Treinamento. Educação Médica. Parede Torácica. Modelos Animais. Suínos.

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