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"Human Reproduction, Development and Growth"
XXV Cycle

Director
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PhD Thesis

"The minimally invasive surgery and regenerative medicine: clinical and experimental aspects"

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Background and aim

The field of pediatric surgery has undergone numerous changes throughout the past few years.

First of all the laparoscopic surgery, introduced with reluctance because many of the instruments were not appropriate for their tiny patients. Finally, the often quoted benefits of smaller scars, less pain, and shorter hospital stays had not been shown to be true in the pediatric patient. However, in the mid 1990s, surgeons began to publish their laparoscopic pediatric cases, showing laparoscopy to be a potential alternative for these patients. When pediatric surgeons began to perform laparoscopic procedures on small children and neonates, they were often forced to use instruments designed for adult patients. In the mid 1990s, 2- and 3-mm instruments were developed, which allowed surgeons to work with greater ease in confined spaces. Around the same time, a neonatal insufflator was also developed. In contrast to adult insufflators, neonatal insufflators deliver CO2 in small, controlled puffs. This technology reduced the risk of over-insufflation that was often associated with using the oversized adult insufflators in small children. Over-insufflation can often be accompanied by a significant increase in end-tidal carbon dioxide, or the measurement of the amount of carbon dioxide in the expired air. If this is not adjusted for by the anesthesiologist, overinsufflation can lead to significant pulmonary complications in already fragile neonates. With these advances, more pediatric surgeons are expanding their repertoire of minimally invasive operations.

Not only are they increasing the number of cases that can be performed laparoscopically, but they are also showing that they can be safely performed on neonates weighing 5 kg and less.

In parallel the regenerative medicine was a relatively new field.

This combining tissue engineering and cell transplantation, with the aim of replacing damaged tissues and organs using living cells.

The regenerative medicine could lead to new ways of repairing or replacing injured organs, even during fetal development and therefore even children could benefit from this exciting field. Moreover, particularly in the field of tissue engineering, there has been a remarkable contribution from academic pediatric surgeons such as Anthony Atala and Joseph Vacanti.

Congenital malformations are major causes of disease and death during the first years of life and most of the time functional replacement of the missing or damaged organ (or tissue) remains an unmet clinical need. Tissue engineering led by advances in two specific fields, cell biology and materials science, has combined to create the perfect biological substitution, while materials science and polymer generation, both of natural and synthetic origin, have had constant evolution.

This thesis reports the results obtained during my PhD course in "Human, Reproduction, Development and Growth" (XXV Cycle) from 2009 to 2012.

During the past 3 years I have been focused my research in these main fields following 3 lines of research:

- Evaluation of changes of intestinal adaptation in animal model of short bowel syndrome and the possibility to increase the intestinal surface used a 3D scaffold as guide for the lengthening of the intestinal wall until complete absorption of the same scaffold

- Study of the possibility of increase of the bladder and urethral surface used an animal model and a 3D scaffold as guide until complete absorption of the same scaffold in bladder
- The standardization of laparoscopic techniques in pediatric patients by multicentric study.

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Capitolo 1

Regenerative medicine and tissue engineering

Regenerative medicine is a relatively new field, combining tissue engineering and cell transplantation, with the aim of replacing damaged tissues and organs using living cells. However, stem cell biology and regenerative medicine could lead to new ways of repairing or replacing injured organs, even during fetal development and therefore even children could benefit from this exciting field. Moreover, particularly in the field of tissue engineering, there has been a remarkable contribution from academic pediatric surgeons such as Anthony Atala and Joseph Vacanti. Tissue engineering led by advances in two specific fields, cell biology and materials science, has combined to create the perfect biological substitution, while materials science and polymer generation, both of natural and synthetic origin, have had constant evolution. The possibility of expanding cells (stem cells) outside the body has been the key driver for most of the recent advances in regenerative medicine. Stem cells are defined as unspecialised or undifferentiated precursor cells with the capacity for self-renewal and the ability to give rise to multiple different specialised cell types. They are present in the embryo, fetus, cord blood and many adult tissues where they maintain their undifferentiated status during an entire lifetime. However, their potential changes with timeThe zygote and its first few divisions are totipotent stem cells (able to generate all the body's tissues together with the placenta and cord blood). At the blastocyst stage, pluripotent embryonic stem (ES) cells can give rise to all lineages

belonging to the three germ cell layers. Later on, during fetal development and in adult life, there are only multipotent cells able to give rise to all lineages belonging to a single germ layer or unipotent cells capable of generating only a single committed phenotype.

While pluripotent ES cells are highly proliferative, multipotent adult stem cells have a limited proliferative capacity and therefore their use for therapy may be impaired. However, the latter have an advantage over ES cells in being usable in an autologous setting and, most importantly, to not be tumorigenic.

The biomaterials according with National Institutes of Health (NIH), as "*any substance (other than a drug) or combination of substances synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system which treats, augments, or replaces tissue, organ, or function of the body.*" With the advent of tissue engineering and regenerative medicine in recent years, the definition has broadened to include "*any material used in a medical device intended to interact with biological systems,*" allowing for structures and combination devices that actively interact with the body to be included in the field. Biomaterials can be synthetic (ie, those made by man) or biological (ie, those produced by a biological system). There are different type of biomaterials: *metal, ceramic and polymer*. The last one was object of our study during the last 3 years. Polymers are the broadest classification of biomaterials.

These long chain, organic molecules are versatile in their composition and properties, finding use in surgical tools, implantable devices, device coatings, catheters, vascular nyurafts, injectable biomaterials, and therapeutics. There are several advantages to using polymers for biomedical applications, including relatively low cost, ease of manufacturing, history of use, and versatility. Selection of the appropriate polymer for a clinical application requires the consideration of

several different parameters to achieve the ideal set of material properties. Biodegradable polymers have evolved over the last 35 years. On implantation, these materials are gradually replaced by regenerating tissue in vivo, breaking down into safe products that are subsequently metabolized and/or eliminated from the body. Poly(lactide-coglycolide) (PLGA), polycaprolactone, polyanhydrides, and polyphosphazenes are common examples of synthetic degradable polymers, whereas starch, chitin, collagen, and glycosaminoglycans are examples of natural degradable polymers.

We focused our attenction to two potencial fields of interest in pediatric age: the short bowel syndrome and neurogenic bladder.

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1.1 Short bowel syndrome (SBS)

Intestinal failure is a chronic condition consisting in the bowel's inability to meet all of the body's nutritional needs, which leads to the need for parenteral nutrition for patient survival and growth.

The surgical short bowel is the most frequent cause of intestinal failure and consequent need for total parenteral nutrition – with all the related risks. The earliest descriptions of SBS in the published pediatric studies cite midgut volvulus and intestinal atresia as the most common etiologies, but more recent data suggest that NEC is the largest contributing agent The symptoms are intestinal malabsorption often correlated with the length and function or the residual bowel.

In older children and adults, multiple resections for Crohn's disease can be implicated, as can long-segment resections for ischemic vascular disease, radiation enteritis, malignancy, trauma, and adhesive obstruction. Other causes for intestinal failure include intestinal motility disorders and mucosal enteropathies.

Accurate estimation of SBS incidence and mortality remains difficult due to variation in the SBS definition between studies, lack of comprehensive follow-up, and inability of tertiary institutions to clearly define their study population because of complex referral patterns. Estimates of incidence and mortality would be helpful in enabling health care personnel to appropriately counsel parents, allocate resources, and plan clinical trials.

SBS can be classified into 3 anatomical subtypes: (1) Small intestine resection with a small bowel anastomosis and intact colon; usually, some ileum is preserved. (2) Small bowel resection with partial colon resection and resulting enterocolonic anastomosis. (3) Small bowel resection with

high-output jejunostomy. Type 1 is the best tolerated subtype with the most optimum adaptive potential. Interestingly, it is also the subtype that is most represented in animal models of SBS. Type 2 is more commonly encountered clinically as NEC commonly affects the ileum and

right colon. Type 3 is the most challenging subtype to manage due to the high fluid replacement requirements Massive jejunal resections, on the whole, are better tolerated than large ileal resections. Massive ileal resections tend to result in an inability to resorb both dietary fluid and fluid secreted by the jejunum to maintain iso-osmolality with the enteric lumen. The tight junctions in the jejunal epithelium are more “leaky,” allowing free transit of fluid and salt across the mucosa to maintain an intraluminal concentration of 70-90 mmol/L. The ileal mucosa is much “tighter” with a net absorption of fluid and salt. Additionally, these resections are associated with impairment of resorption of vitamin B12, bile salts, and fatty acids. In turn, disruption of the enterohepatic circulation and the delivery of unabsorbed bile salts into the colon stimulate colonic secretion and promote motility. In time, the colon may adapt to the delivery of large volume of solutes. It will also augment energy absorption through the metabolism of unabsorbed carbohydrate

into short-chain fatty acids by resident bacteria. There is a clear correlation between intestinal length and patient outcome, but there is much more to the equation than absolute bowel length. The region of intestine remaining, the

primary diagnosis, the functional capacity of the residual intestine, the presence of colon, and the age of the patient are all

relevant.

The period following intestinal resection, so, is of fundamental importance for the adaptive response that ensues, which is characterized by intestinal hyperplasia, defined as intestinal adaptation. What is meant by intestinal adaptation is a set of morphological and functional alterations involving the residual intestine after massive resection. There is an immediate response to resection and the primum movens is the loss of intestinal tissue and the wider the resection, the more intense is the adaptive response.

After extensive small bowel resection, the remaining intestine undergoes compensatory changes to maintain its absorptive function consisting of an increase in small intestinal mucosal thickness, villus length, and crypt depth. Intestinal crypt cells activate pathways of gene expression of adaptation and development resembling the developing immature intestinal tissues.

These changes are interpreted as a homeostatic response to increase the remaining digestive-absorptive surface. A crucial factor of adaptation is the time of changes. When a large part of the intestine is suddenly removed, a prompt response is needed to ensure transepithelial ion fluxes, restore the barrier against bacterial translocation, and restart motility. Timely changes are essential for survival; however, intestinal adaptation, its mechanisms, and times are still largely unknown.

Our endpoint heve been: investigation the segment- and time-related changes in rat short bowel syndrome and intestinal adaptation after implant of 3D scaffold in an animal model of short bowel syndrome.

Animal model of short bowel

Wistar rats weighing between 210 and 270 g were used.

The animals were housed in individual cages, under a 12-hour light-dark regimen and humidity and temperature control.

Surgery was performed on animals anesthetized with diazepam, ketamine, and medetomidine.

The length of the small intestine from Treitz ligament to the ileocecal valve was measured in situ, still attached to the mesentery, under constant tension. Then, 75% of the mid-small bowel was resected, leaving the proximal 12.5% corresponding to the remaining jejunum, and the distal 12.5% corresponding to the remaining ileum. A primary end-toend anastomosis was performed and the length of remaining small

intestine ranged from 13 to 20 cm.

Animals received 5% glucose solution after surgery, wetted food from the second day after surgery, and then were allowed free access to food. Postoperative analgesia and the antibiotic enrofloxacin were given and animals' weight was recorded daily.

The animals were fed a normal standard diet with no restriction on food or water supply for 1 week before surgery.

Before surgery, rats were fasted overnight and weighed.

On the first postoperative day, rats had free access to water and rodent diet.

The animals were killed by CO2 inhalation 2, 7, and 15 days after surgery because adaptive intestinal changes reach a plateau 15 days after intestinal resection in rats

The experimental protocol was approved by the local Ethics Committee of the Ospedale Cardarelli (n. 1292/09/CB; February 3, 2009). The animals were treated and housed according to national and international regulations governing the use of animals in scientific research.

Segment- and time-related changes in rat short bowel syndrome

The aim of the present study was to investigate the segment- and time-related changes in rat short bowel syndrome and construct a 4-dimensional (4D) geometrical model of intestinal adaptation.

The results of this study has been published on J*ournal Pediatric Gastroenterology and Nutrition*, see the manuscript below

ORIGINAL ARTICLE: GASTROENTEROLOGY

Time- and Segment-related Changes of Postresected Intestine: A 4-dimentional Model of Intestinal Adaptation

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ABSTRACT

Objectives: The aim of the present study was to investigate the segment- and time-related changes in rat short bowel syndrome and construct a 4-dimensional (4D) geometrical model of intestinal adaptation.

Methods: Sprague-Dawley rats were divided into 3 groups: 2-day, 7-day, and 15-day postresection groups in which 75% of the jejunoileum was removed. Histological and morphometrical parameters in the remaining proximal to distal intestinal segments, from the jejunum to the distal colon, were comparatively evaluated in the groups. The data were used to construct a 4D geometric model in which villi were considered as cylinders, and their surface area was expressed as cylinder lateral area.

Results: Major adaptive changes were observed in the ileum consisting of an increase in both the diameter of base and the height of villi. A parallel reduction in their number/mm^2 was observed. The resulting ileal architecture was characterized by a limited number of large villi. An opposite pattern was observed in the jejunum whose postresection structure consisted of an increased number of villi. No changes were observed in the colon. Postresection restructuring was early and faster in the ileum than in the jejunum resulting in an increase in absorptive area of 81.5% and 22.5% in the ileum and jejunum, respectively.

Conclusions: Postresection adaptation is intestinal segment–specific because all of the major changes occur in the ileum rather than in the jejunum. Sparing ileal segments during resection may improve the outcome of patients undergoing extensive intestinal resection. Our 4D model can be used to test interventions aimed at optimizing postresection intestinal adaptation.

Key Words: 4-dimensional model, children, intestinal adaptation, intestinal failure, surgical short bowel

(*JPGN* 2012;00: 00–00)

Short bowel syndrome (SBS) is a clinical condition resulting from massive enterotomy and is the most frequent cause of intestinal failure in children. In children with extensive intestinal resection, parenteral nutrition is required for survival, but this procedure is associated with frequent and severe complications (1). The length of the remnant intestine and the presence of the ileocecal valve predict the chance of acquiring intestinal sufficiency (1,2). After extensive small bowel resection, the remaining intestine undergoes compensatory changes to maintain its absorptive function (3–5) consisting of an increase in small intestinal mucosal thickness, villus length, and crypt depth (6,7). Intestinal crypt cells activate pathways of gene expression of adaptation and development resembling the developing immature intestinal tissues (8). These changes are interpreted as a homeostatic response to increase the remaining digestive-absorptive surface (9). A crucial factor of adaptation is the time of changes. When a large part of the intestine is suddenly removed, a prompt response is needed to ensure transepithelial ion fluxes, restore the barrier against bacterial translocation, and restart motility. Timely changes are essential for survival; however, intestinal adaptation, its mechanisms, and times are still largely unknown (10–12). In addition, the role and adaptive pattern of proximal to distal intestinal segments are also unknown.

Menge et al found that the total mucosal surface of ileal segments already was increased in the proximal but not in the distal remnants at the fourth day post-resection (13). We tested the hypothesis that intestinal adaptation in SBS is segment- and time-specific and adaptive changes take place with a specific structural and temporal pattern in proximal to distal intestinal segments. To this aim, we studied the qualitative and quantitative changes of proximal to distal intestinal epithelial architecture, including villus area and volume, at 3 time-points after resection, in a model of extensive small bowel resection. Using the morphometric data obtained in the ileum and jejunum, we constructed a 4-dimensional (4D) geometric model of epithelial structure. The model provides an accurate experimental standard for investigations of postresection structural events and can be used to test the effects of nutrients and drugs on adaptation.

METHODS

Experimental protocol: Wistar rats weighing between 210 and 270 g were used. The animals were housed in individual cages, under a 12-hour light-dark regimen and humidity and temperature control. They were divided into 3 groups: resected animals (n = 19), sham-resected control animals that underwent ileal transection with subsequent end-to-end anastomosis (n = 10), and nonoperated control animals (n = 7). The animals were killed by CO_2 inhalation 2, 7, and 15 days after surgery because adaptive intestinal changes reach a plateau 15 days after intestinal resection in rats (14–16).

Surgery was performed on animals anesthetized with diazepam, ketamine, and medetomidine. The length of the small intestine from Treitz ligament to the ileocecal valve was measured in situ, still attached to the mesentery, under constant tension. Then, 75% of the mid-small bowel was resected, leaving the proximal 12.5% corresponding to the remaining jejunum, and the distal 12.5%

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corresponding to the remaining ileum (16,17). A primary end-to-end anastomosis was performed and the length of remaining small intestine ranged from 13 to 20 cm. Animals received 5% glucose solution after surgery, wetted food from the second day after surgery, and then were allowed free access to food. Postoperative analgesia and the antibiotic enrofloxacin were given and animals' weight was recorded daily.

The animals were fed a normal standard diet with no restriction on food or water supply for 1 week before surgery. Before surgery, rats were fasted overnight and weighed. On the first postoperative day, rats had free access to water and rodent diet.

The experimental protocol was approved by the local Ethics Committee of the Ospedale Cardarelli (n. 1292/09/CB; February 3, 2009). The animals were treated and housed according to national and international regulations governing the use of animals in scientific research.

Tissue sampling: bowel samples were taken 2, 7, and 15 days postoperatively. Six proximal-to-distal intestinal segments were obtained from each animal as follows: antrum (1.5-cm proximal to the pylorus); duodenum (3-cm distal to the pylorus); proximal jejunum (3-cm proximal to the anastomosis in the resected group and 10-cm distal to the ligament of Treitz in the control group); distal ileum (3-cm distal to the anastomosis); right colon (10-cm distal to the cecum); left colon (proximal to the rectum). All of the intestinal segments were weighed, measured, and rinsed with ice-cold saline to remove any luminal contents. Tissue sections measuring 1 cm^2 were cut from each segment along the longitudinal axis and used for histological and morphometric analysis.

Histology: intestinal specimens were fixed in 10% buffered formalin for 24 hours, dehydrated and embedded in paraffin wax using standard techniques. Four-micrometer sections were cut perpendicular to the mucosa, placed on gelatin-coated glass, and stained with hematoxylin and eosin. At least 10 well-oriented sections from each intestinal segment were prepared and evaluated by linear quantitative methods using light microscopy. All of the morphometric and counting procedures were performed in blind by 2 independent investigators. The following parameters were obtained for each segment: total wall thickness (micrometers), total mucosal thickness (micrometers), and inflammatory cells in the mucosa (number of cells/mm^2 of mucosa and degree of inflammatory infiltrate). The glandular height in the antrum was also measured and is expressed in micrometers.

Villus height (in micrometers), number of villi (villi/mm^2 of mucosal length), crypt depth (micrometers), and villus/crypt ratio were recorded in the duodenum, proximal jejunum, and distal ileum. In the small intestine, only villi and crypts cut throughout their length were measured. The distance from the tip to the base of the villus was taken as villus height. The distance from crypt base to villus-crypt junction was taken as crypt depth. Total mucosal thickness was measured in the proximal and distal jejunum by calculating the distance from the tip of the villus to the muscularis mucosae. Small bowel thickness was determined by calculating the distance from the villus tip to the serosal extremity of longitudinal muscle.

An adaptive response consisting of an increase in mucosal mass may occur through an increase in the number or in the size of intestinal villi or both. We tested the hypothesis that the adaptive response is segment-specific and that the specificity is associated with different mechanisms of adaptation. To address this issue, we applied the geometry of cylinders to villi and calculated epithelial surface expressed as cylinder surface lateral area. The formula to calculate the surface area is the following: surface area $= 2(\pi r^2) + (2\pi r) \times h$, where h is the height of the cylinder and r is half the measure of the base. Then we constructed a 4D geometric model using form-Z, version 6.0 software (Auto-Des-Sys Inc, Columbus, OH) by fitting the villi measures to obtain a spatial representation of adaptive changes in proximal to distal segments. This software is a 3D modeling program combining solids and surface modeling and is used for the first time to construct a biological model. The 4th dimension is time.

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Statistical analysis: results are means ± standard deviation with significance determined by analysis of variance test at the $P < 0.05$ level.

RESULTS

General Findings

Two rats died 1 day after surgery. Three animals (1 in the control group and 2 in the 15-day resected group) were excluded from the study because a stricture developed at the anastomotic site. At post-mortem analysis, both showed a dilated bowel proximal to the anastomosis. Mean body weight did not differ significantly before and after intestinal resection or between sham-resected and resected rats 2, 7, and 15 days after surgery. Data from nonoperated rats were also identical to the data obtained with the study groups.

Macroscopical Evaluation

The gut reacted to massive resection with a general adaptive response consisting in catch-up growth of the remaining small intestine. Fifteen days after surgery, the small intestine showed an increase in mass of 48.2% ± 7.6% over baseline. The adaptive changes had a well-defined segmental pattern. Figure 1A shows the major macroscopical findings 15 days post-surgery. Changes were clearly evident in the ileum: its weight/cm^2 was almost 2-fold higher than in sham-resected animals. The stomach, the duodenum, and the jejunum underwent minor, not significant modifications, although there was a consistent trend toward an increase in all 3 segments, which could be interpreted as an expression of minimal adaptation. Finally, the proximal and the distal colonic segments were virtually unchanged after small bowel resection 2, 7, and 15 days after surgery compared with sham-resected and control rats. Total bowel thickness showed a similar segmental pattern with the ileum undergoing the most evident changes (Fig. 1B).

Microscopical Evaluation

At microscopic evaluation, intestinal adaptation consisted of an increase in villus height, crypt depth, and mucosal thickness in the jejunum and ileum. The increase in villus height occurred 7 days after surgery in the ileum, whereas villus adaptation ceased 15 days after surgery in the jejunum (Fig. 1C). The crypt adaptive response was a distinct time-dependent process with a peak at day 15 in both ileum and jejunum (Fig. 1C). Two days after surgery, no changes were found in length and crypt depth, in the jejunum, or in the ileum. Overall, the ileum was the major site of intestinal adaptation; the major modifications were observed 15 days after surgery, and consisted of an increase in both villus height and crypt depth. Villus height and crypt depth were significantly increased also in the jejunum albeit to a lesser extent than in the ileum. There were no substantial changes in the stomach, duodenum, or colon (data not shown).

To evaluate whether the different adaptive responses of intestinal segments were linked to segment-specific restructuring of the intestinal architecture, we analyzed the villus/crypt ratio in all of the intestinal segments. This ratio was conserved in all of the intestinal segments and there was a close overlapping of numerical values between resected and sham-resected animals at 15 days post-surgery (Fig. 2).

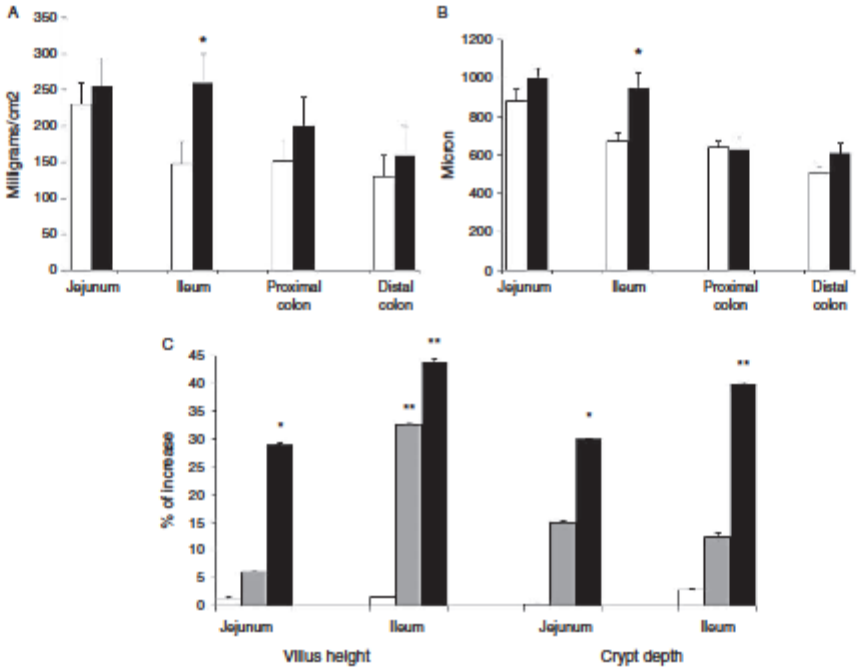

FIGURE 1. A, Total wet weight modifications in different intestinal segments 15 days after extensive small intestinal resection (black bars) compared with sham-resected animals (white bars); $^{*}P < 0.01$ versus sham-resected. B, Total bowel thickness in different intestinal segments 15 days after extensive small intestinal resection (black bars) compared with sham-resected animals (white bars); $^{*}P < 0.001$ versus sham-resected. C, Increase in villus height and crypt depth in jejunum and ileum 2 (white bar), 7 (grey bar) and 15 days (black bar) after extensive small intestinal resection versus sham-resected animals. $^{*}P < 0.05$ versus sham-resected; $^{**}P < 0.01$ versus sham-resected.

Light microscopy did not reveal evidence of inflammatory changes in the ileum or jejunum. This indicates that the observed changes were because of a true compensatory increase in epithelial cell mass, rather than to a change in inflammatory mucosal fluid content.

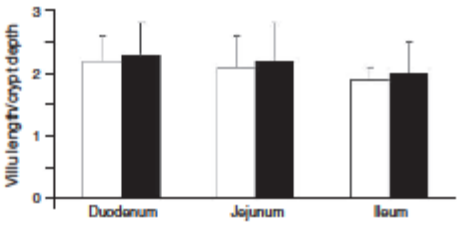

FIGURE 2. Villus/crypt ratio in different small bowel segments 15 days after extensive small intestinal resection (black bars) compared with sham-resected animals (white bars).

Morphometrical Evaluation

The different adaptive pattern in the jejunum and ileum supports the hypothesis that adaptation is the result of distinct mechanisms. To address this issue, we analyzed the segment-specific morphometric changes of the intestinal structure. The number of villi per linear millimeter of mucosa was calculated for each intestinal segment. The area and volume of villi were also measured in all of the segments. Finally, the overall increase of the intestinal surface was calculated for each segment. Minor quantitative and qualitative changes were observed 2 days after surgery, although differences were not significant. Adaptation became progressively more evident at subsequent observations. The number of villi in the ileum was significantly lower in resected animals than in sham-resected animals. In parallel, structural modifications were found in the ileal villus architecture, that is, there was an increase both in the diameter of the villus base and in villus height (Fig. 3). In contrast, the number of villi in the jejunum was similar to that observed in sham-resected animals; however, jejunal villi were longer, whereas the diameter of the villus base remained unchanged (Fig. 3).

We identified a time-related increase in morphometric parameters in the jejunum and ileum (Table 1), which indicates that the time course pattern and the type of architectural restructure

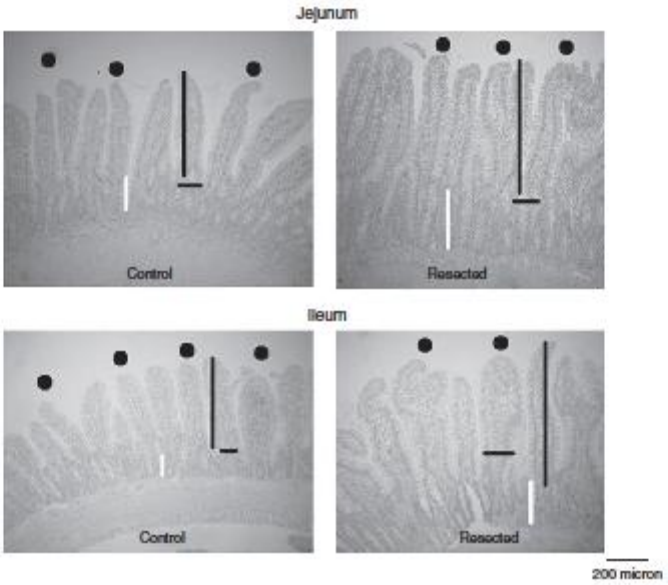

FIGURE 3. Comparative analytical histology of jejunum and ileum 15 days after extensive small intestinal resection compared with sham-resected animals (control). All of the fields are shown at the same magnification (Zeiss, 200× optical magnification). In the jejunum, villus height is increased (black vertical bars). Base diameter is unchanged (black horizontal bars), and crypt depth is increased (white bars). The number of villi is unchanged (black circles). In the ileum, villus height (black vertical bars) and base diameter are increased (black horizontal bars). Crypt depth is (white bars). The number of villi is decreased (black circles).

AQ6

AQ4 were both segment-specific. There were no modifications 2 days after surgery, whereas there was a significant increase in villus height and crypt depth at day 7 post-surgery in the ileum and at day 15 post-surgery in the ileum and jejunum segments. In both segments, the area and the volume of the intestinal surface increased, resulting in an increase of surface area, but the bulk of changes occurred in the ileum, which suggests that the latter possesses the highest adaptive plasticity (Fig. 4). Changes were also faster in the ileum than in the jejunum with an increase of >50% of surface area 7 days after surgery in the ileum versus 5% in the jejunum. The ileal segment located distally to the anastomosis was the major site of adaptive changes, which comprised mainly an increase of total bowel thickness, weight, and volume (Figs. 1 and 4).

Construction of a 4D Model of Segment-specific Intestinal Adaptation

The adaptive changes in jejunum and ileum were associated with a segment-specific restructuring of epithelial architecture. Based on the morphometrical data, a 4D geometric model of adaptation was constructed that provides a view of segment-specific changes (Fig. 5). Compared to sham-resected rats, the number of villi/mm^2 of mucosa did not change in the jejunum, whereas it decreased in the ileum. This observation, together with the changes in bowel thickness, wet weight, height, and diameter of villi, strongly suggests that adaptation takes place in the jejunum and ileum with segment-specific events. In the former, the mucosal

TABLE 1. Comparative morphometric parameters of the adaptive response in jejunum and ileum

	Villus height (mean %± SD)		Crypt depth (mean %±SD)		No. villi/mm^2 (mean %±SD)		Mean absorptive (mean %± SD)	
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
2 days	+1±0.5	0±0.02	0±0.1	+2.8±0.2	−1.4±0.7	−8.1±0.9	+2.54±0.8	+6.5±0.5
7 days	+6±0.1	32.7±0.2*	+15±0.2*	+12.3*±0.9	+1.3±0.2	−19.5±0.5*	+5.06±0.2	+64.97±0.6*
15 days	+29±0.3*	43.9±0.5*	+30±0.1*	+39.8±0.5*	+5.4±0.3*	−20.7±0.1*	+22.54±0.9*	+81.50±0.7*

Data are expressed as % of increase over baseline.
* $P < 0.05$ versus 2 days.

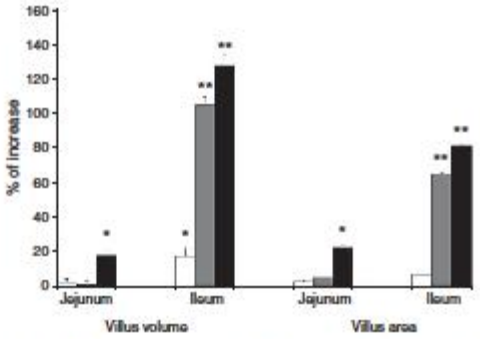

FIGURE 4. Time-course pattern of modifications of villus volume and area in the jejunum and ileum 2 (white bars), 7 (grey bars) and 15 days (black bars) after extensive small intestinal resection versus sham-resected animals. $^{*}P < 0.05$ versus sham-resected; $^{**}P < 0.01$ versus sham-resected.

increase was associated with an increase in villus height, whereas their number and diameter did not change. In contrast, in the ileum, the mucosal increase was the result of a massive increase in villus height and diameter, whereas the villus number decreased (Fig. 5). Villus area and volume were much larger in the distal ileum than in the proximal jejunum; as a result, the nutrient absorptive surface area was strikingly larger in the distal ileum. In contrast, the stomach and colon were not involved in the adaption processes.

DISCUSSION

The total intestinal absorptive surface area is approximately 250m^2 in adult humans; however, there is a functional and structural segmental pattern of the intestine, and distinct intestinal segments have different mechanisms for nutrient absorption and for the transepithelial flux of electrolytes. In SBS, extensive intestinal resection suddenly leads to a dramatic reduction in intestinal surface producing an imbalance of hydroelectrolyte transport and hampered nutrient absorption. Most patients require parenteral nutrition for survival (18,19). In recent years, the anatomic definition of irreversible SBS has changed and, in parallel, the outcome of patients has improved. The longer survival of children with severe SBS provided the opportunity to observe the ability of the remaining intestine to adapt over time (20). Adaptation is affected by the time of refeeding and other clinical variables such as age and nutritional state (21); however, in our rat model, there was a clear segment-specific pattern of adaptation. A major difference in the adaptive responses of jejunum and ileum was the time-related pattern of adaptation. Two days after resection, the mucosal morphology of the rat jejunum and ileum did not differ from that of controls. Adaptation was completed in the ileum within 2 to 7 days, whereas adaptive changes continued in the jejunum at 15 days post-surgery. We do not know whether jejunal changes were completed at 15 days; however, the adaptive response was found to reach a plateau after 2 weeks in similar animal models of short gut (22,23).

The pattern of intestinal adaptation that we observed resembles the intrinsic development of intestine during embryonic development. In human infants, the intestine grows more rapidly during the last trimester of pregnancy, doubling its length by the 40th week or term gestational age (24,25). In contrast, the intestine in rats is immature at birth, but grows rapidly starting from the time of weanling at 18 to 22 days until reaching a plateau at 5 weeks of

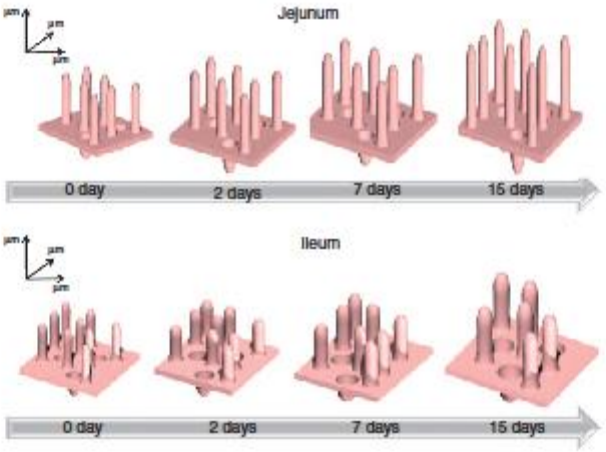

FIGURE 5. Graphic model of the time-related adaptive response observed in the rat short bowel model, showing the mucosal adaptation in the jejunum and ileum. The 4D geometric model was derived using data on villus height and base width and crypt depth as a function of time after bowel resection. It provides a 3D view of the adaptive response after intestinal resection. A differential adaptive response was observed in the jejunum and ileum. In the former, villi height was slightly increased (thin villi pattern). In contrast, in the ileum, the number of villi was decreased, but their volume became very large (large villi pattern).

age (26). In our model, ileal architecture underwent major modifications. A major increase in surface area and volume was associated with a reduction in villi number. This led to an overall increase in villi surface. From the functional point of view, these changes enhance the adaptive response because the ileum is the main site of absorption of liquids, thereby allowing prompt restoration of transepithelial ion fluxes and of nutrient absorption, which obviously is an advantage in terms of postresection structural/functional intestinal modifications.

In an attempt to restore the intestinal functions, the adaptive response is fully functional in terms of the restructuring because there is an increase in absorptive surface area in a relatively short period.

Both drugs and nutrients have been used to stimulate cell growth and proliferation in SBS, namely, insulin (27), growth hormone and GLP-2 (28,29), glutamine, arginine, zinc, and, more recently, lactoferrin (30–32). The 3D model provides a tool to investigate the type and amount of fuel necessary to optimize absorptive changes and promote intestinal sufficiency. The 3D model was approached by other authors. Liao et al (33) constructed a 3D surface model of different gastrointestinal tracts, from the stomach to the colon. Their macroscopic model was developed to study the geometry and the morphology of the visceral organs to evaluate the visceral distention and curvature during stress events. The 3D modeling approach is a quantitative method that could be used as a useful and analytical tool to study the biochemical properties of intestinal mucosa in different physiological and pathophysiological states.

In conclusion, extensive intestinal resection results in a differential adaptive response in the remaining proximal and distal small intestinal segments. The bulk of changes is observed in the ileum distal to the anastomosis, whereas the adaptive response is less evident in the jejunum and does not involve the colon. The responses follow a different time- and segment-related pattern. Our findings show that the ileum plays a major role in postresection adaptation. This is reflected in the better outcome observed in patients with SBS with preserved rather than removed ileocecal valve (34,35). Sparing even small segments of ileum could result in adaptive changes that may be eventually associated with restoration of full intestinal digestive absorptive functions in children undergoing extensive intestinal resection.

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Conclusive remarks

Accurate estimation of SBS incidence and mortality remains difficult due to variation in the SBS definition between studies, lack of comprehensive follow-up, and inability of tertiary institutions to clearly define their study population because of complex referral patterns. Estimates of incidence and mortality would be helpful in enabling health care personnel to appropriately counsel parents, allocate resources, and plan clinical trials. The longer survival of children with severe SBS provided the opportunity to observe the ability of the remaining intestine to adapt over time. Adaptation is affected by the time of refeeding and other clinical variables such as age and nutritional state.

The extensive intestinal resection results in a differential adaptive response in the remaining proximal and distal small intestinal segments. The bulk of changes is observed in the ileum distal to the anastomosis, whereas the adaptive response is less evident in the jejunum and does not involve the colon. The responses follow a different time- and segment-related pattern. Our findings show that the ileum plays a major role in postresection adaptation. This is reflected in the better outcome observed in patients with SBS with preserved rather than removed ileocecal valve. In an attempt to restore the intestinal functions, the adaptive response is fully functional in terms of the restructuring because there is an increase in absorptive surface area in a relatively short period.

Sparing even small segments of ileum could result in adaptive changes that may be eventually associated with restoration of full intestinal digestive absorptive functions in children undergoing extensive intestinal resection.

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Preliminary study of neointestinal rigeneration

The current approaches and knowledge for neointestinal regeneration, unlike intestinal adaptation, are still unsatisfactory. Biodegradable 3D scaffolds, PL/PGA have been used for regenerating the small intestine.

The aim of this study is to evaluate the feasibility of PGA/PL scaffolds for intestinal regeneration in a rat model.

Methods:

50 Wistar rats weighing between 210 and 270 g were used.

The animals were housed in individual cages, under a 12-hour light-dark regimen and humidity and temperature control.

Surgery was performed on animals anesthetized with diazepam, ketamine, and medetomidine.

The length of the small intestine it was not measured in situ, to avoid intestinal manipulation.

A 3D tubulized scaffold was anostomized site-to-site with ileal bowel after a 2 cm of of ileal rection.

Animals received 5% glucose solution after surgery, wetted food from the second day after surgery, and then were allowed free access to food. Postoperative analgesia and the antibiotic enrofloxacin were given and animals' weight was recorded daily.

The animals were fed a normal standard diet with no restriction on food or water supply for 1 week before surgery.

Before surgery, rats were fasted overnight and weighed.

On the first postoperative day, rats had free access to water and rodent diet.

The animals were killed by CO2 inhalation 7, and 15 days after surgery .

The experimental protocol was approved by the local Ethics Committee of the Ospedale Cardarelli (n. 1292/09/CB; February 3, 2009). The animals were treated and housed according to national and international regulations governing the use of animals in scientific research.

Preliminary results

In early experiments, the use of hard silicone stent as tissue scaffold in 11 rats was unsatisfactory for neointestinal regeneration. In later experiments, when a soft silastic tube was used, the success rate increased up to 90.9%. Further analyses revealed that no neointestine developed as macroscopically evaluation has show

10 rats with implant of PL/PGA 3D scaffold died between 6-10 days after surgical procedure.

In 3 rats sacrificed at 7 days an entero- enteric fistula was found.

The macroscopical observation it was not encouraged, but the macroscopic analysis is progress.

1.2 Enterocystoplasty and autoaugmentation: indications and problems

The neurogenic bladder remains as a relevant and unresolved target for regenerative medicine. When patients with spina bifia, spinal cord injury, or other bladder insult develop progressive elevated storage pressure, hydronephrosis, or upper tract injury, surgical reconstruction of the bladder may be necessary to reduce storage pressure and prevent further renal injury.

In fact in conjunction with intermittent catheterization and sphincter-enhancing procedures, detubularized enterocystoplasty imparts additional functional capacity and compliance, and offers the prospect of continence. Reconstructive procedures using intestinal segments have undoubtedly transformed the quality of life of many thousands of patients. However, the benefits of enterocystoplasty carry a price, in the form of significant well-documented complications that include mucus production, stone formation, bacteriuria, metabolic disturbances and intestinal complications. A further concern is that nitrosamines and other carcinogens derived from the interaction of urine and fecal bacteria, which have been implicated in the aetiology of cancers associated with ureterosigmoidostomy, have been identified in the urine of patients who have undergone enterocystoplasty. Whilst there is no evidence of an emerging epidemic of cancer in reconstructed bladders, complacency would be unwarranted in view of the long latency of malignant disease.

The complications of enterocystoplasty can be attributed to the fact that the intestinal epithelium is neither structurally nor physiologically adapted to prolonged exposure to urine. The ideal biomaterial for bladder reconstruction would therefore combine the mechanical properties of a

smooth muscle wall with the barrier characteristics of normal urothelium.

Several alternatives to conventional enterocystoplasty have been explored, which aim to create a reconstructed bladder lined by urothelium.

After many years of experience with augmentation shows evidence of the long-term complications inherent in enterocystoplasty, such as perforations, stone/mucus formation, and cancer risk. Yet, these authors note that, "intestinal cystoplasty still seems to be the gold standard due to the lack of promising alternative options.

Ureterocystoplasty and autoaugmentation

These techniques exploit the use of native urothelium derived either from a grossly dilated ureter or from the bladder itself after excising the overlying detrusor muscle.

Ureterocystoplasty is an attractive and clinically proven concept, but one which is effectively confined to a small minority of patients with gross ureteric dilatation.

Although the long-term functional outcome of autoaugmentation alone has been disappointing, better results have been obtained by combining the exposed autoaugmentation with an overlying segment of demucosalized colon or stomach.

Whilst seromuscular colocystoplasty is a logical approach in relatively normal bladders, it is not suited to small or trabeculate bladders, of the sort commonly encountered in neuropathic dysfunction.

Animal model

The animals were housed in individual cages, under a 12-hour light-dark regimen and humidity and temperature control.

Surgery was performed on animals anesthetized with diazepam, ketamine, and medetomidine.

After midline incision following the vesical ligament, we found the bladder. The bladder will be exteriorized and by a cystocath we will misured its capacity. After a 0,5 x 0,5 cm specimen of bladder will be removed and replaced with o 0,5 x 0,5 cm “2D” scaffold of PCL (polycaproilatone)

Animals received 5% glucose solution after surgery, wetted food from the second day after surgery, and then were allowed free access to food. Postoperative analgesia and the antibiotic enrofloxacin were given and animals’ weight was recorded daily.

The animals were fed a normal standard diet with no restriction on food or water supply for 1 week before surgery.

Tissue engineering and tissue replacement with acellular grafts

The alternative approaches being pursued to find a practical and functional substitute for native bladder tissue can be grouped into the two broad categories of *in vivo* tissue regeneration and *in vitro* tissue engineering .

IN VIVO TISSUE REGENERATION USING ACELLULAR MATRICES

In this approach, which is intended to take advantage of the capability of the bladder to undergo rapid regeneration and repair after acute injury, a biomaterial implanted at the time of surgery becomes cellularized and eventually is assimilated into the tissues of the host bladder. Of all the synthetic and natural materials used to date, decellularized xenogeneic or allogeneic matrices prepared from the submucosa of small intestine and bladder have shown greatest promise.

We waiting for the experimental protocol is approved by the local Ethics Committee of the Ospedale Cardarelli . The animals will be treated and housed according to national and international regulations governing the use of animals in scientific research.

In 6 months 20 Wistar rats weighing between 210 and 270 g will be used.

The animals were housed in individual cages, under a 12-hour light-dark regimen and humidity and temperature control.

Surgery was performed on animals anesthetized with diazepam, ketamine, and medetomidine.

After midline incision following the vesical ligament, we found the bladder. The bladder will be exteriorized and by a cystocath we will misured its capacity. After a 0,5 x 0,5 cm specimen of bladder will be removed and replaced with o 0,5 x 0,5 cm “2D” scaffold of PCL (polycaproilatone)

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analgesia and the antibiotic enrofloxacin were given and animals' weight was recorded daily.

The animals were fed a normal standard diet with no restriction on food or water supply for 1 week before surgery.

Before surgery, rats were fasted overnight and weighed.

On the first postoperative day, rats had free access to water and rodent diet.

The animals were killed by CO2 inhalation 7, and 15 days after surgery.

Conclusive remarks

The use of small-intestinal submucosa (SIS) and bladder acellular matrix (BAM) materials have been investigated in bladder augmentation studies in rodent, canine and porcine models. These studies suggest that both SIS and BAM are biocompatible, biodegradable and facilitate tissue-specific morphological and functional regeneration. Indeed, Kropp *et al.* reported that when SIS was used to augment the bladder after partial cystectomy in dogs, histologically normal bladder tissue regenerated within a month. A more pertinent question is whether the regenerated tissue acquires the functional properties of normal bladder tissue. On this point the evidence is contradictory, as the authors of some quantitative morphometric studies have reported marked graft contraction and decreased muscle content. There are several possible reasons to account for the progressive shrinkage of acellular grafts, including resorption of the matrix, smooth muscle contraction and graft fibrosis. Although the exact cause remains to be established, graft fibrosis is clearly undesirable as it would adversely affect the biomechanical properties and compliance of the regenerated tissue.

The obvious advantage of acellular matrices lies in their potential as an 'off the shelf' solution to reconstructive bladder surgery.

Unfortunately, the processes required to produce a marketable material, e.g. chemical cross-linking and sterilization, may adversely affect the physical properties of the matrix.

The same processes may also deactivate matrix-bound growth factors and other signalling molecules responsible for the regenerative properties of the fresh material.

As yet, there are no published reports that any decellularized matrix has been used in a commercially available form for bladder reconstruction in clinical

urology. In the future, advances in the understanding of the physical and chemical factors responsible for urothelial and smooth muscle cell proliferation, migration, differentiation and function may be exploited in the development of so-called 'smart' biomaterials, in which

biologically active agents are incorporated into acellular natural or synthetic matrices.

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CAPITOLO 2

The role of minimally invasive surgery in pediatric age:

2.1 laparoscopic approach for inguinal hernia: new indications, limits and problems

Inguinal hernia is one of the most common surgical conditions in infants and children. Over the past few decades, inguinal exploration with clear dissection of the hernial sac and secure high ligation of the patent processus vaginalis (PPV) has remained the standard treatment. Inguinal exploration is generally done with a traditional surgical approach. In girls, it can be achieved without any complications, whereas in boys it needs to be performed after separating important elements of the cord and peritoneum and it requires a delicate dissection, in particular, in the first year of life.

At present literature shows few report about some important problems about management of babies affected by inguinal hernia: indication to controlateral exploration, possible repair via laparoscopy in weighing 5 kg or less, indications to laparoscopic approach for incarcerated hernia, and finally the role of laparoscopy in the direct hernia in pediatric patients.

The contralateral access route is widely debated. However, in the first year of life, the possibility of finding a PPV on the contralateral side seems to vary between 50 and 90% of cases. In addition scanty reports exist about the laparoscopic treatment of inguinal hernia in young boys under 1 year of age. The management of the contralateral region in a child with a known unilateral inguinal hernia has been debated for several years. Analyzing the international literature, there are

mainly two procedures to adopt: unilateral inguinal repair and laparoscopic repair. There is no evidence in the international literature about the better procedure to adopt. Although there are numerous articles regarding the data about the incidence of a contralateral patency of the peritoneal-vaginal duct, allowing surgeons to decide whether the inspection or the evaluation of the contralateral region is indicated, the perspective of the child's parents regarding these decisions is rarely reported.

Same problems for hernia repair in infants weighing 5 kg or less: are rarely reported in the international literature the incidence of complications after open inguinal herniotomy among babies weighing 5 kg or less, and described the traditional open herniotomy as a technically demanding procedure that may be associated with an increased risk of hernia recurrence and testicular atrophy.

Recently, few papers reported a preliminary experience in infants of 5 kg using laparoscopic repair. No paper has been published until now about the laparoscopic treatment of premature infants of less than 3 kg.

About the incarcerated hernia the traditional approach is inguinal. However, this technique has several limitations; the dissection of the cord structures is difficult, and the repair of the hernia sac is not easy . Furthermore, inspection of the hernia sac contents after reduction is extremely difficult.

These problems are compounded in infant. Treatment for IIH using open surgery poses unique challenges also for the most experienced pediatric surgeon for the presence of an edematous sac with not well-defined tissue planes.

In addition, open repair of incarcerated inguinal hernia is associated with serious complications .

Misra et al. reported that the postoperative complication rate associated with irreducibility of the hernia can be as high as 50 %, with testicular atrophy occurring in up to 30 % of cases.

About the direct hernia, this is an unusual event in children. Direct hernias are considered rare and usually recurrent after repair of an indirect hernia They are often missed and are correctly diagnosed preoperatively in only 38% of cases. The rarity of this kind of pathology makes the diagnosis difficult Laparoscopy seems to be an excellent diagnostic and therapeutic choice in this rare condition.

Projects

All previous topics were object of multicentric study whit the Centre Hospitalier de Luxembourg, Medical University of Graz, Austria , University Medical Center, Mainz, Germany, Ospedale San Bortolo of Vicenza, Italy.

The data have been published on the international review as follow.

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Laparoscopic Treatment of Inguinal Hernia in the First Year of Life

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Abstract

Background: Laparoscopic inguinal hernia repair is rarely reported in the first year of life. In this article, we report our experience to emphasize the advantages of this procedure in this age range.
Patients and Methods: In a 2-year period, we operated on 315 patients for unilateral inguinal hernia by using laparoscopy. Fifty of 315 patients (15.9%) had less than 1 year of age. This study focused on this group of 50 children (36 boys and 14 girls). The age range was 1–12 months (median, 6.7) with a median body weight of 5.5 kg (range, 3.7–9). As for a technical point of view, we used modified laparoscopic herniorraphy, according to the Montupet technique. After sectioning the sac distally to the ring, the periorifical peritoneum was closed by using a purse-string suture of a nonresorbable suture.
Results: The median operating time was 22 minutes (range, unilateral, 7–30; bilateral, 12–42). All the procedures were performed in a day hospital setting. As for laparoscopic findings in 22 of 50 patients (44%), we found a contralateral patency of the processus vaginalis. In these 22 cases, we performed a bilateral herniorraphy. In 1 girl (2%), we found a coexistence of indirect hernia and a direct hernia on the right side. Both orifices were sutured in laparoscopy. We recorded only 1 minor complication (2%); a problem with needle extraction. With a minimum follow-up of 1 year, we have had only 1 recurrence (1.3%) on 73 herniorraphies performed.
Conclusions: We believe that laparoscopic repair of inguinal hernia in boys under 1 year of age by expert hands is a safe, effective procedure to perform. Its ability to simultaneously repair all forms of inguinal hernias, together with contralateral patencies, has cemented its role as a viable alternative to conventional repair.

Introduction

Inguinal hernia is one of the most common surgical conditions in infants and children.[1] Over the past few decades, inguinal exploration with clear dissection of the hernial sac and secure high ligation of the patent processus vaginalis (PPV) has remained the standard treatment.[2] Inguinal exploration is generally done with a traditional surgical approach.[2] In girls, it can be achieved without any complications, whereas in boys it needs to be performed after separating important elements of the cord and peritoneum, and it requires a delicate dissection, in particular, in the first year of life.[3]

The contralateral access route is widely debated.[4–6] However, in the first year of life, the possibility of finding a PPV on the contralateral side seems to vary between 50 and 90% of cases.[7–9] In addition, the literature reports that about 5–16% of patients operated on for a unilateral hernia need to undergo surgery a second time for a contralateral undetected hernia.[3,9]

Laparoscopy has been used for the diagnosis and/or treatment of pediatric inguinal hernias since the 1990s.[10–12] However, scanty reports exist about the laparoscopic treatment of inguinal hernia in young boys under 1 year of age.[3] The aim of this study was to retrospectively analyze the files of all children under 1 year of age operated on for an unilateral inguinal hernia, using laparoscopy in two italian units of pediatric surgery, to show the advantages of laparoscopic herniorraphy in this age range.

Patients and Methods

In a 2-year period, between January 2006 and January 2008, we operated on 315 patients for unilateral inguinal hernia, using laparoscopy. Fifty of 315 patients (15.9%) were under 1 year of age. This study focused on this group of 50 children.

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In our units, only three surgeons perform laparoscopic herniorraphy, and we analyzed all the procedures performed by these three surgeons in this 24-month period. All three surgeons are skilled laparoscopic surgeons. The age range of patients was 1–12 months (median, 6.7), with a median body weight of 5.5 kg (range, 3.7–9). As for gender, there were 36 boys and 14 girls. All the procedures were performed under general anesthesia. The pneumoperitoneum pressure varied between 6 and 8 mm Hg.

As for a technical point of view, we always used three trocars: an umbilical port of 5–10 mm for the optic of 0 degrees and two 3-mm trocars in triangulation for the operative instruments (two needle drivers and one pair of scissors of 23 cm in lenght). An important technical detail is to position a piece of a 16-Fr nelaton urinary catheter around the cannula of the 3-mm trocars. In this way, we fix the 3-mm trocar cannulas to the skin, thereby avoiding its displacement during the surgical procedure. We used a 4/0 nonresorbable suture with a 3/8 circle needle to suture the sac. As for technical steps that are adopted over time, to perform the herniorraphy, we have used the modified laparoscopic herniorraphy described by Montupet. The technique consists in circumferentially opening the periorifical peritoneum distally to the internal inguinal ring, to pull down the sac to collapse it, and in closing the periorifical peritoneum with a purse-string suture (Figs. 1 and 2). Considering the small diameter of the trocars, the needle was inserted into the abdominal cavity transparietally. As for the extraction of the needle, in the first 15 patients, the needle was extracted, together with the trocar, through the trocar orifice. After a minor complication in 1 patient, the remaining 35 patients underwent needle extraction transparietally.

Results

All the procedures were completed in laparoscopy; no conversion in open surgery was necessary in this series. The median operating time was 22 minutes (range, unilateral, 7–30; bilateral, 12–42). All the procedures were performed in a day hospital setting. As for laparoscopic findings in 22 of 50 patients (44%), we found a contralateral patency of the processus vaginalis. In these 22 cases, we performed a bilateral herniorraphy. As for the side of patency on 22 cases, 14 had a left inguinal hernia with a right patency (63.6%) and 8 a right inguinal hernia with a left patency (36.4%).

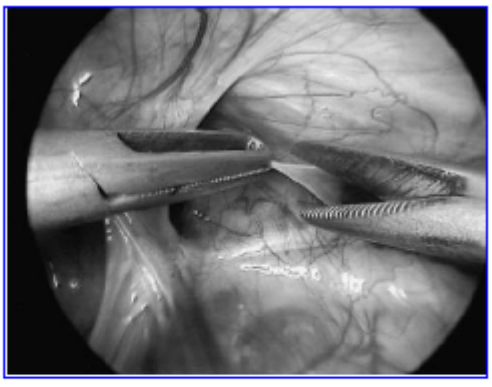

FIG. 1. The first step of the procedure consists in opening circumferentially the periorificial peritoneum.

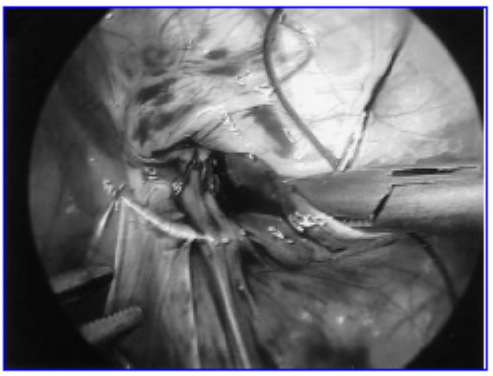

FIG. 2. The periorificial peritoneum is closed at the end of the procedure with a purse-string suture, using a nonresorbable suture.

In 1 girl (2%), we found the coexistence of indirect hernia and a direct hernia on the right side; both orifices were sutured in laparoscopy. Three girls (6%) presented with a dislocation of the ovary into the hernial sac. The ovaries were reduced into the abdominal cavity, and the sac was closed, as previously described. We recorded only 1 minor complication (2%), a problem with needle extraction through the trocar orifice. During the extraction of the needle with the residual suture after trocar extraction, the needle remained blocked in the abdominal wall. The needle was retrieved by using a laparoscopic needle holder and then extracted transparietally.

All children received at least a 1-year follow-up. All patients had a 1-week, 1-month, 6-month, and 1-year follow-up. As for the skin incision, all the 3-mm scars were not visible at 1-year follow-up, except in 2 cases, in which they were slightly discromic. The umbilical scars were not visible in any of the patients. As for the rate of hernia recurrence, we had only 1 recurrence (1.3%) in a total of 73 herniorraphies performed. This premature baby that was operated on for a large, left inguinal hernia (more than 10 mm in diameter) had a recurrence 2 weeks after surgery. He received a redo-surgery, in laparoscopy, 3 weeks after the first procedure. In this case, we performed a two-plane closure. First, we narrowed the internal inguinal ring positioning two separated stitches, between the conjoined tendon and crural arch, and then we closed the periorifical peritoneum with a purse-string suture. No other complications was recorded in our series; in particular, all the testes were of normal size and were well positioned into the scrotum. We have had no iatrogenic ascent of the testes or hydroceles in our series.

Discussion

Inguinal hernia repair is one of the most common operations performed in children. Inguinal exploration has a high success rate and a low complication rate.[2] However, this

treatment is still controversial because of four main aspects: 1) exploration of the asymptomatic contralateral side; 2) incidence of complications related to the possible damage of the vas deferens or spermatic vessels; 3) complications related to surgical technique, such as recurrences of hernia or iatrogenic cryptorchidism, and 4) the possibility of identifying, using inguinal exploration, rare hernias, such as direct or femoral hernias.[13–15] As for the exploration of the contralateral side, in the United State and in France, some pediatric surgeons prefer to perform bilateral approaches in both sexes in patients under 2–3 years of age.[3,16,17] Several articles report that routine bilateral exploration would disclose a contralateral sac in about 50–90% of cases (> 89% in the first year of life), but contend that only a small percentage of these sacs (5–16%) would evolve into clinical hernias.[3,7,8,9,17] In addition, the risk of damaging the vas and vessels is considerably greater in cases of boys under 1 year of age when elements are very small and a thin sac is extremely adherent to them.[9,16,17] However, in open surgery, it is extremely difficult to identify a lesion of the vas deferens, which can occur with a simple compression on it during dissection.

Sparkman states that, even among competent surgeons, there may be a 1.6% incidence of inadvertent removal of a segment of the vas deferens with the hernia sac.[2,17,18] As for the rate of recurrences after inguinal hernia repair when using inguinal exploration, it seems to vary between 0.2 and 0.8%, and whose incidence seems to be higher than 2–3% in boys under 1 year of age.[3,7,16] The incidence of these complications after inguinal open exploration is probably underestimated, especially because the follow-up of a patient who has undergone surgery for an inguinal hernia is rather short, whereas these complications occur much later and may be casually detected during adolescence.[2,9,17,18] For all these reasons, 12 years ago, following the experience of Philippe Montupet, we began to adopt the laparoscopic approach to treat pediatric patients affected by inguinal hernia.[3,9]

At the beginning of our experience, we preferred to avoid treating patients under 8 months of age for technical limitations (3-mm instruments were not available at that time), and because our anesthetists, at that time, had little experience in performing anesthesia for laparoscopy in neonates and infants.[3] Compared to previous experiences reported by our group, we have modified our thoughts on some technical points. First, owing to the easy availability of 3-mm instruments and improved laparoscopic skills, we have begun to operate, in laparoscopy, on a large number of neonates, and, for this reason, our anesthetists have understood that there were no risks, compared to older children, in operating on a boy in the first year of life for an inguinal hernia by using laparoscopy.

In a previous article, we published a series of 933 sacs closed with a recurrence rate of 3.4%. On the basis of our previous experience, we have modified two aspects in our procedure.[8] First, we have adopted the nonresorbable suture to close the sac, and, second, we have standardized the technique by sectioning the sac distally to the internal inguinal ring. In this way, we had only 1 recurrence (1.3%) in this series. This is an important point to emphasize above all else, because we had these results in patients under 1 year of age, in whom the recurrence rate is usually higher than 3–4%.[9,16]

Another point of interest of our study is that we have had no complications related to the dissection phase, mainly because, owing to the magnified view of the surgical field on screen, we avoid touching the vas and vessels, which are so small in this age range.[18–20] Another clear advantage of the laparoscopic procedure to treat the inguinal hernia is to identify and treat rare hernias, as happened in 1 case in our series, with the coexistence of 2 hernias: indirect and direct.[20–23] Probably, the main advantage of treating the inguinal hernia in laparoscopy in the first year of life is the possibility of discovering a contralateral patency (44% in our series) and avoiding the possibility, as can occur after an inguinal herniorraphy, to develop a metachronus, contralateral hernia.[3,9]

In cases of ovarian hernias with an irreducible ovary, in laparoscopy, as had occurred in 3 cases in our series, it is extremely simple to reduce the ovary and close the inguinal defect.[23–25] As for cases of recurrence in our series, we think that in the case of a large, inguinal hernia, with a diameter of the inguinal orifice larger than 10-mm, it is always preferable to perform a two-plane closure, as we have described above.[9] As for the two main criticisms leveled against laparoscopic hernia repair, such as high costs and length of surgery, in our experience, we used all reusable material as in open surgery; second, in our series, operative time was probably shorter or similar to that necessary to repair an inguinal hernia when using the inguinal approach. Among the possible disadvantages of this type of hernia repair, there is the requirement for general endotracheal anesthesia for laparoscopy; however, all children in our series were operated on in a day hospital setting.

Conclusions

We believe that laparoscopic repair of the inguinal hernia in patients under 1 year of age, by expert hands, is a safe, effective procedure to perform. Its ability to simultaneously repair all forms of inguinal hernias (i.e., indirect, direct, combined, recurrent, and incarcerated), together with contralateral patencies, has cemented its role as a viable alternative to conventional repair. However, more patients and longer follow-up are needed to determine the exact recurrence rate in this age range.

Disclosure Statement

No competing financial interests exist.

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Technical Standardization of Laparoscopic Direct Hernia Repair in Pediatric Patients

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Abstract

Background: The aim of this article is to standardize the laparoscopic technique to treat direct inguinal hernia in pediatric patients.
Patients and Methods: In the last 3 years we treated laparoscopically 163 patients with a diagnosis of inguinal hernia. In 7 patients we discovered laparoscopically a direct inguinal hernia. This study is focused on the management of these 7 cases (4 girls and 3 boys; median age 4.6 years). They presented a right defect in 4 cases and a left defect in 3 cases. Six of 7 patients had been already operated for an inguinal hernia and presented a recurrence of the hernia. We used three trocars, 5-mm 0 degree optic, and two 3-mm instruments. In each case, after the resection of the lipoma using the hook cautery, the defect was closed by means of separated stitches. In every case we used the vesical ligament as an autologous patch to reinforce the closure of the defect.
Results: The average operative time was 35 minutes. All the procedures were performed in a day-hospital setting. We had neither conversions nor complications in our series. With a minimum follow-up of 1 year, we had no recurrence.
Conclusions: Laparoscopic identification and repair of direct inguinal hernia in children is a safe and effective procedure to adopt. The key points of the technique are the resection of the lipoma, the closure of the defect using separated, nonabsorbable sutures, and the use of the vesical ligament to reinforce the suture. We believe that in case of recurrence of inguinal hernias after inguinal approach, laparoscopy is the gold standard technique to identify and treat the cause of the recurrence itself.

Introduction

A DIRECT INGUINAL HERNIA is an unusual event in children.[1] Today's knowledge on the incidence of direct hernias is based on old articles published in the international literature and on findings obtained during conventional open surgery.[2,3] Direct hernias are considered rare and usually recurrent after repair of an indirect hernia.[4] In a review by Wright, a direct hernia was present in only 19 cases on more than 1600 hernia operations (1.2%).[1] Direct hernias are rarely identified preoperatively. They are often missed and are correctly diagnosed preoperatively in only 38% of cases.[3,5] The rarity of this kind of pathology makes the diagnosis difficult.[6] Schier et al. reported a series in which they show that probably the incidence of direct inguinal hernia is underestimated.[6,7] Laparoscopy seems to be an excellent diagnostic and therapeutic choice in this rare condition.[8,9] We report our experience in laparoscopic direct hernia repair with the aim of standardizing the technique.

Patients and Methods

In the last 3 years we treated laparoscopically 163 patients with a diagnosis of inguinal hernia. In 7 patients we discovered laparoscopically a direct inguinal hernia. This study is focused on the management of these 7 cases. These 7 patients (4 girls and 3 boys) with an age variable between 3 and 9 years (median 4.6 years) presented a right defect in 4 cases and a left defect in 3 cases. Six of 7 patients had been already operated for an inguinal hernia in another institute via inguinal approach and presented a recurrence of the hernia on the same side. The seventh patient had a general diagnosis of inguinal hernia preoperatively and we discovered the presence of the direct

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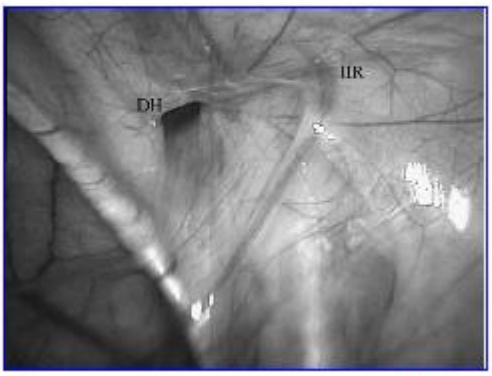

FIG. 1. Laparoscopic appearance of a right DH. DH, direct hernia; IIR, internal inguinal ring.

defect during the laparoscopic procedure. All the patients were operated in general anesthesia with an orotracheal intubation. In each case the bladder was emptied using a Nelaton catheter to prevent the full bladder from covering a medial defect as direct or femoral hernias. Pneumoperitoneum pressure varied between 8 and 11 mm of Hg. We always used three trocars, an optic of 5- or 10-mm 0 degree and two 3-mm instruments. The 20-mm needle (3/8 of circle) was always introduced transparietally with a suture length of 15–18 cm. All the direct defects were larger than 10 mm and in each case we found a big lipoma adherent to the hernia sac (Figs. 1 and 2). The internal inguinal ring was closed bilaterally in all the patients. As for the technical point of view, in every case after the resection of the lipoma using the hook cautery (Fig. 3), the defect was closed by means of separated stitches, using nonabsorbable 2 or 3/0 suture. In each case we used the vesical ligament as an autologous patch to reinforce the closure of the defect (Fig. 4). The use of vesical ligament allows an easy closure of the defect without tension on the defect's borders.

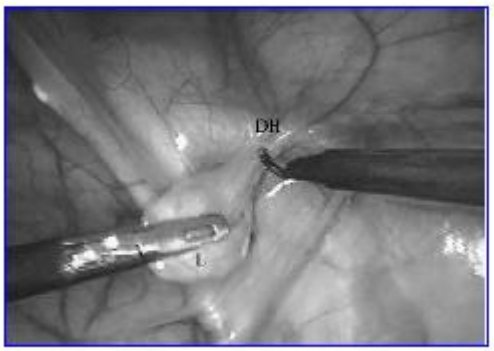

FIG. 3. A key point of the technique is the section of the lipoma (L) using the hook cautery.

Results

All the procedures were completed in laparoscopy without recurring to conversion. The average operative time was 35 minutes (20–60 minutes). All the procedures were performed in a day-hospital setting with an overnight hospitalization. We had neither pre- nor postoperative complications in our series. With a minimum follow-up of 1 year, we had no recurrence in our series, and cosmetic result was excellent in all the patients.

Discussion

Direct inguinal hernia is a rare condition in children.[1] In general, direct groin hernias appear as a recurrence after previous inguinal operations for congenital indirect inguinal hernias.[10,11] Therefore, direct hernias are often missed during the first operation (6/7 cases in our series).[1,2,12] A correct preoperative diagnosis may be difficult and it can be misleading when an inguinal bulge is observed again.[5,7] Recently, articles which focus on laparoscopic inguinal hernia repair are more often published in the international literature and the so-called "rare hernias" are more often identified and treated.[6,7,13,14] We believe, on the basis of our series, that in case of

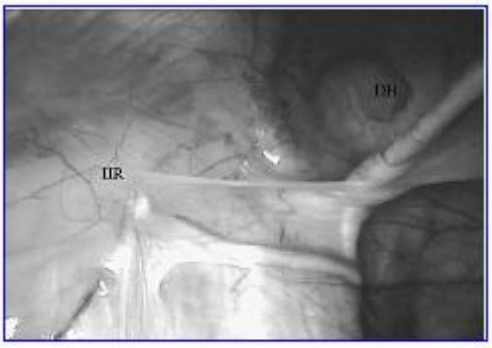

FIG. 2. Laparoscopic appearance of a left DH.

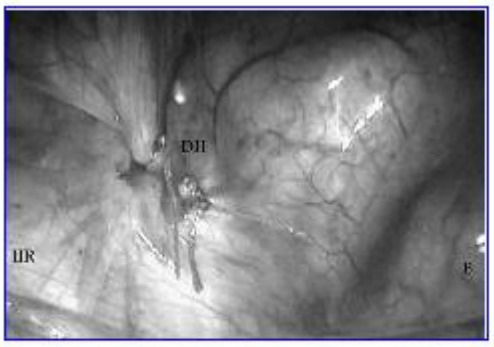

FIG. 4. Laparoscopy view after the closure of the defect.

hernia recurrence after open repair, the cause of recurrence can be a misdiagnosed direct hernia and laparoscopy is the most effective technique to identify and to treat it. We agree that laparoscopic treatment is a safe and valid technique to diagnose and treat recurrent inguinal hernias. It allows an accurate diagnosis of the potential coexistence of direct and indirect inguinal hernias, and it helps to distinguish a true recurrence of an indirect inguinal hernia from a missed direct inguinal hernia (like in 6/7 cases in our series).[7] Further, it avoids an unnecessary exploration of the inguinal canal and the consequent possible risk of iatrogenic injuries to the vas deferens or the spermatic vessels.[2] Based on our 15-year experience on laparoscopic hernia repair, considering that the technique of indirect hernia repair is well-established and standardized, the aim of this article is to standardize the technique of direct inguinal hernia repair.[13] First of all, it is important to remember, for each patient, to empty the bladder before surgery to prevent the full bladder from covering a medial defect as direct or femoral hernias; in our series the bladder was emptied using a Nelaton catheter also if Credè maneuver is as effective as Nelaton use. As for the oblique external hernia, the technique is simple to perform; the first step is the section of the peritoneal vaginal duct distally to the internal inguinal ring circumferentially, and then the closure of the periorificial peritoneum using either a purse string suture or an N-shape suture.[15] As for the direct hernia repair, the technique is different. In fact, first of all in all hernias we found a huge lipoma adherent to the hernia sac; it is wrong to leave the lipoma because it can cause a recurrence of the hernia. For this reason, the reduction of the lipoma into the abdominal cavity and its resection using a hook cautery is fundamental and also easy to perform. As for the defect, the periorificial peritoneum of a direct hernia is more adherent to the defect and it is difficult to perform a purse string suture or an N-shape suture as in the case of oblique external hernia. For this reason it is safer to close the defect using separated stitches. Considering that direct defects, as it happens in our series, are often larger than 10 mm, to avoid tension on the defect's borders after closure, we used the vesical ligament to reinforce the closure. This technique, already described by Lima et al. in 2002, allows to perform a safer closure of the direct defect.[16] In our series, laparoscopy represented a peculiar surgical solution to correct direct inguinal hernia. Another interesting aspect about our study is that all 7 patients were referred to our hospital to be operated in laparoscopy and their parents specifically requested to our group to adopt the minimally invasive technique. It means that laparoscopy techniques are now widespread, also thanks to the Internet, which gives everyone the chance of looking for medical infos. We think that laparoscopy has the advantage of using a uniform, standardized approach for all hernia forms—indirect, direct, femoral, or combinations. This provides, for the first time in surgical history, an objective and reproducible picture/video that is accessible and clear to everyone in the operating room.[6,7] The pictures are easy to interpret for all pediatric surgeons.[6,7] In the previous studies, direct inguinal and femoral hernias had been found less frequently in the open approach than in the laparoscopic approach. Probably some pediatric surgeons had never seen a direct inguinal hernia or a femoral hernia, among hundreds of hernia repairs in the prelaparoscopy era.[6,7,13] Due to the laparoscopic magnification, direct inguinal hernias are easily identifiable and now it is possible to standardize their treatment.[4,6,7]

We believe that direct hernia repair is more demanding, technically, than indirect defect repair; for this reason it is better to treat this pathology only after developing a good skill in intracorporeal knoting. In conclusion, the laparoscopic approach allows for the first time an objective picture of the anatomy and the true incidence of unusual hernias in children.[3,6,7,13] Direct hernias, as our series shows, can be sutured laparoscopically in a safe and efficient manner without recurrence. The key points of laparoscopic direct hernia repair are the resection of the lipoma, the closure of the defect using separated, nonabsorbable sutures, and the use of the vesical ligament to reinforce the suture. We believe that in case of recurrence of inguinal hernias after an inguinal approach, laparoscopy is the gold standard technique to identify and treat the cause of the recurrence itself, which is represented by a direct hernia in a high percentage of cases.

Disclosure Statement

No competing financial interests exist.

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ORIGINAL ARTICLE

Laparoscopic repair of incarcerated inguinal hernia. A safe and effective procedure to adopt in children

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Abstract

Background The purpose of our retrospective study was to describe the efficacy and the advantages of laparoscopic approach to treat incarcerated inguinal hernia (IIH) in pediatric patients.

Methods In a 2-year period, 601 children underwent a laparoscopic inguinal hernia repair, 46 (7.6 %) of them presented an IIH. Our study will be focused on these 46 patients: 30 boys and 16 girls (age range 1 month–8 years).

Results Twenty-one/46 hernias (45.6 %) were reduced preoperatively and then operated laparoscopically (RH), 25/46 (54.4 %) were irreducible and they were operated directly in laparoscopy (IRH). We have no conversions in our series. The length of surgery in RH group was in median 23 min and in IRH group was in median 30 min. Hospital stay was variable between 6 h and 3 days (median 36 h).With a minimum follow-up of 14 months, we had 2/46 recurrences (4.3 %).

Conclusion The laparoscopic approach to IIH appears easy to perform from the technical point of view. The 3 main advantages of laparoscopic approach are that all edematous tissue are surgically bypassed and the cord structures are not touched; the reduction is performed under direct visual control, and above all, an inspection of the incarcerated organ is performed at the end of procedure.

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Introduction

Inguinal hernia remains one of the most common conditions managed operatively by the pediatric surgeon.

Repair in elective conditions is an easy procedure to perform with low risk of complication [1].

On the contrary, inguinal hernia repair in case of incarcerated hernia is known to be a challenging procedure and has been described as one of the most difficult operations in the pediatric surgeon's repertoire [1].

Inguinal hernia repair via inguinal approach has a recurrence rate of 0.2–1 % in the term infants, higher than 2–3 % in the first year of life, while in case of incarcerated hernias, the recurrence rate is of about 15–20 % [2–5].

In the last 15 years, laparoscopy has changed the perception of inguinal hernia with respect to the incidence of contralateral openings, the treatment of recurrences, and the incidence of rare hernias such as direct and femoral hernias [2–6].

Regarding the international literature, a small number of studies have been published on laparoscopic treatment for incarcerated inguinal hernia (IIH).

In a recent paper, Nah et al. [1], comparing laparoscopy and open surgery for IIH repair, state that laparoscopic technique seems to offer more advantages compared to the open technique with a lower incidence of complications.

The objective of our study was to perform a retrospective analysis of the experience, in the laparoscopic IIH repair, of 2 European centers of pediatric surgery to show the advantages of laparoscopy to manage this condition in pediatric patients.

Patients and methods

In a 2-year period in 2 European centers of pediatric surgery, 601 children underwent a laparoscopic inguinal hernia repair, 46 (7.6 %) of them presented an incarcerated inguinal hernia.

Our study will be focused on a retrospective analysis of these 46 patients: 30 boys and 16 girls (age range 1 month–8 years).

All the procedures were performed by only 2 surgeons with a huge experience in laparoscopic surgery.

All the procedures were performed in general anesthesia with orotracheal intubation.

In all the patients, we used 3 trocars (2 or 3 mm instruments).

As for surgical technique, already described by our group in a previous paper, after sectioning the periorificial peritoneum distally to internal inguinal ring, the defect was closed with a 2 or 3/0 non-absorbable multifilament suture (polybutylate-coated polyester) (Fig. 1).

In case of non-reducible hernia preoperatively, the hernia content was gently reduced in laparoscopy with the aid of external manual pressure by the assistant and at the end of procedure, the formerly incarcerated organs were inspected for ecchymosis and peristalsis.

This study received the approval of the Ethical Committee of both centers.

Results

Out of 46, 21 hernias (45.6 %) were reduced preoperatively and then operated laparoscopically (RH) and 25 (54.4 %) were irreducible and were operated directly in laparoscopy without a previous reduction (IRH). For demographic data, see Table 1. The median age at operation was 18 months.

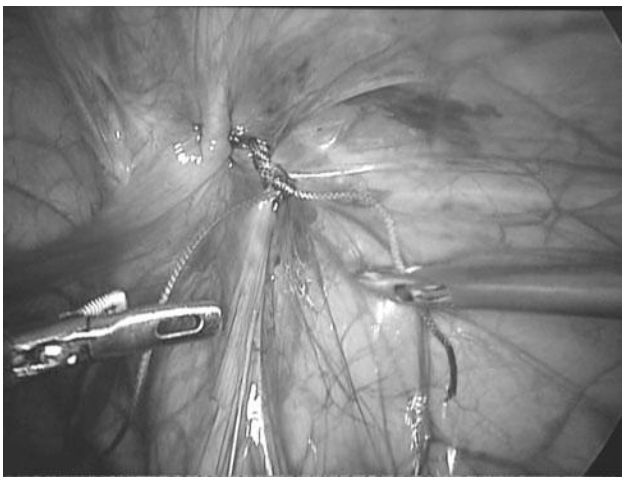

Fig. 1 The appearance of the closed internal inguinal ring at the end of the procedure

Table 1 Demographic data of 46 patients operated in our series

Data analyzed	RH group[a]	IRH group[b]
Number of patients	21	25
Sex	12 boys–9 girls	15 boys–10 girls
Age	1 month–8 year	1 month–8 year
Hernia side	15 right–6 left	19 right–6 left
Weight	4–35 kgs	5–44 kgs
Length of surgery	15–37 min (median 23)	18–41 min (median 30)
Recurrences	1	1

[a] RH group = Hernia reduced preoperatively

[b] IRH group = Hernia not reducible preoperatively and reduced in laparoscopy

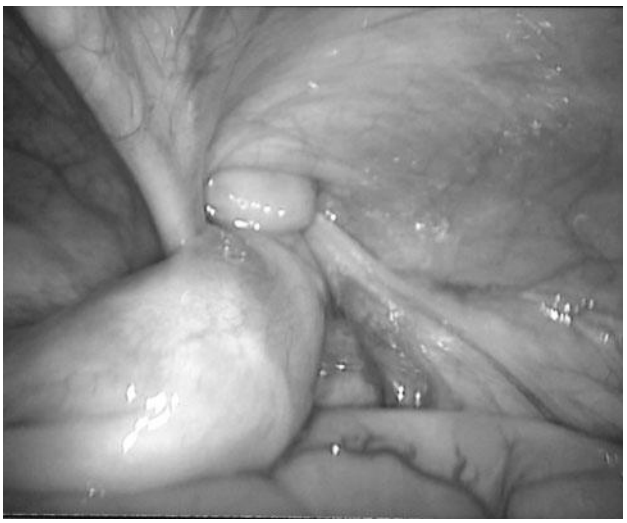

Fig. 2 As for hernia content, in girls, in almost the totality of patients (9/10 in our series), the hernia content is the ovary/annex

In 44 patients, an indirect hernia (oblique external defect) was detected; in 1 patient, a double hernia (femoral/oblique external); and in 1 patient, a direct hernia. In 25 non-reducible hernias (15 boys and 10 girls), the hernia content was: adnex/ovary 9, bowel loops 9, epiploon 5, and appendix 2 (Figs. 2, 3).

All the procedures were completed in laparoscopy without conversions.

The length of surgery in RH group was variable between 15 and 37 min (median 23); in IRH group, the duration was variable between 18 and 41 min (median 30).

Children were offered regular diet 3 h after surgery and were discharged on the same day, with the exception of 3 neonates required to stay 1–2 nights on a monitor, in particular hospital stay was variable between 6 h and 3 days (median 36 h).

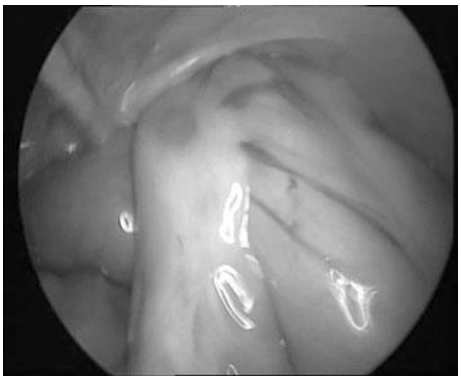

Fig. 3 In 2 cases, the hernia content was represented by the appendix

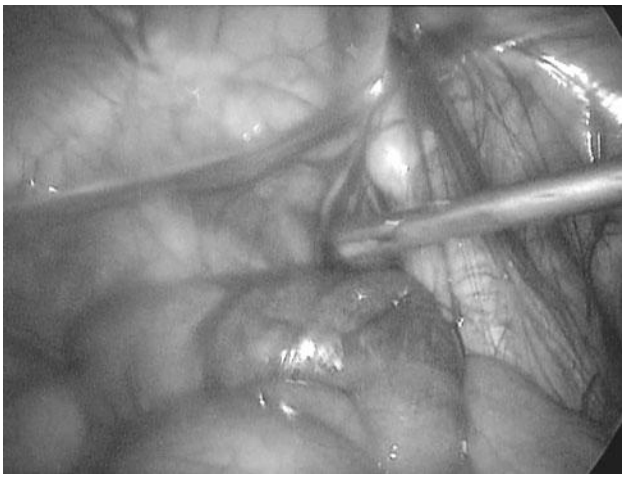

Fig. 4 One of the main advantage of laparoscopic IIH repair is the possibility to perform an inspection of the incarcerated organ at the end of procedure

No complications were recorded in our series.

After reduction, the prolapsed intra-abdominal contents (bowel and ovary) were inspected (Fig. 4). Although looking rather mechanically strained, peristalsis appeared uninhibited in all cases. An obvious mechanical lesion was never seen despite several previous episodes of adequate reductions.

With a minimum follow-up of 14 months (range 14–26 months), we had 2/46 recurrences 4.3 %, both re-operated in laparoscopy.

As for the 2 recurrences, they were 2 infants being 2 and 4 months old. During the laparoscopic re-operation, we found a patency of the peritoneal vaginal duct, which was closed with a suture of non-absorbable material without further follow-up.

In operated boys, all the testes were correctly positioned in the scrotum and we have no testicular atrophy in this study.

Discussion

The traditional approach for incarcerated hernia is inguinal [1, 7]. However, this technique has several limitations; the dissection of the cord structures is difficult, and the repair of the hernia sac is not easy [8].

Furthermore, inspection of the hernia sac contents after reduction is extremely difficult.

These problems are compounded in infant.

Treatment for IIH using open surgery poses unique challenges also for the most experienced pediatric surgeon for the presence of an edematous sac with not well-defined tissue planes.

In addition, open repair of incarcerated inguinal hernia is associated with serious complications [9].

Misra et al. reported that the postoperative complication rate associated with irreducibility of the hernia can be as high as 50 %, with testicular atrophy occurring in up to 30 % of cases [8, 10, 11].

As reported in results section, we have no testicular atrophy in this series.

Using the “conventional” approach, the sac, which is friable, often tears through to the deepinguinal ring, making transfixion of the neck of the hernia difficult [11–13]. This sometimes results in early recurrence of the hernia or in unrecognized lesion of vas deferens during dissection [14, 15].

In addition, the increased risk of testicular ischemia probably is caused by the compression of cord structures within the inguinal canal and by unrecognized damage to the vessels during the herniotomy dissection [9]. Moreover, it is often difficult to reduce the hernia, and there is always the danger of reducing nonviable bowel into the peritoneal cavity [16–18].

As for IIH reduction rate, we have a 45.6 % reduction rate in our series, a lower rate compared with other series already reported in the literature that shows a reduction rate variable between 30 and 80 %. This is probably due because we avoid to perform a too strong reduction in our patients with IIH in order to avoid spermatic cord or loops damages.

As for complications after IIH, Nah reported recently in his comparative study that open inguinal repair presents a higher complication rate (14 %) compared to laparoscopy (4 %) in patients operated for IIH [1]. This author states that avoiding a difficult dissection in the groin and operating under the magnification of the laparoscope would greatly reduce the incidence of vas and vascular injury [8, 19, 20].

The laparoscopic technique appears safe, avoids the difficult dissection of an edematous sac in the groin, allows inspection of the reduced hernia content, and permits the repair of a contralateral patent processus vaginalis if present [21–23].

In fact, the laparoscopic approach to incarcerated inguinal hernia profits from intra-abdominal insufflation, which mechanically widens the internal inguinal ring, thus facilitating reduction.

The procedure allows for direct and unrestricted visual control. All organs involved can be inspected immediately for mechanical damage and for the completeness of reduction. In the same session, the inguinal hernia is repaired laparoscopically [21, 23].

All edematous tissue is surgically bypassed; the cord structures are not touched.

Some interesting considerations can be done on the basis of the results of our study; first of all, it seems that incarcerated hernia is more frequent in boys (65.2 %) than in girls (27 %), and in our study, only in 45.6 % of patients, the hernia was reduced preoperatively, while in 54.4 % the reduction was impossible before surgery.

Probably, this concern was explained by our laparoscopic findings that in the majority of incarcerated hernia, the patency of the internal inguinal ring is very small after reduction.

In fact, the surprising aspect was the small extent of the internal inguinal ring after reduction. As already known from open surgery, evidently, it is not the large opening that produces an increased risk of incarceration and strangulation.

In this series, there is no age limit for correction; in fact, we have operated also patients of 1 month of age, and we think that above all in infants, there are the major advantages for laparoscopic repair, because in this age range, the funicular elements are too small and adherent to hernia sac, and in open surgery, there are important risks of complications (literature reports 30 % of testicular atrophy after inguinal repair of IIH) [21, 23].

As for hernia content, in girls, in almost the totality of patients (9/10 in our series), the hernia content is the ovary/annex, and on the contrary, in boys, hernia content is represented by bowel loops of epiploon also if appendix can be present in the sac as in 2 cases of our series.

After elective inguinal hernia repair in children, recurrence rate is variable between 0.2 and 1 % (this incidence is higher than 2–3 % in boys younger than 1 year), as for elective laparoscopic inguinal hernia repair, recurrence rate is about 1–1.5 %.

In our series of laparoscopic IIH repair, we have 4.3 % of recurrence rate (2/46), this is a higher incidence compared to standard hernia repair (1–2 %), but lower compared to inguinal IIH repair (15–20 %) as reported by Nah and Misra [1, 10].

In addition, we have not had any complications in our series, while the incidence of complications after inguinal repair of IIH reported in the international literature can be higher than 50 % [10, 12, 18, 24].

In conclusion, the laparoscopic approach to incarcerated inguinal hernia appears easy to perform from the technical point of view, probably easier compared to the authors' previous experience using inguinal approach. The 3 main advantages of laparoscopic approach are that all edematous tissue is surgically bypassed and the cord structures are not touched; the reduction is performed under direct visual control, and above all, an inspection of the incarcerated organ is performed at the end of procedure. Our series at the best of our knowledge is the large series of pediatric IIH treated in laparoscopy published in the international literature.

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SHORT COMMUNICATION

Unilateral inguinal hernia: laparoscopic or inguinal approach. Decision making strategy: a prospective study

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Abstract The management of the contralateral region in a child with a known unilateral inguinal hernia is a debated issue among paediatric surgeons. The available literature indicates that the perspective of the child's parents is seldom. This study was performed to evaluate parents' views on this topic. After the Ethical Committee's approval, 100 consecutive patients under 12 years of age with a unilateral inguinal hernia were studied prospectively from March 2010 to September 2010. After an oral interview, a study form was given to the parents about the nature of an inguinal hernia, the incidence of 20 to 90% of a contralateral patency of the peritoneal-vaginal duct and the possible surgical options (inguinal repair or laparoscopic repair). The parents' decision and surgical results were analyzed. Eighty-nine parents chose laparoscopic approach, and 11 parents preferred inguinal exploration. Regarding their motives, all 89 parents requesting laparoscopic approach indicated that the convenience and risk to have a second anaesthesia was the primary reason of their decision. The 11 parents who preferred inguinal approach indicated that the fear of a new surgical technology was their primary reason. Conclusion There is no consensus about the management of paediatric patients with a unilateral inguinal hernia. We believe that a correct decision-making strategy for parents' choice is to propose them the both procedures. Our study shows that parents prefer laparoscopic inspection and repair in the vast majority of cases.

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Keywords Inguinal hernia · Contralateral side · Laparoscopy · Parental views

Introduction

The management of the contralateral region in a child with a known unilateral inguinal hernia has been debated for several years [1]. Analyzing the international literature, there are mainly two procedures to adopt: unilateral inguinal repair and laparoscopic repair [9, 10]. There is no evidence in the international literature about the better procedure to adopt [3, 11, 12].

There are advantages and disadvantages with both approaches. Laparoscopy has the advantage to check and eventually treat a contralateral patency of the peritoneal-vaginal duct (PPVD), but it requires general anaesthesia with orotracheal intubation and a trocar to enter the abdominal cavity [11]. The inguinal approach has been the gold standard for decades, and the anaesthesia is lighter compared to laparoscopy; however, using the inguinal approach, there is the risk of missing a contralateral patency in 20–90% of cases and of a metachronous inguinal hernia in 8–20% of cases [1, 2, 4].

Although there are numerous articles regarding the data about the incidence of a contralateral patency of the peritoneal-vaginal duct, allowing surgeons to decide whether the inspection or the evaluation of the contralateral region is indicated, the perspective of the child's parents regarding these decisions is rarely reported [5, 10]. In our unit, both inguinal and laparoscopic repair are performed, we decided to set up a prospective study to evaluate parents' views on the question regarding their preferred technique to adopt in a child with a known unilateral inguinal hernia.

Patients and methods

After the Ethical Committee's approval, 100 consecutive patients under 12 years of age with a unilateral inguinal hernia seen in our unit were studied prospectively from March 2010 to September 2010. They were 75 boys and 25 girls with a median age of 3.2 years (1 month–12 years). Exclusion criteria were: a bilateral hernia, recurrent hernia, incarcerated hernia and the age limit under 1 month and over 12 years.

At the time of the initial preoperative visit and interview, a study form was given to both parents about the nature of an inguinal hernia, the incidence of 20 to 90% of a contralateral PPVD and the possible surgical options. Three surgical options were discussed with the parents, and they included: repair of the known unilateral inguinal hernia only, repair of the known unilateral inguinal hernia with contralateral inguinal exploration and repair of a PPVD if indicated, or laparoscopic procedure and contralateral laparoscopic repair of a PPVD if indicated.

The parents were given time to read independently the study form, and subsequently, all questions were answered. Additionally, they were told specifically that there was no evidence in the literature on this topic, and in our unit, both procedures (inguinal and laparoscopic) were available, and their preference was requested.

The parents were instructed that both procedures had the similar length of surgery and recurrence rate according to the literature reports. The surgeons never expressed their opinion to avoid influencing the parents' decision, even if often requested by the parents. Two different informed consents were prepared and both parents signed the correspondent informed consent before surgery.

Results

Eighty-nine parents chose laparoscopic approach, and 11 parents preferred inguinal unilateral exploration; no one chose the repair of the known unilateral inguinal hernia with contralateral inguinal exploration and repair of a PPVD if indicated. Regarding their motives, all 89 parents requesting laparoscopic approach indicated that the convenience and risk of having a second anaesthesia was the primary motive of their decision.

As for the 11 parents who preferred inguinal approach, they indicated that the fear of a new surgical technology and/or the insufflation of gas into the abdominal cavity were their primary motives.

As for technical results, all the patients were operated under general anaesthesia, the inguinal group with a laryngeal mask and the laparoscopic group with orotracheal intubation.

As for length of surgery, for the inguinal group, the length of surgery varied from 10 to 30 min (15 median), and for laparoscopic group, the length of surgery varied from 7 to 28 min (17 median). In the laparoscopic group, a contralateral patency was identified and treated in 40 patients (44.9%). In 25/40 cases, the diameter of PPVD was about 5 mm, and in 15/40 cases, about 10 mm. In the laparoscopic group, we identified a direct hernia in two patients (2.2%) (Table 1).

As for laparoscopic technique, we used a 5 or 10 mm 0° optic with two 3-mm trocars in triangulation. We used the laparoscopic repair according to Montupet's technique; after sectioning the periorificial peritoneum distally to the internal inguinal ring, the periorificial peritoneum was closed with a 3/0 suture of non-resorbable material.

In both groups with a minimum follow-up of 1 year, we had no complication and no recurrence of hernia. Cosmetic aspect was good in both groups.

Discussion

Inguinal hernia repair is one of the most common operations performed in children [1, 6, 7]. Inguinal exploration has a high success rate and a low complication rate.

However, this treatment is still controversial because of the four main aspects: (1) the exploration of the asymptomatic contralateral side, (2) the incidence of complications related to the possible damage of the vas deferens or the spermatic vessels, (3) the complications related to the surgical technique, such as recurrences of hernia or iatrogenic cryptorchidism and (4) the possibility to identify, using inguinal exploration, rare hernias such as direct or femoral

Table 1 Summary of the results of our prospective study on a series of 100 patients with unilateral inguinal hernia analyzed

Procedures proposed to the parents	Laparoscopic approach	Unilateral inguinal exploration	Bilateral inguinal exploration
Parental decision	89/100 patients	11/100 patients	0/100 patients
Anaesthesia	Orotracheal intubation	Laryngeal mask	NA
Length of surgery	7–28 min	10–30 min	NA
Contralateral patency	40/89 patients (44.9%)	NA	NA
Complications/recurrence	0/100 patients	0/100 patients	NA

NA data not available

hernias [3, 8, 10]. Several papers report that routine bilateral exploration would disclose a contralateral sac in about 20 to 90% of cases (>89% in the first year of life), but contend that only a small percentage of these sacs (8 to 20%) would evolve into clinical hernias [3, 6].

In the last decade, a lot of papers were published on the results of laparoscopic repair of inguinal hernia, and it seems that this technique gives similar results compared with inguinal repair [3, 10, 12]. However, analyzing the international literature, there is no evidence that one of the two procedures is preferable in paediatric patients with unilateral inguinal hernia [3, 6, 10].

Our surgical team with a large experience in laparoscopic hernia repair, together with our anaesthetists, thought to organize a prospective study to evaluate parents' preference in order to plan a decision-making strategy to adopt in children and infants with a unilateral inguinal hernia. We created a form to be shown to the parents, which described the nature of inguinal hernia and the different surgical treatments to adopt. Then, two different informed consents were created: the first one for laparoscopy and the second for inguinal approach.

We explained to the parents that in laparoscopy there is the possibility of evaluating the patency of contralateral side. In case of patency, we explained to the parents that their child could have developed a metacronous inguinal hernia (for larger PPVD) or a hydrocele (for smaller one), and for this reason, thanks to laparoscopic view, we could close the contralateral size to prevent hernia or hydrocele formation.

This study was approved by the Ethical Committee of our university. A similar study had already been performed by Holcomb III et al. (2004), but in this study, the surgeons did not offer to the parents the laparoscopic repair but only a unilateral hernia repair with laparoscopic evaluation of the contralateral region through the ipsilateral hernia sac [5]. The main characteristic of our study is to offer to the parents the possibility of deciding the technique to adopt for hernia repair after illustrating them the advantages and disadvantages of both procedures.

When given the information about the possibility of a PPVD on the opposite side, in this study, 89% of the parents requested laparoscopic repair (non capisco bene cosa vuoi dire modifica) [3, 10].

Regarding their motives, all 89 parents requesting laparoscopic approach indicated that the convenience and risk of having a second anaesthesia was the primary motive of their decision. As for the 11 parents who preferred inguinal approach, they indicated that the fear of a new surgical technology together with the fear of the insufflation of gas into the abdominal cavity were their primary motives.

We think that, when for a given paediatric pathology, there are different treatments, as in inguinal hernia, it is important before performing the operation to explain to the parents the pathology itself and the different techniques to treat it, and this is an important procedure to be taken also by paediatricians. In fact, we think that the message of our study is extremely important also for paediatricians who are the first to diagnose an inguinal hernia and meet the parents before surgeons. Our study shows that when we presented options regarding the management of a unilateral inguinal hernia, parents preferred laparoscopic inspection and repair in 89% of cases.

Disclosure statement No competing financial interests exist.

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ORIGINAL ARTICLE

Laparoscopic inguinal hernia repair in premature babies weighing 3 kg or less

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Abstract

Purpose This retrospective study aims to evaluate the feasibility, safety and complication rate of laparoscopic inguinal hernia repair for small babies weighing 3 kg or less.

Methods A retrospective analysis was performed on the surgical charts of 67 infants (47 boys and 20 girls) weighing 3 kg or less who underwent laparoscopic hernia repair in a 3-year period. A regular 5-mm scope was used for visualization, and 2 or 3-mm instruments were used for the closure of the inner inguinal ring using 3/0 non-absorbable suture. The median weight at surgery was 2,600 g (range 1,450–3,000 g). All except three were premature.

Results Of the 67 infants, 15 (22.3 %) presented with an irreducible hernia. In three cases of irreducible hernias, we also performed a transumbilical appendectomy at the end of the hernia repair. Minor problems related with anesthesia were noted in four cases. Hernia recurrence was observed in three patients (4.4 %). No cases of testicular atrophy occurred. In 10 boys, we observed 12 cases of high testes, only 4 testes requiring subsequent orchiopexy.

Conclusions Laparoscopic inguinal hernia repair for babies weighing 3 kg or less is feasible, safe and perhaps even less technically demanding than open inguinal herniotomy.

Keywords Babies · Premature Infants · Hernia repair · Laparoscopy

Introduction

Inguinal hernia repair is one of the common surgical procedures performed in pediatric surgery. The last decade has seen a significant controversy concerning the value of laparoscopic hernia repair in children. However, the literature has recently contained an increasing number of reports on laparoscopic hernia repair in children, highlighting an increase not only in the number of cases that have been managed, but also in the number of modifications and technical refinements in the procedure itself [1–8].

Papers focused on hernia repair in infants weighing 5 kg or less are rarely reported in the international literature [9–13].

Nagraj et al. [14] reported the incidence of complications after open inguinal herniotomy among babies weighing 5 kg or less, and described the traditional open herniotomy as a technically demanding procedure that may be associated with an increased risk of hernia recurrence and testicular atrophy.

Recently, few papers reported a preliminary experience in infants of 5 kg using laparoscopic repair [9]. No paper has been published until now about the laparoscopic treatment of premature infants of less than 3 kg.

This retrospective study aims to evaluate the feasibility, safety and complication rate of laparoscopic inguinal hernia repair for premature babies weighing 3 kg or less in two European centers of pediatric surgery.

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Patients and methods

A retrospective analysis was performed on the surgical charts of 67 infants weighing 3 kg or less who underwent laparoscopic hernia repair in two European centers of pediatric surgery in a 3-year period. The median weight at surgery was 2,600 g (range 1,450–3,000 g). All except three were premature.

A regular 5-mm scope was used for visualization, and 2 or 3-mm instruments were used in triangulation to have a better ergonomics.

All the procedures were performed by only two surgeons with a huge experience in laparoscopic surgery and particularly in laparoscopic hernia repair. All the procedures were performed in general anaesthesia with oro-tracheal intubation. As for the technique of laparoscopic hernia repair, after sectioning the peri-orificial peritoneum distally to the internal inguinal ring, the defect was closed with a 3/0 non-absorbable multifilament suture (polybutylate-coated polyester) with a 17 or 20-mm needle. We always introduce the needle transparietally and extract the needle through the umbilicus at the end of the procedure.

Results

Of the 67 infants (47 boys and 20 girls; 55 bilateral, 10 right-sided, 2 left-sided hernias) 15 (22.3 %) presented an irreducible hernia. In these 15 cases of non-reducible hernias (12 boys, 3 girls), the hernia content were: eight bowel loops, five colon/appendix, and two adnex/ovary. In case of non-reducible hernia preoperatively, the hernia content was gently reduced in laparoscopy with the aid of external manual pressure by the assistant, and at the end of procedure the formerly incarcerated organs were inspected to check their vascularisation and peristalsis. In three cases of irreducible hernia, after reduction of the hernia content, the appendix seemed to be not well vascularized and for this reason, we performed a transumbilical appendectomy at the end of hernia repair. The median operative time for hernia repair was 22 min (14–38 min). No serious intraoperative surgical complications occurred. No post-operative infections occurred. Minor problems related with anesthesia were noted in four cases. In particular, in these four cases the babies had a slow recovery after the mioresolution at the end of anesthesia and they were left intubated 1–2 h after the end of procedure.

All the babies started feeding on the same day of procedure, except those for whom we performed an appendectomy. These babies started feeding the day after surgery. All the patients were discharged from hospital 1–4 days after surgery (median 2 days).

After a median follow-up period of 30 months (range 6–52 months), all the children were clinically examined. Hernia recurrence was observed in three patients (4.4 %). All these children were re-operated in laparoscopy. No cases of testicular atrophy occurred. In 10 boys, we observed 12 cases of high testes, only 4 testes requiring subsequent orchiopexy at 1 year of age. It is important to underline that all these 12 patients presented a testis positioned in high scrotal position at the moment of the intervention, but we decided preoperatively together with the parents to correct this condition at 1 year of age.

Discussion

Inguinal hernia is one of the most common surgical conditions in infants and children. Over the past few decades, inguinal exploration with clear dissection of the hernial sac and secure high ligation of the patent processus vaginalis have remained the standard treatment. Inguinal exploration is generally carried out with a traditional surgical approach. However, the literature has recently contained an increasing number of reports on laparoscopic hernia repair in children, highlighting an increase not only in the number of cases that have been managed, but also in the number of modifications and technical refinements in the procedure itself [1–8].

To the best of our knowledge, to date our study represents the largest series of infants weighing 3 kg or less who had inguinal hernias repaired via laparoscopic surgery.

Nagraj et al. [14] recently reported on early follow-up data for a similar group of patients weighing about 5 kg, who underwent an open inguinal operation. These authors state, like many previous authors, that primary inguinal herniotomy in neonates of low weight is a technically demanding procedure and that the procedure is associated with an increased overall rate of complications compared to the rate for older children who underwent the same procedure [15]. The results of the traditional open inguinal herniotomy, at least for children of low body weight, are characterized by significantly high rates for recurrence, testicular hypotrophy, and atrophy or high testes that requires subsequent orchiopexy [16–18].

In the current study, we did not observe a single case of testicular atrophy, although the condition probably is less likely to be caused by the surgical technique than by incarceration [19–21].

High testes requiring subsequent orchiopexy occurred in 5.9 % (4/67 patients) of our boys undergoing laparoscopic hernia repair. It is important to underline, as we already reported in results section, that all these patients presented a testis positioned in high scrotal position at the moment of the intervention but we decided preoperatively together with the parents to correct this condition at 1 year of age as it is reported in the guidelines of the management of undescended testis.

Another interesting finding of our study is that we had no post-operative infections. We think that this is an interesting point to discuss, in fact it is an advantage of laparoscopic hernia repair because laparoscopic scars are located higher compared to inguinal scars that are inside the diaper area, and for this reason they are subject to urine or fecal contamination with a higher infection rate compared to laparoscopic scars that are located outside diaper line in a safer zone. In fact, we reported fewer wound infections compared to the infants of similar age operated through inguinal way (0 vs. 2.3 % by Nagraj et al.) [14]. As for technical point of view, probably, this procedure is easier but technically demanding for the surgeon because he has to be able to knot in a very small space; hence it is useful to perform one or two enemas the day before surgery to empty intestinal loops and to have a larger space into the abdominal cavity to move instruments. As for trocars position, in small infants we do not have a true triangulation between optic and trocars because the two operative trocars are located higher compared to usual position, exactly they are positioned on the umbilical line at the same level of the optic to have more distance from trocars and the internal inguinal ring. As for the needle size, it is difficult to manage large needle in premature infants, probably the needle length has to be maximum of 17–20 mm, because larger needles are difficult to manage with a higher risk of complications. Probably, one of the main advantages of this procedure is in the case of irreducible hernia. In fact thanks to laparoscopic traction and the manual pressure from outside, it is easy to reduce hernia content and in case of problems of bad vascularization of appendix or loops it is easy to exteriorize through the umbilicus the specimen to perform a resection as happened in three cases of appendectomy in our series.

Laparoscopic hernia repair in small babies is not only easier for the surgeon but certainly it is also safer for the patient. In fact the internal ring is reached in the open technique by opening the inguinal canal and dissecting the hernial sac from the "very thin" cord structures. Laparoscopic surgery approaches the internal ring without any dissection of the abdominal wall or spermatic cord structures. In our experience, this approach is the easiest in terms of its technical requirements for infants and babies. In this population of patients, there is a risk of bowel distension, leading to visual restriction and a smaller operative field in which to handle needle-drivers, but pre-operative enemas can solve this problem. Serious intraoperative complications such as bladder injury or rupture, which can occur in open surgery with excessive mobilization of the sac, are unlikely with the laparoscopic technique due to superior visualization of the anatomic structures.

In the cases reported by Miyano et al. and Chung and Yu [22, 23], even after the initial successful surgical management of bladder injury caused by open herniotomy, some children experienced long-term, serious complications and the development of bladders with small capacity. Miyano et al. [22] reported the need for sigmoido-colocystoplasty for bladder augmentation. The same authors concluded that inguinal hernia repair (the open technique) may be difficult, even in the most experienced of hands, and can result in complications, especially in small infants.

Age, size, and weight of the child are not limiting factors for the laparoscopic approach in terms of surgical technique. Anesthetists tend to object to laparoscopy for small children considering the risk of opening right-to-left-shunts due to the increased intra-abdominal pressure. We have reflected on our subjective observations of various surgeons in the years since laparoscopy was first established in our unit. We believe that despite a steep learning curve for our pediatric anesthesiologists, patient safety has increased generally as laparoscopy has become a routine procedure. However, we report minor problems related with anesthesia in four patients in our series. In particular, in these four cases the babies had a slow recovery after the mioresolution at the end of anesthesia, and they were left intubated for 1–2 h after the end of procedure.

As for the three recurrences, they always occurred in boys and they were always located on the medial part of the internal inguinal ring. We reoperated the three patients in laparoscopy and we reclose the internal inguinal ring without further recurrences. The take-home message is that it is important to close well the medial part of the ring in particular the peritoneum between the inner spermatic vessels and the vas, because this is the location of our recurrences.

In conclusion, laparoscopic inguinal hernia repair for babies weighing 3 kg or less is feasible, safe, and perhaps even less technically demanding than open inguinal herniotomy. We found no evidence of testicular atrophy or serious intraoperative complications. The rates for recurrence and secondary high testes were similar or lower to those with the open technique quoted in the international literature [16–18, 24].

In our opinion, the laparoscopic approach seems to be better than the traditional open herniotomy for small babies, at least in terms of overall procedural benefit.

Conflict of interest We have no conflict of interest.

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Conclusive remarks

Inguinal hernia is one of the most common surgical conditions in infants and children. Over the past few decades, inguinal exploration with clear dissection of the hernial sac and secure high ligation of the patent processus vaginalis (PPV) has remained the standard treatment. Inguinal exploration is generally done with a traditional surgical approach. In girls, it can be achieved without any complications, whereas in boys it needs to be performed after separating important elements of the cord and peritoneum and it requires a delicate dissection, in particular, in the first year of life.

The studys reported in this thesis show laparoscopic repair of the inguinal hernia in patients under 1 year of age, by expert hands, is a safe, effective procedure to perform. Its ability to simultaneously repair all forms of inguinal hernias (i.e., indirect, direct, combined, recurrent, and incarcerated), together with contralateral patencies, has cemented its role as a viable alternative to conventional repair. However, more patients and longer follow-up are needed to determine the exact recurrence rate in this age range.

As for direct inguinal hernia, a rare condition in children, and often missed during the first operation (6/7 cases in our series) is better to treat via laparoscopy, only after developing a good skill in intracorporeal knoting. In fact the laparoscopic approach to incarcerated inguinal hernia appears easy to perform from the technical point of view, probably easier compared to the authors' previous experience using inguinal approach. The 3 main advantages of laparoscopic approach are that all edematous tissue is surgically bypassed and the cord structures are not touched; the reduction is performed under direct visual control, and

above all, an inspection of the incarcerated organ is performed at the end of procedure.

I think that, when for a given paediatric pathology, there are different treatments, as in inguinal hernia, it is important before performing the operation to explain to the parents the pathology itself and the different techniques to treat it, and this is an important procedure to be taken also by paediatricians.

Also for babies weighing 3 kg or less is feasible, safe, and perhaps even less technically demanding than open inguinal herniotomy. We found no evidence of testicular atrophy or serious intraoperative complications. The rates for recurrence and secondary high testes were similar or lower to those with the open technique quoted in the international literature.

So age, size, and weight of the child are not limiting factors for the laparoscopic approach in terms of surgical technique.

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2.2 Laparoscopic approach for Varicocele

Varicocele is generally considered the most common correctable cause of male infertility. It is indicated as a factor in about one third of infertile males, being associated with testicular atrophy and abnormal seminal factors .

The term varicocele indicates a dilatation of the testicular veins in the pampiniform plexus caused by venous reflux. This reflux can impair the countercurrent heat exchange mechanism within the spermatic vessels, thus causing an increase in testicular and scrotal temperature. The abnormally high temperature can subsequently lead to a progressive dysfunction of the testicle and epididymis].

Therefore, testicular atrophy and infertility can be direct consequences of varicocele.

Varicocele mainly occurs on the left-hand side (80– 90% of cases). However, bilateral lesions are reported in up to 20% of cases and right-sided lesions in approximately 7% .

The incidence among men is 15%, and it is 20–40% among men presenting to infertility clinics.

Varicocele is a rare disorder in children, with an average reported incidence of approximately 5%. Although rare before puberty (<1%), the incidence of varicocele in postpubertal children is similar to that of adulthood.

Several treatment options have been used, including spermatic vein sclerotherapy or embolization, open inguinal ligation of the spermatic vein, subinguinal microscopic varicocelectomy, and, most recently, laparoscopy, but the ideal method for varicocele treatment is still

controversial . The ideal procedure should perform a varicocelectomy with a low rate of recurrence, hydrocele formation, and testicular atrophy. The indication for varicocelectomy includes testicular volume discrepancy, chronic scrotal pain, and infertility.

Use of Blue Patent Lymphography to prevents hydrocele

Varicocele is a relatively common disorder in children, with an average reported incidence of approximately 15% in the prepubertal group. Varicoceles can lead to testicular hypotrophy and infertility; therefore, surgical treatment is frequently required in the pediatric age group. Multiple methods exist for the treatment of varicoceles, including sclerotherapy and open and laparoscopic surgical ligation of the spermatic vessels. With recent advances in minimally invasive surgery, there have been many reports showed the safety and efficacy of Palomo laparoscopic repair. Whichever treatment is chosen, postoperative complications are fairly common, such as recurrence, persistence, hydrocele, and testicular atrophy. In particular, with Palomo repair the incidence of persistence/recurrence of varicocele seems lower than 5%, and the incidence of postoperative hydrocele seems to be higher than 30%.

The lymphatic-sparing (LS) laparoscopic Palomo procedure of injecting isosulfan blue into the scrotum is one of the surgical options that has gained popularity in the last 10 years because it seems to reduce the occurrence of postoperative hydrocele with better identification of the lymphatic vessels.

However, based on an analysis of the international literature, only small series with a short or an intermediate follow-up have been published.

Togheter the centre of Vicenza we tested the hypothesis that the laparoscopic Palomo procedure with use of blu patent limphography prevent the post-operative hydrocele.

The study see below is published in *Journal Of Laparoendoscopic & Advanced Surgical Techniques* shows long term results.

JOURNAL OF LAPAROENDOSCOPIC & ADVANCED SURGICAL TECHNIQUES
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IPEG 2012 Paper

Blue Patent Lymphography Prevents Hydrocele After Laparoscopic Varicocelectomy: 10 Years of Experience

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Abstract

Purpose: Laparoscopic varicocelectomy according to the Palomo technique is the most common procedure adopted in children with testicular varicocele. This procedure involves the ligation of the internal spermatic cord and is associated with a 3%–5% incidence of recurrence and up to 30% incidence of hydroceles. We sought to determine the impact of lymphatic preservation on hydrocele formation and the success of varicocelectomy.
Patients and Methods: We retrospectively evaluated 396 patients with a mean age of 13.2 years who underwent laparoscopic varicocelectomy. Patients were divided into two groups: those who underwent a lymphatic-sparing (LS) procedure using isosulfan blue scrotal intra-dartoic injection and those who underwent a non-LS (NLS) technique. The incidences of recurrence/persistence and postoperative hydrocele formation requiring surgery or aspiration were analyzed statistically using the chi-squared test.
Results: Of 396 patients, 244 received a laparoscopic LS procedure, and 152 received an NLS operation. The LS patients in whom the lymphatic vessels were not identified (26/244 [10.6%]) were considered NLS repairs. The follow-up was at least 12 months. LS surgery (218 patients) was associated with a decreased incidence of postoperative hydrocele (0/218 [0%] versus 18/178 [10.1%]; chi-squared test = 25.84, difference statistically significant). There was no significant difference in incidence of persistent or recurrent varicocele requiring reoperation following the initial procedure (5/218 [2.2%] versus 5/178 [2.8%]; chi-squared test = 0.41, difference statistically not significant).
Conclusions: Laparoscopic LS varicocelectomy using isosulfan blue is preferable to laparoscopic Palomo repair that does not preserve the lymphatics. It has a significantly lower incidence of postoperative hydroceles and still maintains a low incidence of persistence/recurrence.

Introduction

VARICOCELE IS A RELATIVELY COMMON disorder in children, with an average reported incidence of approximately 15% in the prepubertal group.[1,2] Varicoceles can lead to testicular hypotrophy and infertility; therefore, surgical treatment is frequently required in the pediatric age group.[3,4] Multiple methods exist for the treatment of varicoceles, including sclerotherapy and open and laparoscopic surgical ligation of the spermatic vessels.[5–9] With recent advances in minimally invasive surgery, there have been many reports showed the safety and efficacy of Palomo laparoscopic repair.[1,8–10]

Whichever treatment is chosen, postoperative complications are fairly common, such as recurrence, persistence, hydrocele, and testicular atrophy. In particular, with Palomo repair the incidence of persistence/recurrence of varicocele seems lower than 5%, and the incidence of postoperative hydrocele seems to be higher than 30%.[7,11–13]

The lymphatic-sparing (LS) laparoscopic Palomo procedure of injecting isosulfan blue into the scrotum is one of the surgical options that has gained popularity in the last 10 years because it seems to reduce the occurrence of postoperative hydrocele with better identification of the lymphatic vessels.[14–16] However, based on an analysis of the international literature, only small series with a short or an intermediate follow-up have been published.[17–20] In the current study, we report a large series of children who underwent LS laparoscopic Palomo varicocelectomy for the treatments of varicocele, operated on in two Italian centers of pediatric surgery, and their long-term outcomes.

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FIG. 1. Lymphatic vessels dyed with blue patent.

FIG. 3. Complete lymphatic sparing.

Patients and Methods

We retrospectively reviewed the data of 396 patients who underwent laparoscopic varicocelectomy in two Italian centers of pediatric surgery from November 2000 to November 2010. All the patients were affected by a left varicocele. Patients' ages ranged from 7 to 18 years (median, 13.2 years). All the patients had a Dubin and Amelar Grade III varicocele associated with hypotrophy of the omolateral testis and/or pain at the level of the left hemiscrotum. All the patients underwent an echo-color Doppler examination preoperatively. No specific clinical criteria were used to select patients, and all underwent a laparoscopic Palomo procedure. Since September 2002, we have performed preoperative lymphography with isosulfan blue during laparoscopic Palomo procedures.

Following induction of general anesthesia, about 5 minutes before trocar introduction, 1–2 mL of 2.5% isosulfan blue solution was injected into the intra-dartoic space of the left hemiscrotum, and the skin of the scrotum was gently massaged for about 1 minute. A laparoscopic procedure with three 3- or 5-mm ports was performed. A small peritoneal window was opened laterally to the cord at about 5–6 cm from the internal inguinal ring, and the internal spermatic vessels were identified and ligated by clips separating them from the blue-stained lymphatics (Figs. 1 and 2). Using this technique, it was possible to preserve the main funicular lymphatic vessels (Fig. 3); however, invariably those intimately attached to the internal spermatic veins were sacrificed.

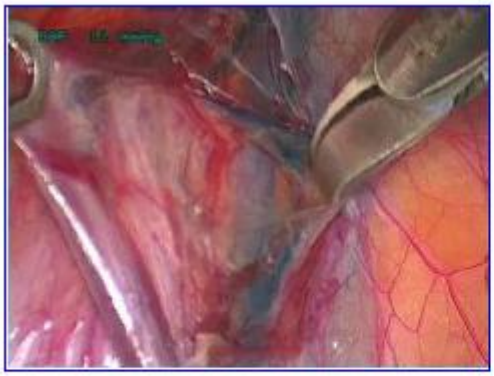

FIG. 2. Lymphatic sparing approach.

The laparoscopic Palomo procedure was successfully carried out in all patients without any conversion or complications. In all patients, mean operative time was 25.7 minutes (range, 10–65 minutes). The left scrotum was blue colored for about 2–3 days, and many children had blue urine for about 48 hours. No adverse effects were recorded related to the isosulfan blue injection. Patients were divided into two groups: those who had undergone an LS procedure and those who had undergone a non-LS (NLS) technique. The incidences of recurrence/persistence, postoperative hydrocele formation, and postoperative hydrocele requiring surgery or aspiration were analyzed statistically using the chi-squared test.

Results

Of 396 patients analyzed, 244 received an LS procedure, and 152 underwent NLS repairs. Of the 244 patients who underwent the LS procedure, 26 (10.6%) had a negative result because lymphatic vessels were not identified and so were considered as "intention-to-treat" patients. These were considered NLS repairs, and their results have been added to those of the NLS group. Therefore, the LS group included 218 patients, and the NLS group included 178 patients.

With a minimum follow-up of 12 months (range, 1–10 years), all the patients have been followed up with a clinical follow-up at 1 week, 1 month, and each year for the first 3 years.

The LS group (218 patients) was associated with a decreased incidence of postoperative hydrocele compared with the NLS group (0/218 [0%] versus 18/178 [10.1%]; chi-squared test=25.84, difference statistically significant). There was no significant difference in incidence of persistent or recurrent varicocele requiring reoperation following LS

procedures (5/218 [2.2%] versus 5/178 [2.8%]; chi-squared test=0.41, difference statistically not significant). No other complications were recorded.

The 18 hydroceles in the NLS group were all managed with repeated scrotal punctures with the patient under local anesthesia; in 13 patients the hydrocele disappeared after three to five punctures. The other 5 patients required a surgical procedure to perform vaginal resection. As for the 10 persistent/recurrence varicoceles, 8 were degree I–II (also at echocolor Doppler study), and they require no further treatment; only 2 patients received a redo laparoscopic procedure by the Palomo technique after a diagnostic phlebography with no further recurrence.

Discussion

At present, several operative techniques are used in the treatment of varicocele.[1,2,7] In analyzing the pediatric international literature, it seems that the laparoscopic Palomo procedure is the technique preferred by the majority of surgeons because it has the advantages of optimal magnification of the vascular structures, minimal residual scars, and, above all, a low incidence of varicocele recurrence/persistence.[2,10,21]

The most common complication of the Palomo technique is postoperative hydrocele as a result of the interruption of the lymphatic outflow from the subservient testis.[7,22] The reports in the literature show a range of hydrocele formation after Palomo varicocelectomy ranging from 5% to 39%.[2,21,22] This variability may be because different authors report different lengths of follow-up. In general, it seems that hydrocele formation may appear from 1 month to 2 years postoperatively.[22]

In 2001 Oswald et al.[20] described a modification of the open Palomo technique using isosulfan blue for preoperative lymphography. Isosulfan blue is an isomer of patent blue, and it is used in breast cancer and other malignancies to detect nodal metastasis. Oswald et al.[20] used this technique to stain lymphatic vessels to prevent their intraoperative ligation.[24]

Other products have been adopted to perform lymphatic identification such as methylene blue, but some authors reported scrotal skin necrosis using this procedure; for this reason the use of methylene blue for lymphography was completely abandoned.[2,6,24] Other authors reported an LS technique of injecting methylene blue dye into testicular tissue, but the consequences for the gonad were not studied.[25] Other reports have already been published in the international literature as for a LS procedure, but all the series are focused on small numbers of patients with a small or intermediate follow-up.[11,18,21,23,24] To the best of our knowledge, our series is the largest series published in the literature on this topic, with 396 patients operated on with a long follow-up of more than 10 years.

From the technical point of view, during laparoscopic Palomo repair, it is really technically difficult to identify lymphatic vessels with certainty because they are too small and too similar to arteries and veins. With an LS procedure, as shown in our series, we are able to identify lymphatics in about 90% of patients, to spare them and to avoid a postoperative hydrocele. In fact, in our series there is a significant difference (chi-squared test=25.84) in hydrocele formation between the LS group (0% developed a hydrocele) compared with the NLS group (11% developed a hydrocele). We already reported in 2006 our preliminary experience with the LS procedure in which we identified 63% of lymphatic vessels compared with about 90% of this series.[24] We think that this difference is due to the fact that we have standardized the procedure of injection.

As for the 10% of patients negative following the LS procedure, we noted that all these 26 patients were patients with a body weight greater than 70 kg. Probably in these patients, considered as adults, the amount of product injected has to be higher compared with the amount that we use in smaller children, and we are working on that premise. In fact, in the patients last operated on not included in this series, this hypothesis seems to be confirmed.[24]

We reported no adverse effects injecting isosulfan blue except the blue coloration of scrotal skin and blue urine for some days in the postoperative period.[20,24] However, it is extremely important to inform parents preoperatively of these minimal adverse effects.

Regarding persistence/recurrence of varicocele, there was no significant difference (chi squared test=0.41) in the incidence of persistent or recurrent varicocele in the two groups analyzed: LS (5/218 [2.2%]) versus NLS (5/178 [2.8%]).

Regarding our operative findings, we noted that after isosulfan blue injection, the majority of lympatics (two or three) are medial to cord, and only small numbers of them (one or two) are anterior to the cord. In our series it was always easy to spare the medial lymphatics. As for the anterior ones, sometime they are too adherent to the cord, and they have to be sacrificed and dipped together with the inner spermatic vessels.

Another interesting finding is that sometime not only the lymphatics became blue, but also we noted that the peritoneum that covers the inner spermatic vessels became blue. However, after the peritoneum is opened lymphatics are clearly visible, and you can spare them easily. As for the timing of injection, we inject the product 5–10 minutes before introducing the first trocar, and lymphatics remained blue for about 30–40 minutes.

Conclusions

We believe that our outcome confirms the ability to perform a microscopic dissection and preserve lymphatics during laparoscopic varicocelectomy. The present series provides further evidence that laparoscopic Palomo varicocelectomy is an effective procedure, and when the lymphatics are spared, the incidence of hydrocele formation and subsequent need for hydrocelectomy can decrease down to zero. Our series shows that it is important to standardize the technique of injection that permits identification of lymphatics in about 90% of cases. The isosulfan blue injection does not seem to influence the recurrence/persistent rate as it is similar in both groups.

Disclosure Statement

No competing financial interests exist.

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Conclusive remarks

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Outcome confirms the ability to perform a microscopic dissection and preserve lymphatics during laparoscopic varicocelectomy. The present series provides further evidence that laparoscopic Palomo varicocelectomy is an effective procedure, and when the lymphatics are spared, the incidence of hydrocele formation and subsequent need for hydrocelectomy can decrease down to zero.

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APPENDIX I

List of publications

- Esposito C, Montinaro L, **Alicchio F**, Scermino S, Basile A, Armenise T, Settimi A. Technical standardization of laparoscopic herniorraphy in pediatric patients. *World J Surg.* 2009 Sep;33(9):1846-50.

- Esposito C, Montinaro L, **Alicchio F**, Savanelli A, Armenise T, Settimi A. Laparoscopic Treatment of Inguinal Hernia in the First Year of Life. *J Laparoendosc Adv Surg Tech A.* 2010 Mar 31.

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- Paternoster M, Graziano V, Settimi A, Di Lorenzo P, **Alicchio F**, Esposito C. Medico-legal observations concerning a mortal case of granulosa cell tumor of the ovary in an 8- Month-old infant. *J Pediatr Surg.* 2011 Aug;46(8):1679-82.
- Esposito C, **Alicchio F**, Giurin I, Castellano M, Settimi A. Technical standardization of laparoscopic direct hernia repair in pediatric patients. *J Laparoendosc Adv Surg Tech A.* 2012 Jan-Feb;22(1):113-6
- Esposito C, De Luca C, **Alicchio F**, Giurin I, Miele E, Staiano AM, Settimi A.Long-Term Outcome of Laparoscopic Nissen Procedure in Pediatric Patients with Gastroesophageal Reflux Disease Measured Using the Modified QPSG Roma III European Society for Pediatric Gastroenterology Hepatology and Nutrition's Questionnaire. *J Laparoendosc Adv Surg Tech A. 2011 Nov 2*
- Esposito C, Giurin I, **Alicchio F**, Farina A, Iaquinto M, Scermino S, Palladino T, Settimi A. Unilateral inguinal hernia: laparoscopic or inguinal approach. Decision making strategy: a prospective study. *Eur J Pediatr. 2012 Jun;171(6):989-91*
- Esposito C, Turial S, **Alicchio** F, Enders J, Castagnetti M, Krause K, Settimi A, Schier F. Laparoscopic repair of incarcerated inguinal

hernia. A safe and effective procedure to adopt in children.*Hernia. 2012 Jul 8.*

- V. Buccigrossi, C Armellino, A. Tozzi, E. Nicastro, C. Esposito, A. Settimi, **F. Alicchio**, S. Cozzolino, A. Guarino Time- and segment-related structural adaptive changes in a rat model of short bowel: a 3D geometric model *Journal of Pediatric Gastroenterology & Nutrition*

- Esposito C, Turial S, Escolino M, Giurin I, **Alicchio** F, Enders J, Krause K, Settimi A, Schier F.Laparoscopic inguinal hernia repair in premature babies weighing 3 kg or less. *Pediatr Surg Int. 2012 Oct;28(10):989-92.*

- Esposito C, Calvo AI, Castagnetti M, **Alicchio** F, Suarez C, Giurin I, Settimi A. Open versus laparoscopic appendectomy in the pediatric population: a literature review and analysis of complications. *J Laparoendosc Adv Surg Tech A. 2012 Oct;22(8):834-9.*

- Chiarenza SF, Giurin I, Costa L, **Alicchio** F, Carabaich A, De Pascale T, Settimi A, Esposito C. Blue patent lymphography prevents hydrocele after laparoscopic varicocelectomy: 10 years of experience. *J Laparoendosc Adv Surg Tech A. 2012 Nov;22(9):930-3*

Scientific contributions to National and International Meetings

11th European Congress of Paediatric Surgery . June 2nd–5th 2010- Berne, Switzerland:

- *C.Esposito,* ***F.Alicchio****, I. Giurin, F. Perricone, A.Savanelli, G. Ascione, E.Miele, A. Staiano, A. Settimi*. **Long-term outcome of laparoscopic Nissen procedure in pediatric patients with GERD mesured using the modified QPSG Roma III ESPGHAN's Questionnaire**

- *C.Esposito, L. Montinaro,* ***F.Alicchio****, F Perricone, A.Basile, T. Armenise, I.Giurin, A.Farina, A. Savanelli, A. Settimi.* **Laparoscopic treatment of inguinal hernia in the first year of life**

43rd Annual Meeting of ESPGHAN, June 9-12, 2010 Istanbul, Turkey:

- *A. Tozzi, V. Buccigrossi, C. Armellino, M. Zobel, C. Esposito,* ***F. Alicchio****, D. Di Napoli, S. Cozzolino, A.Guarino.* **Post-natal gut is able to generate new neurons: a novel finding in a rat model of intestinal resection**

- *V. Buccigrossi, A. Tozzi, C. Armellino, M. Zobel, E. Ruberto, G. De Marco, C. Esposito,* ***F. Alicchio,*** *S. Cozzolino, A. Guarino*. **Post-resection intestinal adaptation shows a time and segment specific pattern: a 3 dimensional study in a rat model of short gut.**

Congres des societies de padiatrie PARIS 16-19 Juin 2010

- *C Esposito,* ***F Alicchio,*** *I Giurin, F Perricone, A Savanelli, G Ascione, E Miele, A Staiano, A Settimi.* **Suivi à long-term dans les enfants opérés par un RGO étudies avec le questionnaire QPSG ROMA III ESPGHAN.**

- *C Esposito,* ***F Alicchio,*** *Montinaro L,* ***I*** *Giurin, F Perricone, A Savanelli, A Settimi .* **Traitment laparoscopique de l' hernie inguinale dans la premiere annee de vie.**

3° Congresso Nazionale Congiunto TORINO 22-25 Settembre 2010

- *C. Esposito,* ***F. Alicchio****, A. Tozzi, V. Buccigrossi, D. Di Napoli, S. Cozzolino,A. Guarino, A. Settimi* **Post-natal gut is able to generate new neurons: a novel fi nding in a rat model of intestinal resection**

- *A. Farina,* ***F. Alicchio****, I. Giurin, F. Perticone, A. Savanelli, C. Esposito, A. Settimi* **E' necessario esplorare il canale inguinale in pazienti con testicolo non palpabile e vasi spermatici che entrano in un anello inguinale interno chiuso?**

- *C. Esposito, A. Savanelli,* ***F. Alicchio****, A. Farina, I. Giurin, M. Iaquinto, F. Perticone,A. Settimi* **Eminefrectomia retroperitoneoscopica con 3 trocars: note di tecnica**

- *C. Esposito, F. Chiarenza, L. Montinaro, L. Mastroianni ,* ***F. Alicchio****, A. Settimi, L. Musi* **Il valore della corretta informazione in laparoscopia pediatrica: il consenso informato laparoscopico**

- *C. Esposito, I. Giurin, F.* ***Alicchio, F****. Perricone, A. Farina, M. Iaquinto, A. Roberti, A. Savanelli, E. Miele, A. Staiano, A. Settimi*

Long-term outcome of laparoscopic Nissen procedure in paediatric patients with Gerd mesured using the modifi ed QPSG Roma III Espghan's questionnaire

- *M. Iaquinto, G. Ascione, A. Savanelli, G. Capano,* ***F. Alicchio****, I. Giurin, F. Perticone, A. Farina, A. Staiano, C. Esposito, A. Settimi* **Gastrostomia vs digiunostomia nei pazienti pediatrici con problemi di alimentazione**

XIX° REUNION du GECI Samedi 9 Octobre 2010 VENISE

- *C.Esposito, L. Montinaro,* ***F. Alicchio****, A. Farina, I. Giurin, M. Iaquinto, F.Perricone, A. Settimi* **Advantages of laparoscopic treatment of inguinal hernia in the first year of life**

- *Esposito C, Savanelli A,* ***Alicchio F,*** *Farina A, Perricone F, Giurin I, Iaquinto M, Centonze A, Settimi A* **The impact of the new hemostatic devices in pediatric patients underwent total or partial retroperitoneoscopic nephrectomy**

BRITISH ASSOCIATION OF PAEDIATRIC ENDOSCOPIC SURGEONS IN ASSOCIATION WITH OTHER EUROPEAN GROUPS & SOCIETIES 15th - 17th November 2010 BERNE, Switzerland

- *C. Esposito, F. Perricone,* ***F. Alicchio****, A. Farina, I. Giurin, M. iaquinto, A. Savanelli, A. Settimi.* **Follow up a lungo termine in pazienti sottoposti ad inter-vento di Fowler-Stephens per testicoli intraddominali**

- *C. Esposito, A. Savanelli ,* ***F. Alicchio****, A.Centonze, F. Perricone, M. Iaquinto, I. Giurin, A. Farina, A. Settimi* **The impact of the new**

hemostatic devices in paediatric patients underwent total or partial retroperitoneoscopic nephrectomy

IPEG's 20th ANNUAL CONGRESS FOR ENDOSURGERY IN CHILDREN 3-7 May 2011 PRAGUE Czech Republic

- *C. Esposito,* ***F. Alicchio****, A. Farina, I. Giurin , F. Perricone, M. Iaquinto, G. Ascione, E. Miele , C. De Luca, A. Staiano , A. Savanelli, A. Settimi.* **Lon g-term outcome of laparoscopic Nissen procedure in pediatric patients with GERD mesured using the modified QPSG ROMA III ESPGHAN 's questionnaire**

- *Esposito C.,* ***Alicchio F.****, Giurin I., Farina A., Iaquinto M., Perricone F., Savanelli A., Centonze A., Settimi A.* **The impact of the new hemostatic devices in pediatric patients underwent total or partial retroperitoneoscopic nephrectomy**

12TH EUROPEAN CONGRESS OF PAEDIATRIC SURGERY15-18 · JUNE · 2011 BARCELONA, SPAIN.

- ***F. Alicchio****, C. Esposito, A. Farina, I. Giurin, M. Iaquinto, A. Tozzi, D. Di Napoli, A. Guarino, A. Settimi.* **Post-natal gut is able to generate new neurons: a novel finding in a rat model of intestinal resection**

- ***F. Alicchio****, C. Esposito, V. Buccigrossi, C. Armellino, MV. Barone, A. Settimi, A. Guarino.* **Polar effects on ion transport and cell proliferation induced by gc-c ligands in intestinal epithelial cells.**

- *C. Esposito, P. Philippe, A. Saxena,* ***F. Alicchio****, A. Settimi, F. Shier* **The advantages of laparoscopic approach to incarcerated inguinal hernia in children: a multicentric survey**

4° CONGRESSO NAZIONALE CONGIUNTO 21-24 Settembre 2011 PADOVA

- *C. Esposito,* ***F. Alicchio****, I. Giurin, I. Calvo, A. Savanelli, G. Ascione, A. Settimi* **Appendicectomia laparoscopica versus open in età pediatrica: review e meta-analisi**

- *T. De Pascale,* ***F. Alicchio,*** *I. Giurin, A. Farina, M. Iaquinto, P. Ricciardi, C. Esposito, A. Settimi* **Comunicazione organizzativa sanitaria e *team working*: Risorse strategiche per il management sanitario. L'esperienza del reparto di chirurgia pediatrica dell'aou federico ii di napoli**

- *Savanelli, I. Giurin, A. Farina, M. Iaquinto, F.* ***Alicchio, F.*** *Perricone, A. Roberti, M. Di Lecce, C. Esposito, A. Settimi* **Trattamento in un tempo dell'ipospadia prossimale con lembo ad isola cutaneo prepuziale-penieno: nostra esperienza**

- *Savanelli, I. Giurin, A. Farina, M. Iaquinto,* ***F. Alicchio****, F. Perricone, M. Escolino, M. Castellano, C. Esposito, A. Settimi* **Prepuzioplastica associata alla uretroplastica: Nostra esperienza. Studio prospettico**

- *C. Esposito,* ***F. Alicchio****, I. Giurin, M. Iaquinto, A. Farina, A. Savanelli, A. Settimi* **Impatto dei nuovi devices emostatici nella chirurgia renale ablativa in età Pediatrica**

- *C. Esposito,* ***F. Alicchio****, A. Farina, I. Giurin, M. Iaquinto, M. Di Lecce, G. Ascione, A. Settimi* **Applicabilita' della fundoplicatio laparoscopica in un paziente con RSPM plurioperato con tetraparesi spastica, portatore di uno shunt ventricolo-peritoneale e di una pompa per il baclofen posizionata nel sottocute dell'emiaddome sinistro**

- *C. Esposito,* ***F. Alicchio****, A. Farina, I. Giurin, M. Iaquinto, M. Escolino, M. Castellano, A. Settimi* **Ernie inguinali rare in eta' pediatrica: un "frequente riscontro" in corso di ernioplastica laparoscopica**

- *C. Esposito****, F. Alicchio****, A. Marte, A. Centonze, A. Farina, I. Giurin, M. Iaquinto, A. Roberti,A. Savanelli, A. Settimi* **Il trattamento del testicolo non palpabile in epoca pre-laparoscopica. Considerazioni su 3 casi operati in laparoscopia**

- *C. Esposito,* ***F. Alicchio****, I. Giurin, M. Iaquinto, A. Farina, A. Savanelli, A. Settimi* **Linfografia pre-laparoscopia: un eccellente metodo per prevenire l'idrocele Post-operatorio dopo intervento di palomo. Esperienza preliminare.**

13TH EUPSA-BAPS JOINT CONGRESS 13-16 · JUNE · 2012 ITALY, ROME

- *I Giurin, A Calvo,* ***F Alicchio****, A Roberti, C Suarez, C Esposito, A Settimi* **open versus laparoscopic appendectomy in pediatric population: review and meta-analysis**

- *C Esposito,* ***F Alicchio****, S Turial, I Giurin, J Enders, A Farina, K Krause, A Settimi, F Schier* **Laparoscopic inguinal herniorrhaphy in babies weighing 3 kg or less. Is it Easier than inguinal surgery ?**

5° CONGRESSO NAZIONALE CONGIUNTO 24-27 NOVEMBRE 2012 MILANO

- *C. Esposito,* ***F. Alicchio****, P. Vecchio, M. Escolino, M. Iaquinto, A. Farina, A. Roberti, I. Giurin, A. Settimi* **Cisti del dotto tireoglosso in età pediatrica. Dieci anni di esperienza**

- *C. Esposito,* ***F. Alicchio*** *, S. Turial, M. Escolino , I. Giurin , A. Roberti , M. Iaquinto, A. Settimi, F. Schier*
  Erniorrafia inguinale laparoscopica in bambini con peso uguale o inferiore a 3 kg. Più semplice della chirurgia inguinale?

- *C. Esposito , F. Alicchio, A. Farina, M. Escolino, I. Giurin, A. Franzese , G. Galloro, M. Musella, A. Settimi* **La chirurgia bariatrica nei pazienti pediatrici obesi: esperienza preliminare**

- *C. Esposito , F. Alicchio , M. Escolino , I. Giurin , A. Settimi* **Problemi medico-legali: la nuova frontiera della chirurgia mini-invasiva pediatrica**

APPENDIX II

Grant proposal

During my PhD, I attended to a Grant Proposals:

Programma di Ricerca Scientifica di Rilevante Interesse Nazionale

(Prin) annualità 2009 ***"I biomateriali nella terapia dell'insufficienza intestinale in un modello animale di intestino corto"***