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Intraperitoneal chemotherapy versus adjuvant chemotherapy for treatment of colo-rectal cancer at high-risk for peritoneal carcinosis

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Abstract

The purpose of this study is to investigate the feasibility and safety of colorectal surgery plus adjuvant intraoperative hyperthermic intraperitoneal chemotherapy in patients at high risk of peritoneal recurrence, but still without pre- or intra-operative evidence of peritoneal spread in terms of length of hospital stay, surgical and medical treatment-related toxicity.

Summary

Aim: prophylactic hyperthermic intraperitoneal chemotherapy (HIPEC) showed promising results in patients with colorectal carcinoma at high risk of recurrence, but still without clinically and radiologically evident signs of peritoneal spread. This study aims to analyze the feasibility of this proactive, early phase, multimodality approach.

Methods: a mono-institutional, prospective, parallel, two-stage phase II trial enrolled 49 patients to standard surgery or surgery plus intraoperative HIPEC. Before the procedure and during surgery patients received intravenous fluorouracil and leucovorin to potentiate oxaliplatin activity. Data analysis included length of hospital stay, surgery duration, type of surgery and chemotherapy-related complications risk score.

Results: no significant difference was seen in the median time spent in the hospital with a median stay of 7 days in both groups (p=0.5720). The surgical procedure median duration was longer in the HIPEC group than in the control one. Side-effects and surgical complications did not cross at any time the Pocock-type boundary for side/effect monitoring (p=0.80, N.S.).

Conclusions: the present prospective study results demonstrate the feasibility and safety of the colorectal surgery plus HIPEC treatment in patients with colorectal cancer patients at high-risk for peritoneal invasion, although clinically and radiologically undetectable.

CHAPTER 1

Background

1.1. Colo-rectal cancer

Globally, colorectal cancer (CRC) is the third most common cancer in males and the second in females, with 1.8 million new cases and almost 861,000 deaths in 2018[1]. Its incidence and mortality rates vary markedly around the world[1].

Once a CRC is suspected the pretreatment clinical staging permits to classify it according the tumor, node, metastasis (TNM) staging system of the combined American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) (eighth edition, 2017)[2].

Surgical resection is the only curative modality for localized colon cancer. The goal of surgery for invasive cancer is complete removal of the tumor, the major vascular pedicle and the lymphatic drainage basin of the affected colonic segment[3]. En bloc resection of contiguous structures is indicated if attachment or infiltration of the tumor into a potentially resectable organ or structure is present.

Approximately 20 to 25% of newly diagnosed colon cancers are metastatic at presentation (synchronous metastasis). The most common distant metastatic sites are the liver, the lungs, the lymph nodes and the peritoneum.

Although major advances in systemic chemotherapy have expanded the therapeutic options for these patients and improved median survival from less than one year in the single-agent fluoropyrimidine era to more than 30 months, fewer than 20%[4] of those treated with chemotherapy alone are still alive at five years and only a few are free of disease, unless resection or ablation of metastases has been performed.

On the other hand, surgery provides a potentially curative option for selected patients with limited metastatic disease, predominantly in the liver and the lung.

The usual recurrence sites include anastomotic site, mesentery or nodal basin, retroperitoneum and

peritoneum[5].

Peritoneal carcinosis is a metastatic deposit on the peritoneal surface throughout the abdominal cavity. The peritoneum is the second most common site of metastasis in patients with colorectal cancer, accounting for 25–35% of all cases of recurrence[6,7]. Among patients with recurrent disease, 5–10% have synchronous disease and 20–50% develop metachronous peritoneal carcinosis (PC)[8-11].

1.2. Pathophysiology of colorectal peritoneal carcinosis

Understanding the mechanism of peritoneal dissemination from the primary tumor plays a key role in the prevention and early detection of PC from CRC. Systemic tumor dissemination is considered to be a multistep process in which tumor cells detach from the primary tumor, acquire motility and evade anoikis (**figure 1**). In both hematogenous and peritoneal spread, free cancer cells (carried by the blood stream or floating in the peritoneal cavity) must evade immune defenses in order to reach host organs. At the site of the host organ, adhesive interactions between the organ and cancer cells are required for the development of metastasis[12]. Specific local environmental factors indicate that peritoneal and hematogenous spread only partially share target adhesion molecules and dissemination processes[13]. Detachment of cancer cells into the free peritoneal space can occur as a result of full thickness invasion through the serosa (T4 stage) or as a consequence of surgery-induced tumor spillage. Once a viable, free cancer cell is present in the peritoneal cavity, adhesion to the peritoneal surface is required in order to ultimately invade the peritoneum, proliferate and produce peritoneal deposits[12]. Upregulation of specific cell surface molecules due to the production of reactive oxygen species and inflammatory cytokines may partially explain higher cancer cell adhesion during the postoperative period[14]. Considering tumor cell adhesion as a key step in the formation of peritoneal deposits, a large literature review has investigated the functional importance of various adhesion molecules and their correlation with clinical outcomes. Currently, only a minority of these targets (CD44, integrin α2β1 and mucin 16 [MUC16]) are supported by scientific data corroborating their implication in peritoneal dissemination in digestive and ovarian cancers. Experimental studies focused on the role of CD44, a cell surface proteoglycan participating in cell–cell interaction, adhesion and migration have underscored the potential role of this protein in the development of PC in gastric, ovarian and pancreatic carcinoma. Other in vivo experiments blocking interaction with mesothelial cells or increasing the delivery of chemotherapy to malignant cells by specific agents show promise for inhibiting peritoneal dissemination. In addition, blocking integrin α2β1 results in poorer cancer cell attachment to the peritoneum in ovarian and digestive cancers and could prevent the

adhesion of CRC cells to the peritoneum. For this purpose, other compounds that diminish integrin β1-chain expression, such as phospholipids, endostatin and simvastatin, might also be effective and warrant further investigation. Likewise, MUC16 is probably implicated in mesothelial cell adhesion, as suggested by experimental studies in ovarian cancer and by a high level of MUC16 in the serum of patients with gastric cancer. Consequently, a better understanding of these interactions and of the mechanisms of peritoneal dissemination could help prevent PC and facilitate the diagnosis of patients at risk of developing PC in the future.

1.3. Treatment options: cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Rationale

Peritoneal carcinosis has historically been regarded as an untreatable disease and, despite advances, has remained a significant challenge for oncologists and surgeons. For many years, patients with PC have been considered to be beyond the realm of curative therapy, but in recent years promising results have been reported in a variety of tumor types using cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC).

CRS-HIPEC is a complex therapeutic modality. It includes an aggressive and extensive surgical procedure and the administration of intraperitoneal chemotherapy with hyperthermia, either in the intraoperative or/and in the early postoperative setting.

In expert hands, the associated morbidity and mortality compares that of other major oncological surgery[15], but this expertise needs to be gained. Awareness of treatment-related toxicity is important and needs to be factored in the patient selection process.

Hyperthermic Intraperitoneal Chemotherapy (HIPEC) is delivered in the operating room once the cytoreductive surgical procedure is finalized and constitutes the most common form of administration of perioperative intraperitoneal chemotherapy. The acronym HIPEC, coined by the group from the Netherlands Cancer Institute, has become the standardized nomenclature for this procedure as a result of the experts' consensus achieved during the Fourth International Workshop on Peritoneal Surface Malignancy (Madrid, 2004)[16].

Intraperitoneal delivery of anti-neoplastic agents for cancer into the abdominal cavity has been attempted since antiquity. In the mid-18th century, English surgeon Christopher Warrik injected a mixture of "Bristol water" and "Claret" (a Bordeaux wine) into the peritoneal cavity of a woman suffering from intractable ascites[17]. The efficacy of this novel method for intraperitoneal drug delivery for peritoneal metastases patients has been slowly developed. Karnofsky and colleagues in 1948 used nitrogen mustard for the palliative treatment of carcinomatous ascites. The efficacy

was such that FDA approval of nitrogen mustard for intraperitoneal administration was granted and has remained in effect until now[18]. However, the rationale for intraperitoneal chemotherapy administration came from pharmacologic research in patients who had cancer spread to peritoneal surfaces. It was recognized that some drugs would be especially appropriate for prolonged retention within the peritoneal space based on their molecular structure[19]. It was Dedrick and colleagues at the American National Institutes of Health who called attention to the potential benefits of intraperitoneal chemotherapy administration of cancer chemotherapy agents especially in ovarian cancer[20]. The studies of Speyer and colleagues clearly identified 5-fluorouracil as an agent with high concentrations within the peritoneal space after intraperitoneal administration as compared to drug levels within the plasma[21]. The rapid metabolism of the 5-fluorouracil after absorption of this drug by the visceral peritoneum within the liver parenchyma resulted in a markedly enhanced exposure of cancer nodules on peritoneal surfaces[22]. Jones and colleagues recognized that a high volume of intraperitoneal chemotherapy solution (belly bath technique) was necessary to adequately distribute the drugs[23]. Ozols and colleagues investigated the pharmacokinetics of doxorubicin and McVee and colleagues the possible benefits of intraperitoneal cisplatin[24-25]. Thanks to the continuous efforts to identify suitable drugs for the administration of intraperitoneal chemotherapy, an extensive list of possible chemotherapeutic agents and their pharmacologic advantages after intraperitoneal administration has been defined[26].

Because of a large molecular size and hydrophobic surface, cancer chemotherapy agents were shown to have a slow clearance from the peritoneal compartment through the lining of the abdomen and pelvis to the body compartment. Moreover, metabolism of the chemotherapy in the body compartment was at all points in time faster than clearance from the peritoneal space. This resulted in a much greater concentration times- time (area under the curve) of the drug in the peritoneal space as compared to concentration times- time measured in the blood. This results in an increased therapeutic effect on cancer nodules on the peritoneal surface and a reduced systemic toxicity[27].

The combination of heat and cytotoxic drugs frequently results in an increased cytotoxicity, beyond that predicted for an additive effect. The synergism between both kinds of treatment is dependent on several factors including increased drug uptake in malignant cells which is due to increased membrane permeability and improved membrane transport. There is also evidence that heat may alter cellular metabolism and change drug pharmacokinetics and excretion, both of which can increase the cytotoxicity of certain chemotherapeutic agents[28]. Additional factors

include increased drug penetration in tissue, temperature dependent increases in drug action and inhibition of repair mechanisms. In many cases, this enhancement of activity and penetration depth of drugs has already been seen above 39-40°C[28].

1.4. Prevention of peritoneal metastases with intra-operative proactive HIPEC treatment after conventional colon-rectal cancer surgery

Recent improvements in the surgical technology of colorectal cancer resection have decreased the incidence of treatment failures, both at the resection site or at a distance from the primary. The benefits of total mesorectal excision have been established and the survival benefit published[29, 30]. This survival advantage has been a result of the absence of tumor contamination within the confines of the pelvis because of a meticulous dissection which maintains a layer of tissue between the primary malignancy and the margins of resection[30]. Also, the benefits of colon cancer resection using wide excision, generous lymphadenectomy and an intact mesocolic resection have been demonstrated. These improvements in surgical technology and therefore in survival are the result of decreased tumor cell contamination resulting from the surgical event itself. A complete absence of tumor cell contamination with primary colorectal cancer surgery has become an absolute requirement of treatment. Any dependence upon systemic chemotherapy to manage resection site disease or peritoneal metastases must be abandoned.

It is important to establish that the mechanism of resection site recurrence and peritoneal metastases is the same. Cancer cells are disseminated either prior to or at the time of the cancer resection. The cancer cells at high density will layer out within the bed of the resection site. Because the surgery has disrupted the peritoneum and created a "sticky surface", a high metastatic efficiency is expected. Single cells disseminated at a distance from the anatomic site of primary cancer resection will progress as peritoneal metastases[31].

One of the most innovative concepts in colorectal cancer in recent years has been that of "patients at high risk of recurrence" and its identification. In approximately 20% of patients with primary colorectal cancer, some clinical findings indicate a high probability of intraperitoneal cancer cell dissemination[32]. These clinical findings show that the primary colorectal cancer surgery, even performed in its most perfect manner with or without systemic chemotherapy, is not a sufficient management strategy.

Honoré *et al*[33] defined patients at risk of developing PC: small peritoneal nodules present in the first surgery (70%), ovarian metastases (60%) and perforated tumor (50%). Positive cytology and T3–T4 mucinous tumors have a risk of 30 to 40%. It is remarkable that positive cytology from

colorectal cancer really worsens the prognosis according to the Lyon's series review with median overall survival (OS) of 19 and 44 months for positive and negative intraperitoneal free cancer cells (p .0.018).A recent review on advanced primary tumors (T4) confirms that T4a tumors are worse than T4b as a prognostic factor for peritoneal metastases development after primaryresection[34]. Sugarbaker[35] defined the risk of peritoneal recurrence according to some clinical and histopathological characteristics of the tumor (**Table 1**).The identification of these groups allowed Segelman *et al.*to develop an individualized prediction model to estimate each patient's risk[36, 37]. In groups 1–4, patients can be considered to have 50–100% incidence of local–regional recurrence and/or peritoneal metastases in the absence of special treatments. Peritoneal metastases discovered and resected at the time of primary colorectal cancer resection will show progression with follow-up in 75% of patients. This occurs even if these metastases are completely removed with the primary intervention[33]. Ovarian metastases have over 60% incidence of other sites of peritoneal dissemination in follow-up. Perforation through the primary cancer at the time of primary cancer resection and a positive margin of resection, usually a lateral margin, indicates a likelihood of local–regional or peritoneal progression in 30 and near 100% of patients, respectively.

Other clinical findings have been shown to place the patient at a lesser risk for local–regional recurrence or peritoneal metastases. Positive peritoneal cytology either before or after colorectal cancer resection, adjacent organ involvement or a cancer-induced fistula, T3 mucinous cancers, T4 cancers or a positive imprint cytology from the primary malignancy, rupture of the cancerous mass, or obstruction at the time of presentation all would have an elevated incidence of local–regional recurrence and peritoneal metastases.

The development of metachronous PC was associated independently with non-R0 surgery ($p < 0.001$), pN2 with lymphadenectomy with less than 12 nodes ($p < 0.001$), pT4 ($p < 0.001$), tumors located in the right colon ($p < 0.002$) and emergency surgery ($p <0.001$). It was possible estimate the risk for each patient. It was very important to identify these patients because they have poor survival rates at 5 years and new strategies are being developed to improve their prognosis[38].

1.5. Data Showing Benefit from Perioperative Chemotherapy in Patients with Primary Colorectal Cancer with Peritoneal Seeding or at High Risk for Peritoneal Seeding

Local–regional recurrence and peritoneal metastases occupy a prominent role in the natural history of gastrointestinal cancer. Intraperitoneal chemotherapy used as a planned part of a

surgical intervention to control local–regional recurrence and peritoneal dissemination from colorectal cancer was proposed by Sugarbaker and colleagues[39-41]. In a phase I/II study, 5-fluorouracil and mitomycin C were administered directly into the peritoneal cavities in the early postoperative period before adhesions had progressed. There was a marked pharmacokinetic advantage of perioperative intraperitoneal chemotherapy with single cancer cells on peritoneal surfaces as the targets of this treatment.

Experience with patients demonstrating peritoneal metastases recognized at the time of primary colon cancer resection came from Washington and was reported by Pestieau and Sugarbaker[42]. They identified five patients who had definitive treatment of peritoneal metastases from colon cancer concomitant with the resection of the primary tumor. At the time of writing their paper, the median disease-free survival of these patients had not been reached and their 5-year survival was 100%. The statistical difference between patients who had perioperative treatment of their peritoneal metastases as compared to those who had delayed management with cytoreductive surgery and early postoperative intraperitoneal chemotherapy (EPIC) was statistically significant ($p < 0.0001$).

Tentes *et al.* has reported their experience on the use of hyperthermic perioperative chemotherapy in patients at high risk for local–regional recurrence. Those were patients with locally advanced T3 or T4 colorectal cancer. Only patients with R-0 resection were randomly assigned to receive HIPEC plus systemic chemotherapy versus conventional treatments. The 5-year survival for the HIPEC group was 100% and 72% for the conventional group ($p = 0.0938$). During follow-up, two patients in the HIPEC group and eight patients in the conventional group were recorded with recurrence ($p = 0.002$). It is important to note that no local–regional recurrence or peritoneal metastases was recorded in the HIPEC group. By contrast, the group treated in a conventional manner showed three patients with local–regional recurrence. These data suggest that perioperative chemotherapy had no effect on the development of distant metastases but exhibited an advantage in eradicating viable cancer cells that were disseminated local–regionally at the time or prior to the colorectal cancer resection[43].

Braam and colleagues reported a total of 72 patients with synchronous peritoneal metastases from colorectal cancer. In 20 patients (27.8%), the primary tumor was resected simultaneously with HIPEC (early referral). In the other 52 patients (72.2%), the primary tumor was resected prior to a reoperative surgery with HIPEC (late referral). During CRS plus HIPEC following late referral, 22 (59.5%) of the 37 anastomoses of the earlier operation were resected, revealing malignancy in 12 patients (54.5%). In the 20late referral (27.8%) patients, a permanent colostomy

was constructed after HIPEC. The relaparotomy rate was higher in patients after a resection of a previous anastomosis (36.4%) compared to 12% in the rest of the patients (p = 0.02). Resection of the primary tumor simultaneously with HIPEC in patients with synchronous peritoneal metastases from colorectal cancer may prevent extended bowel resections and permanent colostomy[44].

To date, the optimal perioperative chemotherapy treatment for prevention of local–regional recurrence and peritoneal metastases has not been determined. It is possible that the best choice is the early postoperative intraperitoneal chemotherapy (EPIC).

This was used by Pestieau and Sugarbaker to achieve good results[42]. Also, in the prevention of peritoneal metastases in gastric cancer, EPIC was shown by Yu *et al.* to be very successful in a prospective randomized controlled study[45].

From a logistical perspective, EPIC may be favored in those patients with unexpected peritoneal metastases who have not signed an informed consent for HIPEC so that they can be treated with full consent in the early postoperative period. It is possible that a single dose of intraoperative chemotherapy (HIPEC) is not as effective as the 5-day intraperitoneal lavage used postoperatively (EPIC). However, EPIC has been shown to be associated with a higher incidence of adverse events but not with a higher incidence of mortality[46].

1.6. Objectives

In relation to the promising results of the first trials launched in the use of prophylactic HIPEC in patients with colorectal cancer at high risk to peritoneal recurrence as well as the consolidated experience in the use of the method that has reduced its morbidity within acceptable limits, we decided to propose in our Center for High Volume Oncology in the Treatment of Colorectal Cancer, a single-center prospective controlled longitudinal cohort study in a consecutive series of patients eligible for surgery followed by intra-perioperative HIPEC comparing the results to a cohort of patients undergoing chemotherapy standard adjuvant after surgery.

The aims of this thesis can be divided into three subsequent steps:

- to evaluate the efficacy and safety of HIPEC during surgery considering the reduction of local recurrence and peritoneal carcinosis compared with standard systemic adjuvant chemotherapy alone. The secondary end-points are the Overall Survival Rate (% OS) and the Disease Free Survival Rate (% DFS) at 6, 12, 18 and 24 months;
- to determine the morbidity (according to Dindo-Clavien) related to the treatment of

open and laparoscopic adjuvant HIPEC;

- cost-benefit analysis of the adjuvant HIPEC method compared to traditional postoperative systemic chemotherapy alone, operating time, average length of hospital stay, re-hospitalization rate and quality of life assessment.

CHAPTER 2

Materials and Methods

2.1. Study design

This study is a mono-institutional, prospective, parallel, two-stage phase II trial. After the approval by the local Ethics Committee of Palermo University (n°10/18 – 14/11/2018), this study was carried out according to the Declaration of Helsinki. All participants included in the study signed a specific informed consent.

Patients with advanced colon cancer or intraperitoneal rectosigmoid cancer (15 cm from the anal verge) with clinical T3/T4 N0-2 M0 stage or perforated colon cancer were prospectively enrolled between January 2019 and December 2020.

Eligibility criteria also included:

- histologically proven adenocarcinoma, cancer with mucinous (MC) or signet ring cell components (SRC);
- age between 18 and 78 years that could undergo major surgery;
- satisfactory cardiopulmonary function with no evidence of myocardial infarction during the previous 6 months;
- ECOG performance status of 0-2;
- normal liver function;
- normal renal function (blood urea < 50 mg/dl and creatinine level < 1.5mg/dl);
- normal white blood cell count (>4000) and platelets (>150.000);
- no major uncontrolled metabolic, cardiovascular or neurologic diseases;
- minimal synchronous PC (nodules < 1 cm in the omentum and close to the primary tumor);
- synchronous ovarian metastases.

PC and ovarian tumor deposits must have been macroscopically wholly resected at the same time as the primary tumor. The exclusion criteria were the following:

- age under 18 years old or over 78;
- the presence of irresectable metastatic disease;
- previous treatment for cancer;
- the presence of a second malignant tumor at high risk for recurrence;
- Karnofsky performance status <50%;
- extensive PC;
- psycosis, drug or alcohol addiction;
- active infection or severe associated medical conditions;
- the presence of diffuse peritonitis;
- pregnancy.

MC was diagnosed when >50% of the tumor comprised a mucinous pattern on histological examination and secreting acini produced extracellular mucin in large amounts.

2.2. Clinical outcomes evaluation

The study endpoints of the first-stage were length of hospital stay, surgical and medical treatment-related toxicity after adjuvant HIPEC.

Safety was reported according to the Dindo *et al.* classification for surgically related complications and to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 4.0 for chemotherapy-related adverse events[47,48]. The surgical complication observation period included 30 days after the surgical procedure. Surgical complications and adverse events occurring within 30 postoperative days or during the same hospital stay were graded from zero to five. Follow-up assessment took place every three months during the first year and every six months later by physical examination, hematological and biochemical examinations, tumor markers (CEA, Ca 19.9), thoracic/abdominal C.T. scan, colonoscopy was performed once a year after the first year of follow-up or as needed according to oncologists' evaluation.

2.3. Treatments

The selection process was divided into two steps. Preoperatively, potential candidates were identified through an intensive workup, including clinical history; physical examination;

colonoscopy; thoracic, abdominal and pelvic computed tomography (CT) scan with venous and oral contrast medium and serum markers (carcinoembryonic antigen, CA19.9). The second step consisted of surgical exploration, with intraoperative pathological confirmation of risk factors for PC. Samples for peritoneal cytology were taken after abdominal exploration. Colon resection was done according to the oncological principles of adequate lymphadenectomy; total mesorectal excision was required for tumor of the middle and lower rectum; tumor deposits on visceral and parietal surfaces were surgically removed and organ resections as surgically needed. Intraoperative pathologic evaluation assessed tumor depth and the histologic feature was mandatory to include patients in the study. In the HIPEC group at the end of the surgery, patients who had signed informed consent and acceptance of receiving intraperitoneal chemotherapy received HIPEC. Only in one patient we performed an early post-operative intraperitoneal chemotherapy (EPIC) to wait for a definitive pathology. EPIC wasn't performed because we share the phenomenon of residual cancer cells which being encapsulated with fibrin probably make these cells less accessible for chemotherapy in an interval of time longer than two weeks after surgery.

HIPEC

The chemotherapy solution is prepared in the pharmacy department and it is sent to the operating room in a closed light-protected bag with appropriate labeling which is handled with double gloves and the integrity of the bag is checked. If the bag is approved there is no risk of direct exposure and it is given to the person responsible for the perfusion, who must check the patient's name, drug and dose delivered against those prescribed.

There are two methods for intraperitoneal administration of hyperthermic chemotherapy: open abdomen technique and closed abdomen technique.

The open method is usually performed by the "Colosseum technique", as described by Sugarbaker. In our center we use the closed technique.

Peritoneal perfusion involves the use of a machine that has the following characteristics (**figure 2**):

- a pump system
- a thermostat or heat exchanger
- integrated systems for temperature, pressure and flow control
- data analysis system using a specific computerized program with real-time display of the parameters and their recording

- an extracorporeal circuit (CEC).

The equipment must be guaranteed by CE regulations. A series of thermometers are also used for the constant evaluation of intra-abdominal temperature.

At the end of the surgery, the tubes for the treatment of chemo-hyperthermia, the drains and the thermal probes (for detecting the temperatures of the perfusion liquid entering and leaving the abdomen) are placed through the abdominal wall. Generally, 4 polyphenestrated tubes are planted, which allow the chemotherapy solution to be introduced and extracted from the cavity at the same time and are positioned in the following ways:

- tube n. 1 introduced below the right costal arch and positioned under the diaphragm and above the upper edge of the liver
- tube n. 2 introduced in the right hypochondrium and positioned in correspondence with the mesenteric root in the epigastrium
- tube n. 3 at the level of the right iliac fossa above pelvis
- tube n. 4 below the left costal arch and positioned in the left subdiaphragmatic region.

Tube n. 2 and n. 3 (in flow) are connected to Y fittings and therefore to the pump of the chemo-hyperthermia machine and will act as an infusion; the others (out flow) will be perfusate recovery drainages. The catheters used for HIPEC are left in place and will serve as drainages for the post-operative course. In some cases, a Jackson-Pratt type drain is added to be maintained postoperatively longer than the drains used for the perfusate. When the closed abdomen technique is adopted, anastomoses and stomoses are usually performed before the laparotomy is closed; therefore, the suture of the wall and the packaging of the stoma must be perfectly sealed in order to avoid leakage of chemotherapy solution during HIPEC. The abdominal wall is closed and the cavity is firstly washed with peritoneal dialysis solution in order to keep the catheters for drug administration and abdominal drainage clean of any blood clots and tissue residues. Subsequently, after verifying that everything is proceeding regularly (conditions of the patient, parameters of the equipment, etc ...), the patient is covered with a cloth and the chemo hyperthermia cycle begins. The suturing of the abdominal wall and the total coverage of the affected area with a surgical cloth prevent the diffusion into the environment of any aerosol produced during the chemotherapy treatment (nebulization). This treatment lasts about 30-60 minutes depending on the neoplasm and during all this time a clinical perfusion scientist is dedicated to checking the procedure. At the end of the intraoperative perfusion, the liquid is

completely aspirated in the abdomen and before reopening the abdominal cavity, a further washing is carried out by recirculating with the Performer LRT about 2 liters of peritoneal dialysis solution for five minutes. According to the patient's BMI, the perfusion circuit is established using either a 5% glucose solution of 2 liters / m2 or physiological solution or according to the Sugarbaker's protocol 1.5% dextrose peritoneal dialysis solution; the choice of solutions does not change the final result, but derives exclusively from the protocols adopted, containing the chemotherapy at the pre-established dose. Flow rates are adjusted to maintain stable temperatures with inflow temperatures not exceeding 42.5 ° C and efflux temperatures not exceeding 41 ° C. The patient's body temperature should not exceed 39.5 ° C using passive (turn off routine warming devices) and active (cooled operating table and cold intravenous and intravescical fluids) methods of cooling when needed. The intraperitoneal temperature is maintained at 41.5 ° C and monitored by thermometers inserted in the subphrenic space and in the pelvic cavity. The use of the Swan-Ganz catheter in place during HIPEC to monitor cardiovascular function is discretionary; currently the use of the "Vigileo ®" allows patient monitoring that can be superimposed on the Swan-Ganz catheter with less risk. The stability of the temperature is directly proportional to the flow of the perfusate which must be maintained between 800 and 1200 mml / min. At the end of the perfusion, of variable duration depending on the drugs and protocols used, the liquid in the abdomen is completely aspirated and the inside of the peritoneal cavity is washed with 2-3 liters of Lactated Ringer's solution. In the immediate postoperative period, washing with a 1.5% dextrose solution is carried out in order to remove fibrin, cells in post-chemotherapy apoptosis and blood residues. The purpose of these washes is to avoid that neoplastic cells not in apoptosis, therefore vital, are harnessed by fibrin and can result in what Sugarbaker calls "the cathedrals of cancer", which could over time result in the recovery of the disease. The postoperative abdominal wash technique is based on three stages:

1. clamp closure of all abdominal drains except the used one as in flow during perfusion.
2. rapid infusion of 1000 cc of solution from the inflow drain
3. reopening of all drains

The postoperative washes are carried out every hour until a clear liquid is obtained or meat washes during the outflow, then continue every 2 hours for the first 12 hours after surgery.

HIPEC is delivered with the closed technique with oxaliplatin at the dose of 460 mg/m2 in 2 l/m2 of dextrose solution over 30 minutes at a flow rate of 2 L/min and a temperature of 43°C. Before the HIPEC procedure and during surgery, patients received intravenous fluorouracil of 400 mg/m2 and leucovorin of 20 mg/m2 to potentiate oxaliplatin activity.

Laparoscopic adjuvant HIPEC

Minimally invasive access to the abdominal cavity is obtained, followed by adhesiolysis if indicated and thorough inspection of the peritoneal surfaces. At least one multiperforated inflow catheter is placed through a 10–12 mm port in Douglas pouch and one multiperforated outflow catheter in the right subphrenic space. The patient's body temperature will be monitored in the oesophagus. All trocars are tightly fixed to the skin to avoid fluid leakage during the procedure. After a total perfusion time of 30 min, the peritoneal fluid is totally suctioned and the abdomen is examined for evidence of tissue injury or bleeding. A suction drain will be left in Douglas pouch for 24 h. The other port sites are closed in a standard fashion (**figure 3**). Postoperative care after simultaneous HIPEC will be according to the primary colonic resection following an enhanced recovery program. After staged laparoscopic HIPEC, patients are fully mobilized at day one with normal diet and will intentionally be discharged from the first to the third day if the institutional discharge criteria are fulfilled. Hematologic parameters will be determined at day 14, followed by start of systemic chemotherapy.

Open adjuvant HIPEC

Open adjuvant HIPEC can be performed simultaneously in patients undergoing primary open resection and staged open adjuvant HIPEC can be performed by re-laparotomy in patients who underwent primary open CRC resection. Besides the access to the peritoneal cavity, the procedure is similar to the laparoscopic approach as described above. Preferably, a closed perfusion is performed rather than a Colosseum technique to have similar pharmacokinetics as a laparoscopic approach. After positioning of the in- and outflow catheters, the abdomen will be closed and subsequently perfusion will be started (**figure 4**). Postoperative care is similar to the laparoscopic approach with an anticipated day of discharge between day two to five if discharge criteria are fulfilled. Hematologic parameters will be determined at day 14, followed by start of systemic chemotherapy.

All the specimens were histopathologically examined. Details about T, N, TNM stage, degree of differentiation and circumferential margins of resection were recorded.

Adjuvant s-CT was administered within 6–8 weeks after surgery, if indicated by medical oncologists, according to international guidelines. During postoperative follow-up, physical examination, thoracic/abdominal CT scan and oncological marker measurements were performed every three months during the first 2 years and every six months thereafter.

Control subjects were retrospectively selected from a retrospective database collecting patients

operated by the same two surgeons in the same period.

2.4. Statistics

The size of this study (two independent samples) was calculated employing a probability of a type-1 error 10% cutoff (alpha 0.1), and a probability of a type-2 error related to the study power (power 1-β) cutoff of 20% cutoff (beta 0.80)[49]. The endpoints were binomial, therefore for an anticipated mean of 3.5 $\pm$ 1.5 in the control group and an assumed 35% increase in complication rate in the experimental arm with an enrollment ratio of 2:1, a total of 49 evaluable patients had to be enrolled, at least 18 and 31 in the experimental group and the control one respectively.

Continuous monitoring for toxicity using a Pocock-type boundary was employed with an event probability (θ) of 0.2 and the desired probability of early stopping of 0.05[50]. The trial will be stopped if the number of dose-limiting toxicities is equal to or exceeds b_n out of n patients with completed follow-up. This boundary is equivalent to testing the null hypothesis; after each patient, the event rate is equal to 0.1, using a one-sided level 0.026846 test. Sequential boundaries were used to monitor the dose-limiting toxicity rate. The accrual will be halted if excessive numbers of dose-limiting toxicities were seen: if the number of dose-limiting toxicities is equal to or exceeds b_n out of n patients with full follow-up. As shown in **table2**, this is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most [probability of early stopping] when the dose-limiting toxicity rate is equal to the acceptable rate [event probability θ].

A descriptive analysis of all included patients was performed. Patient characteristics, tumor characteristics, and operative findings by lymphadenectomy or other surgical maneuvers were compared using Wilcoxon's test for quantitative variables and chi-square or Fisher's exact tests for qualitative variables. The time-dependent comparison was constructed using the Kaplan–Meier method with the log-rank test and the Gehan-Breslow-Wilcoxon test to detect differences between groups.

CHAPTER 3

Results

3.1 Patient population

Overall, 49 patients were enrolled in the study. **Table 3** reports the main clinical and demographic characteristics of the enrolled patients. Eighteen patients were candidate to receive post-surgery HIPEC and 31 patients had surgery only. Fourteen out of the eighteen patients who were candidates for HIPEC were evaluable (83%). Three patients did not reach intraoperatively criteria to receive post-surgery HIPEC. Other patients didn't accept the study, some hadn't normal white blood cell count, in one case a perforation of diaphrammatic peritoneal didn't permit the HIPEC. **Table 3** reports no statistically significant differences between the two groups of patients in terms of gender, median age, the primary tumor site, percentage of patients treated with neoadjuvant chemotherapy or radiotherapy. Nearly one-third of patients had the primary tumor in the ascending colon/hepatic flexure, while the remaining ones had it in the descending colon/splenic flexure or sigmoid colon. Three patients had neoadjuvant chemotherapy and only one pre-operative radiotherapy. Video-laparoscopic surgery was done in 33% and 39% of HIPEC and control groups, respectively. In some patients, it was necessary to combine the resection of other organs with the standard colectomy. Peritoneal washing was cytologically negative in all cases.

HIPEC group: they were 9 women and 5 men, with an average age of 61 (38-76 years). A right hemicolectomy was performed in 5 patients (3 performed in videolaparoscopy and in one case associated with cholecystectomy); 3 patients underwent anterior resection of the rectum with ileostomy packaging; 3 patients with anterior resection of the rectum en bloc with uterus and adnexa; in 1 patient an extended multivisceral resection was performed in addition to the transverse colon and the left colon, the spleen, the pancreas body-tail, the gastric body-antrum, the left kidney and adrenal gland; in 1 patient left hemicolectomy was extended to the transverse colon; 1 patient underwent en bloc anterior rectal resection with cystectomy and prostatectomy.

Control group: it includes 31 patients, 17 men and 14 women, with an average age of 62, who were referred for adjuvant therapy according to local protocols after surgery.

All the surgical procedures met the oncological radicality criteria and were R0. Definitive histological examinations confirmed the pT3-T4 pN0-N2 stage **(figures 5, 6, 7, 8, 9).** No complications were recorded in the postoperative course and the average hospital stay was approximately one week. The absence of complications was recorded in the perioperative up to 30 days.

Patients were referred to adjuvant therapy with XELOX regimen, capecitabine or follow-up alone.

The follow-up controls, carried out up to now according to the protocols of the international guidelines, have all been negative.

The pandemic coronavirus disease 2019 (COVID-19) has reduced the possibility to enroll patients.

3.2 Outcomes

Table 4 shows results in terms of duration of surgical procedure subtracting time dedicated to hyperthermic intraperitoneal chemotherapy procedure, length of hospitalization, surgical complications, side-effects related to chemotherapy and impact of HIPEC on post-surgical chemotherapy. No significant difference was seen in median time spent in the hospital (**figure 10**) with a median stay of 7 days in both groups (Gehan-Breslow-Wilcoxon test p=0.5720, N.S.; Mantel-Haenszel HR 0,0922, 9%% CI 0.4282-2.299). As shown in **figure 11**, the surgical procedure's median duration was longer in the HIPEC group than in the control one (median 192 versus 138 minutes). This difference was statistically significant when the log-rank test was applied (p=0.0037), but not with the Gehan-Breslow-Wilcoxon test (p=0,0810). The rate of surgical complications as well side-effects potentially related to chemotherapy were equally distributed in both groups. Side-effects and surgical complications did not cross at any time the Pocock-type boundary for side/effect monitoring (p=0.80, N.S.). Moreover, HIPEC did not affect subsequent adjuvant chemotherapy safety being toxicity the same in both groups of patients.

None of the patients in the HIPEC group developed recurrence; one patient who had no indication for adjuvant CT during follow-up underwent chest CT showing suspected mediastinal lymphadenopathy with uptake on station 4R lymph nodes at the next PET scan. She was then subjected to c-EBUS-TBNA with histological diagnosis of tumor-free lymph nodes.

To date, the outcome records a 51-year-old patient in massive hepatic metastatic progression and death from cachexia approximately 6 months after surgery and a patient who died of respiratory failure two months after surgery.

In the remnant patients OS and DFR are 100%.

In the control group four patients showed relapse of disease: one developed pulmonary metastases 4 months after the primary surgery and underwent left basal trisegmentectomy in VTS; one developed anastomotic and peritoneal recurrence and died 15 months later the primary surgery; one had hepatic

metastases after 3 months RFTA treatment and one had parietal peritoneal recurrence treated with surgical resection.

CHAPTER 4

Discussion

Between 2010 and 2020 in our center "La Maddalena" were performed about1000 cases of colorectal resection for oncological pathology. In the same decade, the center developed a consolidated experience for various abdominal oncological diseases for which an indication for cytoreductive treatment with HIPEC (carcinosis from ovarian cancer, gastric cancer, etc ...) is recommended. The overall complication assessment, according to DINDO *et al.* classification, recorded 0.05% of mortality (class V). All interventions were performed only by two surgeons with advanced background and expertise in open and laparoscopic surgery. Postoperative colorectal staging recorded approximately 700 cases of pT3 / pT4 pN0-2 (70%). Of this population, to our knowledge, about 15% developed peritoneal carcinosis despite R0 resection and adjuvant treatment, in line with literature data. Metastatic diffusion into the peritoneal surface carries out a very unfavorable prognosis and a dismal quality of life in many patients with recurrent/metastatic CRC[51]**.** CRS plus HIPEC has become a valid treatment option for colorectal PC. A 5-years overall survival (OS) rate of 35-40% has been reported for patients treated by CRS + HIPEC. This strategy is supported by a strong rationale: first, CRS combined with HIPEC improve CRC-PC survival, but most patients are not suitable for this demanding treatment due to extensive peritoneal involvement, systemic metastases and/or poor clinical conditions; second, CRS/HIPEC is maximally effective and safe when small-volume disease is treated; third, in the palliative setting, modern systemic chemotherapy (s-CT) and target agents appear to be less effective for peritoneal metastatic CRC than non-peritoneal metastatic CRC; finally, the absence of symptoms, as well as current limitations of imaging, hamper early diagnosis and treatment[52]. Because of these aforementioned difficulties, for those patients with colo-rectal cancer at high risk for peritoneal carcinosis, although the characterization of their genetic and epigenetic alterations may improve the prognostic model and allow a tailored therapy, difficult to apply in daily surgical practice, it seems justified a "proactive" surgical approach. The use of adjuvant HIPEC has been tested at different time-points, either simultaneously with primary surgery, at the time of second-look surgery after adjuvant systemic chemotherapy or as a staged procedure at 5-8 weeks postoperatively.

Researchers at the National Cancer Centre in Singapore have recently published an updated review of the state of the art of surgical management plus HIPEC for locally advanced CRC[53]. The multicenter COLOPEC study was carried out in nine hospitals in the Netherlands and the primary endpoint was peritoneal metastasis free-survival at 18 months, according to an intention-to-treat[54]. This study followed a pilot study, which reported a clear advantage for adjuvant delayed laparoscopic HIPEC after a median follow-up of 4.5 years in patients with a high risk of peritoneal spread[55]. In the COLOPEC trial, 204 patients with clinical or pathological T4, N0–2, M0 stage or perforated CRC were randomized before surgery in a 1:1 ratio to receive adjuvant systemic chemotherapy alone or HIPEC within 5-8 weeks after primary resection followed by standard adjuvant systemic chemotherapy[54]. Patients were stratified for perforation, stage of the disease, age (<65 years or ≥65 years), and surgical approach (open or laparoscopic). Adjuvant HIPEC consisted of systemic leucovorin potentiated fluorouracil followed by intraperitoneal hyperthermic delivery of oxaliplatin by either laparoscopic or open procedure to allow exploration of the abdominal cavity for peritoneal staging and adhesiolysis when necessary. This technical approach was based on the assumption that second-look surgery, first described more than five decades ago, remains the only method to ascertain the presence of minimal progression at the peritoneal surface[55]. This study showed no statistically significant difference being peritoneal metastasis-free survival at 18-months 80.9% for the HIPEC group versus 76.2% for the control group, respectively. The main criticism is the high proportion (91%) of patients who received delayed adjuvant HIPEC 5 to 8 weeks after primary tumor resection when adhesions and tumor cell entrapment may have limited the drug distribution and effectiveness of HIPEC. Moreover, 9% of the 100 patients in the HIPEC group had peritoneal invasion before delivering HIPEC.

Another recently published prospective open-label, phase III trial PROPHYLOCHIP, carried out in France, failed to improve disease-free survival compared to standard surveillance[56]. This trial enrolled 150 patients affected by CRC who underwent resection of the primary tumor and synchronous peritoneal or ovarian metastases and treated with adjuvant systemic chemotherapy. Patients were randomized to no further therapy or second-look surgery at the end of chemotherapy, plus, if no recurrence, oxaliplatin- or mitomycin-based HIPEC. The study did not reach the primary endpoint being 3-year disease-free survival 53% (95% CI 41-64) in the surveillance group versus 44% (33-56) in the second-look surgery group (hazard ratio 0·97, 95%

CI 0·61-1·56). These results disfavored the use of second-look surgery plus HIPEC in this clinical setting.

Overall, the results of these two-phase III trials challenged the effectiveness of HIPEC protocol with oxaliplatin and raised the question of whether delayed and limited exposure to chemotherapy (only 30 minutes) may negatively affect its antitumor effect. Timing of adjuvant HIPEC is another point of debate since it should be ideally delivered during primary surgical resection to avoid tumor cell entrapment and delayed microscopic disease management. Statistical sample underpowering could be another explanation for the failure to reach study endpoints.

Although surgery plus HIPEC yielded unsatisfactory results in patients with a high PCI score, it showed promising results in patients at high risk of recurrence, but still without clinically evident peritoneal spread. Generally, CRC patients with a low PCI score show better survival rates and lower postoperative morbidity after treatment with surgery and HIPEC[57]. Therefore, the recognition and management of peritoneal invasion as early as possible may play a pivotal role in maximizing therapeutic results and, ultimately, in patients' survival and quality of life[54]. Unluckily early detection of peritoneal invasion is a significant challenge due to the lack of signs and symptoms and the relatively low accuracy of imaging techniques. CT scan may detect only less than 30% of peritoneal deposits with a size <5 mm[58,59]. Recently diffusion-weighted magnetic resonance (DW-MR) has been introduced as a possible imaging method[60].

To date there are two Italian prospective case-control studies to evaluate the feasibility and utility of HIPEC in reducing PC in high-risk CRC patients: the procedures were performed with oxalipatin-based HIPEC and mitomycin plus cisplatin-based HIPEC respectively; in both HIPEC was given at the same time as primary surgery. In the study by Sammartino *et al.*[46] high-risk cases were defined by T3/T4, perforation and mucinous histology. The experimental group underwent carcinosis prevention strategies including complete omentectomy, bilateral salpingo-oophorectomy, hepatic round ligament resection and appendectomy. After 48 months, PC and local recurrence developed significantly less often in the patients who had received prophylactic HIPEC compared to the control group (4% *vs.* 28%) (P<0.03). Patients in the prophylactic HIPEC group also survived longer (median overall survival 59.5 *vs.* 52 months). Despite similar morbidity, Kaplan-Meier survival curves disclosed significantly longer disease-free and overall survival in the prophylactic HIPEC than in the control group (P<0.05 and P<0.04).

In the paper by Baratti *et al.*[61], high-risk cases were defined as T4, synchronous krukemburg tumours and minimal peritoneal disease. Prophylactic HIPEC was with cisplatin and mitomycin-C and correlated to lower PC cumulative incidence [hazard ratio (HR) 0.04, 95% CI, 0.01–0.31; P=0.002] and better overall survival (HR 0.25, 95% CI, 0.07–0.89; P=0.039) and progression-free survival (HR 0.31, 95% CI, 0.11–0.85; P=0.028). Reported morbidities from HIPEC were minimal in both papers and there were no reported mortalities. The preliminary results have also shown that prophylactic HIPEC is feasible with minimal morbidity and does not delay time to adjuvant systemic therapy.

In our study, we report the feasibility and safety of colorectal surgery plus HIPEC in terms of length of hospital stay, surgical and medical treatment-related toxicity in a mono-institutional series of CRC patients high risk of recurrence, but still without evident signs of peritoneal spread who underwent radical surgery. As Sammartino *et al.* did, we chose to include pT3 tumor according to the study of Kojima[62] who assessed that when a pT3 tumor invades the elastic lamina, as it does in almost 40% of patients, the clinical outcome almost always matches with those patients with pT4 cancer. With regard to histology of the tumor, several clinical and post-mortem studies have already suggested that colo-rectal mucinous adenocarcinoma seems to metastasize more frequently to the peritoneum compared with other types of adenocarcinoma. Although the detailed mechanisms of peritoneal metastasis from mucinous colorectal adenocarcinoma are not clear, the production of mucus under pressure might allow cancer cells to separate tissue planes in the bowel wall and more frequently gain access to the peritoneal cavity. In our hands, colorectal surgery plus HIPEC was feasible without an increase in surgical- or chemotherapy-related complications. The median length of hospital stay was not statistically different in patients who received HIPEC and those who did not.

Time spent on the surgical procedure was slightly longer in the HIPEC group than in the control one. This difference reached statistical significance. Even though other authors reported a 14% rate of postoperative complications after adjuvant HIPEC[54], in our experience, both surgical complications and chemotherapy-related toxicity were low. None of the 14 patients showed peritoneal recurrence or distant metastases after a median follow-up of 12 months. Although the patients sample size is too small to draw conclusions about survival outcomes, also due to the pandemic COVID-19 situation, our results are, however, in line with the encouraging results of other studies investigating the role of adjuvant HIPEC in high-risk CRC patients. These data

support the need for a proper patient selection based on clinical criteria if surgeons plan to deliver HIPEC simultaneously with primary or staged resection and if prophylactic resection of target organs may influence outcomes. These criteria include data from pre-operative imaging, histological biopsies, biomarkers and intra-operative findings. However, in our experience, it was difficult to select patients based on clinical staging adequately. A well-defined cT4 stage based on imaging or intraoperative findings frequently turns out to have a pathological T3 stage. Therefore, this proactive management of patients with stage T4, as the only risk factor, could represent an overtreatment as recently suggested[63]. In our HIPEC series only two patients were classified as adenocarcinoma pT3 pN0 and we motivated this possible "over- treatment" with their young age. To eliminate the doubt of Sammartino *et al.*[64] who cannot state whether the good results in terms of peritoneal recurrence and DFS in the HIPEC group depend on the associated surgical procedure, we didn't perform the resection of the target organs because we hypothesized that potential micro-metastases at these sites are sufficiently treated with HIPEC. Three trials, the Italian PROMENADE, the Chinese APEC and the Spanish HIPECT4 are currently investigating the early use of adjuvant HIPEC for locally advanced CRC[65-67].

Conclusions

"*It's what the surgeon doesn't see that kills the patient*" said Sugarbaker[68]. It was this sentence that prompted us to undertake this research project. In our experience colorectal surgery plus HIPEC treatment is safe and feasible, it seems to be a promising strategy for patients with advanced colorectal cancer to prevent the development of peritoneal recurrence and improve prognosis of this group of patients. The goal is to avoid peritoneal disease or to treat it at its earliest stages when citoreduction and HIPEC have the biggest impact. Our data concerning the impact of survival parameters are not available due to follow-up shortness. Further studies are needed to better identify early peritoneal invasion and optimize the role of colorectal surgery plus HIPEC in patients at high risk of peritoneal cancer spread. It is necessary not to stop at the appearance, but to go further.

CHAPTER 5

Tables & Figures

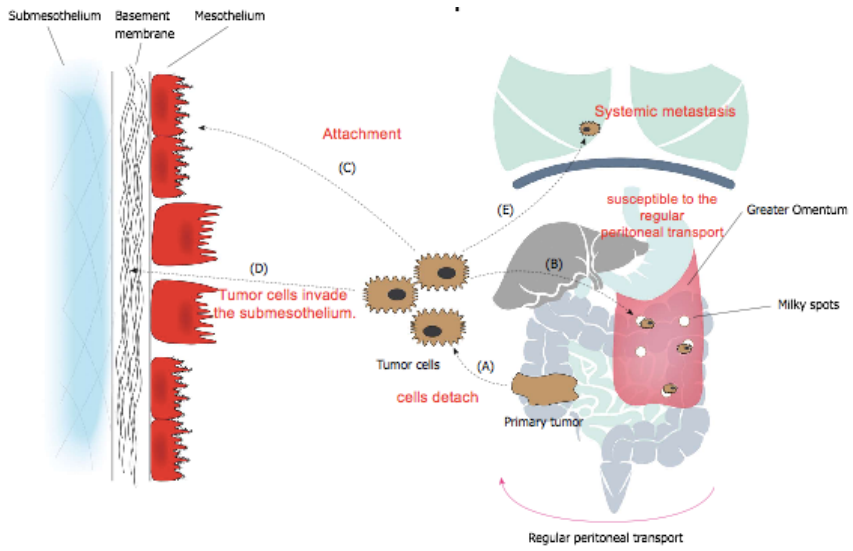


Figure 1. *The peritoneal metastatic cascade*

Pathophysiology of colorectal peritoneal carcinomatosis: the peritoneal metastatic cascade. The emergence of PC is the result of molecular crosstalk between tumor cells and host elements, comprising several well-defined steps. A: Individual or clumps of tumor cells detach from the primary tumor and gain access to the peritoneal cavity. Spontaneous exfoliation of tumor cells from the primary tumor can be promoted by the down-regulation of E-cadherin, increased interstitial fluid pressure, and iatrogenically during surgery; B: The free tumor cells become susceptible to the regular peritoneal transport. Peritoneal transport is due to changes in the intra-abdominal pressure during respiration, gravity and peristalsis of the bowel; which results in a clockwise flow from the pelvis, along the right paracolic gutter and to the subdiaphragmatic space and finally towards the pelvis again; C: Attachment of tumor cells to distant peritoneum occurs via two processes, denominated transmesothelial and translymphatic metastasis. During transmesothelial metastasis, loose tumor cells directly adhere to distant mesothelium through adhesion molecules. During translymphatic metastasis, free tumor cells gain access to the submesothelial lymphatics through lymphatic stomata. Preferential tumor growth in the milky spots of the greater omentum has been observed; D: Tumor cells invade the submesothelium. In areas of absent or rounded (cuboidal) mesothelial cells, tumor cells interact with the laminar network of the basement membrane through integrin-mediated adhesion. Subsequent invasion of the submesothelial tissue occurs via degradation by proteases (MMPs); E: Systemic metastasis. PC: Peritoneal carcinomatosis.

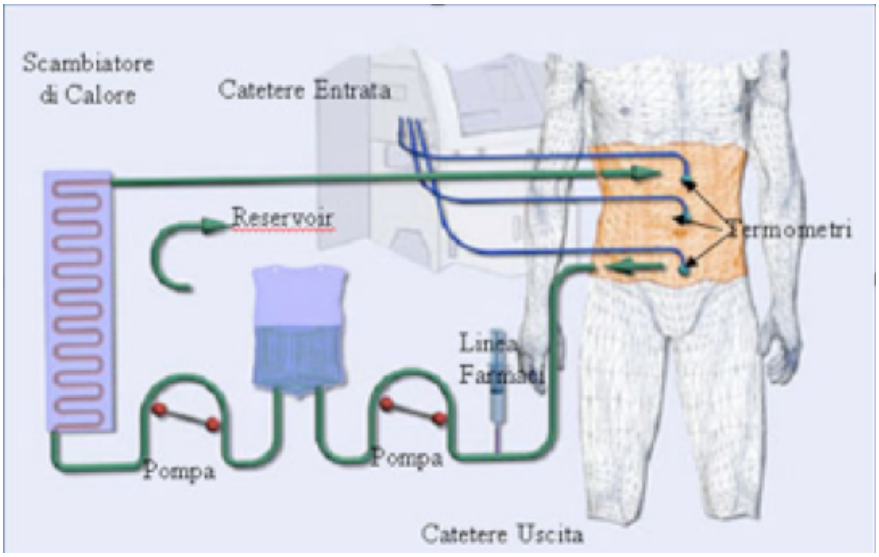


Figure 2. *HIPEC machine*

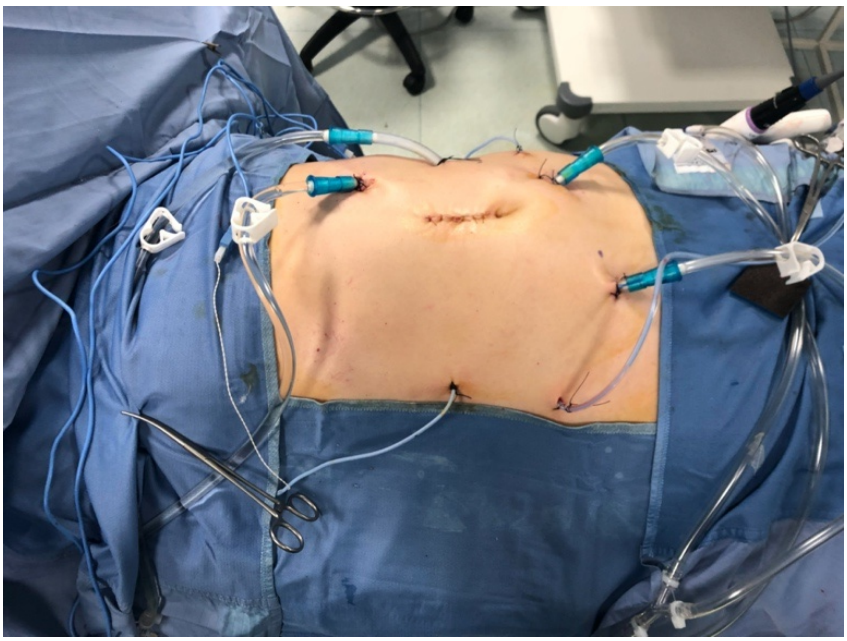


Figure 3. *VLS HIPEC performed*

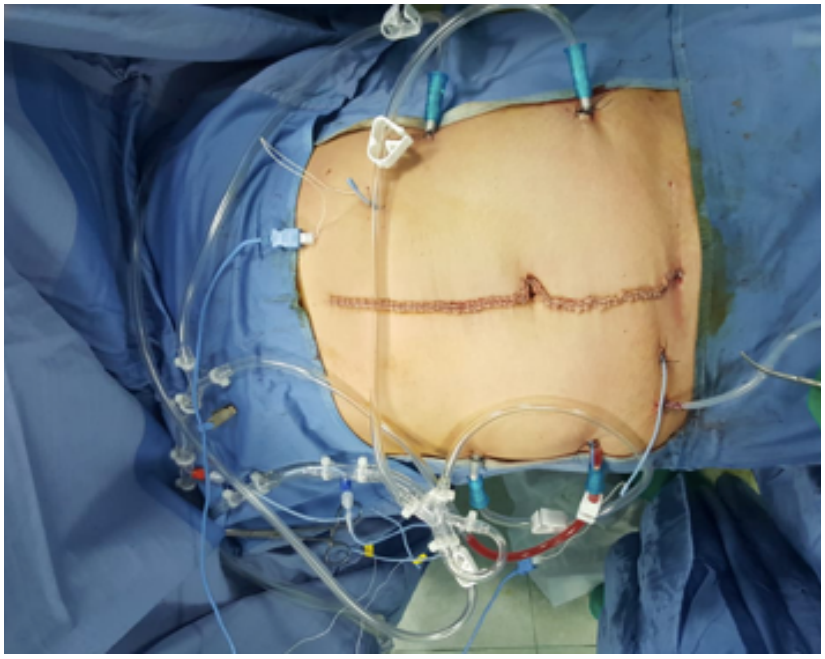
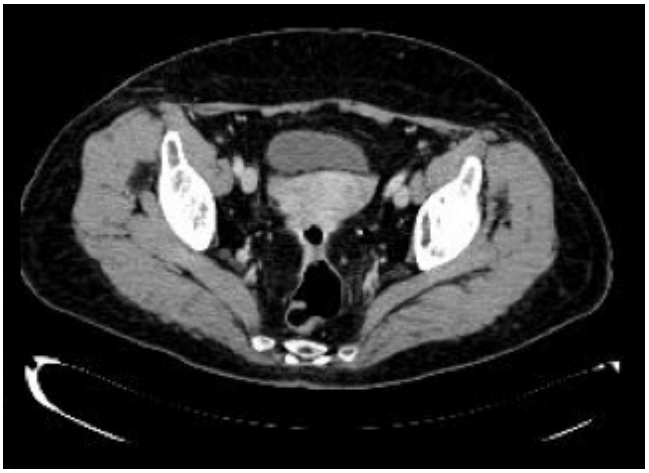


Figure 4. *Open HIPEC performed*



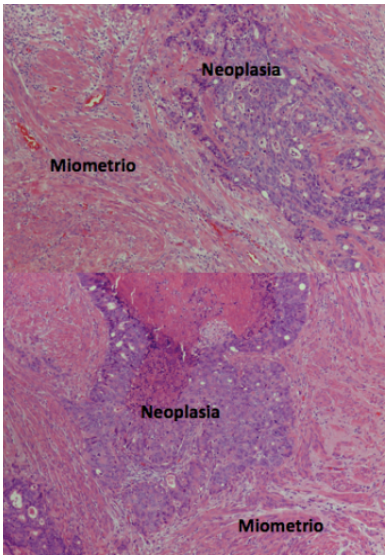
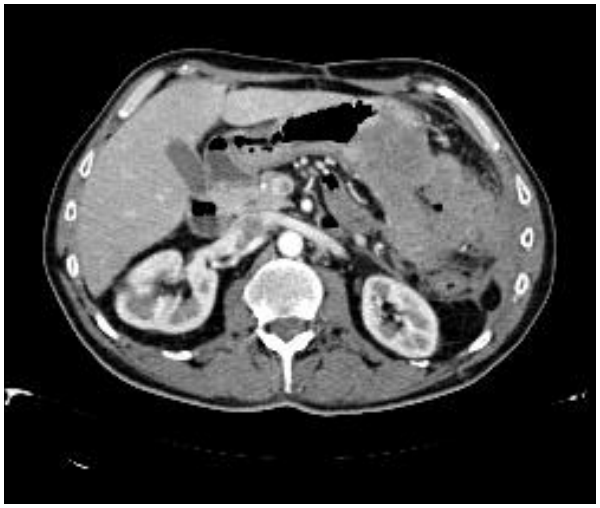


Figure 5 - Case 2. *Pre-operative imaging TC, intra-operative macroscopic imaging of surgical specimen and histopathological detail of a case of colon cancer which infiltrate the uterus pT4bpN1b.*



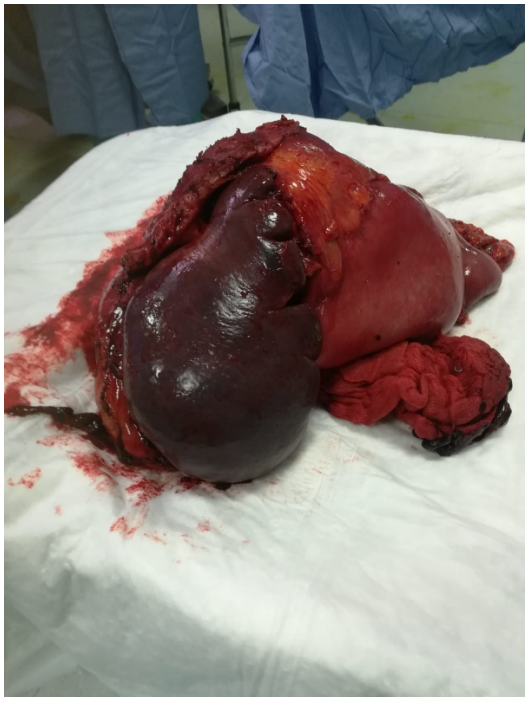


Figure 6 – Case 4. *Pre-operative imaging TC and macroscopic imaging of surgical specimen of a case of colon cancer which required extended multivisceral resection to the transverse colon and the left colon, the spleen, the pancreas body-tail, the gastric body-antrum, the left kidney and adrenal gland required the resection of spleen, stomach and left kidney (pT4b pN0).*

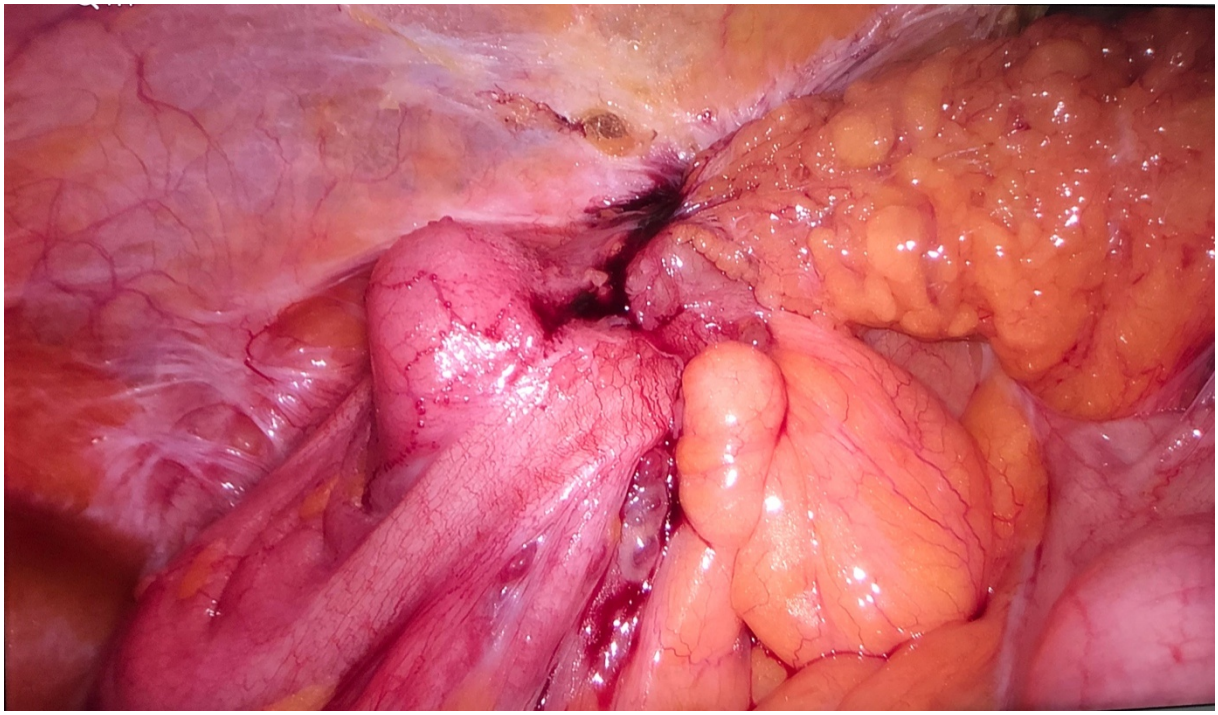
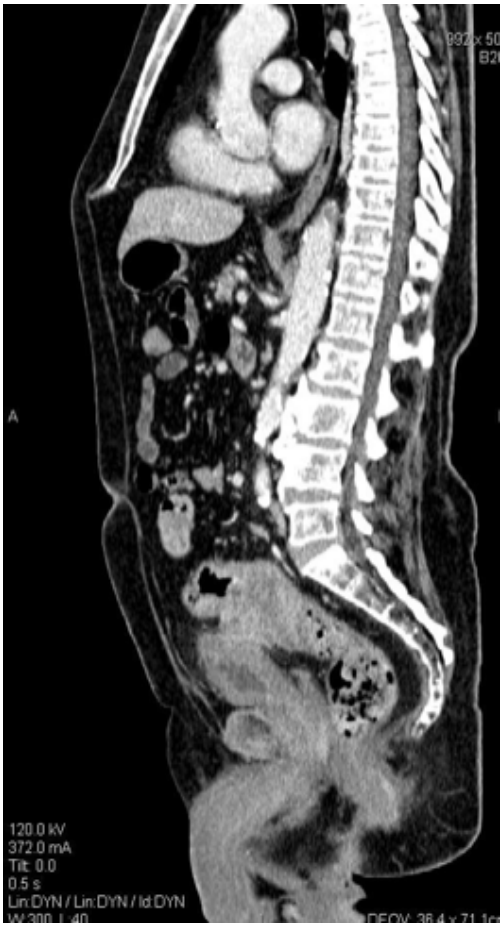


Figure 7 – Case 7. *Intraoperative VLS imaging of right colon cancer which infiltrate the abdominal wall (pT3 pN1a).*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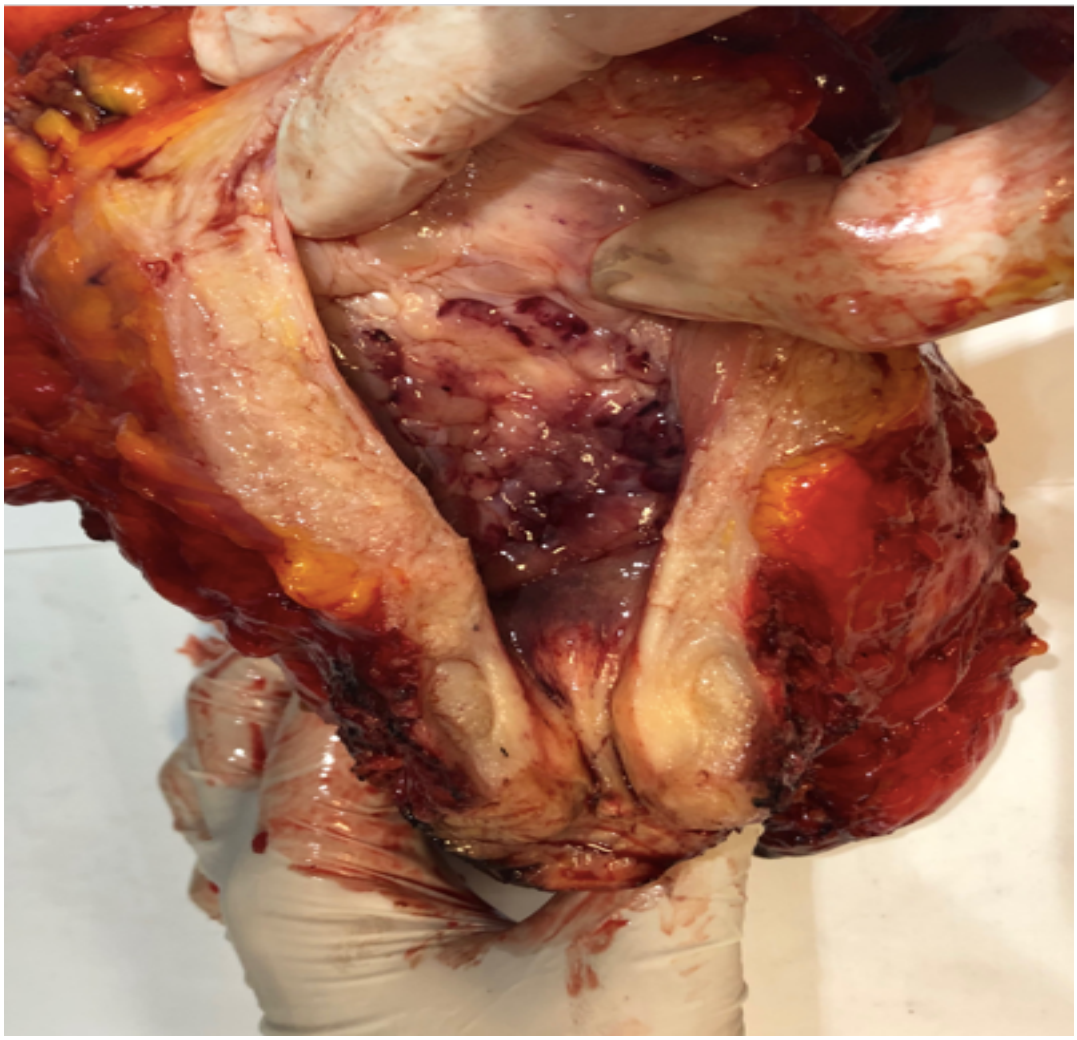

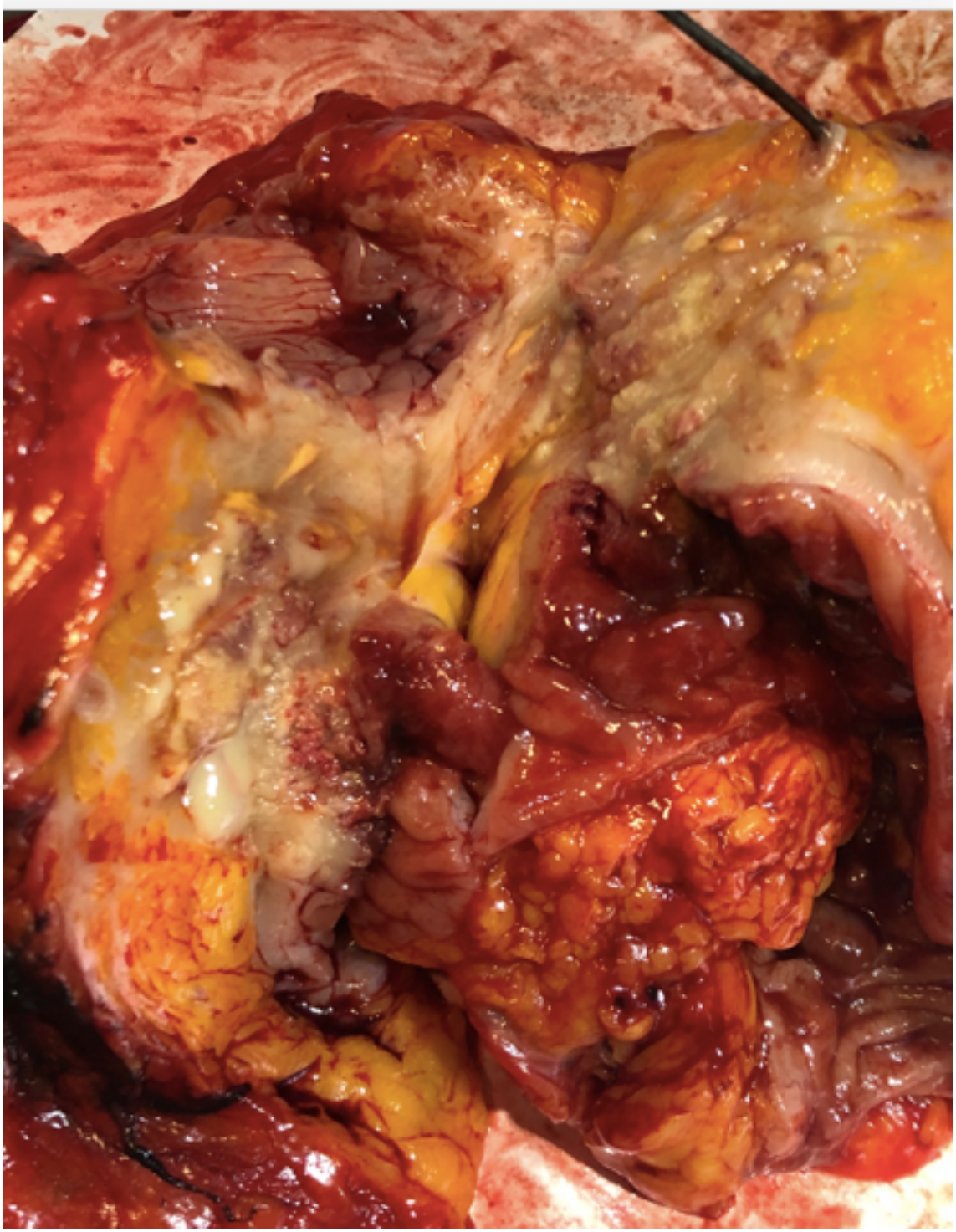

Figure 8 – Case 15. *A case of miss-match between a well-defined cT4 stage based on imaging or intraoperative findings that turns out to have a pathological T3 stage. The surgical procedure included, in addition to colonic resection, cystectomy, prostatectomy and ureterocutaneostomy and Bricker urinary diversion. HIPEC didn't not performed for leucopenia.*

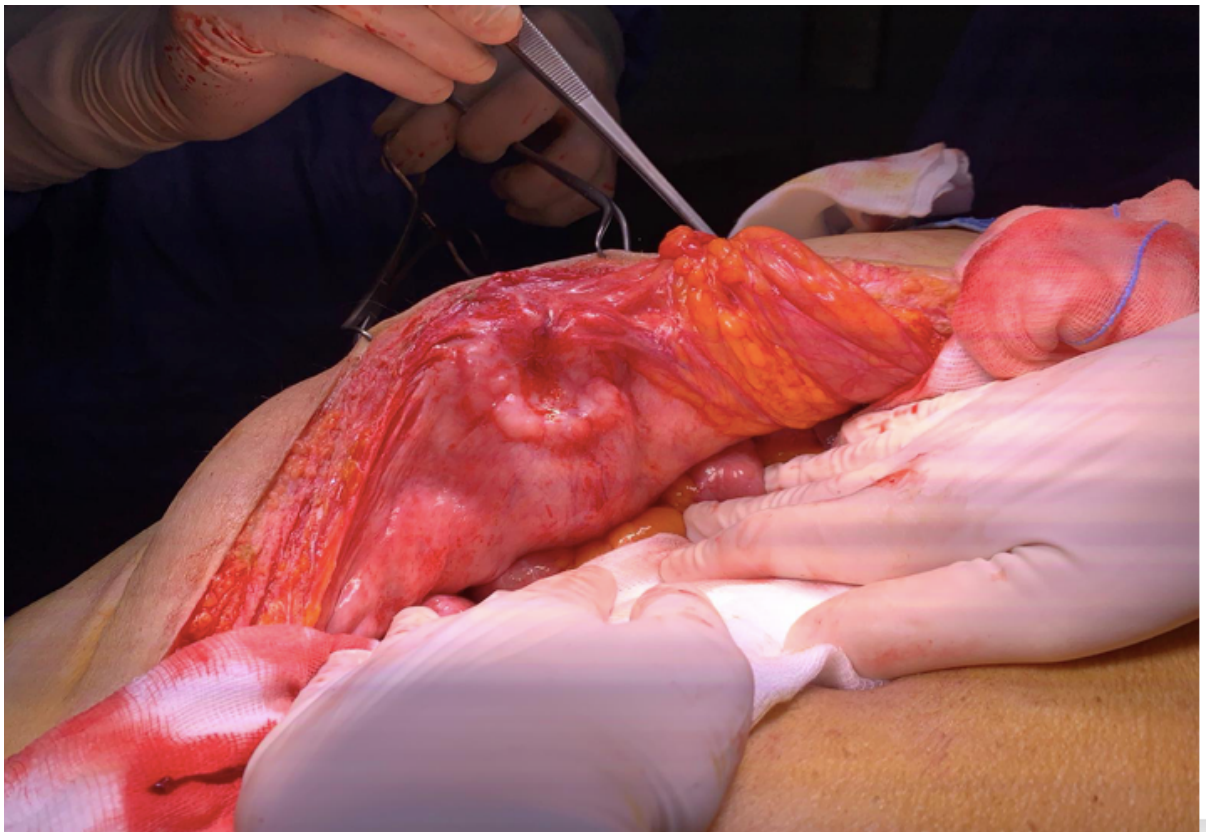

Figure 9 *Resection of abdominal wall metastases 1 year after radical right hemicolectomy pT3 pN0.*

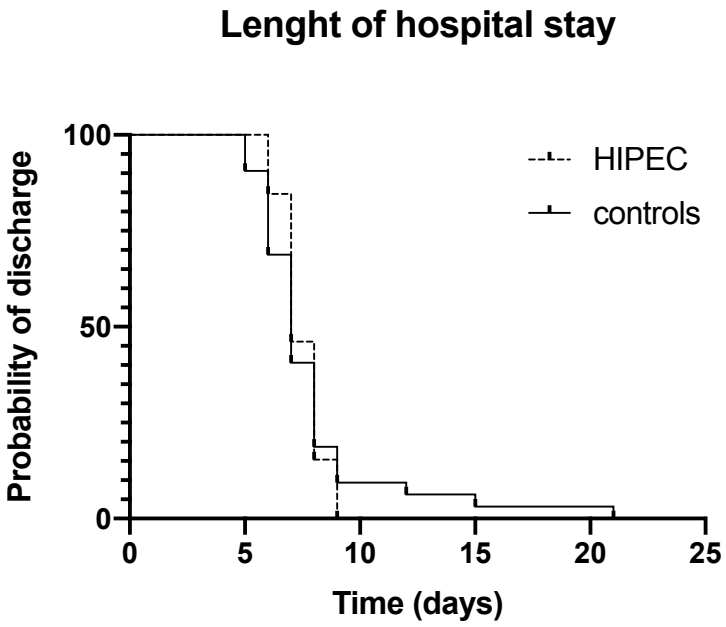

Figure 10

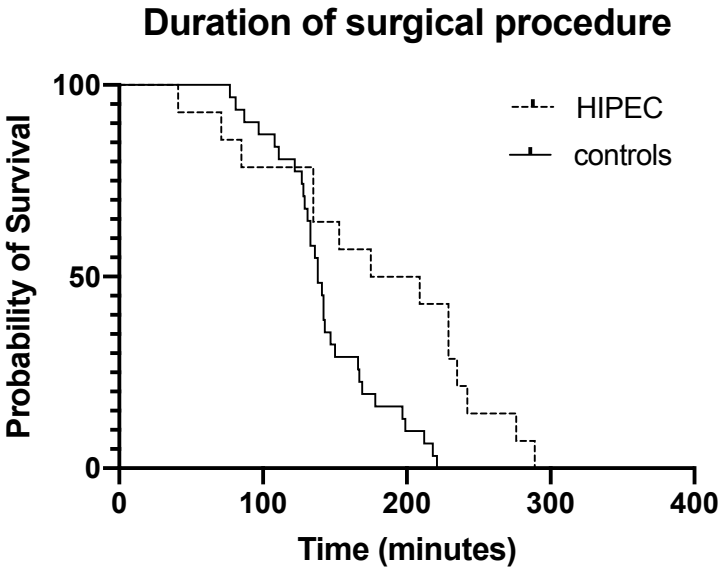

Figure 11

Clinical features	*Estimated incidence of peritoneal metastases observed in follow-up (%)* *Colorectal cancer*
1. Peritoneal nodules detected with primary cancer resection	70
2. Ovarian metastases	60
3. Perforation through the primary cancer (free or localized)	50
4. Adjacent organ or structure invasion	20
5. Signet ring histology by endoscopic biopsy	20
6. Fistula formation	20
7. Obstruction of primary cancer	20
Histopathologic features[o]	
8. Positive margin of resection	80
9. Positive peritoneal cytology before or after resection	40
10. Positive imprint cytology	40
11. Lymph nodes positive at or near the margin of resection	20
12. T3/T4 mucinous cancer	40

[o]Requires intraoperative histopathologic assessment by the pathologist who is a member of the multidisciplinary team

Table 1 *Clinical and intraoperative histopathologic features of the primary cancer as an estimate of the incidence of subsequent local recurrence and/or peritoneal metastases to guide proactive treatment with perioperative chemotherapy at the time of primary colorectal resection.*

Number of patients	n	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Boundary	b_n	-	-	3	4	4	4	5	5	5	6	6	6	7	7

Table 2 *Pocock-type boundary for side/effect monitoring*

		HIPEC	CONTROLS	
N. of patients		18 (100%)	31 (100%)	
N. evaluable patients		15 (83%)	31 (100%)	
Age (years)	Median (range)	58 (46-76)	60 (44-78)	
Gender	Male	5 (36%)	17 (55%)	p=0.275787
	Female	9 (64%)	14 (45%)	
Site of primary	ascending colon*	6 (33%)	8 (26%)	p=NS
	transverse colon	0	1 (03%)	
	descending colon**	3 (17%)	10 (32%)	
	sigmoid/rectal colon	9 (50%)	12 (39%)	
CEA at basal > 4		2 (11%)		
Neoadjuvant CT	Yes	1 (6%)	1 (3%)	
	FOLFOX	1	0	
	XELOX	1	0	
	Capecitabine	0	1	
	None	13	30	
Neoadjuvant R.T.		0	1	
Surgery	Open	12 (67%)	19 (61%)	p=0.059126
	VLS	6 (33%)	12 (39%)	NS
	hemicolectomy	10	17	p=NS
	rectal anterior resection	8	14	
	Ileostomy	6	5	
Other organs resect-ed	Pancreas	1	0	
	gall bladder	1	0	
	uterus/ovary	3	3	
	urinary bladder	2	2	
	Peritoneum	1	3	
	Prostate	1	0	
Peritoneal washing	Negative	14	31	
	Positive	0	0	

Histology	Adenocarcinoma	18 (100%)	31 (100%)	
Post-surgical stage	pT3, N0, M0	5	2	
	pT3, N1a, M0	1	6	
	pT3, N1b, M0	1	11	
	pT3, N2a, M0	2	3	
	pT3, N2b, M0	2	3	
	pT3, N1c, M0	0	1	
	pT3, N2b, M0	0	1	
	pT3, N0,M1 (per)	1	0	
	pT4, N0, M0	0	1	
	pT4a, N1b, M0	0	2	
	pT4b, N0, M0	1	0	
	pT4b, N1a, M0	0	1	
	pT4, N2a, M0	1	0	
	pT4b, N1b, M0	1	1	
	pT4, N2b, M0	1	0	
*Including hepatic flexure; ** Including splenic flexure				

Table 3 *Patients' demographic and clinical characteristics*

		HIPEC	CONTROLS	
Duration of surgery (minutes)	median (range)	192 (71-276)	148 (77-221)	p=0.0037
Post-procedure side-effects°	Nausea/vomiting	1	2	p=NS
	None	14	29	
Hospital stay (days)	median (range)	7 (6-21)	7 (5-15)	p=NS
Complications after discharge		1 §	1 ^	
Adjuvant chemotherapy		11(73%)	25(81%)	p=NS
	FOLFOX	2	4	
	XELOX	7	18	
	CAPECITABINE	2	3	
	none	4 (27%)	6 (19%)	
Percent of planned cycles		90%)	87 (%)	p=NS
Side-effects (≥ grade 3)	Mucositis	2 (18%)	4 (16%)	p=NS
	Diarrhea	2 (18%)	3 (12%)	
	Neutropenia	2 (18%)	6 (24%)	
	Febrile neutropenia	0	0	
	Platelets	0	1	
Delays in chemotherapy	yes	4 (27%)	7 (28%)	p=NS
	no			
Adjuvant R.T.		1	1	

Table 4 *Results*

CHAPTER 6

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CHAPTER 7

Scientific Products

Oral presentation and poster

- ✓ "Low–volume liver surgery centre: perioperative outcome analysis of ten years experience", First Congress "Associazione Italiana Chirurgia Epato-Pancreatica";
- ✓ "PET e carcinoma esofageo: lungimirante visione o suggestivo miraggio", 57° Congresso regionale della Società Siciliana di Chirurgia;
- ✓ "VLS one-way valve: a minimally invasive surgery for surgery for gastroesophageal reflux", International Society of Diseases of the Esophagus Congress;
- ✓ "Thyroidectomy and Laryngo-Pharyngeal Reflux: What's New", International Society of Diseases of the Esophagus Congress.

Publications

- ✓ "How uncomplicated total thyroidectomy could aggravate the laryngopharyngeal reflux disease?", European Archives of Oto-Rhino-Laryngology, Volume 273, Issue 1, pp 197–202;
- ✓ "Surgical treatment of primary gastrointestinal stromal tumors (GISTs): management and prognostic role of R1 resections", Am J Surg. 2019 Dec 10. pii: S0002-9610(19)31557-0;
- ✓ "Sentinel Lymph Node Analysis in Colorectal Cancer Patients using One-Step Nucleic Acid Amplification (OSNA) in combination with Fluorescence and Indocyanine Green", Ann Coloproctol, Edizione 2019 - 35(4):174-180;

- ✓ "Laryngopharyngeal reflux as a potential cause of persistent local neck symptoms after total thyroidectomy", European Archives of Oto-Rhino-Laryngology, doi: 10.1007/s00405-020-06223-0;
- ✓ "Impact of BMI on preoperative axillary ultrasound assessment in patients with early breast cancer", Anticancer Research.