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Analysis of Tumoral Evolution and Prognostic Factors of Multi-Site Hepatic and Peritoneal Colorectal Metastases Processes: from the Animal Model to an International Clinical Study

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SUMMARY

LEGEND

ALPPS Associating liver partition and portal vein ligation

BMDC Bone marrow derived cells

CEA Carcinoembryonic antigen

CD31 Cluster of differentiation 31

c-Met Cellular Mesenchymal and Epithelial transition factor

CRC Colorectal cancer

CRS Cytoreductive surgery

CSF- Macrophage colony-stimulating factor

DSF Disease free survival

EGF Endothelial growth factor

EPC Endothelial progenitor cells

EPO Erythropoietin

FGF Fibrinogen growth factor

GFP Green fluorescent protein

HIF Hypoxia-inducible factor

HPC Hematopoietic progenitor cells

HGF Hepatic growth factor

HIPEC Hyperthermic intraperitoneal chemotherapy

IL- Interleukin

INF- Interferon

LM Liver metastases

LSEC Sinusoidal endothelial cells

NK- Natural killer cell

MMP Metalloproteinase

OS Overall survival

PC Peritoneal carcinomatosis

PDGF Platelet derived growth factor

PlGF Placental growth factor

RFA Radiofrequency ablation

SDF- Stromal cell-derived factor

TAM Tumor-associated macrophage

TNF- Tumor necrosis factor

TGF- Tumor growth factor

VEGF Vascular endothelial growth factor

VEGFR Vascular endothelial growth factor receptor

A. INTRODUCTION

The gold standard of the therapeutic strategy of resectable liver metastases (LM) is surgical resection [1]. Also, cytoreduction, with hyperthermic intraperitoneal chemotherapy (HIPEC), is the only curative treatment for colorectal peritoneal carcinomatosis (PC) [2]. However, surgery in cases of LM associated with extra hepatic metastases is still being debated [3]. LM occur in 25% to 40% of patients who have colorectal cancer (CRC) [1]. After curative liver resection, 70% of patients relapsed despite peri-operative chemotherapy. If a second surgical procedure is not possible, the overall survival (OS) rate is 12-38 months [1]. Chemotherapy improves the operability of liver metastases. In cases of non-resectable metastases, chemotherapy improves the margins of resection, reduce the sizes to facilitate surgery in inaccessible locations, eventually treated the micro-metastases [4-6]. The long-term outcome for patients undergoing chemotherapy alone is poor, with a median OS of 16-17 months in *CAIRO* and *CAIRO2* trials [7], and up to 31 months with intensification of the treatment in TRIBE trials [8]. The same results, in terms of OS, were obtained in *Karoui's* study of 208 patients operated on for primary tumours treated with adjuvant chemotherapy in association with an antiangiogenic factor (Bevacizumab) [9]. Over a period of ten years, the use of effective chemotherapy, when associated with new biotherapies, improved the OS [8,10-12]. Peri-operative chemotherapy, with FOLFOX, also improves OS and Disease Free Survival (DFS) in cases of resectable metastases when the CEA is elevated and the performance status is good [1].

PC occurs in 8 to 20% of patients with CRC [13-16] and is associated with a low survival rate (inferior to 6 months if untreated [17] or barely reaching 12 months with systemic chemotherapy [13,18,19]). Cytoreduction surgery (CRS), with intraperitoneal chemotherapy, including HIPEC, eventually associated with early postoperative intraperitoneal

chemotherapy (EPIC), is accepted as the only potentially curative treatment for PC from CRC origin, leading to a mean DFS of 18.4 months and a mean OS of 27.4 months [2] and up to 63 months in selected patients [20].

The synchronous presence of LM and PC from CRC origin is linked to very poor outcomes and is traditionally considered a contraindication to any surgical approach [21-25]. Recently, *Franko et al.* have shown that the presence of PC, with or without extra-peritoneal metastasis, is a prognostic factor of poor OS for patients with metastatic CRC [26].

However, since 1999, following the encouraging results of the management of liver and peritoneal metastasis from CRC separately, some studies reported a prolonged survival rate after the management of synchronous colorectal LM and PC (reaching 3 years in selected patients) [27-33]. This suggests that PC is not an absolute contraindication to liver surgery and that a curative surgical management of LM and PC may be possible [3,29,30,34,35]. To date, no standard management pathway has been established for patients with simultaneous LM and PC, especially if major surgery (liver and peritoneal resection) has to be performed. So far, the presence of PC has been considered an absolute contraindication against a hepatectomy for LM of CRC. Indeed, the major decision of surgical treatment is justified only if the chances of remission are significant. Yet, if liver and peritoneal metastases associated, mono-centric and limited series published promising results with median OS of up to 3 years (range 3.5-8) after simoultaneous complete resection of LM and PC [30,31,34,35]. Similar results have been reported when there extra hepatic not peritoneal lesions (lung and lymph node) which can be fully resected [3]. Thus, extra hepatic metastases may be a systematic contraindication to liver surgery, except if all the lesions can be resected in a oncological surgery satisfactory.

Cytoreductive surgery and HIPEC for peritoneal metastases combined with liver resection was recently analyzed, in a systematic review, as a possible option [33]. Authors claimed that

patients with metastatic CRC showed a tendency towards increased median OS, after cytoreductive surgery and HIPEC combined with resection of LM, when compared to treatment with modern systemic chemotherapies. At the present time, there is no specific surgical strategy for selecting patients with a high potential for success in view. In our study, we suggested that a surgical curative management of synchronous LM and PC is possible with acceptable mornbi/mortality rate. We analyzed a prospective international cohort of patients with PC associated with LM of CRC. The aim of this study was to describe and assess early outcomes (morbidity/mortality, hospital stay duration) and long-term results (DFS and OS) of patients undergoing liver resection and cytoreductive surgery with HIPEC for concomitant PC and LM. The secondary goal of this study was to identify variables potentially related to poor outcomes, in order to establish future guidelines for the management of those patients and optimize the selection of candidates for surgical treatment.

However, in case of aggressive surgical approach with curative intent, there are no guidelines for choosing which surgery should be done first: should we operate on the liver first or on the peritoneum? The question of the optimal sequence thus remains unsolved. The choice depends primarily on the number, and location, of liver metastases. If liver metastases require minor and uncomplicated resection, liver resection and cytoreduction, with HIPEC, can be performed in one stage. If liver metastases require complex or important resection, in particular on an injured parenchyma (post-chemotherapy), hepatic resection and cytoreduction with HIPEC will be conducted in two stages. A recent publication reports a monogram to help with the selection of a patient for a complex strategy. The strategy is based on the number of liver metastases, the extent of peritoneal metastases evaluated by the peritoneal carcinomatosis index (PCI), as well as the type of surgery [[36]]. Postoperative mortality was 2.7% after liver resection, 4.2% after CRS with HIPEC, and 8.1% after liver resection and CRS with HIPEC. The postoperative 3-4 grade morbidity rate reported was 11% after liver

resection, 17% after CRS and HIPEC, and 41% after liver resection and CRS with HIPEC ($p<0.001$). According to literature [30], the morbidity rate was high but the total number of reported patients with liver and peritoneal concomitant surgery was low (n=37) [37].

To offer a strategy for patients we proposed to associate the cytoreduction with HIPEC for limited liver procedures such as radiofrequency tumor ablation (RFA) or wedge resection. In cases of major liver resection, and peritoneum metastases, we propose to separate the surgery into two procedures (*Lo Dico et al*., ASCO 2013 Abstract n° #113772). The first procedural choice could be liver surgery, or a peritoneal procedure with HIPEC. We postulate that the choice be based on specific technical problems and oncological aspects. Technical problems depend on the type of surgery, including the choice of laparoscopic liver surgical approach, or anti-adhesion barrier film used to limit postoperative adhesion [38]. The oncological aspect depends on the effects of the liver resection on the metastasis growth in liver remnant and, less knows, in extra-hepatic recurrences [39-41]. We assumed that liver regeneration, after liver resection, could promote peritoneal carcinomatosis. To test our hypothesis, we constructed an animal model to mimic the human clinical situation of concomitant liver resection and peritoneum metastases. To mimic the human situation, an immunocompetent animal mouse model was chosen. If the animal model confirmed our hypothesis, we could determine the best sequence for liver and peritoneum surgeries and definitively propose the peritoneal cytoreduction as the first step of the surgical strategy.

Metastatic process and interactions with the microenvironment

In 1889, after scrutinizing more than 900 autopsy records of patients with different primary breast tumors, the English surgeon *Stephen Paget* published the seminal ***'seed and soil'*** hypothesis to explain the non-random pattern of metastasis to visceral organs and bones. According to this hypothesis, he claimed the outcome of metastasis was not due to chance but rather that certain tumor cells (***'seed'***) have specific affinities for the milieu of certain organs (***'soil'***). *Paget* concluded that metastases formed only when the seed and soil were compatible [42]. In accordance, the *Fidler* studies showed that cancer cells can reach the vessels of all organs but the metastases only develop in some organs [43,44]. *Paget* affirmed that the tumor could give metastases in specific territories outside the drainage areas of the primary tumor which differed from the Ewing's theory, according to which the onset of metastases strictly follow the vascular and lymphatic drainage of the primary tumor [Ewing 6th edn.WB Sounders Co.1928]. Some tumor types, such as the uveal melanomas, have a particular tropism for the liver, with a high rate of hepatic localization (93% of a cohort of 1,003 patients) [Collaborative ocular melanoma study (COMS): Arc Oph 2001]. Indeed, one has to take into account the genetic and epigenetic changes in the tumor cells, such as unlimited potential of replication and the acquisition of a phenotype resistance to apopotosis. However, stimulating angiogenesis and the spread of metastases (***metastatic potential***) could be linked to the genetic and epigenetic alterations as well as cellular changes and those of the microenvironment, in extra-peritoneal recurrences. Mediator metastases are organ specific and are not identical to those in the liver, bones, brain, lungs and peritoneum. The metastatic process depends on the intrinsic properties of tumor cells and extrinsic properties of the microenvironment of the original tumor and metastatic sites. A relation, between primary tumor sites and secondary organs, affected by the metastatic process, is evident in all theses

observations. Not all features of metastatic progression can be explained on the basis of the microenvironment.

The metastasis process of the peritoneal carcinomatosis remains unknown. More theories are being developed to explain the tumoral process in the spread of PC. The tumoral process could follow the same manner as in lymphatic or hematologic abdominal cases; or could be the consequence of the exfoliation of neoplastic cells [45], from the primary tumor directly in the peritoneal cavity; it could also be the consequence of the adhesion and invasion of peritoneal tissue by the tumoral cell clusters [46].

Angiogenesis

Angiogenesis refers to the development of neo-vessels from preexisting vessels. ***Physiological angiogenesis*** is a necessary component of tissue repair processes; this phenomenon is closely associated with liver regeneration. ***Pathological angiogenesis*** is responsible for tumor growth and metastasis. The angiogenesis phenomena are necessary in the development of the tumor. In 1971 *Judah Folkman* published the hypothesis (in the "New England Journal of Medicine") that solid tumors were able to induce the growth of new vessels (from pre-existing vasculature) by the secretion of pro angiogenic factors, such as the vascular endothelial growth factor (VEGF), in the tumor microenvironment. These factors will activate quiescent endothelial cells, stimulating them to proliferate and begin a program of morphogenesis. This tumor angiogenesis has long been regarded as the main model of tumor neovascularization, and the process was considered exclusively local for a long time [47-49]. The synthesis and secretion of angiogenic factors establish a capillary network from the surrounding host tissue [50]. However, it has also been suggested that circulating endothelial precursors could be recruited away to the formation of these new vessels [51]. Specific angiogenic molecules can initiate this process and specific inhibitory molecules can

stop it. These molecules, with opposing functions, appear to be continuously acting in concert to maintain a quiescent microvasculature [52].

VEGF is present in the peritoneal cavity and plays an important role in ascites development from ovarian cancer [53]. Pre-clinical study of *Passot et al.* shows that VEGF intravenous and intraperitoneal levels burden increased significantly after CRS, and then decreased progressively ($p<0.005$) [54]. Its presence may have an impact on survival from PC and recurrence following potentially curative surgery [55,56]. In our murine model, to study the growth and development of the metastases, we decided to analyse the angiogenetic process by the marker of the pro-angiogenetic factors (most importantly, the VEGF and its receptor).

The process of new vessels formation is defined as ***sprouting*** and starts with a detachment of pericytes and the dilation of pre-existing vessels. The consequence is an increase in capillary permeability and the degradation of the extracellular matrix (MEC) by the metalloproteinase. The endothelial cells migrate and proliferate in the peri-vascular space; the adhesion of endothelial cells to each other forms a new vascular tube; finally, the fusion of neo-vessels constructs an unstable vascular network [57]. Abnormal vascular remodeling in tumor neovascularization is called *switch angiogenic* and tumor vessels exhibit, not only quantitative, but also qualitative, abnormalities: the blood vessels are winding, overly permeable and unstable; they are surrounded by not-functional pericytes [57-59]. Tumor progresses from a non-angiogenic to angiogenic phenotype based on the imbalance of pro-angiogenic and anti-angiogenic factors. The angiogenic switch is "off" when the effect of angiogenic activators is balanced by that of the inhibitor's factors, and it is "on" when the balance is in favor of angiogenesis [60,61]. Tumor neovascularization is induced by the interaction between tumor cells and cells of the tumor's microenvironment. These new vessels carry oxygen and nutrients essential to cancer cells and, thus, to tumor growth. Capillary blood vessels consist of endothelial cells and pericytes. These two cell types carry

all of the genetic information to form tubes, branches, and whole capillary networks [52]. Endothelial cells exhibit broad fenestral junctions, a discontinuous basal lamina favoring extravasation of tumor cells in the blood flow, which is abnormal with areas of slowed and accelerated flow [62]. This phenomenon generates zones of hypoxia which increases the concentration of HIF-1a [63], and favors the sinuses of the pro-angiogenic factors, VEGF and PlGF [64-68]. HIF is related to increased microvessel density in the adjacent area of necrosis, or hypoxia, and plays a pivotal role in tumor progression [69]. A tumor must continuously stimulate the growth of new capillary blood vessels for the tumor itself to grow. Furthermore, the new blood vessels embedded in a tumor provide a gateway for tumor cells to enter the circulation and to metastasize to distant sites, such as the liver, lungs, or bones.

To confirm the development of aberrant neo-angiogenesis networks, in our model, we studied the vascular density on the endothelial cells by the positive marking of the CD31 and the circulating levels of some cytokines (such as VEGF, HIF-1a and PlGF).

Local invasion of the host stroma by some tumor cells occurs by passing into the circulation: a lymphatic channel, and neo-capillary, offer very little resistance to penetration by tumor cells [70]. Most circulating tumor cells are quickly destroyed. After the tumor cells have survived the circulation, they become trapped in the capillary beds of distant organs by adhering, either to capillary endothelial cells, or to the sub-endothelial basement membrane that might be exposed [71].

The microenvironment

The tumor cells and the ***stroma*** are the two components of tumor tissue. The stroma is a non-tumor tissue from the host and represents a supporting feeder to tumor cells. It consists of tissue structures, vascular and immune cells, and its formation is induced by the presence of cancer cells. It is present in all types of solid cancer tumors as well as in metastases. The

stroma is composed of an *extra-cellular matrix*, a network of proteins and proteoglycans whose role is to ensure cohesion, cell migration and differentiation. It contains ***interstitial matrix*** (mesenchymal cells) including collagens, elastin, fibronectin, glycosaminoglycans and the *basement membrane* (epithelial cells) consisting of collagen IV and laminin [[72]]. The other components of the microenvironment are: endothelial cells and pericytes, which constitute the new vascular network of the tumor ensuring the transport of nutrients necessary to increasing the tumor and immune cells (mococytes, macrophages and neuthrophils).

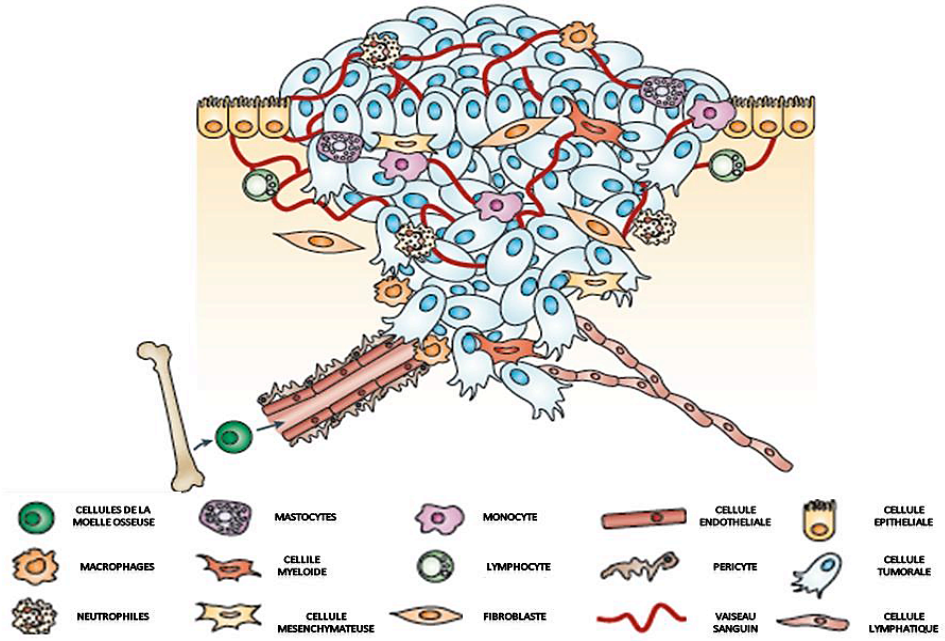

Fig.1: Tumoral microenvironment (adapted from Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer 2009; 9: 239-252).

It is known that peritoneum tissue is rich in lymphatic and blood vessels. What is unclear, is whether the phenomena responsible for the formation and promotion of the micro-environment of extra-peritoneal metastases, are the same for the formation of peritoneal metastases.

Monocytes and macrophages recruited by the tumor, become associated macrophages in the tumor (TAM), and assist in the creation of a microenvironment that promotes angiogenesis,

migration and the growth of malignant cells [73]. TAM can play a dual role in the tumor microenvironment, inhibiting tumor cells but also favoring tumor progression and angiogenesis when cancer cells begin to evade immune surveillance [74].

Metastatic process

The metastatic process involves several steps through which tumor cells spreading from the primary tumor, colonize remote organs [75]. The steps, in part, take place in the primary tumor, partly in the systemic circulation and partly in the distant organs. During this process the cancer cells break away from the primary tumor, enter into the blood circulation (***intravasation***) preferentially occurring in close proximity to perivascular macrophages, interrupting endothelial cell contacts and degrading the vascular basement membrane (***disruption***). This process is mediated by proteases supplied from the cancer cells, macrophages, or both. Then, the cancer cells recognize and adhere to the host body (*adhesion*). In distant organs tissue invasion can be seen in secondary growths as tumor cells spread (*extravasation*) [76,77]. During the intravasation, cancer cell migration is controlled through a paracrine loop involving epidermal growth factor (EGF), colony-stimulating factor 1 (CSF-1) and their receptors, which are differentially expressed on carcinoma cells and macrophages, resulting in the movement of cancer cells towards macrophages. VEGF, and its receptors (VEGFRs), are also involved in the migration and invasion process. A recent in vitro study shows that the depletion of VEGF and its receptors in multiple CRC cell lines led to strong inhibition of the migration and invasion of CRC cells resulting in the reduction in levels of phosphorylated focal adhesion kinase and its upstream regulators such as cMet and the EGF receptor [78]. Additional paracrine loops exist between cancer cells and stromal cells, such as fibroblasts and pericytes, producing the cognate ligand stromal cell-derived factor 1 (SDF1), which contributes to directional cancer cell migration [72]. *Lewis and Pollard* had demonstrated that TAM secretes a number of potent pro-angiogenic growth factors and

cytokines such as VEGF, TNF-α and can affect the invasion of tumor cells on stroma surrounding the degradation of the basement membrane by metalloproteinase production. It also plays a role in immunosuppression by expression of prostaglandin, interleukin-10 and TGF-β, and metastases by the secretion of EGF factor that can guide the tumor cells to the blood vessel [79].

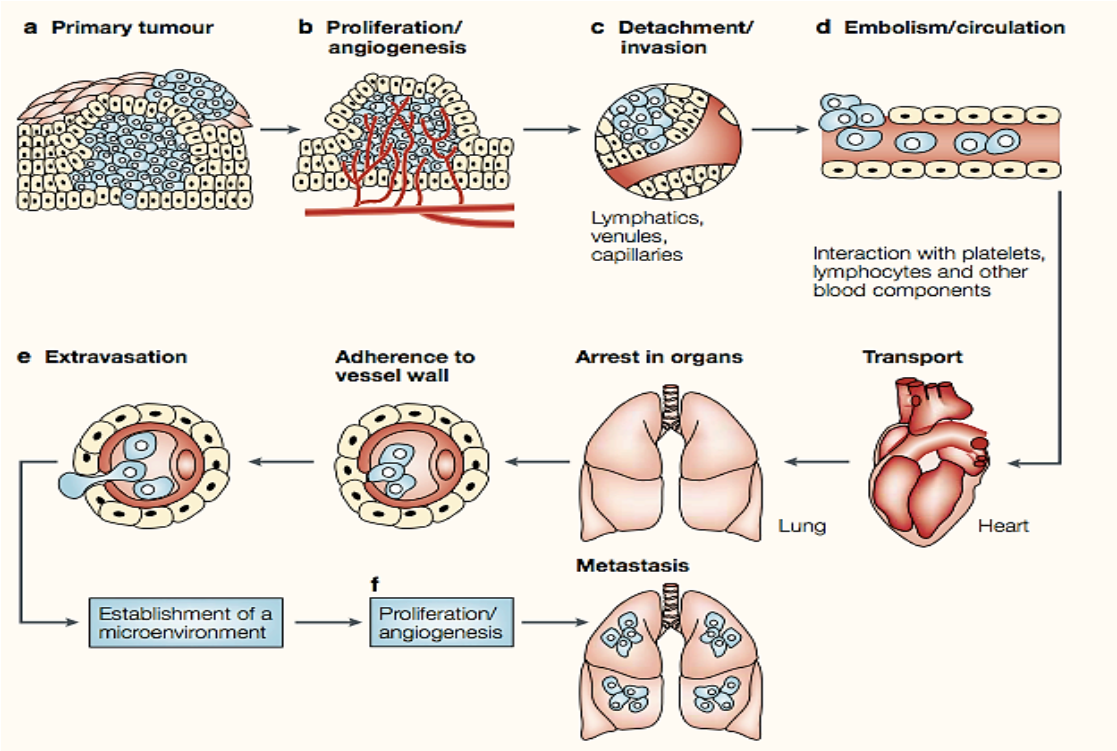

Fig.2 Mechanism of tumor dissemination (adapted from I. Filder. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003; 3:1-6).

During extravasation, tumor cells promoting the cascade of coagulation results in platelet aggregates that increase cancer cell survival through protection from NK cell-mediated lysis. The fibrin clots may also reduce shear forces that can destroy individual circulating cancer cells, and facilitate the slowing, arrest and adhesion of cancer cells, thus increasing their ability to extravasate at a secondary site [72].

This process, established for hepatic recurrences, has not yet been osberved for PC. In fact, the proliferation mechanisms of peritoneal metastases are unknown. After his clinical observations, Sugarbaker hypothesized that the tumoral process in the peritoneum may occur if the neoplastic cells first gain access to the peritoneal cavity and spread transcoelomic by peritoneal fluid [45]. Exfoliating malignant cells derived from the primary cancer can be absorbed through communications with the submesothelial lymphatic network (called ***stoma***) remain entrapped, and then invade the peritoneal surface and proliferate. The lack of knowledge concerning the PC tumoral process has motivated our choice to develop an orthotopic murin model of the intraperitoneal neoplastic cells injection.

Another way described is the entrapment of free cancer cells in the naturally or iatrogenically raw surfaces, like the ovarian surface in the Krukemberg syndrome, or in case of dissection, during abdominal surgery. We postulated that laparotomy plays an important role in the tumoral processes of PC. We studied the sham laparotomy to verify its pro-metastatic effects on the PC growth.

Bone marrow progenitors initiate the "pre-metastatic niche"

Previously, *Asahara* showed, for the first time, that endothelial progenitor bone marrow derived cells (BMDC) were able to move, become incorporated into the vessels and contribute to neovascularization on ischemia sites. It's a vasculogenesis mechanism concept which was previously related to embryogenesis exclusively [48]. Following the work of *Lyden* it was demonstrated that the BMDC could participate in tumor vasculogenesis, tumor growth and neoangiogenesis, in the formation of the pro-metastatic niche and development of metastases [49].

In advanced and metastatic CRC, even after complete resection of the primary tumors, the interaction between tumor and stroma in the tumor microenvironment often promotes cancer

invasion and/or metastasis through chemokine signaling in distant organs such as the liver, lung, lymph node, bone or peritoneum [80]. These chemokines may affect tumor immunity by recruiting several types of BMDC to the tumor microenvironment. Following literature, we decided to analyse the roles played by pro-angiogenic and pro-metastase BMDC progenitors, as well as the growth factors secreted from our animal models concerning PC and liver regeneration.

In 2005, in animal models based on intradermal injection of B16 melanoma cells and Lewis lung carcinoma cells, *Kaplan* showed that VEGF-A, produced by the primary tumor, promotes entry in systemic circulation and mobilization towards metastatic sites derived from BMDC, hematopoietic progenitor cells (HPC) and endothelial progenitor cells (EPC), and prepares the future site of metastasis development [81]. This migration would precede the arrival of the tumor cells. The HPC circulate in the blood, expressing VEGFR-1, and the integrin VLA-4, enabling them to adhere to fibronectin and metalloproteinase degrading the basement membrane, which increases extravasation of HPC in the pre-metastatic niche before the arrival of tumor cells and EPC (VEGFR2+). The EPC can circulate in the blood and are able to migrate to the tumor site where they provide specific functions in neo-angiogenesis [82-84]. All these events result in a change in the local microenvironment for attachment, survival and growth of circulating tumor cells, in which ***angiogenesis*** plays a fundamental role. In a subcutaneous injection of lung cancer cells and in a model of spontaneous breast cancer in transgenic mice, *Gao et al.* showed that the EPC infiltrated the periphery of the avascular pre-metastatic niche and were then incorporated into the lumina of macrovascular metastasis vessels. At this stage the pre-metastatic niche is a ***micrometastasis.*** An angiogenic switch is associated with the progression of micrometastases (<1mm) to macrometastases (≥1mm), during which EPC are recruited to the metastastic foci and contribute luminally to the neovasculature in metastatic lesions [85]. To promote the progression of micrometastases in

macroscopically detectable metastases ***(macrometastases),*** EPC (VEGFR-2+) are recruited secondarily to promote angiogenesis [86]. To continue growing, the micrometastasis must develop an abnormal vascular network [50] and evade destruction by host defenses. Circulating endothelial cells and EPC were considered biomarkers of tumor angiogenesis. For the first time, a non-neoplastic cell population had been identified to be able to promote a future metastatic site. The impact of this evidence was strong: anti-VEGFR1 treatment has been found to prevent the formation of pre-metastatic HPC clusters and metastatic progression [81].

Mechanisms of liver regeneration

The mention of liver regeneration by ***Prometheus*** in Greek mythology indicates that ancient people had noticed the regenerative capacity of the liver. To illustrate this well known liver feature, we mention the work of *Taub et al.* After a 70% hepatectomy in rats, 95% of quiescent hepatocytes (G0 phase) go through mitosis, with a peak of DNA synthesis at H24 and the restoration of 90% of the volume is obtained at H72 [87]. In mice there is a full recovery of the parenchyma in 7 to 10 days [88,89]. On a human level, restoration is achieved from 2 to 6 months in a healthy liver, but the biological function is restored in less than 3 weeks [90]. As the resection of lobes does not induce damage to the remaining liver tissue, partial hepatectomy has long been considered an excellent experimental model for tissue regeneration. The liver regeneration is different that compensatory hyperplasia: in a severely damaged liver, liver stem cells, which have the potential to differentiate into hepatocytes and biliary epithelial cells, proliferate and are assumed to contribute to regeneration. By contrast, the liver does not recover the original lobular structure; the remnant tissue simply increases in size. Each hepatocyte can divide 1 to 2 times, thus allowing the recovery of liver mass, while in a normal liver, little spontaneous mitosis is observed in hepatocytes (mitosis about 1 to 20,000 cells). *Miyaoka et al.* observed in a murine model that increased hepatocyte size

occurs as early as a few hours after a 70% partial hepatectomy, much earlier than their entry into the cell cycle. It peaks at 1 day, suggesting that cell size increase is the first response of hepatocytes to the loss of liver mass. [91]. In this phase hepatocytes dramatically change their gene expression necessary to entry into the cell cycle and prepare for regeneration [92]. A second phase of cellular division concerns the Kupffer and stellate cells at H48, followed by endothelial cells of liver sinusoids (LSEC) at H72. *Ding et al.* have shown that there is a synchronism between hepatocyte and endothelial proliferation [93]. Endothelial cells have a prominent role in hepatocyte proliferation and spatial distribution together with the platelets. A wave of hepatocyte apoptosis follows this DNA synthesis, performing a feedback regulation [94]. Hepatocyte proliferation results in the formation of avascular clusters of 10–14 cells that are not organized in the final architecture of the liver. These clusters are not functional because the core cells are located outside of the oxygen diffusion capillary area [95]. This hypoxia activates the transcription of HIF-1 factor which, in turn, induces the expression of downstream target genes, including VEGF, and VEGFR-1 [96]. Stellate cells produce extracellular matrix on the fourth day to re-establish a connection between hepatocytes and endothelial cells. TGF-α is produced by stellate cells and allows the synthesis of the extracellular matrix. This is in response to the increase in portal pressure after liver surgery because there is a reduction of the vascular bed and portal flow as well as a release of nitric oxide from the liver sinusoidal endothelial cells (LSEC) allowing a sensitization of hepatocytes to HGF [97]. VEGF production by hepatocytes increased during liver regeneration peaking at 48-72 h [98]. Most solid tumors overexpress and secrete VEGF [99]. During liver regeneration, it promotes new vasculature formation from preexisting blood vessels, the proliferation of endothelial cells and regulates the vascular permeability of the LSEC [100,101]. This production is accompanied by an increase of VEGFR-1 expression on hepatocytes and

HPC, and VEGFR-1 and VEGFR-2 expression on the LSEC [95,102,103]. After binding to VEGFR-1 hepatocyte, VEGF can induce autocrine hepatocyte proliferation [98].

Our laboratory animal model, based on hepatic and limb ischemia, developed by *Lim et al.*, shows that the hepatic ischemia and, consequently, liver regeneration, leads to the mobilization of EPC progenitors and enhanced intra-hepatic angiogenesis, which is associated with an increased tumor burden in an animal model of colorectal liver metastasis [104]. Accordingly, we have postulated that the liver regeneration process, consequently after major hepatectomy, could mobilize the progenitor BMDC to promote the increase of tumoral angiogenesis of the PC and, thus, have a pro-metastastic effect.

Cytokines and liver regeneration

In addition to pro-tumoral and pro-angiogenic effets, the cytokines and angiogenic factors play a role in liver regeneration. [105,106]. Usual, in humans, liver regeneration is mainly studied in the context of hepatic resection for liver cancers, liver failure or liver transplant recipients. In the clinical setting, right lobe donor hepatectomy for healthy donors liver transplantation is an ideal model to study liver regeneration after partial hepatectomy. *Sasturkar et al.* affirm that the cytokines and growth factors play prominent roles in liver regeneration for these patients. HGF, IL-6, and TNF-α are involved in upregulation of the early phase, whereas TGF-β1 and IFN are involved in the termination phase of liver regeneration. However, factors such as underlying liver diseases, the degree of portal hypertension, or immunosuppressant use may influence liver regeneration [107]. The expression of HGF increases 6 to 8 hours after partial hepatectomy. It is probably the most important growth factor upregulated during liver regeneration. Secreted by stellate cells, Kupffer cells and LSEC, HGF stimulates the production of the transforming growth factor αTGF, an autocrine growth factor, active on hepatocyte after binding to its tyrosine kinase

receptor [108]. During the tumoral process, HGF accelerates angiogenesis, tumor migration and infiltration [109] and increases the MMP activity and secretion of proteinases which lyse basal membrane and promote metastasis [110]. IL-6 is released from the hepatocytes and Kupffer cells in response to portal-system-carried factors and contributes to the initiation of the cell cycle (G0 to G1) [111]. It induces the transcription of many genes involved in cell division and survival by controlling apoptosis pathways [112]. Several experimental studies have shown that IL-6 is necessary for proper liver regeneration [94,111].

The increased level of local and systemic IL-6 is important for inflammatory processes. In this study, we considered that the IL-6 plays a key role in cancer development and progression; is the common factor between the immuno-regulation and the angiogenesis [113]. Stone et al. analyzing the effect of cancer on platelet counts in human primary tumors and a orthotopic murine model of epithelial ovarian cancer, demostrated that the thrombocytosis was correlated with the number of peritoneal metastases and a shortened survival [114]. In their animal model, platelet counts were strongly correlated with mean BMDC counts. The use of an anti–IL-6 antibody treatment significantly reduced platelet counts in tumor-bearing mice, as well as in patients, and enhanced the therapeutic efficacy of the systemic chemotherapy (Paclitaxel) in their mouse model.

TNF-α secreted by hepatocytes, is a potent regulator in the initiation of liver regeneration. In rodent studies, TNF-α enhances the effects of direct mitogen such as HGF [115]. Antibodies against TNF-α administered at the time of hepatectomy lower the regenerative response [116]. The last step is to stop the proliferation engineered by the TGF-ß1. TGF-ß1 is produced predominantly by hepatic stellate cells [117] and is known to have growth inhibitory effects on liver regeneration. These factors play an exogenous monitoring role on liver regeneration, which enables the adaptation of the size of the liver to its new environment [118]. In experimental studies, after partial hepatectomy, matrix-bound TGF-ß1 is activated and

released into the circulation and thus an increased concentration is detected within 1 hour of partial hepatectomy [89].

In our study, the blood levels of these cytokines were analysed at different times to monitor the phases of liver regeneration.

Pro-metastatic effects of liver surgery

Although portal flow drains the tumor cells through the hepatic parenchima, this metastatic process is not very effective: in a murine model of hepatic melanoma metastases, only 0.02% of the tumor cells injected into the portal system will become metastases [119]. Liver surgery and, consequently, liver regeneration, play a crucial role in tumor recurrence by stimulating tumor cells to proliferate following resection. Clinical studies show that partial hepatectomy for metastases of colorectal cancer is associated with a 60 to 80% tumor recurrence rate and is a major cause of treatment failure [120,121]. Cellular and molecular changes resulting from liver regeneration after hepatectomy contribute to extrahepatic and hepatic recurrences [21].

The extent of hepatectomy is an important factor influencing the tumor growth. To show the effects induce of the liver resction, we have chosen a murine model of 68% of liver parenchyma resection.

In a mouse model *Brandt et al*. analyzed the mechanism behind the development of recurrent malignant lesions after liver resection. The animals were treated with a sham laparotomy without liver injury, a 30% liver resection, or a 70% liver resection. After surgery, the animals received a tumor cell injection into the remaining liver tissue. After a 70% liver resection, the tumor volume, weight and tumor proliferation rate of Ki-67 were significantly increased compared to a laparotomy alone ($p < 0.05$) [122]. Previous experiment for colorectal cancer reported that on mice model liver surgery, 70% partial hepatectomy induced tumor growth and the spread of extrahepatic metastases more than limited 37% hepatectomy. The increased

growth in liver metastases occurred predominantly in the late phase of liver regeneration rather than the early phase [123]. In a rat tumor model, *Mizutani et al.* have shown that resection is a potential promoter for the growth of micrometastases. After surgery, the immediate intraportal injection of hepatocarcinoma AH130 cells resulted in an increased number of hepatic metastases, compared with the control animals that did not undergo liver resection. In contrast, tumor cell injection, 2 weeks after major hepatectomy, revealed no significant differences, compared with controls. In a third group, the removal of half of the caudate lobe resulted in the same number of metastases as in control animals. The authors concluded that the promotion of hepatic metastases was increased in the initial period of active liver regeneration and was proportional to the volume of hepatectomy [124]. *de Jong et al.* suggest that specific factors and phases involved in liver regeneration may influence the growth patterns of residual or dormant micrometastases after 70% liver resection [125]. In an animal model of liver metastases after an injection of GFP-transfected CT-26 cells under capsula in right lobe, *Breitenbuch et al*. observed that RFA increased the metastasis of residual neoplastic cells compared with resection. The reasons for the neoplastic growth, after RFA or liver resection, remain unclear but this model shows the pro-metastatic effect in residual intrahepatic neoplastic cells. Possible explanations may involve factors such as the immunologic and biological effects of heat trauma [126].

Togo et al. showed that the incidence of residual liver and lung metatsases increased after a two–stage hepatectomy, when a complete resection was not achievable after a single hepatectomy [41]. The same result was observed by *Elias et al*. after portal embolization [127]. To confirms the pro-tumoral role of the liver surgery, we created a murine model of major liver surgery to test the effects of consequent liver regeneration in the angiogenesis and proliferation of PC. To confirms the major incidence of the tumoral growth after

hepatectomy, our model was compared to a sham laparotomy and natural history after intra-peritoneal tumor cells injection.

Peritoneum: the first-line of defense in carcinomatosis

The peritoneum acts as a barrier to macromolecules that enter the submesothelial layer and reduce the friction between the visceral organs by the secretion of a surfactant phospholipid by each mesothelial cell. It consists of a monolayer of mesothelial cells supported by a basal membrane that rests on a layer of connective tissue. Laparotomy, laparoscopy, the suture line of digestive anastomoses, and surgical dissection, are the most common causes of breaches of the peritoneal barrier and, consequently, the most frequent sites of peritoneal implants. Because laparotomy stimulates the growth factors associated with the healing phenomena, in our animal model, we analyzed the effects of the sham laparotomy on the growths of the peritoneal metastases growth.

In the case of recurrence after abdominal or pelvic surgery, the cancer cells are often free, dispersed in the peritoneal cavity, sometimes derived from colic or rectal light and they sometimes remain trapped in the anastomotic sites (or in the tissues crushed by the surgical resection).There they are trapped in fibrin and their growth is favored by the growth factors brought on by platelets, neutrophils and monocytes in the injured sites. The result is a peritoneal dissemination of the tumor disease [45,128].

B. RESEARCH PROJECT

1) PART ONE

1a) HYPOTHESIS AND OBJECTIVES

- **Scientific methodology**

The main objective of this study was to evaluate the effect of hepatic surgery on the growth and tumor angiogenesis of peritoneal carcinomatosis. Our hypothesis was that the surgery for resection of liver metastases can accelerate the tumor progression of peritoneal carcinomatosis. In order to test our hypothesis, we decided to study the growth of peritoneal carcinomatosis, in vivo, in murine models of intra-peritoneal grafting of carcinomatosis (CT-26) on BalbC mice after a major hepatectomy.

A preliminary exsperiment was neccessary to develop a reproductible immunocompetent murine model of limited PC to monitor the increase and the proliferation of peritoneal lesions. These results are listed in **Annex 1.**

The secondary objectives were:

1 - To determine the tumor growth of peritoneal carcinomatosis after hepatectomy

2 - To verify whether these results in the mobilization of circulating progenitor cells derived from the bone marrow (Endhotelial and Hematopoietic progenitor cells)

3 – To evaluate the effect induced by surgery on the mobilization of these progenitors

4 - To evaluate the effect of these progenitors on tumor growth.

The approach taken by my PhD thesis was to design animal models that mimic the natural history of human disease, in order to transfer our results to the clinic in a translational way, from patients with peritoneal carcinomatosis and synchronous hepatic metastases.

Annex 2 shows the construction of a murine model of liver regeneration.

1b) RESULTS AND DISCUSSION

- **<u>Article 1</u> (Original article currently submitted to the EJSO)**

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Abstract: Background:
Curative surgery of synchronous peritoneal carcinomatosis(PC) and colorectal liver metastases(LM) has been recently investigated as a feasible option. When synchronous peritoneal and liver resection(LR) is not achievable, the sequence of surgery remains unknown. Our hypothesis was that LR promotes peritoneal growth resulting in a non-resectable PC. The aim was to analyze the effects of major LR and liver regeneration after hepatectomy in a murine model of PC, and the associated angiogenesis.

Methods:
A murine model of colorectal PC in Balb/C mice was developed by intraperitoneal injection of different CT-26 tumor cells concentrations. Five days after the injection, mice were randomized into three groups: 68% hepatectomy group, sham laparotomy and control group without surgical injuries. Results were analyzed on post-operative days 1, 5 and 20. PC was evaluated macroscopically; tumor growth and liver regeneration by immunohistochemistry; angiogenesis by immunofluorescence, circulating progenitor cells, plasmatic cytokines and digestive arterial blood flow velocity measurements.

Results:
A reproducible murine model of limited colorectal PC was obtained. Surgery induced PC increases and promoted neo-angiogenesis. Major hepatectomy influenced tumor growth, in the late phase after surgery, the extent of extra-peritoneal metastasis and the increase of Ki-67expression in the remnant liver.

Conclusions:

This animal model confirms the pro-tumoral and pro-angiogenic role of surgery, laparotomy and major LR, which promotes the increase of angiogenetic factors and their participation in PC growth. These results suggest that peritoneum resection should be the first step in the case of two-step liver and peritoneum surgery for patients with colorectal PC and LM.

Dear Editor,

Please find enclosed a manuscript entitled “Hepatectomy increases hepatic metastatic graft and growthin an immunocompetent murine model of peritoneal carcinomatosis” that we would like to be considered for publication in European Journal of Surgical Oncology.
We believe this paper deserves publication priority because we analysed the effects of an aggressive surgical approach for patients with concomitant liver and peritoneal metastasis from colorectal cancer, trough an immunocompetent animal model of peritoneal carcinomatosis in order to transfer the results to the clinic in a translational way.

Traditionally considered a contraindication to any surgical approach, the synchronous presence of peritoneal carcinomatosis and liver metastases has been recently treated by surgery with curative intent in selected patients. There are currently no specific criteria to select patients with the highest potential for surgical success, nor guidelines concerning the timing of peritoneal and liver surgery, this paper can be a valuable aid in selecting patients.

Our hypothesis was that liver regeneration induce by liver resection may accelerate the tumor progression resulting in a non-resectable peritoneal carcinomatosis. In order to test our hypothesis, we decided to study in vivo the growth of peritoneal carcinomatosis in murine models of intra-peritoneal grafting of carcinomatosis (CT-26 *luciferase*+ cells) in the BalbC mice underwent to the major hepatectomy.
The main objective was to evaluate the effects of hepatic surgery on the growth and tumor angiogenesis of peritoneal carcinomatosis. The secondary objectives were: 1) determine the natural history of peritoneal carcinomatosis after hepatectomy 2) verify whether this results in mobilization of circulating endothelial progenitor cells derived from the bone marrow 3) evaluate the effect of the mobilization of these progenitors, induced by surgery, on tumor growth.

As a highly respected journal, we believe that European Journal of Surgical Oncology is the most appropriate journal for sharing our findings and we hope to consider our manuscript for publication.
All the listed authors have made substantial contributions in conception, analysis and interpretation of data and in the drafting and critical revisions of the manuscript.
This paper is not being considered for publication elsewhere, none of its contents have been previously published in any Language and all authors have read and approved the manuscript.
None of authors have relationships with the pharmaceutical industry.

Thank you in advance for considering our paper, I look forward to earing from you soon.

Best Regards,

Rea Lo Dico

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Study design:	Rea LO DICO, Marc POCARD ……..
Data acquisition:	Rea LO DICO, Marc POCARD, Annemilai TIJERAS-RAMBALLAND, Philippe BONNIN
Quality control of data and algorithms:	All authors ……
Data analysis and interpretation:	Rea LO DICO, Gianluigi LO DICO, Philippe BONNIN, Annemilai TIJERAS-RAMBALLAND
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"All the authors have made a significant contribution to this manuscript, have seen and approved the final manuscript, and have agreed to its submission to the *EJSO*".

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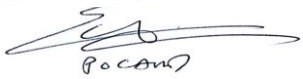

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(Please note that this form must be signed by ALL authors)

Hepatectomy increases hepatic metastatic graft and growth in an immunocompetent murine model of peritoneal carcinomatosis

Running title:

Surgical Strategies in Synchronous Peritoneal and Liver Metastases: Animal Model

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INTRODUCTION

Curative management of liver metastases (LM) is based on surgical resection. However, in 70% of cases, LM will recur despite the use of multimodal and adjuvant chemotherapy[1]. A recent meta-analysis by *Franko et al*. reported an overall survival rate (OS) of 19 months in patients with LM from colorectal cancer (CRC) origin[2]. In the same study, the presence of isolated peritoneal carcinomatosis (PC) was associated with a worse prognosis than other isolated metastases[2]. Synchronous LM and PC from CRC origin was traditionally considered as a palliative disease and a contraindication to the curative surgical approach[3-7]. Extensive surgery with curative intent has been recently investigated as feasible[8]. For some authors, LM is not an absolute contraindication to peritoneal cytoreduction anymore[9]. This aggressive approach is associated with an increased, but acceptable, postoperative severe complications rate[10]. No guidelines currently exist concerning the relative timing of peritoneal and liver surgery. A recent study reported a nomogram to select patients suitable for this complex curative strategy by taking into account the number of LM, the extent of PC and the surgery[11]. However, when curative liver surgery and peritoneal cytoreduction with HIPEC cannot be achieved synchronously, the choice of the main surgical procedure remains unclear. Traditional chemotherapies are effective at varying degrees to control LM but less for PC. Our aim was to evaluate a strategy of liver resection (LR) first[12]. We postulated that the LR, through the consequent liver regeneration process, would promote PC growth. The objective of our study was to analyze the effects of major LR and liver regeneration on PC growth and the associated angiogenesis process, after hepatectomy in an immunocompetent murine model of PC.

METHODS

Cell culture

Luciferase-expressing CT-26 cells (CT-26luc+) were kindly provided by Prof. Lea Eisembach Weizmann (Institute of Science Rehovot, Israel). CT-26luc+ cells were grown as monolayers in DMEM culture medium supplemented with 10% fetal Calf serum (FCS), 5% of antibiotics (penicillin and streptomycin), 5% Fungizone and 5% of HEPES buffer solution, in a humidified incubator at 37°C (5% CO2 and 95% air).

Animals

The protocol used was approved by Local Ethic Committee (Protocol N° 02095.03). Five-week-old murine, hepatitis virus-free, and immunocompetent BALB/c females, weighing 20±0.5 g, (Charles River, Arbresle, France) were housed in our specific pathogen free compliant animal facility. Animals were acclimated for one week before experimentation. Limited PC was obtained testing five different concentrations ($5x10^5$, $2.5x10^5$, $1.25x10^5$, $6.2x10^4$ and $3x10^4$ cells) of intraperitoneal injection of CT-26luc+ cells in 1 mL of DMEM culture medium. PC was quantified, post-mortem, using the Peritoneal Cancer Index (PCI) score[13] adapted to tumor sizes in mice[14,15]. Surgical procedures were performed under isoflurane anesthesia using an oxygen (30%)/nitrous oxides (70%) mixture. After medial laparotomy, partial 68% hepatectomy was performed by removing three anterior hepatic lobes as previously described[16]. During the procedure, 9% saline solution was administered subcutaneously to prevent dehydration. Buprenorphine (0.1 mg/kg) was administered immediately after surgery and every 12 hours for 48 hours to prevent pain. Five days after CT26luc+ injection, mice were randomized into three groups: control animals that only received the CT26luc+ cells injection (Natural History, NH, n=30), animals subjected to major hepatectomy (Liver Surgery, LS, n=30), and sham laparotomy animals (n=30). Animals were euthanized by cervical dislocation. Heparinized blood was obtained from cardiac puncture, and liver, lungs,

abdominal lymph nodes and peritoneal tissue were collected for analysis. To assess the kinetics of tumor growth, after randomization, 10 mice from each group were sacrificed at days 1, 5 and 20.

Tissue analysis

Tissues were immediately fixed overnight in 4% paraformaldehyde and embedded into paraffin or directly embedded into optimal cutting temperature compound (OCT, Tissue-tek O.C.T. Compound Sakura®) to be frozen. Standard Haematoxylin-Eosin (H&E) and Haematoxylin-Eosin-Safran (HES) staining were performed on paraffin-embedded tissue sections for standard histology and PC analysis, respectively. Ki-67 immunohistochemistry (IHC) was performed on frozen tissue sections fixed with ice-cold acetone (90%) using an immunohistochemical stainer (Bond Max, Leica Biosystems, Nussloch, Germany) for standardized procedure. Ki-67 antibody (ab16667, Abcam) was diluted to 1/200 using the ER1 (citrate, PH=6) procedure according to the manufacturer's instruction. IHC slides were then scanned using an Aperio AT Turbo automat. For immunofluorescence, CD31 staining was performed on frozen tissue sections using a Rat/Mouse anti-CD31 primary antibody (1/50, BD Pharmingen, clone MEC13.3). Alexa Fluor 488-conjugated goat anti-rat IgG was used as a secondary antibody (1/200, Life Technologies). Samples were mounted using Dako Fluorescent Mounting Medium (S3023, Dako, Germany). All images were obtained with a Z1 Zeiss microscope (Germany) equipped with a Axiocam Icc 1 camera (Zeiss, Germany). Tumor growth was evaluated by measuring mitotic rate/10 high-power fields (HPF)[17]. Tumor growth was considered as unmodified for $\leq$ 2 mitoses/10 HPF and accelerated for $\geq$5 mitoses/10 HPF. Proliferation was measured as the number of Ki-67-positive cells per field over three different fields per sample. Neo-angiogenesis was evaluated by the number of CD31-positive cells per field over three different fields per sample.

Cytokines

Plasma was obtained by the centrifugation of heparinized blood at 700 rm for 30 min at 4°C. Plasma samples were frozen at -80°C until use. Plasma levels of IL-6, VEGF-A, sVEGFR-1, TGF-β, and TNF-α were measured using sandwich immunoassay methods with commercially available electrochemiluminescent detection systems, plates and reagents (V-PLEX cytokine Plex kits - Meso-Scale Discovery [MSD], Gaithersburg, USA), according to manufacturer's instructions. Other parameters (sVEGFR-2, EGF, sEGFR-1 and HGF) were measured similarly from commercially available (R&D) or homemade antibodies. Briefly, 50 µL of diluted plasma were loaded per well in the MSD plates. The plates were analyzed using the SECTOR Imager 2400. Immunoassays of murine ACE were performed on a Cobas e601 analyzer.

Mobilization of circulating endothelial and hematopoietic progenitor cells

Following hemolysis, bone marrow derived progenitors cells (EPC, Endothelial Progenitor Cells and HPC, Hematopoietic Progenitor Cells) previously isolated by centrifugation on a human Pancoll density gradient from fresh blood samples, were incubated for 30 min at 4°C with fluorescein isothiocyanate conjugated anti-mouse vascular endothelial growth factor receptor VEGFR-2 antibody, phycoerythrin (PE)-conjugated anti-mouse CD-34 antibody, fluorescein isothiocyanate conjugated anti-CD-45 antibody, or immunoglobulin G isotype controls (eBiosciences, Paris, France). The cells were then analyzed by flow cytometry using a BD Biosciences LSRII flow cytometer (BD Biosciences, Le Pont de Claix, France). The positive cells percentage in each sample was calculated using Kaluza flow cytometry analysis software (Beckman Coulter France S.A.S, Villepinte, France).

Measurements of blood flow velocities (BFV)

Ultrasound examinations were performed using an echocardiograph (Acuson S3000, Siemens®, Erlangen, Germany) equipped with a 14-MHz linear transducer (14L5 SP) (7.5MHz) under light

isoflurane anesthesia. Mean blood flow velocities (mBFV) in the celiac trunk, and in the superior mesenteric artery, were repetitively, and non-invasively, measured from the first day to the 4th week after surgery, as previously described[18-21] .

Statistical analysis

Results are expressed as the means +/- standard error (SEM). Multiple groups were compared using the *Kruskal–Wallis test*, followed by the pairwise *Wilcoxon rank-sum test*. *P-values* for multiple comparisons were adjusted using *Holm's method*. A two-sided *P-value <0.05* was considered statistically significant. Repeated measures were analyzed by one-way ANOVA followed by a post hoc unpaired *Student t test*. The relationship between PCI and mBFV was evaluated using the *Pearson correlation moment*. Statistical analysis was performed using *R, the R foundation* (http://www.r-project.org/).

RESULTS

Development of an animal model of limited PC

To mimic human disease, we developed a murine model of PC in an immunocompetent animal (Balb/c) by injecting different concentrations of murine CRC-derived CT-26luc+ cells ($5x10^5$, $2.5x10^5$, $1.25x10^5$, $6.2x10^4$ and $3x10^4$ cells). **Figure 1A** shows a dose-dependent increase in PC along the increase in concentration of CT-26luc+. Using $3x10^4$ CT-26luc+ cells, we obtained a limited PC at day 15 (PCI < 10) without tumor outgrowth at day 20 that would require the euthanasia of the animals for ethical reasons. Therefore, this concentration was used hereafter. As we anticipated an increase in PC growth after partial hepatectomy, we obtained a model of limited PC to be able to study the kinetic of tumor growth in these animals.

PC evolution after partial hepatectomy

At day 1, there was neither macroscopic, nor pathological evidence of PC in any of the three groups. At day 5 however, PC was observed, and pathologically confirmed, in 60% of the mice. PCI was different among the three groups ($p< 0.05$, **Figure 1B**), and there was a trend towards a higher PCI in LS mice when compared to NH animals (3.2±0.8 *vs*. 0.5±0.2, p=0.058). At day 20, there was no difference among the three groups although a trend was observed between LS and NH groups (p=0.07). However, mitotic count at day 20 showed a marked increase in the LS group compared to NH and sham groups (83±8 *vs*. 10±8 cells/field, p=0.0001 and 83±8 *vs*. 27±10 cells/field, p=0.009, respectively), suggesting a higher proliferative index of the tumor after partial hepatectomy (**Figure 1C**). Furthermore, macroscopic analysis of the non-carcinomatosis lesions showed that three mice from the LS group exhibited hemorrhagic ascites and one developed LM diffuse in the remnant lobes, (confirmed pathologically). Neither extra peritoneal metastases, nor hemorrhagic ascites, were observed in the other groups. In addition, no macroscopically liver regeneration was observed in the LS group, even though Ki-67 labeling was significantly increased

in the remaining lobes compared to the liver of the two other groups from day 5 onwards ($p < 0.05$ for all comparisons, **Fig. 2 A-D**).

Circulating progenitor cells

At day 1, early after hepatectomy, there was an increase number of EPC (CD-34+/VEGFR-2+) in LS mice (9.98±1.43) compared to NH animals (7.18±2.16, p=0.033, **Fig. 3A**). EPC number increased at day 5, and a positive trend towards an increase number of EPC in LS animal was observed, although not reaching significance (p=0.075). At day 20, the number of EPC was similar and low in all three groups. There was no difference in the number of HPC cells (CD-34+/CD-45+) in the three groups at any point in time **(Fig. 3B).**

Cytokines

Plasma levels of murine ACE were higher in the LS group compared to the others (NH, p=0.0009 and sham, p=0.006, respectively); ACE levels were also higher in the sham group compared to the NH group (16.6±1.6 *vs*. 9.3±0.3, p=0,0001). The results are showed in **Figure 4L**. VEGF-A, sVEGFR-1, sVEGFR-2 were increased in LS mice compared to the two other groups from day 5 onwards ($p<0.05$ for all comparisons); the same effect was observed for the the pro-inflammatory cytokine IL-6. We observed an increased plasma concentration of the growth factors (EGF, sEGFR-1 and HGF) and TGF-β in the late phase, after hepatectomy, during the hepatic regeneration. There was no difference in the TNF-α level among all groups.

Neo-angiogenesis

At day 20, the number of CD31-positive blood vessels was markedly increased in the PC nodules of LS (90±8) and the sham (86±6) groups when compared to the NH animals (20±7, $p<0.005$ for each comparison). Similar results were observed in the surrounding normal peritoneal tissue. In contrast, the number of CD-31 positive cells were reduced in the remaining hepatic lobes of the LS mice

compared to NH and sham groups ($p<0.05$ for each comparison) while similar in all three at day 1 **(Fig.5).**

Measurements of blood flow velocities (BFV) with Doppler ultrasound

The mean BVF (mBFV) in the superior mesenteric artery was equally increased in NH and LS groups at day 20 from 5.3+/-1.9 to 11.2+/-3.6 cm/s ($p=0.0010$). The mBFV in the coeliac trunk remained stable from day 1 to day 20 in the NH group (8.9+/-2.3 cm/s) while decreasing in the LS group at day 1 after hepatectomy to 5.2+/-1.8 cm/s ($p=0.0077$ LS *vs* NH). Compared to day 1, the mBFV of the LS group increased to 9.5+/-2.4 at day 5 ($p=0.0105$), to 12.7+/-1.8 at day 10 ($p= 0.0021$) and to 14.0+/- 1.9 cm/s at day 20 ($p= 0.0017$). There was a strong positive correlation between mBFV in the coeliac artery and the PCI ($R=0.85$, $p<0.001$). These results are showed in **annex 1**.

DISCUSSION

LR for CRC metastases is associated with a 60 to 80% tumor recurrence rate during a lifetime[1,22]. Recently, management of patients with PC and LM from CRC origin has undergone major improvements, but the surgical approach with curative intent remains controversial. In the case of limited LR, several teams reported concomitant peritoneal and LR[23]. However, if complex or major hepatectomy is required, LR was usually delayed and performed during a second procedure, especially in case of functional parenchyma injured by preoperative chemotherapy. The choice of an optimal surgical strategy has been highly debated. *Togo et al*. showed an increased incidence of residual liver and lung metastases after a two–stages hepatectomy, when a complete resection is not achievable, with a single hepatectomy[24], suggests the pro-tumoral local and systemic effects of the major hepatectomy. Surgical strategies, such as portal vein embolization and two-stage hepatectomy involving liver regeneration, may also be associated with stimulation of tumor growth[25]. *Elias et al*. have shown that the growth rate of liver metastases may increase by eight times compared to normal liver parenchyma after portal vein embolization, suggesting that the process of regeneration has a significant proliferative effect on tumor cells[26]. In line with these previous results, we observed an increase in peritoneal and extra-peritoneal metastases growth after LR that was not observed after the sham laparotomy. These results suggest that liver surgery should not be performed first in patients with LM and PC from CRC origin.

After hepatectomy, cellular and molecular changes secondary to liver regeneration may influence the kinetics of tumor growth[27-29] and contribute to extra-hepatic and hepatic recurrences[30]. It is well established that major LR, and consequently liver regeneration, results in an increased secretion of cytokines, such as IL-6, and angiogenic factors (VEGF) that alter the microenvironment of distant dormant tumor deposits. Growth factors such as HGF, EGF, TGF-α and TGF-β play an essential pivotal role in liver regeneration and induce changes in the microenvironment that stimulates

intrahepatic tumor growth[31]. In this study, the plasmatic concentration of angiogenic cytokines and growth factors reflected the stimulation of liver regeneration with an increase of VEGF and EGF receptors in the proliferative phase followed by the peak of the angiogenic cytokines and growth factors ligands (IL-6, VEGF, EGF, HGF). We found that the presence of a high concentration of angiogenic markers correlated with the high propensity of tumor recurrence in the LS group compared to the sham and NH groups. Furthermore, in preclinical models, the recruitment of EPC contributes to tumor growth, metastases formation and is closely related to the plasmatic level of angiogenic cytokines[32]. After partial hepatectomy, the levels of EPC increase instantaneous[33]. Consequently, the present study shows an increase in EPC recruitment early after hepatectomy at day 1 with a peak at day 5 in the LS group compared to non-liver injured group, confirming the pro-angiogenic role of LR and the direct effect of high plasmatic concentration of angiogenic markers.

The extent of hepatectomy appears to be an important factor to influence tumor growth, incidence and the volume of recurrence[34-36]. We have chosen a model of major hepatectomy to mimic the human condition. Prior results reported that in the mice model of LM from CRC origin, 70% partial hepatectomy induced a superior tumor growth and extent of extra-hepatic metastases than 37% hepatectomy[36,37]. *Castillo et al*. found that a small 42% LR was associated with a significantly increased survival in mice and did not produce enough growth factors to stimulate tumor growth[38]. After major hepatectomy, it seems that the liver shows higher levels of growth factors and cytokines to restore functional liver parenchyma than the cases of smaller hepatectomies[39,40]. In our study, the effect on the growth of PC after major hepatectomy, in line with previous studies, was associated with an increase in macroscopically PCI. Moreover, plasmatic concentration of growth factors was higher after liver surgery than plasmatic levels in not-injured groups.

The surgical trauma and concomitant wound-healing process induces local and systemic reactions resulting in acceleration of tumor development [41-44]. Injury in peritoneum barrier increases exfoliated or free-floating peritoneal tumor cells developing or growing into secondary

malignancies with an increased adhesion of peritoneal implants[45]. Previously, animal models demonstrated that injury sites are a preferential location for tumor recurrence and surgical trauma enhances loco-regional metastasis[46-50]. Interestingly, the influence of surgery on tumor development was not limited to local peritoneal locations. *Raa et al*. showed that in animal models with intraperitoneal injected cells of CRC, the thoracotomy enhanced tumor development in the peritoneal cavity[51]. The severity of trauma was shown to correlate with the amount of tumor load, as laparoscopy (causing minor trauma) induced less loco-regional tumor load compared with laparotomy[41]. In accordance with previous results, in our study, the sham laparotomy induced a significant increase in the PCI compared to the others groups in the early stage after surgery. However, the hepatectomy induced an increase in PC in the LS group at day 20.

Previously, *Leen et al*. showed that LM were associated with an increased ratio of hepatic arterial to total liver BFV measured by Color Doppler Ultrasonography, which suggests that measurement of changes in liver BFV could be used to detect the presence of occult metastases[52] and to identify patients at high risk of hepatic recurrence[53]. In this study, coeliac trunk mBFV changes in accordance with the phases of liver regeneration: BFV was decreased at day 1, due to the reduction in liver mass, and was significantly increased in the late phase after hepatectomy, indicating liver regeneration. The analysis of curvilinear regression between coeliac trunk mBFV and the PCI demonstrated proportional changes. This result reflects the extension of neo-formed tumor vascular networks of PC nodules branched and implanted on the native liver vascular network boosted after hepatectomy.

CONCLUSIONS

Clinical and experimental evidence suggest that liver regeneration may stimulate residual micro- and macro-metastatic disease. In our PC animal model, LR resulted in an increase in PC without

macroscopic liver regeneration. This animal model confirms the pro-angiogenic role of hepatectomy, which promotes the increase of EPC and their participation in the growth of PC. A clear understanding of the underlying processes may help to delay LR to a second procedure, after peritoneal cytoreduction, to minimize the risk of PC growth until a non-treatable stage.

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Figure legend(s)

FIGURE LEGENDES

Figure 1.

A. Peritoneal Carcinomatosis Index (PCI) scores in function of different concentrations of murine CRC-derived CT-26luc+ cells injected: $5x10^5$, $2.5x10^5$, $1.25x10^5$, $6.2x10^4$ and $3x10^4$ cells.
B. Macroscopically Peritoneal Cancer Index (PCI) scores in function of the three groups at different times.
C. Number of mitosis in function of the groups at different times.
NH, Natural History; LS, Liver surgery; Sham, sham laparotomy;
A two-sided *P-value <0.05* was considered statistically significant (*p<0.05; ** p<0.01)

Figure 2.

A. Cellular proliferation: Ki-67 expression in different tissues (magnification x40).
B. The graph shows the Ki-67 expression rate in function of the three groups at different times in the carcinomatosis nodules;
C. The graph shows the Ki-67 expression rate in function of the three groups at different times in the remnant liver parenchyma;
D. The graph shows the Ki-67 expression rate in function of the three groups at different times in the normal peritoneum tissue;
NH, Natural History; LS, Liver surgery; Sham, sham laparotomy;
A two-sided *P-value <0.05* was considered statistically significant (*p<0.05; ** p<0.01)

Figure 3.

The plasmatic concentration of progenitor Derived Bone Marrow Cells (DBMC) in function of the three groups at different times: A. Endothelial progenitor cells (EPC); B. Hematopoietic progenitor cells (HPC).
NH, Natural History; LS, Liver surgery; Sham, sham laparotomy;
A two-sided *P-value <0.05* was considered statistically significant (*p<0.05; ** p<0.01)

Figure 4.

The plasmatic concentration of the Cytokines in function of the three groups at different times: A. Vascular endothelial growth factor (VEGF); B. Vascular endothelial growth factor receptor 1 (VEGFR-1); C. Vascular endothelial growth factor receptor 2 (VEGFR-2); D. Epithelial growth factor (EGF); E, Epithelial growth factor receptor 1 (EGFR-1); F. Interleukin 6 (IL-6); G. Hepatic growth factor (HGF); H. Tumor necrosis factor alpha (TNF-a); I. Tumor growth factor beta (TGF-ß); J. Hypoxia-inducible factor 1alpha (HIF-1alpha); K. Placental growth factor (PlGF); L. Carcinoembryonic antigen (ACE).
NH, Natural History; LS, Liver surgery; Sham, sham laparotomy;
A two-sided *P-value <0.05* was considered statistically significant (*p<0.05; ** p<0.01)

Figure 5.

A. Angiogenesis: vascular CD-31 positive expression in different tissues (magnification x40).
B. The graph shows vascular CD-31 positive expression rate in function of the groups at different times in the carcinomatosis nodules
C. The graph shows vascular CD-31 positive expression rate in function of the groups at different times in the remnant liver parenchyma
D. The graph shows vascular CD-31 positive expression rate in function of the groups at different times in the normal peritoneum tissue (D).
   NH, Natural History; LS, Liver surgery; Sham, sham laparotomy;
   A two-sided *P-value <0.05* was considered statistically significant (*p<0.05; ** p<0.01)

Annex 1.

A. The sequential digestive arterial blood flow velocity measurements in function of two groups (LS, Liver surgery and NH, Natural History): Mean BFV in the superior mesenteric artery (A). Mean BFV in the coeliac trunk (B). Mean BFV in the coeliac trunk followed proportional modifications of PCI, reflexing the extension of the neo-formed tumor vascular networks branched on the native liver vascular network boosted after hepatectomy (C).
   A two-sided *P-value <0.05* was considered statistically significant (*p<0.05; ** p<0.01). ** p<0.01 LS *vs* NH groups at the same time-point; # p<0.05; ## p<0.01 *vs* day 1

Figure(s)

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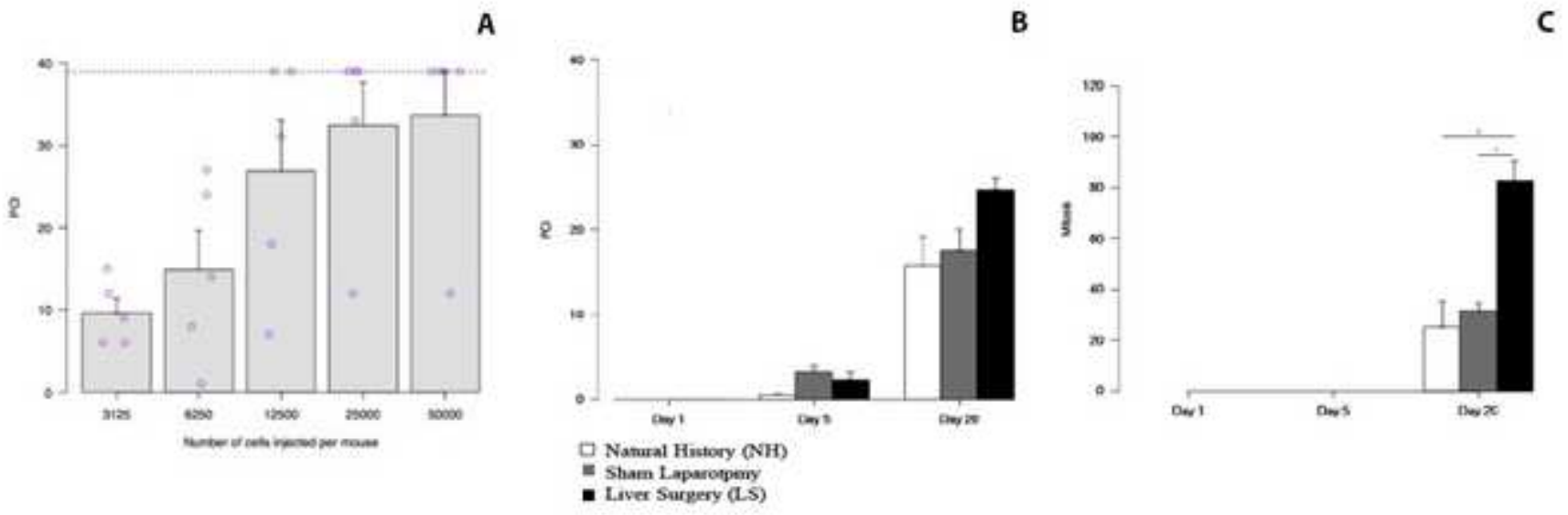

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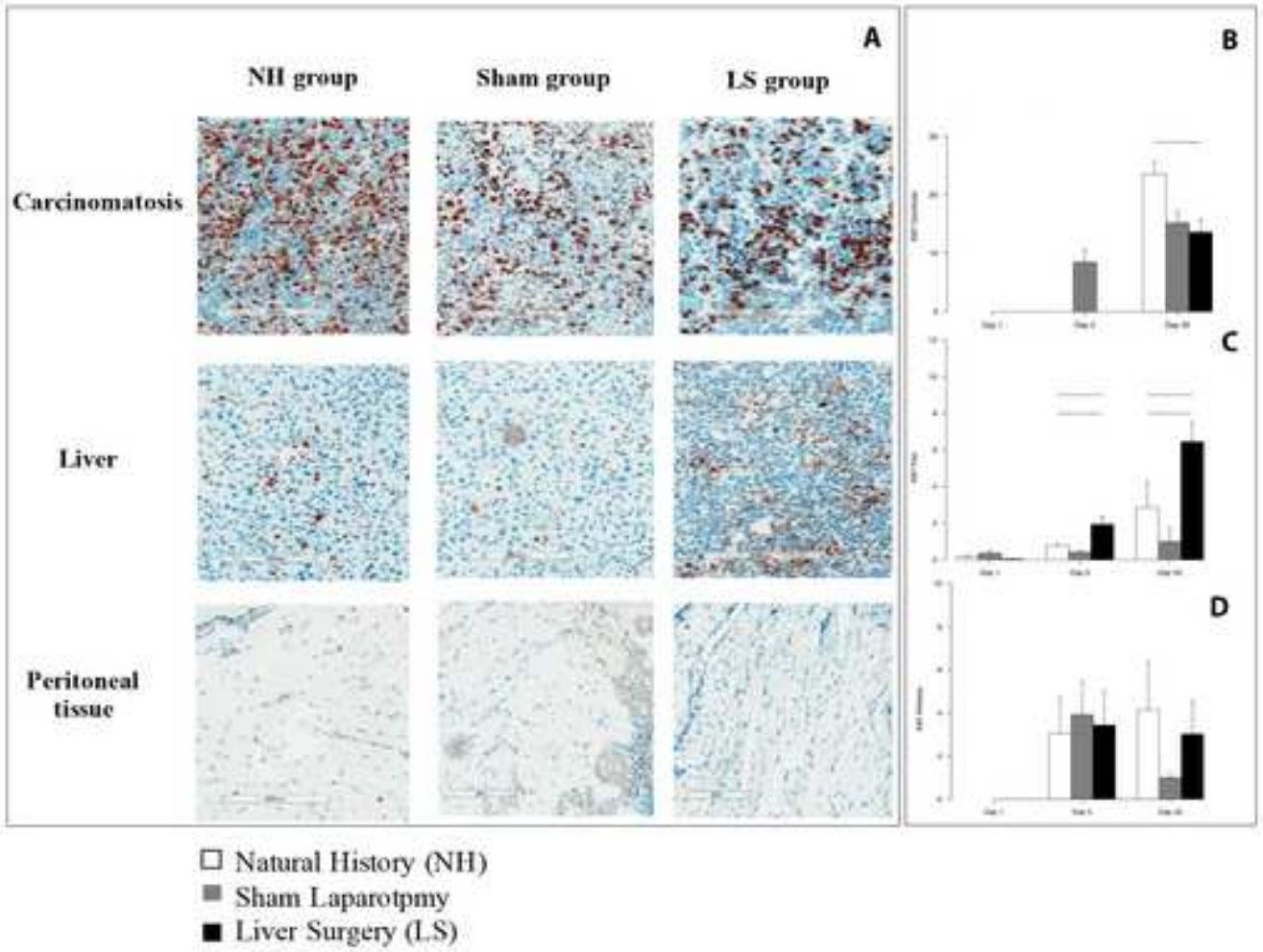

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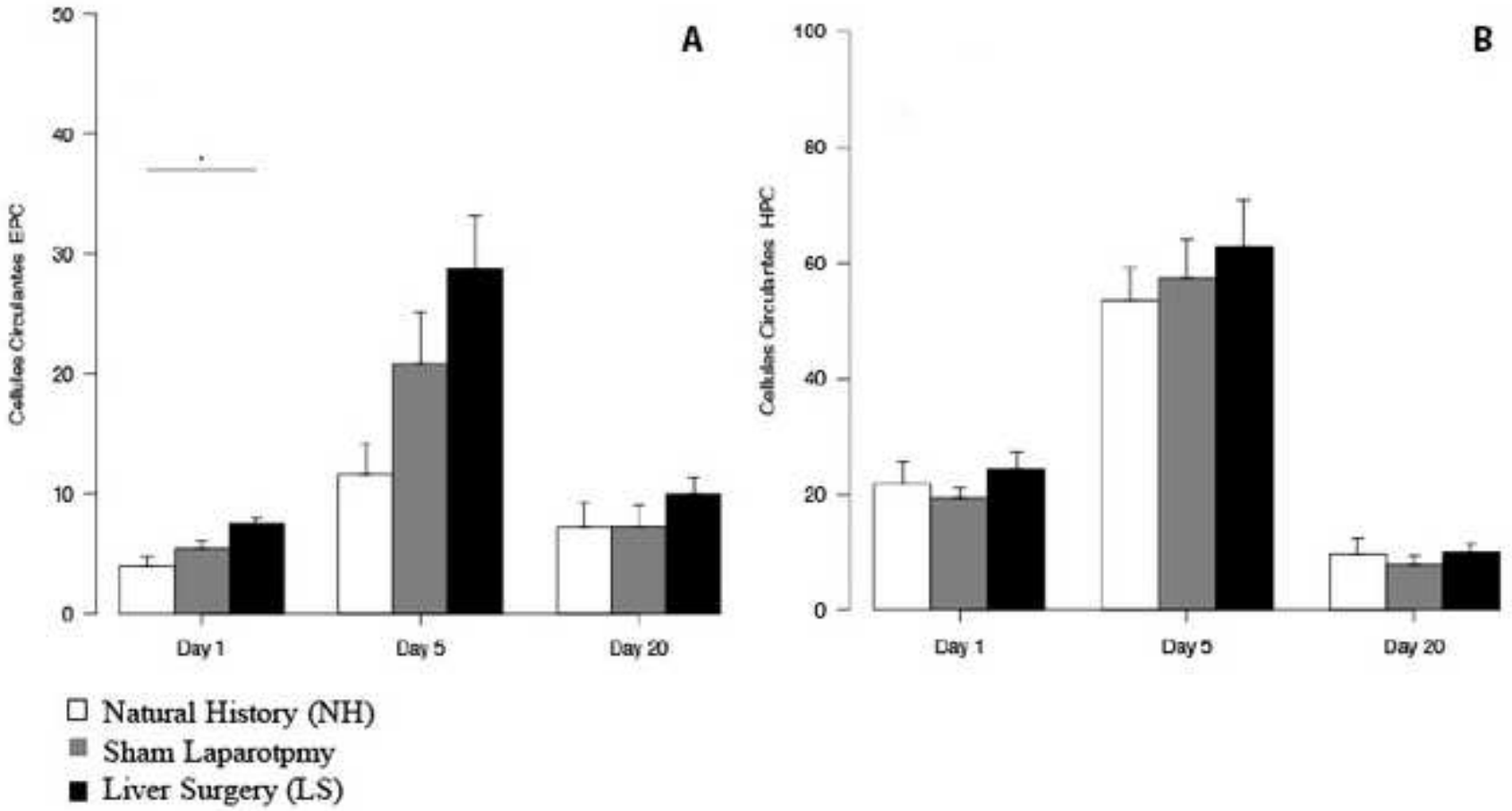

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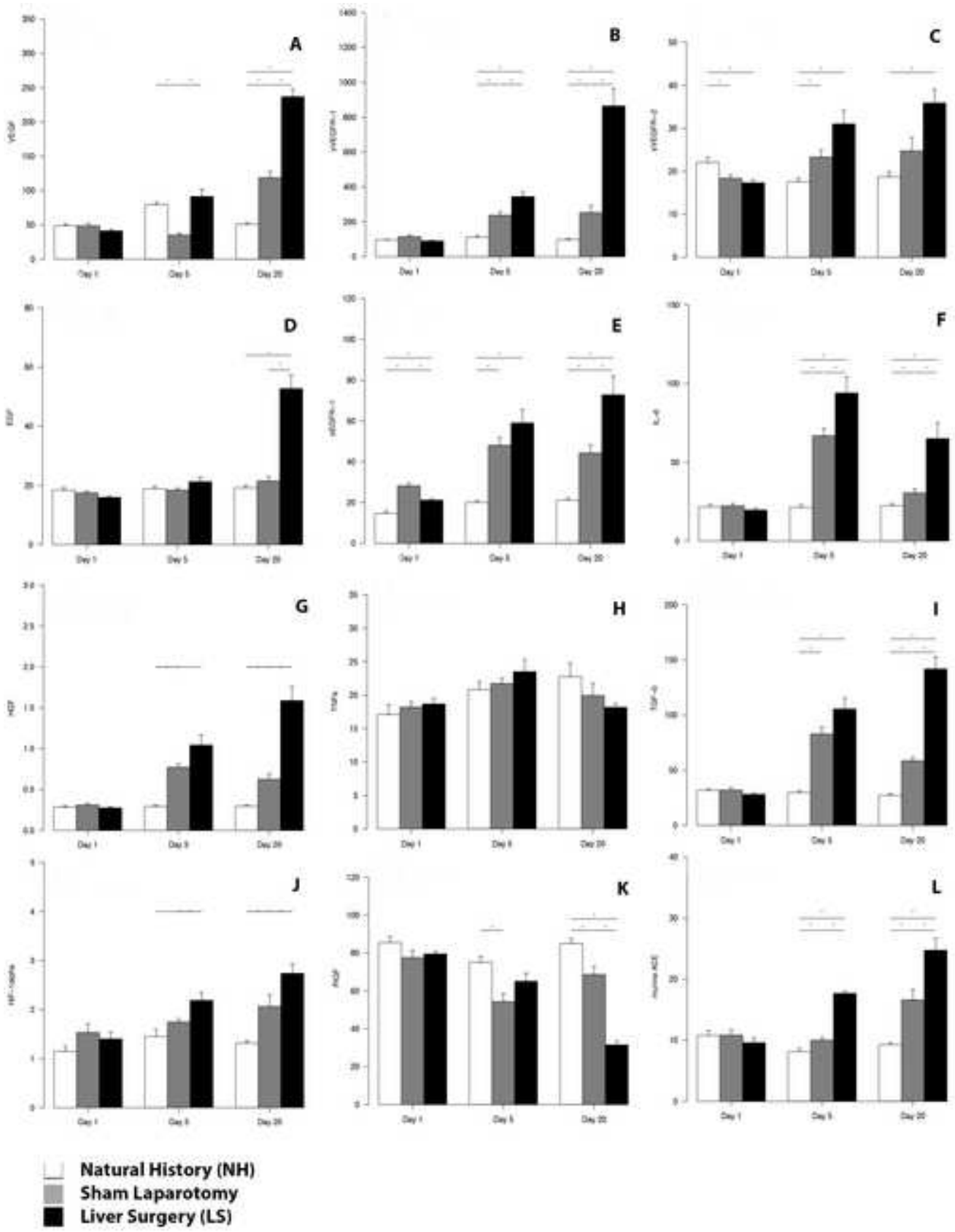

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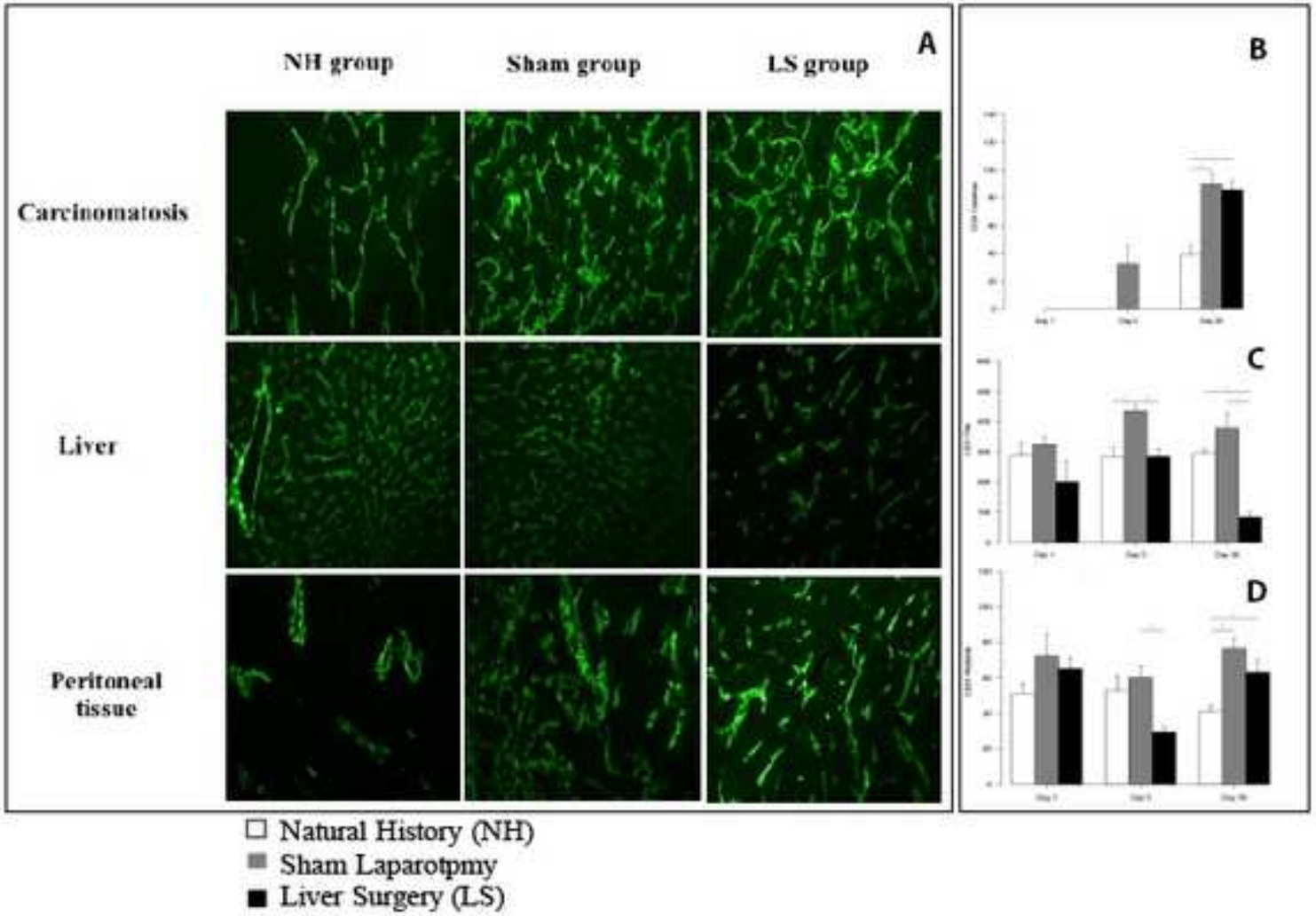

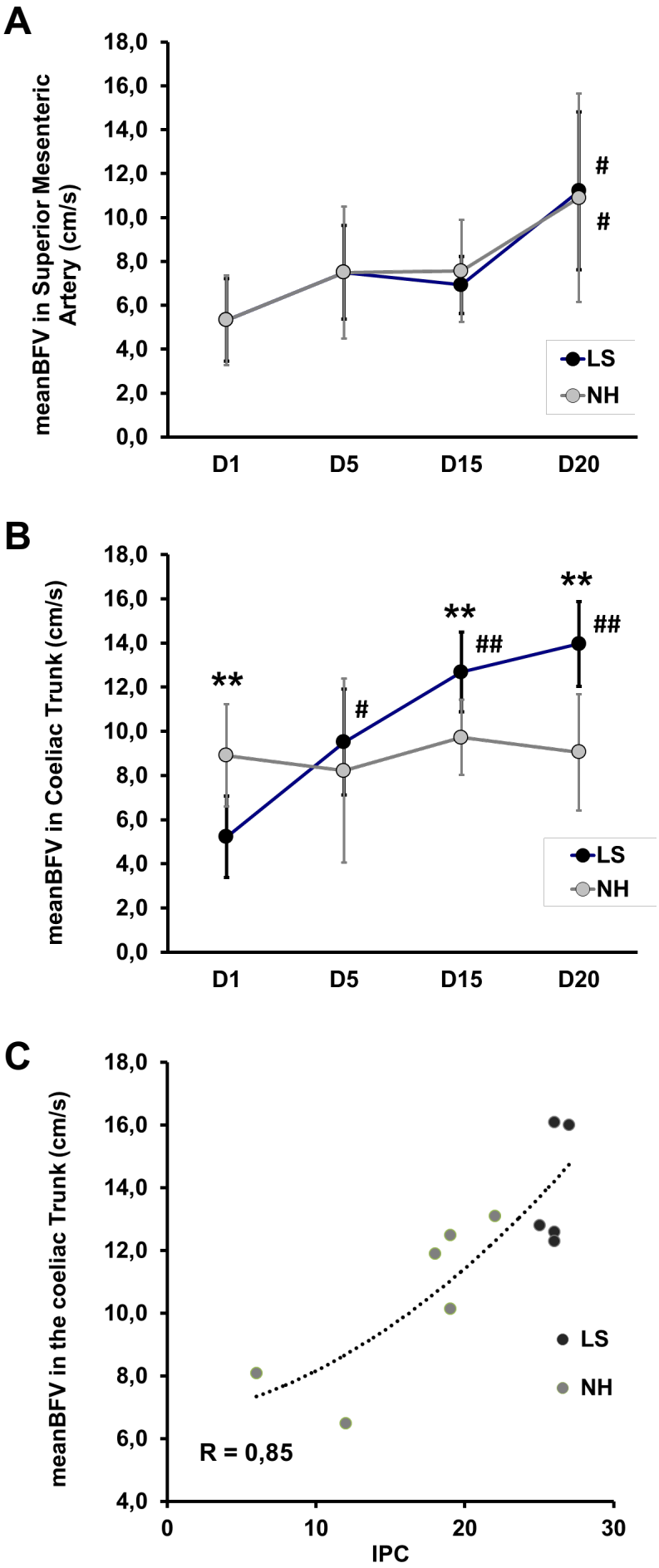
A
meanBFV in Superior Mesenteric Artery (cm/s)
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14,0
12,0
10,0
8,0
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0,0
D1
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LS
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B
meanBFV in Coeliac Trunk (cm/s)
**
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LS
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C
meanBFV in the coeliac Trunk (cm/s)
R = 0,85
LS
NH
0
10
20
30
IPC

*Conflict of Interest statement

Hepatectomy increases hepatic metastatic graft and growth in an immunocompetent murine model of peritoneal carcinomatosis

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Conflicts of interest: All the authors claim to have no conflicts of interest.

1c) ANNEX 1: Preliminary study (not published)

METHODS

- Animals

- Maintenance of colon cancer cell lines

- Construction of a murine model of limited peritoneal carcinomatosis

- Bioluminescence

- Peritoneal Cancer Index (PCI) scores in mice

RESULTS

METHODS

To mimic human situations, and to evaluate PC growth, an immunocompetent animal model of limited murine PC was constructed by intraperitoneal injection of CT-26luc+ cells.

Animals

Mice, immunocompetent BALB/c 5-week-old females weighing 20 ± 0.5 g, of controlled origin (Charles River, Arbresle, France), were housed in an animal laboratory. The laboratory is approved by the ministry Agriculture and Fisheries under the aegis of the Departmental Directorate of Veterinary Services, and their health checks have validated the presence of SPF mice (free of pathogen-specific) especially concerning the absence of virus murine hepatitis. The animals were acclimated for one week before experimentation and used in accordance to the guidelines of the European Ethics Committee (Decree No. 2001-131 of 6 February 2001, related to 86-609-EEC European directive 1986, Project n° 02095.03) as well as the national charter on ethics of animal experimentation established in 2008 (www.enseignementsup-recherche.gouv.fr).

Derivation and maintenance of colorectal cancer cell lines

The CT-26 cell line is derived from a BALB/c mouse colorectal tumor. CT-26 cells have been transfected with a gene coding for *luciférase (luc+)* and kindly provided by Prof. Lea Eisembach Weizmann (Institute of Science Rehovot, Israel). CT-26luc+ cells are grown in monolayers with DMEM culture medium supplemented with 10% fetal Calf serum (FCS), 5% antibiotic (penicillin and streptomycin), 5% Fungizone and 5% of HEPES buffer solution, in an incubator at 37°C (5% CO2 and 95% air).

Construction of a limited peritoneal carcinomatosis murine model

An intra-peritoneal injection of 1mL of decreasing concentrations of CT-26luc+ cells in 5- week-old mice was performed on day 0 on a total of 25 mice (5 in each group): $5x10^5$, $2.5x10^5$, $1.25x10^5$, $6.2x10^4$ and $3x10^4$ cells. To reduce the number of animals (for ethical reasons), we constructed our murine model with 5 mice in each group. The figure 3 shows the logarithmic correlation between the increase of PCI and the different concentrations of tumoral cells injected. The control group did not receive tumor cell or culture medium injections. The animals were euthanized by cervical dislocation on day 20.

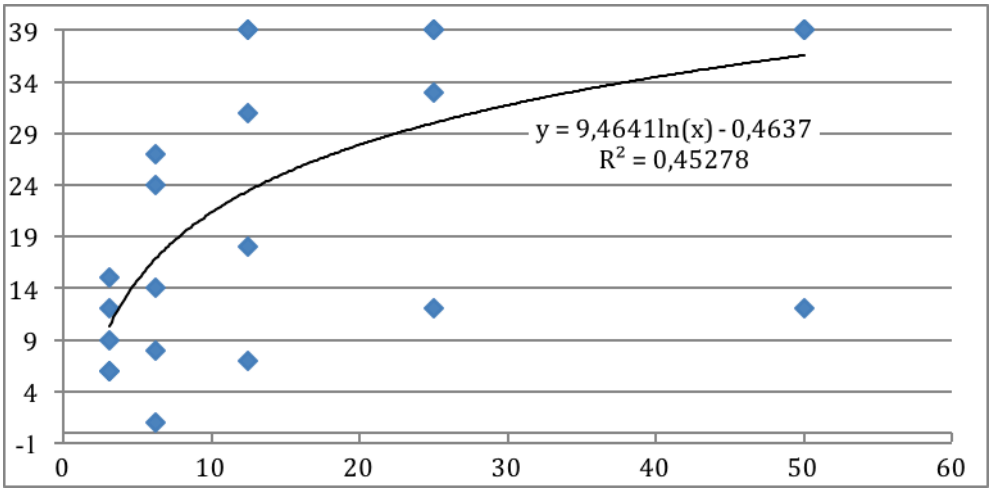

Fig. 3: Correlation between the PCI increase and the different concentrations of CT26 luc+ tumor cells injected in the peritoneal cavity of the murine model. The graph shows a logarithmic correlation.

Bioluminescence

The analysis of PC growth in mice was performed using optical *bioluminescence* for detecting photons emitted in vivo by cells transfected with a gene encoding luciferase with a high sensitivity camera. Bioluminescence imaging was performed using an IVIS Spectrum. Image radiance values were normalized using Living Image (Caliper LifeScience) (fig. 4).

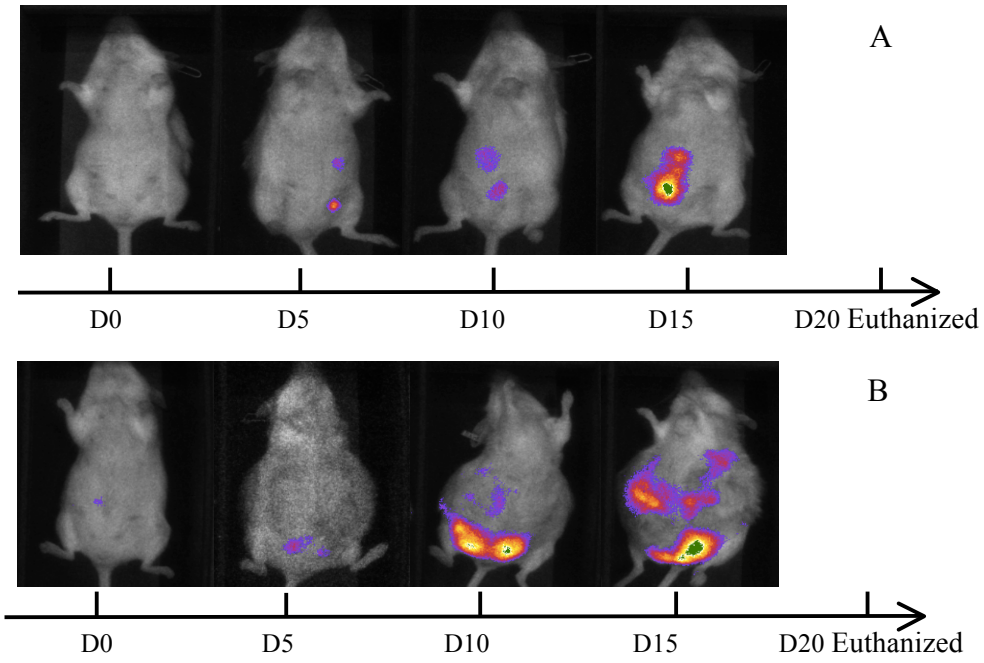

Fig. 4 Detection of the bioluminescence signal in the murine model:

Group 1, injection of $3x10^4$ cells (A); Group 4, injection of $2.5x10^5$ cells (B).

Bioluminescence was first confirmed on CT-26luc+ cells in cultures. A preliminary kinetic study of Luciferin was performed on the 24-well plate suspension to determine the peak signal time after Luciferin administration (Figure 5).

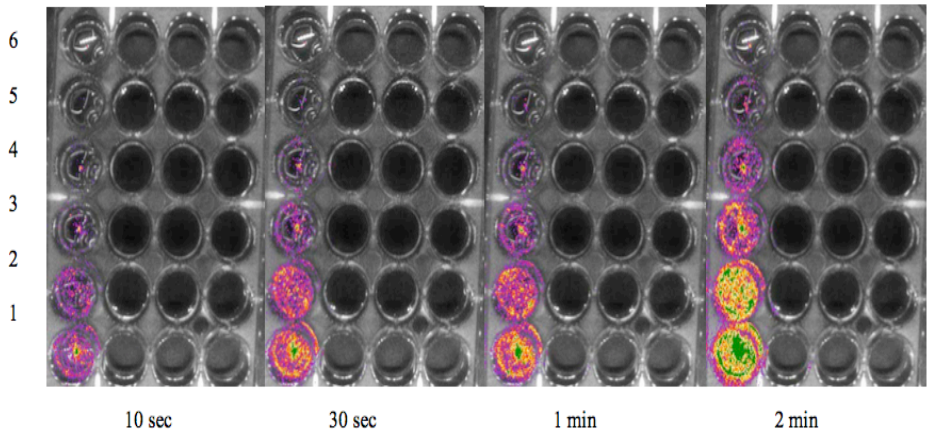

Fig. 5: Preliminary bioluminescence kinetic study:

Decreasing concentration of cells in 24-well plate (well 1-6): 100.000, 50.000, 25.000, 12.500, 6.250, 3.000 cells. Exposure time: 10 seconds, 30 seconds, 1 and 2 minutes.

In the animal model, tumor growth was evaluated on the day of CT-26luc+ cell injection (day 0) and on days 5, 10, and 15 after injections.

Peritoneal Cancer Index (PCI) scores in mice

As in humans, extent of PC was evaluated by the Peritoneal Cancer Index (PCI) [129]. The PCI (range, 1 to 39) allows the assessment of the distribution of cancer throughout the abdomen and pelvis and is calculated by summing the lesion size scores (0 to 3) in the abdominopelvic regions (0 to 13). The PCI was adapted to tumor sizes in mice with the following lesion size scores (fig.6): a tumor smaller than 2.0 mm (lesion size 1), 2.1 to 5.0 mm (lesion size 2), and greater than 5.0 mm or confluence (lesion size 3), as previously described [130,131].

We postulated that the PCI had to be less than 10 at time of liver resection, in our model, to able to detect a peritoneal tumor growth.

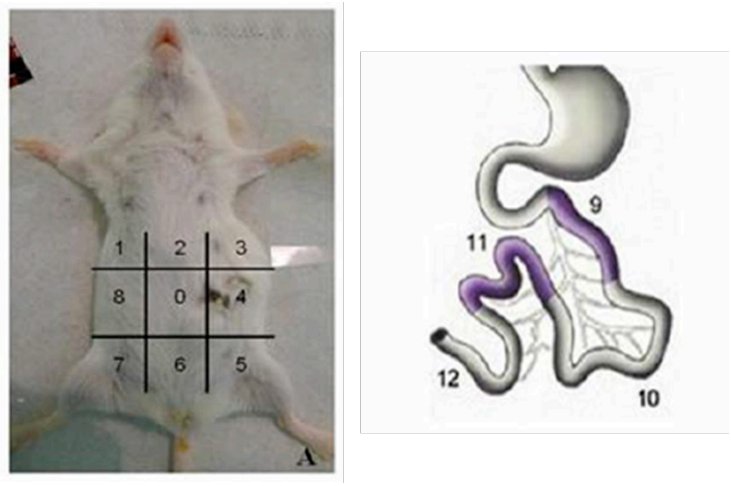

Fig. 6: Peritoneal Cancer Index (PCI) score in mice.

The abdomino-pelvic regions (0 to 13): 0, Umbilical region; 1, Right hypochondrium (RHC); 2, Epigastric region (epigastrium); 3, Left hypochondrium (LHC); 4, Left lumbar region; 5, Right iliac fossa (RIF); 6, Hypogastric region; 7, Left iliac fossa (LIF); 8, Right lumbar region; 9, proximal jejunum; 10, distal jejunum; 11, proximal ileum, 12 distal ileum.

RESULTS

The results obtained from our murine model demonstrate a tumor graft rate in more than 99% of the mice with variable scores in the different groups: limited PC extent without ascites was found in group 1 (mean PCI=10, range 6-15); a moderate carcinomatosis was obtained in group 2 (mean PCI=15, range 1-27); Extensive carcinomatosis associated with the presence of ascites was observed in groups 3, 4 and 5 (mean PCI=26, range 7-39; mean PCI=32, range 12-39; mean PCI=34, range 12-39, respectively). The control group did not show carcinomatosis. Weight was calculated every 5 days and the presence of ascites at the euthanizing of the mouse. Statistically significant differences found between the groups are showed in the Table 1.

	Group 1 5×10^5 **cells**	**Group 2** 2.5×10^5 **cells**	**Group 3** 1.25×10^5 **cells**	**Group 4** 6×10^4 **cells**	**Group 5** 3×10^4 **cells**	***p***
Carcinomatosis (at day 15)	100%	100%	100%	100%	100%	ns
Median PCI **<10** **10-20** **>20**	**34** 1 0 4	**32** 1 0 4	**26** 1 1 3	**15** 2 1 2	**10** 3 2 0	***0,0284****
Weight (gr)	26,2	24,8	22	18,8	19,8	***0,014 ****
CEA (ng/ml)	34,4	38,8	19,24	8,32	3,62	***0,022****
Ascitis	3	4	4	4	3	*ns*
Liver metastases	0	1	0	0	0	*ns*
Death	3	1	0	0	0	*ns*

Table 1: The results from a murine model of peritoneal carcinomatosis obtained with intraperitoneal injection of different tumor cell concentrations.

We performed a non-invasive evaluation of kinetics of the PC progression by bioluminescence. We established a reproducible murine model of limited peritoneal carcinomatosis with a mean PCI <10 on day 15 by an intraperitoneal injection of 3×10^4 cells CT-26Luc+. This concentration was retained for further experimentations.

1d) ANNEX 2: Preliminary study

METHODS

- Construction of a murine model of liver regeneration

RESULTS

METHODS

Construction of a murine liver regeneration model

To mimic the human setting and evaluate the effect of liver regeneration on PC growth, a major liver resection model was constructed. The surgical technique (fig. 7) was proposed previously by *Greene et al.*[88] : partial hepatectomy including the resection of 3 anterior lobes (right upper lobe (RUL 18%), left upper lobe (LUL 15%) and lower left lobe (LLL 35%)) out of a total of 7 lobes (fig. 8). The mice were placed in a closed circuit box and anesthetized with isofluorane (2% in the induction and between 1.5 to 1.8% to maintain the anaesthetic state during the procedure) in a mixture of oxygen (30%) and nitrous oxide (70%) of 2L/min. A medial laparotomy was performed. The liver was then mobilized and gently exteriorized using two cotton swabs. A 6/0 Prolene wire (Ethicon®, Somerville, NJ) was passed around the vascular pedicle of the resected lobe, the suture positioned near the origin of the vessel. Then, three knots were tied off and the parenchyma was divided using scissors. The same procedure was then repeated for the other two anterior lobes of the liver to achieve a resection volume of 68% of the total hepatic parenchyma.

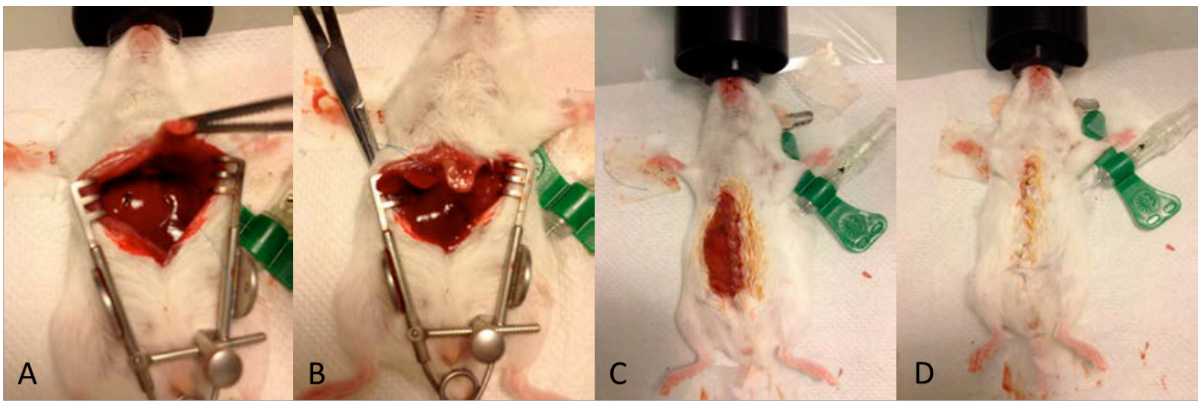

Fig. 7: Hepatectomy stages in mice

From left to right: Opening and exposure of the liver (A), ligature of the vascular pedicle (B), peritoneal and cutaneous closures (C-D).

During the procedure, a 9% saline infusion (4-5ml) was administered subcutaneously to prevent dehydration due to evaporation during laparotomy. An injection of Buprenorphine (0.1 mg/kg) was administered after surgery and then repeated every 12h for a 48h period.

Five days after injection of CT26luc+ cells, the animals were randomized into three groups: in the Liver Surgery group (LS), the animals underwent 68% liver resection (as previously described); in the second group, animals underwent a sham laparotomy (Sham); in the control group, the animals received only injections of intraperitoneal cells, without any other injuries (Natural History, NH).

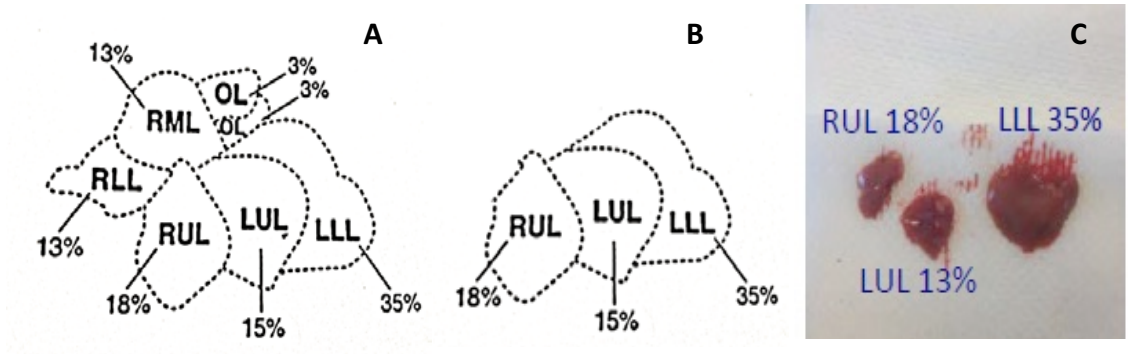

Fig.8: Liver anatomy, hepatic segments

Partial hepatectomy includes resection of 3 anterior lobes (fig. B-C): right upper lobe (RUL 18%), left upper lobe (LUL 15%) and lower left lobe (LLL 35%), above 7 total lobes (fig. A): RML, right median lobe; RLL, right lower lobe; CL, caudate lobe, CLL Caudate lower lobe.

To evaluate neo-angiogenesis and tumor growth, histological and biological analyses were performed. Mitosis count, carried out by Ki-67 marker proliferation, and microvessel density, using a CD-31 endothelial cell marker, were performed in the peritoneal tumor nodules, peritoneal normal tissue, epiploon and remnant liver tissue. Local vascular modification of neo-angiogenesis was quantified using the Doppler index for measurements of blood flow velocities (BFV). Systemic effects of angiogenesis and liver regeneration were quantified using cytokines plasmatic levels and

by monitoring the circulating progenitor BMDC mobilization. The Doppler index and the monitoring of the mobilization methods for the circulating progenitor BMDC are detailed in article 1. All experiments were carried out on the three groups on days 1, 5 and 20 after randomization.

RESULTS

The results of this experiment are described in the **Article 1,** that currently being submitted to the EJSO

2) PART TWO

2a) HYPOTHESIS AND OBJECTIVES

- **Clinical methodology**

In the second part of our study we propose an aggressive approach for patients with concomitant liver and peritoneal metastasis from colorectal cancer, traditionally considered a contraindication to any surgical approach because the disease is considered to be too advanced. This multi-center study is the largest sampling of selected patients with peritoneal carcinomatosis from colorectal cancer and simultaneous liver metastasis treated with cytoreduction, liver resection and HIPEC.

An international database was created by the La Sapienza Department of Surgery in Rome and the BIG-RENAPE group, which brings together French surgical centers specializing in peritoneum pathology. The database has collected 161 cases of combined surgeries with hepatectomy and peritoneal resections with CHIP. The agreement of the Scientific Council of the BIG-RENAPE was obtained to analyze the databases.

At first, the international series was analyzed retrospectively: Early and long-term outcomes were evaluated to select significant clinical prognostic factors. The present study shows that, in expert centers, an aggressive management of multi-metastatic colorectal cancer is feasible, and safe, with an acceptable morbidity rate of 15% and no postoperative mortality.

There are currently no specific criteria to select patients with the highest potential for surgical success, nor guidelines concerning the timing of peritoneal and liver surgery. This paper can be a valuable aid in selecting those patients.

The preliminary results of this study have been submitted and accepted by the 2016 ASCO Annual Meeting (Abstract N° 3558; Subcategory: Advanced Disease, Category Gastrointestinal (Colorectal) Cancer; Citation: J Clin Oncol 34, 2016 (suppl; abstr 3558)). The original article is in the process of submission to the ***Annals of Surgery*.**

2b) RESULTS AND DISCUSSION

- Abstract N°3558 May 2016 Journal of Clinical Oncology (ASCO 2016)

- Article 2 (currently submitted to the Annals of Surgery)

ABSTRACT N°3558 May 2016 Journal of Clinical Oncology (ASCO 2016)

Curative treatment for patients (pts.) with synchronous liver metastases (LM) and peritoneal carcinomatosis (PC) of advanced colorectal cancer (aCRC): A multicenter study of the French Association of Surgery.

Rea Lo Dico, Guillaume Passot, Diane Goere, Clarisse Eveno, Francois Quenet, Marc Pocard; Assistance Publique–Hôpitaux de Paris, Hôpital Lariboisiere, Paris, France; Centre Hospitalo-Universitaire Lyon Sud, Lyon, France; Institut Gustave Roussy, Villejuif, France; Institut de Cancerologie Montpellier, France

Abstract:

Background: Aggressive surgical approaches combining hepatectomy associated to peritoneal resection with curative intent remain controversial in such a setting and almost no data are available on such patients. Pts with synchronous PC and LM are generally considered for exclusive systemic palliative chemotherapy.

The aim of this prospective cohort was to assess morbidity, mortality, disease-free survival (DFS) and overall survival (OS) of peritoneal and liver metastasis of aCRC patients (pts.) treated with an aggressive therapeutic approach combining surgical treatment of liver and peritoneal lesions followed by hyperthermic intraperitoneal chemotherapy (HIPEC).

Methods: All patients registered in the French Association of Surgery prospective database with PC and synchronous LM who had undergone cytoreductive surgery and LM resection followed by intraperitoneal hyperthermic chemotherapy were analysed. The primary endpoint was survival from the time of surgery.

Results: From 1993 to 2015, 146 pts. with PC and LM were analyzed. This is the largest series actually reported. After a mean follow-up of 36 months, the median OS and DFS, were respectively 27,2 and 9.5 months. Postoperative morbidity and mortality was 14.8 and 0%, respectively. In pts. with a complete cytoreductive surgery OS was 29 months (n=), as compared to 4 months in pts. (n=) with incomplete cytoreduction (p=0.0001). Rectal primary tumor, PCI of 13 or more, pN+ status, and more than 3 LM were not identified as independent factors for poor OS.

Conclusions: This multicenter study confirms that prolonged survival can be achieved in selected patients suitable for PC and LM surgery if they underwent multimodality treatment including surgical treatment of PC and LM with curative intent, using intraperitoneal chemotherapy.

Annals of Surgery

Early and long-term outcomes of patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy combined with liver resection for simultaneous liver and peritoneal metastases from colorectal cancer: A multi-center study

--Manuscript Draft--

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Dear Editor,

Please find enclosed a manuscript entitled “Early and long-term outcomes of patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy combined with liver resection for simultaneous liver and peritoneal metastases from colorectal cancer: A multi-center study” that we would like to be considered for publication in Annals of Surgery.

The preliminary results of this study has been submitted and accepted to the 2016 ASCO Annual Meeting (Abstarct N° 3558; Subcategory: Advanced Disease, Category Gastrointestinal (Colorectal) Cancer; Citation**:** J Clin Oncol 34, 2016 (suppl; abstr 3558)).

We believe this paper deserves publication priority because we propose an aggressive approach for patients with concomitant liver and peritoneal metastasis from colorectal cancer, traditionally considered a contraindication to any surgical approach as the disease is considered to be too advanced.

This multi-center study is the largest series of selected patients with peritoneal carcinomatosis from colorectal cancer and simultaneous liver metastasis treated with cytoreduction, liver resection and HIPEC. The present study shows that an aggressive management of multi-metastatic colorectal cancer is feasible and safe with an acceptable morbidity rate of 15% and no postoperative mortality.

There are currently no specific criteria to select patients with the highest potential for surgical success, nor guidelines concerning the timing of peritoneal and liver surgery, this paper can be a valuable aid in selecting patients.

As a highly respected journal, we believe that Annals of Surgery is the most appropriate journal for sharing our findings and we hope to consider our manuscript for publication. All the listed authors have made substantial contributions in conception, analysis and interpretation of data and in the drafting and critical revisions of the manuscript.

This paper is not being considered for publication elsewhere, none of its contents have been previously published in any Language and all authors have read and approved the manuscript.

None of authors have relationships with the pharmaceutical industry.

Thank you in advance for considering our paper, I look forward to earing from you soon.

Best Regards,

Rea Lo Dico

MINI-ABSTRACT

Aggressive surgical approaches combining liver resection and peritoneal cytoreduction with curative intent remain controversial. The aim of this prospective multicenter study was to assess morbidity, disease-free survival and overall survival of patients with PC and LM of CRC treated by combining LR with CRS followed by hyperthermic intraperitoneal chemotherapy.

ABSTRACT

Background: Synchronous peritoneal carcinomatosis (PC) and liver metastases (LM) of colorectal cancer (CRC) are usually treated with palliative systemic chemotherapy. Aggressive surgical approaches combining liver resection (LR) and peritoneal cytoreduction (CRS) with curative intent remain controversial.

Objective: The aim of this prospective multicenter study was to assess morbidity, disease-free survival (DFS) and overall survival (OS) of patients with PC and LM of CRC treated by combining LR with CRS followed by hyperthermic intraperitoneal chemotherapy (HIPEC).

Methods: From 1993 to 2015, 161 patients underwent LR with CRS and HIPEC of curative intent in 18 centers were prospectively registered and analyzed. One hundred and thirty-three patients had simultaneous LR with CRS, 13 had two-stage surgery (peritoneal followed by subsequent LR).

Results: After 24-month mean follow-up, the median OS and DFS were 32.32 [95%CI: 24.57–40.06] and 10.13 [95%CI: 8.85–11.41] months, respectively. The mean number of LM was 2 [range: 1–15]. Postoperative grade III–IV morbidities occurred in 14.9% with no postoperative mortality. Peritoneal carcinomatosis index >12/39 (aHR 1.67; 95%CI 1.05-2.66, *P*=0.03) was identified as the only independent prognostic factors for OS. Completeness of cytoreduction and number of LM>3 were two independent predictive factors (aHR=1.99; 95%CI: 1.02-3.89, *P*=0.04 and aHR=3.32; 95%CI: 1.67-6.63, *P*=0.001, respectively) for DFS. Simultaneous LR with CRS was associated with longer hospital stays compared to two-stage surgery (24 *vs* 15 days, *P*=0.02). The number of LM and the type of LR did not influence the rate of postoperative complications.

Conclusion: This multicenter study is the largest series, to date, confirming the feasibility of combined LR with CRS and HIPEC in selected patients with LM and PC of CRC, resulting in 32 months median OS with limited morbidity.

Early and long-term outcomes of patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy combined with liver resection for simultaneous liver and peritoneal metastases from colorectal cancer: A multi-center study.

Running title:

CRS and HIPEC in synchronous colo-rectal liver and peritoneal metastases

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The study was presented in 2016 at the ASCO Annual Meeting (Abstract N° 3558, Subcategory: Advanced Disease, Category: Gastrointestinal (Colorectal) Cancer, Citation: J Clin Oncol 34, 2016 (suppl; abstr 3558)).

274 words, 2917 words, 2 figures, 4 tables (6 total figures and tables), 53 references

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Presentations:

Contributing authors:

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INTRODUCTION

Liver metastases (LM) occur in 25–40% of patients with colorectal cancer (CRC).[1] Long-term outcomes of patients undergoing systemic chemotherapy (CTH) alone is poor, with a median overall survival (OS) of 16 months reported in the Cairo trial,[2] and up to 31 months achieved with treatment intensification as shown in the TRIBE trial.[3] A recent meta-analysis by *Franko et al*. reported an OS of 19 months in patients with LM of CRC.[4] Curative management of LM is based on surgical resection, although, in 70% of cases, LM will recur despite the use of multimodal and adjuvant CTH.[1]

Peritoneal carcinomatosis (PC) occurs in 8–20% of patients with CRC at the time of diagnosis,[5-8] and is associated with poor survival as low as 6 months if left untreated.[9] The presence of isolated PC in patients with CRC is a prognostic factor of poor OS compared with isolated non-peritoneal metastasis.[4] Using modern systemic CTH, modest improvements in survival can be achieved in 12–16 months.[4,5,10,11] Cytoreductive surgery (CRS) with intraperitoneal chemotherapy, including hyperthermic intraperitoneal chemotherapy (HIPEC) is increasingly accepted as the only potentially curative treatment for PC of CRC origin, achieving a mean disease-free survival (DFS) of 18 months, an OS mean of 27 months[12] and an OS of up to 63 months has been reported in highly selected patients.[13]

Synchronous LM and PC from CRC is traditionally considered a contraindication to any surgical approach as the disease is thought to be too advanced.[14-18] However, smaller pilot series have reported prolonged survival after management of synchronous colorectal LM and PC, reaching up to 3 years in selected patients.[19-26] These suggest that LM is not an absolute contraindication to peritoneal CRS and that a curative surgical management of LM and PC may indeed be possible.[27-29] However, to date, no standard management pathway has been established for patients with simultaneous LM and PC, especially if a major hepatectomy and

an extensive peritoneal CRS have to be performed. Moreover, there are currently no specific criteria to select patients with the highest potential for surgical success, nor guidelines concerning the timing of peritoneal and liver surgery.

The aim of this study was to analyze a prospectively maintained multi-institutional database in order to describe and assess the early outcomes (morbidity/mortality, hospital length of stays) and long-term results (DFS and OS) of CRC patients undergoing liver resection (LR) and peritoneal CRS with HIPEC for concomitant PC and LM. The secondary aim was to identify potential factors, related to poorer outcomes, in order to establish a basis to guide the management of these patients, optimizing the selection of candidates for surgical treatment and determining the best sequence of surgical procedures.

PATIENTS AND METHODS

Patients

A prospective multi-institutional database was established using the French BIG-RENAPE database network for colorectal PC and the Italian database from "La Sapienza" University of Rome. The French network was developed for collecting the data of all French surgical teams performing CRS and HIPEC for primary digestive cancers.[30] This study was carried out in accordance with the precepts established by the Helsinki Declaration. Using the BIG-RENAPE and the Italian databases, we identified all patients treated with LR and CRS with HIPEC between 1993 and 2015 for PC from CRC origin in 17 French, and one Italian, centers. Among these, we identified 161 consecutive patients who had concomitant PC and LM and who underwent LR combined with complete CRS and HIPEC. To be included in the present study the following criteria were established: patients who had undergone complete CRS, HIPEC and LR, with pathological examinations confirming liver and peritoneal metastases from CRC origin. The exclusion criteria were non-CRC origin (appendiceal, gynecological and peritoneal primary malignancies). Ovarian metastases were considered a manifestation of peritoneal disease.[31]

Standardized Data Collection

All background clinical, histological, operative and postoperative data for this study were prospectively collected and entered into a standardized central electronic database. Simultaneous resection was defined as LR during the same operation for PC and separate procedures were defined as two-staged. Pre-HIPEC CTH complications were graded according to the National Cancer Institute's Common Toxicity Criteria.[32] Postoperative complications at 30 days, or until hospital discharge, were graded according to the Dindo-

Clavien criteria.[33] The follow-up period was measured until recurrence for disease free survival (DFS) and until death for overall survival (OS).

Surgical Procedure

The extent of PC was assessed by intraoperative examination and defined according to Sugarbaker's Peritoneal Carcinomatosis Index (PCI).[34] The completeness of cytoreduction (CCR-) score was used to define the volume of PC remaining in the abdomen after CRS, as previously described.[34]

HIPEC was administrated after completion of CRS using an open coliseum or closed technique according to the team's preference, to deliver the chemotherapy agent at 42-43°C for 30-90 min in a closed circuit. The drugs employed were oxaliplatin or mitomycin C, as previously described.[12,35]

LR was performed according to the principles of oncologic radicality. Minor hepatectomy was defined as any LR of less than three hepatic segments, including atypical resection (metastasectomy, segmentectomy and bisegmentectomy) and radiofrequency ablation (RFA) for lesions measuring less than 2.5 cm and located far from the main vessels according to each team's preference. Major hepatectomy was defined as the LR of at least three hepatic segments. The general approach across the centers was that patients requiring minor resections had concomitant LR and peritoneal CRS, whereas in some cases major LR was postponed to be performed after CRS and HIPEC.

Endpoints

The primary endpoints of the analyses were DFS and OS. DFS at 3 years, defined as the time from CRS and HIPEC surgery to relapse, or death, whichever occurred first. Second colorectal cancers are considered as DFS events, whereas non-colorectal tumors are to be

disregarded in the analyses. OS was defined as the time from CRS and HIPEC surgery to the time of death due to any cause. In the case of a two-staged procedure, the CRS procedure date was considered as the first treatment day. The secondary endpoints were completeness of surgical resection, postoperative morbidity/mortality, and duration of hospital stay. Postoperative morbidity/mortality was defined according to the Dindo-Clavien classification.[33] All in-hospital complications were included.

Statistical Analysis

Categorical data were compared by the $\chi 2$ test or the Fisher exact test. For continuous data, the independent-samples *t-t*est was used. Survival rates were estimated using the Kaplan-Meier method. The long-rank test was used to compare survival curves. Follow-up information was available for all patients included in the study until death or censored from 31/12/2015 onwards. Date of tumor recurrence was not available for 20 patients (12.4%). Univariate analyses were conducted using a Cox proportional hazard model to identify potential prognostic factors of survival. To take into account confounders of survival analysis, a multivariate analysis was performed using a Cox proportional hazards model with forward stepwise selection of covariates and with entering and removing limits of $P<0.10$ and $P>0.05$. All statistical analyses were performed using SPSS, version 20.0 (SPSS Inc, Chicago, IL). A *P*-value <0.05 was considered significant.

RESULTS

Patient Characteristics

One hundred and sixty–one consecutive patients from 18 centers were included, 84 of which were female (52.2%). The mean age was 56.5 years (SD: 11.1, range, 26-88 years). Karnofsky Performance Status was 0-1 in all patients. Patient characteristics are summarized in Table 1. Primary tumor locations were the right colon (29.2%), left colon (59.6%), and rectum (6.2%). Primary tumors were well differentiated in 52 patients (32.3%), moderately differentiated in 70 (43.5%) and poorly differentiated in 10 (6.2%). Differentiation was unknown/not reported in 29 cases (18.0%). Lymph node status of the primary tumor was recorded for 141 patients (87.6%) being positive in 104 patients (64.6%) and negative in 37 (23.0%). One hundred and thirty–eight patients (85.7%) were treated with preoperative systemic CTH.

Treatment Related Data

Mean PCI was 9.8 (SD: 7.3, range, 0-39), being ≤12 in 106 patients (data was missing for 5 patients). A complete CRS (CCR-0) was achieved in 144 patients (89.4%), with a CCR-1 in 14 (8.7%) and CCR-2 in 3 patients (1.9%). No CCR-3 was reported. The mean number of LM was 2 (SD: 1.80, range: 1–15). For 75 patients (46.6%) the LM were synchronous of the primary tumor, and metachronous in 71 patients (44.1%). Major LR was performed in 28 patients (17.4%); 117 patients (72.7%) were treated with limited resections: 92 hepatic wedges, 12 hepatic wedges with RFA, 12 RFA alone and 1 hepatic wedge with RFA and intra-arterial chemotherapy. One hundred and thirty-three patients (82.6%) underwent LR simultaneously with CRS and HIPEC, whereas thirteen had a two-staged procedure (8.1%).

Early Outcome

Forty-height complications were observed: abdominal complications (bleeding (n=6), abscesses (n=4), anastomotic (n=9) or bilio-pancreatic leaks (n=4), pancreatitis (n=4), gastroparesis (n=2), cardiac complications (n=2), respiratory complications (acute respiratory distress (n=2), pneumonia (n=7), pleural effusion (n=11), and hematological toxicity (n=12). Severe postoperative morbidity (grades III-IV) occurred in 14.9% (n=24). Surgical interventions for complications were required in 13 patients (8.1%). The mean hospital stay duration was 23 days (SD: 12.5, range, 8-87 days). No postoperative mortality occurred. Simultaneous CRS and LR was associated with a longer postoperative hospital stay than two-staged surgery (24 (SD: 13.1) *vs*.15 (SD: 5.8) days, respectively, *P*=0.02). The number of LM and the type of LR were not identified as being associated with the frequency of severe postoperative complications (Table 2).

Long-term Outcome

The mean follow-up was 24 months (range, 0.2 to 102 months). For patients with complete data (n=156), median OS was 33 months (range, 0.2 to 102 months), with 1-, 3- and 5-year OS rates being 87%, 45%, and 25% respectively (Fig. 1A). Seventy-nine (49%) of the 161 patients died during the follow-up period. For patients with complete data (n=141), the median DFS was 10.1 months (range, 0.3 to 56 months) with 1-, 2- and 3-year DFS rates equating to 44%, 17%, and 8% respectively (Fig. 2A). One hundred and twenty-four patients had cancer recurrence (87.9%) during the follow-up: 19 patients (13.5%) had peritoneal recurrence, 37 patients (26.2%) had extra-peritoneal recurrence and 46 patients (32.6%) had both. Site of recurrence was unknown in 22 patients (15.6%). Among patients with extra-abdominal recurrences, 44 (31.2%) had pulmonary metastases. Among patients with DFS available, relapse occurred within the first year in 74 patients (52.5%). On univariate analysis,

more than 3 LM and Grade III–IV postoperative complications were identified as prognostic factors for lower DFS (Table 3). However, on multivariate analysis, more than 3 LM and CCR-1 resection were the only independent predictive factors for decreased DFS (adjusted Hazard Ratio [aHR]: 3.32, 95%CI: 1.67-6.63, *P*=0.001 and aHR: 1.99, 95%CI: 1.02-3.89, *P*=0.04, respectively) (Table 3). Kaplan-Meier curves of all DFS patients, stratified by number of liver metastases, are shown (Fig 1A, B).

On univariate analysis, male sex, PCI >12 and CCR-1 resection were identified as prognostic factors for lower OS (Table 4). On multivariate analysis, PCI >12 was the only independent predictive factor of decreased OS (aHR 1.67, 95%CI 1.05–2.66, *P*=0.03). Kaplan-Meier curves for OS of all patients and stratified by PCI are shown (Fig 2A,B).

DISCUSSION

Management of patients with liver and peritoneal metastases from CRC has undergone major improvements. Whereas OS did not exceed one year with classic systemic CTH based on 5-FU,[36] oxaliplatin and targeted therapies such as anti-angiogenic or anti-EGFR antibodies (for wild type *RAS*) have allowed extending the OS up to 2 years in selected patients.[4,22,37] Recent studies have suggested that resection of liver and peritoneal metastases combined with HIPEC may increase OS up to 3 years, despite an increased risk of morbidity.[21,25] Recently, the combination of three systemic CTH agents (FOLFOXIRI regimen) have shown an increase OS in metastatic CRC compared to classical CTH regimens[3] for several months and achieve OS similar to extensive surgery. However, the incidence of serious adverse events in patients treated with FOLFOXIRI plus bevacizumab is up to 20.4%,[38] which is comparable to perioperative morbidity of major surgery (5-28% for CRS and HIPEC[16] and 5-20% for extended liver resection).[39-41]

This multicenter study is the largest series of selected patients with PC from CRC and simultaneous LM treated with LR, CRS and HIPEC. The present study shows that aggressive management of multi-metastatic CRC is feasible with an acceptable morbidity rate of 15% and no postoperative mortality. These morbidity and mortality rates are consistent with those reported after LM resection and similar to PC treatment alone.[25,37,42] We believe these low rates of morbidity were achieved by careful selection of patients: if LM required only minor LR, this was usually performed at same time as CRS + HIPEC. However, if LM required complex or major LR, especially on parenchyma injured by preoperative CTH, LM resections were mostly delayed to a second procedure. Interestingly, despite this approach, a major LR was not associated with an increased complication rate. However, the authors suggest that this concept of a two-staged procedure, already used in complex abdominal and liver surgeries, may represent a valuable tool to reduce patient morbidity and mortality rates. Despite this

cautionary note, in light of the present data, it should still be concluded that when both LM and PC are resectable, aggressive surgery improves chances for selected patients to achieve better OS rates.

Despite the encouraging OS rates in our study, almost 64% of patients recurred within the first postoperative year. We also found a significant association between reduced DFS and severe complication occurrences, which is in line with previous studies.[43] However, we also found that OS, surprisingly, was not related to severe morbidity as an earlier recurrence was assumed to be related to shorter survival. However, *Varban et al*. reported similar results.[37] Thus, these data suggest that careful selection of patients, less likely to experience severe postoperative complications, may allow for improved DFS. Unfortunately, we did not identify any risk factors associated with perioperative severe morbidity. Our study sampling is the likely explanation for this. Nevertheless, this association of complications and survival should be attentively considered when selecting any patient with LM and PM for surgery.

The promising long-term results of LM surgery from CRC over the past decade and recent trends towards increasing surgical aggressiveness (as illustrated by iterative resections of LM[44]) formed the rationale for the surgical management of both LM and PC, given that CRS + HIPEC may also achieve excellent outcomes. Some series suggest that relatively long survival may be achieved with aggressive management, including the simultaneous resection of LM and PC.[21,26,45] Previous findings have been confirmed in the present study showing that OS is significantly prolonged: up to 60 months in selected cases. However, a recent meta-analysis of *de Cuba et al*. showed that patients with synchronous PC and LM of CRC seemed to fare less well when compared to patients with isolated PC (pooled HR=1.24, 95%CI 0.96–1.60).[22] Despite this, the authors also showed a tendency towards better OS in carefully selected patients with PC and LM who were treated with curative resection of both sites plus HIPEC compared to treatment with modern systemic CTH alone.

The PCI is considered the most widely used tool to evaluate disease extent in primary or digestive carcinomatosis.[16,46-48] Increased PCI is also recognized as an independent prognostic indicator for long-term outcomes in patients with PC from CRC[49] and an inverse linear relationship between point rise in PCI and OS has been demonstrated.[50] Similarly, we found that PCI was an independent prognostic factor for OS in patients undergoing simultaneous resection of LM and PC. Whilst PCI also influences the likelihood of complete cytoréduction,[11,51] in the present analysis, incomplete cytoreduction only impacted DFS independently (not OS). This finding is not at odds with the literature, as previous studies have shown that a large volume of disease is associated with poor long-term survival even if complete cytoreduction is achieved.[47,49]

The concomitant presence of LM is a poor prognostic factor compared to patients with PC alone.[16,22,45] Furthermore, *Elias et al*. reported that completely resected LM during CRS remained a negative prognostic factor for patients with PC of CRC.[52] However, *Maggiori et al*. suggested that in LM and PC, prolonged survival may still be achieved in highly selected patients with limited peritoneal disease (PCI <12).[26] In our study, we also found that a PCI>12 was associated with a poor OS in both uni and multivariate analysis. Therefore, PCI itself could be a useful criterion for patient selection. In line with *de Cuba et al*., we also believe that, based on current data, there is no evidence to support an exclusion of patients with PC and LM from aggressive, potentially curative, treatment.[22] However, an accurate, extensive preoperative evaluation is mandatory before surgery, and thus a diagnostic laparoscopy may prove useful in avoiding unnecessary surgery in high PCI and simultaneous CRC LM patients.[53]

Despite analyzing prospectively maintained databases, this study is limited by its retrospective design. Furthermore, great heterogeneity in patient selection and operative techniques may have compromised our findings. However, despite these short-comings, this

study represents the largest multicenter series, to date, and the data provided herein forms a basis for future prospective trials.

CONCLUSION

This multicenter study shows that concomitant treatment of CRC dissemination in the liver and peritoneum, confirms the feasibility of combined hepatectomy, CRS and HIPEC, in selected patients with LM and PC of CRC, resulting in 32-month median OS with limited morbidity. The present data supports this aggressive treatment strategy; the exact timing of these individual complex treatment steps remains unknown. Future studies assessing the feasibility of this surgical approach in a prospective, randomized setting (as well as further studies elucidating how best to control disease progression following disease recurrence) would be helpful in furthering the care of CRC patients with advanced stage of disease.

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Figures legends

Figure 1.

A. Disease free survival of 130 patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy combined with liver resection for simultaneous liver and peritoneal metastases from colorectal cancer.
B. Prognostic impact of the number liver metastases (>3) on disease free survival of 130 patients (*P*=0.0001)

Figure 2.

A. Overall survival of 161 patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy combined with liver resection for simultaneous liver and peritoneal metastases from colorectal cancer.
B. Prognostic impact of the peritoneal carcinomatosis index (>12) on overall survival of 151 patients (*P*=0.008)

Table 1. Background and operative characteristics of the study population

Characteristics of population	No. of Patients	(%) of Patients
Demographics		
Sex		
Male	77	(47.8)
Female	84	(52.2)
Age		
<60	88	(54.7)
≥60 yr	73	(45.3)
Primary tumor		
Site		
Rectum	10	(6.2)
Right Colon	47	(29.2)
Left Colon	96	(59.6)
NR	8	(5.0)
Differentiation		
Good	52	(32.3)
Moderate	70	(43.5)
Poor	10	(6.2)
NR	29	(18.0)
Nodal Status		
Positive	104	(64.6)
Negative	37	(23.0)
NR	20	(12.4)
Chemotherapy		
Pre-HIPEC systemic CTH	138	
Yes	23	(85.7)
No		(14.3)

Perionteal Carcinomatosis		
PCI ≤12 >12 NR	 106 50 5	 (65.8) (31.1) (3.1)
Completeness of Cytoreduction CCR-0 CCR-1 CCR-2	 144 14 3	 (89.4) (8.7) (1.9)
Type of HIPEC Oxaliplatin MMC	 103 58	 (64.0) (36.0)
Liver Metastases		
No. of Liver metastases ≤3 >3 NR	 130 16 15	 (80.7) (9.9) (9.3)
Type of liver resection Major (≥3 segments) Minor (<3 segments) NR	 28 117 16	 (17.4) (72.7) (9.9)
Time of liver resection Simultaneous Delayed or two-stage NR	 133 13 15	 (82.6) (8.1) (9.3)
Synchronous ovarian metastases Yes No NR	 10 136 15	 (6.2) (84.5) (9.3)

Surgical data		
Postoperative complications Minor Major	 137 24	 (85.1) (14.9)
Second surgical procedure Yes No NR	 13 141 7	 (8.1) (87.6) (4.3)
Mean hospital stay duration Simultaneous CRS and LR Two-staged surgery	23 (SD:12.5, range 8-87) 24 (SD: 13.1, range 9-87) 15 (SD: 5.8, range 8-26)	

Table 2. Predictive Factors for severe post-operative severe complications (DINDO III or IV) on Univariate Analysis

Variables	No. Patients (N = 161)	Univariate Analysis	
		OR (95% CI)	*P*
Male sex	77/161	0.74 (0.31-1.77)	0.50
Age >60 yr	73/161	0.98 (0.41-2.33)	0.96
Primary Site			
Rectum	10/153	0.61 (0.07-5.07)	0.65
Right Colon	47/153	0.52 (0.21-1.28)	0.16
Left Colon	96/153	1.67 (0.22-1.55)	0.28
Primary differentiation			
Good	52/132	1.91 (0.72-5.06)	0.20
Moderate	70/132	0.61 (0.23-1.63)	0.32
Poor	10/132	0.65 (0.08-5.43)	0.69
N+ status	104/141	0.78 (0.29-2.08)	0.62
Pre-HIPEC systemic CTH	138/161	0.84 (0.23-3.07)	0.79
PCI > 12/39	50/156	1.17 (0.45-3.04)	0.74
CCR-1*	14/158	0.63 (0.16-2.43)	0.50
HIPEC with Oxaliplatin	103/161	0.93 (0.38-2.28)	0.87
Liver metastases >3	16/146	1.35 (0.35-5.18)	0.66
Major liver resection	28/144	1.74 (0.61-4.95)	0.30
Simultaneous liver resection	133/146	0.44 (0.06-3.60)	0.45

The values given are number (%). Variables in bold are statistically significant ($P < 0.05$). CTH indicates chemotherapy. OR indicates odds ratio. *Three CCR-2 patients were excluded from this analyze.

Table 3. Prognostic Factors for Disease Free Survival on Univariate and Multivariate Analyses

Variables	No. Patients (N = 141)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*P*	aHR (95% CI)	*P*
Male sex	70/141	0.94 (0.64-1.37)	0.74		
Age >60 yr	63/141	1.35 (0.92-1.98)	0.13		
Primary Site					
Rectum	9/136	0.74 (0.37-1.47)	0.39		
Right Colon	44/136	0.78 (0.52-1.16)	0.22		
Left Colon	83/136	1.38 (0.93-2.02)	0.11		
Primary differentiation					
Good	47/120	1.24 (0.82-1.88)	0.32		
Moderate	63/120	0.79 (0.52-1.18)	0.25		
Poor	10/120	1.11 (0.54-2.29)	0.78		
N+ status	93/125	0.62 (0.37-1.05)	0.08	1.47 (0.85-2.56)	0.17
Pre-HIPEC systemic CTH	125/141	0.57 (0.32-1.02)	0.06	1.77 (0.84-3.74)	0.14
PCI > 12/39	45/138	0.77 (0.52-1.14)	0.19		
CCR-1*	12/140	0.59 (0.33-1.07)	0.08	1.99 (1.02-3.89)	***0.04***
HIPEC with Oxaliplatin	94/141	1.17 (0.78-1.73)	0.45		
Liver metastases >3	15/130	3.04 (1.59-5.84)	***0.001***	3.32 (1.67-6.63)	***0.001***
Major liver resection	25/130	1.24 (0.76-2.03)	0.39		
Simultaneous liver resection	119/130	1.14 (0.55-2.36)	0.72		
Severe postoperative complication (grade III or IV)	21/141	1.82 (1.01-3.25)	***0.04***	1.69 (0.93-3.06)	0.09

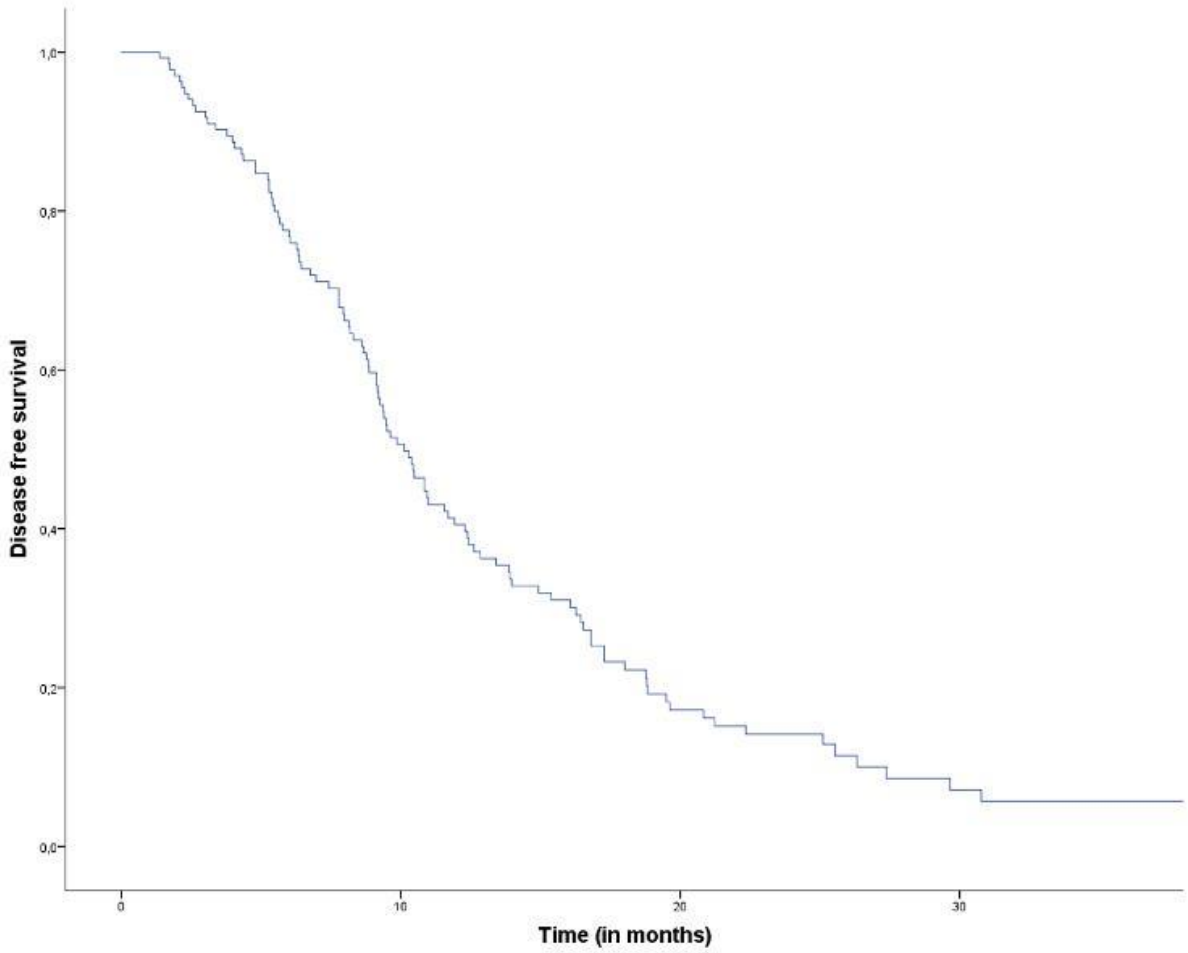
For each variable, the number of patients is reported to the number of patients with data and DFS available. Variables in bold are statistically significant ($P < 0.05$). CTH indicates chemotherapy. HR indicates hazard ratio. aHR indicates adjusted hazard ratio.
*Three CCR-2 patients were excluded from this analyze.

Table 4. Prognostic Factors for Overall Survival on Univariate and Multivariate Analysis

Variables	No. Patients (N = 156)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*P*	aHR (95% CI)	*P*
Male sex	75/156	1.75 (1.11-2.76)	***0.02***	1.60 (0.99-2.58)	0.06
Age >60 yr	70/156	0.77 (0.49-1.20)	0.25		
Primary Site					
Rectum	10/148	1.51 (0.69-3.32)	0.30		
Right Colon	45/148	0.82 (0.49-1.35)	0.43		
Left Colon	93/148	1.06 (0.66-1.70)	0.81		
Primary differentiation					
Good	51/129	1.11 (0.69-1.81)	0.66		
Moderate	67/129	0.88 (0.54-1.43)	0.61		
Poor	10/129	1.36 (0.54-3.41)	0.52		
N+ status	100/136	1.52 (0.83-2.81)	0.18		
Pre-HIPEC systemic CTH	133/156	1.14 (0.66-1.98)	0.64		
PCI > 12/39	50/151	1.85 (1.18-2.92)	***0.008***	1.67 (1.05-2.66)	***0.03***
CCR-1*	14/153	2.04 (1.11-3.72)	***0.02***	1.52 (0.79-2.95)	0.21
HIPEC with Oxaliplatin	99/156	0.85 (0.54-1.34)	0.50		
Liver metastases >3	16/141	1.07 (0.49-2.36)	0.86		
Major liver resection	28/141	0.83 (0.45-1.55)	0.56		
Simultaneous liver resection	129/141	0.64 (0.23-1.77)	0.39		
Severe postoperative complication (DINDO III or IV)	24/156	1.00 (0.52-1.95)	1.00		

For each variable, the number of patients is reported to the number of patients with data and OS available. Variables in bold are statistically significant ($P < 0.05$). CTH indicates chemotherapy. HR indicates hazard ratio. aHR indicates adjusted hazard ratio.
*Three CCR-2 patients were excluded from this analyze.

Figure


Disease free survival
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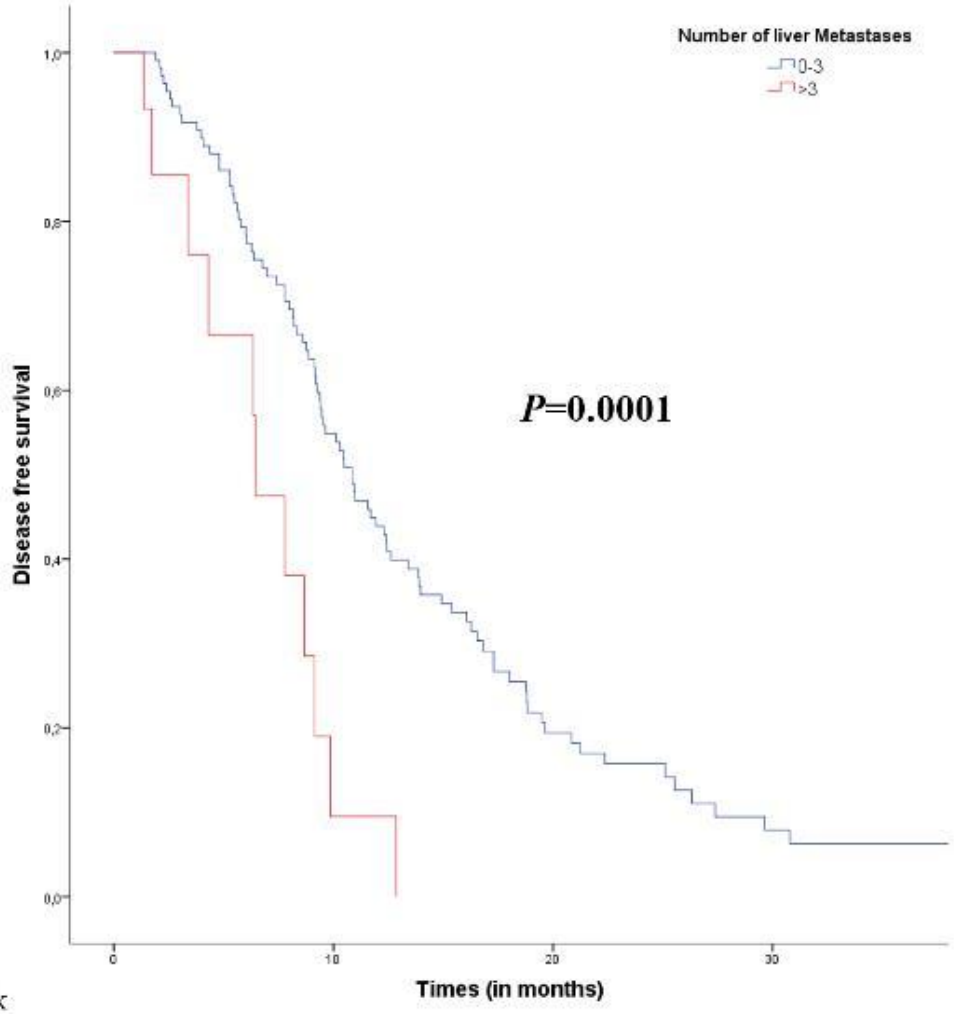
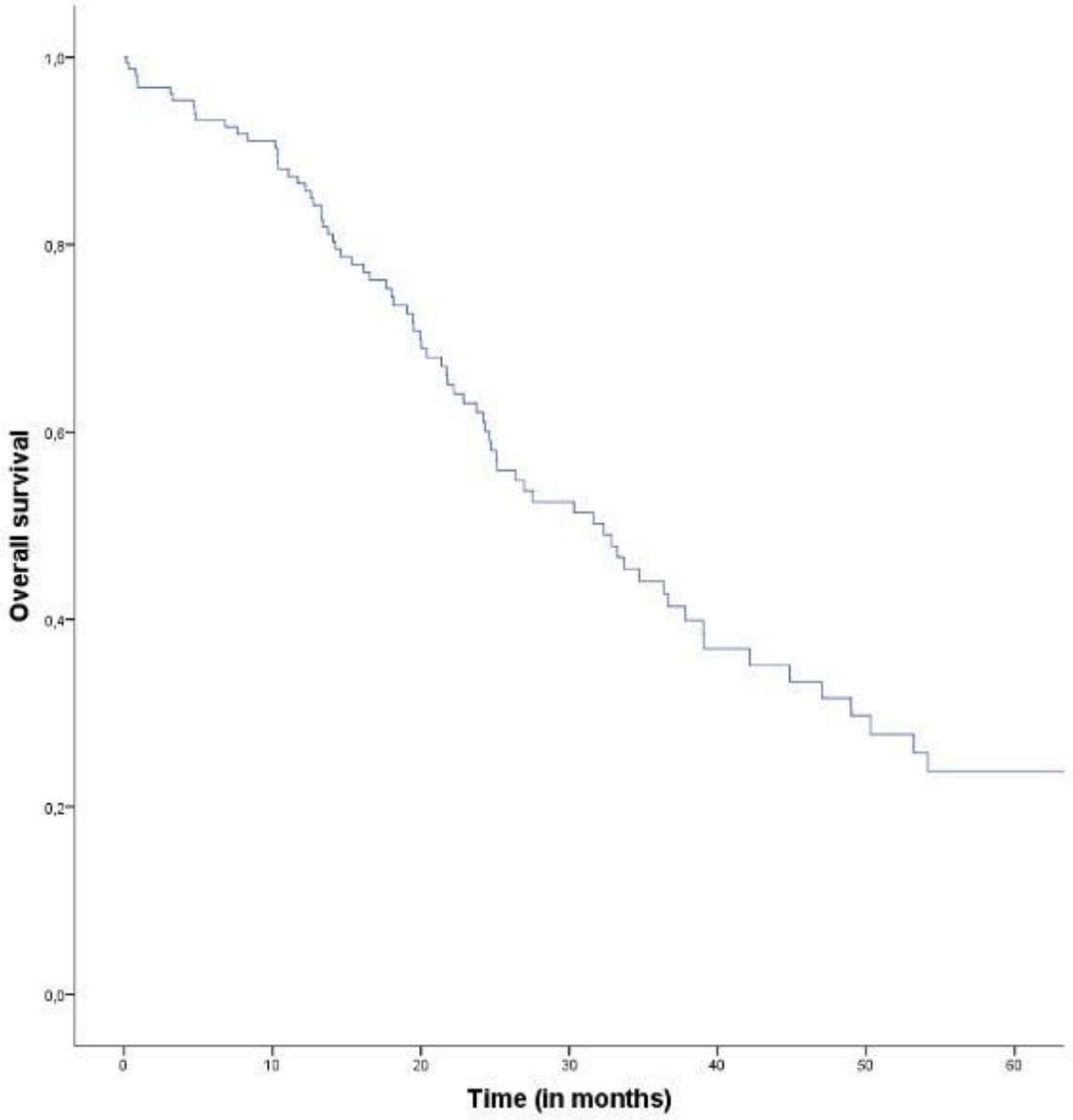
0,8
0,6
0,4
0,2
0,0
0
10
20
30
Time (in months)

Figure

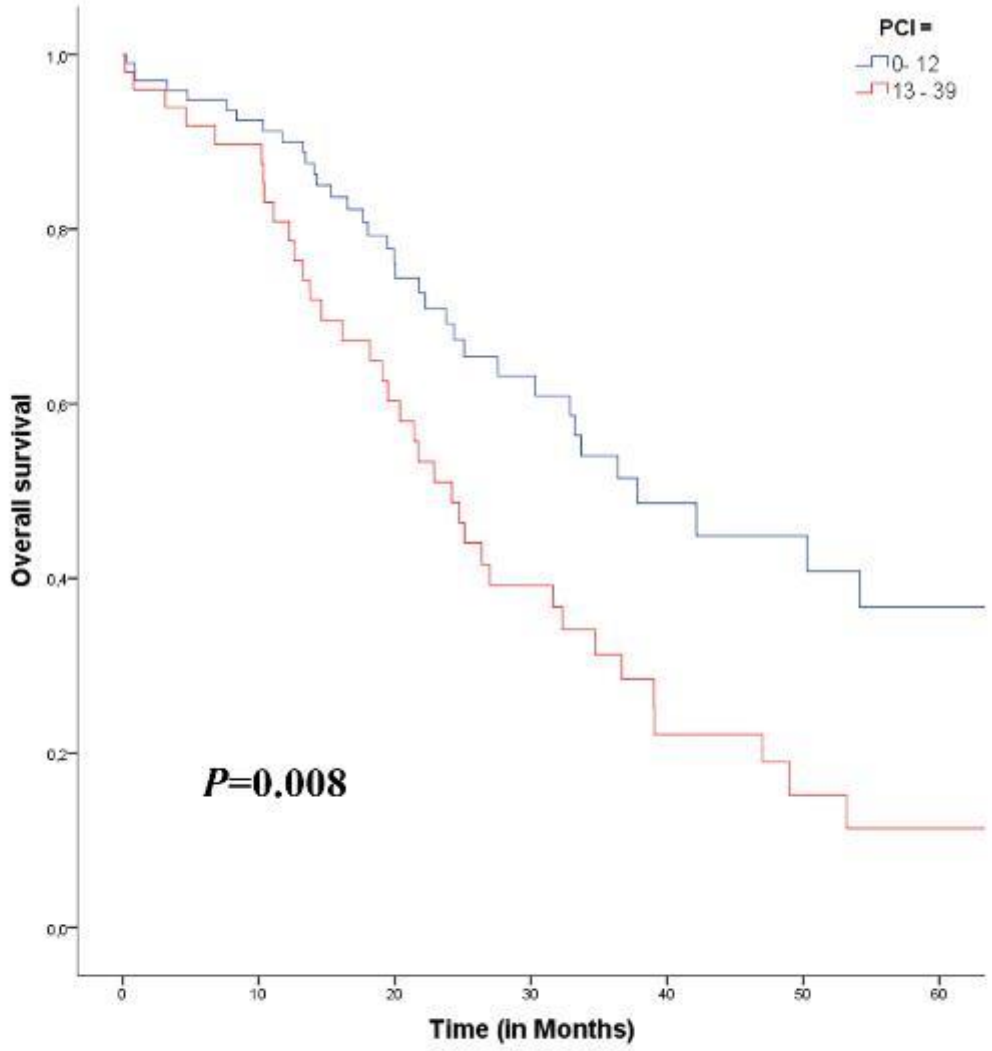


Patients at risk				
0-3 liver metastases	115	55	16	5
> 3 liver metastases	15	1	0	0

Figure


Overall survival
1,0
0,8
0,6
0,4
0,2
0,0
0
10
20
30
40
50
60
Time (in months)

Figure



Patients at risk							
PCI = 0 to 12	101	76	46	28	16	11	7
PCI = 13 to 39	50	41	26	16	7	4	3

Title page

C. CONCLUSION and CLINICAL PROSPECTS

In this thesis, there are three principal questions that we wanted to address:

1. Is it possible to treat patients with synchronous liver and peritoneal metastases, individually accessible, to a surgical treatment with curative intent?

2. In cases of aggressive surgical treatment, what order of surgery should be chosen?

3. In cases of two-step surgery, what is the effect of liver surgery on peritoneal carcinomatosis?

To answer question number one, firstly, the literature data was analyzed (21 studies [3,27-32,35-37,132-143] and 6 reviews [33,35,135,144-146] on this subject have been published to date). Among these, 16 retrospective series exist comparing patients with PC alone *vs* PC plus LM undergoing CRS with HIPEC and or not liver resection: 7 studies note negative impact of concomitant presence of both metastatic sites, 9 studies show no difference. It is clear that most have been pauci-centric studies, often retrospectives, with small samplings of patients, because this aggressive surgical treatment can only be proposed to a very selected number of patients.

However, the studies suggest that this aggressive surgery, with curative intent, is possible. Even though this surgical treatment has been contraindicated for a long time, it currently seems feasible with an acceptable morbidity/mortality rate found at expert centers in peritoneal diseases. Subsequently, we have carried out a multicentric study, motivated by the fruitful Franco-Italian cotutelle between two expert centers in peritoneal diseases (Lariboisiere, Paris and La Sapienza, Rome). A multicentric database, with an increase in the number of patients, was the first result of this cooperation. Our preliminary clinical study of

161 patients, having undergone combined hepatic and peritoneal surgery, shows the feasibility of complex liver and peritoneal surgery. In our study this surgery is associated with a low rate of morbidity, similar to that of major abdominal surgery. The OS of these patients was greater than that OS achieved with systemic CT treatment alone [33,145]. Nevertheless, it should be emphasized, the DFS of our series was poor (10 months: range 0.3 to 56 months); 87.9% of the patients in our study relapsed with recurrences occurring during the first year. A careful selection of patients becomes fundamental if we want to propose an aggressive, curative treatment. Indeed, several factors come into play in this situation which determines the complexity of clinical cases. Firstly, the intrinsic variability related to multisite, hepatic and peritoneal metastases. Although, in our study, we have listed patients able to undergo surgical treatment concerning both metastatic sites, the treatments performed could be very different depending on the localization and, above all, the extent of the disease in the two metastatic sites. The second factor was the variety of chemotherapy used and when it was administered (pre- or post-operatively) and, in some patients, between the hepatic and peritoneal surgeries. The impact of chemotherapy on the OS of these patients is not negligible. Unfortunately, the sampling period was very wide (about 20 years) and much data (concerning the type and timing of chemotherapy administered) was missing. These facts, in our opinion, represent the limits of this study. We have envisaged three possible solutions. Firstly, to improve the patient selection process, in order to collect relevant data concerning the impact of systemic chemotherapy (even if the numbers of patients are reduced). Secondly, to increase the number of patients, through international collaboration, and then carry out sub-group analyzes (types of chemotherapy used, ages of treated, choice of surgery performed first, etc.). The third possibility is to compare data among patients who have received curative surgical treatment and those treated by systemic chemotherapy. *De Cuba et al.* showed, in

their review, that the surgical treatment of liver and peritoneal surgery is associated with a high OS compared to modern systemic chemotherapy [33].

These studies encouraged me to take a short outward mobility where I had the opportunity to work in the Peritonectomy Unit (Kogarah Center, Australia), which is part of the Peritoneal Surface Oncology Group International (PSOGI). The results obtained (under international cooperation) will lead to a prospective database to which a network of expert centers in peritoneal diseases of the PSOGI and BIG-RENAPE (French national clinico-biological database of peritoneal diseases) will participate.

Currently underway:

a) International Partnership between the centers of the PSOGI, which brings international surgical centers, specializing in the pathology of the peritoneum, together. The new prospective database has already collected 423 cases of combined surgery with hepatectomy and peritoneal resection with HIPEC. The outcomes results will be presented at the PSOGI Congress in Paris at September, 8-11 2018, and will be the subject of a first author publication.

b) A propensity score analysis (PSM), of two populations of patients operated on by two different surgical teams to evaluate the morbi-mortality risk of a combined surgical approach: (I) a series of patients undergoing resection of isolated LM (Rome cohort); (II) a series of patients undergoing hepatic resection associated with peritoneal metastases resection (Paris cohort).

In cases of major and complex surgery, carried out in two stages, the proper surgical sequence, between hepatic surgery and peritoneal, remains debatable. No literature offers data helping to make a clear choice. Our animal model was conceived to address to this question.

In our study, to mimic the human situation of concomitant liver resection and peritoneum metastases, and confirm the effects of liver regeneration on peritoneal metastasis growth, we have developed a reproducible animal model with limited peritoneal carcinomatosis and liver regeneration after major hepatectomy. This model was constructed with immune competent mice to mimic the natural human immunity. The model is not perfectly reproducible. Indeed, at each concentration, at least 2 out of 5 mice have a PCI greater than expected value. However, the mean values of each group were respected and allow us to follow the natural evolution of the peritoneal carcinomatosis. For ethical reason we preferred euthanize only 5 mice per group.

The pro-metastatic role of hepatic surgery, and the consequent effects of liver regeneration, are a phenomena noted in basic and clinical studies of the literature [123,147-149]. Therefore, it has been postulated that hepatic surgery, if performed as a first procedure, could stimulate tumor growth in PC. That is because the production of normally contiguous growth factors towards the *restitutio ad integrum* of the hepatic parenchyma is diverted towards the proliferation of tumor cells at the peritoneal level.

Experimental results were obtained on an animal model that we defined for our specific purposes. In our model of major liver surgery, we have analyzed the role of endothelial and hematopoietic bone marrow derived cells on the changes of the pre-metastatic niche as well as on the promotion of the metastatic process. We have found that the concentration rate of tumoral and non-tumoral growth factors and circulating endothelial progenitors, which significantly increase after hepatectomy to assist hepatic regeneration and angiogenesis, play a crucial role in tumor growth by stimulating tumor cells of carcinomatosis tumor cells. Our experimental results confirmed the functional and structural role of the endothelial BMDC.

These cells stimulate the secretion of pro-angiogenic factors to promote the changes of the microenvironment as well as the promotion of a new tumoral vascular network. In our study, the presence, and tumor growth, of PC was monitored, non-invasively, with bioluminescence without sacrificing the mouse. Furthermore, we have established a reproducible technique of non-invasive evaluation of tumoral angiogenesis by Echo-Doppler. Our results confirm the pro-metastatic effect of hepatic surgery on the PC growth. We have also studied the effect of surgery, without liver injury, on the PC growth and we have observed smaller increase of peritoneal PCI compared to the effect after liver surgery. This result confirms the crucial role of the liver regeneration on the tumoral hepatic and extra-hepatic growth. Further analysis, currently in progress, should also identify the mechanisms involved in this process, must importantly the role played by natural and post-operative immunity.

In clinical practice, what is involved concerning the choice of the surgical sequence can be summarized by the following:

1) Carcinological aspects:

- If choosing to start by cytoreduction surgery, we treat the metastatic site in light of a poor prognosis. However, *Cao et al.,* who analyzed patients with metastatic colorectal cancer, showed that there was no difference found between patients undergoing hepatectomy for isolated LM *versus* patients undergoing peritonectomy with HIPEC for isolated PC (37 months for both groups) [150].

- In other cases, after major hepatectomy and under pro-metastatic effects, the PC can rapidly become non-resecable, as evidenced by our murine model of hepatic regeneration. In our department, a pilot study carried out on few patients (n=4) treated with primary hepatectomy, showed a rapid evolution on the PC which quickly became non-resectable.

2) Technical aspects:

- If choosing to start with cytoreduction surgery, we carry out surgery associated with a potentially higher morbidity rate.

- In other cases, the impact of surgery on tumor growth could be lessened in cases of laparoscopy hepatectomy or in case of use of an anti-adhesion barrier film to limit postoperative adhesions [38].

More studies are necessary to establish the best surgical strategy. However, in light of these findings, we can affirm that in a situation with concomitant presence of liver and peritoneal metastases, when combined surgery is not possible, carrying out peritoneal surgery first is associated with lower risk of tumoral growth compared to carrying out liver surgery first. Currently, the peritoneal surgery first represents our therapeutic choice in case of synchronous liver and peritoneal metastases when the combined surgery is not possible.

Future researches will assist these findings. These include:

1) Testing anti-angiogenic drugs in our animal model to establish the role of pro-angiogenic factors produced during liver regeneration after major hepatectomy on the metastatic process and tumoral angiogenesis.

   In metastatic colorectal cancer, several biological agents, such as anti-angiogenic drugs (Bevacizumab, Aflibercept) [9,12] or anti-EGFR (Cetuximab or Panitumumab) [11], are added to the chemotherapy regimen to increase the response to the treatment. In our model we have seen that tumor neo-angiogenesis participates in several stages of the metastatic process, on the carcinomatosis growth as well as on the hepatic regeneration process after major hepatectomy. Contrary to hepatic metastases, the

effect of antiangiogenics in PC is not still clear. It is known that the preoperative use of anti-angiogenics is responsible for an increase in post-operative morbidity/mortality after cytoreduction surgery and HIPEC [151]. Nevertheless, studies by *Glehen et al.* were centered on the interest of pre-operative anti-angiogenics agents administration in cytoreductive surgery and HIPEC with results that show that lower IP VEGF level prior to surgery is associated with improved OS [152]. The use of preoperative intraperitoneal Bevacizumab for patients with a heavy disease load should be considered, especially in colorectal cancers. In the further, it would be interesting to test the anti-angiogenics molecules after major hepatectomy in the mouse to check if the pro-angiogenic and pro-metastatic effects of the hepatic regeneration after hepatectomy are antagonized by the anti-angiogenics. In our model of PC and hepatic regeneration, testing with new treatments, such as anti-angiogenics and immunomodulatory drugs, should, therefore, decrease the risk of peritoneal metastases growth by reducing the concentration of circulating EPC and VEGF levels.

2) Identifying the role of post-operative immunosuppression in our animal model.

Actually, Immunotherapy is a major focus in basic and clinical research. The recent studies of *Voron et al.,* studying the interactions between the immune system and the angiogenetic factors, show that VEGF-A produced by tumors, plays a key role in the development of an immunosuppressive microenvironment and enhances expression of PD-1 and other inhibitory checkpoints involved in CD8+ T cell exhaustion [153,154]. Based on these immunomodulatory properties, in our preclinical model, we could test the effects of the immunosuppression of the antiangiogenic molecules as well as find an efficient combination among anti-angiogenic drugs associated with immunotherapeutic molecules.

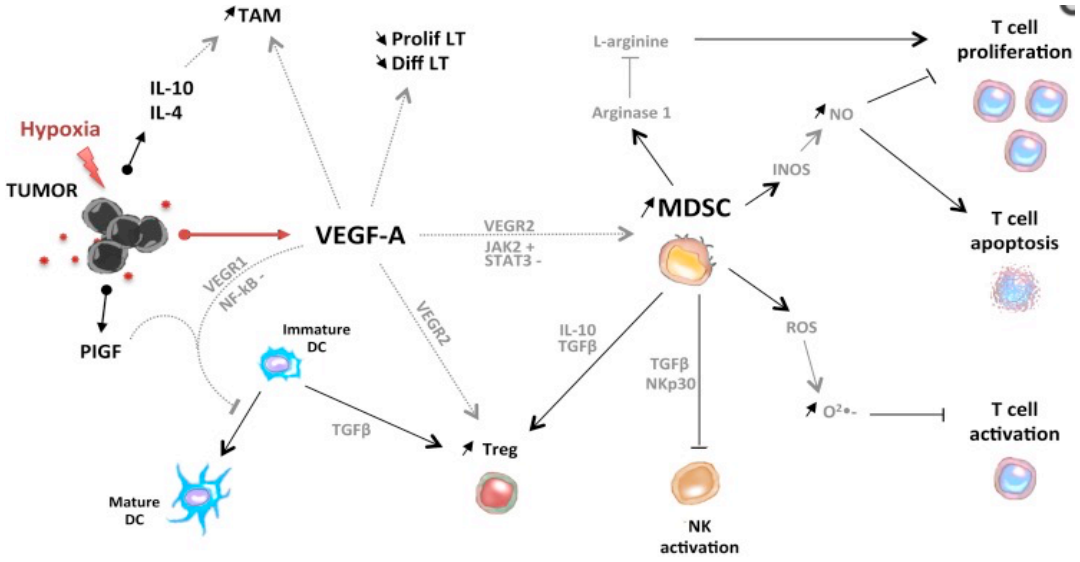


Fig. 9: Pro-angiogenic factors induce the development of an immunosuppressive state in tumors (adapted from T. Voron et al. Control of the Immune Response by Pro-Angiogenic Factors. Front Oncol. 2014; 4: 70).

3) The development of a reproducible model of limited peritoneal and liver metastasis to test the inverse effect of the peritoneal cytoreduction on the liver metastasis growth to confirm the ideal surgical timing in case of two-stage surgery.

Different animal models for liver metastases with immunocompetent rodents have been developed, with various results, to test new treatments: subcutaneous or orthotopic cecal wall grafts, intrahepatic, intrasplenic or intraportal injections [125,149]. The principal problems of the models are their reproducibility, but also their efficiency, the maintenance of the metastatic potential of the tumor cells and the mortality rates due to the operation or anesthesia. For our model, to test the effects of the peritoneal surgery in the liver metastasis growth, we needed to develop a limited liver metastatic model, preferably localized in the same hepatic lobe. A possible solution could be to test the ALPPS (Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy) technique [155] so as to divide the liver parenchyma

before the implant of liver metastases. In mouse, is a complex surgical model probably associated with a high mortality rate.

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ANNEXES

- **Annex 3: Article 3,** Peritoneoscopy evaluation in peritoneal carcinomatosis
- **Annex 4: Article 4,** Cytoreductive Surgery and HIPEC in Elderly
- **Annex 5: Article 5,** Echo Doppler evaluation in peritoneal carcinomatosis of PMP
- **Annex 6: Article 6,** Neoadjuvant bidirectional chemotherapy **(**currently being edited by the authors)

INTRODUCTION

Following is a short selection of works carried out over the last few years, its aim being to understand the development, and the mechanisms of progression, of peritoneal carcinomatosis of digestive origin (as well as the study of possible therapeutic approaches).

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Single-incision flexible endoscopy (SIFE) for detection and staging of peritoneal carcinomatosis

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Abstract
Objective To show the feasibility and the safety of peritoneal carcinomatosis (PC) evaluation by single-incision flexible endoscopy (SIFE) and to compare it to single-incision rigid endoscopy (SIRE).
Background Direct peritoneal visualization, either by laparotomy or laparoscopy, continues to be the gold standard in diagnosing PC. We reported, in animal study, that combining single-incision laparoscopic surgery and flexible endoscopy improved evaluation of the peritoneal cavity in a live porcine model and in four human cadavers.
Methods Patients, undergoing surgical exploration for diagnosis and staging of PC, were included in a prospective study. Using a superiority design a sample size of 47 patients was determined. Through a single incision, a standardized peritoneoscopy was conducted with rigid (SIRE) and with flexible endoscope (SIFE). Primary outcome was the access success rates for the 13 regions of the Peritoneal Carcinomatosis Index (PCI).
Results Overall access to the 13 regions of PCI was successful in 83 % of the cases with SIRE and in 91.1 % with SIFE ($p < 10^{-10}$). SIFE access rates were superior to SIREs' in the regions: R1 (87.2 vs. 61.7 %, $p = 0.002$), R2 (87.2 vs. 66 %, $p = 0.004$), R3 (85.1 vs. 59.6 %, $p = 0.001$) and R6 (80.9 vs. 61.7 %, $p = 0.008$). The mean PCI was higher ($p < 10^4$) with SIFE 12.77 ($\pm$11.97) than with SIRE 11.77 ($\pm$11.63).
Conclusion This prospective, comparative study shows that SIFE was significantly superior to SIRE in the exploration of some difficult-to-access peritoneal areas, located in regions 1, 2, 3 and 6. These two minimally invasive staging procedures are safe, feasible and have to be seen as complementary rather than competing.

Keywords Minimally invasive surgery · Single-incision laparoscopic surgery · Peritoneal carcinomatosis · Peritoneoscopy · Laparoendoscopic single-site surgery

Peritoneal carcinomatosis (PC) was considered a terminal condition with a merely palliative treatment, which included only supportive care, palliative surgery and the best systemic chemotherapy. Since the birth of the concept of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), the management of PC changed dramatically. In fact, it has been proven that CRS/HIPEC improves survival in patients with PC of colorectal origin [1]. CRS with HIPEC framed by systemic chemotherapy is now proposed with curative intention in selected cases of limited PC from colonic and ovarian origin [2–4].

The Achilles heel of CRS and HIPEC is appropriate patient selection, in order to prevent from excessive morbidity and mortality. Among the criteria of patient's

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selection, the evaluation of the extension of the peritoneal disease through the Peritoneal Carcinomatosis Index (PCI) is one of the most important [5]. The PCI is an independent prognostic factor for survival. The lower the PCI, the better the prognosis maybe also due to the fact that a complete cytoreduction becomes more likely [6].

An accurate evaluation of the PCI is therefore required in order to select the patients eligible for CRS and HIPEC. Current imaging methods are not sensitive enough for the diagnosis and staging of limited PC and often do not detect small tumor nodules [7].

Computed tomography scan, which remains the standard imaging modality in the assessment of PC [8], misses 30–45 % of peritoneal nodules or lesions, in particular if these are smaller than 5 mm [9, 10]. Thus, the extent of PC is difficult to evaluate preoperatively, and precise evaluation is most often performed during surgical exploration [11]. Some institutions utilize laparoscopy for that purpose [12–15].

Due to the risk of tumoral spreading through the lateral ports into the abdominal wall muscles [16], we believe that the conventional triangle laparoscopy is not the most suitable option for the evaluation of PC.

It is true that the reported incidence of port site metastases in laparoscopic surgery has declined notably compared with early publications [17]. However, metastatic tumor seeding in surgical scars in the setting of PC in candidates for CRS/HIPEC has not been studied as much. In a recently published study, Nunez et al. [18] showed that one-third (34 %) of the patients with a history of laparoscopic procedure prior to CRS/HIPEC had port site metastases at the time of CRS/HIPEC. This rate reaches 42 %, if laparoscopy was performed for tumor staging purposes.

The occurrence of port site metastases can make the cytoreduction impossible, especially in some etiologies, such as mesothelioma [19]. Moreover, an extensive abdominal wall resection in order to achieve a complete cytoreduction increases significantly the morbidity of the procedure [20].

In our institution, all the peritoneal exploration procedures for diagnosis and staging of PC are performed via single-incision laparoscopic surgery. We called this procedure single-incision rigid endoscopy (SIRE).

We found, however, that the SIRE did not allow to properly explore the whole abdominal cavity. In fact, rigid endoscopy has some limitations in terms of ergonomic and lack of triangulation, due to the coaxial position of the instruments. Moreover, this procedure can be challenging, especially in those patients previously operated on.

We hypothesized that combining single-incision laparoscopic surgery and flexible endoscopy may overcome these pitfalls. We called this procedure single-incision flexible endoscopy (SIFE).

We, therefore, performed an animal study comparing flexible and rigid single-port peritoneoscopy [21]. A standardized exploration of the peritoneal cavity was conducted in a porcine model, using the two techniques, aiming to access 11 elective sites of PC. We found that the overall rate of access to target was significantly higher in SIFE than in SIRE, 98 and 87 %, respectively ($p < 0.001$). Based on these encouraging results, we tried to transpose this new technique to humans.

The aim of this study is to show the feasibility and the safety of the SIFE technique in clinical practice, than to evaluate its diagnostic impact through a comparison between this technique and the rigid endoscopy SIRE.

Materials and methods

This is a prospective study, and all the patients were systematically informed of the aim of the study. Institutional review board approval was obtained from the local ethics committee.

The study was carried out in the Department of Surgical Oncology in Lariboisière Hospital (Assistance Publique Hôpitaux de Paris), which is a tertiary care center for PC.

We included patients, with histologically proven malignant disease, who underwent surgical exploration for diagnosis and staging of PC. The indications were staging of a carcinomatosis already diagnosed with imaging (CT scan and MRI), restaging after neoadjuvant chemotherapy, restaging during follow-up in the case of dubious imaging and restaging after adjuvant chemotherapy.

Through a single incision, a standardized peritoneoscopy was conducted with a rigid optic (SIRE) and with a flexible endoscope (SIFE), in a random order and in a back-to-back manner (i.e., one technique right after the other during the same operation).

Access to peritoneal cavity

Under general anesthesia, and in a supine position, a 25-mm paraumbilical midline incision was made. A sponge-like SILS^{TM} port (Covidien France, Elancourt) was inserted through this incision. The SILS^{TM} port was connected to a standard autoregulated laparoscopic insufflator (Electronic CO_2 Endoflator; Karl Storz Endoscopy, Guyancourt, France) to create and maintain 12 mm Hg CO_2 pneumoperitoneum.

Single-incision rigid endoscopy (SIRE)

A 10-mm-diameter, 60-cm-long, 30° axial optic (27425 P; Karl Storz Endoscopy, Guyancourt, France) and two 5-mm rigid laparoscopic graspers were inserted through the

SILS™ port. A senior surgeon experienced in laparoscopy and in oncologic surgery performed all the SIRE procedures.

Single-incision flexible endoscopy (SIFE)

A 10.8-mm-diameter, 110-cm-long, Fujinon® gastroscope EG-490ZW5 (Fujifilm Medical Systems France, Montigny Le Bretec, France) was inserted through the SILS™ port. The endoscope distal tip could be deflected in four directions: 210° up, 90° down, 100° left, 100° right. If needed, two 5-mm rigid laparoscopic graspers were also inserted through the SILS™ port. Another senior surgeon with 3-year experience in endoscopy performed all the SIFE procedures.

Peritoneoscopy

Standardized exploration of the peritoneal cavity was conducted quadrant by quadrant using the two techniques in random order, aiming to access the 13 regions of PCI as described by Sugarbaker [22].

The procedures were only exploratory, and no extensive dissection was made. The viscerolysis was limited to the essential minimum to avoid iatrogenic lesions. For both techniques, and in order to facilitate access to the different regions when not reachable in supine position, the table was rolled laterally side to side possibly combined with Trendelenburg or anti-Trendelenburg position, up to 30°. These positions were often needed to adequately expose the pelvis and the diaphragmatic domes.

For each technique, we noted whether a complete exploration of each of the 13 regions was possible or not, and then, the PCI was calculated. Evaluation of access to the different regions was based on operators' consensus. An independent nurse scored the results for all the procedures.

Depending on the region explored, the exploration was considered successful if it allowed complete visualization of specific areas and anatomic structures:

- For the Region 0: The greater omentum and the transverse colon.
- For the Region 1: The superior surface of the right lobe of the liver and the under surface of the right hemi-diaphragm to the peritoneal reflection at the level of the coronary ligament of the liver.
- For Region 2: The left lobe of the liver, the falciform ligament, the lesser omentum and the hepatic hilum.
- For Region 3: The spleen, the anterior surface of the stomach and the under surface of the left hemi-diaphragm to the peritoneal reflection at the level of the phreno-splenic ligament.
- For Region 4: The descending colon and the left abdominal gutter.
- For Region 5: The sigmoid colon and the pelvic sidewall lateral to the sigmoid colon.
- For Region 6: The upper rectum, the Douglas pouch, the female internal genitalia with ovaries, tubes and uterus, and the bladder.
- For Region 7: The cecum, the appendix and the right pelvic sidewall.
- For Region 8: The ascending colon and the right abdominal gutter.
- For Region 9: The upper jejunum and its mesentery.
- For Region 10: The lower jejunum and its mesentery.
- For Region 11: The upper ileum and its mesentery.
- For Region 12: The lower ileum and its mesentery.

Outcome parameters and statistical analysis

Primary outcome parameters were the feasibility of the procedure and the access success rates for the 13 regions of the PCI. Evaluation of the successful access to these regions was based on operators' consensus.

Secondary outcomes were the safety of the procedure, the complications and the diagnostic impact defined as the difference in PCI between the two techniques.

For the primary endpoint, a superiority design was used to compare SIFE and SIRE. Using $\alpha = 0.05$ and $1-\beta = 0.8$, and assuming that SIFE has a sensitivity of at least 98 % and SIRE a sensitivity of 87 %, a sample size of at least 47 patients was determined.

Mc Nemar's test was used for comparison of qualitative data, and Student's t test for paired data was used for comparison of continuous variables.

Results

Between October 2009 and October 2012, 50 patients underwent surgical exploration for diagnosis and staging of PC in our institution. Among these patients, 3 were excluded from the study because of the impossibility to access the peritoneal cavity. In the remaining 47 cases, both SIRE and SIFE access to the peritoneal cavity was successfully achieved.

In 45 patients (95.74 %), the SILS™ port was inserted through a para-umbilical midline incision. The two other patients (4.26 %) underwent stoma closure at the same operative time. The lateral hole of the stoma was therefore used to introduce the SILS™ port.

Among the patients enrolled in this study, 25 were male and 22 female. The median age was 54 (range 25–76). The median weight was 68 kg (range 47–103). The median

Table 1 Patients and primary tumor characteristics

Gender	
Male	25 (53.2 %)
Female	22 (46.8 %)
Mean age ± SD (year)	53 ± 11.3
Mean weight ± SD (Kg)	68 ± 12.6
Mean size ± SD (m)	1.72 ± 0.09
Mean BMI ± SD (Kg/m^2)	23 ± 3.66
Previous surgical history *n* (%)	
None	12 (25.5 %)
Laparoscopy	3 (6.4 %)
One laparotomy	18 (38.3 %)
Two laparotomies	8 (17 %)
Three laparotomies or more	6 (6.4 %)
Primary tumor site *n* (%)	
Colorectal	24 (51.1 %)
Stomach	14 (29.8 %)
PMP[a]	3 (6.4 %)
Ovary	2 (4.3 %)
Unknown	2 (4.3 %)
Small bowel	1 (2.1 %)
Appendix	1 (2.1 %)

[a] Pseudomyxoma peritonei

BMI was 22.9 kg/m^2 (range 16.2–36.5). 74.5 % of the patients had previous abdominal surgery. The origin of the suspected carcinomatosis was mostly either colorectal (51.1 %) or gastric (29.8 %). The patients and tumors characteristics are summarized in Table 1.

Navigation into the peritoneal cavity was found to be easy in both techniques. Overall access to the 13 regions of PCI was successful in 83 % of the cases with SIRE and in 91.1 % with SIFE ($p < 10^{-10}$) (Table 2).

Both techniques showed similar access rates to the regions 0, 4, 5, 7, 8, 9, 10, 11 and 12. SIFE access rates were superior to SIREs' in the regions: R1 (87.2 vs. 61.7 %, $p = 0.002$), R2 (87.2 vs. 66 %, $p = 0.004$), R3 (85.1 vs. 59.6 %, $p = 0.001$) and R6 (80.9 vs. 61.7 %, $p = 0.008$).

There was no significant difference for successful access to the different regions of PCI for both SIFE and SIRE, between male and female, and between the different types of carcinomatosis. These rates were also independent from the number of previous laparotomies. The order of procedures (SIFE first or SIRE first) did not significantly influence the results.

The mean peritoneal score for the extent of the peritoneal seeding was significantly higher ($p < 10^{-4}$) with the SIFE procedure 12.77 than with the SIRE procedure 11.77.

The mean difference in PCI was 1 point. The PCI was the same in half of the cases. There was a difference of at least 2 points of PCI, in 25 % of the cases. The maximal difference noted was 5 points.

Patients eligible for HIPEC (PCI < 20) represented 66 % (31/47) of the patients. For this group, the PCI was significantly higher ($p = 0.0005$) with the SIFE procedure (7.10 ± 6.51) than with the SIRE procedure (6.16 ± 5.89). The results were similar for the remaining group of patients with a PCI ≥20, who represented 34 % (16/47) of the total. For this group, the PCI with the SIFE (27.06 ± 7.38) was also significantly higher ($p = 0.005$) than the PCI with the SIRE (25.56 ± 7.38).

Three patients (6.4 %) had an evaluation score of PCI <20 with the SIRE procedure and >20 with the SIFE procedure.

No postoperative mortality was observed. Postoperative complications occurred in two patients (4.3 %) and included an acute acalculus cholecystitis in one case and a postoperative pneumonia in the other. These two Grade II complications evolved well under medical treatment. The mean hospital stay was 2.8 days (range 2–6).

Discussion

This prospective study is the first to compare flexible and rigid endoscopic trans-umbilical exploration of the peritoneal cavity. We show that both techniques allow easy and

Table 2 Access rates to the different regions of peritoneal carcinomatosis by single-incision rigid endoscopy (SIRE) and single-incision flexible endoscopy (SIFE)

	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	Total
SIRE	46/47	29/47	31/47	28/47	43/47	41/47	29/47	42/47	44/47	41/47	47/47	43/47	43/47	507/611
	97.9 %	61.7 %	66 %	59.6 %	91.5 %	87.2 %	61.7 %	89.4 %	93.6 %	87.2 %	100 %	91.5 %	91.5 %	83 %
SIFE	46/47	41/47	41/47	40/47	45/47	42/47	38/47	43/47	45/47	43/47	47/47	43/47	43/47	557/611
	97.9 %	87.2 %	87.2 %	85.1 %	95.7 %	89.4 %	80.9 %	91.5 %	95.7 %	91.5 %	100 %	91.5 %	91.5 %	91.2 %
P[a]	NA[b]	0.001	0.004	0.001	0.48	1	0.008	1	1	0.48	NA[b]	NA[b]	NA[b]	$<10^{-11}$

[a] McNemar's Chi-squared test

[b] Not available—perfect concordance between the two variables

safe minimally invasive navigation into the peritoneal cavity and staging of PC.

Despite major advances in imaging technology in the last few years, the early and adequate detection of peritoneal dissemination remains challenging because of the great variety in size, morphology and location of the peritoneal lesions. Thus, the gold standard in diagnosing PC continues to be the direct peritoneal visualization, either by laparotomy or laparoscopy [11, 23].

Laparoscopic exploration of the abdomen supplements the information provided by the imaging techniques and enables direct visual assessment of peritoneal involvement. It is associated with less pain, shorter hospitalization and quicker time to recovery in comparison with laparotomy [8]. Valle and Garofalo [24] used laparoscopy to stage 97 cases of PC and achieved full laparoscopic PCI assessment in 96/97 cases, while only 2/96 cases were understaged. There was a good correlation between the open successive surgery data and the laparoscopic PCI. Pomel et al. [25] achieved complete cytoreduction in seven of the eight patients who were considered resectable by laparoscopy.

Despite these advantages, there are two major limitations associated with laparoscopy. First, it is technically challenging, especially in patients with extensive prior surgery. In fact, a complete and systematic exploration of the entire abdominal cavity and the direct palpation of the peritoneum are only possible with laparotomy [26].

The second major concern is the risk of port track seeding. In order to prevent this risk, some authors propose to place all laparoscopy trocars in the midline and to resect the scars at the time of cytoreduction [25]. In a recently published study, Nunez et al. [18] showed that 42 % of patients who underwent diagnostic laparoscopy for staging of PC developed port site metastases. This complication was an independent prognosis factor in patients with PC.

In our institution, because of the risk of malignant cells spread through the trocar tract, all the PC-staging procedures are performed via single-incision laparoscopy. This minimally invasive technique allows using three instruments through a single port. Several human series have demonstrated its feasibility, with low morbidity and mortality [27–29].

We found that this procedure was feasible in 94 % of the cases (47/50). Two of the three failures were cases of pseudomyxoma peritonei with extensive PCI. The third case was a patient with PC from colic cancer who had undergone 4 prior laparotomies. In all three cases, the access to the peritoneal cavity was impossible, even after a second upper midline laparotomy, because of thick cancerous adhesions between the small bowel loops and the abdominal wall.

However, this single-incision laparoscopic surgery exploration generates new challenges and magnifies difficulties compared with conventional laparoscopic surgery [30].

The handling of straight instruments in parallel with the laparoscope through a small single-incision decreases the range of movements for the surgeon and complicates the holding of the camera by the assistant [31]. Furthermore, the lack of instrument triangulation increases the complexity of organ exposure and exploration.

In order to overcome these pitfalls, we combined single-incision laparoscopic surgery and flexible endoscopy. We called this technique SIFE (single-incision flexible endoscopy). We showed that this technique consistently allowed comprehensive evaluation of the peritoneal cavity in a live porcine model, as well as in four human cadavers [21]. Some authors had described trans-umbilical endoscopic surgeries, mainly appendectomy and cholecystectomy [32–34]. This is the first study that evaluates this new technique in the detection of PC.

We showed that the flexible endoscope allows better overall access to the 13 regions of PCI than the rigid laparoscope (91.2 vs. 83 %). The access rates to the regions 1, 2, 3 and 6 were statistically superior by SIFE in comparison with SIRE, 87.2 versus 61.7 %, 87.2 versus 66 %, 85.1 versus 59.6 % and 80.9 versus 61.7 %, respectively. These results can be explained by great deflection capacities of the distal tip of the flexible endoscope, which expands visualization possibilities in some areas difficult of access even with the 30° angled laparoscope. These difficult-to-access areas include the peritoneal reflection at the level of the coronary ligament of the liver in the Region 1, the peritoneal reflection at the level of the phreno-splenic ligament in the Region 3, the falciform ligament and the hepatic hilum in the Region 2 and the Douglas pouch in the Region 6.

There was no difference in the access rates to the other regions between the two techniques. The small bowel exploration was excellent with the two techniques. This result seems obvious because of the central position of the small bowel. It is also of major importance due to the fact that an extensive involvement of the small bowel and its mesentery can compromise the feasibility of cytoreductive surgery.

The mean PCI was also significantly higher ($p < 10^4$) with SIFE 12.77 ($\pm$11.97) than with SIRE 11.77 ($\pm$11.63). The results were similar for the patients suitable for HIPEC ($p = 0.0005$), as well as the patients who had a PCI $\geq$20 ($p = 0.005$). In 25 % of the cases, the difference in PCI between the two techniques was at least equal to 2. This fact is of crucial importance, knowing that the PCI is the main prognosis factor of PC. It serves as an estimate of probability of complete cytoreduction and has been found to be an accurate assessment of survival when cytoreductive surgery and intraperitoneal chemotherapy are used as

treatment [22, 35, 36]. The evaluation of the PCI with the SIFE had a therapeutic impact, in that it could help and even change decision making. In our study, three patients (6.38 %) with a PCI evaluation with SIRE <20 had in fact a PCI ≥20 with SIFE and were, therefore, in theory, not eligible for HIPEC.

However, a major limitation was associated with SIFE because of its flexibility. In fact holding a flexible endoscope in a stable position in the peritoneal cavity is quiet difficult. In order to overcome this lack of stability, the assistant surgeon holds the trocar and maintains the torque of the flexible endoscope. Moreover, the SILS^{TM} port system and the abdominal wall thickness allowed a certain degree of stability compatible with a comprehensive and convincing peritoneal exploration. This is not the case of trans-gastric peritoneal exploration, where the lack of stability is due to the thinness of the gastric wall [37]. Voermens et al. [38] showed in a prospective, randomized, controlled study in pigs that trans-gastric NOTES was inferior to laparoscopic surgery for evaluation of PC extension.

The problem of lack of stability may be solved by the use of the flexible tip laparoscope [39]. More studies are needed to evaluate this newly developed technology in detection and staging of PC.

Although SIFE and SIRE demonstrated significantly different results in terms of access rates to the different regions of the PCI, the two techniques should be seen as complementary rather than competing. In fact, SIRE offers interesting capabilities in terms of intraperitoneal navigation, with good overall access to most sites (83 %). It does not require any experience in endoscopy and is therefore feasible by the majority of surgeons. SIFE can be associated with the procedure in order to explore the difficult-to-access areas that we defined.

The last outcome of this prospective study was the safety of the procedure. Because many of the components of the flexible endoscope are temperature sensitive, steam sterilization was not possible, and low-temperature chemical methods, such as liquid chemical germicide, were used. However, the SIFE does not seem to increase the morbidity. In fact, the flexible endoscope was dedicated to the procedure.

Following the guidelines on reprocessing flexible gastrointestinal endoscopes, a high-level disinfection was performed after each procedure [40].

No mortality was observed. There were 2 grade II complications [41], an acalculous cholecystitis in one case and a postoperative pneumonia in the other. The evolution was good in the two cases under medical treatment. Garofalo et al. [42] reported a morbidity rate of 2.04 % in 197 patients who underwent laparoscopic staging of peritoneal surface malignancies (2 cases of infection of the trocar insertion site, one diaphragm perforation and one intraoperative bleeding). We consider that SIRE and SIFE entail a small risk of complications, which is in contrast to exploratory surgery where high mortality (20–36 %) and morbidity (12–23 %) rates are observed in diagnostic laparotomies performed in advanced tumor case series [43].

This prospective study demonstrates that both SIRE and SIFE allow comprehensive evaluation of the peritoneal cavity for detection and staging of PC. Overall access rate to the different regions of PCI was higher with SIFE (91.1 %) than with SIRE (83 %). This difference was due to the fact that SIFE was significantly superior to SIRE in the exploration of some difficult-to-access areas, located in regions 1, 2, 3 and 6. These two minimally invasive staging procedures are safe and feasible. They have to be seen as complementary rather than competing and should be associated in order to appreciate accurately the PCI.

Compliance with ethical standards

Disclosures Fujifilm Medical System Company paid the inscription and the travel to the «United European Gastroenterology Week» which took place in Stockholm in October 2011. Two members of the team were present for poster presentation (Drs. R. Lo Dico and Dr. X. Dray). It is also a partner to the INSERM U965 Unit to study impact of endoscopy on evaluation of peritoneal carcinomatosis. Drs. M. Grienay, A. Dohan, H. Najah and M. Pocard have no conflicts of interest or financial ties to disclose.

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Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Peritoneal Carcinomatosis in the Elderly: A Case-Controlled, Multicenter Study

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ABSTRACT

Objective. This study was designed to identify factors associated with morbidity and mortality in patients older than 70 years who underwent cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) for peritoneal carcinomatosis (PC).

Background. Major surgery is associated with higher morbidity and mortality in elderly patients. For PC, CRS and HIPEC is the only current potential curative therapy, but the risks inherent to this patient population have called its benefits into question.

Methods. We retrospectively analyzed a multi-center database from 1989 to 2015. All patients who underwent CRS and HIPEC for PC were selected and patients older than 70 years were matched 1:4 with a younger cohort

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according to cancer origin, peritoneal cancer index (PCI), and completeness of cytoreduction. Major morbidity and mortality were analyzed.

Results. Of 2328 patients, 188 patients older than aged 70 years were matched with 704 younger patients. Patients older than aged 70 years demonstrated a higher American Society of Anesthesiologist score (≥ASA III 10.8 vs. 6.6 %, $p = 0.008$). There was no difference in overall 90-day morbidity (≥70: 45.7 % vs. <70: 44.5 %; $p = 0.171$); however, patients older than 70 years had significantly more cardiovascular complications (13.8 vs. 9.2 %, $p = 0.044$). Differences between the older and younger cohorts failed to reach significance for 90-day mortality (5.4 and 2.7 %, respectively; $p = 0.052$), and failure-to-rescue (11.6 and 6.1 %, respectively; $p = 0.078$). In multivariate analysis, PCI > 7 (95 % CI 1.051–5.798, $p = 0.038$) and HIPEC duration (95 % CI 1.106–6.235, $p = 0.028$) were independent factors associated with morbidity in elderly patients.

Conclusions. CRS and HIPEC appear feasible for selected patients older than aged 70 years, albeit with a higher risk of medical complications associated with increased mortality.

Peritoneal carcinomatosis (PC) is a common evolution of abdominal cancers and is associated with a poor prognosis without aggressive multimodal therapeutic approaches.[1] Since its origin in the 1990s, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have been increasingly used as curative treatment for several etiologies of peritoneal carcinomatosis.[2,3] Cytoreductive surgery and HIPEC offer the best outcome for pseudomyxoma peritonei and mesothelioma and represent the only current curative treatment for colorectal and gastric PC.[4–7]

The proportion of North Americans and Western Europeans aged 60 years and older is projected to reach 23 and 25 % of their respective total populations by the year 2020.[8] Despite advances in surgical and perioperative care, age remains independently associated with worse short-term outcomes after major oncologic resections.[9] The feasibility of major hepatic, pancreatic, or gastric surgery in elderly populations has been recently described, as had the multidisciplinary approach required to optimize outcomes for CRS/HIPEC, which is associated with morbidity and mortality rates between 20–42 and 0–10 %, respectively.[10–15] This is especially true in the elderly, whose reported post-CRS/HIPEC morbidity and mortality increase to 19.4–56 and 0–18.2 %, respectively.[16–23]

Using a multi-institutional database, we present the largest study of elderly CRS/HIPEC patients to date. Our primary gaol was to identify specific factors associated with morbidity and mortality in this otherwise understudied population.

METHODS

Study Population

Three French national databases encompassing 22 different institutions—RENAPE for rare PC, BIGRENAPE for colorectal and gastric PC, and FROGHI for ovarian PC—were retrospectively queried for all patients aged 70 or older who underwent CRS and HIPEC between 1989 and 2015.

Demographic and illness-specific data displayed in Table 1 were obtained for each patient. For each patient, the following data were extracted: gender, body mass index (BMI), age at time of surgery, origin of PC, method and duration of HIPEC, American Society of Anesthesiology (ASA) score, history of neoadjuvant chemotherapy, extent of PC determined according to the Peritoneal Cancer Index (PCI),[24] and completeness of cytoreduction according to the CC score (CC-0, no residual nodule; CC-1, residual nodes <2.5 mm; CC-2, residual nodules <25 mm; and CC-3, >25 mm), length of surgery, length of stay in hospital and postoperative morbidity and mortality.[25] Postoperative morbidity was graded according to the common toxicity criteria of the National Cancer Institute (NCI-CTC version 3.0).[26] Major complications grade III (severe adverse events) and grade IV (life-threatening adverse events) up to 90 days after surgery were included in our analysis. Surgical complications were defined as those related to the operative site (abscess, fistula, bleeding, collection, or incisional dehiscence) and medical complications grouped according to organ system. Failure-to-rescue was defined as death in a patient with one or more of the defined major complications. The failure-to-rescue rate was calculated as the number of patients who died after a major complication divided by the total number of patients who developed a major complication. This study was performed in accordance with the precepts established by Helsinki declaration.

Perioperative Evaluation and Management

Preoperative workup included a full history and physical, contrast CT scans of the chest, abdomen and pelvis, and preoperative blood work including relevant tumor markers. Eligibility for CRS/HIPEC was determined during multidisciplinary conference involving medical and surgical oncologists, radiologist, anesthesiologists, and pathologists. Following CRS/HIPEC, all patients were admitted to the ICU for at least one postoperative day before transfer to progressively lower acuity units as determined by the healthcare team.

TABLE 1 Comparative clinicopathological, perioperative data for patients undergoing cytoreductive surgery for peritoneal carcinomatosis according to the age (70 years and older and younger than 70 years)

Characteristic	≥70 ($n = 188$)	<70 ($n = 704$)	p value
Median age (range), year	72.47 (70.0–82.6)	56.74 (17.6–70.0)	<0.001
Gender			
Male	66 (35.1)	201 (28.6)	0.081
Female	122 (64.9)	503 (71.4)	
Body mass index (kg/m^2), median (range)	24.4 (15.8–42.4)	23.4 (13.3–45.3)	0.082
Preoperative chemotherapy	114 (60.6)	425 (60.5)	0.980
Nb of preoperative cycle of chemotherapy, median (range)	6 (2–70)	6 (1–44)	0.440
Delay between diagnosis and CRS/HIPEC in months (range)	10.5 (0–156)	10 (2–254)	0.825
Origin of PC			
Colorectal	29 (15.4)	112 (15.9)	1
Ovarian	52 (27.7)	199 (28.3)	
PMP	61 (32.4)	240 (34.1)	
Gastric	6 (3.2)	24 (3.4)	
MMP	25 (13.3)	91 (12.9)	
Serous	9 (4.8)	26 (3.7)	
Appendix	3 (1.6)	6 (0,9)	
Other	3 (1.6)	6 (0.9)	
ASA score			
1–2	132 (89.2)	478 (93.4)	0.008
3–4	16 (10.8)	34 (6.6)	
PCI, median (range)	12 (0–39)	12 (0–39)	0.765
Length of surgery (min), median (range)	300 (150–660)	330 (90–900)	0.096
CC score			
0	136 (73.9)	537 (76.3)	0.637
1	33 (17.9)	123 (17.5)	
2	5 (2.7)	20 (2.8)	
3	10 (5.4)	24 (3.4)	
HIPEC technique			
Open	81 (43.5)	313 (45.4)	0.647
Closed	105 (56.5)	376 (54.6)	
Median hospital stay in days (range)	20 (2–139)	19 (5–164)	0.489
Readmission	30 (26.1)	134[28]	0.675

Values in table are numbers of patients (percentages) unless otherwise indicated

PMP pseudomyxoma peritonei, *MMP* malignant mesothelioma of the peritoneum, *Serous* peritoneal serous carcinoma, *PCI* Peritoneal cancer index, *CC* completeness of cytoreduction score, *HIPEC* hyperthermic intraperitoneal chemotherapy

Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy

All centers performing CRS employ a team of surgeons, anesthesiologists, and nurses specifically trained in this procedure. Following laparotomy, PCI was assessed by exploration of the entire abdominal cavity. Cytoreductive surgery was performed with the goal of achieving complete excision of tumor deposits, affected organs, and the visceral and parietal peritoneum as described by Sugarbaker.[27] The CC score was determined upon completion of CRS via visual assessment of the operative field.

Following CRS, HIPEC was performed by circulating a heated solvent infused with chemotherapeutic medication throughout the abdomen. This was performed using either an open ("Coliseum") or closed technique according to the preference of the operating team. The choice of cytotoxic agent and duration of hyperthermia depended on the origin

TABLE 2 Postoperative morbidity and mortality, and treatment modality

	≥70 (n = 188)	<70 (n = 704)	*p*
Death within 90 days	10 (5.4)	19 (2.7)	0.052
Surgical complications	34 (18.1)	134 (19.0)	0.709
Intra-abdominal abscess	12 (6.4)	47 (6.7)	0.632
Digestive fistula	13 (6.9)	59 (8.4)	0.665
Intra-abdominal bleeding	6 (3.2)	51 (7.3)	0.064
Incisional dehiscence	4 (2.1)	3 (0.4)	0.026
Wound infection	3 (1.6)	3 (0.4)	0.154
Medical complications	77 (41)	270 (38.4)	0.095
Hematologic	23 (12.2)	121 (17.2)	0.591
Hemoglobin <80 g/L (4.9 mmol/L)			
Hemoglobin <65 g/L (4 mmol/L)			
Neutrophil granulocytes <1000/mm^3			
Associated with hyperthermia (> 38.5)			
Platelet <50,000/mm^3			
Cardiovascular	26 (13.8)	65 (9.2)	0.044
Cardiac arrhythmia			
Digestive arterial thrombosis			
Deep venous thrombosis or pulmonary embolism			
Gastrointestinal	21(11.2)	87 (12.4)	0.884
Anorexia needing parenteral alimentation			
Pancreatitis			
Respiratory	31(16.5)	88 (12.5)	0.052
Acute respiratory distress syndrome			
Renal	20 (10.6)	46 (6.5)	0.066
Renal insufficiency needing dialysis	8 (4.3)	15 (2.1)	0.056
At least one complication	86 (45.7)	313 (44.5)	0.171
More than one complication	43 (50)	146 (46.6)	0.595
Treatment			
Interventional radiology	15 (9.4)	71 (10.8)	0.903
Endoscopic	30 (16.6)	77 (11.2)	0.208
Surgery	17 (13.6)	77 (15.8)	0.621

Values are numbers of patients (percentages) unless otherwise indicated

of carcinomatosis. The cytotoxic agents used, alone or in combination, included cisplatin, mitomycin C, oxaliplatin, doxorubicin, and irinotecan. No age-based adjustments to HIPEC regimens were made.

Case-Matching

Each individual subject meeting the above criteria was matched with patients aged <70 years from the same databases according to pathology and similar PCI (±5) and CC scores. To raise power, each subject was matched with up to four younger patients.

Statistical Analysis

Descriptive results were expressed as a number (percentage) for qualitative variables and by mean ± standard deviation (SD) or median (minimum–maximum) for quantitative variables. Comparisons were performed using χ^2 test (or Fisher exact test when conditions for χ^2 were not fulfilled) for qualitative variables and by Student's *t* test (or Mann–Whitney test in cases of non normality) for quantitative variables. Univariate analysis for complications was performed using logistic regression with an adjustment for center. Multivariate analyses were done using a stepwise

TABLE 3 Cause of death for patients ≥70 years

Patient	Age (years)	Malignancy	Cause(s) of death
1	71.9	Mesothelioma	Cerebral hemorrhage
2	70.6	Pseudomyxoma peritonei	MSOF, renal failure
3	72.4	Pseudomyxoma peritonei	Massive intrapulmonary hemorrhage
4	70.3	Cancer primitive of peritoneum	MSOF
5	73.9	Colon	Enterocutaneous fistula, renal failure
6	82.5	Ovarian	Pancreatic leak, renal failure
7	70.3	Colon	renal failure
8	71.7	Colon	ARDS
9	70.5	Pseudomyxoma peritonei	MSOF, pneumonia
10	70.0	Ovarian	Hemorrhagic shock

MSOF multisystem organ failure, *ARDS* acute respiratory distress syndrome

logistic regression model. The significance level was set at $p = 0.05$.

RESULTS

Population

Among 2328 patients treated with CRS and HIPEC for PC, 188 patients were aged 70 years or older. They were matched with a control group of 704 patients. Table 1 reports clinicopathological and perioperative data for the matched cohorts. Peritoneal pseudomyxoma was the most common etiology for patients older than age 70 years. After matching, patients older than age 70 years displayed a significantly higher ASA score ($p = 0.008$). There was no difference regarding hospital stay and readmission.

Postoperative Morbidity and Mortality

Table 2 reports 90-day mortality and morbidity. For patients aged ≥70 years, the 90-day mortality and morbidity were 5.4 and 45.7 %, respectively. Cause of death was preferentially due to medical complications (Table 3). Cardiovascular complications in particular were significantly more prevalent among older patients (13.8 vs. 9.2 %, $p = 0.044$). There was no significant difference in mortality (5.4 vs. 2.7 %, $p = 0.052$, respectively) or morbidity compared with the younger cohort (45.7 vs. 44.5 %, $p = 0.171$, respectively). Similarly, failure to rescue was higher in older patients (11.6 %) than younger (6 %), although this also was not significant ($p = 0.078$). In multivariate analysis, for patients aged ≥70 years, there was no independent factor associated with mortality, but PCI >7 (odds ratio 2.469; 95 % CI 1.051–5.798, $p = 0.038$) and the HIPEC duration (odds ratio 2.626; 95 % CI 1.106–6.235, $p = 0.028$) were independently associated with increased morbidity (Table 4).

DISCUSSION

Our study suggests that, given appropriate patient selection, postoperative mortality and morbidity after CRS/HIPEC is comparable between patients aged >70 years and their younger counterparts. Nonetheless and despite no statistically significant difference, the failure-to-rescue rate was higher in elderly group (11.6 vs. 6.1 %, $p = 0.078$), supporting the prevailing opinion that elderly patients are at a higher risk of death following a complication. The mortality rate in our study was similar to that after major hepatic or pancreatic surgery and the mortality previously reported after CRS/HIPEC in elderly patients.[10–13,16–23] To the best of our knowledge, this study is the largest series of elderly patients undergoing CRS/HIPEC to date and supports the feasibility and the safety of CRS/HIPEC in well-selected elderly patients.

Age is directly related with comorbidities and a reduced capacity to recover after surgery.[28,29] In addition, this increased risk has been argued to be due preexisting medical disease as shown in studies demonstrating greater postoperative complications after noncardiac surgery among patients older than 70 years.[30] Our results support this argument; there was no difference in surgical complications, but medical complications were more frequent for elderly patients and associated with an increased risk of failure-to-rescue. However, this risk may be mitigated by careful patient selection and age-appropriate care, including geriatric consultation, supplemental enteral nutrition, and early rehabilitation placement planning.[31] In the past, age alone has been considered a limiting factor when

TABLE 4 Univariate and multivariate analyses of risk factors for major complications and mortality after CRS/HIPEC for patient ≥70 years

Risk factor	Post-operative complication					Post-operative mortality
	Univariate		Multivariate			Univariate
	p	Odds ratio	95% CI		*p*	*p*
			Lower	Upper		
Age	0.761					0.208
Origin of peritoneal carcinomatosis	0.991					0.468
PCI 0–7 versus >7	0.067	2.469	1.051	5.798	0.038	0.937
PCI 0–10 versus 11–15 versus >16	0.061					0.951
PCI 0–18 versus >18	0.232					0.448
Gilly score 0–1–2 versus 3–4	0.406					0.930
ASA score 1 versus 2 versus 3	0.609					0.451
CCR score 0 versus 1 versus 2	0.217					0.868
CCR score 0 versus 1 + 2	0.151					0.850
Hepatic resection	0.840					0.194
HIPEC technique: close versus open	0.465					0.961
HIPEC duration	0.008	2.626	1.106	6.235	0.028	0.987
HIPEC with cisplatin	0.909					0.191
HIPEC with doxorubicin	0.429					0.959
HIPEC with mitomycin	0.662					0.606

TABLE 5 Reported morbidities and mortalities of CRS/HIPEC in the elderly population

Study (yr)	*N*	Ages	Carcinomatosis origin	Morbidity (%)[a]	Mortality (%)
Alyami (2016)[b]	188	≥70	Colorectal, ovarian, mesothelioma, pseudomyxoma, gastric, other	45.7	5.4
Huang (2015)	124	≥65	Colorectal, mesothelioma, appendix, pseudomyxoma	40	3
Delotte (2015)	15	≥70	Ovarian	20	0
Cascales-Campos (2014)	9	≥75	Ovarian	56	0
Spiliotis (2014)	30	≥70	Colorectal, ovarian, gastric, pseudomyxoma, sarcoma, mesothelioma	50	3.3
Votanopolous (2013)	81	≥70	Appendiceal, mesothelioma, ovarian, colon, gastric	38	13.5
Tabrizian (2013)	35	≥65	Colorectal, ovarian, appendiceal, mesothelioma, pseudomyxoma, gastric	19.4	11.4
Klaver (2012)	24	≥70	Colorectal	33.3	0
Macri (2011)	11	≥65	Colorectal, ovarian, gastric	27.3	18.2
Total (mean %)	517			36.6	6.08

[a] Where reported, Grade I-II morbidity was excluded

[b] Our study

pursuing major abdominal surgery. However, this dogma appears outdated and several recent studies shown that age does not influence the oncologic outcome of surgery and that cancer-specific survival in these patients is similar to that of younger patients.[10,32,33] Therefore, elderly patients might benefit from being offered CRS/HIPEC for PC as well as other major intra-abdominal surgeries.

Our study focused on 90-day outcomes due to the high physiologic impact of this complex surgical procedure. The overall mortality and morbidity in that time period were 5.4 and 45.6 %, respectively, with a 26.1 % risk of readmission. These data are comparable with data reported by Chua et al. for the general population after CRS/HIPEC.[34] Similar morbidity and mortality rates for performing CRS and HIPEC for elderly patients compared with younger patients also have been reported previously (Table 5).[16–23] However, strict selection of the patients appears to be even more important in this population, as demonstrated by as

demonstrated by the strong impact of the PCI on the occurrence of complications. Peritoneal carcinomatosis index >7 and HIPEC duration were two independent risk factor for postoperative complications in the elderly patients. The potential benefit of extended CRS for elderly patients with PCI >7 is questionable.

We recognize that the current study is limited in its retrospective nature and high selection bias of elderly patients who are offered CRS/HIPEC. It does, however, demonstrate the surgical outcomes of CRS/HIPEC procedures across multiple institutions, regardless of primary tumor etiology, on a population that will continue to grow in the future.[8] We believe that rigorous patient selection is, in fact, the key to maintaining low complications rates for older patients. In addition to strict patient selection criteria, both the patient and their families must understand the considerable risks of these procedures as well as the potential impact to their quality of life, which may be negatively impacted for up to 6 months.[35,36] Unfortunately, quality of life data for our experimental group were not available. Nonetheless, appropriately selected patients stand to gain a considerably improved quality of life from this aggressive surgical therapy. Therefore, CRS and HIPEC should not be withheld from elderly patients who stand to improve quality of life and survival, especially when selected in a multidisciplinary fashion and treated at high-volume centers.

CONCLUSIONS

CRS and HIPEC can achieve comparable perioperative outcomes in well-selected patients previously thought too old to undergo these procedures. The results of this study should encourage surgeons to offer potentially curative CRS/HIPEC to elderly patients according to a multidisciplinary preoperative evaluation.

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Prediction of clinical outcome using blood flow volume in the superior mesenteric artery in patients with pseudomyxoma peritonei treated by cytoreductive surgery

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Abstract

Background: Pseudomyxoma peritonei (PMP) is a rare carcinomatosis limited to the peritoneal cavity, mainly supplied by the superior mesenteric artery (SMA). The only curative treatment is cytoreductive surgery (CRS) associated with hyperthermic intraperitoneal chemotherapy. This study aimed to evaluate the ability of blood flow volume (BFV) recorded in the SMA using Doppler ultrasonography pre-operatively to predict the extent and resectability of the disease and post-operatively to assess clinical outcome.
Methods: BFV was measured in the SMA of forty-nine patients before and the year following CRS. Patients were categorized in 3 groups according to clinical and surgical outcomes: group-1 (n = 22): patient with completed CRS, group-2 (n = 16): incomplete resection with slowly progressive disease (alive at 2 years without severe clinical symptoms), group-3 (n = 11): incomplete resection and with severe clinical symptoms or dead within two years.
Results: Pre-operative mean SMA BFV was higher in group-2 (510 mL/min, $p = 0.027$) and in group-3 (572 mL/min, $p = 0.004$) than in group-1 (378 mL/min). After surgery, BFV dropped to normal values (203 mL/min, $p = 0.001$) in group-1, and to 423 mL/min ($p = 0.047$) in group-2. It remained elevated in group-3 (626 mL/min, $p = 0.566$). BFV allowed stratification of 1) resectability before CRS (group-2 and -3 *vs* group-1, area under the ROC curve: 0.794 [0.650–0.939]), and 2) non progression after incomplete CRS (group-3 *vs* group-2, area under the ROC curve: 0.827 [0.565–1.00].
Conclusions: Pre-operative BFV in the SMA correlates with extent and resectability of PMP. After incomplete surgery, post-operative BFV might aid in identifying patients who may benefit of post-operative therapy.

Keywords: Pseudomyxoma peritonei; Cytoreductive surgery; Hyperthermic intraperitoneal chemotherapy; Doppler ultrasonography; Superior mesenteric artery

Introduction

Pseudomyxoma peritonei (PMP) is a rare disease with an estimated incidence of one to two per million per year that is characterized by diffuse intra-abdominal gelatinous ascites.[1] The only curative treatment for PMP consists of a combination of cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC).[2] When resection is complete, this treatment may achieve a 20-year survival rate of 70%.[3,4]

The World Health Organization (WHO) has recently classified PMP into two pathological categories: low and

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high grade tumors. While some studies suggested that the grade correlates with prognosis (overall 5-year survival of 63% for low grade and 23% for high grade respectively),[1–5] others have suggested that recurrence and survival are quite variable for PMP of the same grade.[6]

When complete surgical resection is not achievable, therapeutic options are limited. Many oncologists propose the same chemotherapy regimen as that used in colorectal cancer; especially in case of high grade PMP.[7] Others have suggested the use of anti-angiogenic agents, which have conferred benefits in case studies.[8] However, clinical outcome in PMP is not related to disease grade alone as some patients with high grade disease may have few symptoms and live longer than those with lower grades whose disease may be complicated by compression of digestive structures and severe clinical symptoms leading to death.[6] Low grade PMP is far more frequent than high grade PMP (78 *vs* 22%, respectively), and among patients with low grade PMP, those with slowly progressive disease will benefit of a therapeutic pause, while patients with aggressive disease may benefit of a post-operative chemotherapy and anti-angiogenic agents as high grade PMP.[7,9]

To date, there are no clinical, biological or pathological criteria to assess the activity of PMP and to distinguish between aggressive and less aggressive PMP. Imaging evaluation of patients with PMP is based on morphological examination such as computed tomography or magnetic resonance imaging (MRI), but assessment of progression or stability of the disease is difficult as there are no standardized and not well-established criteria as those used for solid tumors.[10] Numerous studies have demonstrated that Doppler ultrasonography (Doppler-US) of the arteries supplying the alimentary tract is a reliable imaging modality to monitor blood flow velocities (BFVels) or blood flow volume (BFV) in inflammatory bowel disease and hepatic malignancies.[11–13] Moreover, it has been suggested that measurement of the BFVels or BFV in the feeding artery upstream of the tumor vascular network allows for the semi-quantitative analysis of the development or the involution of tumor vasculature.[14,15] Of note, Dohan et al. have demonstrated that Doppler-US of the superior mesenteric artery (SMA) can be used to monitor tumor angiogenesis and response to antiangiogenic therapy after CRS in an orthotopic murine model of PMP.[16]

As blood flow is supplied to PMP mainly from the SMA, we anticipated that the increased blood supply to the PMP would be reflected in BFV in this vessel, which had never been evaluated before. The objectives to the current study were to evaluate the ability of BFV recorded in the SMA by Doppler-US, 1) pre-operatively to predict the extent and resectability of PMP and 2) post-operatively to assess clinical outcome in patients with PMP.

Patients and methods

Patient cohort

After IRB approval, all patients gave their informed consent to be enrolled in this prospective single center study, from October 1st, 2011 to October 1st 2016. Patients were included into the study when they were scheduled to undergo surgical resection of PMP or after surgery. The first step of the surgical procedure was a peritoneal exploration with determination of the peritoneal carcinomatosis index (PCI) followed by the decision to perform HIPEC.[4,17] All patients had a pathological analysis with an evaluation of the tumor grade according to the WHO classification.[5]

The study population comprised 49 consecutive patients, 22 (45%) men and 27 (55%) women, with a mean age of 57 [26–87] years (mean [min–max]). Among these 49 patients, 41 (82%) had SMA BFV measurement during the month (mean: 1.3 months) before the CRS, and 30 (60%) within the 6–12 months following surgery (10.1 [6.1–11.9] months). Twenty two (45%) patients had Doppler-US examination before and after surgery, 19 (39%) only before surgery, 8 (16%) only during follow-up. A group of 14 healthy patients was constituted as a control group to determine the normal values of SMA BFV (Fig. 1).

Classification of patients according to clinical outcome

Patients were distributed into 3 groups according to their clinical and surgical outcome as follows. Completeness of resection was graded according to Sugarbaker's complete cytoreduction (CCR) score as follows: CCR0: no residual tumor; CCR1: residual tumor < 0.25 cm; CCR2: residual tumor between 0.25 and 2.5 cm; CCR3: residual tumor > 2.5 cm.[4]

- *Group-1*: patients with complete resection (CCR0 or CCR1 score), HIPEC and absence of recurrence within two years after surgery or at last follow-up ($n = 22$, *among them, 6 patients had SMA BFV measurements before and after surgery*);
- *Group-2*: patients with incomplete resection (CCR2 or CCR3 score) with slowly progressive disease: alive 2 years after surgery or at last during follow-up without severe clinical symptoms (performance status = 0 or 1), ($n = 16$, *among them, 11 patients had SMA BFV measurements before and after surgery*);
- *Group-3*: patients with incomplete resection (CCR2 or CCR3 score) with severe active progressive disease: dead within 2 years after surgery or with severe clinical symptoms (performance status >1), ($n = 11$, *among them, 5 patients had SMA BFV measurements before and after surgery*).

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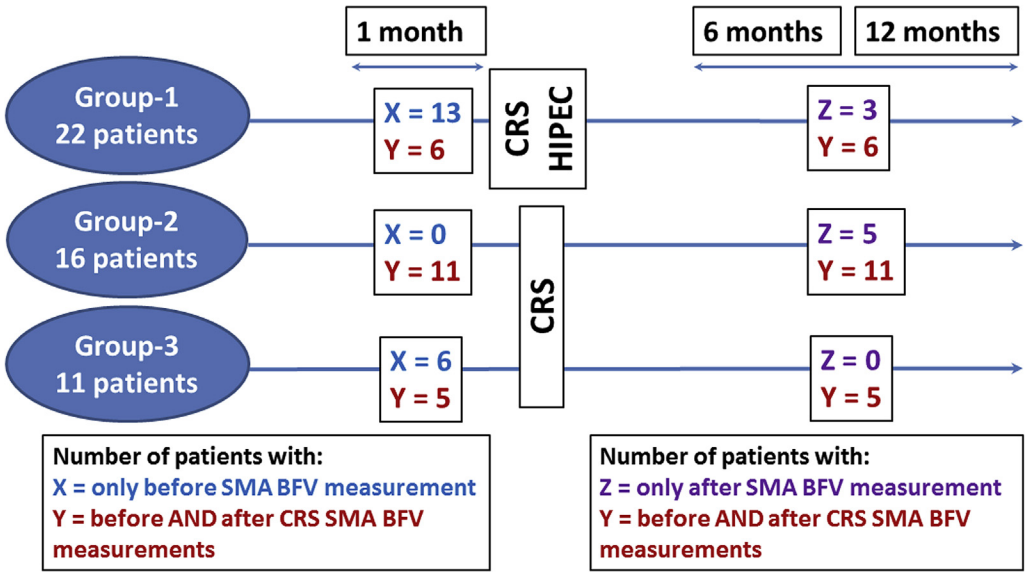

Figure 1. Flow chart of patient enrolled in the study. SMA BFV indicates superior mesenteric artery blood flow volumes, CRS, cytoreductive surgery, HIPEC, hyperthermic intra peritoneal chemotherapy.

Superior mesenteric artery ultrasound imaging

The sonographer was blinded to the clinical and radiological status of each patient; the surgeon and the radiologist were blinded to the SMA BFV. The SMA BFV was not included as an element of the surgical decision or as an element of the follow-up.

Doppler-US was performed at rest in patients in 45° seat-position after an overnight fast using an ultrasound scanner (Acuson S2000®, Siemens, Erlangen, Germany) equipped with a curvilinear transducer type CH4-1 (6-4 MHz) as previously described.[11] The SMA was studied in its long axis in the sagittal plan. M-mode was activated for measurement of inner diameters. Pulsed Doppler was activated for blood flow velocity (BFVel) waveforms acquisition 2–3 cm after the SMA origin. Great attention was given to angle correction application in order to record accurate velocities. Spatial-average-time-average BFVel was calculated from the spectral analysis of the Doppler signal by integrating the area under instantaneous mean velocity curve. BFV was calculated using the following formula: $BFV = [(BFVel\ 0.60).(\pi.(D/2)^2)]$, where BFV is the blood flow volume in mL/min, BFVel is the spatial-averaged-time-averaged mean blood flow velocity in cm/s, and D the SMA diameter in cm. For each patient, BFV was measured five times during quiet breathing and averaged.

Accuracy of the superior mesenteric artery blood flow volume measurement

Repeatability of SMA diameter, BFVel and BFV measurements was investigated in the controls through the calculation of the repeatability coefficient (RC) (British Standards Institution Precision of Test Method).[18] Two series of paired measurements separated by 2 min interval performed by the same investigator were compared according to the formula: $RC^2 = \Sigma Di^2/N$, where N is the sample, Di is the relative (positive or negative) differences within each pair of measures. This coefficient is the standard deviation of the estimated difference between two repeated measurements. The RC values for intra-observer repeatability were 0.05 mm for the inner diameter, 0.4 cm/s for the spatial-average–time-average mean blood flow velocity and 5.0 mL/min for the BFV.

Blood tests

All patients had carcinoembryonic antigen (CEA) serum level measurement before and after surgery. For the 13 patients who were included after surgery, pre-operative CEA was collected retrospectively. Carbohydrate antigen CA125 and CA19-9 were not routinely collected and not available for all patients.

Statistical analysis

Continuous variables are reported as mean [min–max]. The Gaussian distribution of the continuous variables was assessed using the Shapiro–Wilk test. Survival curves in the 3 groups were estimated according to the Kaplan–Meier method. Comparisons between the survivals of the different groups were performed using the Log-rank test. Categorical data were analyzed using the Chi-squared test or Fischer's exact test when appropriate, whereas differences in continuous variables (SMA BFV, and CEA serum level) before and after CRS, according to the patients groups were analyzed with a two-way ANOVA with post-hoc Bonferroni correction and paired or unpaired Student

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t-test. For the 22 patients with both pre- and post-operative value of SMA BFV, an ANOVA for repeated measurements was performed between pre- and post-operative values with patients groups as between-subjects factor. A polynomial regression was calculated between the pre-operative SMA BFV and the PCI. A receiver operating characteristics (ROC) curve of pre- or post-surgical BFV was built and a cut-off value was calculated to discriminate 1) between patients who benefited from completeness of resection from the others or 2) between patients who had severe active progressive disease (group-3) from those with slowly progressive disease (group-2). Areas under curves (AUC) were calculated. Sensitivity and specificity obtained with the respective cut-off BFV values were calculated (MedCal® Software, Mariakerke, Belgium). *P* values < 0.05 were considered significant.

Results

Survival

Overall survival was higher in group 1 than in group 2 ($p = 0.003$), in group-1 than in group-3 ($p < 0.001$) and in group-2 compared to group-3 ($p = 0.021$) (Fig. 2). There was no difference in age, sex ratio and low/high grade ratio between the three groups. PCI were different between group-1 (16 [0–39]) *vs* group-2 (31 [20–39], $p < 0.001$) and group-3 (33 [23–39] *vs* group-1, $p < 0.001$). There was no difference in PCI between group-2 and group-3 ($p = 0.453$). Affected areas counts were also different between group-1 (7 [1–13]), group-2 (12 [9–13], $p = 0.002$) and group-3 (13 [12–13], $p < 0.001$ *vs* group-1), without any difference between group-2 and group-3 ($p = 0.270$). Patients' characteristics are summarized in Table 1.

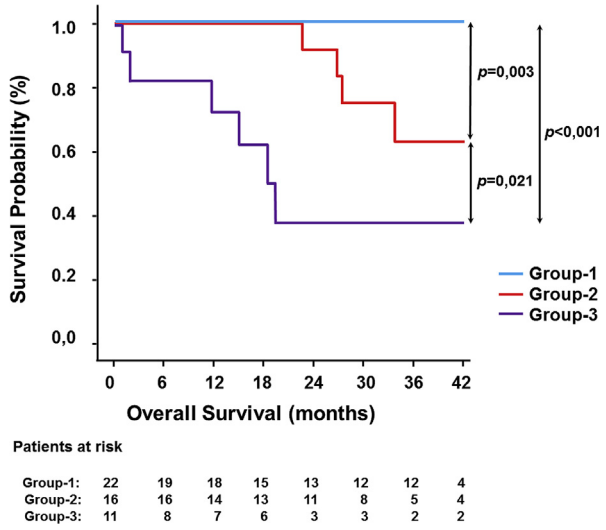

Figure 2. Survival curves in the three different groups of patients. Patients with completed CRS and HIPEC (group-1) were alive after 2 years. Survival of patients with uncompleted CRS and progressive PMP was lower than group-1 (group-3 *vs* group-1, $p < 0.001$), and survival of patients with uncompleted CRS and slowly progressive PMP was also slower than group-1 (group-2 *vs* group-1, $p = 0.003$). Survival was higher in group-2 compared to group-3 ($p = 0.021$).

Pre-operative blood flow volume measurements

Before surgery, BFV in the SMA was lower in group-1 than in group-2 (378 [220–779], *vs* 510 [338–840] mL/min respectively; $p = 0.027$) and that in group-3 patients (572 [265–889] mL/min, $p < 0.001$, Table 1). No difference was observed in baseline BFV between group-2 and group-3 patients ($p = 0.388$) (Fig. 3a). We found a polynomial regression between the baselines BFV and PCI ($R = 0.65$, $p < 0.001$, Fig. 3b). There was no difference in baseline BFV between patients with high histological grade (488 [225–775] mL/min) and patients with low grade (434 [220–889] mL/min, $p = 0.429$).

A ROC curve showed the ability of pre-operative BFV to distinguish resectable (group-1) from unresectable PMP (groups-2 and -3). The area under the curve (AUC) was 0.794 [95%CI: 0.650–0.932]. A pre-operative BFV >450 mL min^{-1} yielded a sensitivity of 68.2% and a specificity of 78.9% for predicting incomplete resection (group-2 or -3) (Fig. 4a).

Post-surgical blood flow volume measurements

In group-1, BFV in the SMA dropped from 378 [200–779] to 214 [150–265] mL/min ($p = 0.001$) after surgery reaching values of healthy controls (236 [179–309] mL/min, $p = 0.091$). In the group-2 the BFV decreased from 510 [338–840] to 406 [265–630] mL/min, ($p = 0.026$). No effect of surgery was found regarding the BFV in group-3 (572 [265–889] before to 645 [528–773] mL/min after surgery, $p = 0.057$). Two-way ANOVA showed a significant interaction between patients groups and pre-/post-operative SMA BFV values ($p = 0.037$) illustrative of the drop in SMA BFV after surgery in group-1 and -2, and the trend of increase in the group-3. For the 22 patients with both pre- and post-operative Doppler-US examinations, the pre- and post-operative SMA BFV modifications were highly significant between the three groups ($p < 0.001$, ANOVA for repeated measurements). In group-2, 5/16 (31%) patients and in group-3, 3/11 (27%) patients had post-operative chemotherapy ($p = 0.360$). The use and the type of chemotherapy was: Folinic acid and 5-Fluorouracil associated to Oxaliplatin (FOLFOX) ($n = 5$) or Irinotecan (FOLFIRI) ($n = 3$). No difference in BFV was found between patients who did and did not receive post-operative chemotherapy.

A ROC curve showed the ability of post-operative BFV to distinguish group-2 from group-3 patients. The AUC was 0.827 [95%CI: 0.565–1.00]. A post-operative BFV >530 mL min^{-1} yielded a sensitivity of 80.0% and a specificity of 93.3% for the diagnosis of aggressive PMP (group-3) (Fig. 4b).

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Table 1
Patients' baseline characteristics, Doppler ultrasonography and laboratory findings.

		Group-1 (n = 22)		Group-2 (n = 16)		Group-3 (n = 11)		One-way or two-way ANOVA, *p* value	
Age (years)	Mean (SD) [range]	56 (14) [26–76]		58 (13) [35–73]		57 (14) [34–87]		0.874	
Gender	Women	15		6		6		0.172	
	Men	7		10		5			
Grade	Low	19		12		9		0.671	
	High	3		4		2			
PCI	Mean (SD) [range]	16 (11) [0–39]		31 (7) [20–39]		33 (6) [23–39]		<0.001	
Affected areas count	Mean (SD) [range]	7 (5) [1–13]		12 (1) [9–13]		13 (1) [12–13]		<0.001	
Delay between diagnosis and surgery (month)	Mean (SD) [range]	34 (56) [0–159]		7 (8) [1–31]		55 (90) [2–258]		0.181	
Post-operative chemotherapy	yes	0		5		3		0.360	
	no	23		11		8			
		Before CRS	After CRS	Before CRS	After CRS	Before CRS	After CRS	Between groups *p* value	After/Before *p* value
SMA BFV (mL/min)	Mean (SD) [range]	378 (151) [220–779]	214*** (46) [150–265]	510 (146) [338–840]	406* (101) [265–630]	572 (184) [265–889]	645 (107) [528–773]	<0.001	0.069
Delay Doppler-US/Surgery (month)	Mean (SD) [range]	1.0 (0.8) [0.3–2.5]	–	1.0 (0.7) [0.2–2.3]	–	0.9 (0.7) [0.2–2.7]	–	0.941	–
Delay Surgery/ Doppler-US (month)	Mean (SD) [range]	–	10.4 (2.1) [7.0–11.9]	–	9.7 (2.3) [5.1–11.8]	–	9.8 (2.3) [6.1–11.6]	0.961	–
ACE (μg/l)	Mean (SD) [range]	41 (87) [1–304]	6 (7) [1–16]	16 (20) [1–50]	82 (241) [1–600]	31 (25) [1–67]	51 (11) [39–60]	0.762	0.581

SD standard deviation, *PCI* Peritoneal Cancer Index, *SMA BFV* Blood flow volume in the superior mesenteric artery, *CRS* cytoreductive surgery; *p < 0.05, ***p < 0.001 after *vs* before CRS.

Blood tests

Pre- and post-operative CEA serum levels were not different among the 3 groups (Table 1).

Discussion

This study demonstrates that pre-operative BFV measured in the SMA with Doppler-US correlates with surgical PCI and might be helpful to predict resectability. SMA BFV measurements showed an interesting predicting value for incomplete resection (sensitivity of 68.2% and specificity of 78.9%). Moreover, we found that post-operative SMA BFV may help discriminate between aggressive and slowly progressive PMP in patients who have incomplete surgical resection, with a sensitivity of 80.0% and a specificity of 93.3%. This technique may help early stratify patients with a poor prognosis and those who might benefit of additional chemotherapy. Conversely, patients with slowly progressive PMP as identified using post-operative BFV measurement may be considered for withdrawal from therapy.

We found that patients who had complete CRS and HIPEC (group-1) had post-operative SMA BFV values similar to those of healthy controls. These patients are assumed to have a very good prognosis. The return to normal BFV values in the SMA after surgery suggests the absence of renewed tumor vascular network development and thus the absence of recurrence of the PMP. Conversely, a persistently high SMA BFV one year after surgery suggests continued progression of the tumor vasculature to meet metabolic needs, thus reflecting recurrence of the disease.

According to Poiseuille's law, local BFV is adjusted by variations of local hemodynamic resistance.[19] When tumor tissues develop on or in an organ, metabolic demands increase, inducing sprouting angiogenesis from the vascular network of the native organ, resulting in an extension of the micro-vascular network downstream from the feeding artery of the affected organ. Local hemodynamic resistance thus falls and the BFV in the feeding artery increases.

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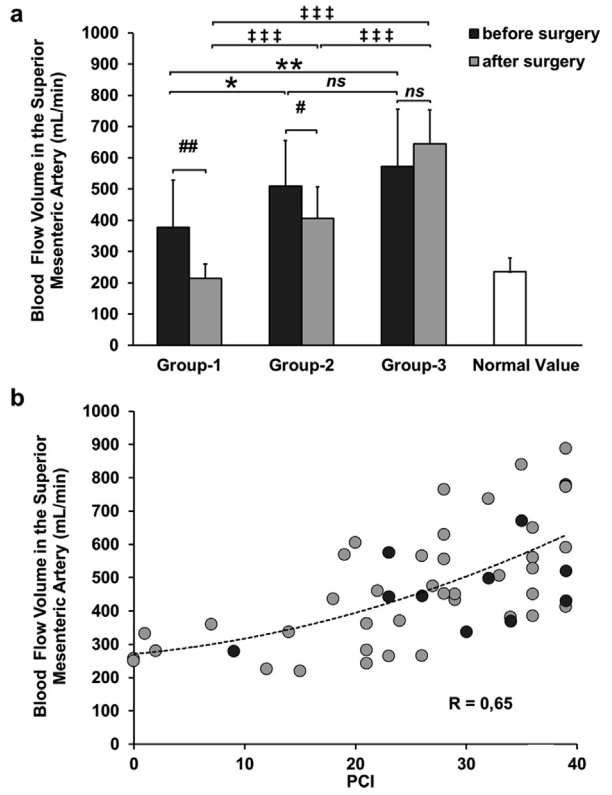

Figure 3. (**a**) Blood flow volume (BFV) in the superior mesenteric artery (SMA) before and after surgery. (*p < 0.05, **p < 0.01, baseline BFV in the SMA related to the patients groups; #p < 0.05; ##p < 0.01, BFV measured before and after surgery; ‡‡‡p < 0.001, BFV measured after surgical treatment related to patients groups). (**b**) Polynomial regression between blood flow volume (BFV) measured in the superior mesenteric artery (SMA) and peritoneal carcinomatosis index (PCI) before surgery. Tumours grade are noted with coloured dot. (● low grade, ● high grade).

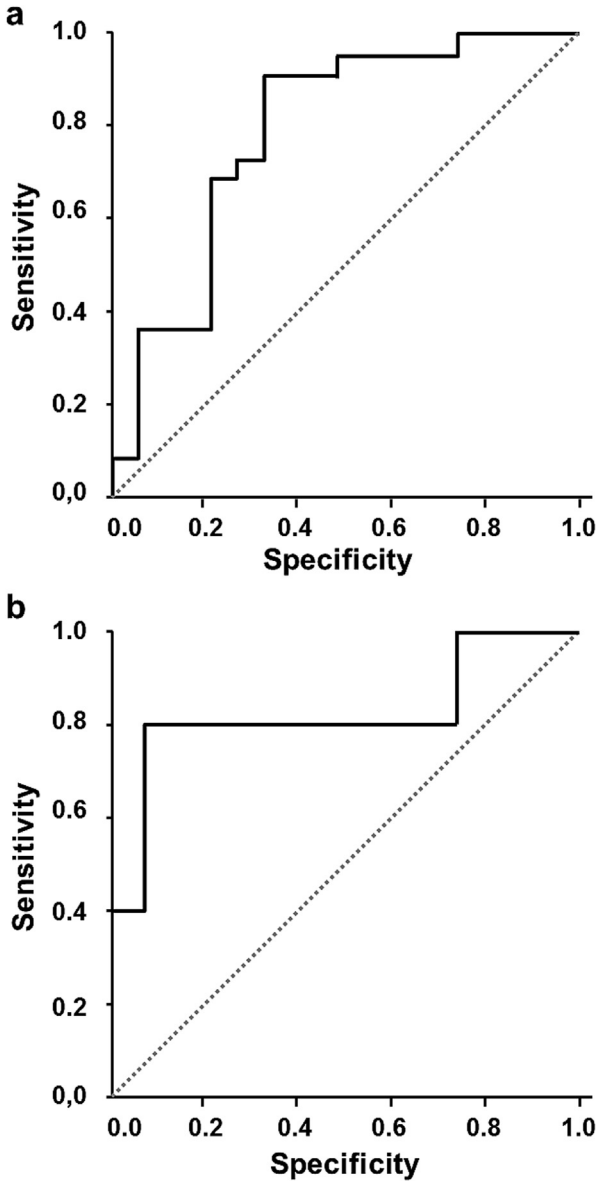

Figure 4. (**a**) Graph shows ROC curves for pre-surgical BFV in differentiating between patients with resectable PMP (group-1) and patients with unresectable PMP (groups-2 and -3). The area under the curve was 0.794 [95%CI: 0.650−0.932]. A pre-operative BFV > 450 mL min^{-1} yielded a sensitivity of 68.2% and a specificity of 78.9%for predicting incomplete resection (group-2 or -3). (**b**) Graph shows ROC curves for post-surgery BFV in differentiating between patients with aggressive PMP (group-3) and patients with slowly progressive PMP (group-2). The area under the curve was 0.827 [95%CI: 0.565−1.00]. A post-operative BFV >530 mL min^{-1} yielded a sensitivity of 80.0% and a specificity of 93.3% for the diagnosis of aggressive disease (group-3).

Although it has been suggested, but only in small-scale studies, that tumor grade correlates with vascularity and degree of enhancement on MRI or degree of glucose uptake on positron emission tomography,[20,21] pre-operative SMA BFV did not correlate with tumor grade in our study. Moreover, it has been suggested that CEA levels may aid follow-up decisions in high grade PMP, but serum CEA levels did not reflect tumor activity, PCI or disease free survival.[22]

There was no difference in survival between patients with low and high grade PMP in our series, but this might be because of the low rate of high grade patients. As PMP is rare and high grade PMP less frequent than low grade PMP, validation of criteria predicting the grade would require a larger number of patients which is rarely achieved in imaging studies.[23]

Unresectable high grade PMP is usually treated with chemotherapy and there is no consensus on the management of unresectable low grade PMP. Our study shows that SMA BFV might be helpful to stratify patients with indication of antiangiogenic treatment (high-perfused PMP).[8,16] Our hypothesis was that a decrease in SMA BFV was related to the decrease in tumor burden and to the decrease of angiogenic activity of the tumor. This functional relationship has been previously shown in a murine model of PMP.[16]

While our cohort was small (49 patients), it was nevertheless sizeable for a single-center prospective study PMP and a rare disease. Our rare cancer group network (RENAPE) treated ~100 patients affected by PMP during the last 10

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years. Follow-up was in some cases compromised by distance from patients home and the hospital. In addition, because of advanced age (>80 years) some patients were excluded from the HIPEC procedure. This could explain why we did not observe recurrence in group-1 patients. The current study should thus be expanded to other reference centers to validate BFV as a biomarker of disease activity, particularly in combination with MRI. Doppler-US offers a quantitative evaluation of SMA BFV. Although the technique is known to be operator-dependent, the application of strict procedural measurement protocols reduces inter-observer variation.

In conclusion, our results suggest that post-operative BFV measured in the SMA with Doppler-US helps differentiate aggressive versus slowly progressive PMP in patients who had incomplete surgical resection. This technique may allow the early identification of patients who may benefit from post-operative chemotherapy and those who have achieved full benefit from therapy (CRS ± HIPEC). Moreover, we found that pre-operative BFV correlates with surgical PCI and might be helpful to predict resectability. Assessment of BFV in the SMA may thus serve as a quantitative biomarker that adds functional information to diagnostic morphological techniques. Doppler-US imaging is a widely accessible, cheap, easy-to-repeat and non-invasive modality that could allow functional evaluation of tumor progression to be included in future decision algorithms and scoring systems. Our study is the first to report measurement of the BFV in the SAM to follow the progression of a peritoneal disease. This study represents the necessary pilot study to establish the proof of concept but needs further validation by multicenter clinical trial.

Conflict of interest statement

The authors have no potential conflicts of interest to disclose.

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Original Article (currently, in reading by the authors)

Neoadjuvant bidirectional chemotherapy combining intraperitoneal Docetaxel with intravenous 5-fluorouracil and oxaliplatin for patients with non-resectable peritoneal carcinomatosis from gastric cancer: the first pilot study in Western countries

Running head:

Neoadjuvant bidirectional chemotherapy for peritoneal carcinomatosis from gastric cancer

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Drafting and editing of manuscript: All authors

Critical manuscript review and approval of final version: All authors

ABSTRACT

BACKGROUND: In patients with synchronous peritoneal spread from gastric cancer, only palliative treatment is proposed. Asiatic surgeons, develop a new concept using bidirectional combination of intraperitoneal and intravenous chemotherapy with high response rate and low incidence of toxicity. We conducted the first pilot study in Western country, using bidirectional combination of European-standard drugs for gastric cancer. The main endpoint was to evaluate the feasibility and safety of this treatement. Secondary endpoint was success of therapeutic strategy reflected by overall survival (OS) and the decrease of 25% of attempted peritoneal cancer index (PCI) evaluated by laparoscopy.

METHODS: All patients with non-resectable peritoneal carcinomatosis (exclusive?) from gastric cancer, confirmed by laparoscopy, underwent a bidirectional chemotherapy using intraperitoneal docetaxel and intravenous 5-fluorouracil (5FU) and oxaliplatin (FOLFOX). Docetaxel at 30mg/m2 administrated intraperitoneally at day 1, 8 and 15 and IV FOLFOX intravenous at day 1 and 15 followed by 7 days' rest, as one course. After three courses, the PCI was evaluated with a second laparoscopy.

RESULTS: We enrolled six consecutives patients. The average age was 47.1 years [range 24-66], performance status ECOG 0-1. The mean PCI was 34 (range 30-39). After one bidirectional cycle, major complications (grade 3/4) occurred in two patients (hematologic and asthenia). One patient had major PCI response, 3 partial PCI response and 2 clinical progressions. The mean PCI decrease to18 (range 12 – 29). The median OS was 10.3 months (range 5-23); 1-year OS rate was 50%. One patient was operated with CC0 resection after major response, with a PFS of X months

CONCLUSION: This pilot study confirms the feasibility and safety of bidirectional treatment with IP and IV chemotherapy for patients with advanced gastric cancer and peritoneal carcinomatosis, resulting in 10.3-months median OS with limited morbidity. The decrease of PCI after one bidirectional cycle is promising. Further phase I-II studies are required for the validation of that strategy.

INTRODUCTION

More than 50% of patients with advanced gastric cancer patients die of peritoneal recurrence. Peritoneal carcinomatosis (PC) is frequent (up to 20%), induces symptoms and often limits treatment options. The median overall survival (OS) of patients with PC from gastric cancer treated with chemotherapeutic agents such as taxanes, platinum salts and 5-fluorouracile (5-FU) is poor, between 3 to 8 months in HER-2 negative tumors (Wagner Cochrane Database Sist Rew 2010, Thomassen IJC 2014). This limited survival did not really increase during the last years, for that new treatment options are required. Several reports have suggested that cytoreductive surgery and peritonectomy procedures combined with hyperthermic intraperitoneal chemotherapy (HIPEC) and/or postoperative intraperitoneal chemotherapy may lead to cure in selected patients with PC from various digestive and extra-digestive cancers (Glehen Lancet Oncol 2004, Armstrong NEJM 2006). However, for PC from gastric origin, the efficiency of this combined procedure remains highly controversial. The experience of few single institutions have reported encouraging survival results, in patients treated with cytoreductive surgery (CRS) combined with intraperitoneal chemohyperthermia (HIPEC) (Glehen Arch Surg 2004, Yonemura Surgery 1996). Moreover, many patients are not candidates to such treatment and consequently treated with palliative systemic chemotherapy only.

In such patients, Asiatic surgeons have recently proposed a new concept using neoadjuvant intraperitoneal and systemic (NIPS) chemotherapy associated with high response rate and low toxicity (Yonemura EJSO 2006, Yonemura JSO 2009). This bidirectional treatement combined intraperitoneally administration of docetaxel and systemic administration of 5-FU or administration of oral S-1. Japan authors claimed that such chemotherapeutic agents comnination, known to be active on gastric cancer, can increase the rate of patients eligible for CRS and HIPEC procedure, and potentially offer curative approaches with acceptable toxicity (Canbay Ann Surg Oncol 2014). However, gastric cancers in Western country have been considered as different from gastric cancer

in Japan in terms of epidemiology and clinical response to the surgery (Kim Medicine (Baltimore) 2016). More, the oral S-1 is considered as inefficient on European Caucasian patient and is not delivered in Europe. We planned a novel therapeutic strategy for Western Country combining administration of IP docetaxel with IV FOLFOX-4 (5-fluorouracil and oxaliplatin) in patients with non-resectable PC from gastric cancer in order to facilitate the setup of a phase I trial. The main endpoint of our pilot study was evaluated the feasibility and safety of this neoadjuvant bidirectional treatement. Secondary endpoint was to evaluate the success of therapeutic strategy reflected by OS and the decrease of 25% of the peritoneal spread evaluated by laparoscopy.

METHODS

Patients

From Mars 2014 and Mars 2016 the patients were enrolled in this first pilot study in Western country. Informed consent, according to the Institutional Guideline, was obtained from all patients. The inclusion criterias were: age between 18 to 65 years; histological evidence of primary gastric adenocarcinoma (endoscopy with biopsie) and PC (confirmed by laparoscopy, synchronous or metachronous); extended carcinomatosis considered non resectable with a PCI index ≥ 15; absence of hematogenous metastasis and remote lymph node metastasis (ovarian metastases were considered as a manifestation of peritoneal disease (Evers Br J Surg 2011); Eastern Clinical Oncology Group (ECOG) score (Luckett Ann Oncol 2011) less than 2; adequate bone marrow, liver, cardiac, and renal function; absence of other severe medical conditions or synchronous malignancy, absence of contraindication for major surgery.

Standardized Data Collection

All patients underwent a total body CT-Scan and gastric endoscopy with multiples biopies to confirm the primitive gastric cancer. PC was diagnosed by systematic biopsy using laparoscopy and the cytologic examination of ascites.

Surgical Procedure

A laparoscopic exploration was performed with a 30° optic by Single Incision Laparoscopy Surgery (SILS) approach (Najah Surg Endosc 2016): a single-port was placed through the umbilicus. The extent of PC was assessed intraoperatively with the Sugarbaker's Peritoneal Cancer Index (PCI) (Jacquet Cancer Treat Res 1996). The assessment of the completeness of the cytoreduction (CCR-), performed at the end of the surgical procedure, as previously described (Jacquet Cancer Treat Res 1996). After the cytological, histological diagnosis and complete evaluation of peritoneal

dissemination, a port system (Bard Port, C.R. Bard Inc., USA) was introduced into the abdominal cavity, the tip placed on the cul-de-sac of Douglas and the port introduced through a 3 cm skin and fascia incision (Kuschnir Clinical Ovarian & Other Gynecologic Cancer 2012).

Bidirectional Chemotherapy

All patients received the bidirectional treatement in the Medical Oncological Departement: Docetaxel at 30mg/m2 was administrated intraperitoneally over 30 min in 1000 ml of saline at day 1, 8 and 15 and IV 5FU and oxaliplatin intravenous at day 1 and 15 followed by 7 days' rest, as one course (Figure 1). A systematic Granulocyte colony-stimulating factor was administered at the investigator's discretion. Before and after bidirectional treatment, 500 ml of saline was injected into the peritoneal cavity through the port, and fluid was recovered for cytology. After three courses, the PCI response was evaluated with a second laparoscopy. If major response was observed and carcinomatosis was evaluated resectable, surgery was performed. If partial response or stability with non resectable carcinomatosis was observed, treatment can be repeat for others three courses, followed by a new laparoscopic evaluation. If progression was observed, patient was proposed for a palliative care (new line of systemic chemotherapy or best supportive care).

Endpoints

The primary endpoint of our pilot study was to evaluate the feasibility and safety of combined bidirectional IV and IP chemotherapy. The secondary endpoints were the OS, the succes of combined therapeutic strategy, the quality of life, postoperative morbidity and mortality. The National Cancer Institute Common Toxicity Criteria (NCICTC) version 2 was applied to evaluate adverse drug reactions (Common Terminology Criteria for Adverse Event" (CTCAE)). OS was defined as the time from the diagnosis of primary tumor to the time of death due to any cause. Success of combined therapeutic strategy was defined by a 25% decrease of PCI betwen two laparoscopy. For evaluation of performance status we used the ECOG score. Quality-of-life (EORTC QLQ-C30) assessment was

performed at two times: at registration of the patients and after the administration of the first course of the bidirectional treatement. The morbidity of the surgical procedure was defined according to the Dindo-Clavien classification (Dindo Ann Surg 2004). All in-hospital complications were included. All patients were followed up until clinical progression and/or death, if it occurred.

RESULTS

Patient Characteristics

Six consecutives patients were included in this pilot study. Four of them were female (66.7%). The average age was 48 years (range 24-66 years). Patient characteristics are summarized in Table 1. Four patients had synchronous PC and the two others had isolated peritoneal recurrence after gastrectomy. Cytology of peritoneal fluid was positive in 5 patients (83%) before the bidirectional treatment. Four patients had ascites at diagnosis (66%). Six patients underwent one complete cycle of NIPS, one patient had a second cycle. Four patients (66%) had second laparoscopy and only one patient a third laparoscopy.

Outcomes

The average PCI during first laparoscopy was 34 (range 30-39). The average PCI during the second laparoscopy decreased to 18, therefore a reduction of 46%, well above to the planned cut-off (Table 2). After the first Bidirectional cycle: one patient (17%) had major response and consequently underwent cytoreductive surgery with HIPEC with curative intent (Figure 2); three patients (50%) had partial response with lower PCI; two patient had progression of disease (33%). One patient underwent a second cycle of NIPS with progression of PC diagnosed during the third laparoscopy. Peritoneal cytology remained positive in the 5 patients with previous positive cytology. Median Follow-up was 12 months. Median overall survival was 10 months (range 5-23 months), 1-year OS was 50%. Two patients died for chronic occlusive symptoms severe malnutrition. Four patients were alive at the time of analysis. During the first cycle, two patients had grade 3/4 complications: one patient had grade 3 bone marrow suppression and one patient severe asthenia (Table 3). Adverse effects occuring during the procedure are summarized in Table 4. After one cycle, ascites decreased in only one patient. Four patients had a good quality of life during the NIPS chemotherapy: one patient an ECOG 0, three patients had an ECOG 1 and two patients an ECOG 2.

DISCUSSION

PC from gastric cancer was considered as a terminal disease (Sadeghi EVOCAPE 1 Cancer 2000). Patients who are not amenable to curative resection generally receive palliative chemotherapy to control related symptoms and improve OS (Wagner Cochrane 2010). Despite new drugs regimen, emerging strategy data and better understanding of tumor biology, OS remains poor in metastatic gastric cancer (Guimbaud JCO, Bang Lancet, Sohn BH Clin Cancer research 2017). Unfortunately, systemic neoadjuvant chemotherapy has never significantly downstaged peritoneal seeding and many consider the presence of carcinomatosis as an inadequate therapeutic option (Kamarag J Clin Oncol 2006).

Cytoreductive surgery (CRS) combined with HIPEC is accepted as the only potentially curative treatment for colorectal PC (Glehen J Clin Oncol 2004, Glehen Cancer 2010, Verwaal Surg Oncol 2008). Similarly, recent studies have suggested that radical resection of macroscopic disease and perioperative chemotherapy to treat microscopic disease could be the potentially curative treatment for advanced gastric cancer with limited carcinomatosis (Yonemura EJSO 2010, Glehen Ann Surg Oncol 2010). The new locoregional therapeutic approaches combining cytoreductive surgery with perioperative intraperitoneal chemotherapy suggest improved survival (Glehen Arch Surg 2004). In a Glehen's study including 159 patients with gastric carcinomatosis treated by cytoreductive surgery followed by intraperitoneal (hypertermic or not) chemotherapy with curative intent, the median OS was 9.2 months and 1-, 3-, and 5-year survival rates were 43, 18, and 13%, respectively. (Glehen Ann Surg Oncol 2010). Moreover, there is still no a standard of therapy for the intraperitoneal treatment for gastric PC. Nevertheless, this large study of Glehen et al. demonstrates that long-term survival for patients with PC from gastric cancer is a realistic endpoint in selected patients and PC should not be considered a terminal event. The high rate of mortality (27.8%) underlines the necessity of strict selection of patient.

A multimodality approach including neoadjuvant systemic chemotherapy followed by surgery appears as a reasonable strategy for the tumour down staging and the early elimination of micrometastases to improve OS. Two randomized trials comparing perioperative chemotherapy with surgery alone, showed efficacy of this approach in resectable gastric cancers (Cunningham NEJM 2006, Ychou JCO 2011). More recently, the FLOT regimen has emerged as as new standard of care in the peri-operative setting (Al Batran, Lancet oncol 2016, ASCO 2017).

Neoadjuvant chemotherapy has been proposed as a treatment modality that would increase the rate of gastric cancer patients with peritoneal seeding who could receive a complete clearing of the peritoneal dissemination (Yonemura Surgery 1996). This concept is close to what is nowadays done for unresectable colorectal liver metastases (Karanicolas Curr Oncol 2014, Kemeny JCO 2009). The approach with neoadjuvant intravenous chemotherapy combined with intraperitoneal chemotherapy without hyperthermia has shown its efficacy with an acceptable toxicity profile in Japanese trials (Yonemura J Clin Oncol 2009, Ishigami Ann Oncol 2010, Imano Eur Surg Res 2011, Fujiwara J Surg Oncol 2012, Fushida Cancer Chemother Pharmacol 2013, Yamaguci Cancer 2013). However, in Caucasian patients, the efficacy of this bidirectional treatment remains to be evaluated. The present pilot study designed to determine the safety and efficacy of neoadjuvant bidirectional chemotherapy (IP and IV) for patients with unresectable gastric carcinomatosis. To the best of our knowedge, no prior report on that topic have been published in western Europe.

The peritoneal cavity acts as a sanctuary against systemic chemotherapy due of the existence of a blood peritoneal barrier consisting of stromal tissue between mesothelial cells and submesothelial blood capillaries. This barrier accounts for a total thickness of 90 μm and inhibits the movement of drugs from submesothelial capillaries to the peritoneal cavity. Accordingly, only a small amount of systemic drugs is capable of penetrating this barrier and passing into the peritoneal cavity so a higher percentage of the administered drugs instead moves to the bone marrow and vital organs other than the peritoneum, resulting in the development of adverse effects (Jacquet Kluwar Academic Publisher

1996). The fundamental goal of IP administration is to maximize the total amount of drug delivered into the peritoneal surface, while minimizing that delivered to the systemic circulation.

The efficacity of IP taxane therapy was demonstrated in phase II studies in advanced gastric cancer (Yonemura J Clin Oncol 2009, Fujiwara J Surg Oncol 2012, Fushida Cancer Chemother Pharmacol 2013). Pharmacocynetic studies shown that IP chemotherapy provides high concentrations of a cytotoxic agent directly to the peritoneal space (Dedrick Cancer chemother Pharmacol 1978, Minchiton Nat Rev Cancer 2006, Yonemura EJSO 2010 Review). Systemic concentrations of drugs are, however, achievable because of absorption of the agent through the peritoneal surfaces. Taxanes (docetaxel and paclitaxel) are hydrophobic with high weitgh molecular drugs. When itraperitoneally administrated, the taxanes are drained from the peritoneum lymphatic stoma into the pleural space (Flessner Am J Physiol 1985, Wang Anat Rec 2010). The taxanes drug molecules, remaining in high IP concentration for 48-72h in contact to the peritoneal nodules, produce antitumoral effect making them the ideal chemotherapic agents for intraperitoneal administration (Table 5).

Morgan et al. established that administration of IP docetaxel can be safely delivered at a dose of 100 mg/m2 every 3 weeks (Morgan Cancer Clin Res 2003). According to phase Ⅰ studies, the recommended doses (RD) of IP docetaxel combined with oral cancer drugs (TS-1) are 45-60 mg/m2 (Fushida Cancer Chemothep Pharmacol 2013, Fujiwara Anticancer Res 2010). Yonemura et al., associating dual IP anticancer drugs, lower the concentration of IP docetaxel to 30 mg/m2 with milde toxicity (Yonemura JSO 2009). However, in our experience, we use a concentration of 30 mg/m2 taxotere to reduce toxicity when administrated in associate with FOLFOX IV. Therefore, we preferred to use a lower dose than that reported in the first papers in literature. In a previous report, adverse effects after NIPS were found in 11% (9/81) of cases, and no chemotherapy related deaths were experienced (Yonemura WJGO 2010). Accordingly, in our study only two patients had grade 3-4 toxicity correlated to systemic chemotherapy. Repetead paracentesys for refractary ascite was necessary for 4 patients with consequent severe malnutrition for one of them. During the

laparoscopies, no abdominal adverse effects were reported except one infection of the catheter; the abdominal pain was controlled with mild analgesics. For that NIPS appears to be a safe method for induction chemotherapy.

In our experience, we have evaluated only the macroscopic response to NIPS with the laparoscopy. Major difference regarding carcinomatosis is that preoperative radiologic evaluation is inefficient to evaluate carcinomatosis (resectability and peritoneal index). For that laparoscopy is mandatory (Valle Eur J Surg Oncol 2006, Najat Surg Endosc. 2016) and used to place a peritoneal access chamber (PAC). If IP chemotherapy is performe during the perioperative period when adhesions have not yet developed, the entire abdominal cavity can be equally treating. The number of NIPS chemotherapy treatments depends on the effect on tumors and the accurate preoperative evaluation of PC is mandatory to propose the secondary complete cytoreduction of PC.

In our study, only one patient had a complete CRS followed by HIPEC. However, in the study of *Yonemura et al.,* 30 of 61 enrolled patients had an operative intervention and 14 of them could be made disease-free with a long-term survival (20.4 and 15 months of OS, respectively) and without major toxicities (Yonemura EJSO 2006). However, in our study we enrolled patients with high volume of carcinomatosis (PIC >15) while the heterogeneity of the population in Asiatic (patients with macroscopic carcinosis and patients with only positive cytology withouth macroscopical carcinosis) was probably responsible to the good results in term of OS.

CONCLUSION

The combination of IV and IP chemotherapy should be considered in patients with carcinomatosis from gastric cancer. Accordingly, the bidirectional chemotherapy appears to be safe and may be the preferred strategy in the pre-operative setting in highly selected patients with young age, ECOG 0-1 status, normal nutritional status and elevated PCI. The NIPS procedure should be evaluated more extensively in phase I-II studies.

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FIGURES AND TABLES:

Table 1. Demographic, clinical and histological characteristic of the patients included

ADK, adenocarcinoma; ACDI adenocarcinoma independent cells; neg, negative; PC, peritoneal carcinomatosis; Pres-NIPS sCT, previous neoadjuvant intra-petitoneal systemic chemotherapy; DOC, docetaxel

Patient	Sex	Age, Years	Histology	Cytology	Her2+	PC Type	Prev-NIPS sCT	NIPS sCT IV	NIPS CT IP
1st	M	66	ADK	neg	neg	Recurrence	3 ECF, 12 Folfox	LV5FU2	DOC
2nd	F	48	ADCI, linitis plastica	+	neg	Synchronous	No	FOLFOX	DOC
3th	M	42	ADCI, linitis plastica	+	1+	Recurrence	8 TEFOX	FOLFOX	DOC
4th	F	45	ADCI, linitis plastica	+	2+	Synchronous	No	FOLFOX	DOC
5th	F	24	ADCI, linitis plastica	+	2+	Synchronous	No	FOLFOX	DOC
6th	F	60	ADCI, linitis plastica	+	2+	Synchronous	8 TEFOX	LV5FU2	DOC

Table 2. Early and long Outcomes for the patients treated by bidirectional treatment

Patient	Cycles of NIPS	IP tolerance	ECOG	1° Laparoscopy PCI (N = 6)	2° Laparoscopy PCI (N = 4)	Results	CRS +/- HIPEC	OS (months)
1st	1	good	1	34	-	Progression	0	15
2nd	1.5	medium	2	30	12	Good response	0	5
3th	1	medium	2	30	-	Progression	0	6
4th	1	good	0	36	13	Good response	CRS + GT+	23
5th	2	good	1	39	29	Partial response	HIPEC	14
6th	1	godd	1	32	18	Good response	Ovariectomy 0	7
Mean				**33.5**	**18**			**10.3**

NIPS, neoadjuvant intra-petitoneal systemic chemotherapy; IP, intraperitoneal; PCI, peritoneal cancer index; CRS, cytoreductive surgery; GT, total gastrectomy;

Table 3. Toxicity during the bidirectional treatment

Toxicity*			
Not IP catheter-related	**Grade 1-2**	**Grade 3-4**	**Total**
Anemia	2	0	**2**
Leucopenia	2	1	**3**
Febrile neutropenia	0	0	**0**
Thrombocytopenia	1	0	**1**
Asthenia	2	1	**3**
Diarrhea	1	0	**1**
Neuropathy	1	0	**1**
Nausea/Vomiting/Dehydration	1	0	**1**
Renal/metabolic	0	0	**0**

* Toxicity was assessed during the the bidirectional treatment according to the National Cancer Institute (NCI-CTC)

Table 4. Adverse effects during the bidirectional treatment

Adverse effects				
Catheter- related	**1° Laparoscopy PCI (N = 6)**	**2° Laparoscopy PCI (N = 4)**	**3° Laparoscopy PCI (N = 1)**	**Total**
IP catheter Infection	1	0	0	**1**
IP catheter blocked	0	0	0	**0**
Acces Problems	0	0	0	**0**
Possibly IP Treatment related	**1° Laparoscopy PCI (N = 6)**	**2° Laparoscopy PCI (N = 4)**	**3° Laparoscopy PCI (N = 1)**	
Other Infection	0	0	0	**0**
Abdominal pain	1	1	1	**3**
Patient refusal	0	0	0	**0**
Bowel complication/ Peritonitis	0	0	0	**0**
Refractory ascites	4	2	1	**7**
Paracentesis	4	1	1	**6**
Severe denutrition	2	0	1	**3**

Table 5. Pharmacocynetic parameters for docetaxel

Docetaxel 40 mg	
Molecular weight (daltons)	861.9
AUC peritoneal/plasma ratio	207*-552
Drug penetration distance in IP administration	NA
Recommended IV dose (mg/m2)	100
Recommended IP dose (mg/m2)**	45-60

* in hyperthermic chemoperfusion; ** combined with oral cancer drugs; AUC, area under curve;

Figure 1. Schema of bidirectional systemic and intrapetitoneal chemotherapy for peritoneal carcinomatosis from gastric cancer

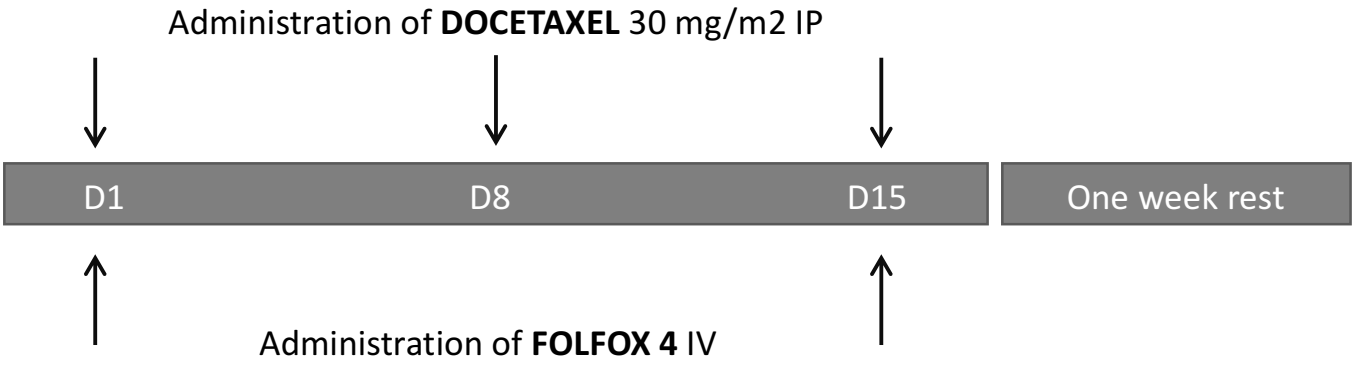
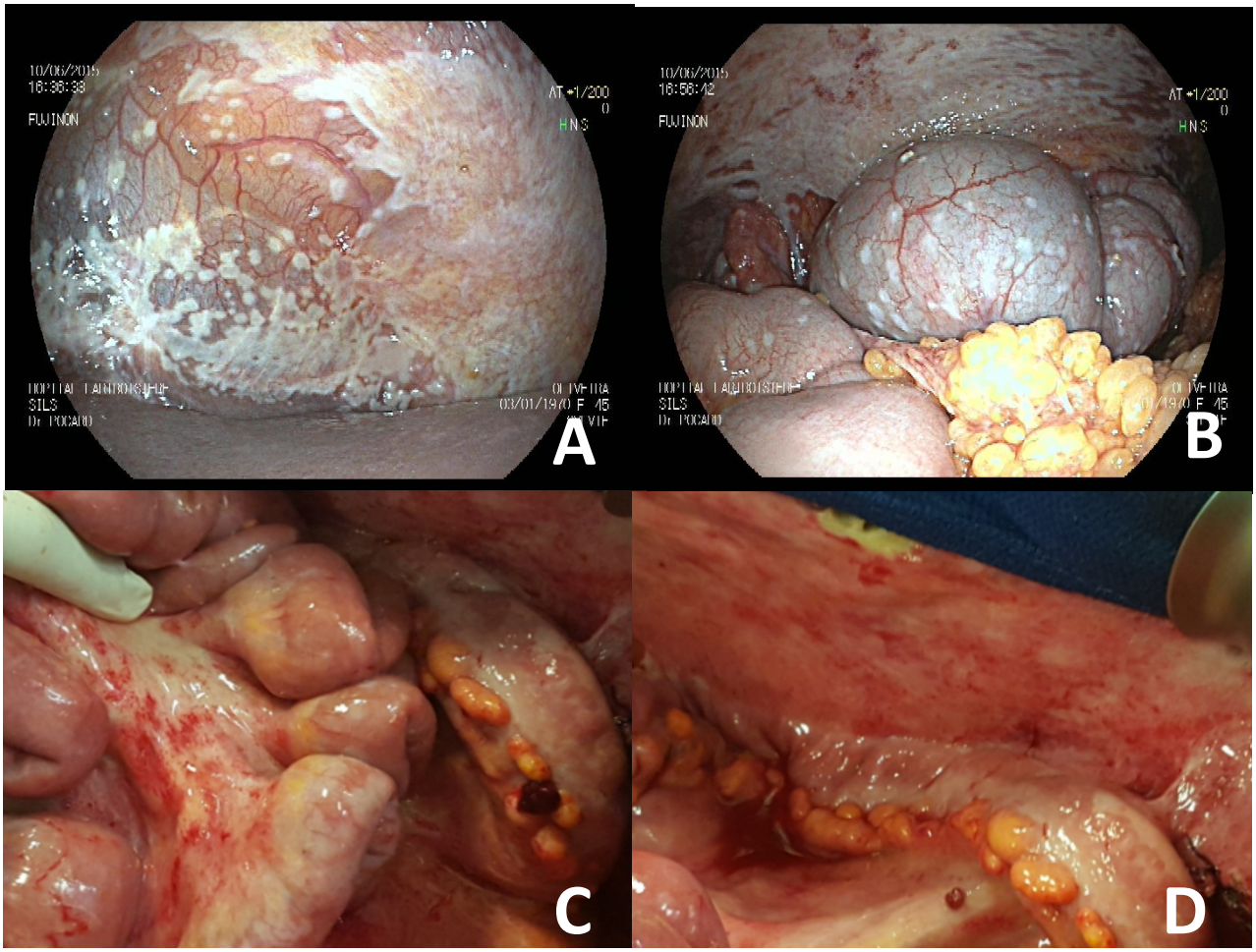


Figure 2. Laparoscopy before and after bidirectional treatment: The first laparoscopy (upper) for staging shows the peritoneal metasatases in the right subphrenic peritoneum (left, A) and the pelvis (right, B). The second laparoscopy (lower), after bidirectional treatment, shows the disappearance of peritoneal metastases in the small bowel (left, A) and in the left parietal peritoneum (right, B).



THESIS SUMMARY

The synchronous presence of liver metastases (LM) and peritoneal carcinomatosis (PC) from colorectal cancer (CRC) is associated with poor outcome and is traditionally considered a contraindication to any surgical approach. However, few series reported a prolonged survival after surgical management, reaching 3 years in selected patients thus suggesting that a curative surgical management may be possible. To date, no standard management pathway has been established, especially if a major liver and peritoneal surgery has to be performed. We postulated that liver regeneration after liver resection could promote PC growth. We constructed an immunocompetent animal model of limited PC. The objective of our study was to analyze the effects of major LR and liver regeneration after hepatectomy on peritoneal carcinomatosis growth and the associated angiogenesis process. Furthermore, we have analyzed a prospective international cohort of patients undergoing synchronous liver resection and cytoreductive surgery with HIPEC. The aim of this study was to describe the outcomes, to identify variables potentially related to poor outcome, in order to establish future guideline for the management of these patients, to optimize the selection of candidates for surgical treatment and determine the best surgical strategy.