FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA - TRABALHO FINAL

MARIA JOSÉ TEMIDO MENDES FERREIRA

CLINICAL AND PATHOLOGICAL FACTORS OF PROGNOSIS AFTER HEPATECTOMY FOR GASTRIC CANCER LIVER METASTASES

Is desmoplastic growth the key to longer survival?

ARTIGO CIENTÍFICO ORIGINAL

ÁREA CIENTÍFICA DE CIRURGIA GERAL

TRABALHO REALIZADO SOB A ORIENTAÇÃO DE:

PROFESSOR DOUTOR HENRIQUE MIGUEL MARQUES BOM BORGES ALEXANDRINO

DR. RUI PEDRO CAETANO MOREIRA DE OLIVEIRA

JANEIRO/2018

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TRABALHO FINAL DO 6º ANO MÉDICO COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

Autores e Afiliações

Autoria: Maria José Temido Mendes Ferreira[1] – mariajosetemido@gmail.com

Orientador: Professor Doutor Henrique Miguel Marques Bom Borges Alexandrino[2,3]

halexandrino123@gmail.com

Co-Orientador: Dr. Rui Pedro Caetano Moreira de Oliveira[4] ruipedrocoliveira@hotmail.com

[1] Faculdade de Medicina, Universidade de Coimbra, Portugal

[2] Clínica Universitária de Cirurgia III – Faculdade de Medicina, Universidade de Coimbra, Portugal

[3] Serviço de Cirurgia A – Centro Hospitalar e Universitário de Coimbra

[4] Serviço de Anatomia Patológica – Centro Hospitalar e Universitário de Coimbra

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Abstract

Introduction**:** Hepatectomy (Hp) might have a definitive role in the treatment of gastric cancer liver metastases (GCLM), due to poor outcomes of other therapies. However, the factors associated with better prognosis that could assist in adequate patient selection are still a matter of debate. Several pathologic factors, such as the growth pattern, have been associated with prognosis in colorectal cancer liver metastases. In spite of this, these factors have never been investigated in GCLM.

Materials and Methods**:** Clinical and pathological review of 19 consecutive patients that underwent surgical resection with curative intent of GCLM between February 1997 and November 2017 at our department. The population is composed of 13 men with a mean age of 66,3±9,9 years. The metastases had a synchronous presentation in 16 patients and were solitary in 11. The mean size was 33,7±23,8mm. The Hp was major in three and synchronous in seven. Major prognostic factors taken into consideration were the patients' gender, age and postoperative course, histopathological characteristics of the primary tumor and of the metastases, as well as timing and extent of hepatectomy. 90-day postoperative morbidity was graded according to the Dindo-Clavien classification. Statistical analysis was done with SPSS™ v. 24.0 (log rank, Kaplan-Meier and Cox regression).

Results**:** Median and 5-year overall survival were respectively 16 months and 21,2%. Major morbidity occurred in four patients, mortality in one. Ten patients developed recurrent disease. Major determinants of better prognosis were: metachronous resection; absence of major morbidity; gastric tumors of antrum or body, earlier T stage and of intestinal type; metastases smaller than 20mm and with desmoplastic growth pattern ($p<0,05$).

Discussion**:** Hp is a valid choice in the treatment of GCLM. Nevertheless, most series have only investigated clinical factors of prognosis. In this study, we confirm several key clinical

factors that are associated with a better prognosis. Moreover, in what is an innovative and so far unreported finding, we observed that desmoplastic growth pattern of the liver metastases, possibly reflecting particular tumor-host interactions, is associated with improved survival.

Conclusion**:** Improvement in survival rates of patients with GCLM is possible with proper selection of patients. Pathologic factors such as growth pattern should prompt further research on tumor-host interactions.

Keywords**:** Liver, metastases, gastric cancer, hepatectomy, histopathology, immunohistochemistry, survival, prognosis.

Introduction

Gastric carcinoma (GC) is the third leading cause of cancer-related death worldwide and the fifth most common malignant tumor [1]. GC shows its highest incidence in Eastern Asia, Eastern Europe and Southern America, particularly in men [2]. In Europe in 2012, there was an incidence of 14,4/100.000 and a mortality of 4,5/100.000 [3]. The aggressiveness of this malignancy is associated with high rates of metastases being the liver, the lungs and the peritoneum the most prevalent sites of dissemination. [4]

Liver metastases are found in up to 37% of the patients with GC after curative gastrectomy [5]. As a matter of fact, the presence of metastatic GC is usually considered a sign of a systemic disease, arguing against the role of surgery with curative intent in the treatment of these patients [6]. GC usually has an aggressive behavior and frequently ends up presenting with relapses, due to the possible presence of micrometastases at the time of surgery [7,8].

Despite the fact that chemotherapy is the advised treatment in advanced, recurrent and disseminated GC and has been the mostly widely used method, it does not achieve long survival [6,9]. Recent literature supports hepatectomy (Hp) as an alternative in selected patients with GCLM, with promising results of 5-year survival rates of about 30% [10,11]. Consequently, Hp in the treatment of gastric cancer liver metastases (GCLM) is still a matter of debate.

Many studies have tried to define the factors associated with longer survival and a more favorable evolution, result of a more indolent biology of the tumor [11–18]. These investigations have been mostly based on clinical variables. However, important pathologic factors, both of the primary tumor and of the metastases, can also be relevant prognostic markers. These factors can be either tumor-related, host-related, or dependent upon the tumor-host interaction. One factor in particular is the growth pattern of the metastases, reflecting

distinct characteristics of the tumor microenvironment. This factor has shown to be relevant in the setting of colorectal cancer liver metastases (CRLM) [19,20].

In our study, we sought to investigate both clinical and histopathological factors, with emphasis on GCLM growth pattern, associated with an improved outcome after Hp. In the future, these factors may assist in the more adequate selection of patients for an aggressive surgical approach to liver-only metastatic gastric cancer. And hopefully, enhanced knowledge on the particular tumor microenvironment could aid in the development of new molecular targets for systemic therapy.

Patients and Methods

Study Design

Review of the clinical and pathological factors of the patients that underwent surgical resection with curative intent of GCLM between February 1997 and November 2017 at *Serviço de Cirurgia A* from *Centro Hospitalar e Universitário de Coimbra* (Head of Department: Professor Doutor Francisco Castro e Sousa [early period] and Professor Doutor Júlio Soares Leite [currently]).

The information was collected from patients' medical records and hospital's database. The study was approved by the institutional ethics committee.

Patients were selected for resection if they had liver-limited disease (excepting the primary tumor), good performance status and had undergone or were undergoing radical gastrectomy with curative intent. In the later experience of our department, from 2011 onwards, adequate response to neoadjuvant chemotherapy was also considered a selection criteria.

Study Population

The population included a total of 19 patients, 13 men and six women. The mean age was 66,3±9,9 years (range 44-79 years); 14 (73,7%) patients were older than 60 years.

The metastases were diagnosed synchronously in 16 (84,2%) patients and metachronously in three (15,8%). Of the patients with synchronous presentation, gastrectomy and hepatic resection were performed in the same procedure in seven (43,8%) patients and performed at different time-points in nine (56,2%). The mean interval between the gastrectomy and the Hp was 6,2±6,7 months and the time interval between the diagnosis and the hepatic resection had a mean of 5,1±6,2 months. A bilobar distribution was found in three patients (15,8%) and the

metastases were limited to one lobe of the liver in 16 (84,2%). The mean size was 33,7±23,8mm.

The primary tumor was multiple in only one (5,3%) – in cardia and antrum - and single in 18 cases (94,7%). In the latter group, the gastric lesion was located in the cardia and fundus in three (16,6%) patients, in the body in ten (55,6%) and in the antrum in five (27,8%). The mean largest diameter was of 5,8±2,5cm (range 1,5-10,5). The T category was T2 in four (21,1%) cases, T3 in seven (36,8%) and T4a in eight (42,1%). Lymph node metastases were absent (N0) in four (21,1%) patients and present in 15 patients (78,9%) as follows: N1 in two (10,5%), N2 in six (31,6%) and N3 in seven (36,8%).

As far as the primary tumor is concerned, neoadjuvant chemotherapy was given to two (10,5%) patients and the other 17 (89,5%) did not receive this treatment modality. No neoadjuvant radiotherapy was performed for the gastric lesions. Adjuvant therapies were performed either as chemotherapy in 12 (63,2%) patients and as radiotherapy in one (5,3%). For the metastases, neoadjuvant chemotherapy was used in 13 (68,4%) patients; the other six (31,6%) did not undergo this therapy. No patient received neoadjuvant radiotherapy. A total of eight patients (42,1%) were given adjuvant chemotherapy after Hp.

The *Association Française de Chirurgie* (AFC) Score was calculated according to Adam *et al.* with a median value of 5 (range 4-9) [21].

Operative Procedures

Our department's technique for Hp has been previously described [22]. Major Hp was performed in three (15,8%) patients and the other 16 (84,2%) underwent minor resection. The procedure was anatomical in ten (52,6%) cases. No laparoscopic resection was performed.

The hepatic pedicle was clamped in five patients (26,3%) with a mean duration of 4,2±9,8 minutes (range 0-34 minutes). Transfusion of red blood cells was performed in three (15,8%) patients and five (26,3%) patients were transfused with plasma.

Postoperative morbidity was defined up to the first 90 days after surgery, graded according to the Dindo-Clavien classification of surgical complications [23] and divided into two groups: no or minor morbidity (no morbidity or grades I and II) and major morbidity and mortality (grades IIIa to V). Posthepatectomy Liver Failure (PHLF) was defined according to the "50-50 Criteria" of Balzan *et al*.[24]: bilirubin level above 2.9mg/dL (50µmol/L) and INR above 1.7 (prothrombin time <50%) on the fifth postoperative day and its severity was graded according to Rahbari *et al*.[25]. Biloma and bile leakage were defined according to Koch *et al*.[26]; and posthepatectomy hemorrhage according to Rahbari *et al*.[27].

Histopathological Analysis

Archive tumor material was examined and reviewed by two experienced pathologists, without knowledge of the patients' clinical data or outcome. Histological examination was performed on Haematoxylin and Eosin (H&E) stained slides observed in light microscope – Nikon Eclipse 50i, and images obtained using a Nikon-Digital Sight DS-Fi1 camera. Immunohistochemical studies were performed on one representative block of the lesion, resorting to avidin-biotin-peroxidase complex detection system and performed on Ventana Marker Platform Bench Mark ULTRA IHC/ISH using the following antibodies: HER-2 (4B5 Ventana, Tucson, AZ- USA), CD44 (SP37, Ventana, Tucson, AZ-USA), CD133 (13A4, Milipore, Temecula, CA-USA) and Ki67 (MIB-1, Dako, Hamburg-Germany).

a) Primary Tumor

The behavior of the gastric malignancy was evaluated according to size, location (esophagogastric junction [EGJ] area, upper portion, middle portion and lower portion),

resection margins, Lauren classification (the tumors were divided into the two types – diffuse and intestinal – or classified as undetermined if none of the above could be applied), World Health Organization (WHO) classification [28], TNM classification, depth of invasion, infiltrative pattern, grading, inflammatory response, lymphatic invasion and perineural infiltration. Immunohistochemistry was performed for Human Epidermal Growth Factor 2 (HER-2) status, expression of cancer stem cells markers (CD44 and CD133) and Ki67 as a marker of proliferation.

The characteristics of the gastric lesion were categorized according to Japanese classification, 3rd edition from Japanese Gastric Cancer Association [29]. Moreover, the histologic types were divided into tubular, papillary, mucinous, mixed, poorly cohesive and non-otherwise specified (NOS).

HER-2 is a receptor with tyrosine kinase activity which have been associated with more aggressive behavior in breast cancer, but which prognostic value in GC is still controversial [30–33]. Its presence, in this study, was detected by immunohistochemistry and graded into 0 and 1+ (negative), 2+ (equivocal) and 3+ (positive – HER-2 overexpression).

Cancer stem cells (CSC) are a population of cells that have the ability to self-replicate and to differentiate into the many heterogeneous types of cells that constitute a tumor [34,35]. CD44 and CD133 are CSC surface markers in gastric cancer [35–40]. Overexpression of these receptors is connected to pathological features like intestinal type, tumor size and grade, serous invasion, advanced stages, lymph node and distant metastases and, as a result, to worse prognosis. [34–36,38,39,41–43]. These glycoproteins were immunohistochemically detected and graded as positive or negative.

Ki67 is only expressed by cells in active phases of the cell cycle. Therefore, it has been widely used as a marker of cellular proliferation of malignancies, but its role as a predictor of

outcome is still controversial [44]. The proliferative ability of the tumor is graded according to the proportion of cells in which Ki67 is detected by monoclonal antibodies. Ki67 proliferative index was counted manually in a printed image after selection of the "hot spot" – field in 400x magnification with the higher signal intensity.

b) Metastases

The investigation of the metastases' specimens was based on the identification of features like: size of the larger lesion, resection margins, growth pattern (GP), inflammatory response, tumor regression grade (TRG), tumor thickness at the tumor-normal interface (TTNI) and HER-2 status. Most histopathological factors studied were adapted from a previous study of our group on pathological markers of prognosis in CRLM [20].

Resection margins were characterized into: R0 (no evidence of residual tumor cells), R1 (microscopic evidence of residual tumor cells) and R2 (macroscopic evidence of residual tumor).

The study of the GP was based on the classification for CRLM of Vermeulen *et al.* and categorized into: desmoplastic pattern (there is a line of stroma with lymphocytes between tumor cells and the healthy liver parenchyma); pushing pattern (the tumor compresses the surrounding hepatocytes leading to their narrowing and forming a plate of elongated cells in a mild inflammatory infiltrate); replacement pattern (there is no compression or inflammatory infiltrate surrounding the tumor – the neoplastic cells only cover the space left by the destroyed hepatocytes in result of the presence of the tumor); and mixed (more than one pattern present).

Tumor regression grade (TRG) and tumor thickness at the tumor-normal interface (TTNI) were firstly studied as a prognostic factor in CRLM [45,46] and we adopted these factors in our study.

As most residual tumor cells after chemotherapy are localized in the periphery of the former tumor, TTNI measures the thickness of remaining tumor.

TRG is another method, originally devised to assess metastases' response to chemotherapy, analyzing their histology after treatment – the ratio between residual neoplastic cells and fibrosis. As described in Rubbia-Brandt *et al.* [46] TRG 1 is characterized by a complete regression of the tumor that is fully replaced by fibrotic tissue; TRG 2 by an high quantity of fibrosis in which the remnant tumor cells are dispersed; TRG 3 has a larger amount of tumor tissue but fibrosis is preponderant; in TRG 4 the area occupied by the tumor is bigger than the fibrotic one and in TRG 5 the tumor did not regress.

HER-2 analysis in the metastases was performed with a similar approach as in the primary tumor: 0 and 1+ (negative), 2+ (equivocal) and 3+ (positive – HER-2 overexpression).

Follow-up

Data to assess the patients' follow-up times was obtained from their medical records and from a national health database. Overall Survival (OS) was defined as the length of time between the hepatic resection and the death of the patient or the last record of the patient being alive. Recurrence was defined as the time between the hepatic surgery and the reappearance of malignant lesions either detected by imaging and/or raised tumor markers. Disease-free Survival (DFS) was measured beginning in the Hp and ending at the time of recurrence or death.

Statistical analysis

The information was analyzed with SPSS™ (version 24.0 for Windows). The survival studies were performed with the Kaplan-Meier method and compared with Log-rank test. Cox-Regression was used to the multivariate analysis. A p value of <0,05 was considered statistically significant.

Results

Postoperative Morbidity

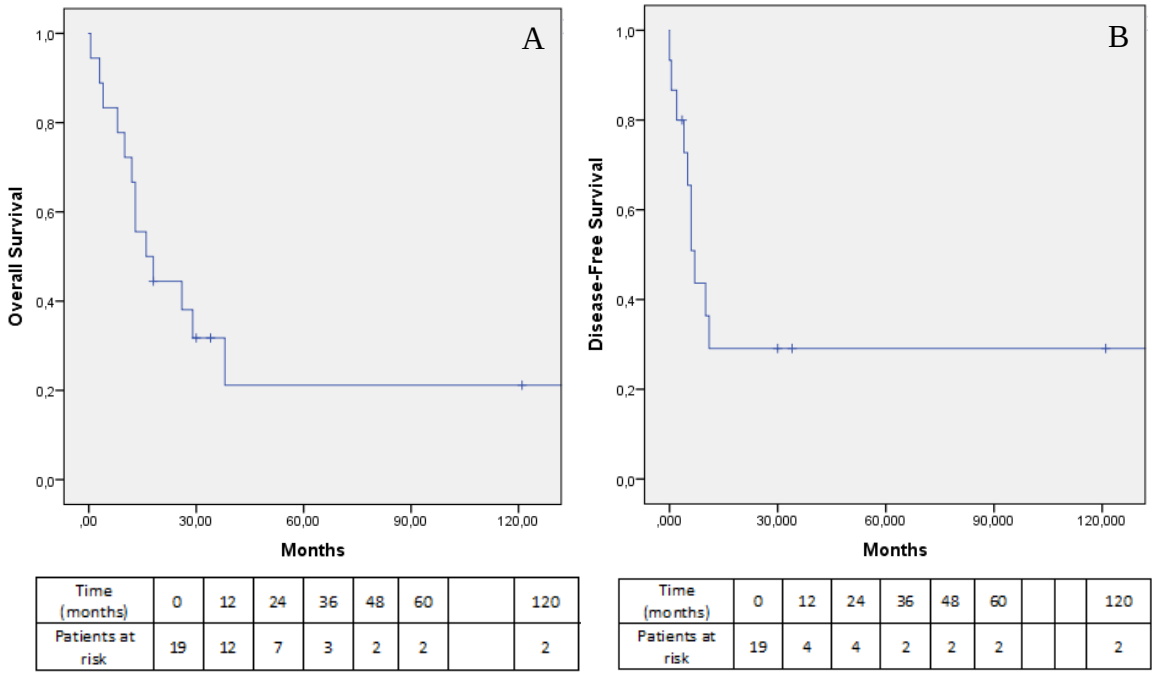
Postoperative mortality occurred in one patient (5,3%) after total gastrectomy, distal esophagectomy and hepatic segmentectomy due to anastomotic leakage, causing severe sepsis and multiple organ dysfunction. Minor morbidity (Dindo grades I and II) was observed in two (10,5%): superficial surgical site infection and intraperitoneal abscess. Major morbidity (Dindo grades IIIa-IVb) was observed in four (21,1%) patients, namely: biliary fistula, biloma, pleural effusion and hemoperitoneum in one case each. Median length of stay was 10 days (range 4-35).

Overall and Disease-Free Survivals

The study had a median follow-up period of 16 months (range 0,5-135). In this period the overall 3- and 5-year survival rates were, respectively, 31,7% and 21,2% (Figure 1). The median overall survival was 16 (range 0,5-135) months.

The disease-free 3- and 5-year survivals were both 29,1% (Figure 1). Recurrent disease was diagnosed in ten (52,6%) patients, having a hepatic location in eight (42,1%), cutaneous in one (5,3%) and peritoneal also in one (5,3%). The recurrent lesions were present in the first six months after the Hp in six (31,6%) cases. There was only one case (5,3%) of reintervention to resect recurrent lesions in the liver.

This disease is known to be the cause of death in eight (42,1%) patients; only one (5,3%) died of other causes; the cause of death is unknown in six (31,6%) patients. In the last follow-up four (21,1%) patients were alive and only one (5,3%) had developed recurrent disease.



Time (months)	0	12	24	36	48	60		120
Patients at risk	19	12	7	3	2	2		2

Time (months)	0	12	24	36	48	60			120
Patients at risk	19	4	4	2	2	2			2

Figure 1. Kaplan-Meier curves of overall survival (A) and of disease-free survival (B) in study population (N=19 patients) undergoing hepatectomy for gastric cancer liver metastases.
A - Median overall survival (OS) was 16 months and 5-year OS was 21,2%.
B - Median disease-free survival (DFS) was seven months and 5-year DFS was 29,1%.

Histopathological findings

The extent of the resection of the primary tumor was determined as R0 in 18 cases (94,7%) and as R1 in only one (5,3%). In the metastatic lesions, the margins were negative in ten patients (52,6%), R1 in five (26,3%) and R2 in one (5,3%).

HER-2 was overexpressed in primary tumor in one (10%) patient and graded as 3+ in four (30,1%) hepatic lesions (Figure 2). In two patients, this receptor was overexpressed in metastases of patients with negative expression in primary tumor.

Immunohistochemistry to detect CD44 was performed in nine patients. One (11,1%) primary tumor was graded as positive (Figure 3).

In three tumors Ki67 was higher than 50%, but this was not statistically significant of prognosis (Figure 4).

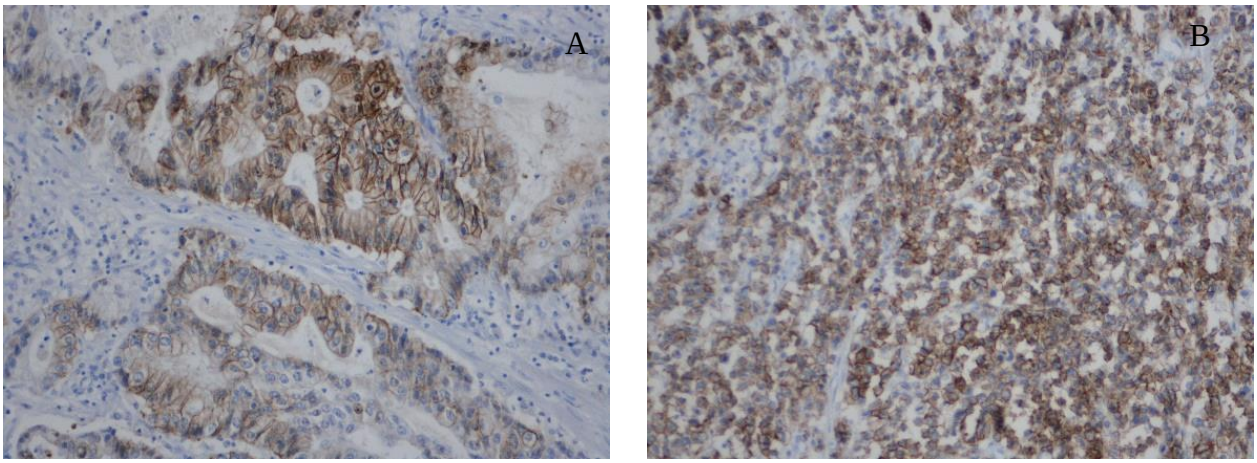


Figure 2. HER-2 overexpression in metastases with complete and basolateral membrane staining in more than 10% of tumor cells.
A - Intestinal type - 200x; B - Diffuse type - 400x.

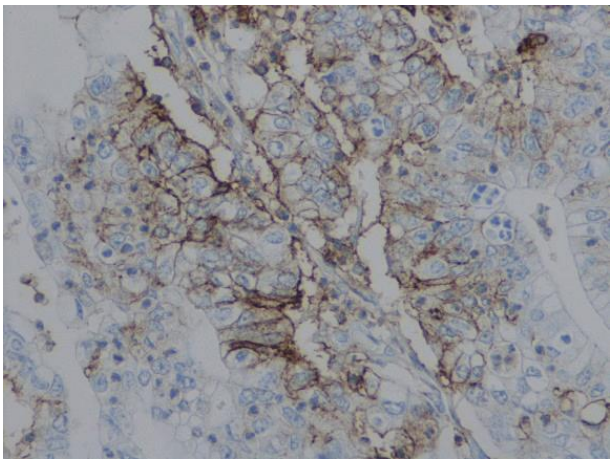


Figure 3. CD44 overexpression in gastric tumor - 200x – membrane staining.

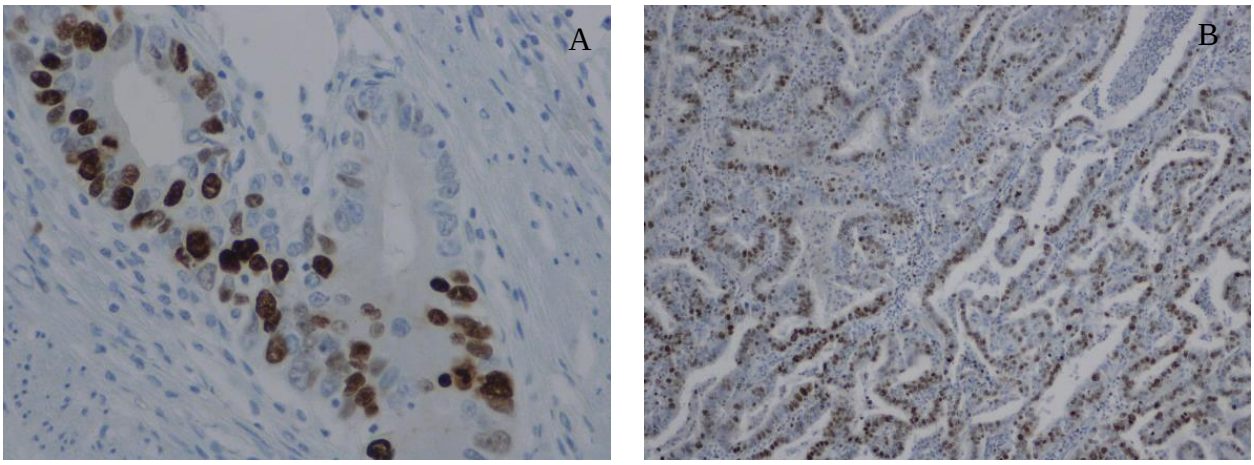


Figure 4. Ki67 proliferative index in gastric tumor (A) 400x and (B) 100x.

The response to chemotherapy in the metastases (TRG 1-4) was detected in ten (76,9%) patients but only one (7,7%) had complete regression of the tumor (TRG 1). There was one (7,7%) case of no regression (TRG 5).

The TTNI was five or more millimeters in eight (61,5%) secondary lesions.

As far as the GP is concerned, it was replacement in six (35,3%), pushing in four (23,5%) and desmoplastic in six (35,3%). There was one (5,9%) case of complete tumoral regression (Figure 5).

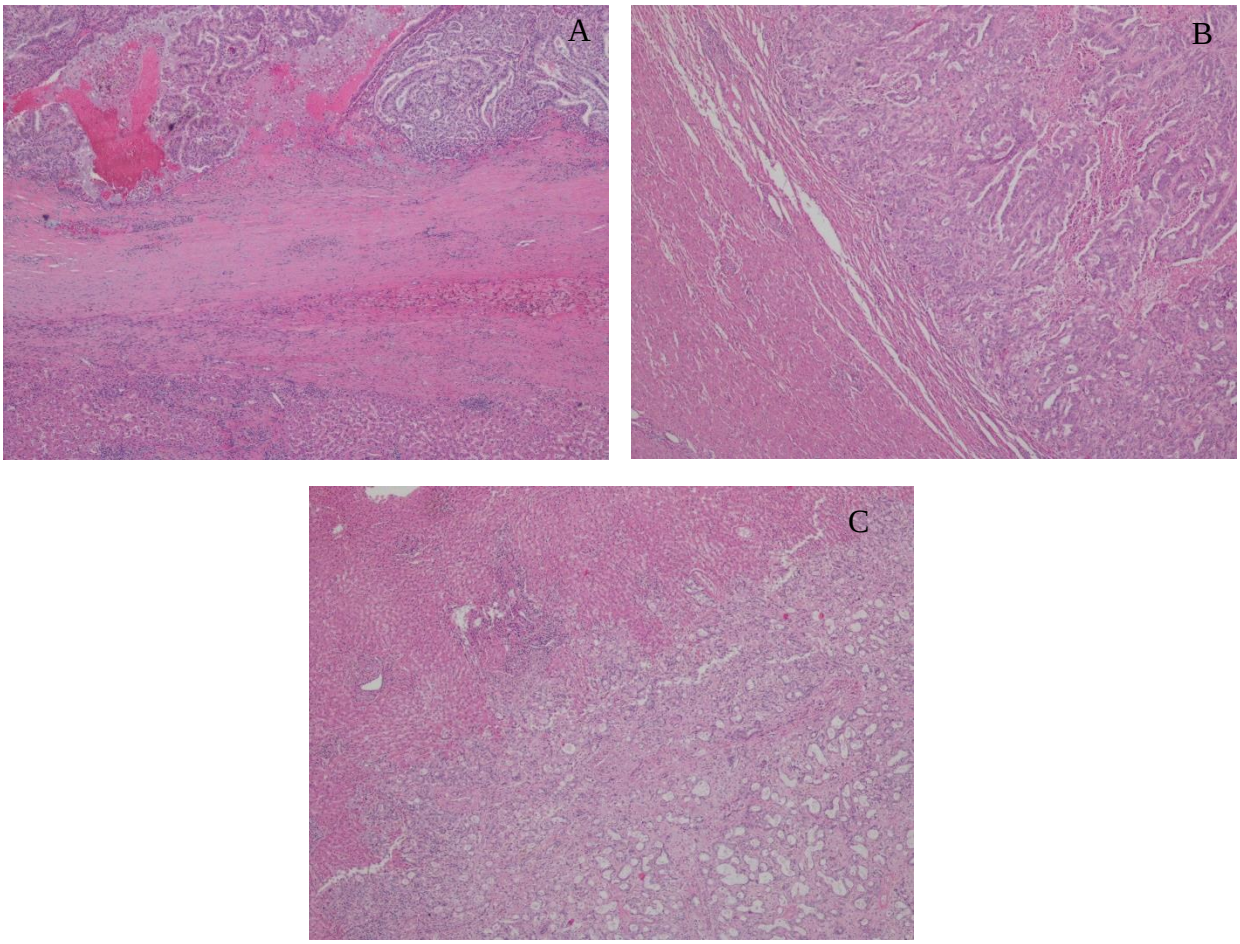


Figure 5 - Growth Patterns in gastric cancer liver metastases.
A - Desmoplastic pattern H&E 40x - there is a band of fibrotic stroma with lymphocytes between tumor cells and the non tumoral parenchyma;
B - Pushing pattern H&E 40x - tumor compresses the surrounding hepatocytes leading to the distortion of the parenchyma;
C - Replacement pattern H&E 40x - the architecture is maintained and neoplastic cells only cover the space left by the destroyed hepatocytes by the presence of the tumor.

Impact of clinical factors in the Disease-Free and Overall Survival

The univariate analysis showed that absence of major morbidity (HR 13,183, p<0,001) and more than eight months between the gastrectomy and the Hp (HR 4,833, p=0,028) were strong predictors of longer OS (Table 1).

Parameters	No. of patients (%)	Overall Survival	
		HR	p
Gender			
Male	13 (68,4%)	0,843	0,359
Female	6 (31,6%)		
Age			
≤60	14 (73,7%)	0,113	0,737
>60	5 (26,3%)		
AFC Score			
<5	5 (26,3%)	0,113	0,737
≥5	14 (73,7%)		
Distribution of metastases			
Unilobar	3 (15,8%)	<0,001	0,988
Bilobar	16 (84,2%)		
Neoadjuvant chemotherapy for metastases			
Yes	13 (68,4%)	0,006	0,936
No	6 (31,6%)		
Timing of Diagnosis			
Synchronous	16 (84,2%)	0,015	0,901
Metachronous	3 (15,8%)		
Interval between Surgeries			
≤8 months	9 (47,4%)	4,833	**0,028**
>8 months	10 (52,6%)		
Timing of Hepatectomy			
Synchronous	7 (36,8%)	2,937	0,087
Metachronous	12 (63,2%)		
Extension of Hepatectomy			
Minor	3 (15,8%)	0,001	0,982
Major	16 (84,2%)		
Type of Hepatectomy			
Anatomic	10 (52,6%)	0,215	0,643
Non-anatomic	9 (47,4%)		
Postoperative Course			
Major Morbidity	5 (26,3%)	13,183	**<0,001**
Minor Morbidity	14 (73,7%)		

Table 1. Clinical predictors of overall survival in study population (N=19 patients) undergoing hepatectomy for gastric cancer liver metastases (Log rank test; statistical significance with p<0,05).

The clinical factors found to be associated with better prognosis of DFS in the univariate analysis were absence of major morbidity (HR 5,069, p=0,024), metachronous resection (HR 4,005, p=0,045), interval between surgeries longer than eight months (HR 6,523, p=0,011).

Impact of Histopathological factors in the Overall and Disease Free Survival

The univariate analysis revealed that the factors, regarding the primary tumor, determinant of a better OS, were location in the medium and lower portions (HR 8,065, p=0,005), papillary and NOS (HR 5,051, p=0,025) histologic types, Lauren intestinal type (HR 13,333, p<0,001), T1 and T2 (HR 4,499, p=0,034) and low grade (HR 5,113, p=0,024). As far as the metastases are concerned, the positive predictors of longer survival were largest lesion smaller than 20mm (HR 4,600, p=0,032) and desmoplastic growth pattern or regression (HR 4,929, p=0,026) (Table 2 and Figure 6).

Parameters	**No. of patients (%)**	**Overall Survival**	
		HR	**p**
Location of primary tumor			
EGJ area and upper portion	4 (21,1%)	8,065	**0,005**
Middle and lower portions	15 (78,9%)		
Lauren type			
Diffuse and Undetermined	3 (15,8%)	13,333	**<0,001**
Intestinal	16 (84,2%)		
Histologic type			
Papillary and NOS	11 (57,9%)	5,051	**0,025**
Other	8 (42,1%)		
Grading of gastric lesion			
G1 and G2	16 (84,2%)	5,113	**0,024**
G3	3 (15,8%)		
Deepness of Invasion			
≤T2	3 (15,8%)	4,499	**0,034**
>T2	16 (84,2%)		
Size of largest GCLM			
≤20	9 (47,4%)	4,600	**0,032**
>20	10 (52,6%)		
Growth pattern			
Desmoplastic or regression	7 (41,2%)	4,929	**0,026**
Infiltrative or pushing	10 (58,8%)		

Table 2. Histopathological predictors of overall survival (Log rank test; statistical significance with p<0,05) in study population (N=19 patients) undergoing hepatectomy for gastric cancer liver metastases.

Papillary and NOS histologic subtypes (HR 11,586 p=0,001), desmoplastic growth pattern or regression of the metastases (HR 6,335, p=0,012) and metastases smaller than 20mm (HR 4,050 p=0,044) were significant good prognostic factors of DFS in the univariate analysis.

Independent predictive negative factors of Overall Survival

Cox regression failed to identify independent predictive factors of improved overall survival. However, both major morbidity and growth pattern approached statistical significance (p=0,065 and p=0,067 respectively).

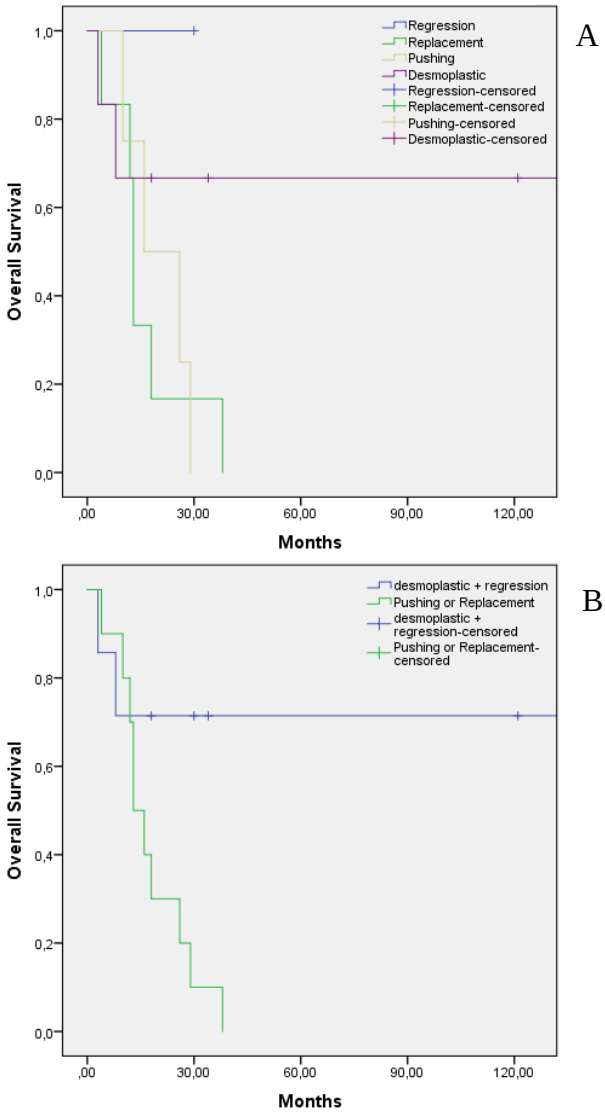


Figure 6. Kaplan Meier curves of overall survival (OS) in study population (N=19 patients) undergoing hepatectomy for gastric cancer liver metastases.
A- Between all the growth patterns;
B - Between desmoplastic and regression (N=7) (associated with better OS - HR 4,929, p=0,026) and pushing and replacement (N=11).

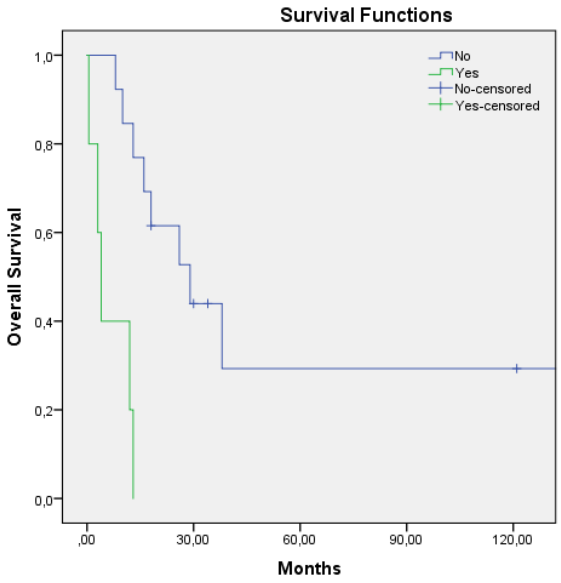


Figure 7. Kaplan Meier curves of overall survival (OS) curves in study population (N=19 patients) undergoing hepatectomy for gastric cancer liver metastases between major morbidity (N=5) and absence major morbidity (associated with better OS - HR 13,183, p<0,001).

Discussion

Resection is a valid option in selected patients with GCLM but the factors associated with improved survival are still under scrutiny. This is confirmed by the median OS and 5-year survival rate of our retrospective series, respectively 16 months and 21,2%, similar to previous studies [13,17]. These results are much better than the 5 months median OS of a study that analyzed the outcomes of GCLM that did not undergo resection [12].

Regarding the clinical prognostic factors, we reported that metachronous resection, in particular with an interval between resections of over eight months, was associated with improved overall survival. A recent systematic review of Markar *et al.* [17] including 991 patients that underwent hepatic resection of GCLM, as well as several other studies [10,12,14,18], failed to prove that metachronous resections was a determinant of a better prognosis. However, these studies showed that solitary metastases and minor resections were predictors of better survival rates, which is in accordance with our finding of improved survival if the size of the largest metastases was less than 20 mm and also in line with the results of Kinoshita *et al.* [10] and Ohkura *et al.* [16].

Posthepatectomy morbidity has already been validated as a poorer prognostic factor after resection for CRLM [47]. Nevertheless, this has not been investigated in most studies regarding GCLM. In our series, major morbidity proved to be a main determinant of the long term outcome, as previously reported by Tatsubayashi *et al.* [11].

An innovative feature of the present study was not only the simultaneous study of clinical and pathological parameters, but also the concurrent investigation of both primary tumor and metastases main pathologic features, including some that had never been investigated before in the setting of GCLM.

Regarding the histopathological analysis of the primary tumor the major predictive factors of a better course in the treatment were Lauren intestinal type, better differentiation, location in the lower portions of the stomach and depth of the primary tumor, in line with preceding observations [10,12,13,18]. We also found that histologic type of the gastric tumor and the growth pattern of the metastases were predictors of outcome.

One of the most interesting findings was related to HER-2. HER-2 expression detection may have a role in the treatment of GCLM patients as it allows targeted therapy. The current guidelines reinforce the testing of this receptor and advise the use of chemotherapy with Trastuzumab (an humanized anti-HER-2 monoclonal antibody) in patients that have HER-2 overexpression, as this combination leads to better prognosis when compared to chemotherapy alone [48,49]. Trastuzumab is the only targeted therapy currently used in the treatment of metastatic GC [50]. The thought-provoking result of different expression between the primary tumor and the metastases may have been caused by the fact that HER-2, in gastric malignancies, has a heterogeneous pattern of expression - being overexpressed in some areas and absent in others. Another cause to this detection may be that the malignancy may alternate HER-2 expression between locations, and may be overexpressed in metastases of negative primary tumors (spatial and temporal heterogeneity). This may be due to the fact that HER-2 positive cells show survival advantage compared to the negative ones, being more capable of inducing secondary disease. GCLM heterogeneity to HER-2 may lead to lack of accuracy in its detection. This poses the question of whether Trastuzumab is useful in the treatment of patients with malignancies with heterogeneous expression. According to calculations of Park *et al.* [51] there was an increment of 72,2% HER-2 positivity in the reassessment of its status in metastatic or recurrent lesions or in repeated biopsies of the primary tumor. Their study also concluded a higher prevalence in the liver of the HER-2

positive metastases. These evidences lead to the conclusion that retesting of HER-2 is crucial in GCLM.

Cancer Stem Cells (CSC) seem to be an essential measure of the tumor aggressiveness [34,35]. There have already been good results in studies that compared chemotherapy and CSC markers' inhibitors versus chemotherapy alone [40]. However, in our study, these markers did not show clinical impact. As a consequence, studies with larger populations are needed to evaluate the prognostic value of these markers.

Ki67 is a marker of tumor proliferation and has been pointed as being associated with higher prevalence in the male gender, worse prognosis in early gastric cancer, location in the stomach, histologic type and grading. [52,53]. It can also reflect tumor heterogeneity, possibly indicating variable responses to therapies. In our work, Ki67 was not statistically significant of prognosis, maybe due to the small N of this population.

The most original and exciting finding of our study was the validation of the growth pattern of GCLM as a predictor of prognosis. To our knowledge, this had never been reported before. Desmoplastic GP was statistically associated with a better prognosis both regarding the OS and DFS, resembling the reality of CRLM's [54]. This is likely due to a more intense immunologic reactivity of the host against the tumor. Thus the host does not act as a passive entity, but, instead tries to contain the metastatic spread. In detail, the growth of the metastases is influenced by features of the primary tumor and by a harmony of paracrine interactions in which stromal and inflammatory cells of the host participate. According to Vermeleun *et al.* [19] this GP is associated with a pro-apoptotic state as it is characterized by two major defense mechanisms: less proportion of endothelial cells – when compared to pushing and replacement patterns – which leads to the activation of programmed cell death in tumor cells; and high density of tumor infiltrating lymphocytes that, together with the

hepatocytes near the rim of fibrotic tissue, express high levels of Fas-ligand, a marker of apoptosis. Apoptosis, not only of the tumor cells, but also of the cells surrounding the metastases, contributes to a more indolent phenotype and protects against the dissemination of the malignancy. Moreover, there is a reaction of the stroma that builds up a pseudocapsule of fibrotic tissue (Figure 8). These defense mechanisms may be decisive in prognosis, not only because they reflect enhanced immunity against the tumor, but because they may allow for a smaller resection margin to result in a potentially curative hepatectomy. This remains to be proven, so far.

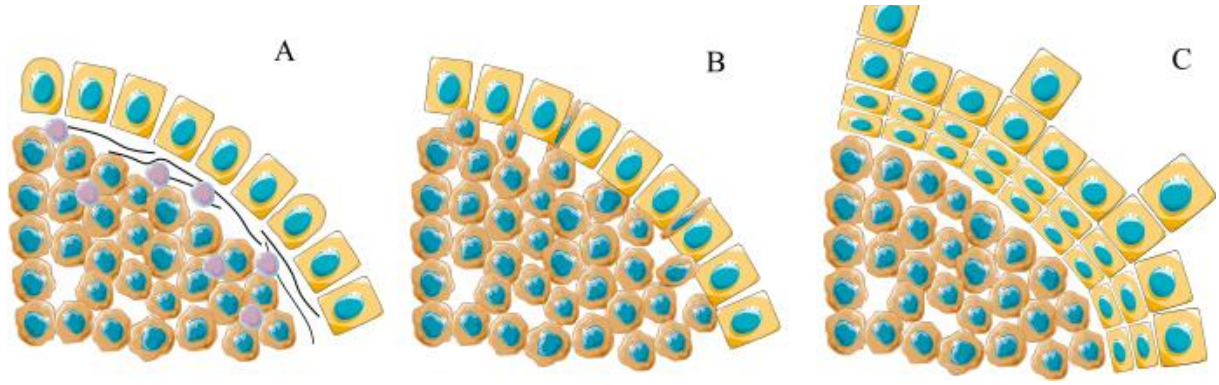


Figure 8. Growth Patterns of liver metastases - adapted from Eyden *et al.* [54].
A - Desmoplastic growth pattern - tumor is separated from the normal parenchyma by a line of fibrotic tissue and tumor infiltrating lymphocytes and hepatocytes near the tumor are in an apoptotic state;
B - Replacement growth pattern - tumor infiltrates the liver without disturbing the normal architecture;
C - Pushing growth pattern - tumor compresses the surrounding hepatocytes disrupting the normal parenchyma.

Future challenges include the discovery of sensitive imaging markers of distinct growth patterns, in particular with Magnetic Resonance Imaging. This would allow a more accurate selection of patients, as well as better preoperative planning. Moreover, molecular mechanisms associated with the different tumor-liver interfaces may lead to the development of future targeted therapies, or even try to predict the GCLM growth pattern using primary tumor characteristics and molecular classification.

However, the study has some limitations: the sample is composed by a small number of patients from a single institution and the design is retrospective which may reflect some bias in the choice of patients and limits the amount of clinical information that may be obtained.

In the future, a prospective completely randomized study would allow the elimination of all the bias present in the recent works about the local treatment of GCLM. Currently, there is an ongoing clinical trial assessing the effect of Bevacizumab in patients with GCLM candidates for liver resection [55].

Conclusion

Hepatic resection is of paramount importance in the treatment of GCLM. Nevertheless, some patients, due to the aggressiveness of this malignancy, will not benefit from resections, making patient selection of the utmost importance.

Selection of patients with less aggressive gastric lesions, with a more indolent dissemination and with a more favorable tumor-host response is likely the key to grant survival advantage when performing hepatectomy. By looking at both primary tumor and metastases in detail, we can safely conclude that the tumor microenvironment – tumor-related, host-related and tumor-host reciprocal interface – is very likely crucial to the prognosis. Desmoplastic growth factor is, for the first time, reported as an important prognostic factor.

Agradecimentos

Agradeço ao Professor Doutor Henrique Alexandrino, por quem tenho uma profunda admiração, quer como professor quer como médico, pelo apoio contínuo e pelo permanente desafio à procura de oportunidade de promover o saber, de ser mais e melhor em Medicina.

Agradeço ao Dr. Rui Oliveira por ser adepto e impulsionador de trabalhos como este, pela constante ajuda, pela infindável disponibilidade e curiosidade incessante.

Agradeço ao Professor Doutor Francisco Castro e Sousa, ao Professor Doutor Júlio Leite e à Dra. Maria Augusta Cipriano por promoverem Serviços onde o trabalho de alunos é, não só possível, como apoiado e estimulado.

Agradeço aos 19 doentes que tornaram este trabalho possível. O melhor cuidado dos nossos doentes é o que deve motivar a procura de novo conhecimento, tendo como fim o seu melhor tratamento.

Agradeço à D. Isabel Faustino por ter sido parte fundamental deste projeto e por toda a paciência, prontidão e excelente trabalho.

Agradeço também a todos os médicos do Serviço de Cirurgia A pelo apoio, não só na construção da base de dados como na resolução de questões que foram surgindo.

Agradeço à Mariana Duque e à Andreia Santos por terem acompanhado este percurso desde o início e pela entreajuda com a qual pude sempre contar.

Agradeço aos meus pais e à minha irmã Mariana por serem o pilar da minha formação, pelo apoio incondicional.

Este trabalho deu origem a várias comunicações em congressos, nacionais e internacionais, com os respetivos resumos publicados, nomeadamente:

1. **Apresentação oral:** "Metástases Hepáticas de Carcinoma Gástrico: Qual o Papel da Ressecção? Revisão Clínica e Patológica da Experiência de um Serviço" - XXXVII Congresso Nacional de Cirurgia, março de 2017;

   Maria José Temido, Henrique Alexandrino, Rui Caetano Oliveira, Luís Ferreira, Ricardo Martins, Marco Serôdio, Mónica Martins, Maria Augusta Cipriano, J. Guilherme Tralhão, Francisco Castro e Sousa

2. **Resumo:** "Metástases Hepáticas de Carcinoma Gástrico: Qual o Papel da Ressecção? Revisão Clínica e Patológica da Experiência de um Serviço" - Suplemento da Revista Portuguesa de Cirurgia, março de 2017;

   Maria José Temido, Henrique Alexandrino, Rui Caetano Oliveira, Luís Ferreira, Ricardo Martins, Marco Serôdio, Mónica Martins, Maria Augusta Cipriano, J. Guilherme Tralhão, Francisco Castro e Sousa

3. **Apresentação oral:** "Clinico-pathologic Predictors of Improved Overall Survival after Resection of Gastric Cancer Liver Metastases" - submetida para o 13th IHPBA (International Hepato-Pancreato-Biliary Association) World Congress;

   Maria José Temido, Henrique Alexandrino, Rui Oliveira, Eva Santos, Luís Ferreira, Ricardo Martins, Marco Serôdio, José Guilherme Tralhão, Francisco Castro e Sousa, Maria Augusta Cipriano, Júlio Soares Leite

4. **Resumo Submetido:** "Clinico-pathologic Predictors of Improved Overall Survival after Resection of Gastric Cancer Liver Metastases" - a aguardar publicação na HPB Journal - Revista indexada, de Fator de Impacto 3.29 (2016).

   Maria José Temido, Henrique Alexandrino, Rui Oliveira, Eva Santos, Luís Ferreira, Ricardo Martins, Marco Serôdio, José Guilherme Tralhão, Francisco Castro e Sousa, Maria Augusta Cipriano, Júlio Soares Leite.

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