Perineural infiltration as a prognostic factor in surgically treated gallbladder cancer

A single center experience and literature review

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Perineural infiltration as a prognostic factor in surgically treated gallbladder cancer. A single center experience and literature review.

INTRODUCTION: *Gallbladder cancer (GBC) is the most incident cancer of the biliary tract with only 5-13% of the sufferers surviving for five years. The aim of this study was to evaluate the prognostic role of perineural invasion (PNI) and its association with several clinicopathological variables in a cohort of surgically treated patients, and through a comprehensive review of the scientific literature.*
MATERIALS AND METHODS: *Twenty-five consecutive patients submitted to curative surgery for GBC from 2008 through 2016 were enrolled. Demographic, clinical and pathological data were retrieved from medical files, and specimens were re-examined by two experienced pathologists. The Pubmed database was searched for articles reporting on perineural infiltration on gallbladder cancer.*
RESULTS: *Perineural invasion was observed in 14 (56%) cases, and it was more frequent in higher pathological stages. A statistically significant association was found with high preoperative serum Ca 19-9 levels. Fourteen (56%) patients died during the follow-up; survival was lower in patients with perineural invasion in comparison to those without, but not statistically significant. Twelve English-language articles reporting on PNI were retrieved and discussed.*
CONCLUSIONS: *Perineural invasion is associated with higher stage and poorer survival in surgically treated GBC patients. In patients with locally advanced GBC resection of the extrahepatic biliary duct and frozen section examination of the distal stump must be taken into consideration, especially in cases of tumor arising from the hepatic side of the gallbladder. In cases without residual disease but with pathological evidence of PNI, a careful follow-up is suggested to early detect recurrences.*

KEY WORDS: Adenocarcinoma, Cancer, Gallbladder, Perineural infiltration, Surgery

Introduction

Gallbladder cancer is the fifth most common gastrointestinal malignancy, and the most incident cancer involving the biliary tract [1-3]. Its incidence is steadily growing in some areas of the globe, and in specific groups of patients such as middle-aged women [4]. This trend, in addition to the high mortality rate in locally advanced cases, makes gallbladder cancer a main health care issue. The estimated 5-year survival rate is currently 5-13% [5]. Surgery is the most effective therapeutic option favoring an increased survival rate in stage I tumors, but poor results in patients with locally advanced disease (0-30%)[6].
Unfortunately, only 15-40% of gallbladder cancer cases are diagnosed in a resectable phase, and wide and inva-

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sive resections are often performed to achieve the highest oncological clearance [6]. Given the need of such invasive surgical approaches, the selection of candidates to surgery, as well as the selection of those to submit to postoperative adjuvant therapies, is of primary importance. On this basis, the identification of histopathological factors predicting the relapse of the disease and, consequently a poor prognosis, is extremely urgent. Perineural infiltration (PNI) seems to be an interesting putative negative prognostic factor in resected gallbladder cancers; nevertheless, the issue has been investigated only in a few reports and no conclusive findings have been achieved, especially in relation to its clinical utility [5,7,8].

In the present article we assessed the prognostic role and clinical usefulness of PNI and its associations with clinicopathological variables in a cohort of surgically treated patients with gallbladder cancer, and through a comprehensive review of the scientific literature.

Materials and Methods

Twenty-five consecutive patients submitted to curative surgery for gallbladder cancer at the Unit of General Surgery 2 of the University of Sassari, Italy, from June 2008 through February 2016, were enrolled. Patients operated on for palliation or in emergency were excluded. Demographic, clinical, and pathological data were retrieved from medical files and reports. Preoperative staging routinely included serous carcinoembryonic antigen (CEA) and CA 19-9 (GICA) determination, as well as total body computed tomography (CT). Magnetic resonance cholangio-pancreatography (MRCP), endoscopic retrograde cholangio-pancreatography (ERCP), and positron emission tomography CT (PET-CT) imaging were employed when necessary. All surgical interventions were performed by experienced surgeons or trainees under supervision. Follow-up data were retrieved from records of periodical outpatient clinical evaluations, and from the cancer registry of the Province of Sassari, Italy. All patients gave their informed consent for each surgical intervention and for the anonymous use of their data for research purposes. The study was approved by the ethics committee of the Local Health Agency.

Surgical specimens were re-examined by two experienced pathologists for the PNI evaluation. Specimens were embedded in paraffin and formalin fixed. New sections were made when necessary, and hematoxylin and eosin staining was carried out in all cases, as well as immunohistochemistry for S-100 protein, in order to better evaluate the PNI (Fig. 1).

Qualitative and quantitative variables were summarized with absolute (percentage) frequencies and means (standard deviations – SD) or medians (interquartile ranges - IQR) according to the parametric distribution, respectively. Chi-square or Fisher exact and t-test or Mann-Whitney tests were used for qualitative and quantitative variables, respectively. Kaplan-Meier survival curves were computed. A p-value less than 0.05 was considered statistically significant. Statistical computations were performed with Stata 13.0 (StataCorp, College Station, TX). For the review of the scientific literature English-lan-

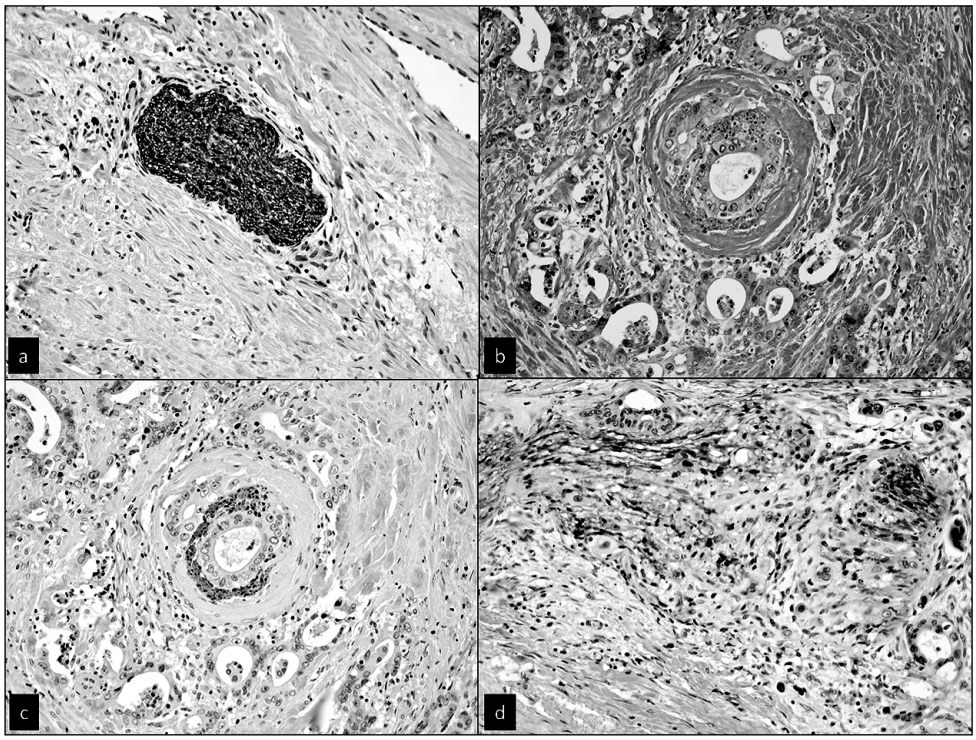

Fig. 1: Pathological evaluation of the cases for the presence of perineural infiltration (PNI); A) Absence of PNI (S100 immunostaining, 10x); B) PNI evidenced with Hematoxylin – Eosin (20x); C,D) Different grades of PNI evidenced by immunohistochemistry with S100 (10x and 20x, respectively).

guage articles published related to PNI in gallbladder cancer were non-systematically retrieved using the Pubmed database. The search terms employed were "perineural invasion" and "perineural infiltration", each of them combined with "gallbladder cancer". Titles and abstracts were evaluated in order to include the most relevant studies. References of the selected articles were cross-checked in order to detect papers missed by the search engine.

Results

Among the 25 patients included, nine (36%) were males, and the median age at diagnosis was 72 (IQR 68-78) years. The main clinical characteristics of the patients enrolled are summarized in Table I. Four (16%) patients had a preoperative American Society of Anesthesiology (ASA) score I, 16 (64%) score II, and five (20%) score III. Ultrasound (US) evaluation was performed in 20 (80%) cases, CT in 21 (84%), MRCP in nine (36%), PET-CT in one (4%) case, and ERCP in three (12%) cases.

TABLE I - *Main demographic and clinical characteristics of the patients enrolled.*

Clinical features	Number/cases available (%)
Sex	
Males	9 (36)
Females	16 (64)
ASA SCORE	
I	4 (16)
II	16 (64)
III	5 (20)
IV	0 (0)
Main comorbidities	
Hypertension	18/25 (72)
Colelithiasis	18/25 (72)
Diabetes	7/25 (28)
Hypothyroidism	5/25 (20)
Hypercolesterolemia	2/25 (8)
COBD	2/25 (8)
High total bilirubin levels	5/23 (22)
TNM stage	
I	2 (8)
II	3 (12)
III	14 (56)
IV	6 (24)
Surgery performed	
Cholecystectomy	2 (8)
Cholecystectomy + liver or wider resection	11 (44)
Resections after cholecystectomy	12 (48)

Only in two (8%) cases a simple cholecystectomy was performed, while in the remaining cases wider resections were necessary. In 12 (48%) cases an initial cholecystectomy was performed in our hospital or in other institutions, and the patients underwent further completion surgery. In one case the patient was affected by a synchronous primary pancreatic cancer and a Whipple resection was performed, along with splenic resection. Postoperative complications were observed in seven (28%) of the cases, and three (12%) patients died within 30 days from surgery. The median hospital stay was 11 (IQR 8-13) days, and the mean follow-up time approximately 51 (range 13-103) months (Table II). Patients with PNI were 14 (56%). Table II describes the clinicopathological differences between those with and without PNI, while Figure 2 shows the survival in patients with and without PNI.

A statistically significant difference was found for the preoperative serum GICA between those with and without PNI. The age of the patients with PNI and their postoperative complications were consistently different, but did not reach statistical significance. Fourteen (56%) patients died during the follow-up; survival was lower in patients with PNI, but the difference was not statistically significant.

Twelve English-language articles reporting on PNI in gallbladder cancer were retrieved for review; Table III summarizes the main findings, which will be now discussed in detail.

Discussion

The involvement of neural spread in gallbladder cancer has been investigated since the 1940s, but it still remains a controversial issue, especially for the usefulness of its prognostic role in the clinical practice. Vadheim et al. in 1944 outlined for the first time the possibility of neural involvement in tumoral spreading in 22% out of 77 cases [9]. Subsequently, Fahim et al. studied 151 gallbladder cancer cases treated at the Mayo Clinic from 1909 through 1950 and evidenced that 23.8% showed neural invasion [10]. These Authors could not assess the clinical significance of their findings, despite they observed that PNI was most often found in advanced Broder's stage patients.

The pathophysiological mechanism of PNI was studied earlier in other than gallbladder cancers; the first description of Ernst and colleagues was published in 1905 [11], and since then this modality of neoplastic invasion has been widely confirmed in several malignancies. Therefore, many surgeons deemed that wide surgical excisions were necessary to include neural structures, and achieve the oncological radicality. It has also been postulated that in advanced cases the spreading within the perineurium was often distant, and thus, impossible to resect completely. This was based on the theory that

TABLE II - *Clinicopathological features of patients with and without perineural infiltration.*

Variables		Total cohort	Missing perineural invasion	Perineural invasion	*p-value*
Median (IQR) age, years		75 (68-78)	68 (61-77)	76 (71-78)	0.09
Male, n (%)		9 (36.0)	4 (36.4)	5 (35.7)	0.97
Median (IQR) hospital stay, days		11 (8-13)	8 (7-12)	12 (9-16)	0.10
GICA, n (%)		8 (32.0)	1 (9.1)	7 (50.0)	0.04
CA-125, n (%)		3 (12.0)	0 (0.0)	3 (21.4)	0.23
CA-153, n (%)		3 (12.0)	0 (0.0)	3 (21.4)	0.23
CEA, n (%)		1 (4.0)	0 (0.0)	1 (7.1)	1.0
Post-surgery staging	I	2 (8.0)	2 (18.2)	0 (0.0)	0.30
	II	2 (8.0)	2 (18.2)	0 (0.0)	
	IIB	1 (4.0)	0 (0.0)	1 (7.1)	
	IIIA	10 (40.0)	4 (36.4)	6 (42.9)	
	IIIB	4 (16.0)	1 (9.1)	3 (21.4)	
	IVB	6 (24.0)	2 (18.2)	4 (28.6)	
Complications, n (%)		7 (29.2)	1 (9.1)	6 (46.2)	0.08
Deaths, n (%)		14 (56.0)	4 (36.4)	10 (71.4)	0.12
Mean (SD) time from surgery to death, days		297.1 (224.4)	259.8 (177.1)	312 (247.8)	0.71
Perineural invasion, n (%)		14 (56.0)	-	-	-

IQR: InterQuartile Range; SD: Standard Deviation

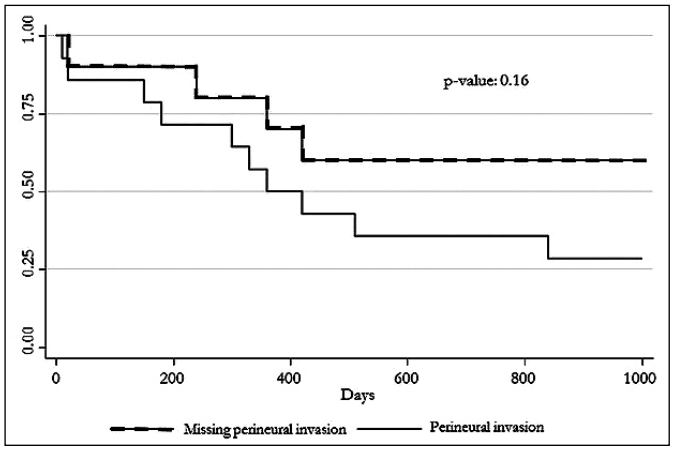

Fig. 2: Time to death of the enrolled cohort according to the perineural invasion.

neoplastic spread occurred by permeation and embolism through lymphatic vessels contained in the perineurium [12]. Nevertheless, subsequent observations showed the lack of lymphatic vessels in nerve tissues, and demonstrated that the tumoral spread occurs within the perineural space. Moreover, it seems that the dimensions of the perineural space are critical, being narrowest in intrahepatic neurons where PNI is generally less extensive, in comparison with extrahepatic nerves [12].

A few studies on the clinical impact of PNI in surgical patients with gallbladder cancer have been performed up to date (Table III)[5,6,9,10,13-20]. PNI seems to occur between 22% and 71% in series including patients with both early and locally advanced disease; percentages are higher in more advanced clinical stages. Shirai et al. studied 78 pT1a stage patients (according to the TNM classification in 1992) and did not detect any PNI cases, while among the 11 patients with pT1b stage disease only one patient showed PNI [13]. These observations support the hypothesis of a more prevalent PNI involvement in advanced stages, as observed in our series and in other reports.

In a later article published by Ouchi et al. PNI was not evaluated singularly, but in association with vascular and lymphatic invasion in 40 patients with surgically treated GBC: they were found in 5/17 and 20/23 long-term and short-term survivors, respectively [8]. Similarly, in the study of Suzuki et al. in 20 patients with pT2 GBC, no PNI cases were found among 16 long-term survivors, and none of the four PNI patients lived after five years from diagnosis [20].

Chijiiwa et al. studied 46 patients with surgically treated GBC; a subgroup of ten patients with early disease and without PNI, showed high survival rates, in agreement with the findings of Shirai et al. [13,14]. Furthermore, in another subgroup of 21 patients with locally advanced disease and extended surgical resections, PNI was found to be related to survival in both uni- and multi-variate regression analysis [14].

In 2002, Yamguchi et al published a study on 68 patients surgically treated for GBC; PNI was found in 71% of the cases and was significantly associated with a poor prognosis, as the 5 year survival rate was consistently lower in PNI patients in comparison with those without [15]. The Authors stated that PNI is a relevant prognostic factor, but not the most important one; indeed, PNI was found also in long-term survivors, in accordance with the data of Ouchi et al. and with our data [8].

TABLE III - *Perineural invasion and its prognostic significance as reported in the scientific literature.*

Author, year.	Number of cases examined	Cases with PNI, n (%).	Prognostic value
Vadheim et al. 1944[9]	77	17 (22)	NA
Fahim et al. 1962[10]	151	36 (24)	NA
Shirai et al. 1992[13]	78 pT1a	0 (0)	NA
	11pT1b	1 (9)	
Chijiiwa et al. 1997[14]	10 T1	0 (0)	NA
	21 advanced	NA	Yes
Yamaguchi et al. 2002[15]	68	48 (71)	Yes
Kaneoka et al. 2003[19]	25 with BDI	21 (84)	NA
Suzuki et al. 2004[20]	20 pT2	4 (20)	NA
Sakamoto et al. 2006[16]	58 without BDI	25 (43)	Yes
Butte et al. 2014[17]	83	35 (42)	NA
Birnbaum et al. 2014[18]	78 T3-T4	63 (81)	Yes
Shindoh et al. 2015[6]	437	89 (20)	NA
Ethun et al. 2017[5]	117	62 (53)	Yes

Interestingly, in the study of Yamaguchi et al. the incidence and extent of PNI was greater at the distal than at the proximal margin of the tumor, and this may be related with the size of the perineural space; the Authors recommend frozen section pathological examination of the distal bile duct during surgery in cases with extrahepatic bile duct invasion (EHBDI) to rule out unexpected distal PNI [15]. This strategy is adopted in our clinical practice, but the number of patients with EHBDI in our series was small to allow remarkable analyzes. Sakamoto et al. studied the association between PNI and the surgical approach in 25 patients with EHBDI [16]. They found survival rates significantly higher in 14 patients who underwent EHBD resection, in comparison with 11 patients who did not (46% vs 0%, p=0.009). Furthermore, Butte et al. demonstrated that PNI was significantly correlated with residual disease, and might be useful in predicting local relapse, and thus, poorer survival [17]. Also in the study of Ethun et al. PNI was related to local residual and distant disease at reoperation in 262 patients [5]. This finding was additionally confirmed by Birnbaum et al. who furthermore proved PNI as a strong prognostic factor for survival in patients with locally advanced GBC [18]. Finaly, Shindoh et al. found that PNI is significantly more frequent in GBCs which arise at the hepatic side of the gallbladder wall, in comparison with those emerging at the free in the peritoneum surface of the organ [6].

Our study did not achieve statistical significance for survival in patients with and without PNI, probably because of the small sample size. Nevertheless, the association of PNI with advanced pathological stages and higher mortality rates seem to occur also in our series. A statistically significant association between PNI and preoperative serum GICA was detected; this finding needs to be further investigated in future studies.

In conclusion, PNI occurs in 22-71% of surgical GBC cases and is associated with the stage of the disease and the poor survival. It is extremely rare in patients with T1 stage disease. In patients with locally advanced GBC the resection of the EHBD must be taken into consideration, especially if the tumor involves the hepatic aspect of the gallbladder, and the distal stump should be examined by frozen section intraoperatively in order to avoid residual disease, which is a stronger negative prognostic factor than PNI. In cases without residual disease but with pathological evidence of PNI, a careful follow-up is suggested in order to early detect possible recurrences.

Riassunto

INTRODUZIONE: Il cancro della colecisti (GBC) è la neoplasia delle vie biliari più frequente con una sopravvivenza a 5 anni di solo il 5-13%. Lo scopo di questo studio era di valutare il ruolo prognostico dell'infiltrazione perineurale (PNI) e la sua associazione con numerose variabili clinicopatologiche in una coorte di pazienti trattati chirurgicamente e attraverso una revisione della letteratura scientifica.

MATERIALI E METODI: Venticinque pazienti consecutivi sottoposti a chirurgia curativa per GBC dal 2008 al 2016 sono stati arruolati nello studio. I dati demografici, clinici e patologici dei pazienti sono stati estratti dalle cartelle cliniche e i pezzi istologici sono stati rivalutati da due anatomopatologi esperti. È stata inoltre effettuata un'analisi della letteratura scientifica mediante ricerca su Pubmed di tutti gli articoli sull'infiltrazione perineurale nel GBC.

RISULTATI: L'infiltrazione perienurale è stata osservata in 14 (56%) casi ed era più frequente negli stadi maggiormente avanzati della malattia. Un'associazione statisticamente significativa è stata rilevata con valori preoperatori elevati del Ca 19-9 sierico. Quattordici (56%) pazienti sono deceduti durante il periodo di follow-up; la sopravvivenza era minore nei pazienti con PNI rispet-

to a quelli senza, tuttavia la differenza non era statisticamente significativa. Dodici articoli in lingua inglese riguardanti la PNI sono stati recuperati e analizzati.
CONCLUSIONI: La PNI è associata agli stadi più avanzati della malattia e a una sopravvivenza peggiore nei pazienti operati per GBC. Nei pazienti con GBC localmente avanzato la resezione delle vie biliari extraepatiche e l'esame al congelatore del margine di resezione distale devono essere presi in considerazione, soprattutto nei casi di neoplasia che insorge sulla parete della colecisti a contatto col fegato. Nei pazienti senza malattia residua ma con evidenza all'esame istopatologico di PNI è auspicabile un attento follow-up per l'identificazione precoce di eventuali recidive.

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