UNIVERSITA' DEGLI STUDI DI VERONA

DIPARTIMENTO DI CHIRURGIA

SCUOLA DI DOTTORATO DI SCIENZE BIOMEDICHE E TRASLAZIONALI

DOTTORATO DI RICERCA IN

SCIENZE CHIRURGICHE E DELLE MALATTIE EPATOBILIARI E PANCREATICHE

CICLO / ANNO (1° ANNO D'ISCRIZIONE) XXV CICLO / 2010

TESI DI DOTTORATO

PROGNOSTIC SYSTEM FOR CHOLANGIOCARCINOMA

S.S.D. MED/18

Coordinatore: Prof. CLAUDIO BASSI

Tutor: Prof. ALFREDO GUGLIELMI

Dottorando: Dott. TOMMASO CAMPAGNARO

INDICE

	N. PAG.
ABSTRACT	3
INTRODUZIONE	6
MATERIALI E METODI	15
RISULTATI	19
DISCUSSIONE	34
CONCLUSIONI	38
BIBLIOGRAFIA	39
RINGRAZIAMENTI	43

PROGNOSTIC SYSTEM FOR CHOLANGIOCARCINOMA

Proposal of prognostic nomogram for perihilar cholangiocarcinoma after surgical resection and comparison with pre-existing staging systems

ABSTRACT

Introduzione

Il colangiocarcinoma perilare (PCC) è un tumore primitivo della via biliare con progressivo incremento di incidenza negli ultimi anni e la sua classificazione, gestione clinica e chirurgica si sta modificando negli anni recenti. Il PCC ha una prognosi infausta ma la resezione radicale chirurgica può prolungare la sopravvivenza.

Sono disponibili diverse classificazioni prognostiche e sistemi stadiativi ma nonostante ciò non esiste accordo diffuso sul loro significato prognostico.

Obiettivo dello studio è di creare un nomogramma con funzione di valutare la prognosi per i pazienti con PCC sottoposti ad intervento chirurgico con intento radicale.

Materiali e Metodi

Un database prospettico di pazienti sottoposti ad intervento chirurgico per colangiocarcinoma in uno stesso reparto di chirurgia è stato la base per valutare le caratteristiche cliniche e patologiche dei pazienti con PCC sottoposti ad intervento chirurgico con intento radicale. Dalle variabili risultate significative all'analisi multivariata è stato creato un modello prognostico per valutare il rischio di mortalità a 3 anni dall'intervento chirurgico. Un nomogramma è stato quindi creato dal modello prognostico. Il nomogramma è stato validato internamente. La capacità di predire la sopravvivenza dopo intervento chirurgico per pazienti con PCC è stata confrontata tra altri quattro sistemi stadiativi o prognostici preesistenti e il tra il nostro nuovo modello prognostico.

Risultati

Da un database di 207 pazienti con colangiocarcinoma, 145 sono stati sottoposti ad intervento chirurgi con intento radicale tra il Settembre 1990 e il Settembre 2012; 75 sono stati classificati come PCC. Sei pazienti

sono stati esclusi dall'analisi perché deceduti nel periodo postoperatorio; 69 pazienti sono stati inclusi nell'analisi dello studio.

Fattori prognostici per la sopravvivenza all'analisi multivariata sono risultati: CA 19.9 preoperatorio >500U/mL, numero di linfonodi prelevati, numero di linfonodi risultati positivi, resezione del caudato, resezione e ricostruzione portale. Dalle variabili significative all'analisi multivariata è stato creato un sistema prognostico, che è riuscito a distinguere i pazienti operati in tre gruppi a seconda del rischio di morire entro 3 anni dall'intervento: basso (<50%), medio (50-75%) o alto (>75%) rischio. Dal sistema prognostico è stato ideato un nomogramma. La curva di calibrazione ha mostrato una buona concordanza tra la sopravvivenza prevista dal nomogramma e la sopravvivenza attesa. Il nostro modello prognostico è stato confrontato con altri sistemi stadiativi pre-esistenti: AJCC/UICC TNM, classificazione di MSKCC, sistema stadiativo JSBS e score prognostico di Kaiser dimostrando una migliore capacità di predire la sopravvivenza (AIC 268.9).

Conclusione

Il nostro nomogramma prognostico ha dimostrato di avere maggiore capacità di predire la sopravvivenza per i pazienti con PCC sottoposti ad intervento chirurgico.

Introduction

Perihilar cholangiocarcinoma (PCC) is primary biliary malignancy increasing worldwide and its classification and management is evolving progressively. Perihilar cholangiocarcinoma has poor prognosis however surgical resection can prolong survival. Prognostic classification and scoring systems are available but there is not general agreement about their prognostic significance.

Aim of this study was to create an effective prognostic nomogram for patients submitted to surgical resection for perihilar cholangiocarcinoma.

Material and Methods

Creation of nomogram was based on consecutive series of 69 patients submitted to surgical resection for perihilar cholangiocarcinoma between 1990 and 2012. Clinical and pathological variables were evaluated. Prognostic model for risk to die within 3 years after surgical resection was created according to variables selected at multivariate analysis. Nomogram was created by prognostic model. Internal validation of nomogram was performed. Predictive accuracy and discriminative capability of our prognostic model was compared with other four prognostic systems for PCC.

Results

From a database of 207 patients with cholangiocarcinoma, 145 were submitted to surgical resection with curative intent between September 1990 and September 2012; 75 were classified as PCC. Six patients were excluded from analysis due to perioperative mortality, thus 69 patients were included in study analysis.

Independent significant prognostic factor for survival at multivariate analysis were increased CA 19.9, number of lymph node harvested, number of positive lymph node, caudate lobe resection and portal vein resection; all these variables were selected for prognostic model. Three group of patients according to low (<50%), medium (50-75%) or high (>75%) risk to die within 3 year were identified by nomogram. Calibration curve for probability of survival showed good agreement between prediction of nomogram and actual observation. Our prognostic model resulted more accurate in predict survival compared to AJCC/UICC TNM staging system, MSKCC classification, JSBS staging system and Kaiser prognostic scoring system (AIC 268.9).

Conclusion

Our prognostic nomogram resulted to be more accurate than previous staging system in predict survival for patients with perihilar cholangiocarcinoma submitted to surgical resection.

INTRODUCTION

Cholangiocarcinoma is malignant transformation of bile duct epithelium; it represents proximally 10% of all primary hepatobiliary cancer [1, 2] . This tumor is the second most frequent primary liver tumor and its incidence is increasing in Western countries [3].

Cholangiocarcinoma is classified as intrahepatic (ICC), perihilar (PCC) and distal type, according to its origin from proximal intrahepatic bile ducts (ICC), from the epithelium of the right or left hepatic ducts at biliary confluence (PCC) or from distal part of common bile duct [1, 4].

Perihilar cholangiocarcinoma, also called Klatskin tumor [5], represent approximately two third of all cases of bile ducts tumors [4] . It was defined as tumor involving biliary confluence, however there are still some difficult in preoperative definition and proper histological classification [6 7].

Different clinicopathological factors have been previously evaluated and related to survival: radical resection, vascular invasion, perineural invasion, tumor size, multifocality, lymph node metastasis, tumor stage, tumor grading and positive resection margins [8-10].

Surgical radical resection (R0) and transplantation in selected cases are still the only therapeutic option to achieve longer survival [11-13] , however prognosis is dismal and rarely 5 years survival exceed 30% [14] .

Staging systems give information about prognosis, stratification of patients according to tumor stage, guide for different type of therapy and allowed to compare different treatments among different institutions over the time [15]. For these reasons, as for others tumors, also for perihilar cholangiocarcinoma were created different staging systems.

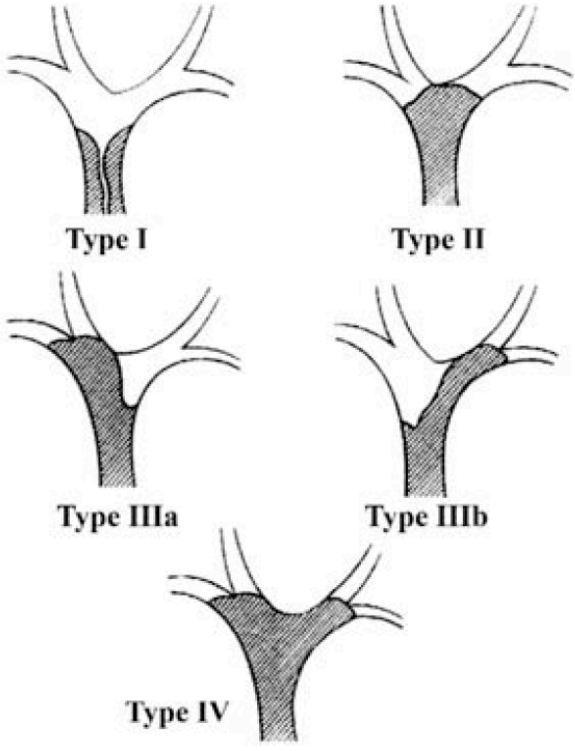
Staging Systems

Different staging systems are available to classify perihilar cholangiocarcinoma: Bismuth-Corlette classification [16], the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM classification [17], Memorial Sloan-Kettering Cancer Center (MSKCC) classification [18], Japanese Society of Biliary Surgery (JSBS) staging system [19], DeOliveira's staging system [20] and Kaiser's Prognostic Scoring System [21].

Bismuth-Corlette Classification.

Bismuth-Corlette classification was introduced in 1975 and modified in 1992 [16, 22]; this classification is focused exclusively on the level and extension of the tumor invasion along the biliary tree (Fig. 1). Tumor is classified as type I (the tumor involves only the common hepatic duct below the confluence of the left and right hepatic ducts), type II (the tumor involves the hepatic bile duct confluence but there without invasion above the confluence), type III (the tumor involves the biliary confluence and is extent along right (type IIIa) or left (type IIIb) hepatic duct), or type IV (the tumor involves both the right and left hepatic ducts and the confluence reaching the secondary intrahepatic biliary system or involves multiple discontinuous sites in the right and left ducts). The Bismuth-Corlette classification was primarily conceived to serve as a guide for surgical strategy (e.g., types I and II indicate local resection, type III indicates associated liver resection, and type IV indicates unresectability), however recent practice in many specialized centres no longer follows the original concept [7, 20]. This system fails to provide other key information such as vascular encasement, lymph node involvement, distant metastases and atrophy of a part of the liver. Thus, it logically does not correlate with patient survival, however in some papers it has be valuated as prognostic factor for survival

Figure 1: Bismuth-Corlette Calssification [16].



TNM Staging System according to UICC/AJCC.

This classification is based on the pathological findings of tumor extension, lymph node involvement and presence of metastases (Figure 2) [17]. In 2010 the 7th edition of AJCC/UICC finally included TNM classification for perihilar cholangiocarcinoma, previously incorporated in extrahepatic bile ducts classification. This classification is a pathological classification and available only after surgical resection. This has little applicability because of low feasibility of surgical operation in this type of patient [7, 23] . This fails to indicate local resectability of the tumor and to distinguish between the various surgical options. Its usefulness in the preoperative setting is thus limited. In literature were validated the different edition of TNM stage also evaluating capability to differentiate prognosis of patients according to different stages [24, 25], however other studies did not reached comparable results [26, 27] . In majority of studies different classes are combined to reach a significant different survival amongst patients. For this reason, this classification has some limitations as prognostic value.

Figure 2: TNM UICC/AJCC Staging System [17].

Primary Tumor (T)

TX	The primary tumor cannot be assessed.
T0	No evidence of a primary tumor
Tis	Carcinoma *in situ*
T1	The tumor is confined to the bile duct histologically.
T2a	The tumor invades the surrounding adipose tissue beyond the wall of the bile duct.
T2b	The tumor invades the adjacent hepatic parenchyma.
T3	The tumor invades unilateral branches of the portal vein or hepatic artery.
T4	The tumor invades the main portal vein or its branches bilaterally, the common hepatic artery, the second-order biliary radicals bilaterally, or the unilateral second-order biliary radicals with contralateral portal vein or hepatic vein involvement.

Regional Lymph Nodes (N)

NX	Regional lymph nodes cannot be assessed.
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis (cystic duct, common bile duct, hepatic artery, and portal vein)
N2	Metastasis to periaortic, pericaval, superior mesentery artery, and/or celiac artery nodes

Distant Metastasis (M)

M0	No distant metastasis
M1	Distant metastasis

Stage Grouping

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2a-T2b	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1-T3	N1	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	N2	M0
	Any T	Any N	M1

Memorial Sloan-Kettering Cancer Center (MSKCC) Classification.

Group from MSKCC proposed a staging system that classifies PCC according to three factors related to the local extension of the tumor, the location of bile duct involvement and the presence of portal vein invasion and hepatic lobar atrophy (Figure 3) [18] . This classification was tested in a series of 225 patients from that institution and showed an accuracy of 86% in the preoperative staging of the local extent of the disease. This staging system attempted to predict resectability, however, there are some limitations: the system does not evaluate the presence of lymph node or distant metastases or the involvement of the artery, the size of the remnant liver is not specified and it was designed exclusively on the basis of the criteria of resectability from a single institution. The concept of resectability is evolving because of the recent developments in aggressive liver surgery with vascular resection and reconstruction and because of new advances in liver transplantation, and for this reason this system have some limitation in applicability. Moreover, also as prognostic factor this classification have some limitation; Zervos et al. did not confirmed the correlation between classification and long-term results [28].

Figure 3: MSKCC Classification. Adapted form Jarnagin et al (2001) [18].

Stage	Criteria
T1	Tumor involving biliary confluence +/− unilateral extension to second-order biliary radicles
T2	Tumor involving biliary confluence +/− unilateral extension to second-order biliary radicles **and** *ipsilateral* portal vein involvement +/− *ipsilateral* hepatic lobar atrophy
T3	Tumor involving biliary confluence + bilateral extension to second-order biliary radicles; **or** unilateral extension to second-order biliary radicles with *contralateral* portal vein involvement; **or** unilateral extension to second-order biliary radicles with *contralateral* hepatic lobar atrophy; **or** main or bilateral portal venous involvement

Staging System according to Japanese Society of Biliary Surgery (JSBS)

Classification of hilar neoplasm formulated by JSBS was published in 2004 (Figure 5) [29]. As TNM of UICC/AJCC, this classification is based on pathological findings. It analyzes tumor extension, lymph node involvement and distal metastases. Patients are classified in five stages: I, II, III, IVA, IVB.

Also this classification have some limitations: useless preoperatively and to define resectability, perihilar cholangiocarcinoma classified along with others extrahepatic tumor of biliary tract, complexity of classification of tumor extension due to inclusion of five parameters (serosa, hepatic parenchyma, pancreas, portal vein and hepatic artery infiltration), classification different from TNM UICC/AJCC regarding lymph node staging and metastases. Thus, because of complexity of this precise staging system it was not diffused internationally.

Figure 5: Japanese Society of Biliary Surgery staging system [29].

pT classification

pT	Contents
pT1	m, fm, hinf0, panc0, pv0, a0
pT2	ss, hinf1, panc1, pv0, a0
pT3	se, hinf2, panc2, pv1,a1
pT4	si, hinf3, panc3, pv2, pv3, a2, a3

Lymph node grouping

Lymph node (site number)	Group		
	Hilar and proximal	Middle	Distal
Infrapyloric LN (6)	pN3	pN3	pN3
LN around the common hepatic artery (8)	pN2	pN2	pN2
LN at the splenic hilum (10)	pN3	pN3	pN3
LN along the splenic artery (11)	pN3	pN3	pN3
LN at the hepatic hilum (12h)	pN1	pN2	pN2
LN along the hepatic artery (12a)	pN1	pN2	pN2
Periportal LN (12p)	pN1	pN2	pN2
Pericholedochal LN (12b)	pN1	pN1	pN1
LN around the cystic duct (12c)	pN1	pN1	pN1
Posterior superior pancreatoduodenal LN (13a)	pN2	pN2	pN2
Posterior inferior pancreatoduodenal LN (13b)	pN3	pN3	pN3
LN along the superior mesenteric artery (14)	pN3	pN3	pN2
Para-aortic LN (16)	pN3	pN3	pN3
Anterior superior pancreatoduodenal LN (17a)	pN3	pN3	pN3
Anterior inferior pancreatoduodenal LN (17b)	pN3	pN3	pN3

Stage grouping

	H(−) and P(−) and M(−) pN0	pN1	pN2	pN3	H(+) and/or P(+) and/or M(+) and any N
pT1	I	II	III	IVa	IVb
pT2	II	III	III	IVa	IVb
pT3	III	III	IVa	IVb	IVb
pT4	IVa	IVa	IVb	IVb	IVb

m invasion limited to the mucosa, *fm* invasion limited to the fibromuscular layer, *ss* invasion limited to the subserosa, *se* invasion of serosal surface, *si* invasion beyond the serosa and invasion of other organs or structures, *hinf0* no direct invasion of the liver, or direct invasion limited to the fibromuscular layer of intrahepatic bile ducts, *hinf1* direct invasion of fibromuscular layer of intrahepatic ducts and/or liver parenchyma which invasion is not more than 5 mm in depth, *hinf2* direct invasion of liver parenchyma, which invasion is 5 mm or more but not more than 20 mm in depth, *hinf3* direct invasion of liver parenchyma, which invasion is 20 mm or more in depth, *panc0* no invasion of the fibromuscular layer of the inferior bile duct, *panc1* invasion of the fibromuscular layer of the inferior bile duct and/or pancreatic parenchyma of which invasion is not more than 5 mm in depth, *panc2* invasion of the pancreatic parenchyma of which invasion is 5 mm or more but not more than 20 mm in depth, *panc3* invasion of the pancreatic parenchyma of which invasion is 20 mm or more in depth, *pv0* no invasion of portal vein, *pv1* invasion of the adventita, *pv2* invasion of the media, *pv3* invasion of the intima, *a0* no invasion of hepatic arteries, *a1* invasion of the adventita, *a2* invasion of the media, *a3* invasion of the intima, *LN* lymph node, *H(−)* no liver metastasis, *H(+)* liver metastasis, *P(−)* no peritoneal metastasis, *P(+)* peritoneal metastasis, *M(−)* no distant metastasis, *M(+)* distant metastasis

DeOliveira's New Staging System for Perihilar Cholangiocarcinoma

Recently was proposed a new classification of perihilar cholangiocarcinoma (Figure 6) [20]. This classification give more information about vascular infiltration and remnant liver volume comparing to previous Staging System; this stage system incorporate Bismuth-Corlette classification and TNM staging 7th edition for perihilar cholangiocarcinoma associated to description of vascular invasion. Aims of this new more precise staging system were to get more radiological and pathological information and to create an international registry. However, there were some limits in this new staging system of perihilar cholangiocarcinoma: first of all this staging is descriptive and did not have availability as prognostic factor, definition of vascular infiltration is precise however non-realistic because vascular invasion is classified like Bismuth-Corlette classifies biliary infiltration and definition of perihilar cholangiocarcinoma was not assessed.

Figure 6: DeOliveira's New Proposed Classification [20].

*"R" indicates right. "L" indicates left. †Based on Bismuth-Corlette classification. ‡Based on the Japanese Society of Biliary Surgery classification. §Based on the TNM UICC/AJCC classification.

Label	Side/Location*	Description
Bile duct (B)†		
B1		Common bile duct
B2		Hepatic duct confluence
B3	R	Right hepatic duct
B3	L	Left hepatic duct
B4		Right and left hepatic duct
Tumor size (T)		
T1		<1 cm
T2		1-3 cm
T3		≥3 cm
Tumor form (F)		
Sclerosing		Sclerosing (or periductal)
Mass		Mass-forming (or nodular)
Mixed		Sclerosing and mass-forming
Polypoid		Polypoid (or intraductal)
Involvement (>180°) of the portal vein (PV)		
PV0		No portal involvement
PV1		Main portal vein
PV2		Portal vein bifurcation
PV3	R	Right portal vein
PV3	L	Left portal vein
PV4		Right and left portal veins
Involvement (>180°) of the hepatic artery (HA)		
HA0		No arterial involvement
HA1		Proper hepatic artery
HA2		Hepatic artery bifurcation
HA3	R	Right hepatic artery
HA3	L	Left hepatic artery
HA4		Right and left hepatic artery
Liver remnant volume (V)		
V0		No information on the volume needed (liver resection not foreseen)
V%	Indicate segments	Percentage of the total volume of a putative remnant liver after resection
Underlying liver disease (D)		Fibrosis Nonalcoholic steatohepatitis Primary sclerosing cholangitis
Lymph nodes (N)‡		
N0		No lymph node involvement
N1		Hilar and/or hepatic artery lymph node involvement
N2		Periaortic lymph node involvement
Metastases (M)§		
M0		No distant metastases
M1		Distant metastases (including liver and peritoneal metastases)

Kaiser's Prognostic Scoring System

A recent study [21] proposed a prognostic scoring system including four variables: patients' years (<62 years vs >62), pathological tumor status from TNM stage (pT1 vs pT2-3-4), radical resection (R0 vs R1-2), adjuvant chemoradiation (yes vs not performed). Zero or one point is assigned for each variable. From personal data set Authors evaluated different variables; variables resulted significant prognostic factors for survival at multivariate analysis were included in the proposal scoring system. Authors created a model with three groups of patients according to number of variables present; this model allowed to stratify patients and to correlate to long-term survival. This is a prognostic scoring system easy to apply, however it has some limitations: it is possible to use only after operation because of pathological variables, lymph node involvement is not include even in literature has strong prognostic value, pathological tumor extension stage was assessed according to TNM UICC/AJCC 6th edition and not to the last 7th edition, definition of Klatskin tumor is vague, chemo-radiotherapy in literature has still a questionable prognostic value, it is performed after surgery and not always could be performed. This stage system needs an external validation with a large cohort of patient.

Aim of this study was to evaluate prognostic factors for survival, create a new prognostic model and compare it with previous staging and prognostic scoring systems for perihilar cholangiocarcinoma.

MATERIAL AND METHODS

Patients and data collection

Patients submitted to surgical resection for cholangiocarcinoma in Division of General Surgery of the University of Verona Medical School between September 1990 and September 2012 were evaluate for this study. Data were collected retrospectively in 2006 and after that prospectively. From database patients with perihilar cholangiocarcinoma (PCC) who underwent surgical resection were analyzed. All patients included in the study underwent surgical resection with curative intent. Patients who deceased in perioperative period (within 30 days after surgery) were excluded from analysis.

All patients signed informed consent before surgery.

Preoperative evaluation

The preoperative evaluation included blood chemistry tests with a complete blood count, PT, aPTT, direct and total bilirubin, albumin, AST, ALT, ALP, GGT, CEA, CA19.9, alpha-fetoprotein and serology for hepatitis viruses (HBV, HCV). The tumor extent was evaluated with ultrasonography, color Doppler ultrasonography and CT or MRI. The differential diagnosis between cholangiocarcinoma and gastro-intestinal tumor liver metastases was made with tumor histology or by the exclusion of other primary tumors by esophagogastroduodenoscopy and colonoscopy.

In patients with obstructive jaundice, the extent of the tumor was assessed using different diagnostic methods during the study period. Between 1990 and 1997, all of the patients underwent percutaneous trans-hepatic cholangiography (PTC) with the placement of single or multiple biliary drains. Subsequently, non-invasive diagnostic methods, such as colangio-pancreatography MRI, were primarily used. All patients with obstructive jaundice (a serum bilirubin level greater than 3 mg/dL) underwent percutaneous biliary drainage to more precisely define the longitudinal extension of the tumor and to reduce the serum bilirubin to less than 3 mg/dL. A percutaneous trahepatic biliary drainage (PTBD) was preferentially positioned only in the future remnant liver. In patients with segmental cholangitis, multiple hepatic drainages of the excluded biliary segments were performed [30] . More recently, endoscopic biliary drainage, CT-PET and diagnostic laparoscopy was introduced in selected patients.

Surgical technique

During surgery, intraoperative ultrasonography was routinely used to confirm the preoperative diagnosis, to evaluate the relationship between the tumor and blood vessels and to evaluate the presence of intrahepatic

metastases. The extent of liver resection was defined according to the Brisbane classification [31] . Common bile duct resection was performed in all cases because of macroscopic involvement by the tumor. In cases of bile duct resection, frozen sections of the bile duct were made intraoperatively.

Lymphadenectomy of the regional LN was classified according to the Japanese Society of Biliary Surgery (JSBS) classification [29]. The LNs of the hepatoduodenal ligament (12 h, 12a, 12p, 12b), the proper hepatic artery (8) and the posterior surface of the pancreatic head (13) were harvested. The paraaortic LNs (16) and the LNs of other stations were retrieved if they were macroscopically suspected for metastases. The surgical LN dissection technique includes the complete dissection of the hilar structures; all fatty tissue of the hepatoduodenal ligament was retrieved and sent to pathologist.

Pathological evaluation

Tumors classification depended on the location of the main tumor and on the presence of histologically proven invasion of the main bile ducts. Tumors with involvement of the hepatic hilus structures requiring resection of the biliary confluence associated with the liver and caudate lobe resection in some cases were defined as peri-hilar cholangiocarcinoma [6, 24, 32].

The microscopic direct invasion of the bile duct wall, the portal pedicle and neural tissue was verified in all surgical specimens; all fatty tissue surrounding the vessels of the hepatoduodenal ligament and from others lymph node stations were analyzed. Metastatic LNs were defined as N+. Invasion of the wall of the major portal or hepatic vein branches and confirmed by the pathological examination was defined as macroscopic vascular invasion. Failed radical resection (R1) was defined as the presence of microscopic disease at resection margins of the specimen (bile ducts and liver).

Postoperative course

Complications were classified according to the Zurich Classification [33]. Complications greater than grade 3a were classified as major. When more than one complication occurred, the higher grade was reported.

Mortality, complication grade 5, was considered as peri-operative mortality if it occurred within 30 days of surgery and it was one of exclusion criteria.

Postoperative treatment and follow up

After surgery, the patients were regularly followed up with blood tests, tumor markers (CEA, CA19.9) and abdominal CT or MRI every 6 months. In cases of suspected recurrence at radiological exams, PET-CT was

performed. Occurrence of disease recurrence, time of recurrence, decease and time of death were recorded.

Tumor stage classification

All cases were classified according to Bismuth-Corlette classification [16], to Memorial Sloan-Kettering Cancer Center (MSKCC) Tumor Staging [18], 7th edition of the TNM UICC/AJCC classification [17], to Japanese Society for Biliary Surgery (JSBS) classification [29] and to Kaiser's prognostic scoring system [21].

Prognostic scoring systems or nomogram not specific for perihilar cholangiocarcinoma were included in the analysis.

Statistical Analysis

The differences between categorical and continue variables were analyzed with a chi-square, Fisher's exact, t-student and Mann–Whitney U tests as appropriated. Overall survival (OS) was defined as the time interval between treatment and death from any cause. Time was censored at the date of last follow-up assessment for patients who were still alive. Survival curves were estimated using the Kaplan-Meier method with the Log Rank test to verify significance of differences. Variables statistically significant at univariate analysis were investigated in the Cox model and by testing (using a likelihood-ratio test) whether the coefficient of the interaction term was significantly different from zero. A p-value lower then 0.05 was considered statistically significant. These statistical analyses and graphical representation were carried out using the R software for statistical computing, v. 2.15.0 (R Development Core Team, 2010), with the following packages: survival, Himsc.

We searched a prognostic clinical model based on multivariate Cox's models in order to classify patients in different classes of increased risk of dead. According to the parameters selected in the multivariate Cox model, we designed a prognostic model of OS. The final model identified was then fitted as a mathematical function, which resulted the risk to die before or equal to 36 months after surgical resection. To make the model more easily accessible in the clinical practice, a nomogram was proposed. For every prognostic factor a partial score was computed. The sum of partial scores gives a total points score that permits to predict the risk to die before or equal to 36 months. In order to confirm the importance of our model we classified patients in low risk (≤50%), medium risk (between 50% and 75%) and high risk (>75%) to die according to predictions based on our clinical model.

The performance of the nomogram was assessed by comparing nomogram-predicted versus observed

Kaplan-Meier estimates of survival probability. Bootstraps with 5000 resamples were used for internal validation.

Patients were categorized according to current four staging systems previously described: TNM stage 7th edition, MSKCC staging system, JSBS staging system, Kaiser prognostic scoring system.

The discriminatory ability of each staging system and of our proposed staging system was examined by expressing the consequences of the Cox model on the basis of the Akaike Information Criterion (AIC).

RESULTS

Clinicopathological Characteristics of Patients

From September 1990 to September 2012, 207 patients underwent surgery for ICC and PCC. After abdominal exploration, 145 patients underwent surgical resection with curative intent.

Seventy-five patients were classified as PCC. Six patients were excluded from analysis because of perioperative mortality; 69 patients were included in the study.

Clinical characteristics are summarised in table 1. Nineteen patients were female (27.5%) and 50 male (72.5). Median age was 66 (range 30-83). Majority of patients were symptomatic (79.7%) at time of diagnosis and symptom most frequent was jaundice (76.8%); 33 patients (47.8%) underwent positioning of biliary drainage. Preoperative median value of CA 19.9 was 95 U/mL with range between 3.5 U/mL and 6835 U/mL. Tumor was classified as Bismut-Corlette type I or type II in 10 cases, type IIIa or IIIb in 17 and 28 cases and type IV in 14 cases. Three patients were submitted to preoperative portal vein embolization because of small remnant liver volume.

Table 1: Clinical characteristics.

		Nr. Cases	**Percentage**
		69	**100%**
Gender	Female	19	27.5
	Male	50	72.5
Age	Median; range	66; 30 - 83	
Symptoms	Present	55	79.7
	Absent	14	20.3
Jaundice	Present	53	76.8
	Absent	16	23.2
Preoperative biliary drainage	Positioned	33	47.8
	Not positioned	36	52.2

Liver status	Normal	56	81.2
	Pathological*	13	18.8
CEA (ng/dL)	Median; range	2.05; 0.2 - 83	
CA 19.9 (U/mL)	Median; range	95; 3.5 - 6835	
Preoperative portal vein embolization	Performed	3	4.3
	Not performed	66	95.7
Bismuth-Corlette type	I	4	5.8
	II	6	8.7
	IIIa / IIIb	17 / 28	24.6 / 40.6
	IV	14	20.3
Neoadiuvant chemotherapy	Performed	4	5.8
	Not performed	65	94.2

* Liver status pathological: cirrhosis, fibrosis, steatosis.

Majority of patients were operated after 2005 (40 after 2005 and 29 before). Surgical variables are summarized in table 2. Resection of only common bile duct (CBD) was performed in 10 patients; all these cases were performed before 2005. Minor liver resections associated to CBD resection were performed in 2 patients (only caudate lobe in one case and caudate lobe plus segment 4 in the other); majority of patients were submitted to major liver resection. Caudate lobe resection was performed in 56 (81.2%) of patients and lymph node dissection in 66 (95.7%) of patients. Resection and reconstruction of hepatic vein or hepatic artery due to vascular infiltration by tumor were performed in 13 and 4 cases respectively. Median operation time was 547 minutes (range 250-780). Postoperative complications occurred in 30 patients and median postoperative length of stay was 19 days with range between 7 and 213 days.

Postoperative complications occurred in 30 patients (43.5%). Complications equal to or greater than grade 3a, excluding grade 5, occurred in 6 (8.7%) patients.

Table 2: Surgical characteristics.

		Nr. Cases	Percentage
		69	**100%**
Operation period	Before 2005	29	42
	After 2005	40	58
Operation type	Bile duct resection only	10	14.5
	Segmentect- / Bisegmentectomy	2	2.8
	Right hepatectomy	17	24.6
	Right trisectionectomy	8	11.6
	Left hepatectomy	27	39.1
	Left trisectionectomy	5	7.2
Liver resection side	Right side	25	43.9%
	Left side	32	56.1%
Liver resection	Associated to CBD resection	59	85.5
	Not performed	10	14.5
Hepatectomy	Minor	2	3.3
	Major	57	96.6
Caudate lobe resection	Performed	56	81.2
	Not performed	13	18.2
Vascular clamping	Without clamping	50	72.5
	Pringle maneuver	14	20.3
	Selective clamping	5	7.2
Lymph node dissection	Performed	66	95.7
	Not performed	3	4.3
Portal vein resection	Performed	13	18.8
	Not performed	56	81.2
Hepatic artery resection	Performed	4	5.8
	Not performed	65	94.2

Operative time (minutes)	Median; range	547; 250 - 780	
Postoperative hospital stay (days)	Median; range	19; 7-213	
Postoperative complications	Occurred	30	43.5
	Not occurred	39	56.5

Pathological data are reported in table 3. Radical resection was obtained in 52 patients (75.4%). Median tumor diameter was of 2.5 cm (range 2-12); tumor grading was G1 and G2 in 16 and 39 cases and G3 and G4 in 13 and 1 case, respectively. Satellites tumor nodules were present in 8 cases. Macroscopic vascular invasion of major brunch of portal vein or hepatic artery or common portal vein or hepatic artery were present in 43 cases (62.3%). Perineural infiltration was present majority of patients (89.9%). Median number of harvested lymph node was 7 (range 1-27); lymph nodes were positive in 27 patients (40.9%) and median number of positive lymph node was 3 (1-10); Pathological tumor classification according to UICC/AJCC TNM 7th Edition were stage as I, IIA and IIB in 32 cases (46.4%) and III and IV in 37 (53.6%) of cases. Patients were classified according to TNM 7th Edition as stage I and II in 25 cases (36.6%), in stage IIIA or IIIB in 23 cases (33.3%) and in stage IVA or IVB in 21 cases (30.4%), respectively.

Median follow up was of 22 months (range 4-107). Median overall survival was 26 months; cumulative proportion survival at 3 and 5 years were 44% and 28% respectively. Disease recurrence occurred in 18 patients; median overall disease free survival was 24 months with disease free survival at 3 and 5 years of 35% and 21% respectively.

Table 3: Pathological characteristics.

		Nr. Cases	**Percentage**
		69	**100%**
Curability	R0	52	75.4
	R1	17	24.6

Tumor diameter (cm)	Median; range	2.5; 2 - 12	
Grading tumor	G1 – G2	16 – 39	23.2 – 56.5
	G3 – G4	13 - 1	18.8 – 1.4
Satellites nodules	Present	8	11.6
	Absent	61	88.4
Macroscopic vascular invasion	Present	43	62.3
	Absent	26	37.7
Microscopic vascular invasion	Present	50	72.5
	Absent	19	27.5
Perineural infiltration	Present	62	89.9
	Absent	7	10.1
Glissonian infiltration	Present	25	36.2
	Absent	44	63.8
Pathological Tumor classification*	I	4	5.8
	IIA – IIB	16 – 12	23.2 – 17.4
	III	21	30.4
	IV	16	23.2
Number lymph node harvested	Median; range	7; 1 – 27	
Lymph node status	N0	39	59.1
	N+	27	40.9
Number lymph node positive	Median; range	3; 1 - 10	
Pathological Node classification*	N0	39	59.1
	N1	19	28.8
	N2	8	12.1
TNM Stage*	I	4	5.8
	II	21	30.4
	IIIA - IIIB	12 - 11	17.4 – 15.9
	IVA	13	18.8
	IVB	8	11.6

* Classification according to UICC/AJCC Cancer Staging System, TNM 7th Edition.

Univariate analysis

Univariate analysis of different clinical variables is reported in Table 4. Only preoperative CA 19.9 and operation performed after 2005 resulted as positive prognostic factors for longer survival; median survival was 42 months for patients with CA 19.9 lower than 400 U/mL and for patients operated after 2005.

Table 4: Univariate analysis of clinical variables collected. CI: Confidence interval.

		Median survival (95% CI)	***3y survival***	***5y survival***	***p-value***
Gender	Female	45.5 (9-81)	52%	41%	0.30
	Male	25 (17-33)	41%	22%	
Age	≤62 years	23 (15-32)	41%	20%	0.65
	>62 years	26 (9-42)	46%	30%	
Liver status	Normal	23 (19-28)	39%	24%	0.17
	Pathological	42 (5-79)	65%	43%	
Symptoms	Present	28 (10-47)	47%	32%	0.27
	Absent	22 (16-29)	34%	17%	
Jaundice	Present	28 (10-47)	47%	32%	0.24
	Absent	22 (15-29)	35%	17%	
Biliary drainage	Positioned	26 (17-35)	44%	37%	0.66
	Not positioned	34 (12-57)	47%	25%	
CEA (ng/dL)	≤4	42 (22-62)	63%	-	0.17
	>4	13 (0-32)	25%	-	
CA 19.9 (U/mL)	≤500	42 (28-56)	64%	33%	**0.004**
	>500	15 (7-22)	-	-	
Bismuth type	I-II	21 (14-28)	33%	0%	0.11
	IIIA-IIIB	36 (15-58)	51%	42%	
	IV	22 (7-37)	32%	16%	
Operation period	Before 2005	20 (15-24)	27%	14%	**0.02**
	After 2005	42 (36-58)	60%	46%	

From surgical variables (Table 5) major hepatectomy, caudate lobe resection, portal vein resection and reconstruction, and lymph node dissection were positive prognostic factors for longer survival. Patients who underwent major hepatectomy associated to common bile duct resection reached longer survival than patients submitted to only CBD resection or to minor liver resection with median survival of 40 and 18 months (p=0.04), respectively. Median survival, when caudate lobe resection was performed, was 36 months instead of 15 months (p=0.05). Median survival of patients without lymph node dissection was 7 months and no one patient reached 3 years survival. Median survival of patients, in whom resection and reconstruction of portal vein was necessary, had a shorter survival with a median survival of 15 months compared to 35 months for patients without vascular resection (p=0.001).

Table 5: Univariate analysis of surgical variables. CBD: common bile duct. S1: caudate lobe. CI: confidence interval.

		Median survival (95% CI)	***3y survival***	***5y survival***	***p-value***
Liver resection	Associated to CBD resection	35 (17-52)	48%	32%	0.07
	Not performed	18 (17-52)	22%	0%	
Liver resection	Right side	29 (7-50)	45%	27%	0.68
	Left side	35 (17-52)	49%	39%	
Hepatectomy	Minor/only CDB	18 (12-24)	27%	0%	**0.04**
	Major	40 (17-52)	48%	35%	
S1 resection	Performed	36 (17-56)	51%	31%	**0.05**
	Not performed	15 (8-22)	16%	0%	
LN dissection	Performed	28 (13-44)	46%	29%	**0.001**
	Not performed	7 (0-15)	0%	0%	
Vascular resection	Performed	15 (3-27)	18%	5%	**0.001**
	Not performed	35 (12-57)	48%	31%	

From pathological variables (Table 6) presence of satellites nodules was related of short term survival, with median survival of 6 months (p=0.001) and no one patient live at 5 years. Pathological tumor stage I and II combined had a median survival of 42 months compared to 15 months of stage III and IV combined (p=0.002). Extension of lymph node dissection results to be a protective prognostic factor: patients with more than 10 LNs harvested had a significantly longer median survival (37 months instead of 19 months) compared to patients with less than 10 LNs harvested (p=0.002). Lymph node status was a significant prognostic factor: patients with positive LN had a shorter survival (15 months instead of 28 months for patients without LN metastasis; p=0.04). Number of positive LN resulted to be a prognostic factor: patients with more than 3 positive lymph nodes had a shorter survival compared to patient without positive lymph nodes or with less than 3 positive lymph nodes, with median survival of 11, 43 and 35 months, respectively (p=0.01). Also lymph node ratio could significantly differentiate prognosis of patients (p=0.01); patients with LNR inferior to 0.25 had a longer survival than patients with LNR greater than 0.25 and similar to patients with LNR equal to 0 (p=0.19). Also pathological node stage (N0, N1, N2) according to TNM UICC/AJCC 7th classification was a strong prognostic factor for survival (p<0.001).

Table 6: Univariate analysis of surgical variables. CBD: common bile duct. S1: caudate lobe. CI: confidence interval.

		Median survival (95% CI)	***3y survival***	***5y survival***	***p-value***
Curability	R0	28 (15-42)	45%	34%	0.12
	R1	21 (10-31)	41%	10%	
Grading tumor	G1 – G2	34 (13-57)	47%	33%	0.35
	G3 – G4	25 (9-40)	35%	23%	
Tumor diameter	≤2 cm	42 (15-68)	52%	36%	0.32
Satellites nodules	Present	6 (0-16)	17%	-	**0.001**
	Absent	35 (16-53)	48%	30%	
Macroscopic vascular invasion	Present	23 (19-28)	38%	34%	0.87
	Absent	42 (17-66)	54%	24%	
Microscopic vascular invasion	Present	35 (20-50)	46%	41%	0.92
	Absent	42 (10-74)	59%	29%	

Perineural infiltration	Present	26 (12-39)	43%	36%	0.35
	Absent	22 (7-38)	40%	0%	
Glissonian infiltration	Present	36 (19-54)	56%	34%	0.96
	Absent	26 (1-56)	48%	31%	
Pathological Tumor stage*	I-II	42 (31-53)	51%	27%	**0.002**
	III-IV	15 (11-26)	16%	11%	
Number LN harvested	<10	19 (13-28)	25%	9%	**0.002**
	>10	37 (NA)	70%	56%	
Lymph node status	N0	28 (19-61)	49%	28%	**0.04**
	N+	15 (11-36)	20%	13%	
Number N+ harvested	0	43 (41-44)	60%	35%	**0.01#**
	1-3	35 (20-50)	48%	39%	
	>3	11 (7-15)	0%	0%	
Lymph node ratio	0	43 (41-44)	60%	35%	**0.01**§
	0-0.25	26 (16-35)	41%	-	
	>0.25	11 (0-31)	0%	0%	
Pathological Noda stage*	N0	43 (41-44)	60%	35%	**<0.001**
	N1	20 (3-37)	25%	-	
	N2	22 (1-19)	22%	0%	

* Tumor and Node classification according to UICC/AJCC Cancer Staging System, TNM 7th Edition.

Positive LN harvested: 0 LN+ vs 1-3 LN+ p=0.74; 0 LN+ vs >3 LN+ **p<0.001**; 1-3 LN+ vs >3 LN+ **p=0.004**.

§ Lymph node ratio: LNR equal to 0 compared to LNR between 0 and 0.25 was not significantly different (p=0.19).

Different scoring systems were evaluated. UICC/AJCC TNM stage 7th edition reached significant different only between stage II and stage IVB (p=0.03), however globally did not correlate with survival. If combined stage I and II compared to stage III and IV, the staging system could reach statistical prognostic value (p=0.03). Different stages according to MSKCC were not statistically different in median survival. Also JSBS does not correlate with survival but could reach significantly different survival between stage II and IVB

(p=0.008). Keiser prognostic scoring system applied to our population was a prognostic value for survival; significantly different survival was reached between stage 6 and 8, with median survival of 42 and 11 months, respectively (p=0.03). Combining group of patients with 6 or 7 points compared to patients with 8 points statistically difference was reached (median survival of 22 months compare to 8 months; p=0.02).

Table 7: Univariate analysis of different scoring systems. TNM: UICC/AJCC scoring system 7th edition. MSKCC: Memorial Sloan-Kettering Cancer Center. JSBS: Japanese Society of Biliary Surgery. CI: confidence interval. NA: not applicable.

		Median survival (95% CI)	***3y survival***	***5y survival***	***p-value***
TNM Stage 7th Eds	I-II	43 (41-44)	66%	33%	**0.03†**
	III-IV	20 (11-28)	25%	20%	
MSKCC Stage	1-2	23 (18-45)	42%	20%	0.49
	3	22 (11-28)	20%	0%	
JSBS Stage	I-II	42 (29-NA)	58%	12%	0.24°
	III-IV	20 (13-26)	25%	0%	
KEISER prognostic scoring system	6-7	22 (20-43)	37%	22%	**0.02#**
	8	8 (3-NA)	13%	0%	

† TNM 7th edition: stage I vs II p=0.49; stage I vs IIIA p=0.67; stage I vs IIIB p=0.89; stage I vs IVA p=0.32; stage I vs IVB p=0.19; stage II vs IIIA p=0.37; stage II vs IIIB p=0.83; stage II vs IVA p=0.13; stage II vs IVB p=0.03; stage IIIA vs IIIB p=0.36; stage IIIA vs IVA p=0.39, stage IIIA vs IVB p=0.88; stage IIIB vs IVA p=0.09; stage IIIB vs IVB p=0.17; stage IVA vs IVB p=0.48.

° JSBS stage: stage I vs II p=0.25; stage I vs III p=0.45; stage I vs IVA p=0.79; stage I vs IVB p=0.21; stage II vs III p=0.16; stage II vs IVA p=0.60; stage II vs IVB p=0.008; stage III vs IVA p=0.37; stage III vs IVB p=0.72; stage IVA vs IVB p=0.59.

Keiser prognostic scoring system: score 6 vs 7 p= 0.38; score 6 vs 8 p=0.14; score 7 **vs 8 p=0.03.**

Independent Prognostic Factors in the Cohort

At multivariate analysis (Table 8) number of harvested lymph node (HR 0.89; 95% CI 0.85-0.93) (p<0.001) and caudate lobe resection (HR 0.33; 95% CI 0.14-0.79) (p=0.01) were positive prognostic factor for survival. Instead, CA 19.9 greater than 500 U/mL (HR 3.38; 95% CI 1.54-7.43) (p=0.002), number of harvested positive lymph node (HR 1.20; 95% CI 0.98-1.48) (p=0.05) and portal vein resection and reconstruction (HR 7.14; 95% CI 2.75-18.76) (p<0.001) were independent prognostic factors for shorter survival.

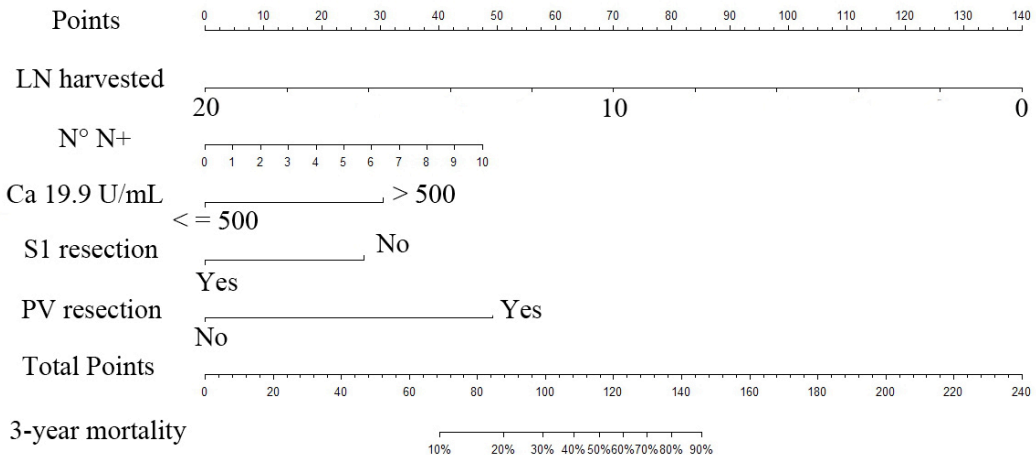
Table 8: Multivariate survival analysis of different variables related to overall survival in perihilar (PCC) cholangiocarcinoma using the Cox proportional hazards model. HR: hazard ratios. CI: confidence interval.

Variables	**HR**	**95% CI**	**p value**
CA19.9 (>500 U/mL)	3.38	1.54 – 7.43	0.002
Number lymph node harvested	0.89	0.85 – 0.93	<0.001
Number positive lymph node harvested	1.20	0.98 – 1.48	0.05
Caudate lobe resection (performed)	0.33	0.14 – 0.79	0.01
Vascular resection and reconstruction (performed)	7.14	2.75 – 18.76	<0.001

Prognostic Nomogram for Overall Survival and comparison to others Prognostic Score

The prognostic nomogram that integrates all significant independent prognostic factors for OS is shown in Fig. 7. For each variable's value of nomogram correspond a partial score and the sum of partial scores allows to predict risk of mortality at 3 years after operation.

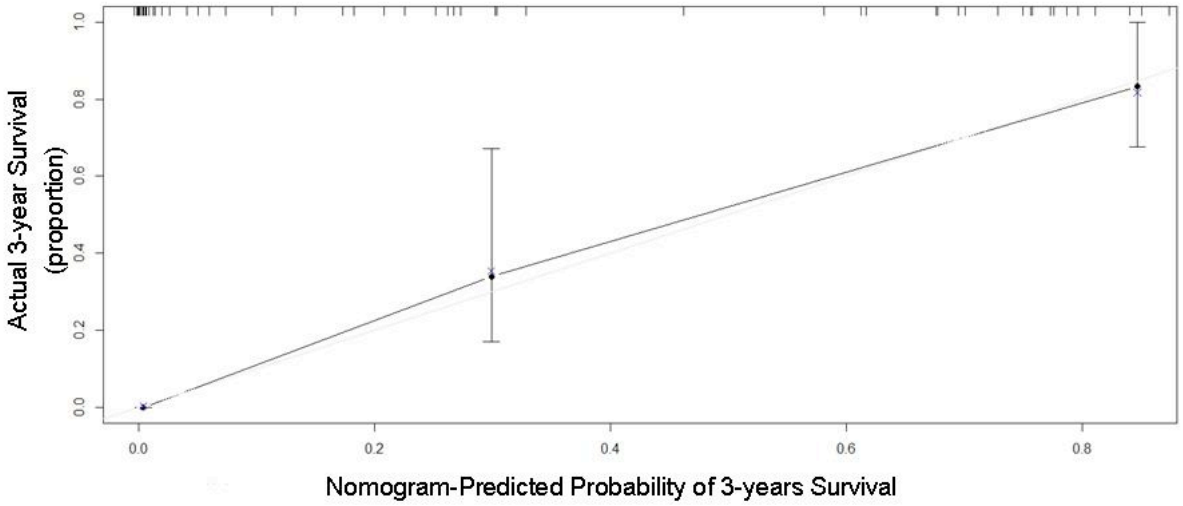
Figure 7. Perihilar cholangiocarcinoma survival nomogram. LN: lymph node. N°N+: number positive lymph node harvested. S1: caudate lobe. PV: portal vein.



According to our nomogram patients were classified in three groups according to probability to die within 3 years: low risk (<50%), medium risk (between 50% and 75%) and high risk (>75%) group of patients.

The calibration plot for the probability of survival at 3 year after surgery showed an optimal agreement between the prediction by nomogram and our cohort of patients (Figure 8).

Figure 8: Calibration curve for predicting patients survival at 3 years after surgical resection. Nomogram-predicted probability of overall survival is plotted on the x-axis; actual overall survival is plotted on the y-axis.



After that we would compare our predicting model with others staging systems. Survival was evaluated stratifying patients according to different staging systems: TNM 7th edition, MSKCC staging system, JSBS staging system and Keiser prognostic scoring system (Figure 9). UICC/AJCC TNM 7th edition staging system (stage I-II versus stage III-IV) and Keiser score (group points 6-7 compared to group 8) significantly differentiate survival. Instead, MSKCC classification and JSBS staging system were not able to significantly differentiate prognosis of our cohort of patients. Our proposed prognostic model could significantly differentiate survival of three different groups of patients according to risk of decease at 3 years. Survival between group of patients with low risk and medium risk and especially between low risk and high risk were statistically significant ($p=0.04$ and $p<0.001$ respectively) (Figure 10) (Table 9). Our proposed prognostic model for perihilar cholangiocarcinoma after surgical resection reached the greater significant value in predicting survival comparing to other staging systems evaluated (AIC 286.9) (Table 9); our model could stratify patients in three groups and good correlate with survival.

Figure 9: Kaplan-Meier survival curves of patients submitted to surgical resection according to different prognostic scoring systems. TNM: AICC/UICC TNM staging system 7th edition. MSKCC: Memorial Sloan-Kattering Cancer Center classification. JSBS: Japanese Society of Biliary Suergery. Kaiser: Kaiser prognostic scoring system.

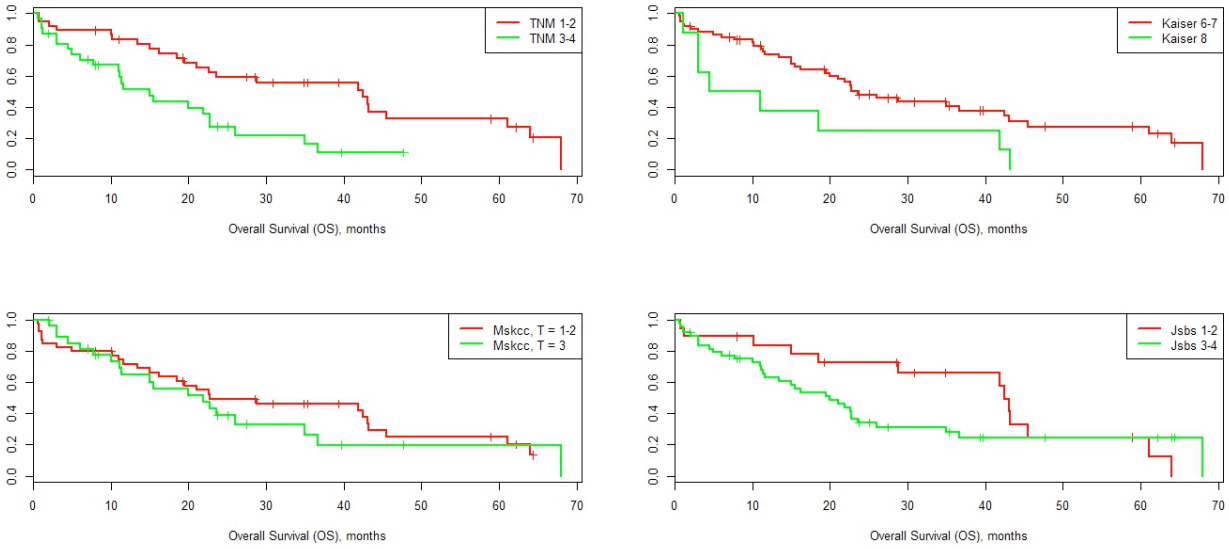


Figure 10. Kaplan-Meier survival curves of patients submitted to surgical resection according to our proposed prognostic system for perihilar cholangiocarcinoma. Low risk: 3 years mortality risk lower than 50%. Medium risk: 3 years mortality risk between 50% and 75%. High risk: 3 years mortality risk greater than 75%. Low vs medium risk: p-value 0.04. Low vs high risk: p-value <0.001.

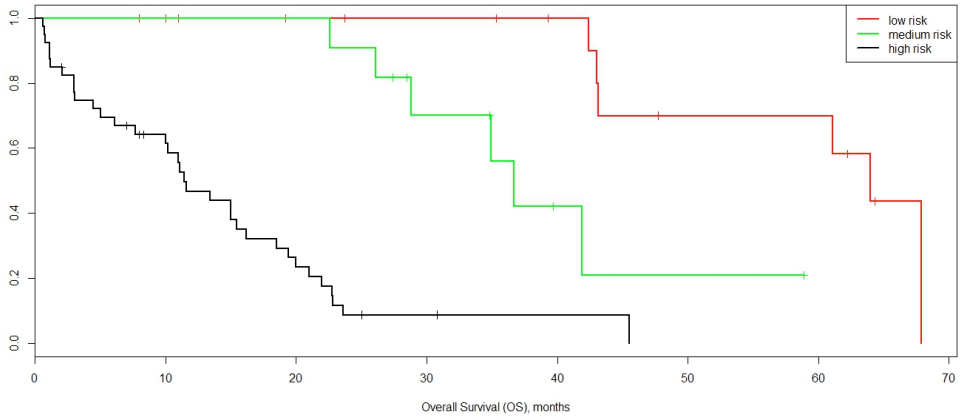


Table 9: Evaluation of different staging systems.

TNM stage: pathological stage according to AICC/UICC staging system. MSKCC: Memorial Sloan-Kettering Cancer Canter classification. Kaiser: prognostic scoring system. JSBS: Japanese Society for bile

Variables	**H.R.**	**95% CI**	**P value**	**AIC**
TNM Stage (I-II vs stage III-IV)	2.60	1.39 – 4.87	**0.002**	306.1
MSKCC Classification (1-2 vs 3)	1.32	0.72 – 2.40	0.361	331.0
JSBS Staging System (I-II vs III-IV)	1.48	0.77 – 2.85	0.24	313.7
Kaiser Prognostic System (6-7 vs 8)	2.66	1.27 – 5.56	**0.02**	310.9
Perihilar Prognostic Classification				268.9
(Low vs Medium Risk)	4.35	1.05 – 17-9	**0.04**	
(Low vs High Risk)	23.71	6.84 – 82.1	**<0.001**	

DISCUSSION

Cholangiocarcinoma is the second most frequent primary liver tumor and its incidence is increased in Western countries in last 30 years [3]. Perihilar cholangiocarcinoma is defined as biliary tumor originating and located in the biliary confluence and extending to extrahepatic biliary tree either to intrahepatic parenchyma [7] . Perihilar cholangiocarcinoma is rarely operable and even when surgical operation is feasible long term survival is dismal but surgical resection is the only chance to cure this aggressive disease [34] . Surgical management is demanding, however recent aggressive surgical operations could reach good long-term survival [34].

Different clinicopathological factors have been previously evaluated and related to survival: radical resection, vascular invasion, tumor size, multifocality, tumor grading, perineural invasion, lymph node metastasis, tumor stage and positive resection margins [10, 26, 35-40] .

In our study we evaluated several clinical and pathological variables of patients submitted to surgical resection with radical intent. From univariate analysis CA 19.9 preoperative value greater than 500 U/mL was a significant prognostic factor for survival reflecting biological behaviour of tumor. CA 19.9 is a tumor marker increased in other gastrointestinal tumors; also in others series of patients with perihilar cholangiocarcinoma was related with poor prognosis [41, 42 43] . CA 19.9 is an easily available test laboratory that could correlate with poor survival and especially it's available preoperatively. Patients submitted to surgical resection after 2005 had a longer survival; this longer survival, as confirmed from other studies, is due to more aggressive surgical treatment [34, 37, 44] . Also from our data, aggressive treatment reach longer survival; indeed, major hepatectomy, caudate lobe resection and lymph node dissection were significant prognostic factor for longer survival. Lymph node metastasis is a strong prognostic factor and was already evaluated in literature [34-36, 45, 46] . Lymph node metastasis is a prognostic factor per se but patients with low value lymph node ratio (<0.25) have survival similar to patients without positive lymph node. For this reasons we suggest to perform an extensive lymph node dissection to better stratify patients. Patients with more than 10 lymph node harvested had a significantly longer survival. In literature extension of lymph node dissection is still under discussion, however recent studies are giving important additional data about that [35 47] .

Cancers are classified in different staging systems. Tumor staging system is important to have a classification of tumor reflecting prognosis, to guide for therapy and to compare results of different case series among various institution and over time. Perihilar cholangiocarcinoma is increasing in last years and clinical and surgical approach is evolving in last years; for this reasons, few classifications are available and

they are still under discussion and modification. Bismuth-Corlette evaluates clinical and radiological extention of tumor along bile ducts and it gives information about operability and extension of surgical resection. This classification was evaluated in different studies however it was not associated with prognostic significance [28]. Also in our study Bismuth classification did not have significant prognostic value for survival. MSKCC classification was proposed to evaluate preoperative respectability, however it did not correspond to current concepts of respectability anymore. The Authors demonstrated value of classification to predict resectability and also to correlate with survival, however different Authors not reached same results in different cohort of patients. Similarly, in our study MSKCC classification did not correlated with prognosis. The last edition of TNM UICC/AJCC classification included a specifically classification for perihilar cholangiocarcinoma, because in previous edition (6th edition) the perihilar tumor was included with extrahepatic bile duct cancer. Pathological tumor stage (pT stage) and TNM stage according to 7th edition were evaluated in literature as prognostic factor [36]. In our study stratifying patients in 4 stages we did not reach significant difference, but comparing stage I-II to stage III-IV it correlates to long term survival. Limitation of TNM stage is that it is not applicable preoperatively because it is based on pathological report. JSBS staging system is a quite complex pathological classification and it has limited application in clinical practice. We classified patients according to JSBS, however it could not predict prognosis. The new proposed staging system for perihilar cholangiocarcinoma is only a descriptive system and up to now it has not a prognostic function.

Recently was published a novel prognostic scoring system for Klatskin tumor by group from Essen [21]. This is the first attempt to create a prognostic scoring system for surgical treatment of perihilar cholangiocarcinoma; it includes clinical (age), pathological (surgical margin and tumor stage) and postoperative variables (adjuvant chemo-radiotherapy). This is an easy and reproducible system, however it includes questionable variables. Radical resection, even in large series, could be obtained only in about 60% of patients [34] and it is not a strong prognostic factor for this type of tumor (studio R+) and incomplete resection (R2) has worst prognosis than R1 resection. Pathological tumor stage I is quite rare for this type of tumor, also in large series [34, 36]. Finally, Authors classified tumor with TNM 6th edition instead of more recent one.

Recently a score system [48] and a nomogram [49] for resected biliary tract cancer were published . In a small series of patients including 2 patients with extrahepatic cholangiocarcinoma and 5 patients with Klatskin tumor, Authors created a point score with preoperative and postoperative blood level of AST, ALT Bilirubin and CA 19.9, symptoms at diagnosis and site of tumor. This is an interesting study, however not specific for perihilar cholangiocarcinoma. In addition, patients with PCC often have jaundice at diagnosis and need preoperative biliary drainage to reduce postoperative complication [30] ; moreover, jaundice increases CA 19.9

blood level [42] . Nomogram proposed by group of van Gulik for patients undergoing resection of extrahepatic cholangiocarcinoma, included lymph node status, microscopic residual tumor at resection margin, tumor differentiation grade. Tumor location was not inserted in the nomogram because it did not independently predicted survival after tumor resection. Nomogram could predict survival better than TNM stage. Even location did not predict survival in this study, perihilar and distal cholangiocarcinoma have different type of surgical management and different prognosis in literature. In our analysis we did not considered these two scoring system and nomogram described, because they are not specific for perihilar cholangiocarcinoma, however in future we could investigate also these interesting aspects.

Nomograms have been developed and shown to be more accurate than conventional staging systems for predicting prognosis [50, 51] . Thus, because of limited variables included in pathological staging systems and lack of prognostic staging systems, we tried to construct a nomogram including clinical and pathological variables for patients submitted to surgical operation for perihilar cholangiocarcinoma. At multivariate analysis CA 19.9 serum level, caudate lobe resection, vascular resection and reconstruction, number of lymph node harvested and number of positive lymph node, confirmed to be strong prognostic factors. We create a nomogram with these five clinical, surgical and pathological variables. Nomogram was able to well predict survival and his prediction was internally validated with calibration curve. From our nomogram we could create a prognostic model stratifying patients in 3 groups with different risk to deceased within 3 years. Applying different staging system to our population TNM staging system and Kaiser's prognostic scoring system were able to predict survival, although our predicting model showed better predictive accuracy for survival. Moreover our nomogram includes important clinical, as CA 19.9, and surgical prognostic factors, otherwise not considered in other staging system. TNM stage was not included in our prognostic model, because lymph node status was a strongest prognostic variable compared to pathological tumor stage. TNM stage system has some limitation in the correct classification of perihilar cholangiocarcinoma and in almost of studies it is able to correlate to survival only if combining different stages.

There are several limitations on this study. First, creation of nomogram and prognostic scoring system was performed from a small cohort of patients and from a single European institution. External validation with more cases should be performed. Second limitation is that also this prognostic scoring system is applicable only after surgical operation, even if we included also clinical variable. Relevant clinical and radiological prognostic factors should be included in a preoperative prognostic scoring system to predict survival and to address best treatment. As for other gastrointestinal tumor, molecular markers are increasing also for cholangiocarcinoma and in future should be incorporate in preoperative staging system. Third, our

nomogram includes caudate lobe resection and portal vein resection-reconstruction; currently caudate lobe has to be resected in every patient with perihilar cholangiocarcinoma submitted to surgical resection, therefore it should not be different from patient to another and probably in the future it will be less strong prognostic factor. Portal vein resection and reconstruction could be a bad prognostic factor due to macroscopic vascular infiltration, however it could not be necessary related with infiltration and moreover Neuhaus theorized “en-bloc” resection of liver with portal vein confluence obtaining longer survival even in patients without portal vein infiltration [52]. Finally, pathological lymph node count after surgical dissection should be standardized because it could be dissimilar in different institutions.

Our nomogram good correlates with survival; it includes prognostic factor related to biological behaviour of tumor (CA 19.9, number of positive lymph node) and surgical characteristics necessary to obtain radical resection (caudate lobe resection, lymph node harvested and portal vein resection). In the future knowledge about specific circulating tumor cells, molecular prognostic factors and new adjuvant chemotherapy has to be developed, therefore to incorporate that in a new prognostic scoring system.

.

CONCLUSION

Perihilar cholangiocarcinoma is an aggressive tumor with bad prognosis and so far surgical resection is the only chance to cure. Our nomogram, including CA 19.9, number of lymph node harvested, number of positive lymph node, caudate lobe resection and portal vein resection, could well predict prognosis of patients after surgical resection and could address adjuvant treatment and follow up.

REFERENCES

1. Nakeeb A, Pitt HA, Sohn TA, et al. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; 224(4):463-73; discussion 473-5.
2. de Groen PC, Gores GJ, LaRusso NF, et al. Biliary tract cancers. *N Engl J Med* 1999; 341(18):1368-78.
3. Khan SA, Toledano MB, Taylor-Robinson SD. Epidemiology, risk factors, and pathogenesis of cholangiocarcinoma. *HPB (Oxford)* 2008; 10(2):77-82.
4. DeOliveira ML, Cunningham SC, Cameron JL, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; 245(5):755-62.
5. Klatskin G. Adenocarcinoma of the Hepatic Duct at Its Bifurcation within the Porta Hepatis. An Unusual Tumor with Distinctive Clinical and Pathological Features. *Am J Med* 1965; 38:241-56.
6. Ebata T, Kamiya J, Nishio H, et al. The concept of perihilar cholangiocarcinoma is valid. *Br J Surg* 2009; 96(8):926-34.
7. Nagino M. Perihilar cholangiocarcinoma: a surgeon's viewpoint on current topics. *J Gastroenterol* 2012; 47(11):1165-76.
8. Kondo S, Hirano S, Ambo Y, et al. Forty consecutive resections of hilar cholangiocarcinoma with no postoperative mortality and no positive ductal margins: results of a prospective study. *Ann Surg* 2004; 240(1):95-101.
9. Nishio H, Nagino M, Nimura Y. Surgical management of hilar cholangiocarcinoma: the Nagoya experience. *HPB (Oxford)* 2005; 7(4):259-62.
10. Guglielmi A, Ruzzenente A, Campagnaro T, et al. Does intrahepatic cholangiocarcinoma have better prognosis compared to perihilar cholangiocarcinoma? *J Surg Oncol* 2010; 101(2):111-5.
11. Lai EC, Lau WY. Aggressive surgical resection for hilar cholangiocarcinoma. *ANZ J Surg* 2005; 75(11):981-5.
12. Ito F, Cho CS, Rikkers LF, et al. Hilar cholangiocarcinoma: current management. *Ann Surg* 2009; 250(2):210-8.
13. Rosen CB, Heimbach JK, Gores GJ. Liver transplantation for cholangiocarcinoma. *Transpl Int* 2010; 23(7):692-7.

14. Nathan H, Pawlik TM, Wolfgang CL, et al. Trends in survival after surgery for cholangiocarcinoma: a 30-year population-based SEER database analysis. *J Gastrointest Surg* 2007; 11(11):1488-96; discussion 1496-7.

15. Sobin LH. TNM: principles, history, and relation to other prognostic factors. *Cancer* 2001; 91(8 Suppl):1589-92.

16. Bismuth H, Corlette MB. Intrahepatic cholangioenteric anastomosis in carcinoma of the hilus of the liver. *Surg Gynecol Obstet* 1975; 140(2):170-8.

17. Edge SB, Byrd DR, Compton CC, et al. AJCC Cancer Staging Handbook. 7th ed. ed. Chicago, IL: Springer, 2010.

18. Jarnagin WR, Fong Y, DeMatteo RP, et al. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; 234(4):507-17; discussion 517-9.

19. Surgery JSoB. General rules for surgical and pathological studies on cancer of the biliary tract. 5th edn. ed. Tokyo: Kanehara, 2003.

20. Deoliveira ML, Schulick RD, Nimura Y, et al. New staging system and a registry for perihilar cholangiocarcinoma. *Hepatology* 2011; 53(4):1363-71.

21. Kaiser GM, Paul A, Sgourakis G, et al. Novel prognostic scoring system after surgery for Klatskin tumor. *Am Surg* 2013; 79(1):90-5.

22. Bismuth H, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 1992; 215(1):31-8.

23. Patel T. Cholangiocarcinoma--controversies and challenges. *Nat Rev Gastroenterol Hepatol* 2011; 8(4):189-200.

24. Nishio H, Nagino M, Oda K, et al. TNM classification for perihilar cholangiocarcinoma: comparison between 5th and 6th editions of the AJCC/UICC staging system. *Langenbecks Arch Surg* 2005; 390(4):319-27.

25. Juntermanns B, Sotiropoulos GC, Radunz S, et al. Comparison of the sixth and the seventh editions of the UICC classification for perihilar cholangiocarcinoma. *Ann Surg Oncol* 2013; 20(1):277-84.

26. Liu CL, Fan ST, Lo CM, et al. Improved operative and survival outcomes of surgical treatment for hilar cholangiocarcinoma. *Br J Surg* 2006; 93(12):1488-94.

27. Hong SM, Kim MJ, Pi DY, et al. Analysis of extrahepatic bile duct carcinomas according to the New American Joint Committee on Cancer staging system focused on tumor classification problems in 222 patients. *Cancer* 2005; 104(4):802-10.

28. Zervos EE, Osborne D, Goldin SB, et al. Stage does not predict survival after resection of hilar cholangiocarcinomas promoting an aggressive operative approach. *Am J Surg* 2005; 190(5):810-5.

29. Surgery JSoB. Classification of Biliary Tract Carcinoma. Japanese Society of Biliary Surgery. Second ed. Tokio: Kanehara & Co. Ltd., 2004.

30. Iacono C, Ruzzenente A, Campagnaro T, et al. Role of preoperative biliary drainage in jaundiced patients who are candidates for pancreatoduodenectomy or hepatic resection: highlights and drawbacks. *Ann Surg* 2013; 257(2):191-204.

31. Strasberg SM. Nomenclature of hepatic anatomy and resections: a review of the Brisbane 2000 system. *J Hepatobiliary Pancreat Surg* 2005; 12(5):351-5.

32. Sano T, Shimada K, Sakamoto Y, et al. Prognosis of perihilar cholangiocarcinoma: hilar bile duct cancer versus intrahepatic cholangiocarcinoma involving the hepatic hilus. *Ann Surg Oncol* 2008; 15(2):590-9.

33. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; 240(2):205-13.

34. Nagino M, Ebata T, Yokoyama Y, et al. Evolution of Surgical Treatment for Perihilar Cholangiocarcinoma: A Single-Center 34-Year Review of 574 Consecutive Resections. *Ann Surg* 2012.

35. Guglielmi A, Ruzzenente A, Campagnaro T, et al. Prognostic significance of lymph node ratio after resection of peri-hilar cholangiocarcinoma. *HPB (Oxford)* 2011; 13(4):240-5.

36. Nuzzo G, Giuliante F, Ardito F, et al. Improvement in perioperative and long-term outcome after surgical treatment of hilar cholangiocarcinoma: results of an Italian multicenter analysis of 440 patients. *Arch Surg* 2012; 147(1):26-34.

37. Saxena A, Chua TC, Chu FC, et al. Improved outcomes after aggressive surgical resection of hilar cholangiocarcinoma: a critical analysis of recurrence and survival. *Am J Surg* 2011; 202(3):310-20.

38. Song SC, Choi DW, Kow AW, et al. Surgical outcomes of 230 resected hilar cholangiocarcinoma in a single centre. *ANZ J Surg* 2012.

39. de Jong MC, Marques H, Clary BM, et al. The impact of portal vein resection on outcomes for hilar cholangiocarcinoma: a multi-institutional analysis of 305 cases. *Cancer* 2012; 118(19):4737-47.

40. Lee JH, Hwang DW, Lee SY, et al. The proximal margin of resected hilar cholangiocarcinoma: the effect of microscopic positive margin on long-term survival. *Am Surg* 2012; 78(4):471-7.

41. Aljiffry M, Abdulelah A, Walsh M, et al. Evidence-based approach to cholangiocarcinoma: a systematic review of the current literature. *J Am Coll Surg* 2009; 208(1):134-47.

42. Marrelli D, Caruso S, Pedrazzani C, et al. CA19-9 serum levels in obstructive jaundice: clinical value in benign and malignant conditions. *Am J Surg* 2009; 198(3):333-9.

43. Hatzaras I, Schmidt C, Muscarella P, et al. Elevated CA 19-9 portends poor prognosis in patients undergoing resection of biliary malignancies. *HPB (Oxford)* 2010; 12(2):134-8.

44. Sano T, Shimada K, Sakamoto Y, et al. One hundred two consecutive hepatobiliary resections for perihilar cholangiocarcinoma with zero mortality. *Ann Surg* 2006; 244(2):240-7.

45. Kitagawa Y, Nagino M, Kamiya J, et al. Lymph node metastasis from hilar cholangiocarcinoma: audit of 110 patients who underwent regional and paraaortic node dissection. *Ann Surg* 2001; 233(3):385-92.

46. Dinant S, Gerhards MF, Rauws EA, et al. Improved outcome of resection of hilar cholangiocarcinoma (Klatskin tumor). *Ann Surg Oncol* 2006; 13(6):872-80.

47. Aoba T, Ebata T, Yokoyama Y, et al. Assessment of nodal status for perihilar cholangiocarcinoma: location, number, or ratio of involved nodes. *Ann Surg* 2013; 257(4):718-25.

48. Berardi R, Mocchegiani F, Pierantoni C, et al. Resected biliary tract cancers: a novel clinical-pathological score correlates with global outcome. *Dig Liver Dis* 2013; 45(1):70-4.

49. van der Gaag NA, Kloek JJ, de Bakker JK, et al. Survival analysis and prognostic nomogram for patients undergoing resection of extrahepatic cholangiocarcinoma. *Ann Oncol* 2012; 23(10):2642-9.

50. Sternberg CN. Are nomograms better than currently available stage groupings for bladder cancer? *J Clin Oncol* 2006; 24(24):3819-20.

51. Touijer K, Scardino PT. Nomograms for staging, prognosis, and predicting treatment outcomes. *Cancer* 2009; 115(13 Suppl):3107-11.

52. Neuhaus P, Thelen A, Jonas S, et al. Oncological superiority of hilar en bloc resection for the treatment of hilar cholangiocarcinoma. *Ann Surg Oncol* 2012; 19(5):1602-8.

ACKNOWLEDGMENTS

Doctor Fabio Bagante for his fancy statistical aptitude.

Doctor Andrea Ruzzenente for his pragmatic view in clinical studies.

Professor Calogero Iacono for his professional and punctual suggestions.