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ORIGINAL ARTICLE

Lymphovascular and perineural invasion as selection criteria for adjuvant therapy in intrahepatic cholangiocarcinoma: a multi-institution analysis

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Abstract

Objectives: Criteria for the selection of patients for adjuvant chemotherapy in intrahepatic cholangiocarcinoma (IHCC) are lacking. Some authors advocate treating patients with lymph node (LN) involvement; however, nodal assessment is often inadequate or not performed. This study aimed to identify surrogate criteria based on characteristics of the primary tumour.

Methods: A total of 58 patients who underwent resection for IHCC between January 2000 and January 2010 at any of three institutions were identified. Primary outcome was overall survival (OS).

Results: Median OS was 23.0 months. Median tumour size was 6.5 cm and the median number of lesions was one. Overall, 16% of patients had positive margins, 38% had perineural invasion (PNI), 40% had lymphovascular invasion (LVI) and 22% had LN involvement. A median of two LNs were removed and a median of zero were positive. Lymph nodes were not sampled in 34% of patients. Lymphovascular and perineural invasion were associated with reduced OS [9.6 months vs. 32.7 months (P = 0.020) and 10.7 months vs. 32.7 months (P = 0.008), respectively]. Lymph node involvement indicated a trend towards reduced OS (10.7 months vs. 30.0 months; P = 0.063). The presence of either LVI or PNI in node-negative patients was associated with a reduction in OS similar to that in node-positive patients (12.1 months vs. 10.7 months; P = 0.541). After accounting for adverse tumour factors, only LVI and PNI remained associated with decreased OS on multivariate analysis (hazard ratio 4.07, 95% confidence interval 1.60–10.40; P = 0.003).

Conclusions: Lymphovascular and perineural invasion are separately associated with a reduction in OS similar to that in patients with LN-positive disease. As nodal dissection is often not performed and the number of nodes retrieved is frequently inadequate, these tumour-specific factors should be considered as criteria for selection for adjuvant chemotherapy.

Keywords

intrahepatic cholangiocarcinoma, lymphovascular invasion, perineural invasion, lymphadenectomy

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Introduction

Cholangiocarcinoma (CC) is the most common primary hepatic malignancy after hepatocellular carcinoma. It affects 5000–8000 individuals per year in the USA[1] and globally accounts for 3% of all gastrointestinal malignancies.[2] Cholangiocarcinoma is an aggressive cancer in which longterm survival is poor as a result of the late presentation of disease and the limited therapies available. Indeed, the overall mortality rate in CC approaches its incidence.[1]

Cholangiocarcinoma is divided into three general categories according to whether the anatomic location of origin of disease is intrahepatic, hilar or distal. Although hilar CC remains the most common type, the incidence of intrahepatic cholangiocarcinoma (IHCC) is rising[3–5] and IHCC currently accounts for 20% of all CC.[5] Many believe that the anatomic location of CC corresponds to specific and distinct tumour biology, as evidenced by separate staging systems for intrahepatic, hilar and distal tumours; therefore, each type of CC should be considered separately. This study focuses specifically on IHCC.

Resection is the mainstay of treatment for IHCC and offers the only opportunity for cure. Resectability rates in IHCC range from 46% to 75%.[6] Improved surgical technique and refinements in patient selection and perioperative care have contributed to increased survival in resectable IHCC.[7] Unfortunately, even after complete resection, 5-year survival in IHCC remains poor (5–43%), indicating that resection alone is not sufficient for most patients.[1,8]

Current chemotherapy regimens are only marginally effective and gemcitabine monotherapy has not been shown to be beneficial in the adjuvant setting.[9] No guidelines for adjuvant therapy for IHCC exist.[10] Recently, the Advanced Biliary Cancer (ABC) Trial demonstrated improved survival in patients with advanced-stage disease who received doublet chemotherapy, consisting of gemcitabine and cisplatin, compared with gemcitabine monotherapy (11.7 months vs. 8.1 months; $P < 0.001$).[11] It is possible that the survival advantage of this more aggressive doublet regimen may translate to the adjuvant setting, but this must be evaluated in a prospective clinical trial. The benefit of adjuvant therapy in resected IHCC will undoubtedly depend on appropriate patient selection.

The presence of lymph node (LN) metastases is commonly utilized to select patients for adjuvant therapy after complete resection of many gastrointestinal malignancies, including IHCC.[12–15] However, portal lymphadenectomy is not routinely performed in IHCC and its use remains controversial.[6,8,16–18] Furthermore, when it is performed, its nodal yield is often inadequate for accurate staging. Given the frequent lack of information regarding nodal status in IHCC, the present study sought to identify a surrogate marker for adverse tumour biology based on the characteristics of the primary tumour. The authors have previously shown lymphovascular invasion (LVI) to be an independent prognostic factor for shortened overall survival (OS) in a single-institution series of resected hilar and intrahepatic CC.[19] The current multi-institution study focuses specifically on prognostic factors in resected IHCC.

Materials and methods

Hepatobiliary resection databases at three institutions (Emory University, University of Wisconsin and Ohio State University) were reviewed for all patients with a diagnosis of IHCC who underwent resection between January 2000 and January 2010. Permission from the institutional review board of each institution was obtained prior to data review. Health Insurance Portability and Accountability Act compliance was ensured.

A total of 58 patients who underwent resection for IHCC were identified. Thirty-five of these patients had been included in a prior analysis that assessed the value of LVI in a single-institution series of intrahepatic and hilar CC.[19] Data for these 35 patients are now reanalysed with updated follow-up, along with data for a different cohort of patients who underwent treatment for IHCC at two other institutions. Overall survival was ascertained through the clinical follow-up documented in each patient's medical record and the Social Security Death Index. Pathology reports were reviewed for important tumour factors that are known to have prognostic value for patient survival. These include tumour size, number and grade, margin status, LN involvement, and the presence of LVI or perineural invasion (PNI).[15,20,21] Patients who did not undergo LN dissection were regarded as node-negative as this is consistent with clinical practice patterns. Perioperative mortality was defined as death within 90 days of operation.

Statistical analysis

Data were analysed using SPSS Version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Kaplan–Meier log-rank survival analysis was used to determine the association of each pathologic factor with patient survival. Univariate Cox regression analysis was performed for adverse tumour factors to determine their association with OS. Factors significant at a level of $P < 0.2$ were included in a multivariate Cox regression model.

Results

A total of 58 patients underwent resection. The median age of the study sample was 66 years (range: 29–89 years); 38 patients (66%) were female. Pathologic and perioperative variables are summarized in Table 1. Thirteen patients had pathologically proven LN disease. Rates of LN positivity ranged from 22% in all patients in this study to 34% in those who underwent LN procurement with pathologic assessment ($n = 38$). Because of the referral patterns of the three institutions, data on adjuvant therapy are limited. Of the 48 patients whose adjuvant treatment status is known, 13 patients

Table 1 Clinicopathologic characteristics of patients with intrahepatic cholangiocarcinoma (n = 58)

Variable	Patients, n (%)	Median (range)
Operative characteristics		
Estimated blood loss, ml		500 (50–4000)
Type of resection		
Left hepatectomy	14 (24%)	
Left lateral sectorectomy	6 (10%)	
Left trisegmentectomy	1 (2%)	
Right hepatectomy	8 (14%)	
Right trisegmentectomy	10 (17%)	
Extended right hepatectomy	13 (23%)	
Other	6 (10%)	
Pathologic characteristics		
Positive margin	9 (16%)	
Tumour size, cm		6.5 (1.3–21)
Tumour size ≥6.5 cm	32 (55%)	
Number of lesions		1 (1–7)
Multiple tumours	12 (21%)	2 (2–7)
No lymphadenectomy performed	20 (34%)	
Lymph node-positive disease	13 (22%)	
Number of nodes retrieved		2 (1–10)
Number of positive nodes		0 (0–5)
Differentiation		
Good	3 (5%)	
Moderate	32 (55%)	
Poor	19 (33%)	
Unknown	4 (7%)	
Lymphovascular invasion	23 (40%)	
Perineural invasion	22 (38%)	
Postoperative course		
Length of hospital stay, days		8 (1–44)
Complications	30 (52%)	
Infectious	15 (26%)	
Bleeding	3 (5%)	
Bile leak	3 (5%)	
Reoperation	3 (5%)	
Perioperative 30-day mortality	6 (10%)	
Perioperative 90-day mortality	8 (14%)	

received chemotherapy and six received radiation therapy. Precise information on the chemotherapy regimen was not available.

Survival analysis

The median follow-up in survivors was 22.0 months (range: 0.4–81.4 months). At the time of last follow-up, 35 patients (60%) had died. Median OS in all patients was 23.0 months (range: 0.3–81.4 months). The results of univariate and multivariate Cox regression analyses for OS are shown in Table 2.

Lymph node involvement resulted in a strong trend towards reduced OS (10.7 months vs. 30.0 months; $P = 0.063$) (Fig. 1a). A subset analysis of patients who did not undergo lymphadenectomy compared with those who were pathologically identified as LN-negative did not demonstrate any difference in survival (54.4 months vs. 21.1 months; $P = 0.234$) (Fig. 1b). Lymphovascular invasion was associated with reduced OS (9.6 months vs. 32.7 months; $P = 0.020$) (Fig. 2a). Perineural invasion was also associated with reduced OS (10.7 months vs. 32.7 months; $P = 0.008$) (Fig. 2b). A representative image demonstrating LVI and PNI is shown in Fig. 3.

Lymphovascular and perineural invasion were statistically more likely to occur within the same tumour specimen [LVI+/PNI+: 24.1%; LVI+/PNI–: 15.5%; LVI–/PNI+: 13.8%; LVI–/PNI–: 46.6% ($P = 0.008$)], but the presence of both LVI and PNI did not portend worse survival compared with the presence of either alone (Fig. 4). There was no association between LN positivity and the presence of LVI [LN+/LVI+: 12.1%; LN–/LVI+: 27.6% ($P = 0.387$)] or between LN positivity and the presence of PNI [LN+/PNI+: 13.8%; LN–/PNI+: 24.1% ($P = 0.095$)]. In a subset analysis of node-negative patients only ($n = 45$), LVI demonstrated a strong trend towards reduced survival (12.1 months vs. 45.8 months; $P = 0.124$) (Fig. 5a). In the same subset of LN-negative patients, PNI was significantly associated with reduced OS (12.1 months vs. 32.7 months; $P = 0.052$) (Fig. 5b). In node-negative patients, the presence of either LVI or PNI was associated with reduced OS (12.1 months) similar to that in patients with node-positive disease (10.7 months) ($P = 0.541$) (Fig. 6). After accounting for adverse tumour factors including tumour size and number, margin status, grade, and LN status, only the presence of LVI or PNI persisted as negative prognostic factors for reduced OS in a multivariate Cox regression analysis (hazard ratio 4.07, 95% confidence interval 1.60–10.40; $P = 0.003$) (Table 2).

Discussion

Cholangiocarcinoma is a deadly disease with an overall mortality rate that is similar to its incidence.[22] Resection offers the only chance for cure, yet 5-year survival in this population remains < 40% in most studies.[23–25] Despite this dismal prognosis, evidence supporting the use of adjuvant chemotherapy after complete resection of IHCC is lacking.[10,15,26] Recently, the ABC-02 Trial demonstrated an improvement in OS in the advanced disease setting with a doublet chemotherapy regimen of gemcitabine and cisplatin.[11] It remains to be determined whether or not this advantage will transfer to the adjuvant setting. As with other malignancies, appropriate selection of patients for adjuvant therapy will be key to maximizing therapeutic benefits while minimizing toxicity.

The presence of adverse pathologic factors, such as LN metastases, is commonly utilized to select patients for adjuvant

Table 2 Factors associated with overall survival (90-day mortality excluded, $n = 50$)

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Positive margin	0.59	0.17–2.00	0.394	–	–	–
Tumour size ≥6.5 cm	1.11	0.49–2.54	0.805	–	–	–
Multiple tumours	1.73	0.77–3.90	0.188	0.91	0.35–2.35	0.839
Poor grade	2.23	0.92–5.42	0.077	2.52	0.94–6.77	0.066
Positive lymph node involvement	2.45	1.05–5.74	0.039	1.90	0.72–5.03	0.197
LVI or PNI	**3.39**	**1.52–7.56**	**0.003**	**4.07**	**1.60–10.40**	**0.003**

HR, hazard ratio; 95% CI, 95% confidence interval; LVI, lymphovascular invasion; PNI, perineural invasion.

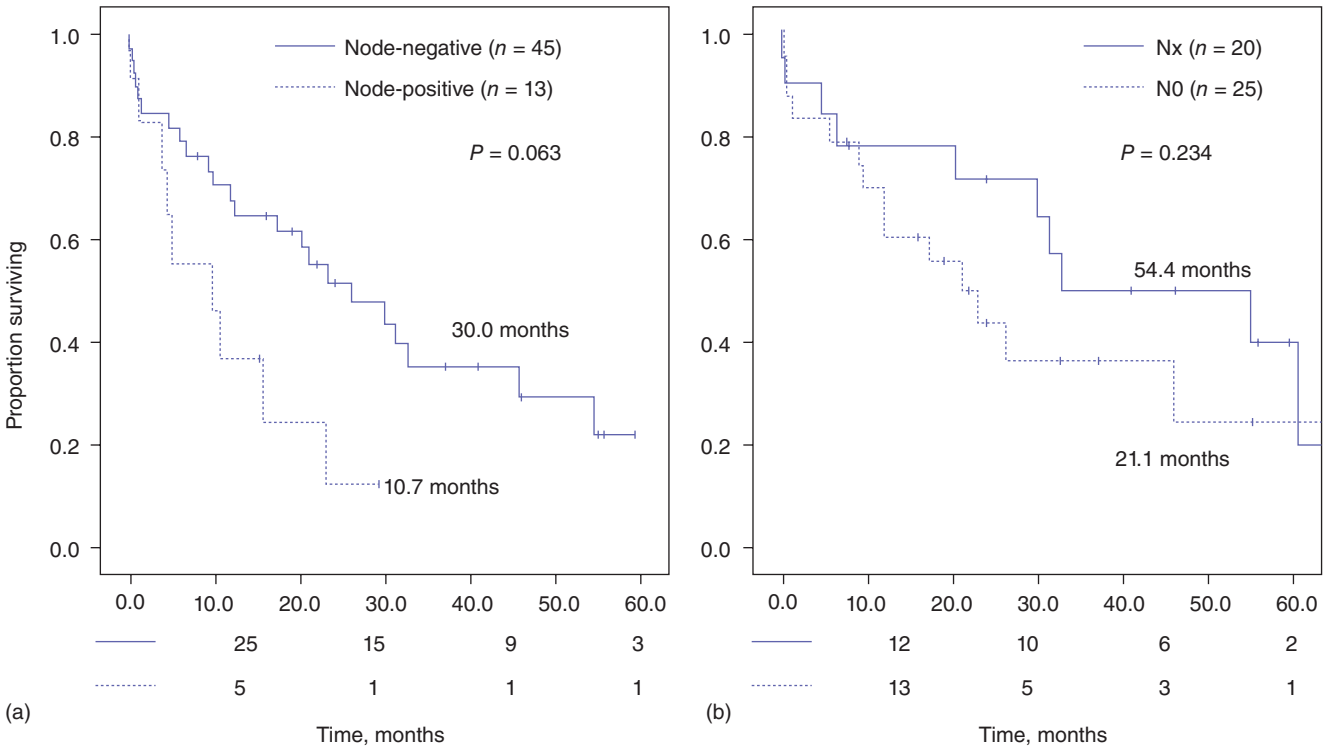

Figure 1 Nodal status and overall survival in (a) node-negative (Nx + N0) vs. node-positive patients, and (b) clinically node-negative (Nx) vs. pathologically node-negative (N0) patients

therapy after complete resection of many gastrointestinal malignancies.[12–14] In IHCC, regional nodal involvement has been shown to be a prognostic factor for reduced OS.[6,17,18,27,28] The 7th edition of the American Joint Committee on Cancer (AJCC) staging system for IHCC takes into account LN involvement[29] as it correlates well with OS.[17,30] In the present study, LN positivity ranged from 22% in all patients to 34% in the subset of patients who underwent LN procurement with pathologic assessment ($n = 38$). This is consistent with a recent multi-institution series that reported LN involvement in the range of 16–30%.[17]

In Western countries, however, portal lymphadenectomy is not routinely performed for IHCC and its value remains controversial.[6,8,16–18] In the clinical setting, patients who do not undergo lymphadenectomy are considered to be LN-negative in decisions on adjuvant therapy. The current study is also subject to this limitation, but it does represent an accurate reflection of clinical practice, particularly as lymphadenectomy is not a standard of care in IHCC. A subset analysis of patients who did not undergo lymphadenectomy compared with those who were pathologically LN-negative did not demonstrate any difference in survival. In fact, patients in whom LN status was unknown displayed a trend towards improved survival (Fig. 1b). Thus, it is unlikely that many of these patients harboured occult LN metastases.

Furthermore, when portal LNs are removed, the median number of nodes retrieved is generally fewer than three.[17–19] This

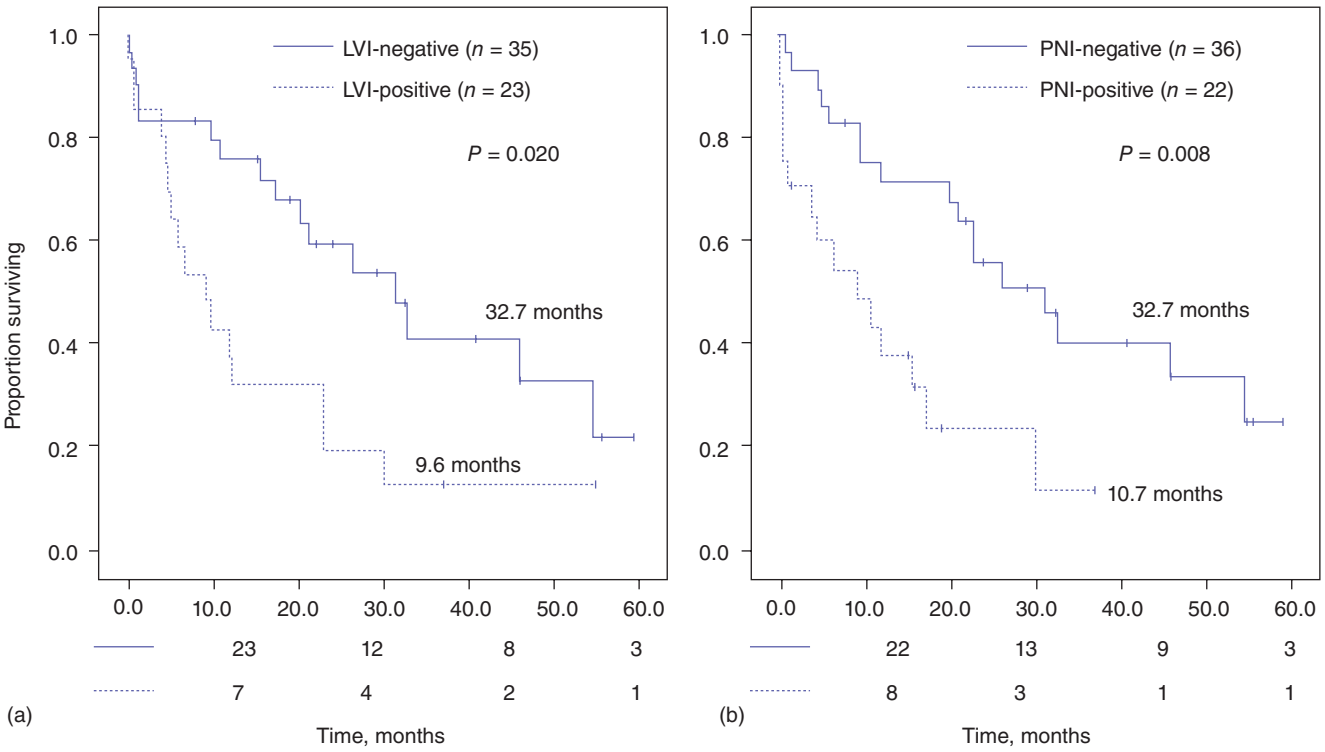

Figure 2 Overall survival in patients with and without (a) lymphovascular invasion (LVI) and (b) perineural invasion (PNI)

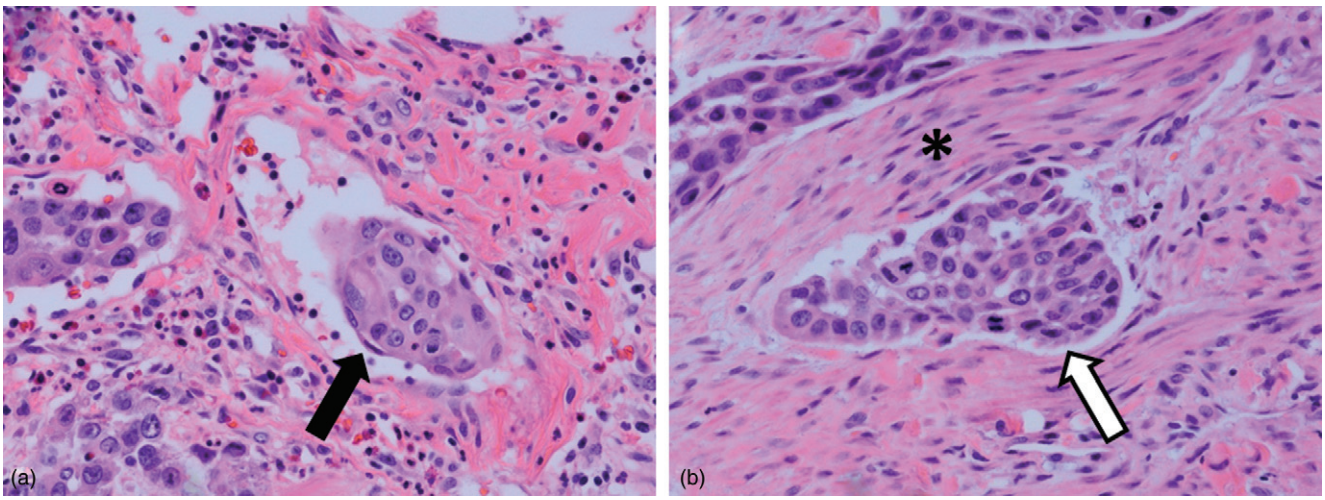

Figure 3 Histopathology shows (a) lymphovascular invasion as demonstrated by tumour cells (black arrow) within a vascular channel and (b) perineural invasion with tumour cells (white arrow) within a neural bundle (*). (Haematoxylin and eosin stain; original magnification ×400)

occurred in the present study, in which the median number of nodes retrieved was only two. There are no specifications within the AJCC guidelines as to the optimal number of nodes that must be retrieved for accurate staging, but it is doubtful that such a small number of nodes will enable a complete and accurate evaluation. Ito *et al*. recently reported that the retrieval of six LNs was necessary for accurate staging of extrahepatic CC.[31] Given these limitations of LN evaluation in IHCC, the aim of the present study was to assess characteristics of the primary tumour that might provide prognostic information similar to that indicated by LN involvement to select patients for adjuvant therapy.

The present authors have previously demonstrated that the presence of LVI is an independent prognostic factor for reduced OS based on a single-institution series of resected CC, which

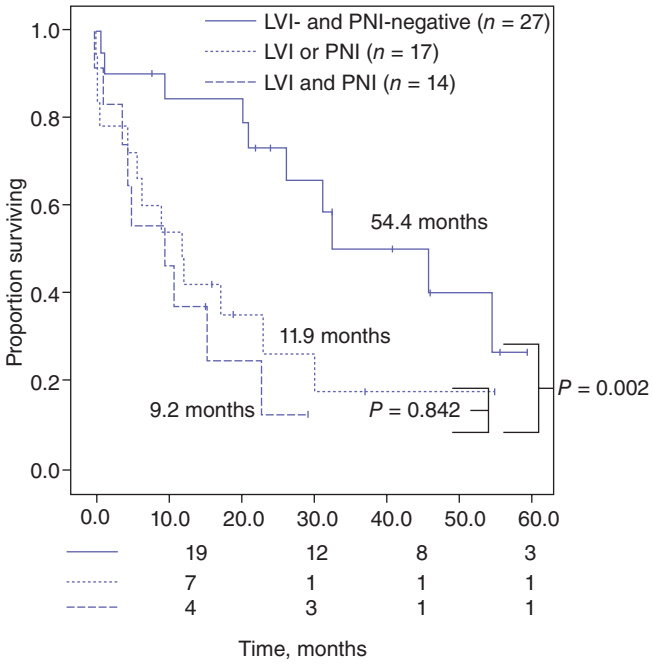

Figure 4 Overall survival in patients with and without lymphovascular invasion (LVI) and perineural invasion (PNI) shows that the effects of LVI and PNI are not additive

included both hilar and intrahepatic disease sites.[19] One of the major limitations of that study was its inclusion of both hilar and intrahepatic disease sites, which many regard as separate entities with distinct tumour biology. The present study is a subsequent investigation that focused only on IHCC. It included 35 patients from the original study, analysed using updated follow-up data, and a different cohort of patients treated at two other institutions. In the current study, which is specific to IHCC, the presence of LVI and/or PNI confers a negative prognostic effect on survival similar to that implied by LN involvement. The authors propose that these characteristics of the primary tumour may be a surrogate for aggressive tumour behaviour and may serve as criteria for selecting for adjuvant therapy. Additionally, the presence of LVI and/or PNI may be used to stratify patient populations in future studies assessing the efficacy of adjuvant regimens.

The concept of LVI as an adverse prognostic marker is not unique to CC. Tumoral lymphovascular involvement significantly correlates with rates of LN metastases in breast cancer,[32,33] endometrial cancer[34,35] and colon cancer[36] and is regarded as a poor prognostic factor for survival.[37,38] In the current series, the presence of LVI did not correlate with the presence of nodal disease. Although this may indicate inadequate LN sampling in IHCC, as previously discussed, it does reflect clinical reality and supports the suggestion that LVI is an independent negative prognostic factor. Studies in node-negative breast cancer support the concept of LVI as an independent poor prognostic factor for OS.[39]

In a small single-institution study of only 22 patients with pathologically node-negative IHCC, Shirabe and colleagues[40] showed a high microvessel count (which is likely to represent a surrogate for LVI) was an independent prognostic factor for poor OS. In the current study, patients who did not undergo portal lymphadenectomy were considered to have LN-negative disease as this is a true representation of clinical practice patterns. Given the rarity of IHCC and the heterogeneity in the performance of LN dissections, a study to assess only histologically assessed LNs is difficult outside the context of a prospective trial. A subset analysis of clinically and pathologically node-negative patients ($n = 45$) in the current study demonstrated a strong trend towards reduced survival in patients with LVI (12.1 months vs. 45.8 months; $P = 0.124$) (Fig. 5a). However, this subset analysis is somewhat limited by the number of patients.

Like LVI, PNI has been implicated as a poor prognostic factor in many cancer types, including squamous cell carcinoma of the head and neck, cancer of the prostate, and colorectal and pancreatic cancers.[41–45] In a large series of hilar and intrahepatic CC, Endo and colleagues showed that the presence of PNI was significantly associated with reduced OS.[46] Several smaller single-institution series that included only patients with IHCC have demonstrated a similar association between PNI and shortened survival, but failed to show the significance of PNI when accounting for other adverse pathologic factors.[8,47] In the authors' recent single-institution study, which included patients with both hilar and intrahepatic CC, PNI marked a trend towards reduced OS, but this did not reach statistical significance.[19] In the current multi-institution study on IHCC, the presence of PNI was found to be significantly associated with a worse prognosis, even after accounting for other known adverse factors, such as large tumour size, multiple tumours, positive resection margin, poor differentiation and LN involvement. Although multiple tumours might be expected to represent poor tumour biology and thus indicate poor survival, most patients included in this series had solitary tumours. Of the 12 patients with multiple lesions, more than half ($n = 7$) had only two tumours. This fact, along with the inherent difficulty of capturing the process of careful patient selection for operation in a retrospective study, is likely to explain the lack of prognostic significance of multiple tumours in this series.

In the current study, lymphovascular and perineural invasion demonstrated a parallel relationship in that, in a given patient, positivity or negativity for one was likely to be reflected by, respectively, positivity or negativity for the other. Studies of lymphatic and blood vessel invasion and PNI in gastric cancer patients have shown similar findings.[48–50] The presence of both factors, however, did not portend a prognosis worse than that implied by the presence of either alone (9.2 months vs. 11.9 months; $P = 0.842$) (Fig. 4). Thus, LVI and PNI both separately and in combination confer equal but independent negative prognostic effects on survival.

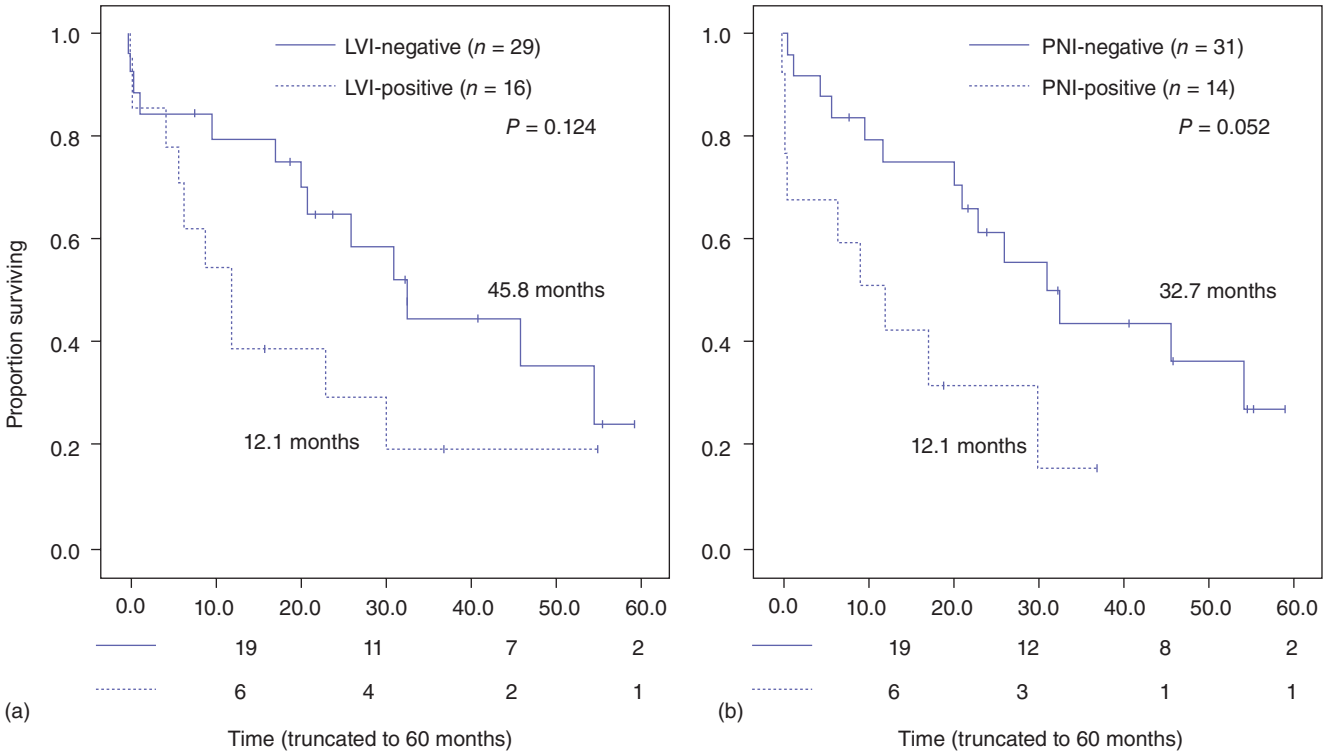

Figure 5 Overall survival in patients without lymph node involvement (n = 45) with and without (a) lymphovascular invasion (LVI) and (b) perineural invasion (PNI)

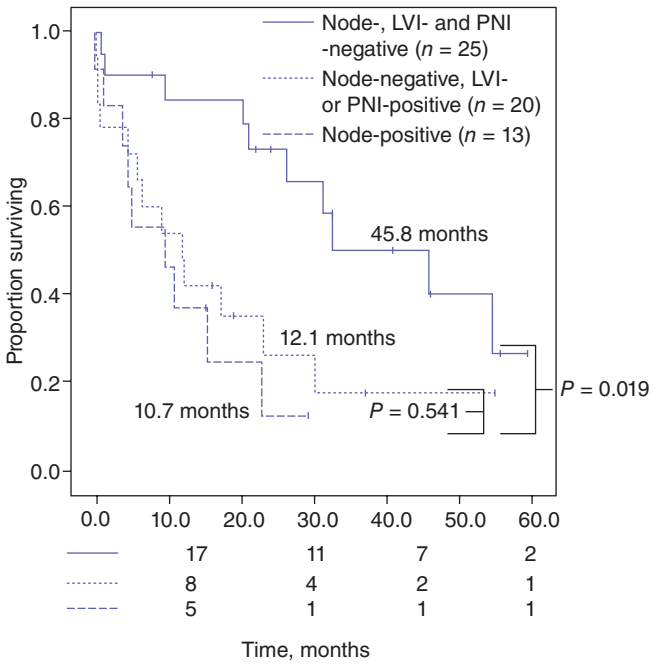

Figure 6 Overall survival in patients with lymphovascular invasion or perineural invasion resembles that in patients with node-positive disease

Both LVI and PNI are routinely assessed in standard pathologic evaluations of resected IHCC. As characteristics of the primary tumour, LVI and PNI are not subject to the variability of LN retrieval. The presence of either LVI or PNI is associated with shortened OS equivalent to that in LN-positive disease. Given the prospect of improved and more efficacious chemotherapy with gemcitabine and cisplatin, the presence of LVI or PNI may represent a reproducible and reliable criterion for selecting patients for adjuvant therapy after resection of IHCC.

Conflicts of interest

None declared.

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