Increasing negative lymph node count is independently associated with improved long-term survival in resectable perihilar cholangiocarcinomas

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Abstract

To evaluate the prognostic value of numbers of negative lymph nodes (NLNs) for patients with perihilar cholangiocarcinomas.

The surveillance, epidemiology, and end results database was used to screen for patients with perihilar cholangiocarcinomas. Kaplan–Meier and Cox regression analyses were used for statistical evaluations. Subsequently, propensity score matching (PSM) was performed to confirm the results.

A total of 938 patients with perihilar cholangiocarcinomas met the inclusion criteria. The cut-off number for the grouping of patients with different numbers of NLNs was 17. Both the univariate and multivariate survival analyses demonstrated that there was a significant improvement in terms of cancer-specific survival for patients with >17 NLNs, compared with patients with ≤17 NLNs. Then, the above results were confirmed via a PSM procedure. Additionally, the independent prognostic value of NLNs was evaluated in subgroup univariate and multivariate analyses of patients with stage I or stage II tumors.

The numbers of NLNs were evaluated and determined to be important independent prognostic factors for the cancer-specific survival of patients with perihilar cholangiocarcinomas.

Abbreviations: AJCC = American Joint Committee on Cancer, LN = lymph node, NLNs = numbers of negative lymph nodes, PNR = ratio of the number of PNs, PNs = positive LNs, PSM = propensity score matching, SEER = surveillance, epidemiology, and end results, TLNs = total number of LNs.

Keywords: negative lymph node count, perihilar cholangiocarcinoma, SEER, survival analysis

1. Introduction

Cholangiocarcinoma is an aggressive adenocarcinoma and constitutes approximately 15% of all hepatobiliary tumors and 3% of all gastrointestinal tumors.[1,2] Depending on its anatomic location, cholangiocarcinoma is classified as intra-hepatic, perihilar, or distal malignancy. Perihilar cholangiocarcinoma constitutes more than 50% of all cholangiocarcinomas. The optimal treatment for perihilar cholangiocarcinoma is surgical resection; however, the early metastasis and large degree of invasion of the major vascular structures at the time of diagnosis make it difficult to perform R0 resections (resectable cases account for <50% of all patients).[3] Even after a curative intent surgery, the 5-year overall survival rate of patients with perihilar cholangiocarcinoma is merely 20% to 45%.[4] Therefore, prognostic related studies in patients with perihilar cholangiocarcinoma have gained a great deal of attention. Along with the surgical margin status, lymph node (LN) status is reportedly a strong predictor of the prognoses of patients with perihilar cholangiocarcinomas.[5] Patients without LN metastases have better prognoses than those with LN involvement.[6–8]

The 7th edition of the American Joint Committee on Cancer (AJCC) staging system, which provides LN classifications according to the location of the LNs involved, is the most commonly used staging method for nodal status. However, several different LN staging methods were evaluated to better predict the survival of patients with gastrointestinal tumors compared with the AJCC staging method, such as the number of positive LNs (PNs), the number of retrieved LNs, and the ratio of them.[5,9,10] The number of PNs and the ratio of the number of PNs (PNR) and the total number of LNs (TLNs) (PNR), were evaluated to be independent prognostic factors for survival, and several studies have evaluated the prognostic value of the number of PNs and the PNR as the new nodal staging methods in patients with perihilar cholangiocarcinoma.[4,11] The TLNs showed a prognostic value in several carcinomas, such as gastric and

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colorectal cancers. However, in patients with perihilar cholangiocarcinomas, it is still controversial as to whether there are any interactions between the TLNs and the prognoses of patients. The 7th edition of the AJCC staging system suggested that patients with perihilar cholangiocarcinomas should have a retrieval of more than 15 LNs, but this suggestion still lacks verifications.

Both the number of PNs and PNR have several limitations for patients without PNs. Additionally, the prognostic value of TLNs, which comprises positive and negative lymph nodes (NLNs), is always confounded by the increasing number of PNs.[12] Therefore, the number of NLNs has been recently proposed as a new indicator of the LN status on survival. The number of NLNs, which has shown a prognostic value in various cancers, has not been studied in patients with perihilar cholangiocarcinomas.[13–15] Hence, our study aimed to elucidate the association between the number of NLNs and the prognoses of patients with perihilar cholangiocarcinomas. To ensure the specificity of the cancers that were evaluated in the study, cancer-specific survival was defined as the primary endpoint.

2. Patients and methods

2.1. Data source

Data from the surveillance, epidemiology, and end results (SEER) database were used for this study. SEER is a public dataset that collects the survival and incidence data of various types of cancers and covers more than 25% of the US population. The SEER data include tumor characteristics, such as primary tumor sites, TNM stagings of tumors, tumor sizes, types of treatment, causes of death, and demographic characteristics, such as the races of patients, the ages of diagnoses and sex. Our study used SEER data from a range of 11 years (from 2004 to 2014). We downloaded data from SEER with the SEER*Stat Software (Version 8.3.4) (https://seer.cancer.gov/seerstat/).

2.2. Patients

Our study was designed to be a retrospective study. The inclusion criteria were

(1) patients who were more than 20 years old,
(2) patients who were diagnosed as having perihilar cholangiocarcinoma, according to the term "008-Bile Ducts Perihilar" of "CS SCHEMA v0204+,"
(3) patients who had histology codes of 8010, 8020, 8070, 8140, 8144, 8160, 8162, 8163, 8260, 8480, 8490, or 8560, with a topographic code of C24.0 or C22.1,
(4) patients who had diagnoses that were not confirmed by a death certificate or an autopsy,
(5) patients with active follow-ups,
(6) patients who were diagnosed from 2004 to 2014, according to the term "year of diagnosis,"
(7) patients who had only 1 tumor and who survived more than 1 month,
(8) patients who did not have distant metastases (the M0 patients),
(9) patients who received intent surgeries, in terms of the combination of "Surg Prim Site" and "Reason no cancer-directed surgery,"
(10) patients who did not receive preoperative radiotherapies, according to the terms of "Radiation" and "Surg/Rad Seq," and

(11) patients who had at least 1 retrieved LN, according to the terms "Regional Nodes Examined."

The demographic characteristics of the patients, such as race, ages at the time of diagnoses, and marital statuses, as well as the tumor characteristics, such as tumor sizes, lateralities of the tumors, grades of the tumors, and stages of the tumors, were all extracted for subsequent analyses. The number of NLNs was calculated by the total number of retrieved LNs subtracted by the number of PNs. The terms "SEER cause-specific death classification" and "SEER other cause of death classification" were used to determine our primary endpoint (the cancer cause-specific mortality).

2.3. Statistical analysis

Statistical analyses were conducted with SPSS (version 23.0). A P-value of $<.05$ was defined to be statistically significant. The total number of retrieved LNs was treated as a categorical variable, and the cut-off number was defined as 15, which was a recommendation of the AJCC staging system. The number of NLNs was treated as a categorical variable, and the cut-off number was determined by using the X-tile program (http://www.tissuearray.org/rimmlab/). The grouping strategy of the X-tile program was to test each number between the range of the NLN counts as the cut-off value, after which the χ^2 score and P-value were calculated by using the tested number as the cut-off value. Eventually, the number with a maximum χ^2 score and a minimum P-value would be suggested to be the final cut-off value. The Kaplan–Meier method (a univariate analysis), along with log-rank tests and Cox regression analyses (a multivariate analysis), were used for the survival analysis.

To make a more objective evaluation of the prognostic value of the NLNs, our study used the PSM method to match the patients with fewer and more NLNs after the calculation of a propensity score for each sample by using a logistic regression model. A matching on the propensity score reduces the possibility of confounding factors by creating a covariate balance between the 2 groups, considering that the score could represent the relationship between the treatment type and multiple characteristics. We performed Kaplan–Meier and Cox regression analyses both before and after the PSM procedure. Then, we performed a subgroup analysis to study the prognostic impact of the NLN counts according to the stages of the tumors.

3. Results

3.1. Patient and demographics details

There was a total of 15,241 patients with perihilar cholangiocarcinomas in the SEER database, and data from 7466 patients who were diagnosed from 2004 to 2014 were extracted. Overall, 1765 patients underwent cancer-direct surgery, and the operation rate was only 23.6%. In the patients who underwent surgeries, 425 patients who received neoadjuvant therapies or who were diagnosed with multiple tumors were excluded. Subsequently, patients who were older than 20 years old and who survived at least 1 month were selected. After screening for patients, in terms of types of follow-up, diagnostic confirmation methods, and other inclusion criteria, 938 patients were finally used for analysis. The characteristics of included patients were shown in Table 1.

Table 1

Characteristics of patients and tumors.

Variables	No. of patients (%) n = 938
Race	
White	723 (77.1)
Black	70 (7.5)
Other	145 (15.4)
Gender	
Male	587 (62.6)
Female	351 (37.4)
Age at diagnosis, yr[*]	66 (23–89)
Marital status	
Married	645 (68.8)
Divorced	67 (7.1)
Separated or single	98 (10.4)
Widowed	102 (10.9)
Unknown	26 (2.8)
Year of diagnosis	
2004–2007	409 (43.6)
2008–2011	354 (37.7)
2012–2014	175 (18.7)
Tumor size, mm	
≤20	365 (38.9)
20–50	362 (38.6)
>50	57 (6.1)
Unknown	154 (16.4)
Grade	
Well differentiated	119 (12.7)
Moderately differentiated	444 (47.3)
Poorly differentiated	272 (28.9)
Undifferentiated	10 (1.1)
Unknown	93 (9.9)
Stage	
IA	124 (13.2)
IB	164 (17.5)
IIA	185 (19.7)
IIB	348 (37.1)
III	117 (12.5)
T stage	
T1	146 (15.6)
T2	264 (28.1)
T3	411 (43.8)
T4	117 (12.5)
N stage	
N0	502 (53.5)
N1	436 (46.5)
Surgery type	
Local excision	253 (26.9)
Extensive surgery	669 (71.3)
Unknown	16 (1.7)
Adjuvant radiotherapy	
No/unknown radiotherapy	612 (65.2)
Beam radiation	326 (34.8)
No. of retrieved LNs[*]	7 (1–65)
No. of NLNs[*]	6 (0–65)

LNs = lymph nodes, NLNs = number of negative lymph nodes.

[*] Values are median (range).

3.2. Identification of optimal cut-offs of NLNs in terms of survival

To study the interactions between the NLN counts and the prognoses of patients, the number of NLNs was converted into a categorical variable. The cut-off number for the conversion was determined by using the X-tile program, which tested each number between the ranges of the NLN counts (0–65) as the cut-off value, in terms of cancer-specific survival. Eventually, the optimal cut-off number of the NLNs was determined to be 17 (Fig. 1). Patients with more than 17 NLNs had a better survival than patients with fewer than 17 NLNs. As shown in Table 2, the difference of the survival rates between the patients with more and fewer NLNs was significant with an increase in the cut-off number. There was a marked heterogeneity between the patients with greater and lower numbers of NLNs, as the cut-off number was 17 ($\chi^2 = 7.11$, $P = .007$). The 3-year cancer-specific survival rate of patients with >17 NLNs was 51.2% versus 38.0% for patients with ≤17 NLNs.

3.3. Prognostic impact of NLNs counts

As shown in Figure 2A, patients with >17 NLNs had a better cancer-specific survival rate than patients with ≤17 NLNs ($P = .007$ for the log-rank test). The median cancer-specific survival rates of patients with ≤17 NLNs and >17 NLNs were 37.0 months and 24.0 months, respectively. Along with the number of NLNs, tumor size, grade of tumor, T stage and N stage were evaluated to be associated with survival in the univariate analysis (Table 3). Factors significant in univariate analysis were entered into multivariate analysis. The number of NLNs was evaluated to be an independently risk factor of cancer-specific survival (hazard ratio [HR], 0.69; 95% confidence interval [CI], 0.52–0.92). Additionally, multivariate analysis revealed the independently prognostic impact of tumor size, grade of tumor, T and N stage. The TLNs was not evaluated to be associated with cancer-specific survival.

3.4. Prognostic impact of NLNs counts after the propensity-matched analysis

To make a more objective evaluation of the prognostic value of the NLNs, we performed a propensity score matching procedure. As described in the methods section, matching based on patients with >17 NLNs was performed. Eventually, 57 patients with >17 NLNs were matched with 57 patients with ≤17 NLNs. Before PSM, the baseline characteristics of tumor size, T stage of tumor and surgery type were not balanced in 2 groups, this situation was significantly improved after the PSM. After the PSM, the univariate and multivariate analysis demonstrated that there was still a significant improvement in terms of cancer-specific survival for patients with >17 NLNs compared with patients with ≤17 NLNs (Fig. 2B and Table 4).

3.5. Prognostic impact of NLN counts according to the stage of tumors

We performed subgroup analysis to study the prognostic impact of NLNs counts according to the stage of tumors. In patients with stage I tumors, the number of NLNs was evaluated to be associated with cancer-specific survival in the Kaplan–Meier analysis. Patients with >17 NLNs had a significantly better cancer-specific survival than patients with <17 NLNs ($P = .010$). This result was persistent in the multivariate analysis. There was a significant decrease in terms of cancer-cause specific mortality for patients with >17 NLNs (HR, 0.36; 95% CI, 0.17–0.73), compared with patients with ≤17 NLNs. The similar results were found out in the analysis of patients with stage II tumors. The optimal cut-off number for the NLNs count was 15, which was
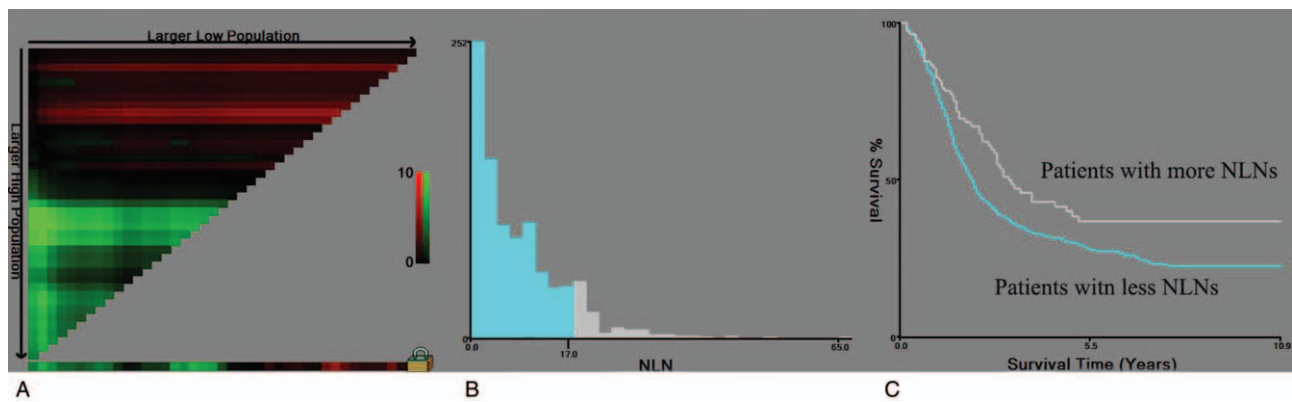
Figure 1. X-tile program plot for number of NLNs in terms of cancer-specific survival. X-tile analysis was done on patient data from the SEER database, equally divided into training and validation sets. X-tile plots of training sets are shown in the left panels (A panel), with plots of matched validation sets shown in the smaller inset. The plot shows the χ^2 log-rank values produced when dividing the cohort with 2 cut-points, producing high and low subsets. The optimal cut-point highlighted by the black circle in the left panels is shown on a histogram of the entire cohort (B panel) and a Kaplan–Meier plot (C panel). P values were determined by using the cut-point defined in the training set and applying it to the validation set. As showed in the B panel, grey and blue represented high and low number of NLNs groups with the cut-off number defined by the left panels. Kaplan–Meier plots (C panel) were generated based on the 2 different groups, patients with high number of NLNs had a significantly better cancer-specific survival rate than patients with low number of NLNs. NLNs = negative lymph nodes, SEER = surveillance, epidemiology, and end results.

determined by the X-tile program. The prognostic value of NLNs was evaluated in both univariate and multivariate analysis. Patients with more NLNs had a better cancer-specific survival than patients with less number of NLNs. However, there was no significant difference of survival between the more and less NLNs count group, in patients with stage III tumors (P = .552). Patients with less number of NLNs even had a slightly better cancer-specific survival than patients with more NLNs (HR, 1.21; 95% CI, 0.64–2.28).

3.6. The sub-stratify analysis according to the TLNs extracted

To avoid the potential influence of the TLNs extracted on the prognostic value of NLNs, we performed the sub-stratify analysis according to the TLNs extracted. Patients were stratified into 2 groups, patients with low total number of retrieved LNs (≤15 total retrieved LNs) and patients with high number of retrieved LNs (>15 total retrieved LNs). There was still a significant better prognosis in patients with high NLNs compared with patients

Table 2

Impact of NLNs on survival.

Cutoff	No.	3-yr cancer cause-specific survival	Log-rank χ^2	P	Cut-off	No.	3-yr cancer cause-specific survival	Log-rank χ^2	P
<2	161	28.8%	6.91	.008	<14	739	38.6%	1.87	.171
≥2	777	41.8%			≥14	199	43.1%		
<3	252	32.9%	5.63	.017	<15	755	38.7%	2.26	.131
≥3	686	42.1%			≥15	183	42.9%		
<4	334	33.7%	3.96	.046	<16	782	38.1%	6.35	.011
≥4	604	43.0%			≥16	156	47.1%		
<5	404	35.8%	3.81	.051	<17	804	38.2%	5.59	.018
≥5	534	42.5%			≥17	134	47.8%		
<6	457	36.6%	3.11	.077	<18	826	38.0%	7.11	.007
≥6	481	42.7%			≥18	112	51.2%		
<7	500	36.6%	3.71	.053	<19	842	38.0%	6.39	.011
≥7	438	43.3%			≥19	96	53.9%		
<8	545	36.0%	4.71	.030	<20	861	38.2%	6.08	.013
≥8	393	44.5%			≥20	77	53.7%		
<9	585	36.7%	3.87	.049	<21	874	38.7%	2.84	.091
≥9	353	44.3%			≥21	64	52.2%		
<10	621	37.7%	1.68	.194	<22	888	39.2%	0.39	.531
≥10	317	43.2%			≥22	50	44.8%		
<11	653	38.9%	0.36	.544	<23	896	39.3%	0.21	.648
≥11	285	40.9%			≥23	42	45.5%		
<12	683	38.7%	0.65	.418	<24	897	39.3%	0.04	.845
≥12	255	41.9%			≥24	41	43.7%		
<13	708	37.8%	1.97	.159	<25	900	39.4%	0.01	.919
≥13	230	44.8%			≥25	38	41.5%		

NLNs = number of negative lymph nodes.
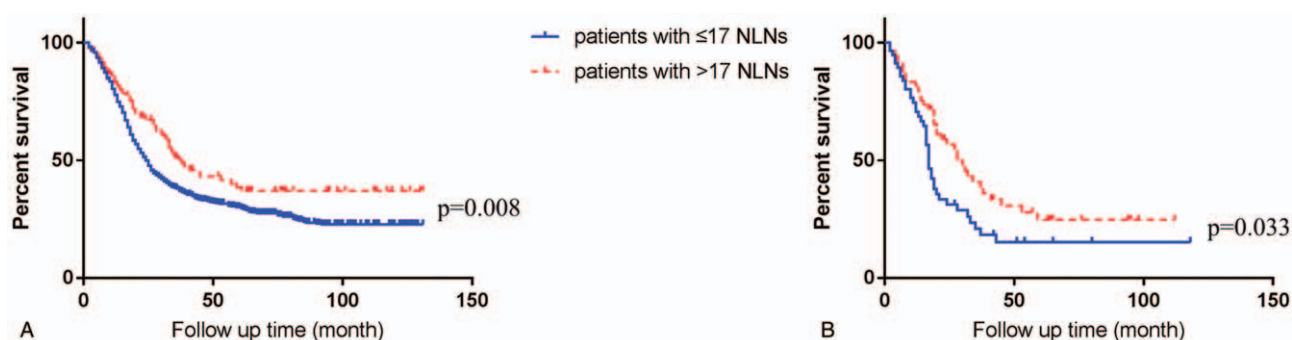
Figure 2. Kaplan–Meier survival analyses showing cancer-specific survival, (A) before the propensity score-matched analysis and (B) after the propensity score-matched analysis in terms of cancer-specific survival.

Table 3

Univariate and multivariate survival analyses of factors associated with cancer-specific survival of patients with perihilar cholangiocarcinomas.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Race						
White	1.00					
Black	1.33	1.00–1.77	.051			
Other	1.12	0.89–1.40	.338			
Gender						
Male	1.00					
Female	1.01	0.85–1.19	.918			
Age at diagnosis						
≤55	1.00					
56–77	1.19	0.96–1.48	.111			
>77	1.28	0.96–1.70	.085			
Year of diagnosis						
2004–2007	1.00					
2008–2011	1.03	0.87–1.23	.701			
2012–2014	0.81	0.60–1.10	.187			
Tumor size, mm						
<=20	1.00			1.00		
20–70	1.26	1.05–1.51	.012	1.12	0.93–1.34	.228
Unknown	1.51	1.21–1.90	<.001	1.42	1.13–1.79	.003
Grade						
Well differentiated	1.00			1.00		
Moderately differentiated	1.18	0.89–1.54	.253	1.11	0.84–1.46	.461
Poorly or Undifferentiated	1.54	1.16–2.05	.003	1.33	0.99–1.77	.051
Unknown	1.23	0.86–1.75	.260	1.17	0.81–1.67	.392
T stage						
T1	1.00			1.00		
T2	1.50	1.13–1.99	.005	1.27	0.95–1.70	.101
T3	2.17	1.67–2.81	<.001	1.72	1.31–2.25	<.001
T4	2.44	1.78–3.35	<.001	1.59	1.14–2.23	.006
N stage						
N0	1.00			1.00		
N1	2.14	1.81–2.53	<.001	1.86	1.56–2.22	<.001
Surgery type						
Local excision	1.00					
Extensive surgery	1.13	0.94–1.35	.209			
Unknown	0.77	0.36–1.63	.491			
Adjuvant radiotherapy						
No/unknown radiotherapy	1.00					
Beam radiation	0.95	0.81–1.13	.581			
No. of LNs						
≤15 LNs	1.00					
>15 LNs	1.02	0.84–1.24	.845			
No. of NLNs						
≤17 NLNs	1.00			1.00		
>17 NLNs	0.68	0.52–0.90	.008	0.69	0.52–0.92	.012

CI = confidence interval, HR = hazard ratio, NLNs = number of negative lymph nodes.

Table 4

Univariate and multivariate survival analyses of factors associated with cancer-specific survival of patients with perihilar cholangiocarcinomas after propensity score matching.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Race						
White	1.00			1.00		
Black	3.06	1.55–6.02	.001	3.50	1.68–7.29	<.001
Other	0.86	0.37–1.99	.731	0.88	0.37–2.12	.792
Gender						
Male	1.00					
Female	0.73	0.46–1.17	.198			
Age at diagnosis						
≤55	1.00					
56–77	1.21	0.68–2.13	.506			
>77	0.69	0.32–1.53	.376			
Year of diagnosis						
2004–2007	1.00					
2008–2011	0.78	0.49–1.25	.316			
2012–2014	0.82	0.32–2.13	.696			
Tumor size, mm						
<=20	1.00					
20–70	1.23	0.77–1.96	.383			
Unknown	0.89	0.34–2.27	.812			
Grade						
Well differentiated	1.00					
Moderately differentiated	0.92	0.46–1.85	.833			
Poorly or Undifferentiated	1.34	0.64–2.81	.438			
Unknown	1.31	0.45–3.83	.621			
T stage						
T1	1.00			1.00		
T2	2.69	0.83–8.72	.100	1.71	0.49–5.85	.396
T3	3.88	1.39–10.84	.009	2.84	0.91–8.78	.072
T4	5.65	1.92–16.64	.002	3.40	1.05–10.93	.041
N stage						
N0	1.00			1.00		
N1	3.26	1.79–5.94	<.001	2.38	1.24–4.58	.009
Surgery type						
Local excision	1.00					
Extensive surgery	0.86	0.47–1.59	.637			
Unknown	1.95	0.25–15.07	.523			
Adjuvant radiotherapy						
No/unknown radiotherapy	1.00					
Beam radiation	0.74	0.45–1.19	.217			
No. of NLNs						
≤17 NLNs	1.00			1.00		
>17 NLNs	0.60	0.68–0.96	.033	0.51	0.31–0.82	.006

CI = confidence interval, HR = hazard ratio, NLNs = number of negative lymph nodes.

with low NLNs. And the cut-off number for NLNs in the above 2 groups (7 for the low TLNs group and 25 for the high TLNs group) were determined by the X-tile program.

4. Discussion

Perihilar cholangiocarcinoma is an aggressive disease, and the prognoses of patients with perihilar cholangiocarcinomas are poor. LN status is reportedly a strong predictor of the prognoses of patients with perihilar cholangiocarcinomas.[11,16,17] Patients are classified as N0 patients if they lacked LN metastases and N+ patients if they had an involvement of LNs, according to the AJCC staging system. The N0 patients have a significantly better survival rate than patients with LN metastases. Therefore, adequately retrieved LNs are essential for the avoidance of understaging, and the number of retrieved LNs is regarded as a predictor of patients' prognoses. The AJCC staging system suggested that patients with perihilar cholangiocarcinomas have at least 15 retrieved LNs; however, this suggestion has yet to be verified. A recent meta-analysis demonstrated that patients who had more than 15 retrieved LNs did not demonstrate an increased survival rate than patients who had fewer retrieved LNs.[18] Furthermore, the prognostic value and optimal number of retrieved LNs are still controversial, and the relevant studies have obtained different conclusions.[11,19,20]

Compared to the TLNs, the number of PNs and the PNR showed convincing prognostic values in several studies of patients with perihilar cholangiocarcinomas. The study of Alfredo et al analyzed a small sample of patients and revealed that a PNR of more than 0.25 was an independent indicator of a

worsened survival.[17] Taro et al concluded that the better staging method of nodal status should be based on the number, and not the location, of PNs[20] Felice et al used a better cut-off number (0.2) to study the prognostic value of the PNR and observed that the PNR was the only independent prognostic factor for overall survival in patients with LN metastases.[11]

Although the number of PNs and the PNR were demonstrated to be better indicators of the impact of LN status on survival, there were limitations when these indicators were used in N0 patients. Additionally, the PNR was unavailable in patients who only had positive LNs. Therefore, the concept of the use of NLNs as indicators was proposed to study the interactions of NLNs with the prognoses of patients. Previous studies have demonstrated that the number of NLNs was an important indicator of the prognoses of patients with colon and esophageal cancers.[12,21] For resectable gastric cancer, the number of NLNs could improve the survival prediction of the PNR, although the prognostic value of NLNs was not significant in the multivariate analysis.[22] However, for patients with perihilar cholangiocarcinomas, it has not been studied whether there are any interactions between the number of NLNs and the prognoses of patients.

The present study screened 938 patients with perihilar cholangiocarcinomas from the SEER database. The N0 patients accounted for 53.5% of all patients; therefore, relying only on the number of PNs and the PNR for predicting the prognoses of patients was insufficient. Both the univariate and multivariate survival analyses revealed that the number of NLNs was significantly associated with the cancer-specific survival of patients. Patients with more than 17 NLNs had a significantly better cancer-specific survival rate than patients with fewer NLNs. Subsequently, the subgroup survival analysis demonstrated the independent prognostic value of the number of NLNs in patients with stage I and II tumors. Additionally, there was no significant difference in terms of cancer-specific survival between patients with >15 and ≤15 retrieved LNs.

There were several hypotheses for the mechanisms underlying the impact of the number of NLNs on prognoses in patients. The first hypothesis was that a greater number of NLNs could be deemed as a marker for the adequacies of surgical and pathological care. Additionally, the number of NLNs was regarded as a basic guarantee for R0 resections and sufficient lymphadenectomies in several studies. Regardless of the surgical and pathological care, either R0 resections or lymphadenectomies could affect the treatment outcomes and prognoses of patients.[22] The second hypothesis was that a greater number of NLNs could guarantee the accurate staging of nodal status, which was demonstrated to be beneficial for the prognoses of patients.[12] The micrometastases in LNs are difficult to discover, and there may be micrometastases in unretrieved LNs. A study by Hendrik et al revealed that approximately 12% of patients with perihilar cholangiocarcinomas had micrometastases in the LNs and that patients with micrometastases had worsened prognoses than patients without micrometastases.[23] Therefore, the third hypothesis was that a greater number of negative LNs indicated that more LNs were resected and retrieved, thus indicating fewer potential micrometastases in the remaining LNs. Furthermore, several anatomic studies have demonstrated that a greater number of NLNs could improve the underlying tumor-host interactions and reset the immunological balance to improve survival.[21]

Several limitations should be noted when interpreting these results. First, although a population-based database was used to screen for the patients, the sample size of our study was still not sufficiently large compared with congener studies for other diseases. Second, several factors were not recorded in the SEER database, such as information regarding adjuvant chemotherapy, surgery details, and areas of lymphadenectomy, among others. Third, the information for radiotherapy in the survival analysis did not contain the details of the protocols, and the SEER database did not provide these data. Fourth, disease-free survival could not be calculated because of the lack of information about local recurrences in the SEER database. Fifth, patients who received preoperative radiation treatments were excluded. However, there may be patients who received radiation treatments in some other centers that were not recorded in the SEER database; thus, the downstaging effect of radiation could not be entirely ruled out. Sixth, the AJCC staging system that was used in the present study was the 6th edition, which was not the most commonly used system in the present day (due to the limitations of the SEER database). Seventh, the sample size was small after PSM, which was because too many clinical factors were balanced in PSM procedure. Finally, we could not collect data that referred to the surgical margin status in the SEER database; this was important in that surgical margin status is an important prognostic factor in patients with resected perihilar cholangiocarcinomas.

In conclusion, the number of NLNs was evaluated to be an important independent prognostic factor for the cancer-specific survival of patients with perihilar cholangiocarcinomas. Patients with greater numbers of NLNs had an increased cancer-specific survival rate compared to patients with fewer NLNs.

Author contributions

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