Assessment of nodal staging and risk factors for nodal involvement in gallbladder cancer: retrospective study

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Abstract

Background:

Nodal assessment in gallbladder cancer (GBC) remains challenging, particularly in incidental GBC. This understages node positive patients resulting in prognostic inaccuracy and insufficient adjuvant treatment. This study aimed to identify risk factors for positive nodes in GBC and compare prognostic discrimination of available nodal staging parameters.

Methods:

This international cohort study assessed GBC resections performed between 1st January 2010 and 31st December 2020. Logistic regression was used to identify risk factors for node-positive status and develop a risk prediction score for positive nodes. Nodal staging models including nodal site, positive node number and positive node ratio were compared for greatest prognostic discrimination in GBC.

Results: A total of 3676 patients underwent GBC resection across 133 centres in 41 countries. T-stage (T2 ,p=0.012, T3, p=0.002, and T4, p<0.001), lymphovascular and perineural infiltration (LVPI, , p<0.001) and tumour differentiation (p<0.001) carried the greatest risk of positive nodes. These three parameters comprised the OMEGA Node Positivity Prediction Score (OMEGA-NOPPS) with C- statistics of 0.81 (95% CI 0.78-0.84, train dataset) and 0.79 (0.73-0.85, test dataset) for identification of node-positive

status, highlighting a 20% or greater increased risk of positive nodes in poorly differentiated tumours with LVPI despite T1 disease.

Conclusions: Data from this large multicentre study confirmed that the number of positive nodes is the most discriminative prognostic model for nodal staging in GBC. The OMEGA-NOPPS stratification provided three simple parameters to risk stratify nodal involvement. Incidental GBC with LVPI and poorly-differentiated tumours, including early T-stages, should be considered for further treatment.

Introduction:

Gallbladder cancer (GBC) is rare but aggressive, and the commonest tumour of the biliary tract[1,2]. There have been no large prospective studies on surgical management of GBC, hence robust evidence for treatment strategies is lacking. Smaller studies lack applicable findings, while cancer registry datasets have insufficient granularity to impact practice.

One key aspect of staging GBC is identifying nodal involvement, which suggests systemic dissemination and indicates adjuvant treatment. The Operative Management of Gallbladder Cancer (OMEGA) international study confirmed nodal stage as a key prognostic marker associated with recurrence-free survival (RFS)[3–5]. Yet lymphadenectomy rates remain low despite international guidelines and patients with incidental GBC (histologically identified following cholecystectomy for suspected benign disease) often had lymphadenectomy omitted at initial surgery [6–10]. The resulting absence of nodal assessment may understage the 3.4% to 20% of T1 tumours reported to have positive nodes[4,11–14]. Better stratification of the risk of nodal involvement could identify patients with early but aggressive tumours and correspondingly higher risk of node positive disease who would benefit from adjuvant treatment. Furthermore there is ongoing debate regarding the optimal nodal parameters to guide prognosis, disputing the widely used 8th AJCC classification in favour of other parameters such as positive lymph node ratios (PLNR) [15–21].

In this publication the large OMEGA study dataset was interrogated to identify the optimal prognostic nodal parameter and to develop a risk score for nodal involvement in GBC.

Methods

Data collection

Collaborating centres were recruited to the OMEGA study by invitation disseminated via emails to all members of the three international HPB associations (the European-African HPB Association, the Americas HPB Association, and the Asia-Pacific HPB Association).

Inclusion and exclusion criteria

Patients were included in this study if they had undergone surgery between 1st January 2010 and 31st December 2020 for either pre-operatively diagnosed or incidental (identified following cholecystectomy for benign disease) GBC. Exclusion criteria were high-grade dysplasia only, metastatic disease at surgery, or macroscopic tumour remaining at the end of resection (R2).

Demographic and pathologic data collection

Clinical parameters, operative details, pathological findings as well as follow-up and survival data were obtained from institutional databases. Comorbidities were incorporated in the Charlson Comorbidity Index (CCI).

Operative details

Extent of surgery was defined as cholecystectomy only, wedge resection (taking the liver margin at the gallbladder bed), resection of liver segments IVb and V, or major hepatectomy (right, extended left or extended right hemihepatectomy). Data was also obtained on specific nodal groups resected, PLNN, total lymph nodes excised (TLNE), extrahepatic bile duct resection (EBDR), resection of additional organs and surgical approach. Complications within 30 post-operative days were classified using the Clavien-Dindo scale[22]. Histology was recorded using the 8th American Joint Committee on Cancer (AJCC) classification (2018) for GBC, incorporating parameters such as differentiation status and lymphovascular or perineural invasion (LVPI)[23].

Follow-up and survival

RFS was defined as the interval between date of surgery and date of first recurrence on imaging.

Ethical approval

Ethical approval was obtained from the Research and Development Office at Cambridge University Hospitals NHS Foundation Trust (the lead site) and the United Kingdom Research Ethics Committee (IRAS ID 285918). Other participating centres obtained further approvals as needed. This study was conducted and reported in compliance with the STROBE guidelines for cohort studies.[18]

Statistical analysis

Factors associated with TLNE were analysed using Poisson and Negative Binomial regression models and data expressed as risk ratios (RR). Binary data were analysed using logistic regression, recursive partitioning classification tree and random forest models. Survival data were analysed using the Cox model and Kaplan Meier plots. Separate multivariable analyses, excluding patients without lymphadenectomy, were employed to assess the association of PLNR and AJCC nodal classification with RFS due to collinearity between those variables. Recursive partitioning classification was also used to select the most suitable cut-off value for PLNN and PLNR. The predictive ability and goodness-of-fit of the prognostic models were compared using the Akaike Information Criterion (AIC) and C-statistic.

To develop the risk prediction model for node involvement, the dataset was split into 80% and 20% train and test sets and performed 10-fold cross-validation repeated 10 times on the train dataset to select hyperparameters for the tree and random forest. The predictive discrimination of the risk score was assessed on the test dataset as a separate validation cohort. The final risk score was derived by multiplying the coefficients of the logistic regression model by 3, rounding to the nearest integer, then adding the transformed coefficient. No data imputation was used and patients were only included in this analysis if data on all the parameters were available. Full statistical methodology is available in Supplementary Data.

Results

The OMEGA study identified 4138 patients who had undergone surgery for GBC in 41 countries across 133 participating centres (Supplementary Figure S1). After excluding 78 patients with only high grade dysplasia and 384 patients with metastatic disease/ R2 resection, 3676 patients were finally included for analysis. Median OS and RFS for the whole cohort were 51.2 (49.3-52.8) months and 35.2 (34.3-36.9) months, with median follow up of 45.3 (24.1-80.5) months[3].

TLNE

Some 3227 patients underwent lymphadenectomy, and 1820 patients had $\geq$ 6 nodes excised. The median TLNE was 5 (interquartile range 2-9). No lymphadenectomy was performed in 48.1% (n=90) and 18.6% (n=75) of patients with T1a and T1b disease respectively. Greater TLNE was associated with T2 tumours and above on multivariable analysis compared to T1a (Table 1, Figure 1a), and poorer TLNE with R1 (margin positive) disease (RR 0.80, 95% CI 0.74-0.88 versus R0). Extent of liver resection showed no association with TLNE, while EBDR was associated with more TLNE compared to no EBDR (RR 1.20 [1.12-1.28]. A laparoscopic approach was associated with less TLNE than an open approach while a robotic approach was associated with more TLNE versus open (RR 0.86 [0.78-0.96] and RR 1.39 [1.11-1.77] respectively). Extending nodal dissection beyond the cystic node to regional (perihilar), coeliac and para-aortic nodes was associated with a 3, 4 and 6-fold increased TLNE respectively,

with no associated increased morbidity or mortality on a separate multivariable analysis (Supplementary Figure S2a). TLNE was not associated with age, gender, comorbidities, or the need for a second operation for disease clearance (Figure 1a).

T-stage

Positive lymph nodes (N+) were identified in 1353 patients across the entire cohort (Table 1). Some 1081 had N1 disease (up to 3 positive nodes) while 272 patients had N2 disease (>3 positive nodes). N+ were predominantly associated with higher T stages on multivariable analysis, but were also present in 2.6% of T1a cases (n=5) and 8.4% T1b cases (n=34, Table 1, Figure 1b). Poorly differentiated tumours and LVPI were also associated with N+ (OR 1.74 [1.31-2.32] p<0.001, and OR 3.25 [2.61-4.06] p<0.001 respectively). Suspected nodal involvement on pre-operative CT, MRI or PET-CT scans was also significantly associated with N+ (OR 2.39 [1.85-3.09], p<0.001). TLNE had a small but significant association with N+ (OR 1.04 [1.03-1.06], p<0.001). Non-regional node dissection was not associated with increased N+ (Supplementary Figure S2b).

Prognostic association with RFS

Higher T-stages, margin positive disease, and N+ had the greatest association with RFS on multivariable analysis (Figure 2). Non-regional node dissection and TLNE had

no significant effect on RFS (Supplementary Figure S3a and b). Survival recursive partitioning tree models identified the PLNN of 3 nodes as the most discriminatory cut-off for RFS , and associated with worse RFS on multivariable analysis ($p<0.001$,Figure 2 and Supplementary Figure S4a). N+ in any nodal group was associated with worse RFS compared to N0 disease; particularly para-aortic nodes ($p<0.001$, Supplementary Figure S5a).

Using the same recursive partitioning methodology as above, a threshold of >0.1 was identified as the best discriminator of RFS for PLNR, and associated with worse RFS on multivariable analysis ($p<0.001$, Supplementary Figures S4c, S5c). PLNR was associated with a 3-fold hazard to RFS when assessed as a continuous variable ($p<0.001$, Supplementary Figure S5d).

Scoring system for node positive status in incidental GBC

The three parameters chosen for the OMEGA-NOPPS, namely T-stage, tumour differentiation and LVPI, had all exhibited significant association with N+ and are routinely available in patients with incidental GBC (Table 3). This analysis utilised 1141 individuals from the main cohort for whom the relevant data were available, split into 80:20 train:test datasets. The train dataset (n=912) exhibited a C-statistic of 0.81 (95% CI 0.78-0.84), sensitivity of 70.6% and specificity of 76.9%. The separate test or

validation cohort showed good generalizability of the model (n=229), with a C-statistic of 0.79 (0.73-0.85), sensitivity of 72.3% and specificity of 74.8%. Calibration curves showed an agreement between predicted and observed outcomes, without signs of overfitting or underfitting (Supplementary Figure S7). Notably, OMEGA-NOPPS quantifies the additional risk conferred by tumour differentiation status and LVPI on top of T-stage.

Discussion

In this multicentre study T-stage, differentiation and LVPI were identified as key risk factors for node positive disease and positive node number was confirmed as the most important prognostic nodal factor for RFS.

This study showed that coeliac and para-aortic nodal dissection could be performed safely although good nodal yields can be obtained with regional lymphadenectomy alone. EBDR was associated with more lymph nodes retrieved, in keeping with some studies, however increasing data indicates that good TLNEs can still be obtained without EBDR, which is no longer routinely recommended in GBC resection due to increased morbidity[7,24–27].

The 8th AJCC classification was introduced in 2018, stratifying GBC nodal stage by PLNN rather than nodal site, however subsequent studies have queried the value of the 8th AJCC classification compared to other staging parameters such as PLNR[16,18,23,28–32]. The 8th AJCC classification had a small but clear advantage over the other parameters in this study, which unlike previous studies combines a large cohort with the necessary granularity and a high lymphadenectomy rate[17,18,28,31,32]. In contrast to other nodal staging prognostic models, the 8th AJCC classification requires no complex calculations and is widely used in histopathological reporting internationally. This makes it feasible, practical, and easily reproducible for prognostication of node positive disease and decisions regarding adjuvant treatment.

This study showed that poorly differentiated tumours with LVPI would carry 20% or higher risk of node positive disease. Cholecystectomy alone is increasingly considered sufficient treatment for T1 tumours, however up to 10% of such patients could harbour positive nodes, overlooked without a lymphadenectomy, and thus not receive adjuvant treatment [6–10,12,14,36]. OMEGA-NOPPS therefore informs consideration of further surgery in early stage tumours, and, for higher scores, may allow consideration of adjuvant treatment without the cost and morbidity of confirmatory lymphadenectomy. Given the encouraging promise offered by ongoing adjuvant and neoadjuvant chemo- and immunotherapy trials in GBC, this is a key opportunity to maximise benefit in a group of patients for whom long-term survival may be possible[37–40].

The 3 parameters in OMEGA-NOPPS are routine histopathology parameters mandated by international histopathological reporting guidelines, readily available in incidental GBC, and easily incorporated in tumour board discussions [41,42] . Additionally, the use of parameters beyond T-stage may counterbalance any inadvertent understaging of tumour extent in cholecystectomy specimens. OMEGA-NOPPS complements existing scores for GBC recurrence, survival and adjuvant treatment, facilitating more personalised prognostication and management[35,43,44]. Importantly, the wide global population within this study should render this score internationally applicable in both high and low incidence areas and allow further validation in a prospective multicentre setting.

The main strength of this study is the large number of patients and centres recruited which allows a truly global picture of the practice of GBC surgery, with a high

lymphadenectomy rate and incorporating a level of detail not available in single-centre cancer registries. Nevertheless, this study has a number of limitations. Given the retrospective nature of the study, specific data on the number of nodes retrieved and exact nodal sites was not always available, and the existence of anatomically continuous nodal chains rendered further detailed breakdown of adjacent nodal subgroups difficult. Lymphadenectomy rates for T1a and T1b disease in this study were much higher than other studies, particularly compared to registry-based data where nodal staging was absent in almost half the patients with T1b disease[10,45]. This may be due to increased lymphadenectomy rates in recent years, but may also represent differences in treatment strategies as well as overall and recurrence-free survival rates between tertiary HPB centres in this study and the more heterogenous data pool in national registries[46]. The exact reason for initial cholecystectomy was not always known, hence it was not possible to separately analyse patients with incidental early stage GBC. OMEGA-NOPPS relies on pathological variables only available post-operatively in cases of incidental gallbladder cancer. Improved imaging techniques and novel molecular biomarkers are necessary for better prediction of nodal metastases for pre-operatively detected GBC and thus a key area for future research.

The data from this study showed that identification of four or more positive nodes has better prognostic discrimination than nodal ratio or more distant positive nodes, highlighting the importance of a thorough regional lymphadenectomy to accurately stage gallbladder cancer. The new OMEGA-NOPPS model provides a simple three parameter score to risk stratify nodal involvement and highlight patients with LVPI and poorly-differentiated incidental GBC who may otherwise be overlooked for further

treatment, ensuring a more nuanced discussion and personalised treatment of this rare and aggressive tumour.

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Figure legends:

Figure 1: A) Multivariable analysis of factors associated with total lymph nodes excised and B) multivariable analysis of factors associated with positive node number.
A) Forest plot and multivariable Negative Binomial regression analysis of factors associated with total lymph nodes excised (TLNE) and B) Forest plot and logistic regression analysis of factors associated with positive node number (N+). EBDR – extrahepatic bile duct resection, HR – hazard ratio, CI – confidence intervals, LVPI – lymphovascular or perineural invasion

Figure 2: Multivariable analysis of factors associated with recurrence-free survival.
Forest plot and multivariable Cox proportional hazard regression analysis of factors associated with recurrence-free survival (RFS). EBDR – extrahepatic bile duct resection, HR – hazard ratio, CI – confidence intervals

Table 1: Demographic and clinical data according to nodal stage

Parameter	N0	N1	N2	Nx	Median TLNE (IQR)
All patients (n=3676)	1874 (51.0%)	1081 (29.4%)	272 (7.4%)	449 (12.2%)	5 (2,9)
Age <60 (n=1044)	550 (52.7%)	316 (30.3%)	86 (8.2%)	92 (8.8%)	5 (4,6)
Age ≥60 (n=2632)	1324 (50.3%)	765 (29.0%)	186 (7.1%)	357 (13.6%)	6 (4,8)
Male (n=1252)	635 (50.7%)	361 (28.8%)	91 (7.3%)	165 (13.2%)	5 (2,9)
Female (n=2424)	1239 (51.1%)	720 (29.7%)	181 (7.5%)	284 (11.7%)	5 (2,10)
CCI <5 (n=2133)	1115 (52.2%)	646 (30.3%)	166 (7.9%)	206 (9.7%)	6 (3,10)
CCI ≥5 (n=1321)	655 (49.6%)	376 (28.4%)	99 (7.5%)	191 (14.5%)	5 (1,9)
Tumour differentiation					
Well (n=837)	522 (62.3%)	155 (18.5%)	27 (3.2%)	133 (15.9%)	5 (1,9)
Moderate (n=1489)	731 (49.1%)	503 (33.8%)	109 (7.3%)	146 (9.8%)	5 (2,9)
Poor (n=795)	305 (38.4%)	290 (36.5%)	114 (14.3%)	86 (10.8%)	6 (2,7)
LVPI present (n=1644)	591 (35.9%)	710 (43.1%)	208 (12.7%)	135 (8.2%)	6 (3,11)
LVPI absent (n=1176)	788 (67.0%)	170 (14.4%)	23 (2.0%)	195 (16.6%)	4 (1,8)
Histology					
T1a (n=187)	92 (49.2%)	4 (2.1%)	1 (0.5%)	90 (48.1%)	1 (0,4)
T1b (n=403)	294 (73.0%)	31 (7.7%)	3 (0.7%)	75 (18.6%)	4 (1,8)
T2 (n=1700)	991 (58.3%)	461 (27.1%)	68 (4.0%)	180 (10.6%)	5 (2,9)
T3 (n=1186)	453 (38.2%)	487 (41.1%)	151 (12.7%)	95 (8.0%)	6 (3,10)
T4 (n=200)	44 (22.0%)	98 (49.0%)	49 (24.5%)	9 (4.5%)	9 (5,15)
R0 (n=3187)	1748 (54.8%)	869 (27.3%)	213 (6.7%)	357 (11.2%)	5 (2,9)
R1 (n=472)	120 (25.4%)	210 (44.5%)	58 (12.3%)	84 (17.8%)	4 (1,8)
Access					
Open (n=3162)	1625 (51.4%)	996 (31.5%)	261 (8.3%)	280 (8.9%)	6 (2,10)
Laparoscopic (n=465)	218 (46.9%)	74 (15.9%)	9 (1.9%)	164 (35.2%)	1 (0,6)
Robotic (n=36)	24 (66.7%)	9 (25.0%)	1 (2.8%)	2 (3.6%)	6 (3,11)
EBDR performed (n=976)	380 (38.9%)	412 (42.2%)	141 (14.4%)	43 (4.4%)	7 (3,12)
EBDR not performed (n=2700)	1494 (55.3%)	669 (24.8%)	131 (4.9%)	406 (15.0%)	4 (1,8)
Extent of resection					
Cholecystectomy alone (n=557)	197 (35.4%)	77 (13.8%)	10 (1.8%)	273 (49.0%)	0 (0,2)
Wedge resection (n=1407)	817 (58.1%)	426 (30.3%)	79 (5.6%)	85 (6.0%)	6 (3,10)
SIVb/V resection (n=1397)	755 (54.0%)	436 (31.2%)	131 (9.4%)	75 (5.4%)	6 (3,9)
Major hepatectomy (n=315)	105 (33.3%)	142 (45.1%)	52 (16.5%)	16 (5.1%)	7 (3,13)
Extent of nodal dissection					
Cystic (n=142)	100 (70.4%)	23 (16.2%)	0 (0%)	19 (13.4%)	1 (1,6)
Regional (n=2332)	1286 (55.1%)	779 (33.4%)	184 (7.9%)	33 (3.6%)	6 (3,9)
Coeliac (n=267)	140 (52.4%)	92 (34.4%)	33 (12.3%)	2 (0.7%)	7 (4,13)
Para-aortic (n=417)	236 (56.6%)	126 (30.2%)	51 (12.2%)	4 (1.0%)	11 (7,17)
Single stage surgery (n=2139)	882 (41.2%)	692 (32.3%)	196 (9.2%)	369 (17.3%)	5 (1,9)
Two stage surgery (n=1537)	992 (64.5%)	389 (25.3%)	76 (4.9%)	80 (5.2%)	5 (3,9)
High prevalence (n=1209)	638 (52.8%)	371 (30.7%)	96 (7.9%)	104 (8.6%)	8 (4,12)
Low prevalence (n=2467)	1236 (50.1%)	710 (28.8%)	176 (7.1%)	345 (14.0%)	4 (1,8)

Abbreviations: TLNE – total lymph nodes excised, CCI – Charlson Comorbidity Index, LVPI - lymphovascular or perineural invasion, EBDR – extrahepatic bile duct resection

Table 2: Discriminative ability of prognostic models for nodal staging

Prognostic model	AIC	C-index (SE)	Model thresholds	HR (95% CI)*	p-value
PLNR continuous	14720.27	0.7581 (0.0069)	N/A	3.00 (2.44- 3.69)	p<0.001
PLNR categorical (>0.1)	14692.51	0.7615 (0.0068)	PLNR ≤0.1	1.00 (Ref)	N/A
			PLNR >0.1	2.22 (1.93- 2.55)	p<0.001
AJCC 7th edition	14694.96	0.7603 (0.0069)	N0	1.00 (Ref)	N/A
			N1	2.19 (1.9- 2.52)	p<0.001
			N2	2.64 (1.9- 3.67)	p<0.001
AJCC 8th edition	14660.80	0.7633 (0.0068)	N0	1.00 (Ref)	N/A
			N1	2.04 (1.76- 2.36)	p<0.001
			N2	3.66 (2.95-4.53)	p<0.001

*Hazard ratios (HR) imported from individual multivariable analyses using each of the prognostic models, as per Supplementary Figures 2 and 3. Abbreviations: AIC – Akaike Information Criterion, CI – confidence intervals, PLNR – positive lymph node ratio.

Table 3: OMEGA Node Positivity Prediction Score (OMEGA-NOPPS) stratification for nodal involvement in gallbladder cancer. As an example, a patient with a T1b tumour would be expected to have a 11.9% risk of N+ if they had a well-differentiated tumour without LVPI, but a 50% risk with a poorly-differentiated tumour and LVPI.

Individual parameters and scores	
T stage	
T1a	0
T1b	3
T2	5
T3	7
T4	10
Tumour differentiation	
Well	0
Moderate	1
Poor	2
Lymphovascular or perineural invasion	
No	0
Yes	4
Final scores and associated risk	
Final risk score	**Probability of positive nodes**
0-2	Up to 6.5%
2-4	6.5 – 11.9%
4-6	11.9 – 20.9%
6-8	20.9 – 33.9%
8-10	33.9 – 50.0%
10-12	50.0 – 66.1%
12-14	66.1 – 79.1%
14-16	79.1 – 88.1%

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