ORIGINAL ARTICLE

Defining a minimum number of examined lymph nodes improves the prognostic value of lymphadenectomy in pancreas ductal adenocarcinoma

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Abstract

Background: Lymph node (LN) metastasis is associated with decreased survival following resection for pancreatic ductal adenocarcinoma (PDAC). In N0 disease, increasing total evaluated LN (ELN) correlates with improved outcomes suggesting patients may be understaged when LNs are undersampled. We aim to assess the optimal number of examined lymph nodes (ELN) following pancreatectomy.

Methods: Data from 1837 patients undergoing surgery were prospectively collected. The binomial probability law was utilized to analyze the minimum number of examined LNs (minELN) and accurately characterize each histopathologic stage. LN ratio (LNR) was compared to American Joint Committee on Cancer (AJCC) guidelines.

Results: As ELN total increased, the likelihood of finding node positive disease increased. An evaluation based upon the binomial probability law suggested an optimal minELN of 12 for accurate AJCC N staging. As the number of ELNs increased, the discriminatory capacity of alternative strategies to characterize LN disease exceeded that offered by AJCC N stage.

Conclusion: This is the first study dedicated to optimizing histopathologic staging in PDAC using models of minELN informed by the binomial probability law. This study highlights two separate cutoffs for ELNs depending upon prognostic goal and validates that 12 LNs are adequate to determine AJCC N stage for the majority of patients.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death in the United States, with a cumulative 5-year overall survival (OS) rate that remains less than 9%.[1,2] This is largely attributed to tumor biology, as well as limitations in all phases of care, including early diagnosis and a lack of effective systemic therapies.[3] In 2018, the 8th edition of the American Joint Committee on Cancer (AJCC) TNM Staging

Manual was released with revised N staging guidelines that convey greater prognostic accuracy for patients with resected PDAC.[4,5] Patients with disease in lymph nodes (LNs) are newly divided into two groups. In N1 disease, 1–3 LNs contain metastatic foci of disease and in N2 disease there are more than 3 involved LNs identified.[4] This updated staging system has been validated, relative to the 7th edition staging guidelines in PDAC, in an analysis across several high-volume PDAC centers.[6]

An accurate prognostic assessment that relies upon the number of positive lymph nodes is inherently dependent on the total number of examined lymph nodes (ELN) in a given specimen. The techniques of surgical lymphadenectomy and histopathologic specimen dissection both clearly play a role. Nevertheless, the use of a minimum number of lymph nodes examined has been suggested as a surrogate marker of quality control to accurately stage patients.[7,8] The College of American Pathologists recommends that a minimum of 12 LNs be examined at the time of pancreaticoduodenectomy (PD). These guidelines are based upon literature that retrospectively suggests a minimum number of ELNs (minELN) that ranges between 12 and 17 for a pancreatic head tumor and at least 20 for a body and tail carcinoma.[9–11] The biologic rationale for a disparate number of ELNs for head tumors, as opposed to body or tail tumors, and accounts for such a broad range of minELN remains obscured. Taken in aggregate, undersampling of LNs remains a potential error that may impact prognosis.

One alternative prognostic strategy proposed by Berger and colleagues is using the positive lymph node ratio (LNR) as an adjunct to AJCC N stage.[12] Support has grown for an expanded utilization of LNR to accurately characterize post-surgical prognosis.[11,13,14] LNR has proven to be a valuable indicator for risk stratification and survival prediction in a variety of malignancies, including gastric cancer, rectal cancer, esophageal cancer, breast cancer, and melanoma.[15–19] The role of the total number of ELNs required to accurately characterize LNR needs to be further investigated.

The purpose of this study is to critically evaluate the impact of the number of ELNs on the prognostic capacity of AJCC N staging and LNR, and to identify the optimal value of minELN using a novel modeling technique based upon the binomial probability law. This approach is particularly well suited for use in evaluating lymphatic metastases in cancer patients for three core reasons. First, the pathologic evaluation of lymph nodes for disease involvement can be viewed as a series of repeated investigations or tests (i.e. each node is evaluated separately). Second, these repeated tests can have just two discrete outcomes (i.e. metastases identified versus no metastases identified). Lastly, the evaluation of each lymph node is done independently such that the findings of one test (lymph node #1 for example) does not impact the outcome for the next test (lymph node #2 is evaluated independently). In this work, prospectively collected histopathologic data are utilized alongside this modeling technique to derive a proposed minELN required for optimal histopathologic staging in PDAC. These techniques are applied in the context of optimizing minELN for accurate determination of N stage and, separately, LNR.

Methods

Patient population and data source

Clinicopathologic data were prospectively collected for all patients undergoing pancreatectomy at Johns Hopkins Hospital (JHH) between January 2000 and November 2018. Patients with a final diagnosis of PDAC were enrolled. Patients who received neoadjuvant therapies before surgery were excluded as the impact of neoadjuvant therapy on the prognostic value of LN evaluation remains unclear and requires dedicated study.[20]

Demographic and clinical data including age, gender, medical history (hypertension, diabetes, and hyperlipidemia), and adjuvant chemotherapy were collected. Pathologic details included tumor grade, presence of perineural or lymphovascular invasion, margin status, examined positive lymph nodes, total ELN, and tumor size.

Lymph node assessment and standard protocol

Surgical resection included en bloc extirpation of the locoregional draining lymph node basins. Pathologists dissected all peripancreatic soft tissues and grossly identify lymph node candidates to be submitted for histologic examination. As an institutional standard, a second pathologist or pathology assistant was required to repeat the dissection of all available tissues if fewer than 12 nodes were grossly identified by the first. There was no institutional standard defining a maximal number of LN candidates identified during the course of tissue dissection. Each candidate node was fixed, sectioned and stained with hematoxylin and eosin for microscopic evaluation. Each node was assessed for tumor spread and defined as a positive node in the event of finding adenocarcinoma cells, of any size or number, within the identified nodal tissue.

Treatment strategy and follow-up

Patients enrolled in this study underwent a surgery-first approach to PDAC with perioperative outcome measures captured prospectively.[21–23] Adjuvant chemotherapy regimens were broadly defined as FOLFIRINOX or modified-FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan), single-agent therapies (capecitabine or gemcitabine alone), or gemcitabine-based regimens (predominately gemcitabine and nab-paclitaxel). Oncologic outcomes were followed through July 2019. OS was calculated from the date of surgical operation. For actuarial survival assessment, patients were censored at the last follow-up date. The date of death was obtained from medical records, local obituary search or the Social Security Death Index.

Statistical analysis

LNR was derived by dividing the total number of positive lymph nodes by the number of ELNs. This ratio was expressed as the

probability of metastasis for each node (a value between zero and one) and quartile classification of LNR was performed to identify unique points of inflection with X-tile (Yale University, New Haven, CT, USA).[24] The probability to detect at least one metastatic LN in a node-positive patient was modeled according to the binomial probability law, formulated as[11,25]:

$$P = 1 - (1 - p)^n$$

In this formula, p is the LNR value, n is the number of ELN and P represents the probability of detecting at least 1 positive lymph node in a node-positive patient. The minELN was then defined as the cutoff of LN yield consistent with a 95% probability to detect at least 1 positive lymph node ($P = 95\%$). Kaplan–Meier survival curves were constructed using R version 3.5.2 (Bell Laboratories, Murray Hill, NJ). Actuarial differences were evaluated with log-rank testing. A Cox proportional-hazards regression model was used for univariate and multivariate analyses. Continuous variables were expressed as medians and compared using the Mann–Whitney U test or Kruskal–Wallis test, as appropriate. Categorical variables were presented as frequencies and percentages, and analyzed by Pearson Chi-square test, or Fisher's exact test, as appropriate. Receiver operating curves are created in R and interpreted using an area under the curve (AUC) analysis. Statistical significance was accepted in a 2-tailed analysis with a p value <0.05.

Results

Patient characteristics and nodal status according to updated AJCC guidelines

Over nearly two decades, 1837 patients met inclusion criteria. The median age was 68 years (IQR 60–75) and 51.9% were male. Hypertension and diabetes mellitus were common comorbid medical conditions. Nodal stage (both AJCC and LNR) was associated with the surgical procedure performed, tumor grade, surgical margin, perineural invasion, lymphovascular invasion and tumor size (Table 1, Supplementary Tables 1 and 2). Patients who underwent PD were more likely to have positive nodes, higher N stage and a higher LNR stage than those who had distal pancreatectomy (DP). The median number of ELN for the entire cohort was 19 (IQR 14–26). In this surgery-first cohort, lymph node metastasis was common (76.3%). The median number of ELNs in patients with positive lymph nodes was 20 and the median number of positive nodes found per patient was 3. The median number of ELNs in node-negative patients was 18 ($p < 0.001$).

An evaluation of N stage (AJCC 8th edition) demonstrates that 23.7% patients were classified as N0, 40.0% patients as N1 (1–3 nodal metastasis) and 36.3% patients as N2 (>3 nodal metastasis). Clinicopathologic features stratified by N staging are presented in Supplementary Table 1. The number of ELNs was found to be higher for patients with N2 disease (21) versus N0 and N1 disease (18, $p < 0.001$). In this cohort, the median number of positive nodes was 6 (IQR 5–9). These data support the notion that patients with N2 disease typically have had a greater number of ELNs.

Evaluating LNR as a method to reduce errors from inadequate LN sampling

To mitigate the dependence of accurate LN staging on adequate total ELN, some studies have suggested the use of LNR. In our cohort, the median LNR was 0.118 (IQR 0.028–0.261). In the node-positive patients, the median LNR in this study was 0.227. Using X-tile, LNR was stratified into four subgroups with the proposed nomenclature LNR0 ($LNR = 0$), LNR1 ($0 < LNR \leq 0.1$), LNR2 ($0.1 < LNR \leq 0.3$) and LNR3 ($0.3 < LNR \leq 1$). The median number of ELNs across prognostic subgroups varied significantly from LNR0, 1, 2, to 3, with 18.0 (IQR 13.0–23.0), 23.0 (IQR 16.0–31.0), 20.0 (IQR 15.5–26.0) and 17.0 (IQR 13.0–22.0), respectively. These data suggest that undersampling of peripancreatic nodes, a proposed factor of N stage migration in the AJCC system may similarly contribute to LNR quartile groupings as well. The clinicopathologic characteristics according to LNR staging are displayed in Supplementary Table 2.

Defining a minimum number of ELN for appropriate nodal staging

Applying the binomial probability function to discriminate between N0 and N1/N2 with 95% confidence, the minELN needed in PDAC specimens was 12 (binomial law calculation of 11.6). When this theory was applied to a hypothetical patient with only one metastatic node expected (and a LNR cutoff value of 0.1, i.e. low burden metastases), the model suggests that the minimum number of lymph nodes examined to reach a 95% probability of detection was 29 (binomial law calculation 28.4). The discrepancy between the minELN to detect any positive node for the average patient (12) and the minELN for a patient with only one node expected to be positive (29) was driven by the significant number of patients found to have greater than one positive node. In keeping with this model, as the predicted LNR increased to 0.3, reflecting poorer disease biology and more frequent detection of lymphatic spread, the number of ELNs to reach 95% probability of accurate characterization decreased to 9 (binomial law calculation 8.4).

Oncologic outcomes and cohorting based on ELN thresholding

The median OS of the entire cohort was 21.0 months and the 1-, 3- and 5-year OS rates were 73.3%, 29.3%, and 16.3%, respectively. In patients with node negative disease, the median OS was no different with fewer than 12 ELNs when compared to 12 or more ELNs (35 v. 38 months). With node-positive disease, the median OS of patients with less than 12 ELNs was significantly

Table 1 Clinicopathologic data stratified by nodal status

Parameters	Total ($N = 1837$, %)	Node-negative ($n = 436$, %)	Node-positive ($n = 1401$, %)	*P* value
Age (years)				**0.027**
≤65	749 (40.8)	158 (36.2)	591 (42.2)	
>65	1088 (59.2)	278 (63.8)	810 (57.8)	
Gender				0.096
Female	884 (48.1)	225 (51.6)	659 (47.0)	
Male	953 (51.9)	211 (48.4)	742 (53.0)	
Hypertension				0.950
No	1081 (58.8)	256 (58.7)	825 (58.9)	
Yes	756 (41.2)	180 (41.3)	576 (41.1)	
Diabetes				0.642
No	1418 (77.2)	333 (76.4)	1085 (77.4)	
Yes	419 (22.8)	103 (23.6)	316 (22.6)	
Hyperlipidemia				0.531
No	1782 (97.0)	421 (96.6)	1361 (97.1)	
Yes	55 (3.0)	15 (3.4)	40 (2.9)	
Surgery type				**<0.001**
PD	1472 (80.1)	292 (67.0)	1180 (84.2)	
DP	264 (14.4)	109 (25.0)	155 (11.1)	
TP	101 (5.5)	35 (8.0)	66 (4.7)	
Tumor grading				**<0.001**
Well	81 (4.4)	35 (8.0)	46 (3.3)	
Moderate	998 (54.3)	258 (59.2)	740 (52.8)	
Poor and undifferentiated	758 (41.3)	143 (32.8)	615 (43.9)	
Chemotherapy				0.160
No	563 (30.6)	132 (30.3)	431 (30.8)	
Yes	1005 (54.7)	228 (52.3)	777 (55.5)	
Unknown	269 (14.6)	76 (17.4)	193 (13.8)	
Margin				**<0.001**
R0	1329 (72.3)	376 (86.2)	953 (68.0)	
R1	508 (27.7)	60 (13.8)	448 (32.0)	
Perineural invasion				**<0.001**
No	180 (9,8)	85 (19.5)	95 (6.8)	
Yes	1634 (88.9)	342 (78.4)	1292 (92.2)	
Unknown	23 (1.3)	9 (2.1)	14 (1.0%)	
Lymphovascular invasion				**<0.001**
No	681 (37.1)	309 (70.9)	372 (26.6)	
Yes	1010 (55.0)	100 (22.9)	910 (65.0)	
Unknown	146 (7.9)	27 (6.2)	119 (8.5)	
Tumor size				**<0.001**
≤2 cm	356 (19.4)	136 (31.2)	220 (15.7)	
>2, ≤4 cm	1156 (62.9)	261 (59.9)	895 (63.9)	
>4 cm	325 (17.7)	39 (8.9)	286 (20.4)	

Bold = *P* value meets definition for statistical significance.

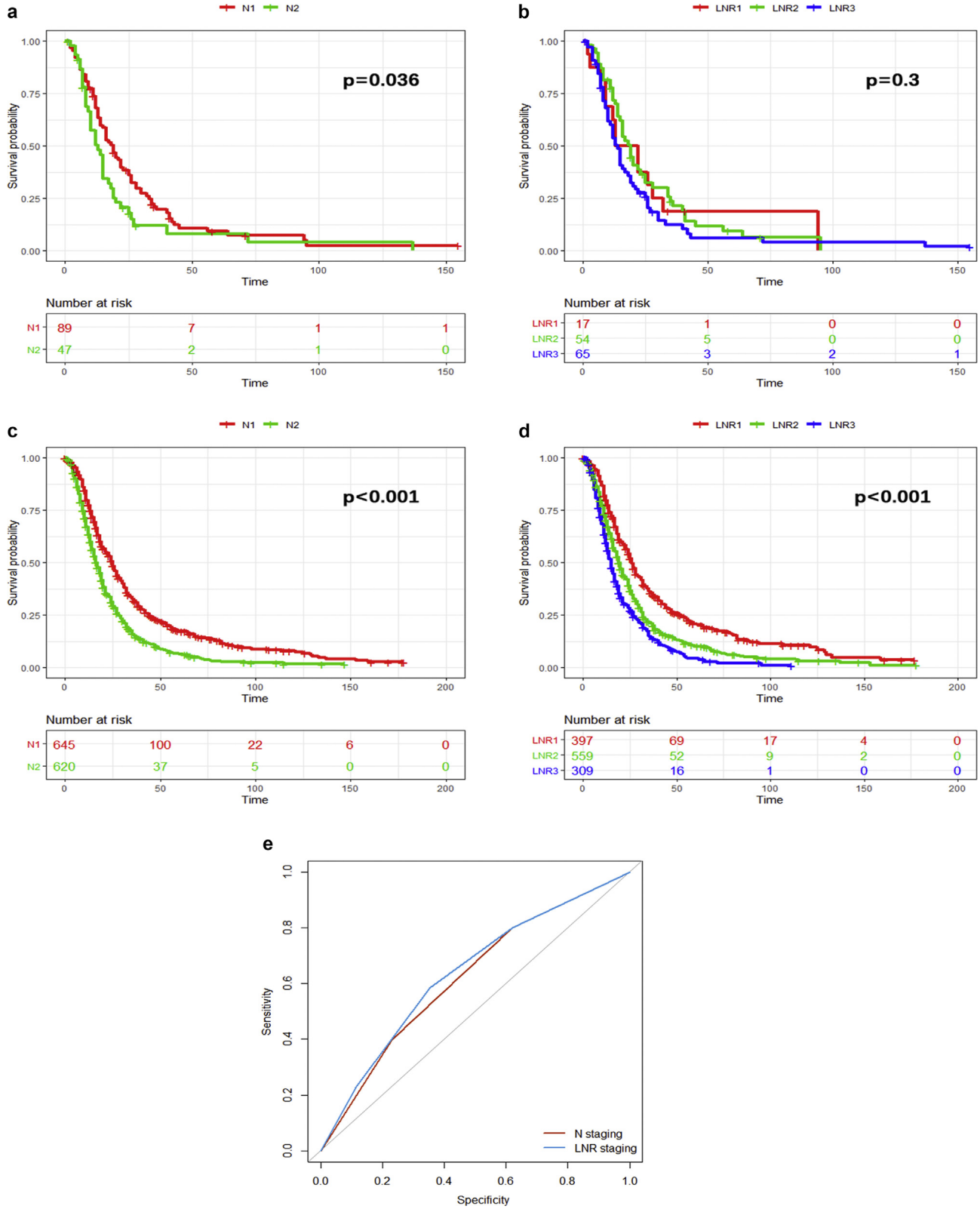

Figure 1 Kaplan–Meier curves showing overall survival for patients with (a and b) fewer than 12 and (c and d) 12 or more examined lymph nodes. The better predictive value for prognosis of (e) LNR staging showed by receiver operating characteristic curves. LNR, lymph node ratio

different to those with 12 or more ELNs (16 v. 19 months, $P = 0.015$). N stage determination (N1 v. N2) was accurate in stratifying oncologic outcomes even when finding fewer than 12 ELNs (19 v. 13 months, $P = 0.036$; Fig. 1a). The use of LNR in this subgroup did not add prognostic value (Fig. 1b). As the number of ELNs increase to 12 or more, the prognostic value of N stage was maintained (Fig. 1c) and an additional prognostic value emerges that supports LNR cohorting (Fig. 1d). When prognostic value was assessed using an AUC analysis, the LNR appears marginally superior to N stage (0.637 versus 0.621, $P < 0.001$, Fig. 1e; Supplementary Fig. 1A and B). Notably here, however, the AUC value failed to approach values typically seen when optimal modeling thresholds are reached (typically 0.8 and higher).

Table 2 The univariate and multivariate analysis for overall survival in the entire cohort

Parameters	No. of patients (*n*, %)	OS				
		Univariate *P* value	Multivariate *P* value (N stage)	Hazard ratio (95% CI)	Multivariate 2 *P* value (LNR)	Hazard ratio (95% CI)
Age (years)		**0.002**				
≤65	749 (40.8)		–	Ref.		Ref.
>65	1088 (59.2)		**0.001**	1.202 (1.080–1.338)	**0.001**	1.204 (1.082–1.340)
Tumor grading		**<0.001**				
Well	81 (4.4)		–	Ref	–	
Moderate	998 (54.3)		**0.014**	1.427 (1.073–1.897)	**0.008**	1.468 (1.104–1.952)
Poor and undifferentiated	758 (41.3)		**<0.001**	1.888 (1.415–2.519)	**<0.001**	1.940 (1.453–2.591)
Chemotherapy		**<0.001**				
No	563 (30.6)		–	Ref.	–	Ref.
Yes	1005 (54.7)		**<0.001**	0.496 (0.441–0.557)	**<0.001**	0.501 (0.446–0.563)
Unknown	269 (14.6)		0.189	0.897 (0.763–1.055)	0.135	0.884 (0.751–1.039)
Margin		**<0.001**				
R0	1329 (72.3)		–	Ref	–	
R1	508 (27.7)		**<0.001**	1.325 (1.180–1.486)	**<0.001**	1.302 (1.159–1.462)
Perineural invasion		**<0.001**				
No	180 (9,8)		–	Ref	–	
Yes	1634 (88.9)		**<0.001**	1.603 (1.304–1.970)	**<0.001**	1.550 (1.261–1.905)
Unknown	23 (1.3)		0.348	1.290 (0.758–2.193)	0.321	1.308 (0.769–2.224)
LVI		**<0.001**				
No	681 (37.1)		–	Ref	–	
Yes	1010 (55.0)		**0.045**	1.135 (1.003–1.284)	**0.021**	1.155 (1.022–1.306)
Unknown	146 (7.9)		0.241	1.139 (0.916–1.416)	0.223	1.144 (0.921–1.422)
Tumor size		**<0.001**				
≤2 cm	356 (19.4)		–	Ref.	–	
>2, ≤4 cm	1156 (62.9)		**0.001**	1.269 (1.102–1.462)	**0.001**	1.266 (1.099–1.459)
>4 cm	325 (17.7)		**<0.001**	1.650 (1.384–1.966)	**<0.001**	1.632 (1.369–1.946)
N stage		**<0.001**				
0	436 (23.7)		–	Ref		
1	734 (40.0)		**<0.001**	1.308 (1.128–1.516)		
2	667 (36.3)		**<0.001**	1.992 (1.698–2.337)		
LNR stage		**<0.001**				
0	436 (23.7)				–	Ref
1	414 (22.5)				**0.034**	1.195 (1.013–1.409)
2	613 (33.4)				**<0.001**	1.633 (1.398–1.908)
3	374 (20.4)				**<0.001**	2.091 (1.753–2.494)

Bold = *P* value meets definition for statistical significance.

Prognostic value of nodal assessment and impact of ELN

Both univariate and multivariate analysis showed that the AJCC N stage and the proposed LNR staging guidelines were useful as independent prognostic biomarkers for OS (Table 2, Supplementary Fig. 2A–C). As guided by our analysis above, we focused next on an evaluation of nodal staging systems as the number of ELN reaches a threshold of ≥29. Similar to other patient cohorts, the median OS of patients with nodal metastasis was significantly shorter than those without nodal metastasis (23 vs. 41 months, $P < 0.001$; Fig. 2a). The N stage subgrouping maintained prognostic significance in these high ELN patients (Fig. 2b). The LNR proved to be similarly valuable in these high ELN patients with median OS varying between 13 months for LNR3 to 41 months in LNR0 (Fig. 2c). When evaluating prognostic capacity using the AUC model, the AJCC N slightly outperformed LNR staging (Fig. 2d). Again here the AUC value did not approach 0.8 or above.

When the number of ELNs was in the range of 9–29, the value of LNR appeared marginally superior to AJCC N staging by AUC analysis (Fig. 3a, values below 0.8). The superiority of the LNR strategy was, however, driven by patients with relatively high LNR burden. AJCC N stage cutoffs, for example, demonstrated a median OS between 16, 23 and 35 months, for N2, N1 and N0 disease respectively ($P < 0.001$; Fig. 3b). When the LNR was examined, the median OS between each LNR stage maintained statistical significance and provided additional prognostic data for high LN positive patients (Fig. 3c).

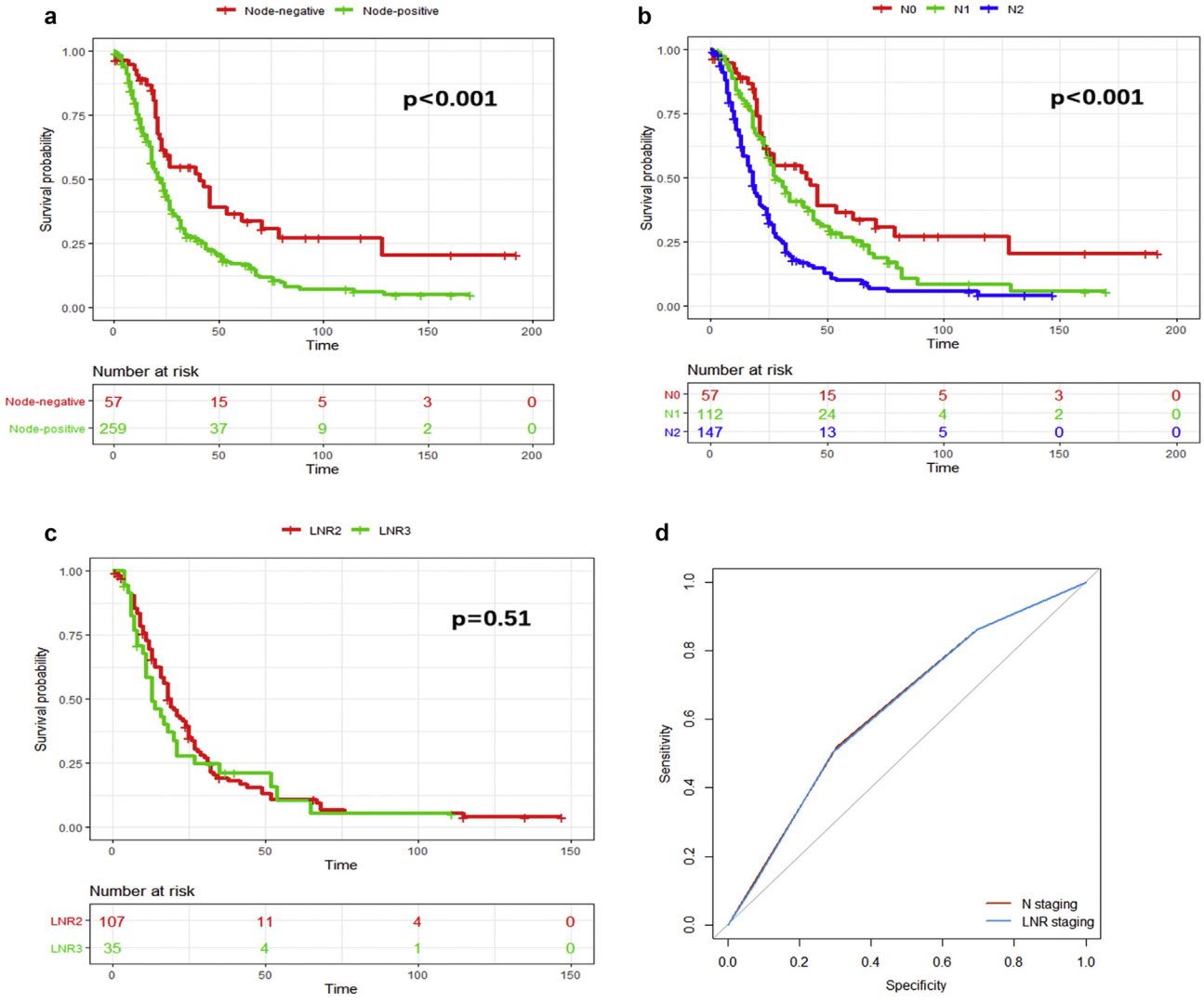

Figure 2 Kaplan–Meier curves showing worse overall survival for patients with (a) positive lymph node metastasis or (b) higher AJCC N stage, (c) but no difference in higher LNR staging, when the examined lymph nodes reach a threshold of ≥29. Marginally better predictive value for prognosis of (d) AJCC N stage showed by receiver operating characteristic curves. AJCC, American Joint Committee on Cancer; LNR, lymph node ratio

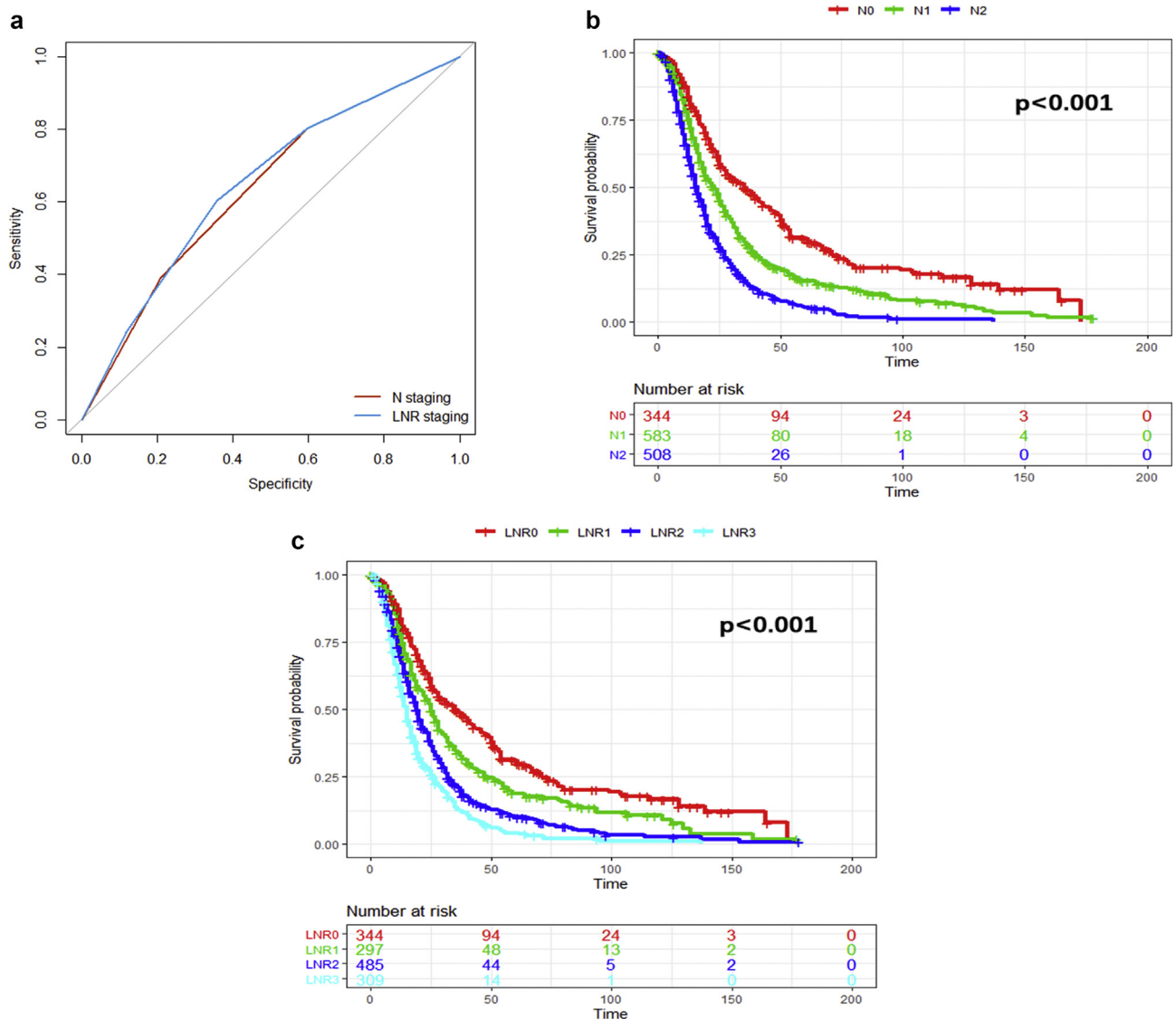

Figure 3 Improved predictive value for prognosis of (a) LNR staging showed by receiver operating characteristic curves. Kaplan–Meier curves showing worse overall survival for patients with (b) higher AJCC N stage or (c) higher LNR staging when the number of examined lymph nodes in the range of 9–29. AJCC, American Joint Committee on Cancer; LNR, lymph node ratio

When the number of ELN was 9 or fewer, the value of utilizing LNR as an alternative to AJCC N stage was no longer maintained. In this cohort, neither AJCC N stage (specifically with N1 and N2, $P = 0.139$; Fig. 4a) nor LNR staging demonstrated statistical significance (Fig. 4b). Only lymph node status (positive or negative) showed a significant prognostic value ($P = 0.003$; Fig. 4c).

Discussion

The prognostic value of lymph node metastases in resected PDAC remains a cornerstone of histopathologic staging of each patient's disease.[8,26,27] Nevertheless, the minELN required during pathologic evaluation to accurately characterize N stage of disease remains an area of debate. Most prior work, for example, has focused on comparing AJCC N stage with LNR while noting sampling bias as a limitation in the work. Herein, we utilize a foundational concept of probability theory, the binomial probability law, to underpin a novel analysis of LN staging in PDAC and address the limitations of prior work directly. Our data confirm that a larger number of ELNs is associated with an increased N stage and support the notion that understaging may be a source of stage migration in PDAC.

As work continues in efforts to find consensus on minELN, the effects of administering neoadjuvant therapies remains similarly controversial. Arrington and colleagues reviewed their

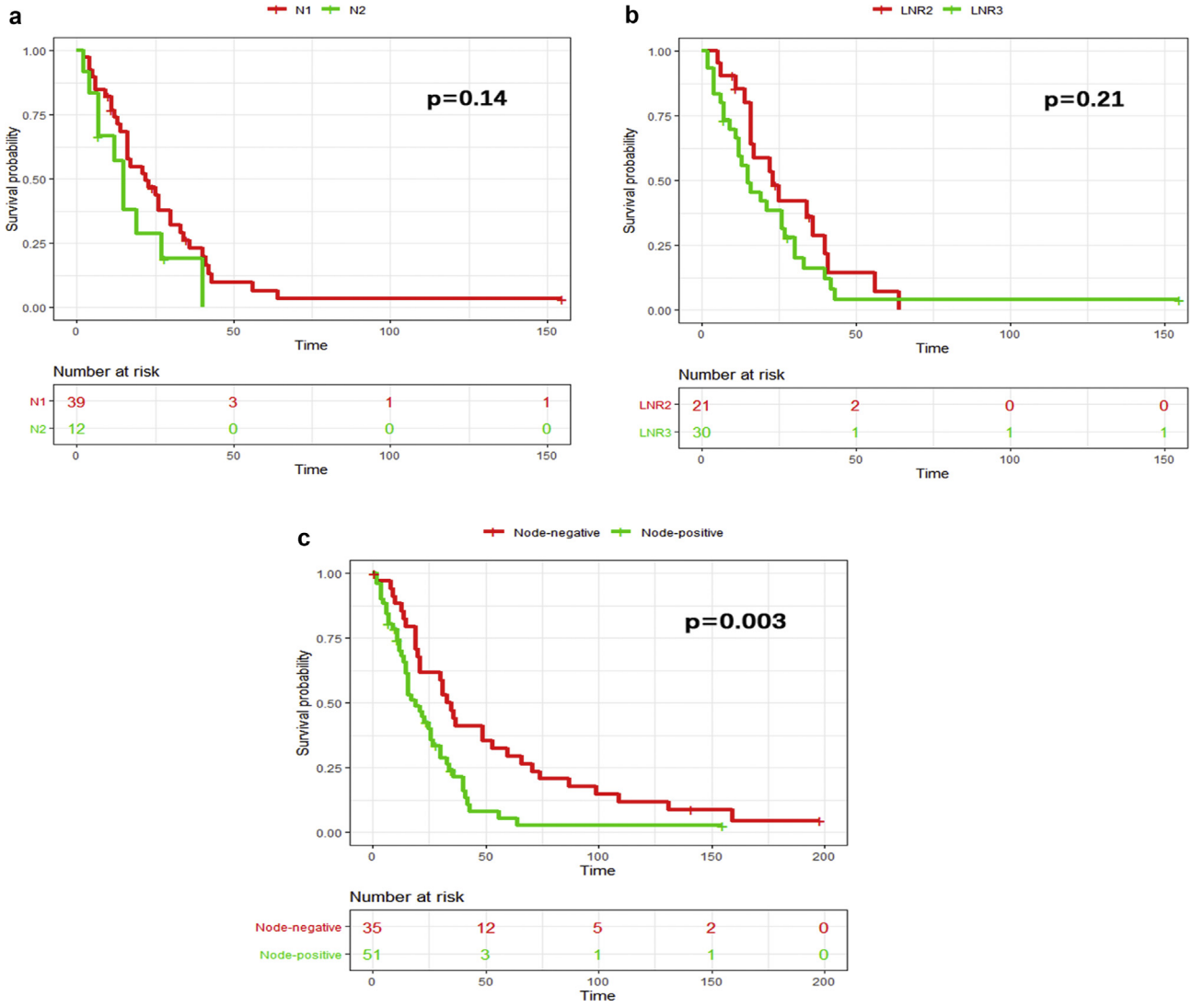

Figure 4 Kaplan–Meier curves showing no significant difference in overall survival for patients with (a) higher AJCC N stage, (b) higher LNR staging when ELNs are 9 or fewer. LN positive disease (versus LN negative) when ELN are 9 or fewer retains prognostic value (c)

experience in a mixed population, with some receiving neoadjuvant therapy, and concluded that 18 LNs were required to capture 90% of cases with any nodal metastasis.[28] This finding exceeds the current recommendations and may be difficult to generalize to centers in which dedicated pancreatic pathologists are not available. We have found that, in the absence of neoadjuvant therapy, 12 nodes are required according to binomial probability modeling to accurately characterize AJCC N0 versus N1/N2 in 95% of patients. This finding supports the College of American Pathologists' recommendation that a minimum of 12 lymph nodes should be sampled.[29] Our data do suggest, however, that understaging when 12 lymph nodes are examined may occur in as many as one in twenty patients and identifying the patient cohort with just one node positive may require more ELNs. Similarly, our data suggest that the minELN may vary based upon the type of surgical resection performed. While as few as 12 ELN may be adequate for patients undergoing pancreaticoduodenectomy, as many as 18 are modeled to be required in patients undergoing distal pancreatectomy and splenectomy. The work done here using this novel method confirms prior retrospective analysis suggesting the minELN is 12–15 in resected pancreatic head carcinoma and as many as 20 in pancreatic body/tail carcinoma.[9,11]

The use of LNR, as opposed to AJCC N stage, has been proposed as a tractable strategy to mitigate the influence of variable ELN sampling on final N stage assessment.[30,31] Berger (12) and Pawlik (32) each reported on the relative value of LNR as prognostic biomarkers.[12,32,33] Subsequent studies showed excellent discrimination of LNR in OS.[8,34–36] Previously, we also reported that LNR could be pivotal prognostic predictor for

resected patients with an analysis of patients from the Surveillance, Epidemiology, and End Results (SEER) database and proposed a nomogram containing LNR as a superior prognostic model with excellent performance in discrimination and risk stratification.[7] The effect of total number of ELN on LNR, however, is a concept that has not previously been investigated. Herein we demonstrate that the minELN required when using a LNR ratio approach is, in fact, greater than the number needed to confidently determine patients as AJCC Stage N1 (as opposed to N0). When contemplating a change from the AJCC N stage classification to one based upon LNR, it is important to note that the relative value of the proposed change is impacted by other markers of prognosis such as surgical margin status, tumor size (T-stage), tumor grade, and perineural or lymphovascular invasion. Thus, as the incidence of other poor prognostic features increases the relative value of LNR over simple N stage determination may be diminished.[37–39]

Most prior work focused on the role of either AJCC N stage or LNR in predicting survival with an eye towards generalization in the absence of consensus regarding the minELN. We have utilized principles of binomial distribution to derive an optimal minELN in both AJCC N staging and LNR determination. As expected, the minELN value is dependent, in part, on the staging system selected (AJCC N stage versus LNR) and the expected LNR in a given population. The proposed LNR system is internally validated using our clinical cohort in an actuarial survival analysis. Our findings suggest that LNR is superior to AJCC N stage in discriminating survival after resection for PDAC in most patients. This includes patients who have a large range of ELNs (>9 but <29) on final pathologic analysis. When the number of ELN $\geq$29, the power of LNR staging was diminished when compared to AJCC N stage, and when fewer than 9 LNs were available for analysis, simple nodal status (N0 v. N1) may be the better choice.

There are several notable limitations that are inherent to this work. These data were analyzed retrospectively from a single center's prospectively collected database and are likely not generalizable to all cohorts. The time period over which these data were collected, nearly two decades, also exposes the dataset to limitations with regards to the routine use and type of adjuvant chemotherapies administered. A multicenter, controlled, prospective study may be required to ultimately confirm these findings and establish a prospectively derived value for minELN across the population. The LNR values derived from our X-tile analysis may not be applicable following neoadjuvant therapy. Owing to the controversy in neoadjuvant therapy for resectable PDAC and challenges in identifying a large number of lymph nodes in the specimen after neoadjuvant therapy, another specific assessment for PDAC patients with neoadjuvant therapy is ongoing. Importantly, the bias of both surgeons and pathologists in harvesting LNs for assessment cannot be overcome in this retrospective work. As an example, it may be appropriate to alter the LN harvest performed during pathologic grossing based upon factors such as obvious and bulky lymphadenopathy versus those with microscopic nodal tissues. The generalizability of this approach is also limited by the practical difficulty of reaching a cutoff of 12 ELNs in many pancreatic specimens. This limitation appears to be greater in patients who proceed to the operating room following neoadjuvant therapies and should be the focus of future work.

Finally, an additional assumption in this work was inherent to the mathematical model utilized, namely, the assumption that any lymph node has an equal probability of being positive as any other for each specific patient. This assumption is contrary to the principles established in several other disease sites (such as breast cancer or melanoma) where the concept of a sentinel lymph node carrying the greatest prognostic value has been validated. Here we look to the current discussion in the literature, and in practice, regarding the optimal surgical lymph node dissection during pancreatectomy and the technical capacity to identify a sentinel node in PDAC. First, in contrast to breast cancer, it does not appear that sentinel node techniques would be a tractable method to address the remaining questions that our computational model poses.[40] As the modern high-volume surgical setting matured, the performance of an extended lymphadenectomy in the retropancreatic soft tissues was found to be safe but added no oncologic benefit and is not currently recommended.[41,9] Nevertheless, outcomes were not cohorted according to LN status in this retroperitoneal space. With modern chemotherapeutics, nodal spread to the retroperitoneal tissues, particularly station 16 nodes, appears to confer a particularly poor prognosis.[42] It remains to be seen if the indexed inclusion of these nodes specifically, or others clearly outside the regional nodes recommended by expert consensus, would alter our analysis here.

Conclusion

Accurate characterization of nodal disease, whether AJCC N stage designation or determination of a LNR, remains a cornerstone of modern oncologic care and informs postsurgical prognosis-based decision making. We present a novel approach to modeling lymph node metastasis based upon the binomial probability law in order to evaluate the frequency with which patients may be understaged according to current guidelines in patient care. In modeling the burden of disease in patients undergoing a surgery-first approach, we have derived the minELN required for accurate characterization of AJCC N stage in resected PDAC. Twelve LNs are required, at a minimum, to capture 95% of patients with N1 disease. As the number of ELNs increased, the discriminatory capacity of alternative strategies to characterize LN disease burden exceeded that offered by AJCC N stage. Specifically, the LNR was a powerful prognostic staging system when ELN value was between 9 and 29. When the ELN count exceeded 29, the accuracy and performance of AJCC N stage was marginally superior to LNR. When fewer than 9 LNs were available for analysis, simple positive or negative LN status

was the better choice. Ultimately, these data support the current recommendations from the College of American Pathologists that 12 LNs are required for adequate nodal staging in PDAC. Finally, this work lays out a method for derivation of minELN that relies upon a probability-based algorithm. This model may be utilized in future to derive the minELN value as the shift towards neoadjuvant therapy continues and paradigms of clinical care change.

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Author contributions

Study conception and design: Pu, Gao, Yu, Burkhart.

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Analysis and interpretation of data: Pu, Gao, Yu, Burkhart.

Drafting of manuscript: Pu, Gao, Thompson, Yu, Burkhart.

Critical revision: Pu, Gao, Chen, Thompson, Hruban, Burns, Wolfgang, He, Yu, Burkhart.

Conflict of interest

None declared.

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Appendix A. Supplementary data

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