Perineural invasion predicts for locoregional failure in patients with oesophageal adenocarcinoma treated with neoadjuvant chemoradiotherapy

Ankur K Patel ,[1] Xueliang Pan,[2] Diana M Vila,[1] Wendy L Frankel,[3] Wei Chen,[3] Kyle A Perry,[4] Robert E Merritt,[5] Desmond M D'Souza,[5] Evan J Wuthrick,[1] Terence M Williams[1]

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2020-206424).

[1]Department of Radiation Oncology, Ohio State University James Cancer Hospital, Columbus, Ohio, USA
[2]Department of Biomedical Informatics, Ohio State University College of Medicine, Columbus, Ohio, USA
[3]Department of Pathology, Ohio State University Wexner Medical Center, Columbus, Ohio, USA
[4]Division of General and Gastrointestinal Surgery, Ohio State University Wexner Medical Center, Columbus, Ohio, USA
[5]Division of Thoracic Surgery, Ohio State University Wexner Medical Center, Columbus, Ohio, USA

Correspondence to
Dr Terence M Williams, Department of Radiation Oncology, Ohio State University James Cancer Hospital, Columbus, OH 43210, USA; Terence.Williams@osumc.edu

Received 1 January 2020
Revised 6 March 2020
Accepted 21 March 2020
Published Online First 21 April 2020

To cite: Patel AK, Pan X, Vila DM, *et al*. *J Clin Pathol* 2021;**74**:228–233.

ABSTRACT

Aim The prognostic significance of perineural invasion (PNI) in oesophageal adenocarcinoma (EAC) is unclear. We examined the association of PNI with clinical outcomes in patients treated with neoadjuvant chemoradiotherapy (nCRT) and surgery.

Methods We performed a single institutional retrospective study. We evaluated the association of PNI with locoregional recurrence-free survival (LRFS), distant metastasis-free survival, disease-free survival (DFS) and overall survival using log-rank and Cox proportional hazard modelling.

Results 29 out of 73 patients (40%) had PNI at the time of surgery. The median follow-up was 20.1 months. The median DFS was 18.4 months for patients with PNI vs 41.3 months for patients without PNI (p<0.05). The median LRFS was 23.3 months for patients with PNI and median not reached for patients without PNI (p<0.01). In a multivariate model including age and pathological variables, PNI remained a significant independent predictor of LRFS (HR 0.20, 95% CI 0.07 to 0.60; p=0.004).

Conclusions For patients with EAC treated with nCRT, PNI found at the time of surgery is significantly associated with worse LRFS. Our data support attempts to validate this finding and perhaps testing the role of adjuvant therapy in patients with PNI.

INTRODUCTION

Oesophageal cancer is a highly lethal malignancy, responsible for over 400 000 deaths annually worldwide.[1] Trimodality therapy (neoadjuvant chemoradiotherapy (nCRT) followed by oesophagectomy) is the standard of care for locally advanced, resectable oesophageal cancer. Randomised trials and a meta-analysis have shown trimodality therapy improves margin-negative resection rates, locoregional and distant control, disease-free survival (DFS) and overall survival (OS) compared with surgery alone in both oesophageal squamous cell carcinoma (ESCC) and oesophageal adenocarcinoma (EAC).[2–4] However, despite advances in management, 5-year survival remains poor at 39%.[2] Given the poor prognosis of oesophageal cancer and the rising incidence of EAC in the USA and worldwide, it is increasingly important to understand the pathological basis of EAC and prognostic factors associated with poor outcomes.

Multiple studies have examined prognostic factors in oesophageal cancer. One factor examined in various contexts is perineural invasion (PNI). PNI is the process of neoplastic invasion of nerves and tumour spread along nerve sheaths. PNI is an important pathological factor in many malignancies, including those of the pancreas, stomach, colon, rectum, prostate, head and neck, and biliary tract.[5] While the definition of PNI has varied in the literature, an often-cited definition by Batsakis in 1985 broadly defines PNI as tumour cell 'invasion in, around, and through peripheral nerves'.[6]

Studies examining the significance of PNI in EAC independent of other adverse pathological features have shown conflicting results and have included patients with EAC and/or ESCC treated with multiple preoperative approaches. Analyses in patients with EAC have alternatively shown PNI to be a significant and a non-significant predictor of OS[7 8] and DFS on multivariate analysis (MVA).[9–11] Similar findings have been reported in studies examining PNI in patients with ESCC[12 13] and in cohorts of patients with both EAC and ESCC.[14–16] Few studies have analysed PNI in patients who underwent preoperative therapy, even after the addition of nCRT to the standard of care for oesophageal cancer. As PNI is a marker for invasive cancer that has the potential to spread both locally and to distant sites,[5] it is important to understand how preoperative therapy aimed at improving local control affects the incidence of markers for local invasion such as PNI and whether PNI retains any prognostic significance in patients who are treated in the modern era with nCRT. Additionally, it is important to understand how PNI affects outcomes in EAC specifically given that adenocarcinoma is the predominant histology in the USA.[17] The majority of studies examining the incidence and prognostic significance of PNI analysed patients who did not undergo any preoperative therapy[8 10 12 15 16] and in patient populations with ESCC[12 13] or mixed cohorts with both EAC and ESCC.[14–16]

The objective of this study was to examine the prognostic significance of PNI in a cohort reflecting the current population of patients with oesophageal cancer. Thus, we examined PNI in patients with locally advanced EAC treated with nCRT and oesophagectomy in order to determine whether PNI is an independent prognostic factor for poor outcomes and if PNI could be used as a risk factor

to assess the need for additional treatment after surgery. In addition, we summarise studies evaluating the prognostic utility of PNI in EAC.

MATERIALS AND METHODS

Patient selection

We reviewed records of consecutive patients with oesophageal cancer treated with nCRT and oesophagectomy at our institution from 1996 to 2015. We collected data pertaining to demographics, clinical presentation, staging, treatment, pathology and outcomes. Patients were included if the oesophagectomy specimen revealed EAC; patients with ESCC or an undifferentiated histology were excluded. Patients who experienced a pathological complete response (pCR) to nCRT were excluded as we were interested in determining whether PNI in the residual tumour specimen after nCRT predicted for outcomes.

Staging and treatment

Clinical staging was performed with endoscopy, oesophageal ultrasonography, CT and positron emission tomography (PET). Tumours were staged according to American Joint Committee on Cancer (AJCC) guidelines, eighth edition, based on tumour invasiveness, lymph node involvement and presence of metastasis. Patients staged initially with sixth or seventh editions were restaged using the eighth edition. When available, patients underwent PET/CT prior and after chemoradiotherapy. All patients underwent chemotherapy with a platinum-based agent in combination with fluorouracil or a taxane. All patients underwent concurrent external beam radiotherapy, with the majority receiving 50.4 Gy in 1.8 Gy daily fractions, 5 days per week (for 5–6 weeks), with a median dose of 50.4 Gy (range 36–50.4). Typically, the primary tumour and involved lymph nodes were treated to 50.4 Gy, and the non-involved regional lymph nodes were treated to 45 Gy. Patients underwent oesophagectomy 5–10 weeks after completing nCRT. Patients underwent follow-up and restaging scans per the treating physicians.

Pathological analysis

Pathologists specialising in gastro-oesophageal malignancies examined surgical specimens using AJCC criteria. Procedures for submission of specimens were consistent throughout the study period, and the entire tumour bed was submitted for all cases. For this study, two pathologists (WLF and WC) reanalysed all specimens for the presence of PNI and pathological tumour (pT) and pathological nodal (pN) stages according to AJCC eighth edition guidelines. Other features examined included histology, tumour size, number of lymph nodes involved, grade, PNI, tumour regression grade (TRG), circumferential resection margin (CRM) status, lymphovascular space invasion (LVSI), distal margin status, as well as other features included in the standard reporting protocol for oesophageal cancer specimens as determined by the College of American Pathologists. At our institution, PNI is defined as the presence of neoplastic cells in the perineural space and is identified using H&E staining (figure 1).

Statistical analysis

Differences in characteristics between PNI-positive and PNI-negative patients were compared using the χ^2 test for categorical variables and the two-sample t-test for continuous variables. Sensitivity analysis was conducted to confirm the results with proper data transformation. Locoregional recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS) were defined as time from the date of surgery to the date of locoregional or distant recurrence, last follow-up, or death from any cause. Disease-free survival (DFS) was defined as the time from surgery to the date of any relapse or death, whichever occurred first. OS was defined as time from date of diagnosis to the date of death. Patients who were disease-free and alive at the last follow-up were censored at the last follow-up date. Patients with recurrence but alive at the last follow-up were censored at the last follow-up date for OS analysis only. Univariate analysis (UVA) of variables associated with LRFS, DMFS, DFS and OS was conducted using Kaplan-Meier analysis and log-rank tests. MVA of variables associated with LRFS, DMFS, DFS and OS were performed using Cox proportional hazard modelling with backward stepwise selection, where the significance level to enter the model was 0.25 and to stay in the model was 0.15. Any variable with $p<0.10$ on UVA for a given endpoint was entered into the multivariate model for that specific endpoint. All statistical analyses were performed using SAS V.9.4 and SPSS V.25.

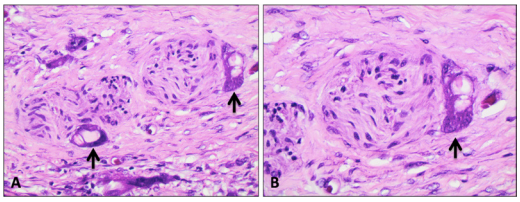

Figure 1 Histological microphotograph of perineural invasion in EAC (H&E stain). (A) EAC tumour cells (arrows) invading perineural space, ×400. (B) Higher-power microphotograph showing the malignant glands invading the perineural space of the nerve (arrow), ×630. EAC, oesophageal adenocarcinoma.

RESULTS

Patient characteristics

One-hundred-twenty consecutive patients with oesophageal cancer treated with nCRT and oesophagectomy during the study period were identified. Of 120 patients, 108 patients had EAC; 11 patients with ESCC and one patient with an undifferentiated histology were excluded from further review. Of the 108 patients with EAC, 21 patients experienced a pCR and were excluded from analysis as we were interested in determining whether PNI in the residual tumour specimen after nCRT predicted for outcomes. A further 14 patients were excluded due to unavailable specimen accession numbers. This left a total of 73 patients with EAC treated with nCRT followed by surgery for final analysis. Median follow-up was 20.1 months (range 2.7–179.2 months). Of the 73 patients in the final analysis, 29 (39.7%) had PNI at the time of surgery. Demographic, clinical and treatment-related characteristics of PNI-positive versus PNI-negative patients are shown in table 1. PNI-positive patients had shorter median follow-up (14.0 vs 26.4 months, p=0.021), more clinical T3–T4 tumours (69.0% vs 38.6%, p=0.009) and were less likely to have undergone transhiatal oesophagectomy (24.1% vs 40.9%, p=0.029).

Pathological characteristics after nCRT

Pathological features from oesophagectomy specimens are shown in table 2. PNI-positive patients had more adverse pathological features than PNI-negative patients. There was a statistically significant difference between PNI-positive and PNI-negative patients with regard to pT stage of 3 or 4 (90% vs 34%, respectively; p<0.001), pN positivity (59% vs 25%, respectively; p=0.004), tumour size (maximum tumour dimension

Table 1 Demographic, clinical, pathological and treatment-related characteristics of PNI-positive and PNI-negative groups

Characteristic	PNI-positive	PNI-negative	P value
Median follow-up in months (min–max)	14.0 (2.7–82.0)	26.4 (3.9–179.2)	0.021*
Gender			0.159
Female	4 (13.8%)	2 (4.5%)	
Male	25 (86.2%)	42 (95.5%)	
Median age at diagnosis (min–max)			
(median=63 for whole cohort)	66.0 (41–78)	63.0 (42–77)	0.548
Clinical T stage			0.009*
1–2	4 (13.8%)	17 (38.6%)	
3–4	20 (69.0%)	17 (38.6%)	
Unknown	5 (17.2%)	10 (22.7%)	
Clinical N stage			0.378
N0	11 (37.9%)	21 (47.7%)	
N+	17 (58.6%)	21 (47.7%)	
Unknown	1 (3.4%)	2 (4.5%)	
Biopsy grade			0.216†
1	0 (0.0%)	3 (6.8%)	
2	9 (31.0%)	8 (18.2%)	
3	14 (48.3%)	21 (47.7%)	
Unknown	6 (20.7%)	12 (27.3%)	
Gastric Involvement			0.356
Yes	13 (44.8%)	15 (34.1%)	
No	16 (55.2%)	29 (65.9%)	
Pre-nCRT PET SUV_{max} (median=12.05)			0.082
≤12.05	8 (27.6%)	16 (36.4%)	
>12.05	14 (48.3%)	10 (22.7%)	
Unknown	7 (24.1%)	18 (40.9%)	
Radiation dose			0.065
50.4 Gy	22 (75.9%)	24 (54.5%)	
<50.4 Gy	7 (24.1%)	20 (45.5%)	
Neoadjuvant chemotherapy type			0.181
Platinum–taxane	10 (34.5%)	9 (20.5%)	
Platinum–5FU	19 (65.5%)	35 (79.5%)	
Induction chemotherapy type‡			0.269
Platinum-taxane	2 (6.9%)	4 (9.1%)	
Platinum-5FU	9 (31.0%)	6 (13.6%)	
No induction chemo	18 (62.1%)	34 (77.3%)	
Post-nCRT/pre-operative PET SUV_{max} (median=5.6)			0.139
≤5.6	8 (27.6%)	15 (34.1%)	
>5.6	13 (44.8%)	10 (22.7%)	
Unknown	8 (27.6%)	19 (43.2%)	
Surgery type			0.029*§
THE	7 (24.1%)	18 (40.9%)	
TTE	15 (51.7%)	23 (52.3%)	
Minimally Invasive	2 (6.9%)	3 (6.8%)	
Robot-assisted	5 (17.2%)	0 (0.0%)	

*P<0.05.
†P compares all biopsy grades.
‡Induction chemotherapy refers to chemotherapy given before nCRT.
§P compares all surgery types.
5FU, fluorouracil; nCRT, neoadjuvant chemoradiotherapy; PET, positron emission tomography; PNI, perineural invasion; SUV_{max}, maximum standardised uptake value; THE, transhiatal oesophagectomy; TTE, transthoracic oesophagectomy.

>2.4 cm in 66% vs 30%, respectively; p=0.005), LVSI (38% vs 7%, respectively; p=0.001), CRM positivity (28% vs 0%, respectively; p<0.001) and TRG of 3 (48 vs 7%, respectively; p<0.001).

Survival and outcomes

Sixteen of 29 patients (55%) in the PNI-positive group had recurrence at any site (locoregional and/or distant) vs 19 of 44 (43%) in the PNI-negative group. PNI-positive patients had a worse median DFS compared with PN-negative patients (18.4 vs 41.3 months, respectively; p=0.037) (figure 2A). Eleven of 29 patients (38%) in the PNI-positive group recurred locoregionally vs 6 of 44 (14%) in the PNI-negative group. The median LRFS was 23.3 months for PNI-positive patients and median not reached for PNI-negative patients (p=0.002) (figure 2B). Thirteen of 29 patients (45%) in the PNI-positive group recurred at a distant site vs 18 of 44 (41%) in the PNI-negative group. The median DMFS was not statistically different between PNI-positive and PNI-negative patients (23.2 vs 41.3 months, respectively; p=0.18). The median survival for the entire cohort was 42.8 months and was not statistically different between PNI-positive and PNI-negative patients (31.8 vs 50.1 months, respectively; p=0.18).

PNI as a prognostic factor on UVA

UVA was conducted to evaluate whether PNI and other demographic, clinical, treatment and pathological variables predicted for survival outcomes (table 3). Variables included sex, age at diagnosis, clinical T and N stage, biopsy grade, gastric involvement, pre-nCRT PET maximum standardised uptake value (SUV_{max}), post-nCRT/preoperative PET SUV_{max}, pT and pN stage, PNI, LVSI, surgery type, CRM status and TRG. Lack of PNI was

Table 2 Pathological features after neoadjuvant chemoradiotherapy of PNI-positive and PNI-negative groups

Characteristic	PNI-positive	PNI-negative	P value
pT stage			<0.001*
1–2	3 (10.3%)	28 (63.6%)	
3–4	26 (89.7%)	15 (34.1%)	
Unknown	0 (0.0%)	1 (2.3%)	
pN stage			0.004*
N0	12 (41.4%)	33 (75.0%)	
N+	17 (58.6%)	11 (25.0%)	
Maximum tumour dimension (median=2.4 cm)			0.005*
≤2.4 cm	9 (31.0%)	26 (59.1%)	
>2.4 cm	19 (65.5%)	13 (29.5%)	
Unknown	1 (3.4%)	5 (11.4%)	
Pathological grade			0.452
1	1 (3.4%)	5 (11.4%)	
2	15 (51.7%)	22 (50.0%)	
3	12 (41.4%)	15 (34.1%)	
Unknown	1 (3.4%)	2 (4.5%)	
LVSI			0.001*
Yes	11 (37.9%)	3 (6.8%)	
No	17 (58.6%)	39 (88.6%)	
Unknown	1 (3.4%)	2 (4.5%)	
Distal margin			0.817
Negative	28 (96.6%)	42 (95.5%)	
Positive/close†	1 (3.4%)	2 (4.5%)	
Circumferential margin			<0.001*
Negative	21 (72.4%)	44 (100.0%)	
Positive	8 (27.6%)	0 (0.0%)	
Tumour regression grade			<0.001*
1	5 (17.2%)	25 (56.8%)	
2	10 (34.5%)	16 (36.4%)	
3	14 (48.3%)	3 (6.8%)	

*P<0.05.
†Close margin defined as ≤0.1 cm.
LVSI, lymphovascular space invasion; pN, pathological N stage; PNI, perineural invasion; pT, pathological T stage.

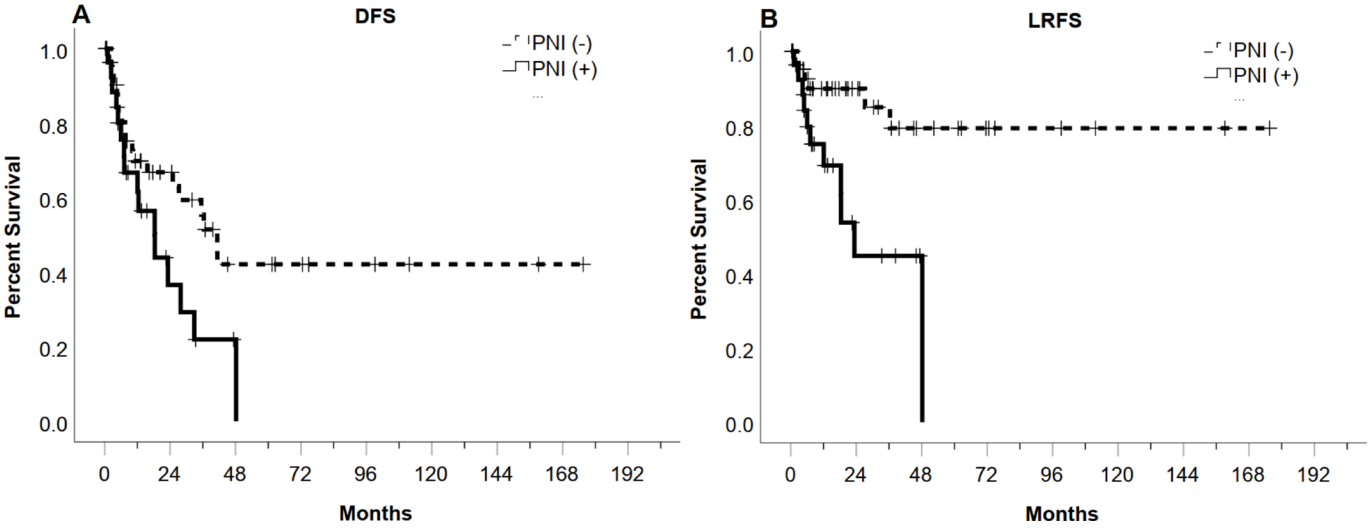

Figure 2 Survival outcomes between PNI-positive and PNI-negative groups. (A.) DFS between PNI-positive and PNI-negative groups. (B) LRFS between PNI-positive and PNI-negative groups. DFS, disease-free survival; LRFS, locoregional recurrence-free survival; PNI, perineural invasion.

associated with improved DFS (HR for recurrence 0.49, 95% CI 0.25 to 0.97; p=0.040) and improved LRFS (HR 0.22, 95% CI 0.08 to 0.62; p=0.004). There was no significant difference in OS or DMFS between PNI-positive and PNI-negative patients. Patients with pT stages 1 and 2 had significantly improved DFS (HR 0.44, 95% CI 0.21 to 0.90; p=0.024) and trend for improved LRFS (HR 0.33, 95% CI 0.11 to 1.03; p=0.06) compared with patients with pT stages 3 and 4, with a trend for improved OS (p=0.16) and DMFS (p=0.10). Patients with pN stage 1 or higher (ie, node-positive) had worse DFS (HR 2.55, 95% CI 1.31 to 4.98; p=0.006) but similar LRFS (HR 2.14, 95% CI 0.82 to 5.56; p=0.12) compared with node-negative patients. Patients who had a primary tumour SUV_{max} less than the median (12.05) on pre-nCRT PET/CT scan had improved

Table 3 Univariate analysis of PNI and select covariates for OS, DFS, LRFS and DMFS

	OS		DFS		LRFS		DMFS	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Sex Female versus male	0.90 (0.27 to 2.93)	0.86	1.80 (0.70 to 4.71)	0.22	1.38 (0.32 to 6.06)	0.67	1.61 (0.56 to 4.62)	0.38
Age (years) <63 versus ≥63	0.36 (0.18 to 0.73)	0.004*	0.98 (0.49 to 1.93)	0.95	0.98 (0.37 to 2.59)	0.97	0.89 (0.43 to 1.85)	0.76
cT stage T1–T2 versus T3–T4	1.16 (0.52 to 2.63)	0.56	0.75 (0.34 to 1.66)	0.47	0.43 (0.12 to 1.60)	0.22	0.78 (0.34 to 1.81)	0.56
cN stage N+ versus N0	0.76 (0.39 to 1.48)	0.42	1.74 (0.86 to 3.53)	0.12	2.85 (0.91 to 8.93)	0.06	1.56 (0.74 to 3.29)	0.24
Biopsy grade G1–G2 versus G3	0.52 (0.22 to 1.22)	0.10	0.73 (0.33 to 1.62)	0.36	0.85 (0.26 to 2.84)	0.83	0.76 (0.32 to 1.77)	0.47
Gastric involvement Yes versus no	1.83 (0.88 to 3.78)	0.10	0.67 (0.34 to 1.30)	0.23	0.58 (0.22 to 1.50)	0.25	0.68 (0.33 to 1.38)	0.28
Pre-nCRT PET SUV_{max} <12.05 versus ≥12.05	0.63 (0.25 to 1.60)	0.33	0.52 (0.23 to 1.17)	0.12	0.74 (0.21 to 2.55)	0.61	0.38 (0.16 to 0.92)	0.029*
Preop PET SUV_{max} <5.6 versus ≥5.6	0.8 (0.32 to 1.96)	0.62	0.85 (0.39 to 1.87)	0.75	0.59 (0.19 to 1.87)	0.36	0.96 (0.41 to 2.21)	0.98
pT stage T1–T2 versus T3–T4	0.61 (0.31 to 1.21)	0.16	0.44 (0.21 to 0.90)	0.024*	0.33 (0.11 to 1.03)	0.06	0.54 (0.25 to 1.13)	0.10
pN stage N+ versus N0	1.43 (0.74 to 2.74)	0.29	2.55 (1.31 to 4.98)	0.006*	2.14 (0.82 to 5.56)	0.12	1.94 (0.95 to 3.93)	0.07
PNI No versus yes	0.64 (0.33 to 1.24)	0.18	0.49 (0.25 to 0.97)	0.040*	0.22 (0.08 to 0.62)	0.004*	0.61 (0.30 to 1.26)	0.18
LVSI No versus yes	1.11 (0.46 to 2.69)	0.69	0.59 (0.27 to 1.27)	0.23	0.35 (0.13 to 0.98)	0.052	0.74 (0.32 to 1.74)	0.65
Surgery type THE versus TTE THE versus mTTE	1.30 (0.67 to 2.53) 2.05 (0.47 to 8.95)	0.46 0.23	0.95 (0.45 to 1.98) 0.90 (0.25 to 3.20)	0.91 0.80	1.25 (0.45 to 3.48) 1.74 (0.21 to 14.16)	0.67 0.58	1.08 (0.49 to 2.37) 0.81 (0.23 to 2.92)	0.81 0.85
CRM Negative versus positive	0.48 (0.20 to 1.15)	0.10	0.69 (0.24 to 1.98)	0.49	0.72 (0.16 to 3.16)	0.66	0.89 (0.27 to 2.96)	0.85
TRG 1–2 versus 3	0.45 (0.22 to 0.91)	0.027*	0.48 (0.22 to 1.03)	0.06	0.39 (0.14 to 1.08)	0.07	0.61 (0.27 to 1.40)	0.24

*P<0.05.
cN, clinical N stage; CRM, circumferential resection margin; cT, clinical T stage; DFS, disease-free survival; DMFS, distant metastasis-free survival; LRFS, locoregional recurrence-free survival; LVSI, lymphovascular space invasion; mTTE, minimally invasive transthoracic oesophagectomy; nCRT, neoadjuvant chemoradiotherapy; OS, overall survival; PET, positron emission tomography; pN, pathological N stage; PNI, perineural invasion; pT, pathological T stage; SUV_{max}, maximum standardised uptake value; THE, transhiatal oesophagectomy; TRG, tumour regression grade; TTE, transthoracic oesophagectomy.

Table 4 Multivariate analysis of PNI and select covariates for OS, DFS, LRFS and DMFS

	OS		DFS		LRFS		DMFS	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
PNI No versus yes					0.20 (0.07 to 0.60)	0.004*		
pN stage N+ versus N0			2.44 (1.24 to 4.80)	0.010*			2.69 (1.10 to 6.58)	0.030*
TRG 1–2 versus 3	0.49 (0.24 to 0.99)	0.048*						
Pre-nCRT PET SUV_{max} <12.05 versus ≥12.05							0.36 (0.15 to 0.88)	0.026*
Age (years) <63 versus ≥63	0.38 (0.19 to 0.76)	0.007*						

Any variable with p<0.10 on UVA analysis for a given endpoint was entered into the multivariate model for that specific endpoint.
*P<0.05.
DFS, disease-free survival; DMFS, distant metastasis-free survival; LRFS, locoregional recurrence-free survival; nCRT, neoadjuvant chemoradiotherapy; OS, overall survival; PET, positron emission tomography; PNI, perineural invasion; pT, pathological T stage; SUV_{max}, maximum standardised uptake value; TRG, tumour regression grade; UVA, univariate analysis.

DMFS (HR 0.38, 95% CI 0.16 to 0.92; p=0.029). However, this finding is of limited value given 25 of 73 patients did not have PET/CT scans as their diagnosis predated the PET era.

PNI as an independent prognostic factor on MVA for LRFS

We performed MVA to evaluate the prognostic significance of PNI and other variables on survival outcomes (table 4). Lack of PNI remained a significant independent predictor of LRFS (HR 0.20, p=0.004) but not DFS. In addition, pN positivity predicted worse DFS (HR 2.44, p=0.010), while pre-nCRT PET SUV_{max}<12.05 predicted improved DMFS (HR 0.36, p=0.026). Age less than the median of 63 (HR 0.38, p=0.007) and TRG of 1–2 (HR 0.49, p=0.048) portended improved OS. Pathological T stage and LVSI did not remain significant predictors of any clinical outcomes examined (LRFS, DMFS, DFS or OS).

DISCUSSION

This study demonstrates the value of PNI as a prognostic factor in patients who receive nCRT for locally advanced EAC. On MVA, PNI remained an independent significant predictor of LRFS when incorporating clinical or pathological variables. Interestingly, although PNI-positive patients had a substantial imbalance compared with PNI-negative patients with regard to more pT3-4 tumours (90%, n=26/29), pathological node positivity (59%, n=17/29) and CRM positivity (28%, n=8/29), the presence of PNI still remained an independent prognostic factor portending worse LRFS. Thus, we feel the presence of PNI warrants further evaluation as an independent prognostic factor for locoregional recurrence in additional datasets.

Few studies to date have examined PNI as a prognostic factor in locally advanced EAC treated with nCRT. Many studies have examined the relationship between PNI and outcomes in oesophageal cancer, but do not apply to today's patients with EAC treated with nCRT. These studies suffer from the following limitations: (1) the majority of patients receiving upfront resection rather than nCRT, which is the current standard of care; (2) examining a mixed population of patients receiving primary resection or some form of preoperative therapy; and (3) examining patients with ESCC or mixed populations of ESCC and EAC.

Online supplementary table 1 summarises 10 retrospective studies, including this study, examining the prognostic significance of PNI in EAC. The incidence of PNI in the other nine studies ranged from 5% to 52% (median, 13%). The average incidence of PNI in these studies is 23.2%, which is lower than our observed incidence of 40%. This may be due to interobserver variability and the fact that esophagectomy specimens were specifically reviewed for the presence of PNI for this study by pathologists specialising in gastro-oesophageal malignancies. Based on our analysis, even with the advent of nCRT to improve local control, the incidence of PNI has not decreased.

Only one study found PNI to be an independent significant predictor of OS in EAC on MVA.[14] However, they did not report the mechanism by which OS was improved (eg, improvement in DFS, LRFS or DMFS), suggesting that confounding variables may explain differences in OS as suggested by the authors. Additionally, only seven patients underwent nCRT, and three adverse features (PNI, venous invasion and lymph vessel invasion) were combined into a single variable on MVA, making it difficult to definitively conclude the statistical significance of PNI as an independent prognostic factor. Four studies did find a statistically significant correlation between PNI and OS on UVA.[7 8 15 18] However, in two of these studies,[8 15] few patients underwent preoperative therapy, and in another study, it is not clear what preoperative modalities patients received.[18] One study did find a significant correlation between PNI and OS on UVA (p=0.05) and a trend for significance on MVA (p=0.07) in patients with EAC. However, these findings were reported when examining the entire cohort of patients, not restricted to the patients who received neoadjuvant therapy. In a subset analysis of the 64% of patients who received neoadjuvant therapy (the majority of which received nCRT), the authors found that PNI was not significantly correlated with OS.[7]

One study found PNI significantly correlated with DFS in EAC on MVA. However, in this study, patients underwent only chemotherapy preoperatively.[9] Vošmik *et al* found no correlation between PNI and DFS in a cohort of patients with EAC treated with nCRT.[11] Wayman *et al* found PNI was not a significant predictor of DFS on MVA, although PNI was significant on UVA, similar to our findings.[10] However, this study included patients with gastric adenocarcinoma, and patients did not undergo preoperative therapy.

There is scant evidence for associations between PNI and locoregional recurrence. Our analysis found PNI correlated with LRFS on MVA (p=0.004). No previous study to our knowledge has demonstrated the correlation between PNI and locoregional recurrence in patients with EAC treated with nCRT, which demonstrates the novelty of our findings. Shaikh *et al* examined a mixed cohort of patients with EAC and ESCC treated with nCRT and found no correlation with local or regional failure.[19]

Our analysis shows that in patients with EAC undergoing nCRT to improve local control, PNI is an independent significant predictor for LRFS, and thus, PNI may be a useful predictor of LRFS in patients who receive nCRT.

One of the strengths of our study is its focus on EAC. EAC was once a rare histology of oesophageal cancer in the USA, but since the 1990s, EAC has surpassed ESCC as the predominant histology of oesophageal cancer. Currently, ~60% of newly diagnosed oesophageal cancers are adenocarcinomas.[17] Previous studies examining the significance of PNI included patients with ESCC or cohorts with mixed histology (EAC and ESCC). Our analysis in patients with EAC is more applicable to today's patients with oesophageal cancer. A second strength of our study is that we analysed patients who underwent modern treatment with nCRT. Previous studies examining PNI included patients who underwent upfront resection. Only a few studies analysed patients who received neoadjuvant treatment, and among these, fewer yet examined patients who received radiation as part of their preoperative therapy. Our analysis in patients treated with standard of care nCRT followed by esophagectomy again provides data that are more relevant for prognosticating patients in the modern era.

One limitation of this study is its retrospective nature. A second limitation is its small sample size, due to specific patient inclusion criteria, such as excluding 19% of patients who experienced a pCR after nCRT. This was done so that we could examine the significance of PNI in patients with residual disease after nCRT and raise the question of whether the presence of PNI could be used to decide on adjuvant therapy after surgery. Finally, patients over a long study period were included in this analysis, spanning a time when principles in the management of oesophageal cancer have evolved. However, 89% of patients received current NCCN-recommended doses of radiotherapy and 100% received current NCCN-recommended concurrent chemotherapy regimens, which reflects a patient cohort treated with modern nCRT. Finally, while surgical techniques may have changed during our study period, a recently published randomised trial showed no difference in oncological outcomes between open and minimally invasive techniques.[20]

In summary, we find PNI is an independent significant predictor of LRFS in patients with EAC treated with nCRT and esophagectomy. This finding identifies a subgroup of patients at increased risk of locoregional recurrence and argues for consideration of testing adjuvant therapies after resection in this high-risk population. Validation of our findings on additional datasets is warranted.

Take home messages

- Studies examining the prognostic significance of perineural invasion (PNI) in oesophageal adenocarcinoma (EAC) have shown conflicting results.
- Few studies have examined the significance of PNI in a modern cohort of patients treated with neoadjuvant chemotherapy and radiation (nCRT) and esophagectomy.
- PNI is an independent predictor of locoregional failure in EAC after nCRT and esophagectomy.
- Validation of the prognostic significance of PNI and testing adjuvant therapies in patients with PNI is warranted.

Handling editor Dhirendra Govender.

Contributors AP, DMV, EJW and TW participated in project conceptualisation. AP, DMV and XP participated in data curation, methodology and formal analysis. AP participated in writing the original draft. WLF and WAC participated in methodology, and writing, including review and editing. TW, EJW, KAP, REM and DMDS participated in writing, including review and editing.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This retrospective review was approved by the institutional review board with a waiver of informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iD

Ankur K Patel http://orcid.org/0000-0002-2961-9304

REFERENCES

1 Ferlay J, Soerjomataram I, Dikshit R, *et al*. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359–86.
2 Tepper J, Krasna MJ, Niedzwiecki D, *et al*. Phase III trial of trimodality therapy with cisplatin, fluorouracil, radiotherapy, and surgery compared with surgery alone for esophageal cancer: CALGB 9781. *J Clin Oncol* 2008;26:1086–92.
3 Gebski V, Burmeister B, Smithers BM, *et al*. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. *Lancet Oncol* 2007;8:226–34.
4 van Hagen P, Hulshof MCCM, van Lanschot JJB, *et al*. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012;366:2074–84.
5 Liebig C, Ayala G, Wilks JA, *et al*. Perineural invasion in cancer: a review of the literature. *Cancer* 2009;115:3379–91.
6 Batsakis JG. Nerves and neurotropic carcinomas. *Ann Otol Rhinol Laryngol* 1985;94:426–7.
7 Torres C, Turner JR, Wang HH, *et al*. Pathologic prognostic factors in Barrett's associated adenocarcinoma: a follow-up study of 96 patients. *Cancer* 1999;85:520–8 http://www.ncbi.nlm.nih.gov/pubmed/10091725
8 Paraf F, Fléjou JF, Pignon JP, *et al*. Surgical pathology of adenocarcinoma arising in Barrett's esophagus. Analysis of 67 cases. *Am J Surg Pathol* 1995;19:183–91.
9 Noble F, Nolan L, Bateman AC, *et al*. Refining pathological evaluation of neoadjuvant therapy for adenocarcinoma of the esophagus. *World J Gastroenterol* 2013;19:9282–93.
10 Wayman J, Bennett MK, Raimes SA, *et al*. The pattern of recurrence of adenocarcinoma of the oesophago-gastric junction. *Br J Cancer* 2002;86:1223–9.
11 Vošmik M, Laco J, Sirák I, *et al*. Histopathologic features are more important prognostic factors than primary tumour location in gastro-oesophageal adenocarcinoma treated with preoperative chemoradiation and surgery. *Pathol Oncol Res* 2018;24:373–83.
12 Chen J-W, Xie J-D, Ling Y-H, *et al*. The prognostic effect of perineural invasion in esophageal squamous cell carcinoma. *BMC Cancer* 2014;14:313.
13 Torres CM, Wang HH, Turner JR, *et al*. Pathologic prognostic factors in esophageal squamous cell carcinoma: a follow-up study of 74 patients with or without preoperative chemoradiation therapy. *Mod Pathol* 1999;12:961–8.
14 Lagarde SM, Phillips AW, Navidi M, *et al*. The presence of lymphovascular and perineural infiltration after neoadjuvant therapy and oesophagectomy identifies patients at high risk for recurrence. *Br J Cancer* 2015;113:1427–33.
15 Tachezy M, Tiebel A-K, Gebauer F, *et al*. Prognostic impact of perineural, blood and lymph vessel invasion for esophageal cancer. *Histol Histopathol* 2014;29:1467–75.
16 Khan OA, Alexiou C, Soomro I, *et al*. Pathological determinants of survival in node-negative oesophageal cancer. *Br J Surg* 2004;91:1586–91.
17 Rubenstein JH, Shaheen NJ, Epidemiology SNJ. Epidemiology, diagnosis, and management of esophageal adenocarcinoma. *Gastroenterology* 2015;149:302–17.
18 Kim T, Grobmyer SR, Smith R, *et al*. Esophageal cancer--the five year survivors. *J Surg Oncol* 2011;103:179–83.
19 Shaikh T, Zaki MA, Dominello MM, *et al*. Patterns and predictors of failure following tri-modality therapy for locally advanced esophageal cancer. *Acta Oncol* 2016;55:303–8.
20 Mariette C, Markar SR, Dabakuyo-Yonli TS, *et al*. Hybrid minimally invasive esophagectomy for esophageal cancer. *N Engl J Med* 2019;380:152–62.