**Pathological lymph node regression following neoadjuvant chemotherapy predicts recurrence and survival in esophageal adenocarcinoma: A multicentre study in the United Kingdom**

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**Context Summary:**

**Key objective:**

What is the effect of pathological Lymph node (LN) regression on survival in patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy followed by surgical resection and what is the optimum categorization of LN response?

**Knowledge generated:**

LN regression was an independent predictive factor for survival using a three-point classification system (complete LN response, partial LN response and poor/no LN response). This classification was quick and simple to use for the reporting pathologist and demonstrated high inter-observer agreement. Patients with a complete or partial LN response had better survival than those with poor/no response, and patients with a complete LN response had equivalent survival to those with negative LNs. This association was independent of primary tumor response, which was discordant in a substantial number of patients.

**Abstract**

**Background:** There is limited evidence regarding the prognostic effects of pathological lymph node (LN) regression after neoadjuvant chemotherapy for esophageal adenocarcinoma, and a definition of LN response is lacking. This study aimed to evaluate how LN regression influences survival after surgery for esophageal adenocarcinoma.

**Methods:** Multicentre cohort study of patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy followed by surgical resection at five high-volume centres in the United Kingdom. LNs retrieved at esophagectomy were examined for chemotherapy response and given a LN regression score (LNRS) – LNRS 1, complete response; 2, <10% residual tumor; 3, 10-50% residual tumor; 4, >50% residual tumor; 5, no response. Survival analysis was performed using Cox regression adjusting for confounders including primary tumor regression. The discriminatory ability of different LN response classifications to predict survival was evaluated using Akaike’s information criterion and Harrell’s C-index.

**Results:** 17,930 LNs from 763 patients were examined. LN response classified as: *complete LN-response* (LNRS 1 ≥1 LN, no residual tumor in any LN; n=62, 8.1%), *partial LN-response* (LNRS 1-3 ≥1 LN, residual tumor ≥1 LN; n=155, 20.3%), *poor/no LN-response* (LNRS 4-5; n=303, 39.7%), or *LN negative* (no tumor/regression; n=243, 31.8%) demonstrated superior discriminatory ability. Mortality was reduced in patients with complete LN-response (HR 0.35, 95%CI 0.22-0.56), partial LN-response (HR 0.72, 95%CI 0.57-0.93) or negative LNs (HR 0.32 95%CI 0.25-0.42) compared to those with poor/no LN-response. Primary tumor regression and LN regression were discordant in 165 patients (21.9%).

**Conclusion:** Pathological LN regression following neoadjuvant chemotherapy was a strong prognostic factor and provides important information beyond pathological TNM staging and primary tumor regression grading. LN regression should be included as standard in the pathological reporting of esophagectomy specimens.

**Introduction**

The prognostic importance of tumor and lymph node (LN) downstaging after neoadjuvant treatment in patients with esophageal adenocarcinoma is well described 1,2. Tumor regression grading classifications such as the one described by Mandard et al. 3 are widely used in the pathological assessment of response to neoadjuvant treatment and have been shown to have prognostic value in this patient group 4–8. However, these classification systems only consider pathological response in the primary tumor and ignore response in locoregional LNs. Several single-centre studies evaluating pathological LN regression have recently suggested improved survival and reduced rates of tumor recurrence in patients exhibiting a LN response, independent of other prognostic variables including primary tumor regression 9–11. Furthermore, there is emerging evidence of a discrepancy in response between primary tumor and metastatic LNs in up to 30% of patients 9,10. It has been hypothesised that this discrepancy may be a result of variation in chemosensitivity between different clonal populations of cancer cells within the tumor microenvironment 9,12. However, there is no consensus on how best to define pathological LN response, with numerous different classifications previously utilised, making standardisation and comparison between studies difficult.

The primary aim of this study was to evaluate the effect of pathological LN regression on survival for esophageal adenocarcinoma in a multicentre setting. Secondary aims were to establish the optimum categorization of LN response that predicts survival and examine the association between primary tumor response and LN regression.

**Methods**

***Design***

This was a multicentre cohort study based on an ethically approved, prospectively maintained database of consecutive esophago-gastric resections performed at five high-volume tertiary centres in the United Kingdom - Guy’s and St Thomas’ National Health Service (NHS) Foundation Trust, The Royal Marsden NHS Foundation Trust, University Hospitals Birmingham NHS Foundation Trust, University Hospital Southampton NHS Foundation Trust, and Belfast Health and Social Care Trust. Patients treated with neoadjuvant chemotherapy followed by surgical resection with curative intent for histologically confirmed adenocarcinoma of the esophagus or esophago-gastric junction between 1st January 2000 and 31st August 2020 were included. Tumors of the esophago-gastric junction were categorised using the Siewert classification 13. The exposure was pathological response to neoadjuvant chemotherapy in LNs. Outcome measures were overall survival (primary) and disease-free survival (secondary). Inter-observer agreement between reporting pathologists was also evaluated.

***Clinical management***

All patients underwent standard staging investigations that included endoscopy, contrast enhanced computed tomography and fluorodeoxyglucose positron emission tomography (FDG PET). Additional staging investigations, e.g. endoscopic ultrasonography/laparoscopy were used for selected cases. All patients were discussed in a specialist esophago-gastric multidisciplinary team meeting and those with tumors staged greater than cT1 cN0 were considered for neoadjuvant treatment. Chemotherapy practice evolved during the study period following the publication of large, multicentre randomised trials 14–16. Patients received regimens that reflected these trial protocols including platinum based dual agent chemotherapy (e.g. cisplatin and fluorouracil, ‘*OEO2’* 15), epirubicin containing platinum based triplet chemotherapy (e.g. epirubicin, cisplatin and fluorouracil/capecitabine, ‘*MAGIC’* 14) and ‘*FLOT’* 16 (fluorouracil, oxaliplatin, leucovorin and docetaxel)*.* Patients who received neoadjuvant chemoradiotherapy or extended neoadjuvant chemotherapy as part of an advanced disease protocol were excluded. Trans-thoracic or trans-hiatal esophagectomy or extended total gastrectomy were performed by either open or minimally invasive approach. All adjuvant treatment strategies were permitted as decided by local multi-disciplinary consensus (e.g. chemotherapy, chemoradiotherapy, none).

***Pathological analysis***

Tumors were staged according to the Union for International Cancer Control (UICC) TNM version 8 17. Pathological primary tumor regression was evaluated using the Mandard classification from 1 (complete response) to 5 (no response) 3.

LNs were processed according to Royal College of Pathologists guidelines 18. This involved embedding the LN as one piece if smaller than 5mm in diameter; larger LNs were sliced at 3 mm intervals and completely embedded. One tissue section from each block was stained with Haematoxylin/Eosin following a standard laboratory protocol. Pan-cytokeratin immunohistochemistry was not used. Slides containing all LNs from each resection specimen were reviewed independently by one of six senior (greater than five-years’ experience) histopathologists following a standardised reporting protocol. Patients with incomplete slide sets (i.e. all LNs not available for regression analysis) were excluded.

***Classification of lymph node regression***

The optimal categorisation of LN regression was evaluated by comparing the accuracy of different classifications to predict survival. These classifications (*Table 1, supplementary table 1)* were chosen based on previously published studies 5,9–11 and examined whether classifying patients by ‘best’ or ‘worst’ responding LN better predicted survival.

Evidence of a LN response comprised areas of fibrosis, mucin pools or necrotic foci within the LN parenchyma. Other causes for fibrosis within a LN (e.g. sarcoidosis) were discounted. A LN regression score (LNRS) was determined for each LN according to the ratio of the area of residual viable tumor to the total area of the nodal ‘tumor bed’ (total area of fibrosis, mucin pools or necrosis) (*Figure 1*): LNRS 1, regression only (complete response); LNRS 2, less than 10% viable tumor; LNRS 3, 10 – 50% viable tumor; LNRS 4, more than 50% viable tumor; and LNRS 5, viable tumor with no evidence of response. LNs without viable tumor or evidence of regression were recorded as ‘negative LN’.

To evaluate inter-observer agreement for reporting LN regression between pathologists, a representative sample of 71 digitised LN slides were distributed to all senior pathologists from participating centres who had evaluated LN regression for this study as well as an independent expert with experience in LN regression (6 in total). Each LN was then independently scored as LNRS 1-5 or negative as described above. Overall agreement, agreement for each individual LNRS and agreement using a 5-point (LNRS 1-5) and 3-point (LNRS 1 ‘*complete response*’; LNRS 2-3 ‘*partial response*’; LNRS 4-5; ‘*no response*’) classification system was evaluated.

***Statistical analysis***

Clinicopathological characteristics were compared using the Chi-squared test.

Overall survival was defined as time from surgery to death from any cause or date of last outpatient department visit. Disease-free survival was defined as time from surgery to cancer recurrence, death or date of last outpatient department visit. Tumor recurrence was classified as locoregional (within the resection bed, locoregional LN or anastomosis), systemic (haematogenous, distant LN or peritoneum) or mixed. Mortality and recurrence data was obtained from electronic healthcare records. Survival curves were created using the Kaplan-Meier method, with subgroups compared using the log-rank test. Crude and adjusted survival analyses were performed using Cox proportional hazards regression adjusting for age (continuous), sex (male/female), chemotherapy regimen (OEO2/MAGIC/FLOT/other), LN yield (continuous), clinical stage (cT1-2 N0, cT0-2 N1-3, cT3-4 N0 or cT3-4 N1-3), tumor grade (well/moderately differentiated or poorly differentiated), presence of lympho-vascular invasion (yes/no) and primary tumor response (Mandard 1/Mandard 2-3/Mandard 4-5). These confounders were defined based on directed acyclic graphs 19.

To identify the optimum categorisation of pathological LN response for predicting survival, the discriminatory ability of different response classifications was evaluated using Harrell’s concordance index (C-index) 20 and Akaike’s information criterion (AIC) 21. Harrell’s C-index measured the probability of concordance between expected and observed outcomes in a model and AIC evaluated how well the model fits the data. A model (classification) with higher C-index and lower AIC values were considered to have better discriminatory ability. A C-index of 1.0 indicated perfect predictive ability and 0.5 indicated no ability. Following selection of the optimum pathological LN response classification, internal validation to evaluate model overfitting was performed using the split-sample method (70% training set and 30% test set) and bootstrap method with two thousand samples 20,22,23. Inter-observer agreement between pathologists was evaluated using Fleiss’ Kappa statistic for multiple responders 24 and results compared using the Z-test. A Kappa score 0.61-0.80 indicated substantial agreement and 0.81-1.00 indicated near perfect agreement 25.

P-values <0.05 were considered to be statistically significant. Statistical analysis was performed using IBM SPSS statistics (IBM Corp, IBM SPSS statistics Version 28.0. Armonk, NY: USA) and GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA).

**Results**

***Patient and treatment characteristics***

In total, 17,930 LNs from 763 patients were analysed (*Table 2*). Median age was 63 years, and the majority were male (n=658, 86.2%). Most patients received MAGIC chemotherapy (n=595, 78.0%), although some received FLOT (n=89, 11.7%), OEO2 chemotherapy (n=66, 8.7%) or alternative regimens (n=13, 1.7%). The majority of patients underwent trans-thoracic esophagectomy (n=557, 73.0%) with the remainder undergoing trans-hiatal esophagectomy (n=179, 23.5%) or extended total gastrectomy (n=27, 3.5%).

***Classification of lymph node response***

The prognostic ability of several different LN response classifications to predict survival were evaluated (*Table 1*). The performance of all classifications was relatively similar, however, the discriminatory ability of one classification was slightly superior when evaluated using both AIC and Harrell’s C-index. This system classified LN regression by best responding LN into *complete LN response* (LNRS 1 in at least one LN, no residual tumor in any LN), *partial LN response* (LNRS 1-3 in any LN, residual tumor in at least one LN) and *poor/no LN response* (LNRS 4-5). Based on its high C-index and low AIC combined with its relative simplicity compared to alternative classifications, potentially improving reproducibility and supporting implementation in clinical practice, this classification system was used for analysis.

***Pathological characteristics***

Overall, 217 out of 763 patients (28.5%) demonstrated a pathological LN response (*table 2, Supplementary Figure 1*), 62 (8.1%) had a complete LN response and 155 (20.3%) had a partial LN response. There were 303 patients (39.7%) who demonstrated poor/no LN response and 243 (31.8%) who had negative LNs with no evidence of previous tumor involvement. Patients with complete LN response or negative LNs were significantly less likely to have an advanced (pT3-4) pathological T-stage, lymphovascular invasionor positive resection margin than those with a partial LN response or poor/no response. Patients treated with neoadjuvant FLOT more frequently demonstrated LN regression compared to those treated with OEO2 or MAGIC regimen chemotherapy (38.2% FLOT vs 28.4% MAGIC vs 13.6% OEO2, *p=0.004*) (*Supplementary Table 2*).

Less than half of the patients demonstrated a pathological response (Mandard 1-3) in the primary tumor (n=322, 42.2%). Patients who demonstrated a LN response were more likely to demonstrate a pathological response in the primary tumor (complete LN response 78.7% vs partial LN response 45.5% vs poor/no LN response 22.7%, *p<0.001*). However, 97 out of 431 (22.5%) patients demonstrated a LN response in the absence of response in the primary tumor, and 68 out of 293 (23.2%) demonstrated a primary tumor response in the absence of response in LNs. Excluding patients with only negative LNs, there was a discordance between primary tumor and LN response in 165 out of 514 patients (32.1%). Ten patients (1.3%) had missing primary tumor regression data.

***Inter-observer agreement***

Six histopathologists independently scored 71 representative LNs (*Table 3*). Overall agreement using the 5-point LNRS scale was good (κ=0.76, 95% CI 0.74-0.79). However, agreement was significantly improved by instead using a 3-point classification system (κ=0.87, 95% confidence interval [CI] 0.84-0.91; Z = -4.62, p<0.001).

***Survival***

Kaplan-Meier (*Figure 2*) and Cox regression survival analysis (*Table 4*) demonstrated improved overall survival in patients with a complete LN response (hazard ratio [HR] 0.35; 95% CI 0.22-0.56), partial LN response (HR 0.72; 95%CI 0.57-0.93) and negative LNs (HR 0.32; 95%CI 0.25-0.42) compared to LN poor/non-responders when adjusted for confounding factors including regression in the primary tumor. Similar results were observed for disease-free survival (complete LN response, HR 0.32 95%CI 0.20-0.51; partial LN response, HR 0.76 95%CI 0.60-0.96; LN negative, HR 0.31 95%CI 0.24-0.41). Internal validation using both the split-sample and bootstrap methods demonstrated similar prognostic performance and model discrimination using this classification of LN response.

Rates of tumor recurrence were highest in patients who demonstrated poor/no LN response (LN negative 23.0% vs complete LN response 19.4% vs partial LN response 50.3% vs poor/no LN response 66.7%, p<0.001). This was true for both locoregional and systemic recurrence. Patients who demonstrated a pathological response in the primary tumor had improved survival compared to primary tumor non-responders (overall survival Mandard 1, HR 0.49 95% CI 0.27-0.91; Mandard 2-3 HR 0.70 95% CI 0.55-0.87).

Stratified analysis evaluating the effect of LN regression on survival in different pathological nodal stages greater than ypN0 (*Figure 2 C-E, supplementary Table 3*) demonstrated a trend towards improved survival in LN responders for all ypN categories. Evaluation of patients with evidence of LN regression without any completely regressed LNs i.e. LNRS 2-3 in any LN and none with LNRS 1 (*Figure 2 F*), demonstrated improved survival compared with poor/non-responders despite no pathological LN downstaging according to the TNM classification. Kaplan-Meier survival analysis for different chemotherapy regimens (*Supplementary Figure 2*) revealed similar patterns of survival for patients treated with MAGIC chemotherapy and FLOT. Subgroup survival analysis evaluating LN regression in primary tumor responders (Mandard 1-3) and non-responders (Mandard 4-5) (*Figure 2 G-H, supplementary Table 3*) demonstrated improved survival in LN responders (LNRS 1-3) and LN negative patients compared with LN non-responders (LNRS 4-5) for both primary tumor responders and non-responders.

**Discussion**

In this cohort of patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy prior to surgical resection, LN regression was an independent predictive factor for survival. Patients with a complete or partial LN response had better survival than those with poor/no response, and patients with a complete LN response had equivalent survival to those with negative LNs. This association was independent of primary tumor response, which was discordant in a substantial number of patients.

This was a large, multi-centre study evaluating the prognostic importance of LN regression in patients with esophageal adenocarcinoma. Several previous studies have evaluated this topic, however, they have been low-volume and single-centre in design 9–11. Some methodological issues deserve attention. This study allowed for long-term follow-up of a large cohort of patients with esophageal cancer treated at five high-volume specialist institutions. Although data were collected prospectively, the observational design made it difficult to exclude confounding. However, the results were adjusted for key prognostic factors to counteract this. Data from five institutions were included which increased the power of the study. All centres had similar demographic patient populations and utilised similar evidence-based protocols for multi-disciplinary perioperative decision-making, reflective of UK practice, which reduced heterogeneity. Although chemotherapy protocols evolved over the study period, stratified analysis revealed similar patterns of survival for both MAGIC and FLOT chemotherapy. Furthermore, the higher rates of LN regression observed in patients who received FLOT supports the findings of previous studies which have demonstrated improved rates of primary tumor pathological response in patients treated with FLOT compared to MAGIC chemotherapy 26,27. No previous studies have evaluated the effect of FLOT on LN regression despite this regimen becoming the standard of care in many Western institutions following the publication of the FLOT4-AIO trial 16. The proposed classification of LN response demonstrated good internal validity using the split-sample and bootstrap methods. However, in order to further evaluate its performance in clinical practice, external validation in an independent cohort is required. This is an intended area of future work.

Although assessment of primary tumor regression is a core data item in The Royal College of Pathologists standards for reporting esophago-gastric cancers, LN regression is not currently part of any regression grading system and is therefore not routinely reported in most UK centres 18. Despite recent attempts 28,29, there is no national or international consensus regarding which tumor regression grading system should be used and this decision is therefore left to individual units, with significant variations in practice observed 30. Furthermore, as noted in the recent HERO study, the definition of response is an area which conspicuously lacks consensus 29. This is further complicated by the pathological evaluation of LNs since an individual patient may exhibit varying degrees of regression in different nodes. This study evaluated several classifications and definitions of LN response aiming to standardise pathological reporting of LN regression. Results suggest that classifying response by best responding LN and using a 3-point system (complete LN response, partial LN response and poor/no LN response) was the best predictor of tumor recurrence and survival. A 5-point classification system similar to primary tumor regression Mandard score was also considered, however, results of inter-observer analysis suggested significantly improved agreement using a 3-point system. Utilisation of this system is therefore likely to improve the generalisability of these results, reproducibility on pathological analysis and, furthermore, its relative simplicity is more sympathetic to the time constraints on already pressurised pathology resources. The results of this study support the argument for standardised evaluation and documentation of LN regression during the pathological reporting of esophageal adenocarcinoma specimens.

Pathological nodal stage is widely accepted to be one of the strongest predictive factors for survival in esophageal adenocarcinoma. A recent study using pooled data from the UK MRC OEO5 and STO3 trials has argued that primary tumor and LN regression analysis does not provide additional prognostic information over and above this 31. However, results from this study suggest that a survival benefit was observed in patients who exhibited a LN response for all ypN stages, including patients who remained ypN3 despite a LN response. Furthermore, this benefit was maintained even in patients who were not ‘downstaged’ using conventional TNM staging classifications (for example those who demonstrated a LN response without any completely regressed LNs). LN regression is therefore likely to be a more useful measure of systemic efficacy of neoadjuvant chemotherapy than pathological nodal stage since it considers these factors and may also provide additional prognostic information in relation to the treatment of extra-nodal micro-metastases. The fact that systemic tumor recurrence is significantly lower in LN responders further supports this hypothesis. Since decisions regarding adjuvant oncological treatments are often based on evidence of a response to neoadjuvant therapy, the additional prognostic information obtained by evaluating LN regression can support clinical decision making when determining whether to continue with the same chemotherapy regimen after surgery or consider alternative strategies (e.g. immunotherapy 32). This benefit is further emphasized when considering the discrepancy between primary tumor and LN response in a significant number of patients, and the survival benefit observed in patients who were LN responders despite being primary tumor non-responders. Further research is needed to determine whether LN regression may provide an additional surrogate marker for systemic treatment efficacy and prognosis in patients treated with newer peri-operative treatments and there is a strong argument for its inclusion in future trials evaluating neoadjuvant or adjuvant treatment strategies.

Although this study evaluated LN regression in patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy, some centres routinely utilise chemoradiation protocols (e.g. CROSS 2) in this patient group, and the optimum neoadjuvant strategy remains debated. Rates of pathological regression in the primary tumor are generally considered to be higher following chemoradiation compared with chemotherapy, although this does not necessarily translate into improved survival 33. Two previous studies evaluating LN regression in patients with esophageal cancer (adenocarcinoma and squamous cell carcinoma) treated with neoadjuvant chemoradiation have demonstrated reduced mortality in patients with histomorphological features of LN regression (central necrosis or fibrosis) 34,35, suggesting that LN regression may be prognostic regardless of neoadjuvant strategy. Furthermore, pathological LN regression has also recently been evaluated in other tumor groups treated with neoadjuvant therapy (gastric, breast and rectal carcinoma) with results similarly demonstrating improved survival in patients exhibiting a LN response 36–38.

In conclusion, pathological LN regression analysis provides important prognostic information beyond currently reported pathological TNM staging and primary tumor regression grading in patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy followed by surgical resection. The three-point system proposed (complete LN response, partial LN response and poor/no LN response) is quick and simple to use for the reporting pathologist and shows very high inter-observer agreement. LN regression should be included as standard in the pathological reporting of esophagectomy specimens.

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**Table 1.** Results of Akaike’s information criterion (AIC) and Harrell’s C-index analysis evaluating the discriminatory ability of different lymph node response classifications to predict survival in patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy prior to surgical resection.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Overall survival** | | **Disease-free survival** | |
|  | **Crude** | **Adjusted a** | **Crude** | **Adjusted a** |
| **Classification by best LNRS** b |  |  |  |  |  |
| *Response* (LNRS 1-3) vs *No-response* (LNRS 4-5) vs *Negative LNs* | AIC | 5142.06 | 5492.69 | 5080.52 | 5428.20 |
| C-index | 0.667 | 0.710 | 0.668 | 0.715 |
| *Response* (LNRS 1-2) vs *No-response* (LNRS 3-5) vs *Negative LNs* | AIC | 5142.00 | 5493.33 | 5080.41 | 5429.42 |
| C-index | 0.665 | 0.708 | 0.667 | 0.711 |
| *Complete response* (LNRS 1, no residual tumor)vs *Partial response* (LNRS 1-3, residual tumor) vs *No response* (LNRS 4-5) vs *Negative LNs* | AIC | **5121.91**\* | **5463.93**\* | 5070.69 | **5413.26**\* |
| C-index | **0.676**\*\* | **0.715**\*\* | **0.679**\*\* | 0.720 |
| **Classification by worst LNRS** |  |  |  |  |  |
| *Response* (LNRS 1-3) vs *No-response* (LNRS 4-5) vs *Negative LNs* | AIC | 5132.21 | 5472.64 | 5077.31 | 5419.51 |
| C-index | 0.660 | 0.712 | 0.666 | 0.720 |
| *Response* (LNRS 1-2) vs *No-response* (LNRS 3-5) vs *Negative LNs* | AIC | 5128.32 | 5466.27 | 5075.42 | 5416.25 |
| C-index | 0.656 | 0.710 | 0.664 | 0.718 |
| *Complete response* (LNRS 1, no residual tumor)vs *Partial response* (LNRS 1-3, residual tumor) vs *No response* (LNRS 4-5) vs *Negative LNs* | AIC | 5129.27 | 5467.81 | 5075.16 | 5416.00 |
| C-index | 0.663 | 0.712 | 0.668 | 0.720 |
| **Other classifications** |  |  |  |  |  |
| *Response* (total number LNRS 1-3 > LNRS 4-5) vs *No-response* (total number LNRS 4-5 < LNRS 1-3) vs *Negative LNs* | AIC | 5124.79 | 5473.73 | **5069.57**\* | 5417.66 |
| C-index | 0.669 | **0.715**\*\* | 0.669 | 0.719 |
| *Response* (LNRS 1-3, no LNRS 4-5)vs *Mixed response* (LNRS 1-3 and LNRS 4-5) vs *No response* (LNRS 4-5, no LNRS 1-3) vs *Negative LNs* | AIC | 5122.41 | 5465.76 | 5072.27 | 5415.90 |
| C-index | **0.676**\*\* | **0.715**\*\* | 0.678 | **0.721**\* |

a Adjusted for age (continuous), sex (male or female), chemotherapy regimen (OEO2, MAGIC, FLOT or other), LN yield (continuous), clinical stage (cT0-2 N0, cT0-2 N1-3, cT3-4 N0 or cT3-4 N1-3), differentiation (well-moderate or poor), lymphovascular invasion (yes or no), primary tumor response (Mandard 1, Mandard 2-3, Mandard 4-5 or not recorded)

b Lymph node regression score

\* Best performing model when assessed using AIC or Harrell’s C-index

\*\* Joint best performing model when assessed using AIC or Harrell’s C-index if 2 or more classifications had equivalent results

**Table 2.** Clinicopathological and treatment characteristics stratified by pathological lymph node response to neoadjuvant chemotherapy in patients having undergone resection for esophageal adenocarcinoma.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *N=763* | | **Negative nodes**  *n=243* | **Complete response** a  *n=62* | **Partial**  **Response** b  *n=155* | **Poor / no**  **Response** c  *n=303* | *p-value* |
| Median age at operation | | 65 years | 65 years | 64 years | 64 years | *p=0.07* |
| Sex | Male | 211 (86.8%) | 58 (93.5%) | 134 (86.5%) | 255 (84.2%) | *p=0.26* |
| Female | 32 (13.2%) | 4 (6.5%) | 21 (13.5%) | 48 (15.8%) |
| Chemotherapy regimen | MAGIC a | 181 (74.5%) | 43 (69.4%) | 126 (81.3%) | 245 (80.9%) | ***p=0.004*** |
| OEO2 b | 27 (11.1%) | 2 (3.2%) | 7 (4.5%) | 30 (9.9%) |
| FLOT c | 32 (13.2%) | 14 (22.6%) | 20 (12.9%) | 23 (7.6%) |
| Other | 3 (1.2%) | 3 (4.8%) | 2 (1.3%) | 5 (1.7%) |
| Tumor location | Esophagus | 52 (21.4%) | 9 (14.5%) | 20 (12.9%) | 53 (17.5%) | *p=0.31* |
| Siewert type 1 | 89 (36.6%) | 20 (32.3%) | 56 (36.1%) | 112 (37.0%) |
| Siewert type 2 | 96 (39.5%) | 31 (50.0%) | 69 (44.5%) | 128 (42.2%) |
| Siewert type 3 | 6 (2.5%) | 2 (3.2%) | 10 (6.5%) | 10 (3.3%) |
| Operation | Trans-thoracic esophagectomy | 167 (68.7%) | 44 (71.0%) | 122 (78.7%) | 224 (73.9%) | *p=0.07* |
| Trans-hiatal esophagectomy | 70 (28.8%) | 14 (22.6%) | 25 (16.1%) | 70 (23.1%) |
| Extended total gastrectomy | 6 (2.5%) | 4 (6.5%) | 8 (5.2%) | 9 (3.0%) |
| Median lymph node yield | | 21 | 24 | 25 | 21 | ***p=0.03*** |
| cT stage | cT0-2 | 46 (18.9%) | 3 (4.8%) | 12 (7.7%) | 32 (10.6%) | ***p<0.001*** |
| cT3-4 | 197 (81.1%) | 59 (95.2%) | 143 (92.3%) | 271 (89.4%) |
| cN stage | cN0 | 85 (35.0%) | 9 (14.5%) | 18 (11.6%) | 74 (24.4%) | ***p<0.001*** |
| cN+ | 158 (65.0%) | 53 (85.5%) | 137 (88.4%) | 229 (75.6%) |
| ypT stage | ypT0-2 | 142 (58.4%) | 37 (59.7%) | 43 (27.7%) | 70 (23.1%) | ***p<0.001*** |
| ypT3-4 | 101 (41.6%) | 25 (40.3%) | 112 (72.3%) | 233 (76.9%) |
| ypN stage | ypN0 | 243 (100%) | 62 (100%) | 0 (0%) | 0 (0%) | *N/A* |
| ypN+ | 0 (0%) | 0 (0%) | 155 (100%) | 303 (100%) |
| Primary tumor regression | Mandard 1 | 31 (12.8%) | 14 (22.6%) | 7 (4.5%) | 1 (0.3%) | ***p<0.001*** |
| Mandard 2-3 | 105 (43.2%) | 34 (54.8%) | 63 (40.6%) | 67 (22.1%) |
| Mandard 4-5 | 103 (42.4%) | 13 (21.0%) | 84 (54.2%) | 231 (76.2%) |
| *Not recorded* | *4 (1.6%)* | *1 (1.6%)* | *1 (0.6%)* | *4 (1.3%)* |
| Tumor grade | Well / moderate | 147 (60.5%) | 31 (50.0%) | 75 (48.4%) | 133 (43.9%) | ***p=0.002*** |
| Poor | 96 (39.5%) | 31 (50.0%) | 80 (51.6%) | 170 (56.1%) |
| Lymphovascular invasion | No | 171 (70.4%) | 52 (83.9%) | 68 (43.9%) | 91 (30.0%) | ***p<0.001*** |
| Yes | 72 (29.6%) | 10 (16.1%) | 87 (56.1%) | 212 (70.0%) |
| Resection margin | R0 | 196 (80.7%) | 55 (88.7%) | 93 (60.0%) | 136 (44.9%) | ***p<0.001*** |
| R1 | 47 (19.3%) | 7 (11.3%) | 62 (40.0%) | 167 (55.1%) |
| Recurrence | No recurrence | 187 (77.0%) | 50 (80.6%) | 77 (49.7%) | 101 (33.3%) | ***p<0.001*** |
| Any recurrence | 56 (23.0%) | 12 (19.4%) | 78 (50.3%) | 202 (66.7%) |
| *Locoregional recurrence* | 9 (3.7%) | 2 (3.2%) | 11 (7.1%) | 23 (7.6%) |  |
| *Systemic recurrence* | 16 (6.6%) | 2 (3.2%) | 30 (19.4%) | 80 (26.4%) |  |
| *Mixed recurrence* | 12 (4.9%) | 3 (4.8%) | 18 (11.6%) | 49 (16.2%) |  |
| *Recurrence location not*  *recorded* | 19 (9.8%) | 5 (8.1%) | 19 (12.3%) | 50 (16.5%) |  |
| Death | Alive | 154 (63.4%) | 38 (61.3%) | 60 (38.7%) | 64 (21.1%) | ***p<0.001*** |
| Not alive | 89 (36.6%) | 24 (38.7%) | 95 (61.3%) | 239 (78.9%) |

a Includes all platinum based dual agent chemotherapy regimen (e.g. cisplatin and fluorouracil)

b Includes all epirubicin containing platinum based triplet chemotherapy regimens (e.g. epirubicin, cisplatin and fluorouracil)

c fluorouracil plus leucovorin, oxaliplatin, and docetaxel

**Table 3.** Results of interobserver agreement analysis evaluating agreement between pathologists (N=6) for reporting lymph node regression in 71 lymph nodes from patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy prior to surgical resection.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **% agreement** | **Fleiss’ Kappa** | **95% Confidence Interval** |
| **5-point classification** |  |  |  |
| *LNRS a 1* | 93.3% | 0.96 | 0.90-1.02 |
| *LNRS 2* | 82.2% | 0.80 | 0.74-0.86 |
| *LNRS 3* | 62.7% | 0.60 | 0.54-0.66 |
| *LNRS 4* | 72.7% | 0.61 | 0.55-0.67 |
| *LNRS 5* | 77.8% | 0.77 | 0.71-0.83 |
| *Negative node* | 100% | 1.00 | 0.94-1.06 |
| **Overall agreement** | **84.1%** | **0.76** | **0.74-0.79** |
| **3-point classification** |  |  |  |
| *Complete nodal response b* | 93.3% | 0.97 | 0.90-1.02 |
| *Partial nodal response c* | 81.5% | 0.87 | 0.74-0.86 |
| *Poor / no nodal response d* | 95.2% | 0.88 | 0.74-0.86 |
| *Node negative e* | 100% | 1.00 | 0.94-1.06 |
| **Overall agreement** | **90.7%** | **0.87** | **0.84-0.91** |

a Lymph node regression score

b LNRS 1 ≥1 LN, no residual tumor in any node

c LNRS 1-3 ≥1 LN, residual tumor ≥1 node

d LNRS 4-5

e No evidence of tumor or regression

**Table 4.** Hazard ratios (HR) with 95% confidence intervals (CI) of survival in patients treated with esophageal adenocarcinoma treated with neoadjuvant chemotherapy followed by surgical resection.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *N=763* | | **Overall survival** | | **Disease-free survival** | |
| **Crude**  *HR 95% CI* | **Adjusted** a  *HR 95% CI* | **Crude**  *HR 95% CI* | **Adjusted** a  *HR 95% CI* |
| **Lymph node regression** | Poor / no nodal response | 1.00 | 1.00 | 1.00 | 1.00 |
| Complete nodal response | **0.24** (0.16-0.37) | **0.35** (0.22-0.56) | **0.22** (0.15-0.34) | **0.32** (0.20-0.51) |
| Partial nodal response | **0.63** (0.49-0.80) | **0.72** (0.57-0.93) | **0.67** (0.54-0.84) | **0.76** (0.60-0.96) |
| Negative nodes | **0.24** (0.19-0.31) | **0.32** (0.25-0.42) | **0.24** (0.18-0.30) | **0.31** (0.24-0.41) |
| **Tumor regression** | Mandard 4-5 | 1.00 | 1.00 | 1.00 | 1.00 |
| Mandard 2-3 | **0.50** (0.41-0.61) | **0.70** (0.55-0.87) | **0.50** (0.40-0.61) | **0.70** (0.56-0.88) |
| Mandard 1 | **0.24** (0.14-0.40) | **0.49** (0.27-0.91) | **0.22** (0.13-0.40) | **0.54** (0.31-0.96) |
| Not recorded | 0.45 (0.20-1.01) | 0.60 (0.26-1.39) | 0.44 (0.20-1.00) | 0.66 (0.28-1.53) |

a Adjusted for age (continuous), sex (male or female), chemotherapy regimen (OEO2, MAGIC, FLOT or other), LN yield (continuous), clinical stage (cT0-2 N0, cT0-2 N1-3, cT3-4 N0 or cT3-4 N1-3), differentiation (well-moderate or poor), lymphovascular invasion (yes or no), primary tumor response (Mandard 1, Mandard 2-3, Mandard 4-5 or not-recorded)

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**Figure 1.** Histological images of haematoxylin and eosin-stained lymph nodes representative of each lymph node regression score (LNRS). **a** Complete nodal response / LNRS 1: a lymph node with tumor bed composed of central necrosis; there is no residual viable tumor. **b** Partial nodal response / LNRS 2: a lymph node with a tumor bed of mostly acellular mucin pools with one small groups of viable tumor cells comprising less than 10 per cent of the total tumor bed area. **c** Partial nodal response / LNRS 2: a lymph node with a tumor bed of mostly central necrosis with several small groups of viable tumor cells comprising less than 10 per cent of the total tumor bed area. **d** Partial nodal response / LNRS 3: a lymph node with a tumor bed of mostly acellular mucin pools; there is viable tumor making up 10–50 per cent of the tumor bed area. **e** Poor / no nodal response / LNRS 4: a lymph node with a large tumor bed area composed of mostly of viable tumor with several acellular mucin pools; There is over 50 per cent viable tumor, but with recognizable features of regression. **f** Poor / no nodal response / LNRS 5: a lymph node completely replaced by metastatic tumor showing no clear signs of regression

**Figure 2.** Kaplan-Meier survival analysis evaluating pathological lymph node (LN) response for overall (A) and disease-free survival (B) and overall survival stratified by pathological nodal stage (C-E), LN regression score (LNRS) (F) and primary tumor regression (G-H) in patients with esophageal adenocarcinoma treated with neoadjuvant chemotherapy followed by surgical resection. The significance of the difference was calculated using the log-rank test.

**Supplementary Table 1.** Lymph node response classification definitions and rationale for inclusion.

|  |  |
| --- | --- |
| **Response category** | **Definition and rationale** |
| **Classification by best LNRS** a | **Patients classified by best LNRS (i.e. if varying degrees of regression identified in different LN, patients were classified by best responding LNRS)** |
| *Response* (LNRS 1-3) vs *No-response* (LNRS 4-5) vs *Negative LNs* | **Response:** LNRS 1-3 in any LN.  **No response:** LNRS 4-5 in any LN, no LNRS 1-3 in any LN.  **Rationale:** Similar to commonly utilised definition of primary tumor response. Previously utilised in studies evaluating LN regression. 9-10. |
| *Response* (LNRS 1-2) vs *No-response* (LNRS 3-5) vs *Negative LNs* | **Response:** LNRS 1-2 in any LN.  **No response:** LNRS 3-5 in any LN, no LNRS 1-2 in any LN.  **Rationale:** Variability in literature regarding whether patients with primary tumor Mandard scores of 3 are responders or non-responders 5. Therefore, alternative classification of LNRS 3 as non-responders of interest. |
| *Complete response* (LNRS 1, no residual tumor)vs *Partial response* (LNRS 1-3, residual tumor) vs *No response* (LNRS 4-5) vs *Negative LNs* | **Complete response:** LNRS 1 in any LN and no residual tumor in any LN.  **Partial response:** LNRS 1-3 in any LN, residual tumor in at least 1 LN.  **No response**: LNRS 4-5 in any LN, no LNRS 1-3 in any LN.  **Rationale:** Of interest to establish whether there is a survival benefit in patients with evidence of LN regression who remain ypN positive (i.e. partial responders). |
| **Classification by worst LNRS** | **Patients classified by worst LNRS (i.e. if varying degrees of regression identified in different LN, patients were classified by worst responding LNRS)** |
| *Response* (LNRS 1-3) vs *No-response* (LNRS 4-5) vs *Negative LNs* | **Response:** LNRS 1-3 in any LN, no LNRS 4-5 in any node.  **No response:** LNRS 4-5 in any LN  **Rationale:** Comparison with best LNRS classifications above to establish whether classification by best or worst responding LN better predicts survival. |
| *Response* (LNRS 1-2) vs *No-response* (LNRS 3-5) vs *Negative LNs* | **Response:** LNRS 1-2 in any LN, no LNRS 3-5 in any node.  **No response:** LNRS 3-5 in any LN.  **Rationale:** Comparison with best LNRS classifications above. |
| *Complete response* (LNRS 1, no residual tumor)vs *Partial response* (LNRS 1-3, residual tumor) vs *No response* (LNRS 4-5) vs *Negative LNs* | **Complete response:** LNRS 1 in any LN and no residual tumor in any LN.  **Partial response:** LNRS 1-3 in any LN, no LNRS 4-5 in any node and residual tumor in at least 1 LN.  **No response**: LNRS 4-5 in any LN.  **Rationale:** Comparison with best LNRS classifications above. |
| **Other classifications** | |
| *Response* (total number LNRS 1-3 > LNRS 4-5) vs *No-response* (total number LNRS 4-5 < LNRS 1-3) vs *Negative LNs* | **Response:** Total number LN with LNRS 1-3 > Total number LN with LNRS 4-5.  **No response:** Total number LN with LNRS 4-5 > Total number LN with LNRS 1-3.  **Rationale:**  Similar to concept of pathologic lymph node ratio. Evaluating whether ratio of LN which demonstrate a pathological response (LNRS 1-3) to no response (LNRS 4-5) predicts survival. |
| *Response* (LNRS 1-3, no LNRS 4-5)vs *Mixed response* (LNRS 1-3 and LNRS 4-5) vs *No response* (LNRS 4-5, no LNRS 1-3) vs *Negative LNs* | **Response:** LNRS 1-3 in any LN, no LNRS 4-5 in any LN.  **Mixed response:** LNRS 1-3 in any LN and LNRS 4-5 in any LN.  **No response:** LNRS 4-5 in any LN, no LNRS 1-3 in any LN.  **Rationale:** Exploring cohort of patients who demonstrate a mixed LN response (i.e. some LN which demonstrate a response and others that don’t) to see whether this predicts survival. |

a Lymph node regression score

**Supplementary Table 2.** Number of chemotherapy cycles and pathological response rates in lymph nodes and the primary tumor for different neoadjuvant chemotherapy regimens in patients having undergone resection for esophageal adenocarcinoma.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **OEO2**a  *n=66* | **MAGIC** b  *n=595* | **FLOT** c  *n=89* | **Other**  *n=13* | *p-value* |
| **Number of chemotherapy cycles** | 1 cycle | 2 (3.0%) | 8 (1.3%) | 0 (0%) | 0 (0%) | ***p<0.001*** |
| 2 cycles | 52 (78.8%) | 31 (5.2%) | 0 (0%) | 0 (0%) |
| 3 cycles | 8 (12.1%) | 477 (80.2%) | 1 (1.1%) | 5 (38.5%) |
| ≥4 cycles | 3 (4.5%) | 77 (12.9%) | 88 (98.9%) | 3 (23.1%) |
| Not recorded | 1 (1.5%) | 2 (0.3%) | 0 (0.0%) | 5 (38.5%) |
| **Lymph node response** | Negative nodes | 27 (40.9%) | 181 (30.4%) | 32 (36.0%) | 3 (23.1%) | ***p=0.004***  *FLOT vs MAGIC p=0.01* |
| Complete nodal response | 2 (3.0%) | 43 (7.2%) | 14 (15.7%) | 3 (23.1%) |
| Partial nodal response | 7 (10.6%) | 126 (21.2%) | 20 (22.5%) | 2 (15.4%) |
| Poor / no response | 30 (45.5%) | 245 (41.2%) | 23 (25.8%) | 5 (38.5%) |
| **Primary tumor response** | Mandard 1 | 2 (3.0%) | 34 (5.7%) | 15 (16.9%) | 2 (15.4%) | ***p<0.001***  *FLOT vs MAGIC p<0.001* |
| Mandard 2-3 | 18 (27.3%) | 199 (33.4%) | 48 (53.9%) | 4 (30.8%) |
| Mandard 4-5 | 45 (68.2%) | 353 (59.3%) | 26 (29.2%) | 7 (53.8%) |
| Not recorded | 1 (1.5%) | 9 (1.5%) | 0 (0%) | 0 (0%) |

a Includes all platinum based dual agent chemotherapy regimen (e.g. cisplatin and fluorouracil)

b Includes all epirubicin containing platinum based triplet chemotherapy regimens (e.g. epirubicin, cisplatin and fluorouracil)

c fluorouracil plus leucovorin, oxaliplatin, and docetaxel

**Supplementary Table 3.** Hazard ratios (HR) with 95% confidence intervals (CI) of survival in patients having undergone neoadjuvant chemotherapy following by surgical resection for esophageal adenocarcinoma stratified by pathological nodal stage and primary tumor regression.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | ***Overall survival*** | | ***Disease-free survival*** | |
|  |  | **Crude**  *HR (95% CI)* | **Adjusted a**  *HR (95% CI)* | **Crude**  *HR (95% CI)* | **Adjusted a**  *HR (95% CI)* |
| **ypN1** *(n=192)* | Poor/no LN response b | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| LN response c | **0.73** (0.49-1.08) | **0.87** (0.56-1.34) | **0.85** (0.59-1.23) | **0.95** (0.63-1.44) |
| **ypN2** *(n=150)* | Poor/no LN response | 1.00 | 1.00 | 1.00 | 1.00 |
| LN response | **0.55** (0.36-0.84) | **0.64** (0.38-0.97) | **0.52** (0.34-0.79) | **0.57** (0.34-0.88) |
| **ypN3** *(n=116)* | Poor/no LN response | 1.00 | 1.00 | 1.00 | 1.00 |
| LN response | **0.60** (0.39-0.91) | **0.59** (0.38-0.94) | **0.65** (0.43-0.97) | **0.61** (0.39-0.96) |
| **Primary tumor responder (Mandard 1-3)** (n=323) d | Poor/no LN response | 1.00 | 1.00 | 1.00 | 1.00 |
| LN response | **0.51** (0.34-0.76) | **0.57** (0.36-0.90) | **0.50** (0.34-0.73) | **0.56** (0.36-0.87) |
| Negative nodes | **0.27** (0.17-0.41) | **0.30** (0.18-0.48) | **0.24** (0.16-0.37) | **0.27** (0.17-0.44) |
| **Primary tumor non-responder (Mandard 4-5)** (n=432) d | Poor/no LN response | 1.00 | 1.00 | 1.00 | 1.00 |
| LN response | **0.61** (0.46-0.81) | **0.66** (0.49-0.88) | **0.66** (0.50-0.86) | **0.68** (0.52-0.90) |
| LN negative | **0.31** (0.22-0.43) | **0.34** (0.24-0.48) | **0.31** (0.22-0.42) | **0.35** (0.25-0.49) |

a Adjusted for age (continuous), sex (male or female), chemotherapy regimen (OEO2, MAGIC, FLOT or other), LN yield (continuous), clinical stage (cT0-2 N0, cT0-2 N1-3, cT3-4 N0 or cT3-4 N1-3), differentiation (well-moderate or poor), lymphovascular invasion (yes or no), primary tumor response (responder, non-responder or not-recorded)

b Lymph node regression score (LNRS) 4-5

c LNRS 1-3

d Not adjusted for primary tumor response

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**Supplementary Figure 1.** Study flowsheet.



**Supplementary Figure 2.** Kaplan-Meier survival analysis evaluating overall survival for pathological nodal response stratified by neoadjuvant chemotherapy regimen in patients treated with esophageal adenocarcinoma treated with neoadjuvant chemotherapy followed by surgical resection. The significance of the difference was calculated using the log-rank test.