# Towards a more personalized approach in the treatment of esophageal cancer focusing on predictive factors in response to chemoradiation

Wang, Da

CHAPTER 7

# Neoadjuvant therapy reduces the incidence of nodal micrometastases in esophageal adenocarcinoma.

Da Wang †
Justin K. Smit †
Eva Zwaan
Christina T. Muijs
Henk Groen
Harry Hollema
John Th.M. Plukker

† Both authors contributed equally to the manuscript.

American Journal of Surgery 2013 Nov;206(5):732-8

## Abstract

***Background***

We evaluated the impact of neoadjuvant chemoradiotherapy (CRT) on nodal micrometastases (NMM) in esophageal adenocarcinoma (EAC) patients with histologically negative nodes (y)pN0.

***Methodes***

Out of 48 consecutively treated patients with neoadjuvant CRT, we selected 20 EAC ypN0 patients (group 1). These patients were matched with 20 pN0 EAC patients who had surgery alone (group 2). Harvested (y)pN0 lymph nodes were examined immunohistochemically (anti-CK8/18(CAM 5.2)) according to a validated sentinel node protocol. A third group (n=11) staged as ypN1 after neoadjuvant CRT was employed as a control group.

***Results***

Upstaging to NMM+ occurred in two patients (10%) in group 1 and in eight patients (40%) in group 2 (P=0.028). Disease free (DFS) and overall survival (OS) in NMM+ patients in group 1 was worse compared to NMM- patients (P=0.014 and P=0.003), but comparable with the ypN1 patients (n=11).

***Conclusion***

A 30% reduction of NMM+ was obtained after neoadjuvant treatment in (y)pN0 patients. NMM+ after CRT had as negative an impact on survival as in ypN1 patients. These data warrant further investigation in larger prospective datasets.

## Introduction

Despite recent advances in cancer treatment, patients with esophageal cancer (EC) still have a relatively poor prognosis. More than 80% of the EC patients present with a locally advanced tumor and nodal involvement or metastatic disease at the time of diagnosis. It is known that histologically proven nodal involvement (pN1), total number of resected, number of tumor positive nodes and lymph node ratio (number of involved/number of examined nodes) are independent prognostic factors for overall (OS) and disease free survival (DFS) 1-4. The importance of an adequate nodal resection is subjected to the strong prognostic value of the number of resected nodes on the outcome 3. As demonstrated in some studies an extended two-field nodal resection should be recommended, usually through a transthoracic (TT) route 5, 6. However, even patients with histological node negative (pN0) status will develop (early) locoregional recurrences which can be explained by the presence of nodal micrometastases (NMM) 7, 8. These NMM are not detected by routine hematoxylin and eosin (H&E) methods, but usually immunohistochemically with antibodies against cytokeratins specific for epithelial tissue 8-10. Patients with pN0 tumors, but with NMM, have a significantly worse survival rate than those without NMM. These NMM can be divided into isolated tumor cells (ITC) and micrometastases (MM) 10. MM have a worse effect on survival than ITC 7, 8, 10, 11.

Neoadjuvant treatment is currently the standard of care in the experienced centers 12-16. The rationale behind this is that tumor downstaging/sizing and elimination of NMM leads to improved resectability and curability rates 12, 13, 17. The improvement on survival rates after chemoradiotherapy (CRT) in a neoadjuvant setting is considerable, between 10-15% 12, 13. Previous research has shown that response to neoadjuvant CRT reduces NMM in esophageal cancer 17. But these studies are scarce and little has been published regarding the rate and the effect of neoadjuvant CRT on NMM in pN0 esophageal cancer patients. Therefore we evaluated the effect of CRT on NMM in ypN0 esophageal cancer patients after neoadjuvant treatment compared to pN0 in the surgery alone group.



## Patients and methods

***Patients***

A total of 380 patients with histological proven esophageal cancer were identified in the prospective database of our tertiary referral medical center. All patients had a surgical resection with a curative



513774-L-bw-wang
Processed on: 19-9-2017

intent. From 2005 onwards, patients were treated with neoadjuvant treatment in a RCT trial or as standard procedure. The preoperaitive diagnostic workup, the surgical procedure and/or surgical team and follow-up did not change during the study period. In total 332 patients received surgery alone and 48 received neoadjuvant CRT followed by surgical resection. According to national guidelines no ethics board review was required for the present study (www.ccmo.nl). Archival tissue was handled according to the Dutch Code for proper use of Human Tissue (www.federa.org).

***Matching and construction of surgery alone and ypN1 groups***

From our prospective database one group of 20 consecutive adenocarcinoma (AC) patients who received neoadjuvant CRT and staged histologically as ypN0 were selected (group 1). This group was matched on cT-stage or best-case lower cT-stage match with 20 AC patients with pN0 who were treated with surgery alone (group 2). Age was not a matching criterion. Consequently patients from group 1 were only treated in the period after 2005 and patients from group 2 during both periods. Additionally a third group which consisted of all 11 AC patients treated with neoadjuvant CRT but classified as ypN1 after routine pathologic evaluation, was used as a control group for the NMM+ patients in group 1 in the analyses. For the analyses we could therefore include 51 patients in this study.

***Staging procedure***

The diagnostic staging procedure consisted of endoscopic ultrasonography (EUS) with fine needle aspiration (FNA) of suspected lymph nodes, 16-64 multidetector Computed Tomography (md-CT) scans of the neck, chest and abdomen, and cervical echographic examination. In case of T2-T4a tumors, or involved regional lymph nodes (N+), 18-F-fluorodeoxyglucose positron emission tomography (18-F-FDG-PET) was performed to exclude distant disease 18.

After staging in accordance with the Union for International Cancer Control TNM 7th edition, all patients were discussed by a multidisciplinary tumor board for an adequate treatment planning 19, 20.

***Pre-operative treatment***

The neoadjuvant chemoradiotherapy regimen consisted of radiotherapy with a total dose of 41.4-45 Gy in daily fractions of 1.8 Gy, five times per week (n=30). Patients received concurrent chemotherapy, which consisted of 5 weekly courses of paclitaxel (50 mg/m2) and carboplatin (area under the curve=

2). One patient received a neo-adjuvant chemotherapy scheme consisting of 3 courses of epirubicin, cisplatin, capecitabine (ECC).

***Surgical procedure***

The patients were operated by two experienced surgeons at our center. All patients underwent a standard radical resection through a transthoracic approach en-bloc with an extended 2-field nodal dissection, as described in detail in a previous study of our group 21. These nodes were located in the mediastinum and the abdomen, including the nodes at the celiac trunk, along the common hepatic artery and a.lienalis at the upper border of the pancreas and the proximal para-aortic regional nodes.

***Lymph node examination***

All identified lymph nodes, which were obtained from the surgical specimen by the standard pathology procedures, were embedded in paraffin blocks and evaluated microscopically by routine H&E staining. For the purpose of this study, all lymph nodes in group 1 and group 2 were reconfirmed as pN0 by an experienced pathologist (HH).

***Reassessment of lymph nodes***

Reassessment of lymph nodes was performed according to a sentinel lymph node sectioning protocol 22. Each lymph node was sectioned at four different levels at a distance of 100μm. After H&E staining was performed and showed to be negative, immunohistochemical (IHC) staining was carried out using anti-CK8/18 (CAM 5.2) to detect NMM. CAM 5.2 is a monoclonal IgG2 antibody reacting against keratins 8/18 present in most adenocarcinomas 23. NMM are considered as tumor deposits ≤ 0.2 mm and > 0.2 mm. The slides were blindly evaluated independently by two researchers. In case of disagreement a third judgment by an experienced pathologist was decisive.

***Pathologic response assessment***

Pathologic response was classified according to the 5-tier, so called Mandard criteria and divided into three subcategories: complete response ([CR], Mandard 1), partial response ([PR], Mandard 2-3) and hardly any response or non-response ([NR], Mandard 4-5) 24.

***Follow-up***

Patients were seen for regular follow up according to national guidelines at 4 to 8 weeks after completion of treatment, every 3 months in the first year, every 4-6 months in the second and third year and annually up to 5 years or until death. This follow-up regimen remained unchanged during the study period. Further radiological investigations were performed based on clinical suspicion of recurrent disease. A recurrence site was defined as local (esophageal bed), regional (lymph nodes) or distant metastases.

***Statistics***

OS was defined as the time interval between the starting date of the neoadjuvant chemoradiotherapy or surgery and documentation of the day of death or last follow-up. DFS, locoregional recurrence-free survival (LRFS) and distant recurrence-free survival (DRFS) were determined from the starting date of treatment to documented date of first recurrence, last follow-up or death of any cause

Categorical data were assessed using Pearson's Chi Square test. Continuous data Mann-Whitney U test. The DFS and OS were calculated according to the Kaplan-Meier method and compared with the Log-Rank test. P-values <0.05 were considered as statistically significant. All data were collected and analyzed using Statistical Package for Social Sciences (SPSS) version 18.0 (Chicago, IL, USA).

## Results

***Patient characteristics between the two groups***

Patient characteristics were equally distributed, except for age for which the groups were not matched (table 1). Patients in the neoadjuvant group (group 1) were significantly younger than those treated with surgery alone (group 2: P=0.019). The tumors were mainly located in the distal part of the esophagus (90% in both groups 1 and 2) and mainly staged as cT3 (80% and 60%, respectively) at time of surgery. None of the patients had distant metastases at the start of their treatment and microscopic radicality (R0) was achieved in all patients. The clinicopathological characteristics of the ypN1 group are displayed in table 2. The post-operative mortality was 0% in all three groups (group 1, group 2 and ypN1).

Table 1: Clinicopathological characteristics of the patients with adenocarcinoma of the esophagus in preoperative treatment and surgery alone group.

**Table 1: Clinicopathological characteristics of the patients with adenocarcinoma of the esophagus in preoperative treatment and surgery alone group.**

| **Characteristic** | neoadjuvant (n=20) group | Surgery (n=20) alone group | P value |
|---|---|---|---|
| Gender | | | |
| Male / Female | 14 / 6 | 15 / 5 | NS |
| | | | |
| Age | | | |
| Mean (years) | 60.3 | 68.2 | 0.019 |
| | | | |
| Localization | | | |
| *Mid/upper* | 0% | 0% | NS |
| *Distal (Siewert I)* | 90% (n=18) | 90% (n=18) | |
| *GEJ (Siewert II)* | 10% (n=2) | 10% (n=2) | |
| | | | |
| Subtype adenocarcinoma | | | |
| *Intestinal and/or barret** | 85% (n=17) | 95% (n=19) | NS |
| *Non-intestinal and/or diffuse type (Singlet cell)$* | 15% (n=3) | 5% (n=1) | |
| | | | |
| cT-stage | | | |
| *T1* | 0% | 10% (n=2) | NS |
| *T2* | 20% (n=4) | 30% (n=6) | |
| *T3* | 80% (n=16) | 60% (n=12) | |
| *T4* | 0% | 0% | |
| | | | |
| cN1-stage | 65% (n=13) | 30% (n=6) | 0.027 |
| | | | |
| pN+-stage | 0% | 0% | NS |
| | | | |
| cM1-stage | 0% | 0% | NS |
| | | | |
| Pathologic response | | | |
| *Non response* | 20% (n=4) | - | |
| *Partial response* | 35% (n=7) | - | |
| *Complete response* | 45% (n=9) | - | |

Abbreviations: NS= not significant, GEJ= Gastroesophageal junction

*: Arising from either barret or characterized as intestinal type adenocarcinoma

$: Characterized as either singlet cell or non-intestinal (diffuse) type adenocarcinoma

**Table 2: Clinicopathological characteristics of patients in the control group of ypN1 patients. Abbreviations: GEJ= Gastro-esophageal junction**

| **Characteristic** | ypN1 (n=11) |
|---|---|
| Gender | |
| Male / Female | 10 / 1 |
| | |
| Age | |
| Mean (years) | 63 (38-74) |
| | |
| Localization | |
| *Mid/upper* | 0% |
| *Distal* | 82% (n=9) |
| *GEJ* | 18% (n=2) |
| | |
| cT-stage | |
| *T1* | 0% |
| *T2* | 9% (n=1) |
| *T3* | 73% (n=8) |
| *T4* | 18% (n=2) |
| | |
| cN1-stage | 64% (n=7) |
| | |
| Pathologic response | |
| *Non response* | 46% (n=5) |
| *Partial response* | 54% (n=6) |
| *Complete response* | 0% |

***Response rate***

In the neoadjuvant group 9 patients (9/20; 45%) had a CR, 35% (n=7) a PR and 20% (n=4) a NR.

***Reduction of nodal micrometastases after neoadjuvant treatment***

All resected lymph nodes were histologically evaluated. The total number of evaluated lymph nodes was 533; 251 in group 2 with surgery alone and 282 in group 1 with neo-adjuvant treatment. The median number of resected nodes in group 2 was 13 (5-20) versus 15 (4-20) (P=0.527) in group 1. The median resected lymph nodes in the ypN1 group (n=11) was 12 (5-30), which was comparable with groups 1 and 2 (P=0.632)

Consequently, more patients in the surgery alone group were upstaged due to positive NMM as compared to the neoadjuvant group; 40% (8 out of 20 patients) versus 10% (2 out of 20 patients), respectively (P=0.028, figure 1).

There was a trend (P=0.050) towards less NMM+ positive nodes in the neoadjuvant group (3 out of 282; 1%) compared with the surgery alone group (9 out of 251; 3.6%).
Interestingly, when the data were analyzed more carefully (table 1), the reduction of NMM+ in the neoadjuvant group is even greater since this group contains more cN1 positive (pre-treatment nodal staging) tumors compared to the surgery alone group (65% versus 30%, P=0.027)

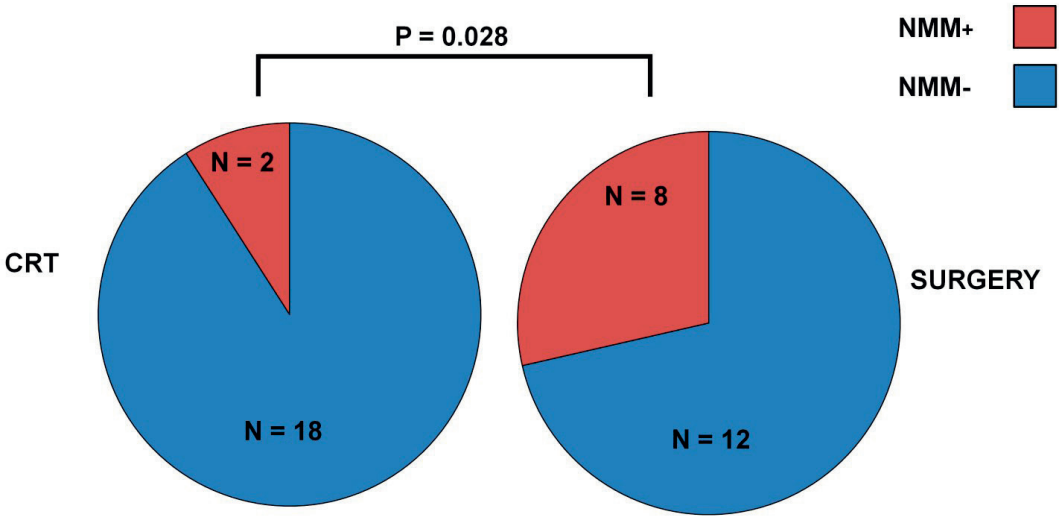


**Figure 1: Lower incidence of nodal micrometastasis (NMM) in (y)pN0 patients after neoadjuvant treatment (CRT group; n=20) compared to pN0 in surgery alone (n=20) group.**

The number of patients with NMM was significantly lower in the CRT group (n=2) compared to the surgery alone group (n=8). P-value = 0.028.

***Localization of positive NMM in the neoadjuvant group***

All three NMM positive nodes in the two upstaged patients were located in the radiation planning-field (para-esophageal region). Interestingly, both patients responded well to neoadjuvant treatment with a classification of pathologic CR (Mandard 1) and pathologic PR respectively.

***Effect of nodal micrometastases on prognosis***

In the neoadjuvant group, the 2 NMM positive patients clearly showed a worse median DFS (figure 2a) compared to the 18 NMM negative patients (P=0.013), with 8 months in NMM+ patients and not yet reached in NMM- patients.

The median OS was also significantly lower in NMM+ patients compared to NMM- patients (P=0.001), with 14 months in NMM+ patients and not yet reached in NMM- patients (figure 2b). Furthermore, we analyzed the survival in a group of ypN1 patients (n=11) as a control because of the relatively small number of NMM+ patients (n=2). As shown in figure 2c and figure 2d, the median DFS and OS of the ypN1 patients were comparable with the NMM+ patients, with 13 months and 24 months respectively (P=0.014 and P=0.003). The DFS and OS did not differ between the neodjuvant and surgery group (P=0.269 and P=0.388). NMM positivity did not have an effect on the OS (P=0.812) in the surgery alone group, but did show a difference towards worse DFS for NMM+, which did also not reach statistical significance (P=0.160).

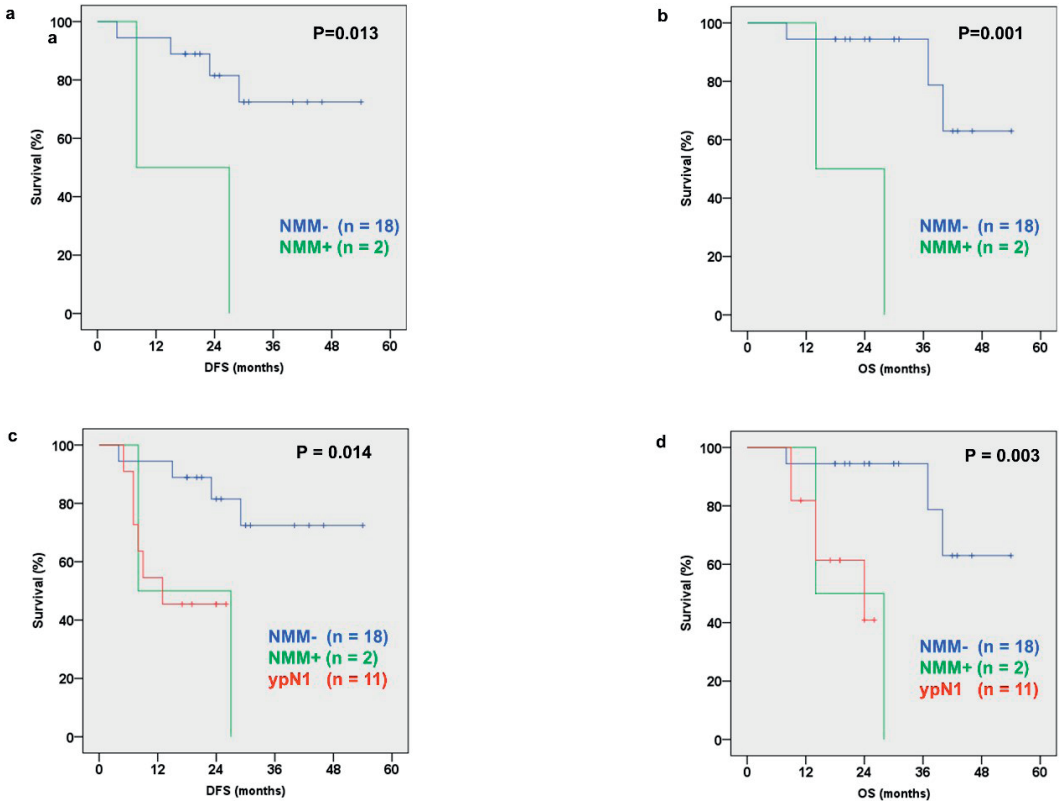


**Figure 2: Survival in NMM+ patients (n=2) compared to NMM- patients (n=18) in the neoadjuvant group. Survival of the NMM+ patients compare to ypN1 patients (n=11).**

A. Disease-free survival (DFS) was significantly worse in NMM+ patients compared to NMM- patients (P=0.013).

B. Overall survival (OS) was significantly more poor in NMM+ patients compared to NMM- patients (P=0.001).

C. Disease-free survival (DFS) was significantly worse in NMM+ patients and ypN1 compared to NMM negative patients (P=0.014).

D. Overall survival (OS) was significantly worse in NMM+ patients and ypN1 compared to NMM negative patients (P=0.003).





513774-L-bw-wang
Processed on: 19-9-2017

## Discussion

Currently, neoadjuvant chemoradiotherapy followed by surgical resection is considered as standard of care for resectable tumors of the esophagus in stage II-III patients 12-16. This is strengthened by the favorable results for the CRT-arm in a recent Dutch RCT (CROSS-study) 25. It is postulated that the favorable results for CRT in the neoadjuvant setting, are achieved by tumor downstaging and downsizing, resulting in higher microscopic radical resection rates (R0 resections) 25.

Another postulated effect of pre-operative treatment is a reduction of micrometastatic disease explaining the smaller burden of disease observed during follow up of these patients 13-16. Even though there is evidence showing reduction in micrometastatic disease after pre-operative CRT in patients who achieved a complete response to the CRT, no data have been published about the exact reduction of micrometastatic disease in esophageal cancer patients 17. Therefore, the present study adds important information to the effect of neoadjuvant treatment on the prevalence and clinical relevance of NMM. Moreover, in the neoadjuvant group we observed a reduction in upstaging (from ypN0 to ypNMM+) of 30% compared to the surgery alone group. In absolute numbers we also observed a trend towards a reduction in NMM+ lymph nodes in the neoadjuvant group compared to the surgery alone group. This reduction was even more apparent when considering the fact that in the pre-treatment phase patients in the neoadjuvant group had significantly more cN1 tumors (65%) compared to surgery alone patients. There are two aspects, which could potentially have an impact on clinical decision-making. One is that even after responses to neoadjuvant treatment an extended nodal dissection should not be omitted, based on the presence of NMM even in these patients. Moreover the two patients (n=2) in the neoadjuvant group with NMM+ were both responders to the pre-operative CRT. Indeed one of these patients had a complete response (Mandard 1). As the NMM positive lymph nodes were located in the para-esophageal region and therefore within the irradiation field. Secondly the information of this study may have an impact on future adjuvant trials for a more appropriate stratification based on NMM disease. Even after routine pathology evaluations, we should be aware of the presence of NMM and the potential impact on outcome. Excluding NMM at least immunohistochemically may increase the rigor of determining cases either true node positive or node negative. Our results should also encourage us and other study groups to validate future data in prospective analyses regarding its true clinical relevance.

Although the Kaplan-Meier survival estimation of the analyzed neoadjuvant group is relatively small (n=20), the two NMM+ patients had a significantly worse DFS and OS compared to the NMM negative (n=18) patients. Furthermore, we performed an analysis on a control group of 11 ypN1 patients, and found that the DFS and OS were comparable to NMM+ patients. This is in line with previous studies that showed a worse prognosis when NMMs were present 7-10. Additionally, even though the surgery alone group also represents a relatively small sample size of 20 pN0 patients, the upstaging expressed by the rate of 40% in NMM positive patients was in line with a previous study at our institute by Heeren et al. 8 consisting of 60 pN0 patients, where the upstaging rate was 30%. Heeren et al. used exactly the same antibody (anti- CAM 5.2, DAKO, Carpinteria, CA, USA) in their study as described in the present study.

A limitation of the current study is the relatively small size of 51 patients, which may induce a form of sample bias, which possibly reduces the impact of the present study. To reduce this type of bias, we carefully matched the neoadjuvant group (n=20) with a control group of 20 patients (surgery alone). All patients had adenocarcinoma of the distal/GEJ esophagus and were (y)pN0 after routine pathologic evaluation. We also used the cT status in the matching procedure as it is known that there is a strong correlation between increasing depth of primary tumor invasion and the presence of nodal disease, even in submucosal disease 2, 6, 21, 26, 27. Furthermore, we included a control group of 11 ypN1 patients in the survival analyses in the neoadjuvant group according to NMM status.
Response to neoadjuvant treatment, specifically pCR, strongly predicts an increased survival and is one of the key criteria to evaluate the success of the given treatment 28-30. In the present study, the given pre-operative treatment regimens provided good responses, with 35% (n=7) PR rate and 45% (n=8) CR rate.

In conclusion, a 30% reduction of NMM positivity was obtained after neoadjuvant treatment in ypN0 patients. NMM+ after CRT had an equal negative impact on DFS and OS as in ypN1 patients. Based on the presence of NMM (10%) after neoadjuvant CRT, within the irradiation field, we still advocate a standard nodal dissection even in patients with good responses. Furthermore, the data from this study warrants caution when considering patients ypN0 after routine pathological examination with H&E staining and the data should also be reconfirmed preferably in a larger prospective dataset.

## Conflict of interest statement

None.

## Acknowledgments

Supported by: GUIDE post-graduate school of Medicine, University of Groningen. The funding source had no role in this study.

We would like to thank Peter W.K. Nagle MSc for correcting English grammar.

## References


[1] Hsu WH, Hsu PK, Hsieh CC, et al. The metastatic lymph node number and ratio are independent prognostic factors in esophageal cancer. J Gastrointest Surg 2009;13:1913-1920.

[2] Mariette C, Piessen G, Briez N, et al. The number of metastatic lymph nodes and the ratio between metastatic and examined lymph nodes are independent prognostic factors in esophageal cancer regardless of neoadjuvant chemoradiation or lymphadenectomy extent. Ann Surg 2008;247:365-371.

[3] Altorki NK, Zhou XK, Stiles B, et al. Total number of resected lymph nodes predicts survival in esophageal cancer. Ann Surg 2008;248:221-226.

[4] Smit JK, Pultrum BB, van Dullemen HM, et al. Prognostic factors and patterns of recurrence in esophageal cancer assert arguments for extended two-field transthoracic esophagectomy. Am J Surg 2010;200:446-453.

[5] Hagen JA, DeMeester SR, Peters JH, et al. Curative resection for esophageal adenocarcinoma: analysis of 100 en bloc esophagectomies. Ann Surg 2001;234:520-30; discussion 530-1.

[6] Lerut T, Nafteux P, Moons J, et al. Three-field lymphadenectomy for carcinoma of the esophagus and gastroesophageal junction in 174 R0 resections: impact on staging, disease-free survival, and outcome: a plea for adaptation of TNM classification in upper-half esophageal carcinoma. Ann Surg 2004;240:962-72; discussion 972-4.

[7] Zingg U, Montani M, Busch M, et al. Prognostic influence of immunohistochemically detected lymph node micrometastasis and histological subtype in pN0 oesophageal cancer. Eur J Surg Oncol 2009;35:593-599.

[8] Heeren PA, Kelder W, Blondeel I, et al. Prognostic value of nodal micrometastases in patients with cancer of the gastro-oesophageal junction. Eur J Surg Oncol 2005;31:270-276.

[9] Prenzel KL, Holscher AH, Drebber U, et al. Prognostic impact of nodal micrometastasis in early esophageal cancer. Eur J Surg Oncol 2012;38:314-318.

[10] Thompson SK, Ruszkiewicz AR, Jamieson GG, et al. Isolated tumor cells in esophageal cancer: implications for the surgeon and the pathologist. Ann Surg 2010;252:299-306.

[11] de Boer M, van Deurzen CH, van Dijck JA, et al. Micrometastases or isolated tumor cells and the outcome of breast cancer. N Engl J Med 2009;361:653-663.

[12] 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.

[13] Sjoquist KM, Burmeister BH, Smithers BM, et al. Survival after neoadjuvant chemotherapy or chemoradiotherapy for resectable oesophageal carcinoma: an updated meta-analysis. Lancet Oncol 2011;12:681-692.

[14] Allum WH, Stenning SP, Bancewicz J, et al. Long-term results of a randomized trial of surgery with or without preoperative chemotherapy in esophageal cancer. J Clin Oncol 2009;27:5062-5067.

[15] Burmeister BH, Smithers BM, Gebski V, et al. Surgery alone versus chemoradiotherapy followed by surgery for resectable cancer of the oesophagus: a randomised controlled phase III trial. Lancet Oncol 2005;6:659-668.

[16] Cunningham D, Allum WH, Stenning SP, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006;355:11-20.

[17] Prenzel KL, Konig A, Schneider PM, et al. Reduced incidence of nodal micrometastasis after major response to neoadjuvant chemoradiation in locally advanced esophageal cancer. Ann Surg Oncol 2007;14:954-959.

[18] Kole AC, Plukker JT, Nieweg OE, et al. Positron emission tomography for staging of oesophageal and gastroesophageal malignancy. Br J Cancer 1998;78:521-527.

[19] Rice TW, Blackstone EH, Rusch VW. 7th edition of the AJCC Cancer Staging Manual: esophagus and esophagogastric junction. Ann Surg Oncol 2010;17:1721-1724.

[20] Sobin LH. TNM, sixth edition: new developments in general concepts and rules. Semin Surg Oncol 2003;21:19-22.

[21] Smit JK, Pultrum BB, van Dullemen HM, et al. Prognostic factors and patterns of recurrence in esophageal cancer assert arguments for extended two-field transthoracic esophagectomy. Am J Surg ;200:446-53.

[22] Weaver DL. Pathology evaluation of sentinel lymph nodes in breast cancer: protocol recommendations and rationale. Mod Pathol 2010;23 Suppl 2:S26-32.

[23] Makin CA, Bobrow LG, Bodmer WF. Monoclonal antibody to cytokeratin for use in routine histopathology. J Clin Pathol 1984;37:975-983.

[24] Mandard AM, Dalibard F, Mandard JC, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. Cancer 1994;73:2680-2686.

[25] van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-2084.

[26] Ancona E, Rampado S, Cassaro M, et al. Prediction of lymph node status in superficial esophageal carcinoma. Ann Surg Oncol 2008;15:3278-3288.

[27] Holscher AH, Bollschweiler E, Schroder W, et al. Prognostic impact of upper, middle, and lower third mucosal or submucosal infiltration in early esophageal cancer. Ann Surg 2011;254:802-7; discussion 807-8.

[28] Berger AC, Farma J, Scott WJ, et al. Complete response to neoadjuvant chemoradiotherapy in esophageal carcinoma is associated with significantly improved survival. J Clin Oncol 2005;23:4330-4337.

[29] Donahue JM, Nichols FC, Li Z, et al. Complete pathologic response after neoadjuvant chemoradiotherapy for esophageal cancer is associated with enhanced survival. Ann Thorac Surg 2009;87:392-8; discussion 398-9.

[30] Schneider PM, Baldus SE, Metzger R, et al. Histomorphologic tumor regression and lymph node metastases determine prognosis following neoadjuvant radiochemotherapy for esophageal cancer: implications for response classification. Ann Surg 2005;242:684-692.