Clinical Science

Neoadjuvant therapy reduces the incidence of nodal micrometastases in esophageal adenocarcinoma

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KEYWORDS: Esophageal cancer; Nodal micrometastases; Neoadjuvant treatment

Abstract

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

METHODS: 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 3rd group (n = 11) staged as ypN1 after neoadjuvant CRT was used as the control group.

RESULTS: Upstaging to NMM+ occurred in 2 patients (10%) in group 1 and in 8 patients (40%) in group 2 (P = .028). Disease-free and overall survival rates in NMM+ patients in group 1 were worse compared with NMM- patients (P = .014 and P = .003, respectively) but comparable with ypN1 patients (n = 11).

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

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Despite recent advances in cancer treatment, patients with esophageal cancer (EC) still have a relatively poor prognosis. More than 80% of 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), the total number of resected lymph nodes, the number of tumor-positive nodes, and the 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 adequate nodal resection is subjected to the strong prognostic value of the number of resected nodes on the outcome.³ As shown in some studies, extended 2-field nodal resection should be performed, usually through a transthoracic route.⁵,⁶ However, even patients with histologically node-negative (pN0) status will develop (early) locoregional recurrences, which can be explained by the presence of nodal...
micrometastases (NMMs). These NMMs are not detected by routine hematoxylin-eosin (H&E) methods but usually immunohistochemically with antibodies against cytokeratins specific for epithelial tissue. Patients with pN0 tumors and NMM have a significantly worse survival rate than those without NMM. These NMMs can be divided into isolated tumor cells (ITCs) and micrometastases (MMs). MM have a worse effect on survival than ITCs.

Neoadjuvant treatment is currently the standard of care in experienced centers. The rationale behind this is that tumor downstaging/sizing and the elimination of NMM leads to improved resectability and curability rates. Previous research has shown that the response to neoadjuvant CRT reduces NMM in esophageal cancer. Unfortunately, these studies are scarce, and little has been published regarding the rate of CRT on NMMs in pN0 esophageal cancer patients. Therefore, we evaluated the effect of CRT on NMM in ypN0 esophageal cancer patients after neoadjuvant treatment compared with pN0 in the surgery-alone group.

Patients and Methods

Patients

A total of 380 patients with histologically proven esophageal cancer were identified in the prospective database of our tertiary referral medical center. All patients had surgical resection with curative intent. From 2005 onward, patients were treated with neoadjuvant CRT in a randomized controlled trial or as standard procedure. The preoperative diagnostic workup, the surgical procedure and/or surgical team, and follow-up basically 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. Archival tissue was handled according to the Dutch Code for the proper use of Human Tissue.

Matching and construction of surgery-alone and ypN1 groups

From our prospective database, 1 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 3rd group that 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 included 51 patients in this study.

Staging procedure

The diagnostic staging procedure consisted of endoscopic ultrasonography with fine-needle aspiration of suspected lymph nodes; 16 to 64 multidetector computed tomographic scans of the neck, chest, and abdomen; and cervical echographic examination. In case of T2 to T4a tumors or involved regional lymph nodes (N+), 18-F-fluorodeoxyglucose positron-emission tomographic scanning was performed to exclude distant disease. After staging in accordance with the Union for International Cancer Control TNM 7th edition, all patients were discussed by a multidisciplinary tumor board for adequate treatment planning.

Preoperative treatment

The neoadjuvant CRT regimen consisted of radiotherapy with a total dose of 41.4 to 45 Gy in daily fractions of 1.8 Gy 5 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 neoadjuvant chemotherapy scheme consisting of 3 courses of epirubicin, cisplatin, and capecitabine.

Surgical procedure

Two experienced surgeons at our center performed the operations. All patients underwent 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. These nodes were located in the mediastinum and the abdomen, including the nodes at the celiac trunk, the nodes along the common hepatic artery and the arteria lienalis at the upper border of the pancreases, and the proximal para-aortic regional nodes.

Lymph node examination

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

Reassessment of lymph nodes

The reassessment of lymph nodes was performed according to a sentinel lymph node sectioning protocol.
lymph node was sectioned at 4 different levels at a distance of 100 µm. After H&E staining was performed and shown to be negative, immunohistochemical staining was performed using anti-CK8/18 (CAM 5.2) to detect NMMs. CAM 5.2 is a monoclonal immunoglobulin G2 antibody reacting against keratins 8/18 present in most ACs. NMMs with deposits less than or equal to .2 mm and greater than .2 mm to less than or equal to 2 mm were considered as ITCs and MMs, respectively. The slides were blindly evaluated independently by 2 researchers. In case of disagreement, a 3rd judgment by an experienced pathologist was decisive.

Pathologic response assessment

The pathologic response was classified according to the 5-tie, so-called Mandard criteria and divided into 3 subcategories: complete response ([CR], Mandard 1), partial response ([PR], Mandard 2–3), and hardly any response or nonresponse (Mandard 4–5).

Follow-up

Patients were seen for regular follow-up according to national guidelines at 4 to 8 weeks after the completion of treatment, every 3 months in the first year, every 4 to 6 months in the 2nd and 3rd year, and annually up to 5 years or until death. This follow-up regimen remained unchanged during the study period. Further radiologic investigations were performed based on a 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 CRT or surgery and the documentation of the day of death or the last follow-up. DFS, locoregional recurrence-free survival, and distant recurrence-free survival were determined from the starting date of treatment to the documented date of the first recurrence, the last follow-up, or death of any cause.

Categoric data were assessed using the Pearson chi-square test and continuous data using the Mann-Whitney U test. DFS and OS were calculated according to the Kaplan-Meier method and compared with the log-rank test. P values less than .05 were considered to be statistically significant. All data were collected and analyzed using Statistical Package for Social Sciences version 18.0 (SPSS Inc, Chicago, IL).

Results

Patient characteristics between the 2 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: \( P = .019 \)). 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 the 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 clinicopathologic characteristics of the ypN1 group are displayed in Table 2. Postoperative mortality was 0% in all 3 groups (group 1, group 2, and ypN1).

Response rate

In the neoadjuvant group, 9 patients (9/20 [45%]) showed a CR, 35% (n = 7) a PR, and 20% (n = 4) a nonresponse.

Reduction of NMMs 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 neoadjuvant treatment. The median number of resected nodes in group 2 was 13 (5 to 20) vs 15 (4 to 20) (\( P = .527 \)) in group 1. The median resected lymph nodes in the ypN1 group (n = 11) was 12 (5 to 30), which was comparable with groups 1 and 2 (\( P = .632 \)).

Consequently, more patients in the surgery-alone group were upstaged because of positive NMM compared with the neoadjuvant group (40% [8/20 patients] vs 10% [2/20 patients], respectively [\( P = .028 \)], Fig. 1). Fig. 2A displays an NMM-positive node in a patient in the neoadjuvant group. There was a trend (\( P = .050 \)) toward less NMM+ positive nodes in the neoadjuvant group (3/282 [1%]) compared with the surgery-alone group (9/251 [3.6%]).

Interestingly, when the data were analyzed more carefully (Table 1), the reduction of NMM+ in the neoadjuvant group is even greater because this group contains more cN1-positive (pretreatment nodal staging) tumors compared with the surgery-alone group (65% vs 30%, \( P = .027 \)).

Localization of positive NMM in the neoadjuvant group

All 3 NMM-positive nodes in the 2 upstaged patients were located in the radiation planning field (paraesophageal region). Interestingly, both patients responded well to neoadjuvant treatment with a classification of pathologic CR (Mandard 1) and pathologic PR, respectively.

Effect of nodal MMs on prognosis

In the neoadjuvant group, the 2 NMM-positive patients clearly showed a worse median DFS (Fig. 2A) compared with the 18 NMM-negative patients (\( P = .013 \)), with 8
months in NMM+ patients and not yet reached in the NMM− patients.

The median OS was also significantly lower in NMM+ patients compared with NMM− patients (P = .001), with 14 months in NMM+ patients and not yet reached in the NMM− patients (Fig. 2B). Furthermore, we analyzed 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 Fig. 2C and D, the median DFS and OS of the ypN1 patients was comparable with the NMM+ patients, with 13 months and 24 months, respectively (P = .014 and P = .003). DFS and OS did not differ between the neoadjuvant and surgery groups (P = .269 and P = .388, respectively). NMM positivity did not have an effect on OS (P = .812) in the surgery-alone group but did show a difference toward worse DFS for NMM+, which did also not reach statistical significance (P = .160).

Table 1  Clinicopathologic characteristics of patients with AC of the esophagus in preoperative treatment and the surgery-alone group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Neoadjuvant group (n = 20)</th>
<th>Surgery-alone group (n = 20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>14/6</td>
<td>15/5</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>.019</td>
</tr>
<tr>
<td>Mean (y)</td>
<td>60.3</td>
<td>68.2</td>
<td></td>
</tr>
<tr>
<td>Localization (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid/upper</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Distal (Siewert I)</td>
<td>90 (n = 18)</td>
<td>90 (n = 18)</td>
<td></td>
</tr>
<tr>
<td>GEJ (Siewert II)</td>
<td>10 (n = 2)</td>
<td>10 (n = 2)</td>
<td></td>
</tr>
<tr>
<td>Subtype AC (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal and/or Barrett*</td>
<td>85 (n = 17)</td>
<td>95 (n = 19)</td>
<td>NS</td>
</tr>
<tr>
<td>Nonintestinal and/or diffuse type (signet cell)†</td>
<td>15 (n = 3)</td>
<td>5 (n = 1)</td>
<td></td>
</tr>
<tr>
<td>cT stage (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>10 (n = 2)</td>
<td>NS</td>
</tr>
<tr>
<td>T2</td>
<td>20 (n = 4)</td>
<td>30 (n = 6)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>80 (n = 16)</td>
<td>60 (n = 12)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>cN1 stage (%)</td>
<td>65 (n = 13)</td>
<td>30 (n = 6)</td>
<td>.027</td>
</tr>
<tr>
<td>pN+ stage (%)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>cM1 stage (%)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Pathologic response (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponse</td>
<td>20 (n = 4)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>35 (n = 7)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>45 (n = 9)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

AC = adenocarcinoma; CR = complete response; GEJ = gastroesophageal junction; NS = not significant; PR = partial response.
*Arising from either Barrett or characterized as intestinal type AC.
†Characterized as either singlet cell- or nonintestinal (diffuse)-type AC.

Table 2  Clinicopathologic characteristics of patients in the control group of ypN1 patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ypN1 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>10/1</td>
</tr>
<tr>
<td>Age</td>
<td>63 (38–74)</td>
</tr>
<tr>
<td>Localization (%)</td>
<td></td>
</tr>
<tr>
<td>Mid/upper</td>
<td>0</td>
</tr>
<tr>
<td>Distal</td>
<td>82 (n = 9)</td>
</tr>
<tr>
<td>GEJ</td>
<td>18 (n = 2)</td>
</tr>
<tr>
<td>cT stage (%)</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>9 (n = 1)</td>
</tr>
<tr>
<td>T3</td>
<td>73 (n = 8)</td>
</tr>
<tr>
<td>T4</td>
<td>18 (n = 2)</td>
</tr>
<tr>
<td>cN1 stage</td>
<td>64 (n = 7)</td>
</tr>
<tr>
<td>Pathologic response (%)</td>
<td></td>
</tr>
<tr>
<td>Nonresponse</td>
<td>46 (n = 5)</td>
</tr>
<tr>
<td>PR</td>
<td>54 (n = 6)</td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
</tr>
</tbody>
</table>

CR = complete response; GEJ = gastroesophageal junction; PR = partial response.

Comments

Currently, neoadjuvant CRT followed by surgical resection is considered the standard of care for resectable tumors of the esophagus in stage II to III patients. This is strengthened by the favorable results for the CRT arm in a recent Dutch randomized controlled trial (CROSS study). 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).
Another postulated effect of preoperative treatment is a reduction of micrometastatic disease explaining the smaller burden of disease observed during the follow-up of these patients.\textsuperscript{13–16} Even though there is evidence showing a reduction in micrometastatic disease after preoperative CRT in patients who achieved a CR to the CRT, no data have been published about the exact reduction of micrometastatic disease in esophageal cancer patients.\textsuperscript{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\textsuperscript{+}) of 30\% compared with the surgery-alone group. In absolute numbers, we also observed a trend toward a reduction in NMM\textsuperscript{+} lymph nodes in the neoadjuvant group compared with the surgery-alone group. This reduction was even more apparent when considering the fact that in the pretreatment phase patients in the neoadjuvant group had significantly more cN1 tumors (65\%) compared with surgery-alone patients. There are 2 aspects that 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 2 patients (n = 2) in the neoadjuvant group with NMM\textsuperscript{+} were both responders to preoperative CRT. Indeed, 1 of these patients had a CR (Mandard 1).

![Figure 1](image1.png) A lower incidence of NMM in (y)pN0 patients after neoadjuvant treatment (CRT group, n = 20) compared with pN0 in the surgery-alone (n = 20) group. The number of patients with NMMs was significantly lower in the CRT group (n = 2) compared with the surgery-alone group (n = 8) (P = .028).

![Figure 2](image2.png) Survival in NMM\textsuperscript{+} patients (n = 2) compared with NMM\textsuperscript{−} patients (n = 18) in the neoadjuvant group. Survival of the NMM\textsuperscript{+} patients compared with ypN1 patients (n = 11). (A) DFS was significantly worse in NMM\textsuperscript{+} patients compared with NMM\textsuperscript{−} patients (P = .013). (B) OS was significantly more poor in NMM\textsuperscript{+} patients compared with NMM\textsuperscript{−} patients (P = .001). (C) DFS was significantly worse in NMM\textsuperscript{+} patients and ypN1 compared with NMM\textsuperscript{−} patients (P = .014). (D) OS was significantly worse in NMM\textsuperscript{+} patients and ypN1 compared with NMM\textsuperscript{−} patients (P = .003).
NMM-positive lymph nodes were located in the paraesophageal region and, therefore, within the irradiation field. Second, 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 pathologic evaluations, we should be aware of the presence of NMM and the potential impact on the outcome. Excluding NMM at least immunohistochemically may increase the rigor of determining cases as 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 2 NMM+ patients had significantly worse DFS and OS compared with the NMM-negative (n = 18) patients. Furthermore, we performed analysis on a control group of 11 ypN1 patients and found that DFS and OS were comparable with NMM+ patients. This is in line with previous studies that showed a worse prognosis when NMMs were present. 

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., consisting of 60 pN0 patients in which the upstaging rate was 30%. Heeren et al used exactly the same antibody (anti-CAM 5.2; DAKO, Carpinteria, CA) 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 that 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 AC of the distal/gastroesophageal junction esophagus and were ypN0 after routine pathologic evaluation. We also used the cT status in the matching procedure because it is known that there is a strong correlation between increasing the depth of primary tumor invasion and the presence of nodal disease, even in submucosal disease. Furthermore, we included a control group of 11 ypN1 patients in the survival analyses in the neoadjuvant group according to NMM status.

The response to neoadjuvant treatment, specifically pathologic Complete Response (pCR), strongly predicts a worse prognosis when NMMs were present. Heeren et al used exactly the same antibody (anti-CAM 5.2; DAKO, Carpinteria, CA) in their study as described in the present study. 

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 warrant caution when considering patients ypN0 after routine pathologic examination with H&E staining, and the data should also be reconfirmed, preferably in a larger prospective datasets.

Acknowledgments

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References


