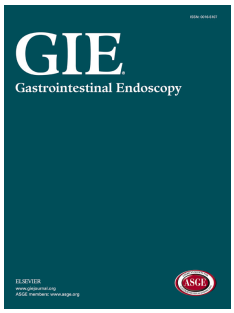

Analysis of metastases rates during follow-up after endoscopic resection of early "high-risk" esophageal adenocarcinoma

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Analysis of metastases rates during follow-up after endoscopic resection of early "high-risk" esophageal adenocarcinoma

Short title: metastases in early esophageal adenocarcinoma

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Conflicts of interest

EN, SvM, SM, LB, MJ, LAH, AA, BS, ES, WC, AK, SV, EV, WN, JW, MH, TT, RP declared to have no disclosures relevant to this manuscript. BW received financial support for IRB-approved research from C2Therapeutics/Pentax Medical and Aqua Medical. JB received financial support for IRB-approved research from C2Therapeutics/Pentax Medical, Medtronic, and Aqua Medical.

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Contributors

EN, SM did data acquisition. EN, SM, JB, and RP coordinated study and did data collection. EN, SM, RP and JB did the statistical analysis. SM, JB, RP contributed to data analysis and interpretation of data in research group meetings. SM, LB, MJ revised histopathology specimens. EN drafted the manuscript. SM, JB, RP co-authored the manuscript. EN, SvM, SM, LB, MJ, BW, LAH, AA, BS, ES, WC, AK, SV, EV, WN, JW, MH, TT, JB, RP participated in annual meetings and were responsible for treatment of patients in their center. All authors critically edited, read, and approved the final manuscript.

RP affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; no important aspects of the study have been omitted; there were no discrepancies from the study as originally planned.

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Abstract

Background and Aims

After endoscopic resection (ER) of early esophageal adenocarcinoma (EAC), the optimal management of patients with high-risk histological features for lymph node metastases (LNM) (i.e., submucosal invasion, poor differentiation grade, or lymphovascular invasion (LVI)), remains unclear. We aimed to evaluate outcomes of endoscopic follow-up after ER for high-risk EAC.

Methods

For this retrospective cohort study, data was collected from all Dutch patients managed with endoscopic follow-up (endoscopy, endoscopic ultrasound) after ER for high-risk EAC between 2008 and 2019. We distinguished 3 groups: intramucosal cancers with high-risk features, submucosal cancers with low-risk features, and submucosal cancers with high-risk features. Primary outcome was the annual risk for metastases during follow-up, stratified for baseline histology.

Results

A total of 120 patients met the selection criteria. Median FU was 29 months (IQR 15-48). Metastases were observed in 5/25 (annual risk 6.9%; 95% CI 3.0-15), 1/55 (annual risk 0.7%; 95% CI 0-4.0) and 3/40 (annual risk 3.0%; 95% CI 0-7.0) in high-risk intramucosal, low-risk submucosal, and high-risk submucosal cancers, respectively.

Conclusions

Whereas the annual metastasis rate for high-risk submucosal EAC (3.0%) was somewhat lower than expected in comparison with previous reported percentages, the annual metastasis rate of 6.9% for high-risk intramucosal EAC is new and worrisome. This calls for further prospective studies and suggests that strict follow-up of this small subgroup is warranted until prospective data are available.

Keywords: esophageal adenocarcinoma, histopathological risk factors, endoscopic therapy, metastases

Introduction

Endoscopic resection (ER) is established as first-choice treatment for early esophageal adenocarcinoma (EAC) without histopathological risk factors of lymph node metastases (LNM). Multiple studies have demonstrated excellent efficacy and safety of ER as an alternative to surgery for these lesions, also in long-term analyses(1–3). Nevertheless, after radical ER of a tumor with histopathological risk factors for LNM, optimal management is still unclear. These risk factors include submucosal invasion (T1b), poor tumor differentiation grade (G3), and lymphovascular invasion (LVI). Nowadays the indication for endoscopic therapy has extended to tumors invading into the superficial submucosa (<500 microns; sm1) with good to moderate differentiation grade and do not display LVI. For these sm1 tumors without high-risk features the risk of LNM is <2% (4,5) and strict endoscopic follow-up is an accepted alternative to esophagectomy (6,7). A small number of – mainly surgical – studies have assessed the LNM rates in patients with deep submucosal invasion (i.e., ≥500 microns; sm2/3), and/or poor differentiation grade, and/or LVI, reporting a wide range of LNM rates between 16 and 46% (5,8,9). Therefore, ER is considered insufficient treatment for these patients and surgery is still advised. However, these LNM rates are mainly based on historic surgical studies, in which the invasion depth and other risk features of tumors in the surgical specimen may have been less accurately reported compared to ER specimens. Since histologic assessment of surgical specimens is based on relatively large cuts of 5mm, invasion depth may have been underestimated if the deepest part of infiltration was not included in slides cut for histological assessment. Accurate assessment of histological risk factors was also less relevant, since the esophagectomy had already been performed and presence or absence of these risk factors would not influence

further management. A number of more recent endoscopy-based studies show a LNM risk for submucosal EAC with high-risk features of 0-37%, during median 23-63 months of follow-up, which is lower than reported in the surgical series, rendering an invasive esophagectomy possibly unnecessary in a subset of patients (5,10–12). Comparatively less is known about the risk of LNM for intramucosal EAC with high-risk features. This disparity drives heterogeneous clinical decision-making and patient management. An alternative to immediate adjuvant surgery may be to survey patients after ER of an EAC with high-risk features, and limit further treatment such as chemoradiotherapy and/or surgery to those patients with proven LNM during follow-up. This would require additional evidence about the long-term safety of this conservative strategy from prospective cohort studies.

Aim of this study was to assess the outcomes of patients who underwent radical ER for an EAC with high-risk histological features without metastases at baseline, who were followed up endoscopically.

Patients and methods

The study included patients from the Barrett Expert Center registry (BEC registry) (Netherlands Trial Register, NL7039), which has been described in detail earlier (13). In summary, this registry represents outcomes for all patients who underwent endoscopic treatment for Barrett neoplasia in the Netherlands from 2008 onwards. Dutch Barrett care is uniquely organized in nine BECs with treatment provided by jointly trained endoscopists and pathologists. The BECs adhere to a common endoscopic management protocol and gather several times a year to safeguard homogeneity. Furthermore, since every patient in the Netherlands receives treatment

in one of the BECs, data on treatment and outcomes of all patients treated for Barrett neoplasia are registered in this uniform, nationwide database. Patients diagnosed with EAC and histological risk factors after ER with negative deep resection margins were counseled for endoscopic management or surgery depending on age, comorbidity and preference following national guidelines (14,15). None of the included patients participated in the prospective PREFER study (NCT03222635). Our study partly overlaps with two earlier reports from our group (n=52) (5,11).

Study population

For this study, we included all patients who underwent endoscopic resection of an EAC with high-risk histological features, with tumor negative deep resection margins, between January 2008 and October 2019. We distinguished three histological subgroups:

- T1a EAC with high-risk features (T1a-HR) was defined as intramucosal adenocarcinoma, with poor differentiation grade (G3), and/or LVI.
- T1b EAC with low-risk features (T1b-LR) was defined as submucosal cancer with superficial invasion in the submucosa (<500 microns; sm1), well to moderately differentiated (G1-G2), without LVI.
- T1b EAC with high-risk features (T1b-HR) was defined as submucosal cancer with either deep invasion in the submucosa (≥500 microns; sm2/3), and/or poor differentiation grade (G3), and/or LVI presence.

Exclusion criteria were: i) Tumor positive deep resection margin (R1) ii) Residual lesion not amendable to re-ER at the first endoscopy after initial ER; iii) Metastases (LNM or distant metastases) diagnosed at baseline; iv) Referral for surgery or chemoradiotherapy directly after ER.

Histopathological evaluation

Histological evaluation of all ER specimens was performed by experienced BE pathologists. After tissue fixation, specimens were cut into 2-3mm strips, processed to paraffin blocks, cut into 4µm slides, and stained with hematoxylin and eosin (H&E) and for p53 expression. Hereafter, the four following histological features were assessed: 1. Tumor infiltration depth, with submucosal invasion measured in microns (i.e., <500 microns was sub classified as sm1; ≥500 microns as sm2/3). In the majority, immunohistochemistry using desmin and/or pankeratin staining was performed on a blank H&E slide with deepest submucosal tumor invasion; 2. Tumor differentiation grade(16); 3. Presence of LVI (including D2-40 staining in most cases); 4. Status of vertical resection margins and lateral resection margins in case of en-bloc resection. Three experienced Barrett pathologists independently revised histopathology of all included T1a cases to ensure that there was no submucosal invasion.

Baseline staging

The joint treatment protocol did not prescribe a standard procedure for baseline staging after ER. Generally, patients underwent endoscopy and endoscopic ultrasound (EUS) +/- 6 weeks after ER to assess presence of residual intraluminal neoplasia and locoregional lymph nodes. Lymph nodes that appeared suspicious as assessed by the treating physician were sampled using fine-needle aspiration (EUS-FNA). In addition, computed tomography (CT) scan of the thorax and abdomen, or a positron-emission tomography (PET)/CT scan was often performed, to evaluate for the presence of distant metastases.

Follow-up and re-treatment

Endoscopic follow-up was performed in the BEC and intervals were determined by the treating physician since no strict protocol was available. Follow-up consisted of 3-6 monthly endoscopy ± EUS and FNA in case of suspicious lymph nodes. To guarantee endoscopic imaging quality, most patients were sedated, and high-quality, high-definition endoscopes were used with virtual chromoendoscopy next to normal white light endoscopy. The Barrett segment was described using the Prague C&M classification(17). Targeted biopsies or direct endoscopic resection were/was performed in case any mucosal irregularity was detected. These irregularities were described using the Paris classification(18). In addition, random biopsies following the Seattle protocol were taken from the (remaining) flat Barrett segment. (PET-)CT-scans were performed in some cases during follow-up, at the discretion of the treating physician. Residual Barrett epithelium was generally kept under surveillance at least one year after ER because of the relatively higher LNM risk in the first 1-2 years after resection of a high-risk lesion. Hereafter, eradication treatment of the residual Barrett was initiated in most patients, per physician's discretion.

Endpoints

Primary endpoint:

Annual risk for metastases during endoscopic follow-up, stratified for baseline histopathological risk group.

Secondary endpoint:

Tumor-related mortality and overall mortality during follow-up. Tumor related mortality was defined as death directly or indirectly caused by EAC (e.g. due to EAC treatment complications).

Data collection and management

Medical interns in the final year of their degree collected endoscopy, pathology, and imaging data in standardized form in all BECs. All patients with endpoints and an additional 70-80% were double-checked by dedicated research fellows (all MDs). Missing data and illogical values were completed and corrected where possible. All authors had access to the study data and reviewed and approved the final manuscript.

The BEC registry (13) was merged with the non-public microdata from Statistics Netherlands to record date and cause of death.

Statistics

Data analysis was performed using the SPSS statistical software package (version 25, SPSS Inc, Chicago, IL, USA) and Rstudio for windows (version 3.6.1). Continuous variables were presented as mean with standard deviation (SD) or median with interquartile range (IQR) for normally distributed or skewed data, respectively. Categorical variables were presented as counts with percentages and 95% confidence intervals (CI). Length of follow-up was calculated from the date of baseline ER to the most recent endoscopy, EUS or scan. Annual risk for metastases was calculated as the number of patients with metastases divided by the total follow-up duration in years. Since competing risks were significant in this cohort, we created cumulative incidence curves performing Fine and Gray survival analysis. The time-to-event analysis was time between baseline ER and occurrence of the event of interest

(progression to LNM/ distant metastases or EAC-related death), the competing risk (unrelated death), or censoring (the last follow-up endoscopy).

Patient and Public Involvement

Patients and public were not involved in the research.

Ethics

The Institutional Review Board of the Amsterdam University Medical Centers declared that the registry was not subject to the Medical Research Involving Human Subjects Act and waived the need for formal ethical review and patient-informed consent. Patients were approached through an opt-out card with the possibility to object against participation in the registry.

Results

Patient cohort

Between January 2008 and June 2019, 1,569 patients underwent ER for a neoplastic lesion in a Barrett segment (patient flowchart and pie chart in Figure 1 and supplementary Figure 2, respectively). There were 120 patients that met our inclusion criteria, baseline characteristics are presented in Table 1. Included patients were subdivided into T1a-HR (25/120; 21%); T1b-LR (55/120; 46%); T1b-HR (40/120; 33%).

Baseline staging and investigations during follow-up

The majority of patients underwent baseline staging examinations prior to initiation of endoscopic follow-up (78% EUS and/or CT-scan) (Table 2).

The median duration of follow-up in all 120 patients was 29 months (IQR 15-48) after baseline ER. Stratified for risk group, the median follow-up duration was 35 months (IQR 22-53) for T1a-HR; 30 months (IQR 18-48) for T1b-LR, and 23 months (IQR 12-50) for T1b-HR (Table 2). Overall, the median number of endoscopies was 5 (IQR 3-7) with 2 EUS (IQR 0-5) per patient. Analyzing results over time, the number of follow-up EUS appeared to increase over time, especially for T1a-HR EAC (median 1 EUS per patient in 2008-2011 versus 3 in 2017-2019).

Additional (PET-)CT scan was performed in 28 patients (23%) during follow-up (median 1, IQR 1-1). Per histological subgroup, (PET-)CT was performed in 4/28 (14%) T1a-HR patients, 7/28 (25%) T1b-LR patients and 17/28 (61%) T1b-HR patients.

In total, twenty-one (18%, 95%CI 12-25) patients were diagnosed with a visible intraluminal recurrence during regular endoscopic follow-up. The median time to intraluminal recurrence was 10 months (IQR 9-20).

Lymph node metastases- and distant metastases detected during follow-up

Overall, nine patients (7.5%, 95% CI 3.5-14) were diagnosed with metastatic disease (LNM (n=4, 3.3%) and/or distant metastases (n=5, 4.2%)) during median 29 months of follow-up, corresponding to an annual risk of 2.7% [95% CI 0.5-7.1]. Metastases were detected after median 27 months (IQR 23-38).

In five patients, metastases were detected as part of routinely performed FU examinations; 4 of these patients had regional LNM and 1 patient was found to have liver metastases. In the remaining four patients in whom metastases were detected, additional examinations were carried out because of symptoms. These detected 1

patient with regional LNM and 3 patients with distant metastases. For the latter group, EUS had been performed median 9 months (IQR 7-11) prior to onset of symptoms. All 9 patients with metastases had undergone baseline EUS and/or CT without evidence of metastases. In 3/9 (33%) patients, there was also occurrence of intraluminal recurrence at the time of metastatic disease detection. The first patient underwent re-ER for a LR T1b EAC (same as the initial tumor). The second patient underwent re-ER for a visible lesion with HGD (initial histopathology showed HR T1b), as well as third patient (initial histopathology HR T1a).

Figure 2 shows the cumulative incidence curves for progression to LNM or distant metastases during follow-up stratified for baseline histology group.
After resection of T1a-HR, 5/25 (20%) patients developed metastases during median 35 months (IQR 22-53) of FU (annual risk 6.9%, [95% CI 3.0-15]). Median time to metastases in this group was 31 months (IQR 25-64).

For patients with T1b-LR, 1/55 (2%) patient developed metastases during median 30 months (IQR 18-48) of FU (annual risk 0.7%, [95% CI 0-4.0]). Time to metastases in this group was 22 months.

Among the T1b-HR patients, 3/40 (8%) developed metastases during median 23 months (IQR 12-50) of FU. The annual risk was 3.0% [95% CI 0-7.0]. Median time to metastases was 24 months.

Table 3 displays histopathological features of these patients per risk group.

EAC-related- and unrelated mortality during follow-up

Of the 9 patients with metastases, 5 developed distant metastases and died. Overall, the risk for EAC-related death was 5.8% (95% CI 2.4-12) during median 70 months (IQR 55-126).

The remaining 4 patients with metastases had LNM and were additionally treated with curative intent, of which two patients were treated successfully (i.e., one patient with neo-adjuvant CRT and esophagectomy, and one patient with definite CRT). The two other patients died due to treatment complications, one patient due to complications after esophagectomy, and one patient due to severe radiation pneumonitis. Supplementary Table 1 shows an extensive overview of all patients with metastases including outcomes.

Mortality not related to EAC was 13% (95% CI 8.0-21) during median 33 months, and patients died median 34 months (IQR 20-61) after baseline. Figure 3 shows the cumulative incidence curves for EAC-related versus non-related mortality and Figure 4 shows the cumulative incidence of progression to LNM or distant metastases compared to unrelated death during follow-up, which indicates that the probability to die from unrelated causes was higher than the probability to develop metastases during FU. Finally, Table 2 provides a summary of all patients, including outcomes mentioned in previous paragraphs.

Discussion

This study includes outcomes of all 120 patients who underwent endoscopic follow-up after radical endoscopic resection of an EAC with histopathological risk features for lymph node metastases in the Netherlands. In total, 9/120 (7.5%) patients developed metastases during a median follow-up of 29 months (IQR 15-48). The

cohort was subdivided in T1a with high-risk features, T1b with low-risk features, and T1b with high risk features in the initial endoscopic resection specimen. The annual risks for metastases for the histological subgroups during follow-up were 6.9% (95%CI 3.0-15), 0.7% (95%CI 0-4.0), and 3.0% (0-7.0) respectively. EAC-specific related mortality and non-related mortality were 5.8% and 13% during 70 (IQR 55-126).

Our results regarding metastases rates in the T1b-LR group are in line with previously published endoscopy-orientated studies. A study that analyzed long-term outcomes showed a metastasis rate of 2% in patients with T1b-LR EAC during 60 ± 30 months FU (10). Our analysis – also showing a metastasis rate of 2% during a median follow-up duration of 30 months – confirms the data supporting endoscopic management for patients with a T1b-LR EAC. Metastases rates in patients with T1b-HR EAC (3/40, 8% during 23 months of FU) were at the lower side of the spectrum compared with existing endoscopic literature (i.e., rates differ between 0-37% during 23-63 months FU) (5,10–12). In comparison with our study, the previous reported studies focused on submucosal EACs only, whereas the current study also includes intramucosal EAC with high-risk features. Furthermore, some studies included patients with a positive deep resection margin in their cohort, whereas this study only includes tumor negative deep resection margins. In addition, in most previous reported literature, metastases rates were analyzed for patients who underwent ER with or without subsequent surgery, whereas our study focuses on metastasis rate after ER during endoscopic follow-up. Our study partly overlaps with two previous reports from our group (5,11).

An explanation for the observed low metastases rates of T1b-HR EACs in this study is that in contrast to previous surgical series, all T1b cancers had to be

amendable to ER in the first place, endoscopic resection had to result in negative deep resection margins and staging after ER could not show (locoregional) metastases. In this regard, five patients who were found to have metastatic disease at baseline staging on EUS-FNA and/or PET-CT were excluded resulting in a subgroup with a lower metastasis risk compared to surgical retrospective studies without a pre-selection excluding these high-risk cases. There was one T1b-HR patient with LNM found during subsequent surgery after radical ER for a baseline staged N0M0 EAC in this study.

Although we cannot compare the metastasis rate of T1a-HR patients with other literature, we found the annual metastasis rate of 6.9% (5/25, 20%) surprisingly high, especially when compared to the T1b cases in this cohort. As this was unexpected, the T1a cases were reviewed by expert pathologists to confirm the diagnosis.

There is scarce knowledge regarding the individual histologic risk factors for metastases (i.e. deep submucosal invasion, poor differentiation grade, lymphovascular invasion).

One study assessed LNM rates in surgical specimens shortly after ER for T1a-HR EAC (n=5; 0/5 patients LNM)(19). The same study also analyzed patients with T1b EAC and poor differentiation grade or LVI, showing that – although not significant – the highest odds ratio for nodal involvement was for LVI (OR 5.2), followed by poor differentiation grade (OR 3.0), independent of invasion depth. A second study assessed clinical and histological variables associated with survival of T1a and T1b EAC patients after endoscopic treatment *with or without subsequent esophagectomy*. Patients with metastasis at baseline and positive resection margin

were – other than in our study - not excluded. Older age, deep margin involvement and presence of LVI were associated with decreased (tumor free) survival (HR, 1.67; 95% 1-3, P .009)(20). To assess independent predictors of survival of endoscopic versus *surgically* treated T1b EAC patients, Otaki et al. built a Cox proportional hazards model and concluded that having one more high-risk histologic feature (i.e., deep margin positivity, LVI, poor differentiation) was associated with decreased survival, compared with the group without any high-risk features. The 5-year survival was higher in patients treated surgically. However, as illustrated by the differences in age and comorbidity score between both groups, patients with poorer life expectancy were followed up endoscopically and were not treated with esophagectomy, leading to a biased comparison of overall survival in favor of surgery(21). Another recently published study developed a prediction tool that estimated the risk of metastases in patients with T1b EAC, also combined with other histopathological risk factors. The highest risk was found in EAC with LVI (subdistribution hazard ratio of 2.95)(22). In our study, 23 patients had LVI of which 5 (22%) were diagnosed with metastases. On the other hand, 4/97 (4%) patients without LVI developed metastases. These data seem to suggest that LVI and poor differentiation grade strongly affect the metastasis risk. However, the number of events in our study was too low to further analyze risk of lymph node metastases for individual histological risk factors. In addition, comparing our study results with other literature is difficult because of discrepancy in in- and exclusion criteria and study aims.

Several limitations of this study must be addressed. First, the retrospective setting of this study could have resulted in selection and information bias. In addition, this was a pre-selected cohort, in which frail and/or elderly patients with a higher likelihood of dying of causes not related to EAC were more likely to have been offered endoscopic

FU instead of surgery. This may play a role in our higher non-EAC related mortality rate (13%) vs EAC-specific related mortality (5.8%). Furthermore, different ER techniques were used during the years. Especially Endoscopic Submucosal Dissections (ESD) have clearly caught up from 2018 and onwards. This may make the cohort less homogeneous. Second, baseline and follow-up strategy was heterogeneous due to lack of strict guidelines and policy changes over time and the median number of EUS per patient was low. This may have led to unjustified inclusion of patients who actually already had metastases at baseline. In addition to this, metastases that developed during FU may have been missed, since median time to detection of metastases (27 months) was comparable to overall median FU duration (29 months). Eventually, nine patients were diagnosed with metastases during FU in our study. Due to heterogeneous FU, the moment of detection – and therefore the stage and the possibility to initiate curative treatment – may be less reliable. Nonetheless, we still found 4/9 patients that developed LNM only, which were detected at curable stages. Two of these 4 patients, died of treatment complications, which indicates the complex trade-off between these competing strategies. Despite the small majority in this cohort having distant metastases at detection, we feel that stringent follow-up after radical resection of early high-risk EAC – performed by dedicated endoscopists only, following strict guidelines when to conduct EUS-FNA – remains a valid strategy in a subset of patients.

Third, this cohort is preselected and contains small numbers per LNM risk group; therefore, it is not suitable to perform comparative or predictive analysis on lymph node metastases regarding specific (histopathological) features or types of (subsequent) endoscopic treatment in this study.

Fourth, histopathology review was only performed for HR-T1a cases. Finally, the follow-up duration of median 29 (IQR 15-48) months is relatively short. Although studies have shown that the majority of metastases are found during the first two years of follow-up, only 4/9 metastases in this study were detected within 24 months FU (9,23). As mentioned before, this might be a consequence of heterogeneous FU.

Strong points of this study are the uniquely harmonized setting of the BECs with care provided by jointly trained endoscopists and pathologists, and registration in a uniform database. This study reflects current clinical practice since some patients with high risk EAC are deemed unfit for surgery or prefer endoscopic management. These patients are offered endoscopic management after extensive informed consent by both gastroenterologist and surgeon. This study adds value to the available literature, since it describes the largest cohort of endoscopic management outcomes in early high-risk EAC, including HRT1a patients. It reflects a *clean* cohort of patients that underwent *radical* ER with subsequent endoscopic follow-up, with a rather long median follow-up duration after treatment. In comparison to other studies assessing metastases in high risk EAC, the number of included patients is reasonably large.

Our study provides additional data regarding metastasis risk during endoscopic follow-up of patients with early esophageal adenocarcinoma with histological risk factors. Whereas the observed annual metastasis rate for T1b-HR EAC (3.0%) is somewhat lower than expected in comparison with previous reported percentages, the observed annual metastasis risk of 6.9% forT1a-HR EAC is new and worrisome. Our findings and optimal management strategies for these patients warrant further prospective evaluation (PREFER study, NCT03222635).

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Table 1 Baseline characteristics of 120 patients included in follow-up analysis

Patients	All	HR T1a	LR T1b	HR T1b
Total, n (%)	120	25 (21)	55 (46)	40 (33)
Age, years (p25-75)	74 (66-81)	74 (66-82)	76 (69-80)	73 (65-82)
Male, n (%)	99 (83)	21 (84)	43 (78)	35 (88)
Esophageal characteristics				
Barrett length, cm (p25-75)				
Circumferential	2 (0-5)	2 (1-5)	2 (0-5)	2 (0-5)
Maximal	4 (2-7)	4 (3-8)	5 (2-7)	4 (2-6)
Paris classification (primary component)*				
0-Ip	6 (5)	1 (4)	0	4 (10)
0-Is	32 (27)	2 (8)	8 (15)	15 (38)
0-IIa	65 (55)	12 (48)	29 (53)	11 (28)
0-IIb	7 (6)	3 (12)	3 (6)	1 (3)
0-IIc	8 (7)	1 (4)	4 (7)	2 (5)
Size lesion, mm diameter (p25-75)**	20 (15-30)	20 (20-30)	20 (15-40)	20 (15-30)
Endoscopic resection				
ER technique, n (%)				
Multiband Mucosectomy	83 (70)	20 (80)	41 (75)	22 (55)
Endoscopic Cap Resection	10 (9)	2 (8)	5 (9)	3 (7)
Endoscopic Submucosal Dissection	24 (21)	3 (12)	9 (16)	12 (38)
Histopathological examination ER specimen				
Infiltration depth, n (%)				
T1m3	25 (21)	25 (100)	-	-
T1sm1 (<500µm)	70 (58)	-	55 (100)	15 (38)
T1sm2/3 (≥500µm)	25 (21)	-	-	25 (62)
Differentiation grade, n (%)				
Good (G1)	24 (20)	-	19 (35)	5 (12)
Moderate (G2)	54 (45)	1 (4)	36 (65)	17 (43)
Poor (G3-4)	42 (35)	24 (96)	-	18 (45)
LVI, n				
Absent	97 (81)	16 (64)	55 (100)	26 (65)
Present	23 (19)	9 (36)	-	14 (35)

**Missings, n=2 (1,7%)*

***Missings, n=17 (14%)*

Table 2 Summary of patients during follow-up divided per risk group

N=120	**Follow-up, months (IQR)**	**Number of endoscopies (IQR)**	**Number of EUS (IQR)**	**LNM/DM during follow-up N (%)**	**Annual metastasis risk during follow-up (95% CI)**	**Time to metastasis, months (IQR)**	**Tumor related death N (%)**
HR T1a (n=25)	35 (22-53)	6 (3-9)	1 (0-4)	5 (20%)	6,9% (3-15)	31 (25-64)	4 (16%)
LR T1b (n=55)	30 (18-48)	4 (2-7)	1 (0-3)	1 (2%)	0,7% (0-4)	22 (NA)	1 (2%)
HR T1b (n=40)	23 (12-50)	5 (3-8)	5 (2-8)	3 (8%)	3,0% (0-7)	24 (NA)	2 (5%)

Table 3 Histopathological features of patients with metastasis detected during follow-up disaggregated per risk group

N=120	**HR T1a (n=25)**			**LR T1b (n=55)**	**HR T1b (n=40)**			**Total**
Histopathological risk factors >	**G3/4 & LVI+**	**G3/4**	**LVI+**	**Sm1**	**Sm1 & LVI+**	**Sm2/3 & G3/4**	**Sm2/3 & G3/4 & LVI+**	
Number of patients with LNM+	1	1	0	1	1	0	0	4
Number of patients with LNM+ and DM+	1	1	1	0	0	1	1	5
Total number of pts with metastases	2	2	1	1	1	1	1	9
	5/25 (20%)			**1/55 (2%)**	**3/40 (8%)**			**9/120 (7,5%)**
Total number of patients with these high risk factors	8/25	16/25	1/25	55/55	6/40	6/40	3/40	

Supplementary Table 1. Overview of patients with metastases during follow-up

Total n=9	**LR T1b/ HR T1a / HR T1b**	**Histopathology at baseline**	**EUS at baseline (y/n)**	**Imaging at baseline (y/n)**	**FU policy**	**Timeline ER → LN M/DM**	**When & how detected**	**Location metastasis**	**Therapy**	**TNM stage**	**Final outcome**
Patients with Lymph Node Metastases (all negative deep resection margins at baseline)											
1. LNM+	LR T1b	T1b sm1 G2 LVI-	y	Yes, PET-CT	EUS 6-monthly GDS 3-monthly	22 months	Regular FU EUS (FNA)	Truncal node mass	CRT & surgery	ypT0N0M0	Died after surgery due to complications (4 months after surgery)
3. LNM+	HR T1b	T1b sm1 G2 LVI +	y	No	EUS/ GDS 3-monthly	6 months	Regular FU EUS (FNA)	1 mediastinal node	CRT & surgery	ypT1aN0M0	Successful CRT/surgery; +1y after therapy
2. LNM+	HR T1a	T1a m3 G2 LVI+	y	Yes, CT	EUS/ GDS 3-monthly	27 months	Patient complaints (weight loss, hoarseness)> CT	1 mediastinal node	CRT	ypT0N1M0	Successful CRT; +2y after therapy
4. LNM+	HR T1a	T1a m3 G3 LVI-	y	Yes, CT	GDS 3-monthly EUS only 2x (baseline &	41 months	Planned EUS (FNA) after MRI liver for other reasons	1 mediastinal node	CRT	pT0N1M0	Died 4 days after last radiation therapy (complicated course

					discovery LNM)						with radiation pneumonitis)
Patients with Distant Metastases											
5. LNM/ DM+	HR T1a	T1a m3 G2 LVI+	y	No	GDS annually	86 months	Patient complaints (icterus, weight loss) > CT and liver biopsy	Multiple organs (liver, bones, lungs, omentum)	Palliative care	pT0N2 M1	Died
6. LNM/ DM+	HR T1a	T1a m3 G2 LVI +	y	Yes, PET-CT	EUS/ GDS 3-monthly	31 months	Regular FU EUS (FNA) & CT	Liver	Palliative care	pT0N1 M1	Died
7. LNM/ DM+	HR T1a	T1a m2 G3 LVI-	y	Yes, CT	EUS/ GDS 3-monthly	23 months	Patient complaints (weight loss and abdominal pain) > PET-CT	Liver	Palliative care	pT0N1 M1	Died
8. LNM/ DM+	HR T1b	T1b sm2/3 G3 LVI+	y	Yes, CT	EUS/ GDS 3-monthly	34 months	Patient complaints (ileus, ascites) > CT	Omentum	Palliative care	pT0N1 M1	Died
9. LNM/ DM+	HR T1b	T1b sm2 G3 LVI-	y	Yes, CT	EUS/ GDS 3-monthly	24 months	Regular FU EUS (FNA) + CT	First mediastinal nodes, later distant metatasis in lungs	CRT in 2017. 2y after CRT: lung metastasis found on FU CT > palliative care	ypT0N2M1 (2019)	Died

1

2

Supplementary Table 2. Baseline staging examinations per histopathological risk group

	HR T1a (n=25)	LR T1b (n=55)	HR T1b (n=40)
Nr. of patients with baseline EUS + CT-scan	13 (52%)	21 (38%)	33 (82%)
Nr. of patients with baseline EUS only	4 (16%)	11 (20%)	6 (15%)
Nr of patients with baseline CT-scan only	-	4 (73%)	1 (3%)
Total	17 (68%)	36 (65%)	40 (100%)

Figure legends

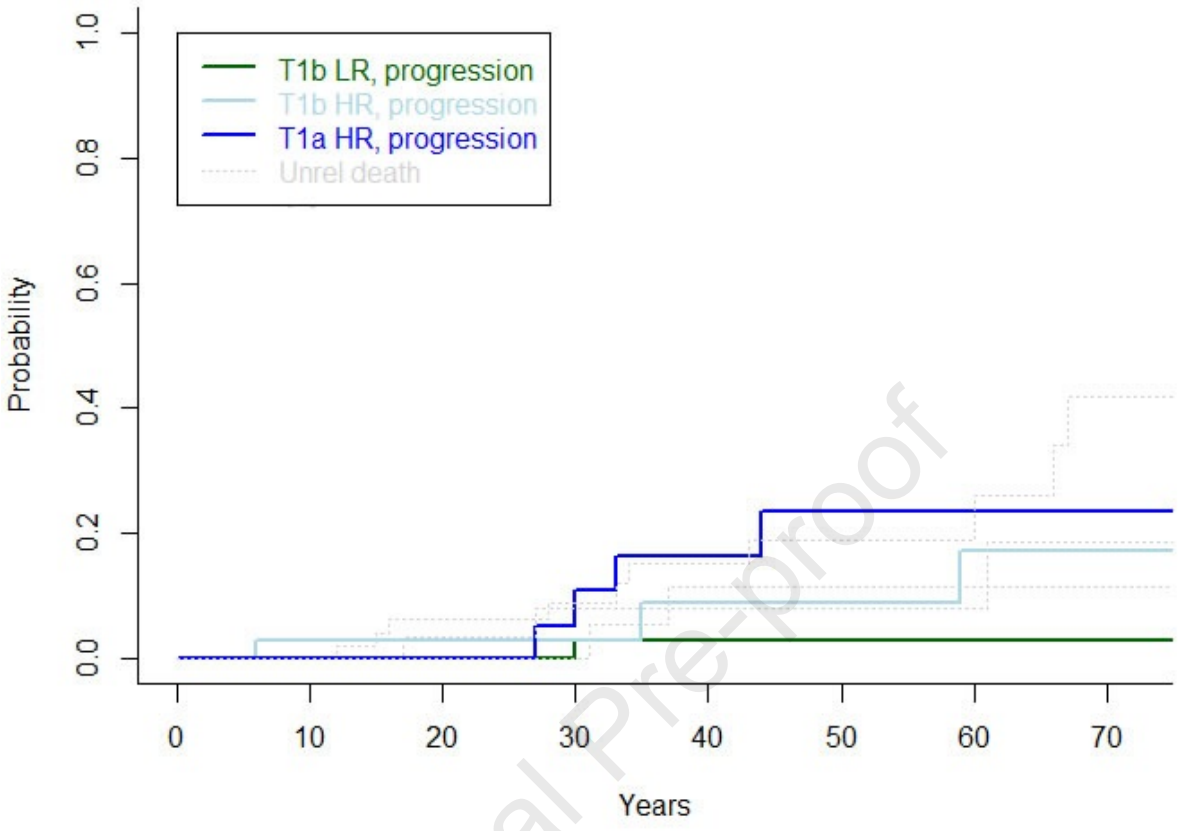
Figure 1. Flow of patients representing the selection of the study cohort. Numbers i-iv state our exclusion criteria as mentioned in the manuscript. Abbreviations: BE, Barrett esophagus; (n)CRT, (neoadjuvant) chemoradiation therapy; EAC, esophageal adenocarcinoma; ER, endoscopic resection; T1a-LR, mucosal tumor with low-risk histopathological features such as no lymphovascular invasion and good or moderate differentiation grade; T1a-HR, intramucosal tumor with high-risk histopathological features such as lymphovascular invasion or poor differentiation; T1b-LR, submucosal tumor with superficial invasion in the submucosa (<500 microns; sm1), well to moderately differentiated, without lymphovascular invasion; T1b-HR, submucosal tumor with either deep invasion in the submucosa (≥500 microns; sm2/3), and/or poor differentiation grade, and/or lymphovascular invasion presence.

Figure 2. Cumulative incidence curves for progression to metastases per histopathological risk group

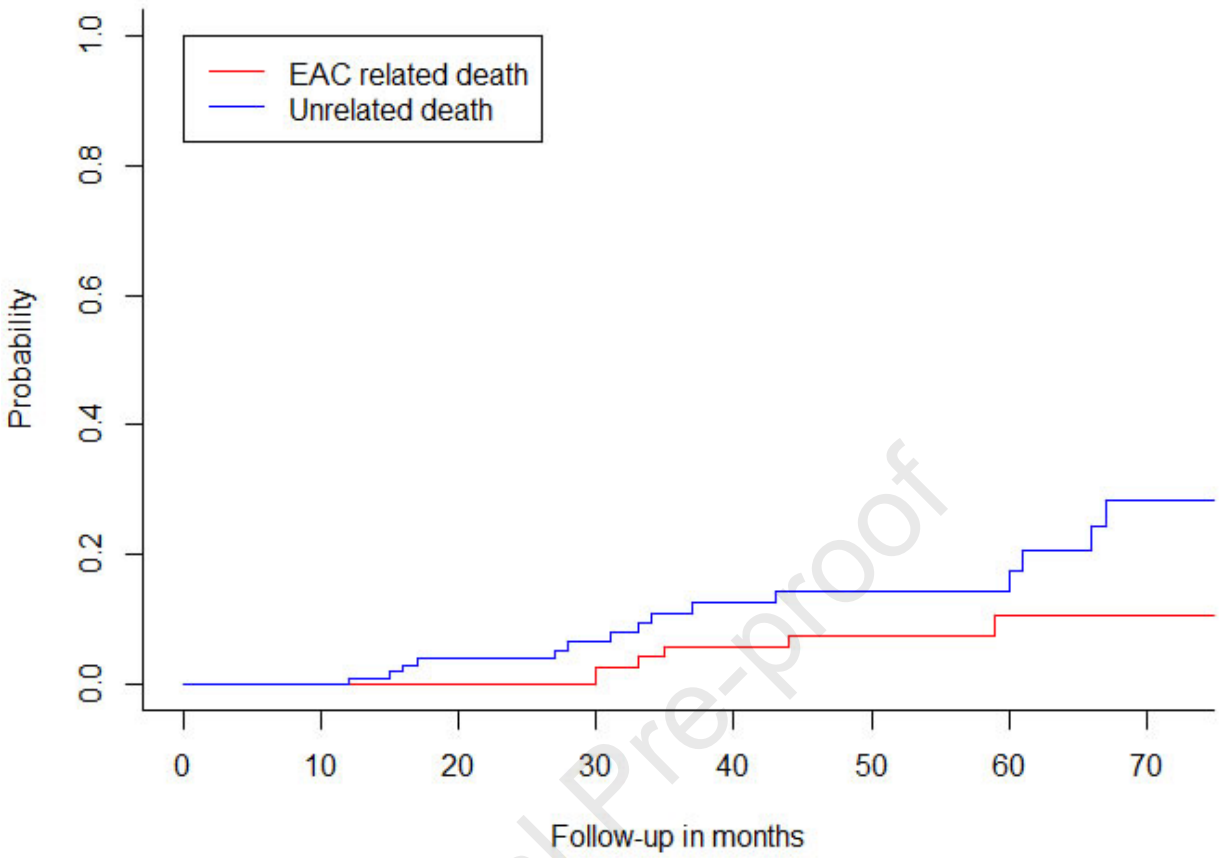
Figure 3. Cumulative incidence curves for EAC related versus unrelated death

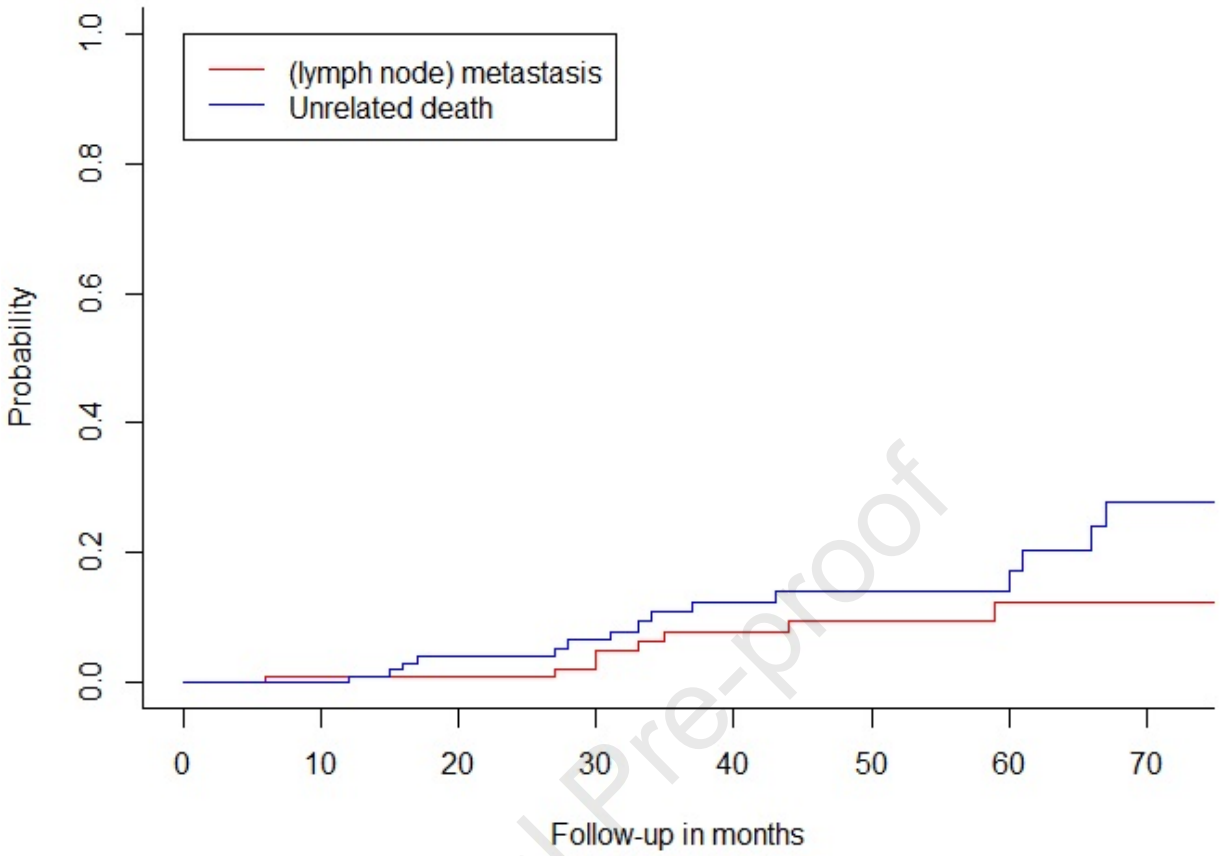
Figure 4. Cumulative incidence curves for lymph node metastases versus unrelated death

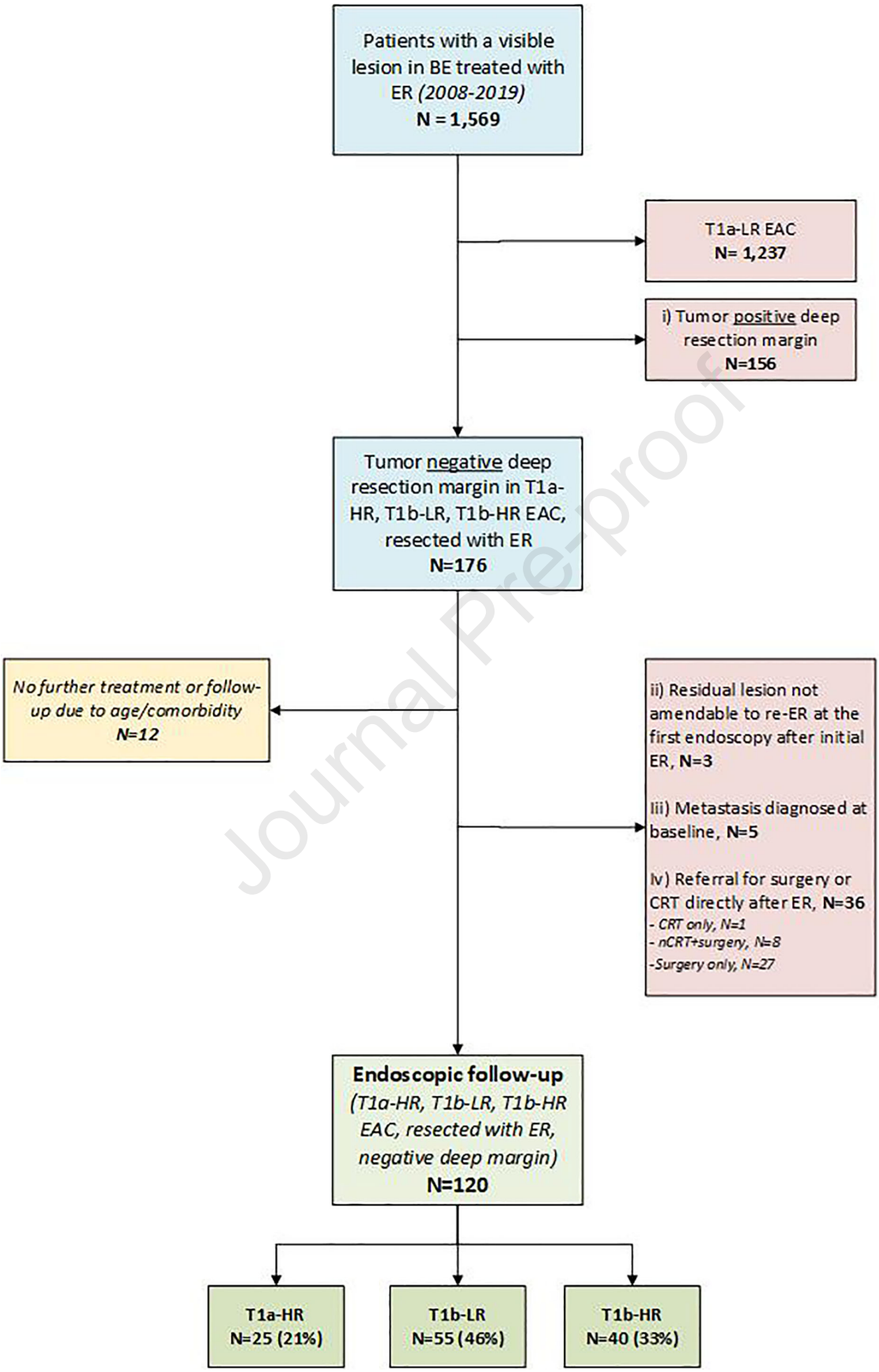
Supplementary Figure 1. Barrett Expert Center patient population (2008-2019)



Nr of Patients	120	101	74	48	33	21	12
Months FU	0	12	24	36	48	60	72







Abbreviations:

BEC – Barrett Expert Center
CI – Confidence interval
CT- Computed tomography
ER – Endoscopic resection
EAC – Esophageal adenocarcinoma
EUS – Endoscopic ultrasound
FNA – Fine needle aspiration
HR-T1a – High-risk mucosal
HR-T1b – High-risk submucosal
LR-T1b – Low-risk submucosal
LNM – Lymph node metastasis
LVI – Lymphovascular invasion
PET – Positron emission tomography

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