Endoscopy International Open

Reassessment reveals underestimation of infiltration depth in surgical resection specimens with lymph-node positive T1b esophageal adenocarcinoma

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Conflict of Interest: M.I. van Berge Henegouwen is consultant for Viatris, Johnson & Johnson, B. Braun, Stryker and Medtronic. All fees paid to institution.

R.E. Pouw is consultant for Medtronic BV and MicroTech Europe and has received speaker fee from Pentax.

The other authors have no conflicts of interest to report.

Abstract:
Background
Endoscopic resection (ER) has proven effective and safe for T1 esophageal adenocarcinoma (EAC). However, uncertainty remains concerning risk-benefit return of esophagectomy for submucosal lesions (T1b). Surgical series in past decades have reported significant risk of lymph node metastasis (LNM) in T1b EAC, but these rates may be overestimated due to limitations in histological assessment of surgical specimens. We aimed to test this hypothesis by reassessing histological risk features in surgical specimens from T1b EAC cases with documented LNM.

Methods
A retrospective cross-sectional study (1994-2005) was conducted. Patients who underwent direct esophagectomy without prior neo-adjuvant therapy for suspected T1b EAC with LNM were included. Additional tissue sections were prepared from archival tumor blocks. A consensus diagnosis on tumor depth, differentiation grade, and lymphovascular invasion (LVI) was established by a panel of experienced pathologists.

Results
Specific depth of submucosal invasion (sm1 to sm3) was not specified in 10/11 archival case sign-out reports. LVI status was not reported in 7/11 cases. Following reassessment, one patient was found to have deep tumor invasion into the muscularis propria (T2). The remaining 10/11 patients exhibited deep submucosal invasion (sm2-3), with five showing ≥1 additional risk feature (poor differentiation and/or LVI).

Conclusion
Our findings highlight the potential for underestimating tumor depth of invasion and other high-risk features in surgical specimens. Despite the limited cohort size, our study confirmed a consistent high-risk histological profile across all cases. Caution is warranted when extrapolating LNM risk data from historic heterogeneous cross-sectional surgical cohorts to the modern ER era.

Thieme

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Introduction

Significant advancements in therapeutic endoscopic techniques have ushered in a paradigm shift in the treatment of T1 esophageal adenocarcinoma (T1 EAC) in recent decades. The approach to treating T1 EAC has notably evolved from traditional surgical methods to the adoption of endoscopic resection (ER) procedures. [1, 2] To determine the most appropriate treatment strategy for a patient with T1 EAC, it is necessary to assess the risk of lymph node metastases (LNM), which is associated with histological risk factors such as tumor invasion depth, differentiation grade and lympho-vascular invasion (LVI).

For patients with T1 EAC confined to the mucosa (T1a) without histologic risk factors such as poor differentiation and/or LVI, the risk of LNM is minimal (<1%). Therefore, the majority of centers worldwide have accepted ER for this indication as the primary treatment modality replacing esophagectomy. This transition is favorable, as it avoids the high complication rates associated with esophagectomy. [2, 3] In case tumor invasion reaches into the submucosal layer (T1b) of the esophagus, esophagectomy with lymph node dissection is often still recommended, based on concerns over the risk of LNM. [4-6] This recommendation is based on surgical literature, which reported LNM in T1b EAC in up to 46%. [7, 8] In contrast, studies reporting LNM in T1b EAC patients treated with less invasive ER have presented a considerably lower LNM risk for T1b EAC, ranging from 0-26%, with higher rates in deeper invading cancers (sm2/3), and/or with additional histological risk factors such as poor differentiation and LVI. [9-12] These LNM rates are based on endoscopic follow-up data, as well as on studies in which patients underwent surgery after R0 ER of T1b EAC.

Given these observed discrepancies in published LNM risk between the surgical and endoscopic literature, we hypothesized that one of the explanations may be that LNM rates for T1b EAC may have been overestimated in surgical series due to differences in histopathological assessment. First, the

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surgical studies were conducted during an era when subtyping T1b EAC into sm1, sm2 and sm3 was not routine practice, since it did not bear clinical consequences. Moreover, pathological assessment of surgical specimens might allow less detailed assessment with regards to tumor infiltration depth when compared to ER specimens. This putative disparity might arise from sectioning surgical specimens into relatively wider slides, which may inadvertently lead to underestimating tumor invasion depth and the potential oversight of other histological risk factors (as illustrated in **Figure 1**).

We aimed to test this hypothesis by reassessing histological risk factors, currently used in risk stratification, in surgical specimens of cases with lymph node-positive T1b EAC.

Material and Methods

Study design

This study utilized a cross-sectional design based on data extracted from the Nationwide Network and Registry of Histo and Cytopathology in the Netherlands (*PALGA Foundation*). The inclusion period spans from 1994-2005, with the gradual integration of endoscopic mucosal resection (EMR) into clinical practice starting in 2001 and becoming standard practice after 2005. This historical cohort was selected to maximize patient inclusion, given the limited cases of surgical resection for this indication during the specified period, and to have long-term follow-up outcomes available. From the database, patients with T1b EAC and LNM treated at Amsterdam UMC location AMC were identified. Patients were included if the diagnosis was based on surgical resection specimens, in patients without prior ER and/or neo-adjuvant chemo-/radiotherapy.

Histological reassessment

For each included case, the pre-existing 5mm tumor slides of the surgical specimens were retrieved from the archive and evaluated by an expert gastrointestinal pathologist (SM) to select the slide with

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deepest tumor invasion. Additional 5µm slides were prepared from the relevant slide and stained with Hematoxylin and Eosin (H&E). Consequently, the slides were digitized for reassessment, anonymized and stored on a secure server (Philips IntelliSite Pathology Solution 3.2).

An international panel consisting of three experienced gastrointestinal pathologists (SM, MJ and MV) was formed. All three pathologists have extensive experience in assessing Barrett's neoplasia. A consensus meeting was convened where the reference panel collectively established consensus diagnoses for all cases. Tumor reassessment was done, according to the WHO classification for tumor grading [13], for:

1) Infiltration depth; classified as invasion into the submucosa (T1b; sm1 (<500µm), sm2/3 (≥500µm)) or muscularis propria (T2);

2) Differentiation grade; divided into well (G1), moderate (G2), poor (G3), or no differentiation (G4);

3) Presence or absence of lymphatic and/or vascular invasion (LVI).

The panel was blinded to patient, treatment, and documented pathology characteristics.

Study endpoints

The primary endpoint of this study was the number of cases upstaged from T1b to T2 invasion after reassessment. The secondary endpoints included the presence of other histological risk factors (i.e. G3-4 and/or LVI) after reassessment, and disease-specific mortality as clinical outcome.

Results

Historic assessment of tumor infiltration and risk features

Between 1994 and 2005, Amsterdam UMC location AMC treated a total of 47 patients with surgery for pT1b EAC. Among them, 13 patients were diagnosed with LNM, and had not undergone prior ER or neo-adjuvant chemo-/radiotherapy, meeting the study inclusion criteria. However, for one case, pathology slides were irretrievable from the archives. Another patient was excluded during the revision process due to inadequate tissue samples caused by sectioning artifacts and lack of full face assessment. All reasons for and numbers of exclusion are demonstrated in **Figure 2**.

Eleven cases were included, all male, with a median age of 68 years (IQR 61-72) at the time of surgery. 10/11 (91%) patients underwent trianshiatal esophagectomy and 1/11 (9%) underwent transthoracic esophagectomy. The median number of resected lymph nodes was 6 (IQR 5-14). The diagnoses based on the histological resection specimens are listed in **Table 1**. Only in one case, the specific depth of submucosal invasion was specified (case 8; sm2). In 5/11 (45%) cases, besides submucosal invasion, at least one additional histopathological risk feature was present (i.e. either ≥G3 a/o LVI). However, LVI status was not explicitly reported in seven cases.

Reassessment tumor infiltration and risk features

The consensus diagnoses, including assessment of the additionally prepared slides, showed discrepancy in 9/11 cases with the initial pathology diagnoses of the surgical resection specimen following standard pathology procedures of that time. Histological details are provided in **Table 1**.

<u>Primary endpoint</u>

1/11 cases was found to have T2 tumor infiltration, agreed by all three pathologists. Detailed histological images of this case are provided in **Figure 3**. The remaining ten cases were all diagnosed with deep submucosal invasion (sm2-3).

<u>Secondary endpoints</u>

Other histological risk factors for LNM

In 6/11 cases, at least one additional risk feature for LNM was identified (**Table 1**). Specifically, two cases showed an upgrading of tumor differentiation from G2 to G3, while two other cases were downgraded from G3 to G2. Tumor differentiation remained unchanged in the remaining cases. Regarding LVI, the vast majority of cases that were initially reported with LVI maintained this diagnosis upon reassessment, with one exception. Additionally, one case reported as lacking LVI was shown to demonstrate LVI during reassessment (case 3).

Disease-specific mortality

After surgery, patients underwent clinical follow-up during median 36 (IQR 11-52) months, measured until the last recorded hospital contact moment, date of recurrent event, or date of death. At the time of data collection, all eleven patients had passed away. Five patients died due to disease-related causes: 1/5 patient deceased in-hospital due to surgical complications; 4/5 patients died due to recurrent disease during follow-up, diagnosed median 18 (IQR 7-33) months after surgery. This subset included the patient whose revision revealed an upstaged tumor invasion to T2. The other three cases were all classified as moderately differentiated T1b tumors with deep submucosal invasion (sm2-3), and one patient additionally had LVI (**Table 1**).

Discussion

Although a number of retrospective studies have demonstrated that endoscopic follow-up in selected patients with T1b EAC is a safe approach [10, 11, 14, 15], there is still an ongoing discussion about this approach with some advocating surgery for this indication. In this discussion, surgical proponents often cite LNM risks of up to 46% to favor surgical esophagectomy with lymph node dissection. [7-9] However, we feel that these high rates of LNM in surgical literature may be an overestimation due to potential underreporting of invasion depth, due to less accurate histological assessment, and by

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collating all cases with submucosal invasion into a joint T1b category without differentiating between superficial and deep submucosal invasion. To test this hypothesis, this study focused on the reassessment of surgical specimens in lymph-node positive T1b EAC cases.

This study was the first to reassess T1b EAC specimens directly treated by surgery by preparing additional cuts to assess the deepest point of invasion, to mimic the histological assessment as currently done for ER specimens. Reassessment indeed demonstrated invasion into the muscularis propria (T2) in one case, and in addition deep submucosal invasion (sm2/3) in all other cases. Furthermore, in addition to deep invasion, six cases demonstrated at least one other histological risk factor for LNM, such as LVI and/or poor differentiation. Even though this is a highly selected high-risk cohort with cases preceding the introduction of ER and standardized neo-adjuvant regimens (e.g., FLOT and CROSS protocols [16, 17]), and situated in an era where transhiatal surgery predominated, the substantial disease-specific mortality observed in almost half of our patients (5 out of 11) highlights the inconsistency of surgery as a definitive cure for T1b EAC patients. In the current era of patient-tailored management strategies, it is crucial to weigh this knowledge alongside morbidity scores, which can be as high as 60% even in high-volume centers, in the shared decision-making processes with patients. [18]

Although today there is no reliable prediction model available to gauge risk of LNM based on clinical and histological characteristics, it is established that the risk is related to the presence of submucosal invasion depth, LVI and poor differentiation, especially if multiple risk factors are present. [19, 20] The differential impact of these risk factors remains to be determined. Older studies, when assessing LNM risk through surgical specimens, typically did not report separately on the presence of different risk factors, nor was the exact submucosal invasion depth reported on. [21, 22] Furthermore, in these historical surgical cohorts, where transhiatal esophagectomy was the prevailing procedure with a limited lymph node harvest, there might have been a potential underestimation of the rate of LNM.

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Taking all this into account, it is currently acknowledged that these cohorts are too heterogeneous, and such studies should therefore be disregarded when discussing the risk-return benefit of surgery in patients with T1b EAC, especially those treated for low-risk T1b EAC. In more recent studies examining surgical specimens for LNM risk, authors commonly carry out histopathological revision of tumors in surgical resection specimens, subdividing into depths of invasion (sm1-sm3) and considering additional risk factors. [20, 23] However, these studies do not involve additional deeper cuts to mimic assessments of ER specimens, potentially missing the deepest point of invasion or overlooking additional risk factors.

Although reassessment did corroborate our hypothesis in one case, we acknowledge that the number of reviewed cases to rigorously assess our hypothesis was very small. Also, we only included patients with LNM and not T1b cases without LNM, which could have increased the study cohort. However, since there are multiple research efforts ongoing in the field of watchful waiting after ER of T1b EAC, we felt that any effort to enlarge the reassessment cohort by identifying more cases and including cases without LNM, preparing additional tissue cuts and revision by an expert pathology panel, would not be appropriate. Given the limited evidence supporting our hypothesis, an alternative and plausible explanation for the disparity in LNM rates might be attributed to a benign historical selection bias. As the field has progressively matured, studies on this topic have gained increased attention and gradually transitioned to higher quality, potentially influencing reported rates of LNM. Another explanation of better outcomes after endoscopic treatment of T1b EAC in recent literature may be found in the fact that high-resolution endoscopy and virtual chromoendoscopy enables endoscopists to identify more subtle T1b lesions. In the past, these lesions were more likely to be identified at a more endoscopically visible and therefore more advanced stage. The current studies may have included a more favorable group also including earlier stage T1b lesions, compared to older series that were more likely to over-represent more advanced T1b lesions.

We advocate for a more nuanced discussion regarding the decision-making process between initiating endoscopic follow-up and opting for additional surgery in selected patients diagnosed with T1b EAC. There are compelling reasons to think that LNM rates derived from surgical specimens, may be an overestimation of the true risk of LNM in existing reports, due to limitations of histological assessment and selection bias due to inclusion of more advanced T1b cases. This perspective gains support from recent findings in endoscopy-focused studies, which employ more precise histological assessment techniques and report LNM rates in a lower percentage of patients. As ongoing large prospective studies (PREFER trial; NCT03222635) progress, they are expected to provide more accurate and reliable LNM rates that will contribute to a better-informed understanding of the optimal management strategies for T1b EAC patients.

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Table 1. Overview of 11 cases with surgically resected metastatic T1b EAC

Case	Year of Surgery	Surgical Procedure	LNs Positive/ Dissected	Clinical outcome	Reported Diagnosis	Revised Consensus Diagnosis (Expert Panel)
1	2002	Transhiatal	5/19 (N2)	Recurrence-free follow-up	T1sm G2 LVI+	T1sm3 G2 LVI+
2	2000	Transhiatal	3/5 (N1)*	Disease-specific death (surgical complication)	T1sm G3 LVI+	T1sm3 G3 LVI+
3	1996	Transhiatal	1/3 (N1)	Recurrence-free follow-up	T1sm G2 LVI not reported	T1sm2 G2 LVI+
4	2000	Transhiatal	3/5 (N2)	Recurrence-free follow-up	T1sm G2 LVI not reported	T1sm3 G2 LVI-
5	2002	Transhiatal	1/6 (N1)	Disease-specific death (metastatic disease)	T1sm G2 LVI not reported	T1sm3 G2 LVI-
6	1994	Transhiatal	2/6 (N1)	Disease-specific death (metastatic disease)	T1sm G2 LVI not reported	T1sm2 G2 LVI-
7	1999	Transthoracic	1/7 (N1)	Recurrence-free follow-up	T1sm G2 LVI+	T1sm3 G3 LVI-
8	1999	Transhiatal	8/14 (N3)	Disease-specific death (metastatic disease)	T1sm G3 LVI+	T1sm2 G2 LVI+
9	1995	Transhiatal	1/14 (N1)	Disease-specific death (metastatic disease)	T1sm G3 LVI not reported	**T2** G2 LVI-
10	2005	Transhiatal	1/3 (N1)	Recurrence-free follow-up	T1sm2 G2 LVI not reported	T1sm2 G3 LVI-
11	1999	Transhiatal	1/10 (N1)	Recurrence-free follow-up	T1sm G2 LVI not reported	T1sm2 G2 LVI-

*Adhering to the AJCC 5th Edition Cancer Staging Fifth edition (1997)

M. mucosae

Submucosa (1)

(2)

(3)

M. propria

Patients identified who underwent surgery for T1b EAC (1994-2005)
N=47

Excluded

N=7 Prior endoscopic resection

N=26 Surgically staged N0

N=1 Synchronous esophageal squamous cell carcinoma

Patients eligible
N=13

N=1 Histology slides irretrievable

N=1 Inadequate tissue sampling

Study cohort
N=11

A

Archival section

B

Deeper level (this study)

C

T2 invasion