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Original Article

Significant variation in histopathological assessment of endoscopic resections for Barrett's neoplasia suggests need for consensus reporting: propositions for improvement

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SUMMARY. **Endoscopic resection (ER) is an important diagnostic step in management of patients with early Barrett's esophagus (BE) neoplasia. Based on ER specimens, an accurate histological diagnosis can be made, which guides further treatment. Based on depth of tumor invasion, differentiation grade, lymphovascular invasion, and margin status, the risk of lymph node metastases and local recurrence is judged to be low enough to justify endoscopic management, or high enough to warrant invasive surgical esophagectomy. Adequate assessment of these histological risk factors is therefore of the utmost importance. Aim of this study was to assess pathologist concordance on these histological features on ER specimens and evaluate causes of discrepancy. Of 62 challenging ER cases, one representative H&E slide and matching desmin and endothelial marker were digitalized and independently assessed by 13 dedicated GI pathologists from 8 Dutch BE expert centers, using an online assessment module. For each histological feature, concordance and discordance were calculated. Clinically relevant discordances were observed for all criteria. Grouping depth of invasion categories according to expanded endoscopic treatment criteria (T1a and T1sm1 vs. T1sm2/3), ≥1 pathologist was discrepant in 21% of cases, increasing to 45% when grouping diagnoses according to the traditional T1a versus T1b classification. For differentiation grade, lymphovascular invasion, and margin status, discordances were substantial with 27%, 42%, and 32% of cases having ≥1 discrepant pathologist, respectively. In conclusion, histological assessment of ER specimens of early BE cancer by dedicated GI pathologists shows significant discordances for all relevant histological features. We present propositions to improve definitions of diagnostic criteria.**

KEY WORDS: **adenocarcinoma, Barrett's esophagus, digital slide review, endoscopic resection, interobserver agreement, lymph node metastasis.**

INTRODUCTION

Diagnostic endoscopic resection (ER) is the cornerstone of endoscopic management in patients with early Barrett's esophagus (BE) neoplasia. Based on the histological assessment of tumor invasion depth, differentiation grade, lymphovascular invasion, and margin status of the resection, the risk of lymph node metastases or local recurrence is assessed. In case of high risk on lymph node metastases or local recurrence, current guidelines advise surgical resection instead of endoscopic management. Given the differences in risk of morbidity and mortality, and impact on quality of life between endoscopic

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management and surgical esophagectomy, this is a pivotal decision and adequate histological assessment of the ER specimen is therefore of the utmost importance. However, little is known about the observer agreement of histological assessment of esophageal ER specimens.[1,2]

In the Netherlands, endoscopic treatment of BE neoplasia is centralized in BE expert centers.[3,4] Besides dedicated endoscopists, these centers employ dedicated GI pathologists with extensive experience in BE neoplasia. Since 2015, these pathologists constitute a national advisory panel for review of BE biopsies diagnosed as indefinite for dysplasia or low-grade dysplasia. By means of structured self-assessment programs and consensus meetings, homogeneous histological assessment of these biopsies is ensured.[5–7] Despite an almost perfect interobserver agreement between the pathologists for assessment of BE dysplasia,[8] it is unknown if this group of expert pathologists is as concordant when it comes to assessment of ER specimens of early BE cancer.

The aim of this study was to evaluate diagnostic concordance of 13 BE expert pathologists in histological assessment of ER specimens of early BE cancer and to evaluate causes of discrepancy.

METHODS

The medical ethical committee of the Amsterdam University Medical Centers waived the need for approval for this study.

Case selection and scanning

From the pathology archives at Amsterdam University Medical Centers, ER specimens of early BE cancer were selected, enriched for one or more of the following features: (i) submucosal invasion; (ii) poor differentiation grade; (iii) lymphovascular invasion; and/or (iv) tumor involvement of the basal margin of the specimen. Of these cases, a single representative cross section was selected. Eventually, the case set consisted of 62 cases with the following predefined number of features: (i) 15 cases with submucosal invasion; (ii) 14 cases with poor differentiation; (iii) 13 cases with lymphovascular invasion; and (iv) 13 cases with tumor involvement of the basal resection margin. Eight cases contained multiple features. This enriched case selection was supplemented with 15 reference cases that had a maximum depth of invasion of m3.[9] All specimens were pinned down on cork or paraffin and processed according to standard Dutch and European guidelines.[4]

Per case, three stainings (H&E, desmin, and an endothelial marker [D2–40 or CD-31]) were fully digitalized, using a slide scanner with a x20 microscope objective (Slide, Olympus, Tokyo, Japan). Images were made available for viewing through the virtual slide system 'Digital Slidebox 4.5' (Slidepath, Leica Microsystems, Dublin, Ireland). Subsequently, these virtual files were incorporated in a secure, online, custom-built histological assessment module.

Assessors

The assessors were 13 GI pathologists employed at one of the eight Dutch BE expert centers, with a median ER assessment experience of 7 years (25–75% percentile: 7–15). Three pathologists assessed a median of four ER specimens per week over the last year, three pathologists assessed one, and three pathologists less than one per week.

Histological assessment criteria

Before starting the assessment round, histological assessment criteria of ER specimens were defined.[10,11] Depth of invasion was scored as m1 (high-grade dysplasia), m2 (intramucosal adenocarcinoma confined to the lamina propria), m3 (intramucosal adenocarcinoma with infiltrative growth into the duplicated or original muscularis mucosae), sm1 (depth of invasion $\leq$500 µm), sm2 (500–1000 µm), or sm3 (>1000 µm).[9] In cases with submucosal invasion, deepest point of invasion in relation to the muscularis mucosa was measured in micrometers. Differentiation grade was scored as 'well', 'moderately', or 'poorly' differentiated.[12] According to this WHO classification, a well-differentiated tumor is defined as having >95% gland formation ('G1'), a moderately differentiated tumor has 50–94% gland formation ('G2'), and a poorly differentiated tumor has 0–49% gland formation ('G3'). Lymphovascular invasion was defined as 'tumor cells inside a lymphatic or blood vessel'. Diagnostic possibilities were 'no', 'yes', or 'suspicious for invasion'. Currently, there is no consensus or evidence-based data on the definition of the clear 'deep margin' on endoscopic resections throughout the gastrointestinal tract.[11] For basal margin status assessment in this study, we defined an R1 basal margin as 'tumor touching inked basal resection margin', and an R0 margin as 'no tumor touching ink'.

Online digital histological assessment

During a course of 8 weeks, pathologists assessed the cases independently and in a random order. First, pathologists documented the following information on a digital case record form: presence or absence of the aforementioned histological features, and exact invasion depth in micrometers in case of submucosal invasion (see also Appendix I). Histological features could be measured in scrollable slides at any magnification, representing daily diagnostic practice. Measurements were calibrated automatically, directly related to the zoom level. Study conditions were equal across all slides and all pathologists assessing them. Second, they delineated and annotated the histological features present on a static image of

the H&E slide, to be used in the separate group discussions.

Group discussion and redefinition of diagnostic criteria

After all pathologists had assessed all cases, three group discussions were held in which all cases without a majority diagnosis (see below) for any of the four histological parameters were discussed with the whole group.

Preprocessing analysis

Homogeneity of the group of pathologists was evaluated by looking at clinically relevant deviations (e.g. when a single pathologist diagnosed deep submucosal invasion while all other pathologists diagnosed the invasion depth to be limited to the mucosa). These deviations were given 'penalty points', in which three penalty points were given if a pathologist was the only one to be discordant (1 vs. 12), two penalty points if the discordance was 2 versus 11, and one penalty point if the discordance was 3 versus 10. For lymphovascular invasion, a discordant diagnosis of 'suspicious' was awarded with 50% of the penalty points. For basal margin status, a diagnosis 'not assessable' was also given penalty points when it deviated from the majority vote.

Outcome measurements

A pathologist's diagnosis was considered 'concordant' if his/her diagnosis on a histological parameter matched that of the majority of the pathologists included in the analysis and 'discordant' if his/her diagnosis differed from the majority. Concordance was expressed as the proportion of cases with unanimous agreement (13 pathologists) or a majority diagnosis (12 out of 13, 11/13, or 10/13 pathologists). Concordance was first reported considering all potential diagnostic categories as separate entities. Furthermore, diagnostic categories were grouped into clinically relevant subgroups. For depth of invasion, grouping was based on standard endoscopic treatment criteria of early BE neoplasia (i.e. 'mucosal' [m1-m2-m3] *vs.* 'submucosal' [sm1-sm2-sm3]) and on the expanded criteria (i.e. m1-m2-m3 plus sm1 *vs.* sm2-sm3). For differentiation grade, diagnoses of well-differentiated and moderately differentiated cancers were combined versus poorly differentiated cancers. For lymphovascular invasion, the diagnostic categories 'yes' and 'suspicious' were grouped. For basal margin status, 'not assessable' was kept separate from the R0 and R1 category.

RESULTS

Preprocessing analysis of homogeneity

Figure 1 shows the penalty points of the pathologists for depth of invasion, tumor differentiation, lymphovascular invasion, and basal margin status. Based on the total number of penalty points per pathologist, we identified four pathologists as outliers and excluded their assessments from the analyses of this study.

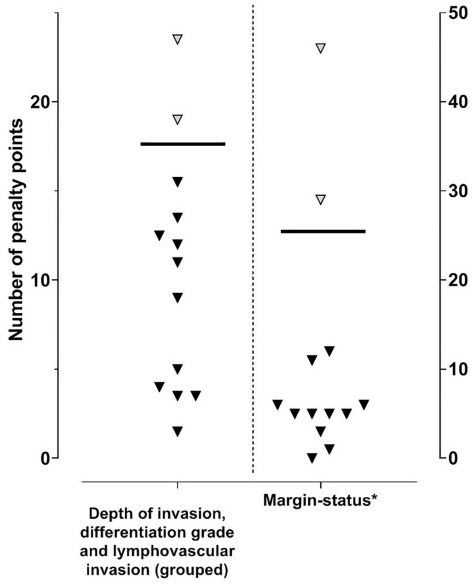

Fig. 1 Penalty points for clinically relevant deviations compared to the majority diagnosis of the participating 13 pathologists. *Margin status depicted separately due to the diagnostic category 'not assessable'. **Gray symbols denote pathologists whose assessments were excluded from the analysis of the study.

Overall concordance

Table 1 shows the percentage of cases with different levels of concordance of nine pathologists for diagnosing depth of invasion, differentiation grade, lymphovascular invasion, and basal margin status. Combining diagnostic categories into clinically relevant subgroups improved concordance, yet for all four histological parameters, a significant number of cases remained where one or more pathologists disagreed with the majority vote of the others.

Concordance for depth of invasion and causes of discrepancy

For depth of invasion, guideline-based subgrouping of mucosal versus submucosal cancers was associated with unanimous agreement in 34/62 cases (55%). When we used the expanded criteria (i.e. grouping sm1 cancers with mucosal cancers), the number of discordant cases decreased significantly, with unanimous agreement in 49/62 cases (79%). In the cases without unanimous agreement, this was due to fragmentation of the muscularis mucosae, artifacts, and unequivocal interpretation in angle of measurement between muscularis mucosae and deepest tumor infiltration. In the group discussion, the pathologists observed that the exact measurement of depth of invasion depends on three factors: (i) the deepest point of submucosal invasion in the cross section; (ii) interpretation of the

Table 1 Number and percentage of cases with different levels of concordance of 9 pathologists in 62 endoscopic resection specimens of early Barrett's cancers

	Number of pathologists in agreement				
Depth of invasion	9 out of 9 (%)	≥8 out of 9 (%)	≥7 out of 9 (%)	≥6 out of 9 (%)	≥5 out of 9 (%)
6 categories: m1-m2-m3-sm1-sm2-sm3	12 (19%)	26 (42%)	38 (61%)	50 (81%)	57 (92%)
'mucosal' versus 'submucosal'	34 (55%)	46 (74%)	54 (87%)	57 (92%)	62 (100%)
'mucosal+sm1' versus 'deep submucosal'	49 (79%)	51 (82%)	55 (89%)	58 (94%)	62 (100%)
Differentiation grade					
3 categories: G1-G2-G3	5 (8%)	18 (29%)	34 (55%)	47 (76%)	61 (98%)
Well/moderate differentiation versus poor differentiation	45 (73%)	46 (74%)	54 (87%)	59 (95%)	62 (100%)
Lymphovascular invasion					
2 categories ('no' vs. 'yes' and 'suspicious')	36 (58%)	47 (76%)	55 (89%)	59 (95%)	62 (100%)
Margin status					
R0 versus R1	42 (68%)	54 (87%)	58 (94%)	61 (98%)	62 (100%)

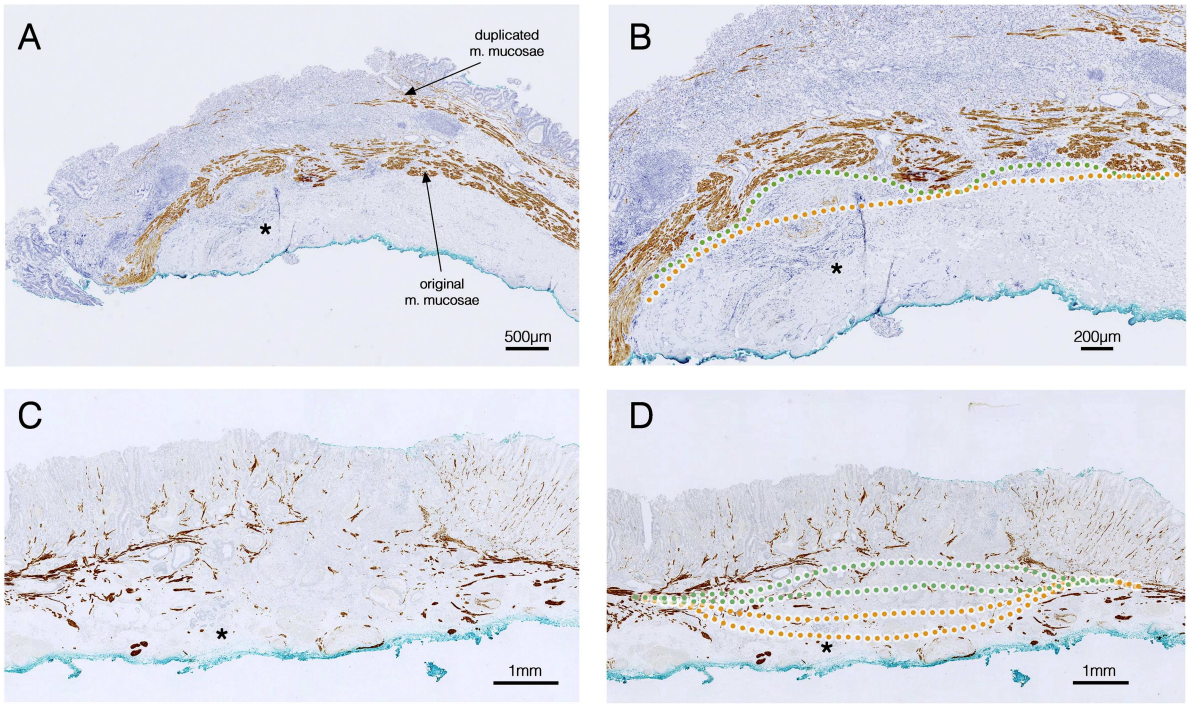

Fig. 2 Tumor infiltration depth is measured by a perpendicular line drawn from the deepest reaching muscle fibers of the original muscularis (m.) mucosa to the deepest tumor cells. Interpretational differences on its course result in assessment discordances of submucosal infiltration depth. (A) Desmin stain visualizes the slight undulating appearance of an intact original m. mucosa (asterisk representing deepest submucosal tumor infiltration). (B) Assessment discordances due to interpretation differences on the deepest muscle fibers of the m. mucosae in relation to tumor infiltration depth (asterisk). Three pathologists measured perpendicular to the (area around the) yellow line (range 300–450 µm, sm1), six pathologists perpendicular to the (area around the) green line (range 542–700 µm, sm2). After discussion, it was measured according to the yellow line (sm1). (C) Desmin stain visualizes a fragmented original m. mucosa (asterisk representing deepest submucosal infiltration). (D) Same desmin stain resulting in sm1 when measured perpendicular to the area between yellow lines (range 40–450 µm, four pathologists), in sm2/3 when measured perpendicular to the area between green lines (range 650–1000 µm, five pathologists).

original course of the muscularis mucosae in the area where the cancer invaded the muscularis mucosae; and (iii) the angle of measurement between these two (Fig. 2).

Concordance for differentiation grade and causes of discrepancy

The distinction between well-/moderately differentiated cancers (G1–G2) and poorly differentiated cancers (G3) showed unanimous agreement in 45/62 cases (73%). In the group discussions, it was observed that most discordances were caused by differences in relating the volume of the observed poorly differentiated focus to the estimated total volume of the tumor, and not by discordances in evaluating the architectural changes of that particular focus (Fig. 3).

Concordance for lymphovascular invasion and causes of discrepancy

Unanimous agreement in the diagnosis of lymphovascular invasion was observed in 36/62 cases (58%). Most discordances reflected interpretational

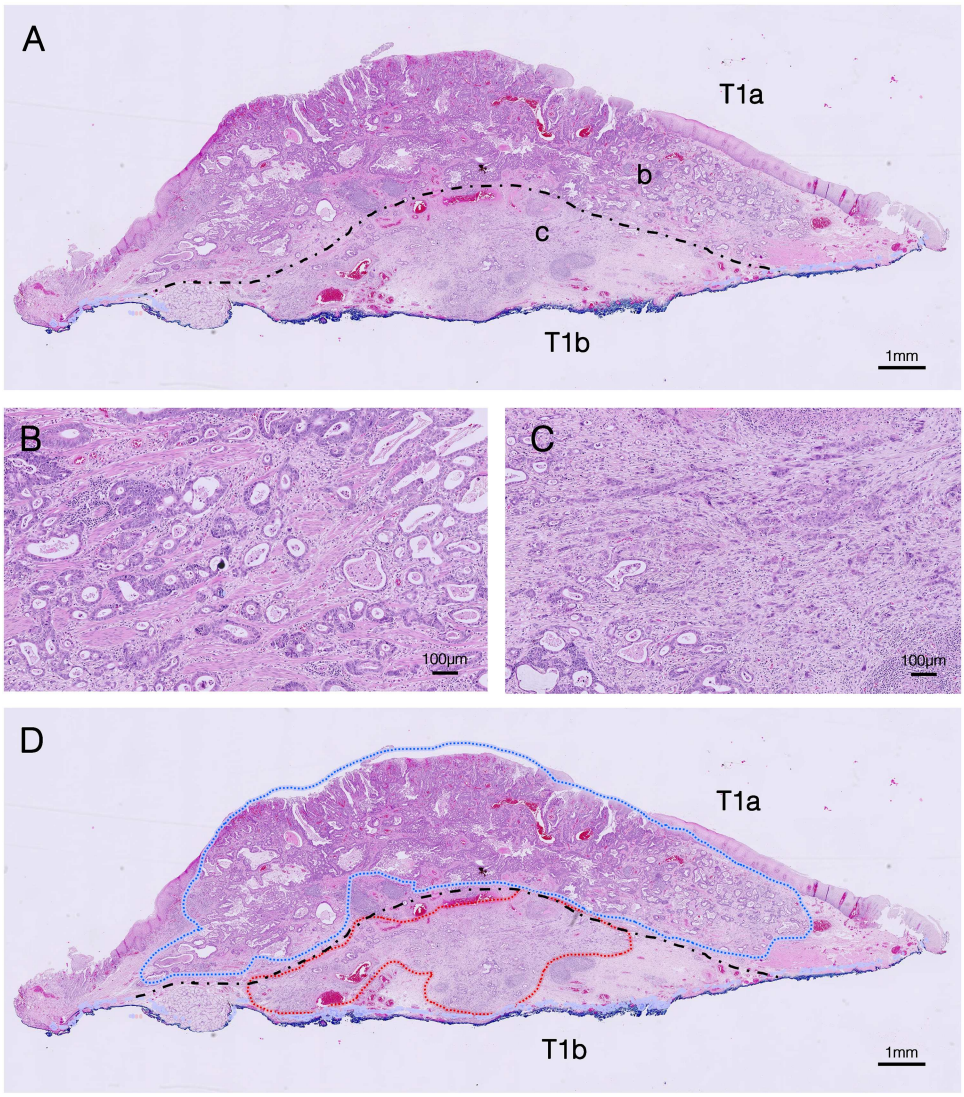

Fig. 3 Heterogeneous differentiation patterns and distribution, leading to assessment discordances in tumor grading. (A) HE stain shows intramucosal and submucosal tumor distribution (black dotted line represents original m. mucosa). The intramucosal component (T1a, blue) comprises 70% of tumor volume. The submucosal component (T1b, red) comprises 30%. (B) HE stain shows intramucosal tumor (T1a), uniformly well differentiated. (C) HE stain shows submucosal tumor (T1b) showing heterogeneous differentiation with >50% poor differentiation, rendering this clinically most relevant, submucosal tumor component a grade (G)3 tumor. (D) HE stain shows total volume of poor differentiation (intra- and submucosal combined) is <50% of tumor volume, grading the total tumor G2. After discussion, it was graded G3 due to clinical relevance (originally G2 by seven pathologists, G3 by two).

differences about whether small clusters of tumor cells were actually located within a vascular structure (20/26 cases, 76%) or whether minute foci morphologically truly consisted of tumor cells (3/26 cases, 12%). Only a minority of discordances reflected overlooked foci (3/26 cases, 12%; Fig. 4).

Concordance for basal margin status and causes of discrepancy

Unanimous agreement was reached in 42/62 cases (68%). Discordances were caused by differences in growth pattern interpretation in 13/20 cases or by artifacts (including curling of the lateral margin) in 7/20 cases (Fig. 5).

Propositions on assessment of ER specimens

During the group discussions that were organized to reach consensus on each feature for each case, and to evaluate causes of discrepancy, a series of propositions (Table 2) was established to aid pathologists when assessing ER specimens in clinical practice.

DISCUSSION

The key finding of this descriptive study is that all histological features, which according to guidelines warrant a subsequent esophagectomy, have significant interobserver variability. The main reason for this is that despite existing guidelines, the exact

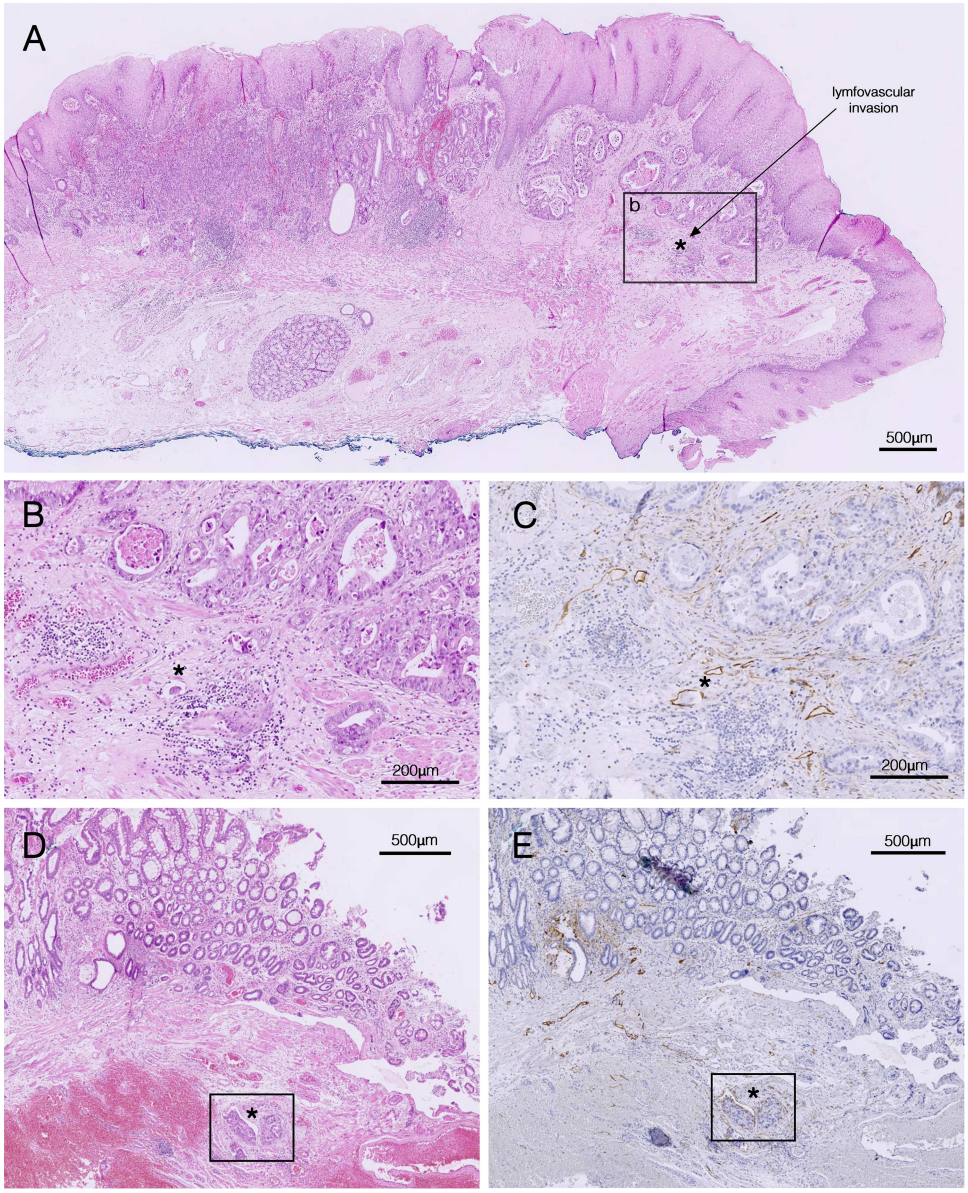

Fig. 4 Subtle and distinct examples of lymphovascular invasion. (A) HE stain shows a small focus of lymphovascular invasion (box and asterisk), originally diagnosed 'not present' by six pathologists and 'present' by three. (B and C) Zoomed HE stain of lymphovascular invasive focus (asterisk), confirmed with vascular marker in C. (D and E) HE stain of distinct vascular invasion (box and asterisk) in D, confirmed with vascular marker in E (originally diagnosed as 'present' by nine pathologists).

histological interpretation of these features leaves room for subjective assessment, for which relatively logical new propositions might be appropriate.

It should be noted that our set of ER specimens was purposely enriched for histological risk features; the aforementioned percentages of discordance, therefore, cannot be considered to reflect the frequency of discordant diagnoses for all ER specimens of BE neoplasia. The concordance will likely be higher for those ER specimens with mucosal cancer, which constitute the majority of ER resections in BE and in which features such as poor tumor differentiation, lymphovascular invasion, and tumor involvement of the basal margin are relatively rare. For example, Worrell et al. reported discordances between two GI pathologists in depth of invasion, presence of lymphovascular invasion, and tumor grade in 48%, 25%, and 44%, respectively, while cases with a positive resection margin or interpretation difficulties due to tangential cutting were excluded. Therefore, the rate of discordances in this study is likely to be higher.[2] Gotink et al. reported high discordances for submucosal invasion assessment, even though assessment criteria had been established beforehand. Moreover, discordances increased as depth of invasion increased from mucosal to submucosal invasion.[1] Both studies therefore confirm our presumptions.

More specifically for depth of invasion, most discordances in our study reflected the difficulty of distinguishing m3-sm1 cancers. This distinction,

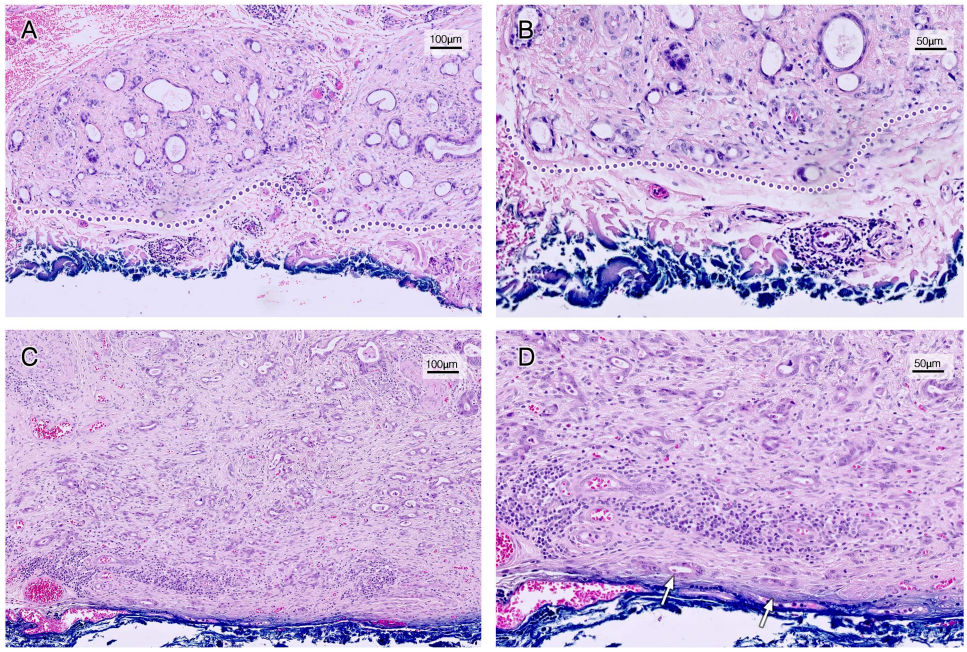

Fig. 5 Basal margin involvement by tumor. (A and B) Tumor located along the invasive front, with a minor fibrous zone (denoted by purple dotted line, width approximately 100 µm) not involved by tumor between tumor area and resection margin (diagnosed R0 by five pathologists and R1 by four). After discussion, margin status was deemed R0. (C and D) Tumor located along the invasive front, with subtle presence of atypical glands within the ink (white arrows, uniformly diagnosed R1).

however, may be clinically less relevant since patients with an sm1 cancer without G3 differentation or lymphovascular invasion are accepted more and more as candidates for endoscopic treatment.[13–15] By grouping m3 and sm1 cancers, the presence or absence of (borderline) submucosal invasion becomes less of an issue; the focus shifts to measuring the exact depth of invasion in those cases with evident (deeper) submucosal invasion. This might not only simplify grading of challenging cases but also could further improve concordance between pathologists.

For differentiation grade, >50% of tumor volume should be poorly differentiated to grade a tumor as G3.[16] However, the WHO grading system is based on surgical resection specimens with advanced, bulky tumors, and one may question if this definition is appropriate when assessing early neoplastic lesions. For example, an early BE cancer with a 5-cm diameter intramucosal (T1a) component, containing a 0.5-cm focus of submucosal invasion with poorly differentiated features, is graded as G1, whereas a 0.5-cm intramucosal (T1a) cancer with a similarly sized poorly differentiated intramucosal component is classified as G3. For other histological features (i.e. submucosal invasion), we refrain from using cutoff values based on a part of the entire lesion, instead scoring the most advanced component. In our opinion, it would be logical to apply the same principle to scoring the differentiation grade of ER specimens. To both underscore the worst biological properties of early cancer present and improve interobserver variability, we propose to label the poorest differentiation grade present (Table 2).

In practice, the decision to proceed to an esophagectomy is a joint effort, made in a multidisciplinary team meeting (Table 2).[15]

Our study included pathologists with a high exposure to BE neoplasia. In addition, over the last 4 years, all pathologists have participated in a structured self-assessment program with face-to-face group meetings to build a national digital review panel for BE biopsy cases diagnosed 'indefinite for dysplasia' and 'low-grade dysplasia'.[5–7] Regarding ER specimens, there was a range of years of experience and the number of ER specimens assessed within this group. To prevent possible outlier effects, we artificially improved the assessment homogeneity in the current study by excluding the four pathologists with the highest number of penalty points for clinically relevant deviations. In our opinion, our results therefore reflect the performance of expert BE pathologists. We speculate that for pathologists with less exposure to BE neoplasia, variability in the assessment of clinically relevant histological features may be present at an even higher rate.

This descriptive study has a number of unique features. It is the first study to assess the diagnostic concordance on ER specimens of such a large, homogenous group of expert pathologists on a large number of digitalized ER cross sections. This study is part of a joint training program for pathologists working at the Dutch BE expert centers. It is designed to guarantee the quality and uniformity of histological assessment for all Dutch patients treated endoscopically for early BE cancer. Our study has a number of

Table 2 Recommendations for histopathological assessment of endoscopic resection specimens of early Barrett's cancer

Feature	Description
General	1. When a histological feature in an endoscopic resection specimen potentially pushes the patient from endoscopic management to the need for a surgical esophagectomy or additional chemoradiotherapy, we propose expert review by at least one other expert pathologist. 2. We propose to discuss every case with equivocal high-grade features in a multidisciplinary meeting with a gastroenterologist, pathologist, radiologist, surgeon, and oncologist in attendance, to decide on further treatment and/or follow-up.
Depth of invasion	3. In case of invasion of tumor into the submucosa: we advise to measure depth of invasion in micrometers, perpendicular from the lower margin of the muscularis mucosae to the deepest point of tumor invasion. Variability in this measurement is caused by: (i) uncertainty in the identification of the deepest point of invasion of tumor cells; (ii) uncertainty concerning the exact position of the lower margin of the muscularis mucosae; and (iii) interpretation of the angle with which these 2 points are connected. To reduce uncertainty, we propose the following measures: a. Obtain multiple additional cuts of the deepest point of invasion for optimal evaluation. b. Use antidesmin immunohistochemical staining to highlight the course of the muscularis mucosae. c. In case of destruction of the muscularis mucosae by tumor, use a virtual line representing the presumed course of the lower margin of the muscularis mucosae as starting point for the measurement of depth of invasion (Figure 2). d. If infiltration depth into the submucosa is not unequivocal, perform multiple measurements and report a range of uncertainty (e.g. '250–350 micrometers').
Differentiation grade	According to WHO for tumors of the digestive tract[12]: • G1 ('well'): >95% gland formation • G2 ('moderate'): 50–94% gland formation • G3 ('poor'): up to 49% gland formation 4. In case of heterogeneous differentiation grades, we propose to grade the tumor according to the poorest differentiation grade, irrespective of its relative volume.
Lymphovascular invasion	5. We propose this definition: 'the unequivocal presence of tumor cells in a blood- or lymph vessel'. a. In case of uncertainty for a single area suspicious for lymphovascular invasion, obtain additional cuts and parallel immunohistochemistry using an endothelial marker with a preferentially circumferential staining result to consider the area positive. b. If possible, differentiate lymphovascular invasion from invasion in blood vessels using immunohistochemistry (CD31 vs. D2–40 staining). c. Report lymphovascular invasion as focal (1–2 foci) or multifocal (≥3 foci).
Basal margin status	6. We propose a 3-tiered definition: a. If tumor infiltrates into the submucosa but does not touch the inked basal resection margin, the exact distance from the deepest tumor border to the basal resection margin should be measured (in micrometers) and mentioned in the final report. b. Obtain additional cuts and a keratin immunohistochemical stain in case this margin is less than 100 micrometers; if the growth pattern is poorly differentiated; or if substantial cauterization of the basal resection margin is present. If the additional cuts are negative for 'tumor touching ink', we propose the basal margin to be deemed free of tumor (R0). c. An R1 resection is defined as tumor invading into the inked basal resection margin ('tumor touching ink'), after evaluation of deeper cuts and performance of additional immunohistochemistry, irrespective of the growth pattern.

limitations. The cases were preselected based on H&E slides and only a single cross section per case with a limited number of additional stainings was used for the study assessments. Second, one pathologist aided in the selection of cases and also assessed the study set. A 'wash-out' period of 1 year was taken into account for this pathologist. Lastly, we are aware of the fact that we did not yet validate the propositions in Table 2.

In conclusion, the histological assessment of ER specimens shows significant variability even among expert BE pathologists. This can be partially overcome by categorizing the assessments for depth of invasion into clinically relevant groups. For the other features, the diagnostic criteria may require further specification. In many BE expert centers, the assessment of BE neoplasia is performed by a single pathologist. To assist the aforementioned required review, a digital review platform may facilitate the exchange of digitalized microscopic images. To improve general knowledge in this difficult field, excellent explanatory reviews exist in which diagnostic challenges are assessed.[10,11]

AUTHOR CONTRIBUTIONS

Study concept and design: M.J. van der Wel, S.L. Meijer, J.G. Tijssen, J.J.G.H.M. Bergman. Acquisition of data: M.J. van der Wel, E. Klaver, L.A.A. Brosens, K. Biermann, M. Doukas, C. Huysentruyt, A. Karrenbeld, F.J.W. ten Kate, G. Kats-Ugurlu, J. van der Laan, I. van Lijnschoten, F.C.P. Moll, G.J.A. Offerhaus, A.H.A.G. Ooms, C.A. Seldenrijk, M. Visser, J.G. Tijssen, S.L. Meijer, J.J.G.H.M. Bergman. Analysis and interpretation of data: M.J.

van der Wel, E. Klaver, J.G. Tijssen, S.L. Meijer, J.J.G.H.M. Bergman. Drafting of the manuscript: M.J. van der Wel, E. Klaver, S.L. Meijer, R.E. Pouw. Critical revision of the manuscript: M.J. van der Wel, E. Klaver, J.J.G.H.M. Bergman, K. Biermann, M. Doukas, C. Huysentruyt, A. Karrenbeld, F.J.W. ten Kate, G. Kats-Ugurlu, J. van der Laan, I. van Lijnschoten, F.C.P. Moll, G.J.A. Offerhaus, A.H.A.G. Ooms, C.A. Seldenrijk, M. Visser, J.G. Tijssen, S.L. Meijer, J.J.G.H.M. Bergman, R.E. Pouw. Study supervision: S.L. Meijer, J.J.G.H.M. Bergman.

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DISCLOSURES

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Appendix I: Online case record form for documentation of diagnostic feature assessments per case

Diagnostic features

1. Differentiation grade (according to WHO classification)[12]
    a. Well
    b. Moderate
    c. Poor
    d. Undifferentiated
2. Vascular invasion:
    a. No
    b. Yes
    c. Suspicious
3. Radicality basal margin:
    a. R0
    b. R1
    c. Not assessable
4. **Assessment**
5. Diagnosis of case (AJCC):
    a. T1m1/T1m2/T1m3/T1sm1 (depth approx. xx um)
    b. T1sm2 (depth approx. xx um)
    c. T1sm3 (depth approx. xx um)
    d. Other
        i. High-grade dysplasia
        ii. Squamous cell carcinoma
        iii. Not assessable
6. **Images**
7. Quality of scan:
    a. In focus
    b. Out of focus
8. **General**
9. Comments