Optimizing histopathologic evaluation of EMR specimens of Barrett's esophagus–related neoplasia: a randomized study of 3 specimen handling methods

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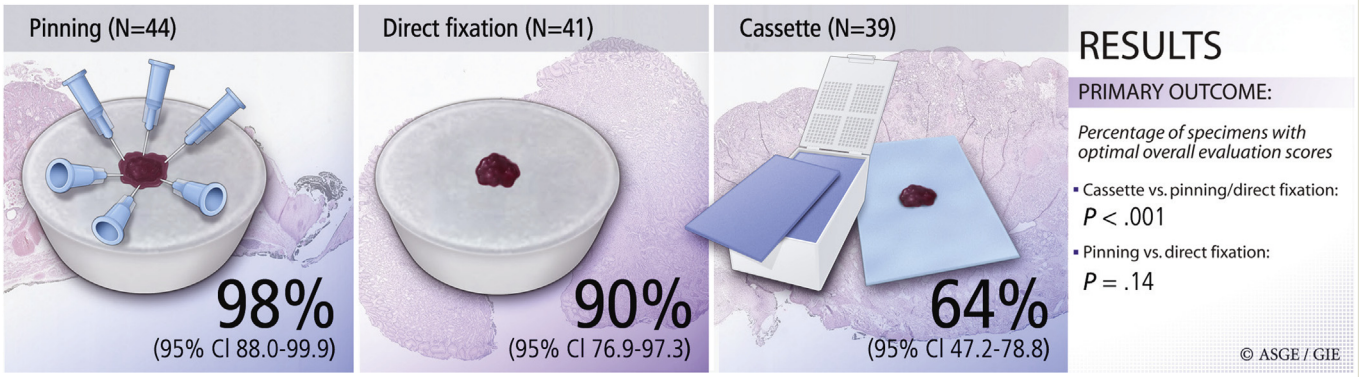
GRAPHICAL ABSTRACT



Background and Aims: Endoscopic resection is the cornerstone of treatment of Barrett's esophagus (BE)-related neoplasia. However, accurate histopathologic evaluation of endoscopic resection specimens can be challenging, and the preferred specimen handling method remains unknown. Therefore, the aim of our study was to compare 3 different specimen handling methods for assessment of all clinically relevant histopathologic parameters and time required for specimen handling.

Methods: In this multicenter, randomized study EMR specimens of BE-related neoplasia with no suspicion of submucosal invasion during endoscopy were randomized to 3 specimen handling methods: pinning on paraffin using needles, direct fixation in formalin without prior tissue handling, and the cassette technique (small box for enclosing specimens). The histopathologic evaluation scores were assessed by 2 dedicated GI pathologists blinded to the handling method.

Results: Of the 126 randomized EMR specimens, 45 were assigned to pinning on paraffin, 41 to direct fixation in formalin, and 40 to the cassette technique. The percentages of specimens with overall optimal histopathologic evaluation scores were similar for the pinning method (98%; 95% confidence interval [CI], 88.0-99.9) and for no handling (90%; 95% CI, 76.9-97.3) but were significantly lower (64%; 95% CI, 47.2-78.8) for the cassette technique ($P < .001$). Time required for specimen handling was shortest when no handling method was used ($P < .001$ vs pinning and cassette).

Conclusions: Both pinning on paraffin and direct fixation in formalin resulted in optimal histopathologic evaluation scores in a high proportion of specimens, whereas the cassette technique performs significantly worse, and its use in clinical daily practice should be discouraged. Given the significantly shorter handling time, direct fixation in formalin appears to be the preferred method over pinning on paraffin. However, the latter needs to be confirmed in larger studies with inclusion of all EMR specimens. (Clinical trial registration number: ISRCTN50525266.) (Gastrointest Endosc 2019;90:384-92.)

(footnotes appear on last page of article)

Endoscopic resection (ER) is the cornerstone of treatment of Barrett's esophagus (BE)–related neoplasia. All visible lesions should be removed for adequate histopathologic evaluation and staging.[1,2] However, accurate histopathologic evaluation of ER specimens can be challenging. One factor in the ability to assess the ER specimen is how the specimen is handled before the assessment. For instance, a proper orientation of the specimen is essential to discern lateral from vertical (deep) resection margins, and artifacts induced by tissue handling might hamper optimal evaluation.[3,4]

Currently, 2 ways of specimen handling are frequently used. First, an ER specimen can be pinned on paraffin or cork using needles, with subsequent fixation in formalin. Most centers in Western Europe and Eastern Asia use this pinning method.[5-7] With this technique, attention must be paid to not create tension on the specimen to avoid artifacts.[7-9] Overextension of the tissue and placement of the pins in neoplastic areas can cause destruction of the tissue and needle artifacts and may influence the accuracy of the histopathologic diagnosis.[5] Moreover, the pinning method is more time consuming, is cumbersome, and involves the use of sharp material. The second method of specimen handling is direct fixation of ER specimens in formalin without prior tissue handling or orientation. This method is popular in other parts of the world, for instance in the United States.[10] Direct fixation in formalin may affect the accuracy of the histopathologic assessment because ER specimens tend to shrink by fixation with formalin and tend to curl because of contraction of the muscle fibers of the muscularis mucosae.[11]

Because of disadvantages of existing methods for specimen handling, a new technique has been developed. This new method, referred to as the cassette technique (Boston Scientific, Marlborough, Mass), comprises a small box in which the ER specimen can be stored (Fig. 1). Possible advantages of this method are that it is faster and provides and easier specimen orientation in comparison with the pinning method without derogating the histopathologic evaluation.

To the best of our knowledge, studies on specimen handling techniques and their effect on the ability to evaluate all relevant histopathologic parameters of the resected specimen have never been performed. Therefore, the aim of this study was to compare 3 different methods of specimen handling for the ability to enable optimal overall evaluation of all clinically relevant histopathologic parameters of ER specimens with no suspicion of submucosal invasion and the time required for specimen handling. The 3 different handling methods are the pinning method, direct fixation in formalin, and the cassette technique.

METHODS

Study design

This multicenter, randomized study was performed in 3 hospitals in the Netherlands with a tertiary referral function for the treatment of patients with BE. The Medical Ethics Review Committee of the Academic Medical Center Amsterdam evaluated the study protocol and stated that the Medical Research Involving Human Subjects Act does not apply to this study (reference no. W15_172#15.0209). The study was registered in the International Standard Randomised Controlled Trial Number (ISRCTN) registry with reference number ISRCTN50525266. The article was written according to the CONSORT guidelines for reporting parallel group randomized studies.[12] Full study protocol is available on request by the principle investigator.

Endoscopic resection

High-definition endoscopes were used for this study. The Barrett segment and the lesion were carefully inspected; the Prague C&M criteria and lesion characteristics (location, size, circumference, Paris classification) were documented. ERs were performed with the Captivator EMR device (Boston Scientific) or the Duette multiband mucosectomy device (Cook Medical, Limerick, Ireland). Patient management on the endoscopy ward and clinical decision-making were conducted according to standard of care in all participating hospitals.

Specimen selection

Visible BE lesions with a poor tumor differentiation if known beforehand were excluded from randomization. Specimens in which 1 or more EMRs were performed on areas with suspicion of submucosal invasion during endoscopy were also excluded from randomization. Suspicion of submucosal invasion was based on thorough endoscopic inspection (detailed description provided elsewhere[13]), taking into account endoscopic features suggestive for submucosal invasion (ie, Paris classifications 0-I, IIc, and 0-III[14-16]). EMR specimens of lesions with no suspicion of submucosal invasion or remaining specimens after exclusion of EMR specimens with suspicion of submucosal invasion were selected for randomization. In case of a piecemeal resection, multiple specimens were randomized per patient.

Specimen handling

After EMR was completed and the specimens with suspicion of submucosal invasion excluded, eligible EMR specimens were randomized to 1 of 3 EMR specimen handling methods: the pinning method, direct fixation in formalin with no prior handling, and the cassette technique (Fig. 1). The cassette is a small box, available for clinical daily practice, in which an ER specimen can be enclosed in between 2 sponges after stretching it out on paper. By closing the cassette, gentle pressure is applied on the specimen during the process of formalin fixation, which prevents curling of the lateral margins of the resection specimen. The pinning method comprises smooth stretching of the ER specimen and pinning the specimen

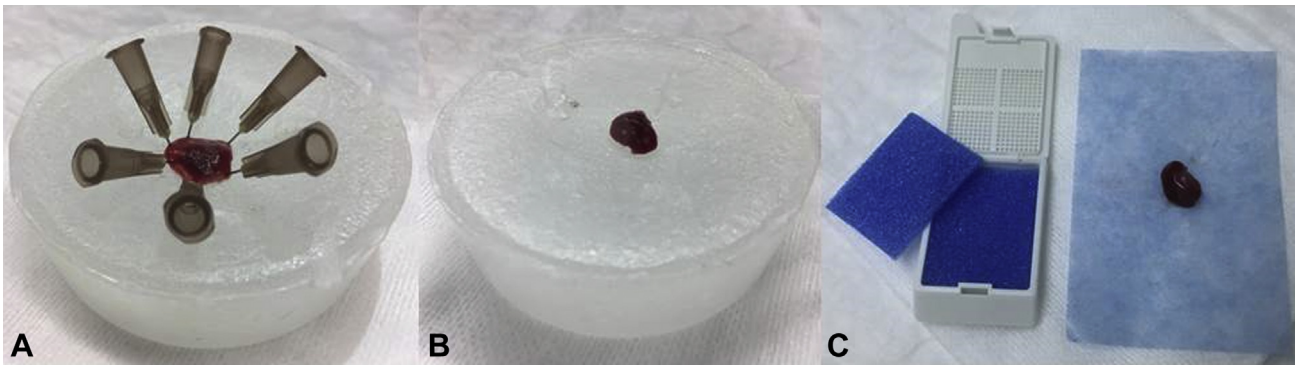


Figure 1. Images of all 3 specimen handling techniques. **A,** Pinning on paraffin. **B,** Direct fixation in formalin. **C,** Cassette technique.

out on cork or paraffin. Direct fixation of the ER specimen in formalin requires no handling at all. Time required for specimen handling was defined as the time of starting the specimen handling procedure until the specimen was fixed in formalin.

Randomization

After ER all specimens were collected and temporarily stored on a gauze in a random order. Specimens with suspicion of submucosal invasion during endoscopy were kept separately and excluded from randomization. To each eligible EMR specimen a number was allocated by the endoscopist. Block randomization with variable block sizes of 3 and 6 was performed 1:1:1 to 3 parallel specimen handling groups, stratified per hospital. The blocked randomization list was created using sealed envelope (https://www.sealedenvelope.com/simple-randomiser/v1/lists) and incorporated in an online randomization tool in REDCap.[17] Randomization was performed after ER in the endoscopy room by the research nurse.

Histopathologic analysis

All EMR specimens were fixed in buffered formalin (10%), embedded in paraffin, and stained with hematoxylin and eosin after 1 of the 3 handling techniques. The dissection plane created by ER was marked with ink at the pathology department after fixation with 1 of 3 specimen handling methods. The specimens were cut perpendicular to the surface in slices of 2 to 3 mm and oriented. The specimens were completely enclosed. On each specimen with tumor invading the submucosa, immunohistochemistry staining for desmin, alpha smooth muscle actin, CD34, and D2-40 were done to accurately assess the clinically relevant parameters like depth of invasion and lymphovascular invasion.

First, all specimens were evaluated by dedicated GI pathologists in participating centers according to standard clinical practice to guide further treatment strategies and clinical decision-making. Thereafter, 2 dedicated BE GI pathologists (G.M.R. and C.A.S.) revised all EMR specimens for the purpose of this study, blinded for the specimen handling method used. All specimens were scored for a total of 12 parameters on a 5-point Likert scale (Research Pathology Form, Appendix 1, available online at www.giejournal.org). Of these, 5 were considered to reflect the clinically relevant issues for optimal histopathologic assessment (ie, vertical resection margin, lateral resection margins, tumor differentiation grade, tumor infiltration depth, and lymphovascular invasion). The remaining 7 parameters were related issues that might account for the possible differences in scores on the clinically relevant parameters (ie, curling of the lateral margins, ability to discern lateral from vertical margins, crushing of the specimen, damaged superficial tissue layers, crushing and damage interfering with the ability to assess the specimen, and orientation of the specimen). In addition to these 12 parameters, pathologists were asked to score the *overall ability* to assess all relevant histopathologic parameters on a separate 5-point Likert scale.

All 5-point Likert scales were rearranged so that a score of 5 was optimal and a score of 1 least optimal. Scores from 1 to 4 were defined as suboptimal. Consensus between pathologists, when needed, was reached by average.

Outcome parameters

The primary endpoint of this study was the percentage of specimens with an optimal score on a 5-point Likert scale (score of 5) for the *overall ability* to assess all relevant histopathologic parameters versus the percentage of specimens with suboptimal scores (scores 1-4). Secondary endpoints were (1) the percentages of specimens with optimal scores (vs suboptimal scores) on a 5-point Likert scale for the ability to adequately assess the vertical (deep) resection margin, lateral resection margins, tumor differentiation grade, tumor infiltration depth, and lymphovascular invasion and (2) the time necessary for handling of the ER specimen.

Data management

Study data were collected and managed using REDCap electronic data capture tools hosted at St Antonius Hospital. REDCap (Research Electronic Data Capture) is a

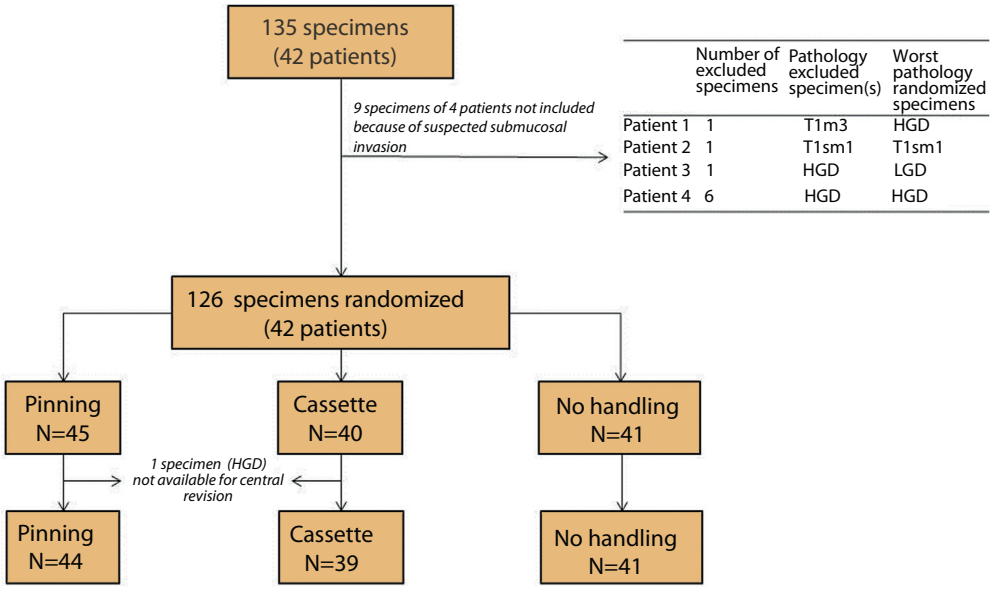


Figure 2. Flowchart of specimen inclusion and randomization. *HGD*, High-grade dysplasia; *LGD*, low-grade dysplasia.

secure, web-based application designed to support data capture for research studies, providing an intuitive interface for validated data entry, audit trails for tracking data manipulation and export procedures, automated export procedures for seamless data downloads to common statistical packages, and procedures for importing data from external sources.[17]

Statistical procedures

No formal sample size calculation was conducted for this study because there were no previously published data available on this subject. Randomization of 40 EMR specimens per specimen handling method was considered sufficient. IBM SPSS statistics version 21.0.0 for Windows (SPSS, Chicago, Ill) and R version 3.3.2 for Windows were used for statistical analyses. All statistical tests were 2-tailed, and statistical significance was defined as $P < .05$. For descriptive statistics, continuous variables with a normal distribution were reported as mean with standard deviation, and median with interquartile range (IQR) was used for variables with a skewed distribution. Pearson-χ^2 test was used to analyze nominal data and compare proportions.

RESULTS

Specimen characteristics

In total, 126 EMR specimens of 42 patients (for patient characteristics refer to Supplementary Table 1, available online at www.giejournal.org) were randomly assigned to 1 of 3 specimen handling groups. Specimens were randomized between February 2016 and March 2017, and histopathologic evaluation was finalized by March 2018. In 4 patients, 9 specimens were not included for randomization because of suspicion of submucosal invasion during endoscopy (Fig. 2).

Of the 126 randomized EMR specimens, 45 were assigned to the pinning method, 41 to direct fixation in formalin, and 40 to the cassette technique (Table 1). On histology, submucosal invasion was present in 5 of the 126 randomized specimens (4.0%). Of these specimens, 2 had a poor tumor differentiation and none had positive deep (vertical) resection margins or lymphovascular invasion. Two specimens (1 cassette with high-grade dysplasia and 1 pinning method with high-grade dysplasia) were not available for central revision during the period of histopathologic evaluation; thus, 124 specimens were eligible for the analyses (Fig. 2).

Primary outcome: overall histopathologic evaluation scores

Overall, 85% of specimens (105/124) had an optimal score for the ability to assess all relevant histopathologic parameters (5 on a 5-point Likert scale, Fig. 3). The percentages of specimens with an overall optimal score were 98% (95% confidence interval [CI], 88.0-99.9) for the pinning method and 90% (95% CI, 76.9-97.3) for no handling method, both significantly higher compared with 64% (95% CI, 47.2-78.8) for the cassette technique ($P < .001$, Table 2).

Secondary outcomes: evaluation scores per histopathologic parameter and required handling time

For the cassette technique, in comparison with both the pinning method and no handling method, significantly

TABLE 1. Baseline characteristics of endoscopic resection specimens per specimen handling group (n = 124)

	Total (n = 124*)	Pinning on paraffin (n = 44)	No handling (n = 41)	Cassette (n = 39)	*P* value
Invasion depth					.84
Nondysplastic Barrett's epithelium	21 (17)	8 (18)	9 (22)	4 (10)	
Low-grade dysplasia	33 (27)	11 (25)	10 (24)	12 (31)	
HGD	38 (31)	13 (30)	10 (24)	15 (38)	
T1m2	3 (2)	1(2)	1 (2)	1 (3)	
T1m3	24 (19)	9 (21)	8 (20)	7 (18)	
T1sm1	4 (3)	2 (4)	2 (5)	0 (0)	
T1sm2	1 (1)	0 (0)	1 (2)	0 (0)	
Differentiation grade					.63
Well	16 (13)	7 (16)	4 (10)	5 (13)	
Moderate	11 (9)	3 (7)	5 (12)	3 (7)	
Poor	5 (4)	2 (4)	3 (7)	0 (0)	
Not applicable	92 (74)	32 (73)	29 (71)	31 (80)	
Lymphovascular invasion	0 (0)	0 (0)	0 (0)	0 (0)	1.00
Deep resection margins free of tumor	124 (100)	44 (100)	41 (100)	39 (100)	1.00

Values are n (%).
HGD, High-grade dysplasia.
*Two specimens (1 cassette with HGD and 1 pinning method with HGD) were not available for central revision during the period of histopathologic evaluation; thus, 124 specimens were eligible for the analyses.

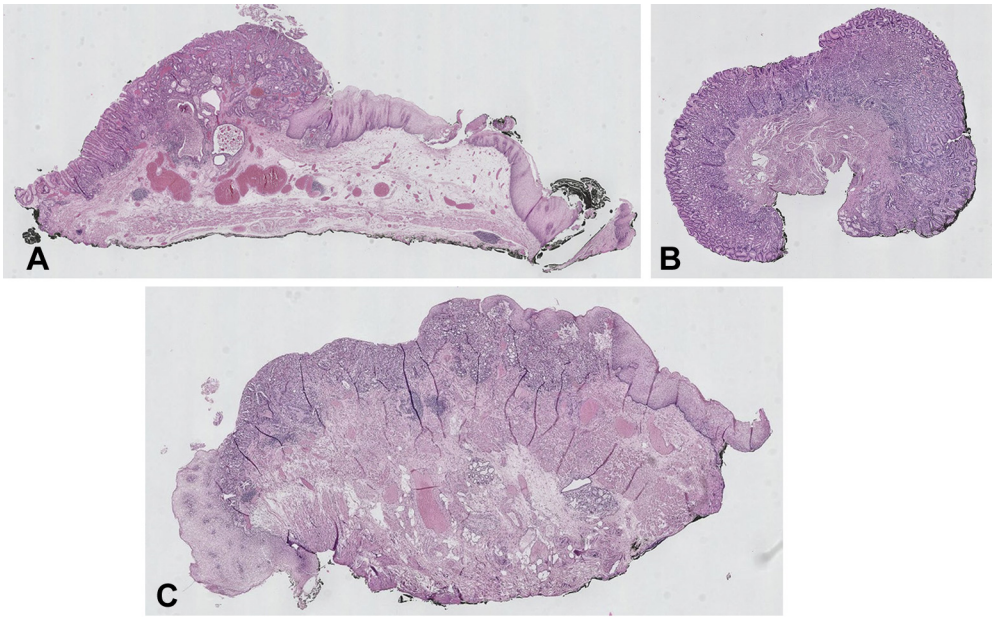


Figure 3. Histopathologic images of endoscopic resection specimens with optimal evaluation scores handled with 3 different specimen handling methods (H&E staining, orig. mag. ×1.25). **A,** Specimen handled with the pinning method. **B,** Specimen directly fixated in formalin. **C,** Specimen handled with the cassette technique.

lower percentages of optimal scores versus suboptimal scores were reported for the evaluation of the ability to discern the lateral from the vertical margin ($P = .001$), the ability to assess the deep/vertical margin ($P = .005$), and the ability to assess the lateral margins ($P = .002$). The 3 handling methods did not result in significantly different ability to optimally assess the orientation, depth of tumor invasion, tumor differentiation, and presence of lymphovascular invasion (Table 2 and Supplementary Table 2, available online at www.giejournal.org). Although

TABLE 2. Ability to assess all relevant histopathological parameters (n = 124)

	Pinning on paraffin (n = 44)	No handling (n = 41)	Cassette box (n = 39)	*P* value
Primary outcome: optimal ability to assess all relevant histopathologic parameters (score of 5 on a 5-point Likert scale)				
Overall optimal score	43 (97.7)	37 (90.2)	25 (64.1)	<.001
				P vs C, *P* < .001
				P vs NH, *P* = .14
				C vs NH, *P* = .005
Frequency of evaluation scores				
Score of 5 (optimal)	43	37	25	
Score of 4	0	2	6	
Score of 3	1	2	5	
Score of 2	0	0	3	
Score of 1 (disastrous)	0	0	0	
Secondary outcomes: optimal ability to assess histopathologic parameters separately (score of 5 on a 5-point Likert scale)				
Discern lateral from vertical margin	40 (90.9)	32 (78.0)	22 (56.4)	.001*
Deep vertical margins	42 (95.5)	37 (90.2)	28 (71.8)	.005*
Lateral margins	40 (90.9)	35 (85.4)	24 (61.5)	.002*
Depth of tumor invasion	42 (95.5)	40 (97.6)	36 (92.3)	.55
Tumor differentiation	44 (100)	42 (100)	39 (100)	1.00
Lymphovascular invasion	44 (100)	42 (100)	39 (100)	1.00

Values are n (%). For the frequency of all evaluation scores for the secondary outcomes please refer to Supplementary Table 2 (available online at www.giejournal.org).
P, Pinning on paraffin; *C,* cassette technique; *NH,* no handling method.
*Significant for both the pinning method and no handling method vs the cassette technique.

crushing of the specimen and damage to the superficial layers did not differ significantly between the 3 handling methods, needle artifacts were observed in 26 specimens handled with the pinning method (59%), significantly more artifacts than those reported by the blinded pathologists for the 2 other, nonpinning handling methods ($P < .001$). For no handling 3 artifacts (7%) were reported and for the cassette technique 4 artifacts (10%), 2 of these as possible needle artifacts and the other 5 as tearing and processing artifacts. Inking of the dissection planes was visible in all ER specimens.

The time required for specimen handling was shortest when no handling method was used (median, .18 minutes; IQR, .10-.30). Both the pinning method and the cassette technique resulted in significantly longer median handling times: 1.0 minute (IQR, 1.0-1.89) for the pinning method and 1.1 minute (IQR, .5-1.45) for the cassette technique ($P < .001$; no handling vs pinning or cassette technique).

DISCUSSION

Histopathologic evaluation of ER specimens of BE-related neoplasia determines the need for additional surgical treatment. ER is considered sufficient treatment for lesions with absence of all risk factors for lymph node metastasis (submucosal invasion >500 μm, lymphovascular invasion, poor tumor differentiation) and tumor negative vertical (deep) resection margins.[18] When 1 of these risk factors is present or if 1 of these parameters cannot be evaluated correctly and thus remains uncertain, current guidelines recommend esophagectomy. Esophagectomy is an invasive surgical procedure associated with high morbidity and mortality.[19,20] Therefore, optimal specimen handling and histopathologic evaluation are important components in optimizing patient care.

Different specimen handling methods can be used after ER. Remarkably, there is little evidence for and no general agreement on the preferred specimen handling method that results in an optimal ability to assess all relevant histopathologic parameters and is least time consuming. Guidelines on management of BE-related neoplasia recommend the pinning method as the preferred specimen handling strategy or make no recommendations at all.[2,6,7,21-24]

This multicenter, randomized study is the first to compare 3 different specimen handling methods for enabling optimal histopathologic evaluation and time required for specimen handling. Our study population was a preselected group of patients with a predicted curative ER. Specimens were only included if there was no suspicion of submucosal invasion during endoscopy, and in all specimens the vertical/deep resection margins turned out to be free of tumor. Optical diagnosis of submucosal invasion of Barrett's neoplasia is challenging. Besides Paris

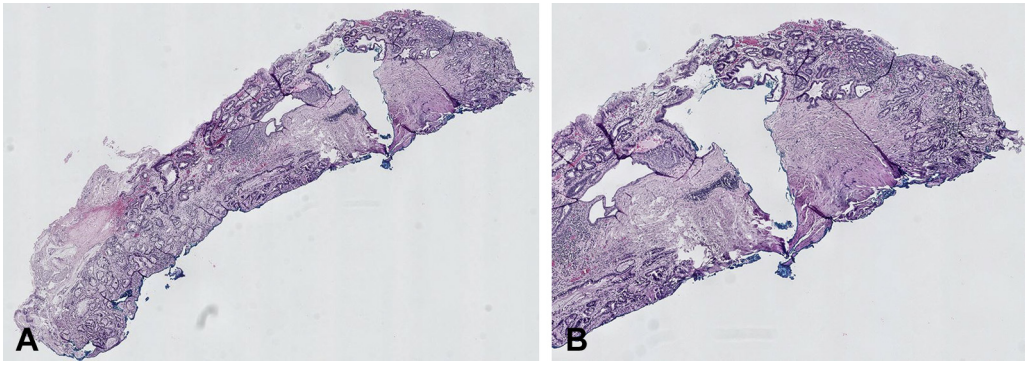


Figure 4. Histopathologic image of a needle artifact (pinning method) through the tumor (H&E staining). **A,** Overview image of the endoscopic resection specimen (orig. mag. ×.25). **B,** Detailed image of the needle artifact invading the tumor area (orig. mag. ×10).

classifications (0-I, 0-IIc, and 0-III), no clear set of endoscopic predictors for the presence of submucosal invasion is reported in the current literature.[13-16] The fact that only 1 lesion with a primary Paris 0-Is classification and 4 lesions with a secondary Paris 0-IIc classification were included in our study mirrors the endoscopists' intention to prevent inclusion of specimens with submucosal involvement.

For this selected population, application of both pinning on paraffin and direct fixation in formalin resulted in high percentages of specimens with an optimal overall histopathologic evaluation score, respectively 98% (95% CI, 88.0-99.9) and 90% (95% CI, 76.9-97.3). The large overlap in CIs indicates that the difference in favor of the pinning method is not statistically significant. However, this result must be interpreted with caution and needs to be confirmed in larger studies because with increasing sample size this difference might become significant. The cassette technique resulted in a significant lower percentage with an optimal score (64%, $P <$ .001), and its use in clinical daily practice should therefore be discouraged. This inferiority was caused by curling of the lateral margins and subsequent deterioration of the assessment of both the deep and lateral margins. A possible explanation for the curling of the lateral margins is that the cassettes do not properly accommodate the regular shape of an ER specimen. Its fixed size and height might result in a stretched middle part but leave too much space for the thinner lateral parts of the specimen, resulting in squeezed curled lateral margins.

In addition to enabling optimal histopathologic evaluation, another aspect of interest is the time required per specimen handling method. As expected, we found that the required handling time per specimen was significantly shorter for direct fixation in formalin compared with pinning on paraffin. Because EMR procedures often consist of multiple resections, the significant shortening of the handling time would favor direct fixation in formalin. Moreover, the use of sharp material for the pinning method, with not only a risk of harming oneself but also damaging the resected specimen, is another argument in favor of direct fixation in formalin. In our study we observed 26 needle artifacts in the specimens handled with the pinning method (59%). Although the frequent presence of needle artifacts did not result in a low percentage of optimal evaluations for the pinning method, in 1 specimen the needle went right through the tumor (Fig. 4). Preferably, this risk is completely eliminated by not using needles at all. These secondary arguments would favor direct fixation in formalin over pinning on paraffin, when comparability for enabling optimal histopathologic evaluation is confirmed.

Specimens with no suspicion of submucosal invasion were selected specifically, because while conducting this study we found it unethical to risk suboptimal histopathologic evaluation for specimens with submucosal invasion. The depth of submucosal invasion, among others, determines whether endoscopy is the definitive treatment or if the patient should be referred to surgery. However, it might well be that direct fixation in formalin would also enable optimal histopathologic evaluation for lesions *with* submucosal invasion: 5 specimens in our study did show submucosal invasion (4 showed T1sm1, 1 showed T1sm2). Of these, 2 were randomized to the pinning method and 3 to direct fixation in formalin. Regardless of the presence of submucosal invasion, all had optimal overall histopathologic evaluation scores. However, based on the current study we cannot draw any conclusions for the optimal specimen handling method for lesions with suspicion of submucosal invasion. Our results hold for lesions with a predicted limited invasion depth on endoscopy.

Lately there is a tendency to resect BE lesions with suspected submucosal invasion en bloc by means of endoscopic submucosal dissection, whereas visible lesions with no suspicion of submucosal invasion can be treated with EMR.[25] Pinning on paraffin or cork should still be considered the preferred handling method for endoscopic submucosal dissection specimens, because these are often much larger, but substantiating evidence is lacking.

This study is the first to assess the optimal specimen handling method for EMR specimens of BE-related neoplasia for both enabling optimal histopathologic

evaluation and minimizing required handling time. In this multicenter, randomized study, procedures were performed in tertiary referral centers by experienced endoscopists, and resected specimens were evaluated for the primary and secondary endpoints by 2 dedicated BE GI pathologists blinded for the specimen handling methods used.

We are aware that our research also has certain limitations. The first limitation is the selection of patients with visible BE lesions with no suspicion of submucosal invasion, which influences the generalizability of our findings. All specimens with possible submucosal invasion were excluded from this study. In daily practice EMR is also used for lesions *with* possible submucosal invasion, and therefore direct extrapolation of our results to all specimens resected by means of EMR is not possible. Second, because of the selection applied, all vertical resection margins were free of tumor, and we do not know how positive vertical resection margins would have influenced the ability for assessment of all histopathologic parameters. Inclusion of specimens with positive resection margins might require evaluation of additional parameters, such as cautery artifacts and tissue inking, which were not assessed as histopathologic parameters in the current study. Third, all endoscopies were performed in BE expert centers by highly experienced endoscopists, which might account for the high accuracy in excluding specimens with submucosal invasion. This might be more challenging for less-experienced endoscopists. Additionally, all resection specimens were evaluated by dedicated BE GI pathologists according to common practice in the Netherlands. Fourth, no formal sample size calculation was performed because of nonexisting previous data on this subject. As previously indicated, the difference in optimal histopathologic evaluations between pinned and directly fixated specimens might become statistically significant after increasing the sample size. Moreover, the clinical relevance of suboptimal histopathologic evaluation scores remains uncertain. Ideally, these scores would be related to clinically relevant outcomes, for example, local recurrence. However, local recurrence is very rare in patients with visible BE lesions treated with ER, which makes such a study rather impossible. Last, during the period for histopathologic evaluation 2 of 126 specimens were unavailable for central revision by the 2 dedicated BE GI pathologists despite repeated request. The findings of the original pathology reports are included in the article, and we consider the missing data to be of minimal influence on our results.

What should be the impact of the current study on clinical practice? Based on our findings, we would discourage the use of the cassette technique for EMR specimen handling. Direct fixation in formalin appears to be justified for handling of EMR specimens *without* suspicion of submucosal invasion because it is fast, easy, and enables optimal histopathologic evaluation. Nevertheless, comparability of the pinning method and direct fixation in formalin for enabling optimal histopathologic evaluation first needs to be confirmed in larger studies. In clinical daily practice EMR is also performed for visible lesions *with* possible submucosal invasion. Therefore, future larger studies on the comparison of the pinning method and direct fixation in formalin should include all EMR specimens, regardless of possible submucosal invasion. In BE lesions with a *high* suspicion of submucosal invasion we believe endoscopic submucosal dissection is the preferred resection method, and derived specimens should be pinned on cork or paraffin, although direct evidence of superiority of this method over direct fixation in formalin is lacking.

In conclusion, both pinning on paraffin and direct fixation in formalin result in optimal histopathologic evaluation scores in a high proportion of specimens. The cassette technique performed significantly worse, and its use in clinical daily practice should be discouraged. Given the significantly shorter handling time, direct fixation in formalin appears to be the preferred method over pinning on paraffin. However, the latter needs to be confirmed in larger studies with inclusion of all EMR specimens to optimize extrapolation to clinical daily practice.

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REFERENCES

1. Weusten B, Bisschops R, Coron E, et al. Endoscopic management of Barrett's esophagus: European Society of Gastrointestinal Endoscopy (ESGE) position statement. Endoscopy 2017;49:191-8.
2. American Gastroenterological Association; Spechler SJ, Sharma P, et al. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084-91.
3. Lauwers GY, Ban S, Mino M, et al. Endoscopic mucosal resection for gastric epithelial neoplasms: a study of 39 cases with emphasis on the evaluation of specimens and recommendations for optimal pathologic analysis. Mod Pathol 2004;17:2-8.
4. Mojtahed A, Shimoda T. Proper pathologic preparation and assessment of endoscopic mucosal resection and endoscopic submucosal dissection specimens. Tech Gastrointest Endosc 2011;13:95-9.
5. Geramizadeh B, Owen DA. Handling and pathology reporting of gastrointestinal endoscopic mucosal resection. Middle East J Dig Dis 2017;9:5-11.
6. Japan Esophageal Society. Japanese classification of esophageal cancer, 11th edition: part I. Esophagus 2017;14:1-36.
7. Nederlandse Vereniging van Maag-darm-leverartsen. Richtlijn Barrett-oesofagus. 2018. Available at: http://www.mdl.nl/richtlijnen2?noCache=214;1484584659. Accessed November 22, 2018.
8. Vieth M, Kiesslich R, Takubo K. Histopathology of endoscopic resection in the gastrointestinal tract. In: Conio M, Siersema P, Repici A, et al, editors. Endoscopic mucosal resection. Hoboken: Wiley-Blackwell; 2008. p. 154.
9. Bejarano PA, Berho M. Examination of surgical specimens of the esophagus. Arch Pathol Lab Med 2015;139:1446-54.

10. Greene CL, Worrell SG, Attwood SE, et al. Emerging concepts for the endoscopic management of superficial esophageal adenocarcinoma. J Gastrointest Surg 2016;20:851-60.
11. Stolte M, Dostler I. Good handling and pathological examination of endoscopic resections of early Barrett's cancer. Diagn Histopathol 2012;18:498-502.
12. Schulz KF, Altman DG, Moher D, et al. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. PLoS Med 2010;7:e1000251.
13. Swager A-F, Curvers WL, Bergman JJ. Diagnosis by endoscopy and advanced imaging of Barrett's neoplasia. Cham, Switzerland: Springer; 2016. p. 81-98.
14. Peters FP, Brakenhoff KPM, Curvers WL, et al. Histologic evaluation of resection specimens obtained at 293 endoscopic resections in Barrett's esophagus. Gastrointest Endosc 2008;67:604-9.
15. Endoscopic Classification Review Group; Diebold MD, Fujino M, et al. Update on the Paris classification of superficial neoplastic lesions in the digestive tract. Endoscopy 2005;37:570-8.
16. Pech O, Gossner L, Manner H, et al. Prospective evaluation of the macroscopic types and location of early Barrett's neoplasia in 380 lesions. Endoscopy 2007;39:588-93.
17. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377-81.
18. Weusten B, Bisschops R, Coron E, et al. Endoscopic management of Barrett's esophagus: European Society of Gastrointestinal Endoscopy (ESGE) position statement. Endoscopy 2017;49:191-8.
19. Ell C, May A, Pech O, et al. Curative endoscopic resection of early esophageal adenocarcinomas (Barrett's cancer). Gastrointest Endosc 2007;65:3-10.
20. Damhuis RAM, Wijnhoven BPL, Plaisier PW, et al. Comparison of 30-day, 90-day and in-hospital postoperative mortality for eight different cancer types. Br J Surg 2012;99:1149-54.
21. Shaheen NJ, Falk GW, Iyer PG, et al. ACG clinical guideline: diagnosis and management of Barrett's esophagus. Am J Gastroenterol 2016;111:30-50.
22. Evans JA, Early DS, Fukami N, et al. The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. Gastrointest Endosc 2012;76:1087-94.
23. Pimentel-Nunes P, Dinis-Ribeiro M, Ponchon T, et al. Endoscopic submucosal dissection: European Society of Gastrointestinal Endoscopy (ESGE) guideline. Endoscopy 2015;47:829-54.
24. Koop H, Fuchs K, Labenz J, et al. S2k-Leitlinie: Gastroösophageale Refluxkrankkheit unter Federführung der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten (DGVS). Z Gastroenterol 2014;52:1299-346.
25. Terheggen G, Horn EM, Vieth M, et al. A randomised trial of endoscopic submucosal dissection versus endoscopic mucosal resection for early Barrett's neoplasia. Gut 2017;66:783-93.

Abbreviations: BE, Barrett's esophagus; CI, confidence interval; ER, endoscopic resection; IQR, interquartile range.

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CURLING OF LATERAL MARGINS

2. Concerning the lateral margins of the resection specimen, on a scale of 1 (not at all) to 5 (completely), to what extend are the margins curled up?

- ◯ 1: not at all
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: completely

3. Concerning the lateral margins of the resection specimen, on a scale of 1 (not at all) to 5 (completely), to what extend are you able to discern the lateral margin from the vertical / deep resection margin?

- ◯ 1: not at all
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: completely

MASHING / SQUEEZING / CRUSHING

4. Concerning the entire specimen, on a scale of 1 (not at all) to 5 (extremely), to what extend is the specimen mashed / squeezed / crushed?

- ◯ 1: not at all
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: extremely

5. Concerning the possible mashing / squeezing / crushing of the specimen, on a scale of 1 (not at all) to 5 (completely), to what extend does this interfere with your ability to assess the specimen for all relevant parameters?

- ◯ 1: not at all
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: completely

SPECIMEN SURFACE

6. Concerning the surface of the resection specimen, on a scale of 1 (not at all) to 5 (extremely), are the superficial tissue layers damaged?

- ◯ 1: not at all
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: extremely

7. Concerning the possible damage to the superficial tissue layers of the specimen, on a scale of 1 (not at all) to 5 (extremely), to what extend does this interfere with your ability to assess the specimen for all relevant parameters?

- ◯ 1: not at all
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: extremely

SPECIMEN ORIENTATION

8. Concerning the orientation of the specimen, on a scale of 1 to 5, do you think the specimen is appropriately orientated or is it tangentially orientated?

- ◯ 1: nice, appropriate orientation
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5: extremely tangential orientation

EVALUATION OF ALL RELEVANT HISTOPATHOLOGICAL PARAMETERS

9. On a scale of 1 (disastrous) to 5 (optimal), how would you grade your ability to assess the vertical (deep) resection margin of the specimen?	◯ 1: disastrous ◯ 2 ◯ 3 ◯ 4 ◯ 5: optimal
10. On a scale of 1 (disastrous) to 5 (optimal), how would you grade your ability to assess the lateral resection margins of the specimen?	◯ 1: disastrous ◯ 2 ◯ 3 ◯ 4 ◯ 5: optimal
11. On a scale of 1 (disastrous) to 5 (optimal), how would you grade your ability to assess the depth of tumor invasion in the specimen?	◯ 1: disastrous ◯ 2 ◯ 3 ◯ 4 ◯ 5: optimal
12. On a scale of 1 (disastrous) to 5 (optimal), how would you grade your ability to assess the tumor differentiation (in case of carcinoma) or the degree of dysplasia in the specimen?	◯ 1: disastrous ◯ 2 ◯ 3 ◯ 4 ◯ 5: optimal
13. On a scale of 1 (disastrous) to 5 (optimal), how would you grade your ability to assess (lympho)vascular tumor invasion in the specimen?	◯ 1: disastrous ◯ 2 ◯ 3 ◯ 4 ◯ 5: optimal

OVERALL GRADING OF ABILITY TO ASSESS THE SPECIMEN

14. On a scale of 1 (disastrous) to 5 (optimal), how would you grade the ability for the overall assessment of the specimen for all relevant histopathological parameters?	◯ 1: disastrous ◯ 2 ◯ 3 ◯ 4 ◯ 5: optimal

Appendix 1. Research pathology form for central histopathologic revision

SUPPLEMENTARY TABLE 1. Baseline characteristics per patient (n = 42)

Characteristics	Value
Patient	
Male	35 (83.3)
Mean age, y (standard deviation)	68.5 (9.2)
Worst known pathology before EMR	
Low-grade dysplasia	7 (14.3)
HGD	26 (61.9)
Esophageal adenocarcinoma	8 (19.0)
No previous pathology	1 (2.4)
Endoscopy	
Median Prague score (IQR)	C 1 (0-4) M 4 (3-7)
Hiatal hernia (median length, 3 cm; IQR, 2-3)	39 (92.9)
Median lesion length, cm (IQR)	1.5 (1-2)
Median circumferential extent of lesion (IQR)	1/4 (1/8-1/3)
Main Paris type component	
0-Is	1 (2.4)
0-IIa	36 (85.7)
0-IIb	5 (11.9)
Secondary Paris type component	12 (28.6)
0-IIa	1 (2.4)
0-IIb	7 (16.7)
0-IIc	4 (9.5)
EMR type	
Captivator multiband mucosectomy	30 (71.4)
Duette multiband mucosectomy	12 (28.6)
Median number of ER specimens per patient (IQR)	2 (1-4)
Clinical diagnosis per patient, based on histopathologic evaluation of all resection specimens	
Invasion depth	
Nondysplastic Barrett's epithelium	1 (2.4)
Low-grade dysplasia	3 (7.1)
HGD	15 (35.7)
T1m2	4 (9.5)
T1m3	15 (35.7)
T1sm1	3 (7.1)
T1sm2	1 (2.4)
Differentiation grade	
Well	12 (28.6)
Moderate	8 (19.0)
Poor	3 (7.1)
Not applicable	19 (45.2)
Lymphovascular invasion	0 (0)
Deep resection margins free of tumor	42 (100)

Values are n (%) unless otherwise defined.
HGD, High-grade dysplasia; *IQR,* interquartile range; *ER,* endoscopic resection.

SUPPLEMENTARY TABLE 2. Ability to assess all relevant histologic parameters (n = 124)

	Pinning on paraffin (n = 44)	No handling (n = 41)	Cassette box (n = 39)	*P* value
Optimal overall score (5 on a 5-point Likert scale) vs suboptimal score (<5) to assess all relevant histological parameters	43 (97.7)	37 (90.2)	25 (64.1)	<.001
Frequency of all evaluation scores				
Score of 5 (optimal)	43	37	25	
Score of 4	0	2	6	
Score of 3	1	2	5	
Score of 2	0	0	3	
Score of 1 (disastrous)	0	0	0	
Curling of the lateral margins	22 (50)	19 (46.3)	1 (2.6)	<.001
Frequency of all evaluation scores				
Score of 5 (not at all)	22	19	1	
Score of 4	20	9	8	
Score of 3	1	4	14	
Score of 2	1	2	3	
Score of 1 (completely curled up)	0	7	13	
Ability to discern lateral from vertical margins	40 (90.9)	32 (78.0)	22 (56.4)	.001
Frequency of all evaluation scores				
Score of 5 (completely)	40	32	22	
Score of 4	1	0	1	
Score of 3	0	1	3	
Score of 2	1	1	2	
Score of 1 (not at all)	2	7	11	
Crushing of the specimen	23 (52.3)	21 (51.2)	19 (48.7)	.95
Frequency of all evaluation scores				
Score of 5 (not at all)	23	21	19	
Score of 4	19	16	14	
Score of 3	0	4	4	
Score of 2	1	0	0	
Score of 1 (extremely)	1	0	2	
Crushing interfering with the ability to assess the specimen	36 (81.8)	32 (78.0)	30 (76.9)	.85
Frequency of all evaluation scores				
Score of 5 (not at all)	36	32	30	
Score of 4	1	0	3	
Score of 3	0	1	2	
Score of 2	1	2	2	
Score of 1 (completely)	6	6	2	
Damaged superficial tissue layers	39 (88.6)	36 (87.8)	30 (76.9)	.27
Frequency of all evaluation scores				
Score of 5 (not at all)	39	36	30	
Score of 4	4	4	9	
Score of 3	1	1	0	
Score of 2	0	0	0	
Score of 1 (extremely)	0	0	0	

(*continued on the next page*)

SUPPLEMENTARY TABLE 2. Continued

	Pinning on paraffin (n = 44)	No handling (n = 41)	Cassette box (n = 39)	*P* value
Damage interfering with the ability to assess the specimen	42 (95.5)	38 (92.7)	32 (82.1)	.10
Frequency of all evaluation scores				
Score of 5 (not at all)	42	38	32	
Score of 4	2	3	7	
Score of 3	0	0	0	
Score of 2	0	0	0	
Score of 1 (extremely)	0	0	0	
Orientation of the specimen	42 (95.5)	40 (97.6)	37 (94.9)	.81
Frequency of all evaluation scores				
Score of 5 (nice, appropriate)	42	40	37	
Score of 4	1	0	1	
Score of 3	1	1	0	
Score of 2	0	0	1	
Score of 1 (extremely tangential)	0	0	0	
Ability to assess deep vertical margins	42 (95.5)	37 (92.7)	28 (71.8)	.005
Frequency of all evaluation scores				
Score of 5 (optimal)	42	37	28	
Score of 4	2	1	4	
Score of 3	0	2	1	
Score of 2	0	0	3	
Score of 1 (disastrous)	0	1	3	
Ability to assess lateral margins	40 (90.9)	35 (85.4)	24 (61.5)	.002
Frequency of all evaluation scores				
Score of 5 (optimal)	40	35	24	
Score of 4	2	2	8	
Score of 3	0	2	1	
Score of 2	1	1	4	
Score of 1 (disastrous)	1	1	2	
Ability to assess depth of tumor invasion	42 (95.5)	40 (97.6)	36 (92.3)	.55
Frequency of all evaluation scores				
Score of 5 (optimal)	42	40	36	
Score of 4	2	0	3	
Score of 3	0	1	0	
Score of 2	0	0	0	
Score of 1 (disastrous)	0	0	0	
Ability to assess tumor differentiation	44 (100)	42 (100)	39 (100)	1.00
Frequency of all evaluation scores	N/A	N/A	N/A	
Ability to assess lymphovascular invasion	44 (100)	42 (100)	39 (100)	1.00
Frequency of all evaluation scores	N/A	N/A	N/A	

N/A, Not applicable.