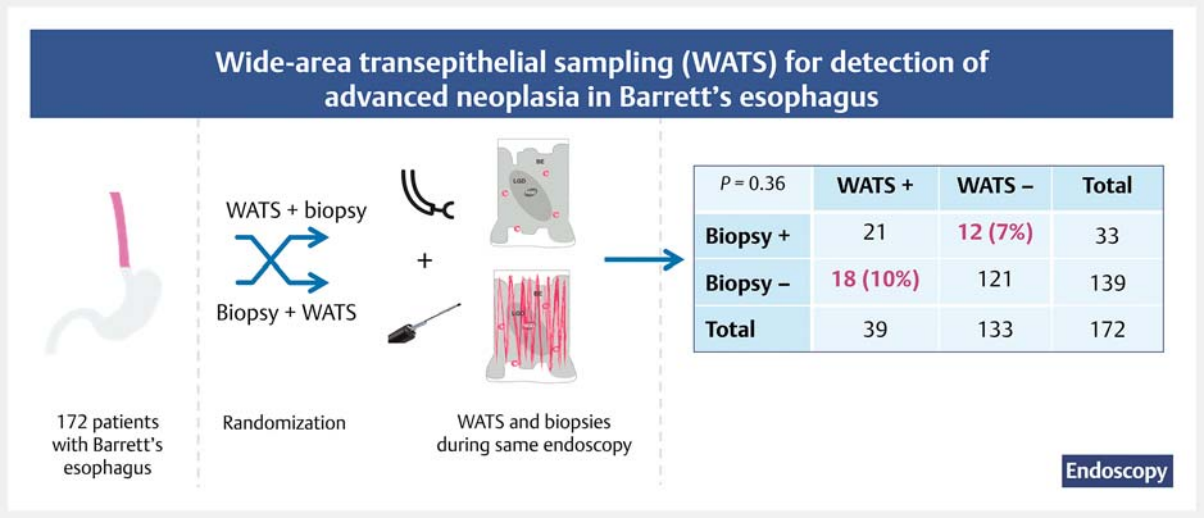
Wide-area transepithelial sampling with computer-assisted analysis to detect high grade dysplasia and cancer in Barrett's esophagus: a multicenter randomized study

GRAPHICAL ABSTRACT



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ABSTRACT

Background Current surveillance for Barrett's esophagus (BE), consisting of four-quadrant random forceps biopsies (FBs), has an inherent risk of sampling error. Wide-area transepithelial sampling (WATS) may increase detection of high grade dysplasia (HGD) and esophageal adenocarcinoma (EAC). In this multicenter randomized trial, we aimed to evaluate WATS as a substitute for FB.

Methods Patients with known BE and a recent history of dysplasia, without visible lesions, at 17 hospitals were randomized to receive either WATS followed by FB or vice versa. All WATS samples were examined, with computer assistance, by at least two experienced pathologists at the CDx Diagnostics laboratory. Similarly, all FBs were examined by two expert pathologists. The primary end point was concordance/discordance for detection of HGD/EAC between the two techniques.

Results 172 patients were included, of whom 21 had HGD/EAC detected by both modalities, 18 had HGD/EAC detected by WATS but missed by FB, and 12 were detected by FB but missed by WATS. The detection rate of HGD/EAC did not differ between WATS and FB ($P=0.36$). Using WATS as an adjunct to FB significantly increased the detection of HGD/EAC vs. FB alone (absolute increase 10 % [95 %CI 6 % to 16 %]). Mean procedural times in minutes for FB alone, WATS alone, and the combination were 6.6 (95 %CI 5.9 to 7.1), 4.9 (95 %CI 4.1 to 5.4), and 11.2 (95 %CI 10.5 to 14.0), respectively.

Conclusions Although the combination of WATS and FB increases dysplasia detection in a population of BE patients enriched for dysplasia, we did not find a statistically significant difference between WATS and FB for the detection of HGD/EAC as single modality.

Introduction

Patients with Barrett's esophagus (BE) undergo regular endoscopic surveillance to detect dysplasia in the early stages when it is amenable to endoscopic treatment to prevent progression to invasive esophageal adenocarcinoma (EAC).

Because dysplasia is often not visible during endoscopy, there is general agreement that forceps biopsy sampling according to the Seattle protocol is currently the best method for detection of dysplasia in the absence of visible lesions. This involves random forceps biopsies (FBs) obtained from four quadrants at 2-cm intervals along the BE segment, using large-capacity forceps. However, dysplasia and early cancer may be patchy or focal and, as a result, can be missed by FBs. FBs are therefore associated with sampling error and missed dysplasia. Furthermore, the Seattle protocol is time-consuming, especially in longer BE segments. Studies have shown that approximately half of gastroenterologists do not adhere to the Seattle protocol [1].

Wide-area transepithelial sampling (WATS) with computer-assisted 3 D analysis is a novel, brush biopsy technique used to broaden the area of sampled BE. The WATS brush has long hard bristles and is abrasive, so it enables deep transepithelial specimens to be obtained. Use of the brush is relatively easy and not time-consuming. Tissue obtained by WATS undergoes computerized neural network analysis that helps identify the areas on the slide that are most likely to represent neoplastic change. These areas are then presented to a pathologist for evaluation and diagnosis.

Previous studies have shown a 2 %–42 % absolute increase, or 42 %–329 % relative increase, in the detection of high grade dys-

plasia (HGD) or EAC when WATS is used as an adjunct to FB [2–6]. However, these studies had some methodological limitations. For example, patients with visible lesions were included; WATS was primarily assessed as an adjunct to FB instead of as a replacement procedure; and/or studies had a primary focus on the detection of intestinal metaplasia (IM) and not dysplasia.

Therefore, the purpose of this study was to assess the value of WATS as a replacement for FBs for the detection of HGD/EAC in BE patients without visible lesions. This was the first prospective randomized study of this nature in a referral population of BE patients with a history of dysplasia.

Methods

This was a prospective multicenter randomized study that enrolled patients under endoscopic surveillance for BE with a history of dysplasia or mucosal adenocarcinoma at 17 participating medical centers (**Table 1 s**, see online-only Supplementary material). The institutional review boards of all participating centers approved the study and informed consent was obtained from all patients. An on-site initiation visit with training on the protocol and the WATS procedure was planned at all sites prior to the start of the study and the first 10 cases per endoscopist were not included in the study analysis.

Study population

We included patients scheduled for a regular imaging endoscopy for BE, with a history of BE-associated neoplasia, either:

- BE in the absence of visible lesions with either low grade dysplasia (LGD) or HGD diagnosed on FBs (▶ **Fig. 1**); or
- Flat BE after prior endoscopic resection (ER) for a visible lesion with the resection specimen showing LGD, HGD, or mucosal EAC with good-to-moderate differentiation, without lymphovascular invasion, and with negative resection margins. The minimum time between ER and randomization was 6 weeks.

Exclusion criteria were: age < 18 years; BE length < 2 cm circumferential extent or > 10 cm maximum extent; prior ablation therapy; and history of esophageal surgery other than fundoplication.

Randomization and index endoscopy

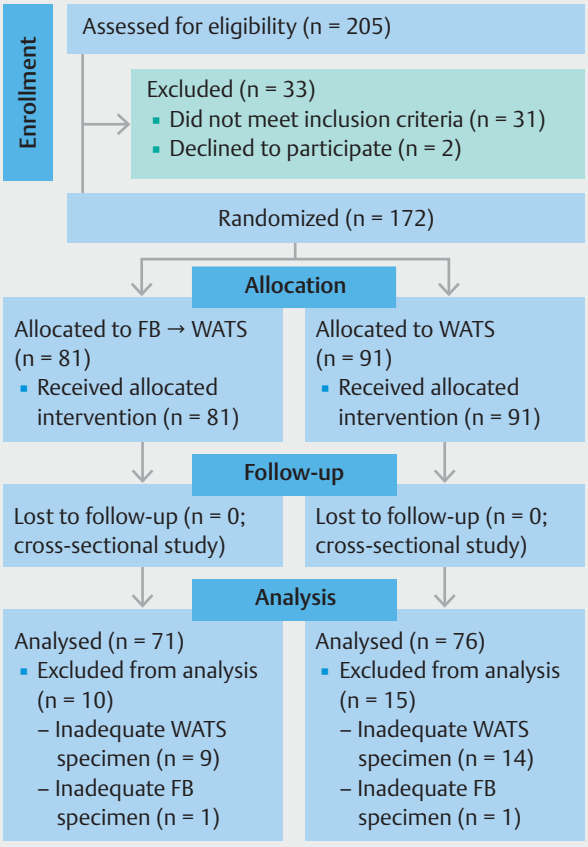
Eligible patients were randomized by a computer-generated system into two groups: FB sampling followed by WATS; or WATS followed by FB. WATS and FB were performed during the same endoscopy.

Random forceps biopsies

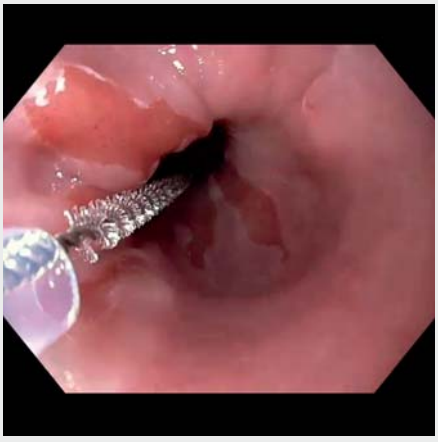
Four-quadrant FB specimens were obtained using a standard biopsy forceps according to the Seattle protocol at 2-cm intervals along the complete length of the BE segment.

WATS brushing

Two WATS brushes were obtained for every 5 cm segment of BE, starting at the gastroesophageal junction and moving proximally through the entire BE segment. Bristles were placed against the mucosal surface and the brush was rotated and re-



▶ **Fig. 1** Patient study flow diagram. FB, forceps biopsy; WATS, wide-area transepithelial sampling.



Video 1 Video showing the wide-area transepithelial sampling (WATS) procedure, with the WATS brush inserted through the working channel of the endoscope and brushing performed until pinpoint bleeding is observed.
Online content viewable at:
https://doi.org/10.1055/a-1949-9542

peatedly passed back and forth until pinpoint bleeding was observed (▶ **Video 1**). The first brush was smeared on a glass slide and fixed for Papanicolaou (PAP) staining and its bristles were cut and placed inside the vial. The process was repeated with the second brush for the same BE area and this brush was placed directly into the vial without first being smeared on a slide. In patients with BE of > 5 cm in length, two new brushes were used for the next 5 cm of BE.

Pathology

All specimens, regardless of tissue acquisition technique, were categorized as either LGD, HGD, or intramucosal EAC according to previously published criteria for FB [7] and WATS [13] (▶ **Fig. 2**).

Pathologists received the specimen along with only a random study number and were blinded to the patient's history, demographics, endoscopic findings, histologic findings, and outcome of the other technique.

Forceps biopsy

The local expert pathologist in each study center performed the initial assessment. All local pathologists worked in BE expert centers and were experienced in the diagnosis of BE and related neoplastic complications. Next, an independent pathologist with expertise in gastrointestinal pathology located at a central European pathology laboratory (G.H.) interpreted the specimens.

Where there were discrepancies, a third BE expert pathologist (S.M.) reviewed the sample to achieve a consensus diagnosis (defined as the diagnosis rendered by two of the three pathologists).

WATS

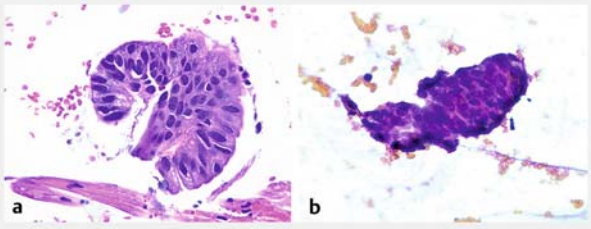
The WATS brush sample that was stained with a modified PAP stain was evaluated with computer-assisted 3 D tissue analysis using neural networks specifically optimized for the esophageal mucosa. The computer is capable of detecting very small numbers of atypical cells on the slide. The images of the most atypical cells were then displayed on a high resolution video monitor in order for a final diagnosis to be made by the pathologist.

All WATS specimens selected by the computer were analyzed by two central pathologists from a single central laboratory (CDx Diagnostics, Inc.), both of whom have extensive experience in WATS evaluation. The pathologists made the diagnosis independently from each other. Where discrepancies occurred, a third pathologist reviewed the case to produce a consensus diagnosis.

When WATS is used for regular clinical care, and not as in the current study, a single pathologist reviews the slides selected by the computer and, when there is dysplasia, a second pathologist is requested, as per national guidelines.

Study end points

The primary end point of this study was the concordance/discordance between the detection of BE-associated HGD/EAC using WATS brushing and FB.



▶ **Fig. 2** Images showing high grade dysplasia diagnosed using the WATS^{3D} brush on: **a** cell block; **b** smear.

Secondary end points were: (i) the adjunctive value of WATS, defined as the absolute and relative increases in detection when WATS is added to FB; (ii) the effect of the order of WATS and FB on the outcome; (iii) procedure time; and (iv) related complications.

Statistical analysis

The intention-to-treat (ITT) analysis included all patients who underwent randomization. The per-protocol (PP) analysis included all patients with an adequate specimen for histologic analysis.

Normally distributed continuous variables were summarized using mean and SD; skewed variables were summarized using median and interquartile range (p25–p75). Categorical variables were summarized using counts and percentages. All confidence intervals were reported at the 95 % level. The McNemar test was used to evaluate the primary outcome. Student's *t* test was used to compare normally distributed variables.

The relative difference was defined as the extra cases found with WATS, divided by the total cases found with FB; the absolute difference as the extra cases found with WATS divided by all study patients. The number needed to test (NNT) was the number of patients required to undergo WATS as an adjunct to FB in order to detect one additional case of HGD/EAC, as compared with the use of FB alone.

The order of randomization was evaluated by the difference in absolute detection of HGD/EAC between the two study arms (i. e. WATS then FB, or FB then WATS).

The primary outcome was evaluated stratified by study site (**Table 2 s**) but, because all sites were highly experienced with BE screening and surveillance, we did not adjust for study center in the analysis.

Sample size

In a recent US multicenter study, the diagnostic yield of WATS was four times greater than the yield of FB [6]. For this reason, we designed this study to test the superiority of WATS compared with FB in detecting HGD/EAC by a superiority margin. We therefore hypothesized that statistically significant differences in detection rates between WATS and FB might not be of interest unless the difference were greater than a threshold (i. e. the smallest difference in proportions considered by our research team to be a clinically significant difference). Our sample size

was based on an anticipated improvement in detection rate with WATS from 27 % to 55 %, a superiority margin of 0.15 (equivalent to observation of a detection rate of at least of 0.42 for WATS), with 80 % power and a significance level of 0.05. This led to a target sample size of 147 participants. This sample size calculation was made for independent samples. The sample size for a paired study can be approximated by the independent calculation in many practical situations. Generally, this assumption produces overestimates of sample size [8].

Results

Between November 2017 and February 2019, 205 patients were initially screened for eligibility, 172 of whom fulfilled the inclusion criteria and underwent randomization (the ITT population) (▶ **Fig. 1**; ▶ **Table 1**). Patients had prior diagnoses of LGD in flat BE (n = 68; 40 %), HGD in flat BE (n = 32; 19 %), and flat BE after prior ER of a visible lesion (n = 72; 42 %). The latter group of patients had undergone prior ER for removal of LGD (n = 3; 5 %), HGD (n = 21; 29 %), or mucosal EAC (n = 48; 66 %).

In total, 25 patients were excluded from the ITT analysis owing to inadequate specimens on either WATS (n = 23) or biopsy (n = 2). The WATS excluded cases all suffered from either air-drying artefact, as a result of either improper application of cells onto the slide, and/or poor application of PAP staining on the slide. The inadequate FBs were the result of insufficient tissue for diagnosis.

In 30 out of 172 cases (17 %), there was a discrepancy between the local "on-site" pathologist and the "central" pathologist regarding the FB diagnosis for the presence or absence of HGD/EAC. Of these 30 cases, 19 were diagnosed with HGD/EAC by the central study pathologist, but with nondysplastic BE (NDBE)/LGD by the local onsite pathologist. In 11 of 30 cases, the central pathologist diagnosed NDBE/LGD, whereas the on-site pathologist diagnosed HGD/EAC. These 30 cases were then reviewed by the third pathologist, as per the protocol. The final "consensus" diagnosis of these cases was as follows: 12 HGD/EAC and 18 NDBE/LGD.

For WATS, there was a discrepancy between the two initial readings for the presence or absence of HGD/EAC in 2/172 cases (1 %), both of which were scored as no HGD/EAC by the third reviewer.

Yield of HGD/EAC detection

In the ITT analysis, we found no statistically significant difference in the yield of HGD/EAC detection with either WATS or FB ($P = 0.36$) (▶ **Table 2a**; **Table 2 s**).

The PP analysis also showed no significant difference in the detection of HGD/EAC between the two modalities ($P = 0.12$) (▶ **Table 2a**).

Discrepant cases between WATS and FB

Discrepant cases were evaluated in the PP analysis. The 18 WATS-positive/FB-negative cases had a mean (p25–p75) BE segment of C3M6 (C1–6; M4–8). The referral diagnoses were flat LGD (n = 7; 39 %), flat HGD (n = 2; 11 %), or a visible lesion that was resected prior to the study (n = 9; 50 %). The worst diagnoses with FB for these patients were LGD (n = 15; 83 %) and IM (n = 3; 17 %) (▶ **Table 3**).

The nine WATS-negative/FB-positive patients had a mean (p25–p75) BE segment of C2M5 (C0–3; M3–5). The referral diagnoses were flat LGD (n = 3; 33 %), flat HGD (n = 3; 33 %), or a visible neoplastic lesion that was resected prior to the study (n = 3, 33 %). The worst diagnoses on WATS samples were LGD (n = 3; 33 %) and IM (n = 6; 67 %).

Yield of HGD/EAC detection with addition of WATS

Considering WATS as an adjunct to FB, the absolute increase in HGD/EAC detection was 10 % (18/172; 95 %CI 6 % to 16 %; $P < 0.001$). The relative increase was 55 % (18/33; 95 %CI 36 % to 72 %; $P < 0.001$). The detection rate increased from 19 % (33/172; 95 %CI 14 % to 26 %) to 30 % (52/172; 95 %CI 23 % to 38 %). In our study population, the NNT to detect one additional case of HGD/EAC was 10 (172/18).

▶ **Table 1** Baseline characteristics of the 172 patients with Barrett's esophagus (BE) who fulfilled the inclusion criteria and underwent randomization (the intention-to-treat population).

	Total	FB then WATS (n = 81)	WATS then FB (n = 91)
Mean age (SD), years	68 (8)	68 (8)	69 (8)
Sex, male, n (%)	144 (84)	68 (84)	76 (84)
Worst neoplasia before inclusion, n (%)			
▪ Flat LGD	68 (40)	26 (32)	42 (46)
▪ Flat HGD	32 (19)	20 (25)	12 (13)
▪ Visible lesion already removed with endoscopic resection	72 (42)	35 (43)	37 (41)
Esophagitis, Los Angeles grade, n (%)			
▪ None	163 (95)	76 (94)	87 (96)
▪ A/B	8 (5)	4 (5)	4 (4)
▪ C/D	1 (1)	1 (1)	0 (0)
Hiatal hernia grade, n (%)			
▪ Small (< 2 cm)	50 (29)	29 (36)	21 (23)
▪ Medium (2–4 cm)	81 (47)	31 (38)	50 (55)
▪ Large (> 4 cm)	37 (22)	17 (21)	20 (22)
▪ Missing value	4 (2)	4 (5)	0 (0)
BE extent, mean (SD), cm			
▪ Circumferential	3 (3)	3 (3)	3 (3)
▪ Maximum	5 (3)	5 (3)	5 (3)

FB, forceps biopsy; WATS, wide-area transepithelial sampling; LGD, low grade dysplasia; HGD, high grade dysplasia.

▶ **Table 2** Yield of high grade dysplasia/esophageal adenocarcinoma for forceps biopsy (FB) and wide-area transepithelial sampling (WATS) on

a intention-to-treat analysis

		WATS		
		Positive	Negative	Total
FB	Positive	21	12	33
	Negative	18	121	139
	Total	39	133	172

b per-protocol analysis

		WATS		
		Positive	Negative	Total
FB	Positive	21	9	30
	Negative	18	99	117
	Total	39	108	147

a P = 0.36. **b** P = 0.12.

Impact of randomization

In the study group "FB-WATS", absolute detection increased by 11 % (9/81; 95 %CI 6 % to 21 %). In the study group "WATS-FB", the absolute increment of HGD/EAC detection was 10 % (9/91; 95 %CI 5 % to 18 %). Differences between study groups were not statistically significant (1 %; 95 %CI −8 % to 10 %; *P* = 0.49).

Time

The mean (95 %CI) procedural times for FB and WATS were 6.6 (5.9 to 7.1) and 4.9 (4.1 to 5.4) minutes, respectively. The mean difference between FB and WATS was 1.6 minutes (95 %CI 1.01 to 2.25; *P* < 0.001). The procedure time for WATS and FB combined was 11.2 (10.5 to 14.0) minutes. When WATS was added to FB, the mean additional time was 4.8 minutes (95 %CI 4.4 to 5.2; *P* < 0.001).

Complications

No complications related to the procedure occurred.

Discussion

In this prospective multicenter randomized study, we aimed to evaluate whether WATS could replace FBs in the detection of HGD or cancer in BE. For the primary end point of our study, we demonstrated that there was no significant increase in the detection of HGD/EAC for WATS as compared with FB. While WATS detected an additional 18 HGD/EAC cases that were missed with FB (i. e. WATS positive/FB negative), 12 other cases with HGD/EAC on the FB were missed with WATS (WATS negative/FB positive). Among the additional cases that were detected with WATS, the majority (i. e. 83 %) were diagnosed with LGD on FB.

Considering WATS as an adjunct to FB, our study confirmed the findings of prior studies, with a significant improvement in the detection of HGD/EAC seen, with a relative increase of 55 % and a NNT of 10 patients in our enriched study population. The previous studies comparing WATS with FB for the detection of HGD/EAC, all had the aim of establishing the value of WATS as an adjunct to FB. In 2011, Anandasabapathy and Johanson reported outcomes for a smaller brush and an older generation computer system [2, 3], and Vennalaganti recently published outcomes using the same generation device as in the current study [4]. The relative yield of dysplasia detection increased significantly in all studies with 42 % [2], 88 % [3], and 329 % increases [4]. The relative increase of 55 % in the current study lies well within this spectrum and confirms prior findings that, if WATS is used as an adjunct to FB, the detection of HGD or EAC increases significantly. However, the addition of WATS to FB would also increase costs and procedure times, so cost-effectiveness studies would be relevant.

Our primary aim was to evaluate WATS as a replacement for FB. In this regard, as well as the extra cases found with WATS (WATS positive/FB negative), the cases missed with WATS (WATS negative/FB positive) are also relevant. WATS detected an additional 18 cases in our study (10 % of the study population), but 12 other cases (7 %) were WATS negative/FB positive. Overall, the difference was not statistically significant with a *P* value of 0.36. It should be noted that, of the extra cases detected with WATS, the vast majority (83 %) were found to have LGD

▶ **Table 3** Detection of intestinal metaplasia (IM), low grade dysplasia (LGD), or high grade dysplasia (HGD)/esophageal adenocarcinoma (EAC) by forceps biopsy (FB) and wide-area transepithelial sampling (WATS) on per-protocol analysis.

		WATS			
		HGD/EAC	**LGD**	**IM**	**Total**
FB	HGD/EAC	21	3	6	30
	LGD	15	37	9	61
	IM	3	19	34	56
	Total	39	59	49	147

in the FB, meaning these patients already had an indication for repeat endoscopy within 6 months or consideration for ablative therapy based on their FB findings.

Although it was not the primary aim of the prior studies to evaluate WATS as a replacement for FB, which it was in our analysis, comparisons can be made. Anandasabapathy reported, in an enriched study population, that 16/151 cases (11 %) were WATS + /FB−; yet more patients were WATS−/FB + (23/151; 15 %). Johanson, in a screening population, reported 14/1183 (1.2 %) WATS + /FB− cases and 11/1183 (0.9 %) WATS−/FB + cases. The outcomes reported by Vennalaganti in an enriched US study population appeared to differ: 23/160 (14 %) were WATS + /FB−; yet only 1/160 (0.6 %) were WATS−/FB + . One major limitation of the latter study that may have contributed to this discrepancy was the inclusion of patients with a visible lesion that might have been brushed but not sampled with the FB protocol. Codipilly, in a recent meta-analysis, reported that WATS^{3D} was negative for dysplasia in 62.5 % of cases where FB identified dysplasia [6].

WATS has several clinical advantages over FB. WATS is easier to perform and decreases the time of the procedure, and it leads to one sample rather than multiple samples each with their own costs. Therefore, one may argue that WATS could replace FB because of its secondary advantages. In this case, a noninferiority study would require 1952 patients in an enriched study population to prove, with 80 % power, that a 5 % difference in dysplasia detection was not statistically significant [9]. This number would be even higher in an actual BE screening population, where the baseline risk for HGD or cancer is lower. Although our study was not designed as a noninferiority trial, the similar rates of dysplasia detection in combination with the secondary advantages may suggest that there could be a role for WATS to replace FB in the future because of its secondary advantages.

This study has important strengths. It is the first study that systematically compared WATS as an alternative to FB for the detection of BE-related dysplasia in a well-defined study population and in the absence of clearly visible lesions. The study was conducted in 17 international expert centers by dedicated endoscopists, after on-site training and after 10 lead-in cases that were not used for the study analysis. All pathologists in the current study were experienced with BE neoplasia, but all FB were reviewed by a single central pathologist. Where there was discordance, a third reading was performed for adjudication, providing a high quality gold standard for histology.

Several limitations in our study may have biased the detection rates of WATS and/or FB. First, there is no gold standard for HGD/cancer. Pathologists that provided the WATS diagnosis derived from a single central laboratory (CDx Diagnostics, Inc.). This may potentially have led to a different, either lower or higher, threshold for the diagnosis of HGD/EAC than for FB. Therefore, it is uncertain whether a WATS + /FB− HGD diagnosis represents comparable risk for progression to (advanced) EAC as an FB diagnosis of HGD. Because most patients in the current study underwent ablation therapy, there is no follow-up data available. Interestingly, a recent study assessed progression rates to HGD/EAC of an initial WATS diagnosis of either NDBE, crypt dysplasia, or LGD in 4545 patients who were followed for a mean of 2 years. Annual progression rates of 0.1 %, 1.89 %, and 3.47 % were found for baseline NDBE, crypt dysplasia, or LGD, respectively, which are comparable to progression studies performed with FB [10]. A new study with long-term endoscopic follow-up for a WATS + /FB− diagnosis of HGD has been initiated to evaluate this further.

We attempted to minimize the uncertainty for the histologic diagnosis with the use of a consensus diagnosis and by standard WHO criteria for dysplasia, regardless of the type of tissue acquisition technique used. Pathologists still disagreed on the distinction between HGD/EAC versus NDBE/LGD based on the FB specimen in 17 % of patients, a finding in line with the prior literature [11, 12].

In the FB-WATS arm, endoscopists may have avoided – consciously or unconsciously– the FB lesions when using the WATS brush, fearing that the brush would stick in the wound. This may have led to bias towards a lower detection rate for WATS. It may also be that FBs were concentrated in the region previously biopsied with dysplasia according to the Seattle Protocol, resulting in a higher detection rate for FBs.

It has been proven that adherence to the Seattle protocol is poor, leading to lower rates for dysplasia detection [1]. All participating endoscopists and pathologists were experts in the field and all adhered strictly to the Seattle protocol. Furthermore, the expert setting of both endoscopists and pathologists is highly sensitive for detecting dysplastic lesions. This may have caused bias towards a higher neoplasia detection rate for FBs. In a less experienced setting, WATS may potentially detect more dysplasia as it may compensate for a lower lesion detection rate and sampling. A repetition of the current study in a community setting would be valuable to show whether the implementation of an AI algorithm in combination with wide-area sampling could raise community practice to the expert level.

Patients in the current study had a recent diagnosis of dysplasia and the results are not generalizable to a NDBE screening population. We initiated this study in an enriched population for efficiency reasons, of note WATS and/or FB had no direct implications for these patients because an indication for ablation therapy already existed. The real clinical relevance therefore is probably lower in a low prevalence surveillance population.

It has to be noted that, despite prestudy training of all sites and investigators, a significant number of WATS brushes turned out to be inadequate owing to air drying problems caused by late fixation for the PAP stain. In a newer WATS generation, the PAP stain is replaced with ThinPrep solution to eliminate any potential technical errors, which may prevent air-drying issues in the future. The risk of technical errors still needs to be taken into consideration when endoscopists want to replace standard FB with WATS, because an inadequate specimen would lead to another endoscopy being required.

The hypothesis that WATS would detect two times as much dysplasia as FBs and that the dysplasia detection rate for FBs would be 27 % was too optimistic. The calculated sample size was however an overestimation because it was based on independent outcomes.

In conclusion, this multicenter randomized study confirmed that the combination of WATS as an adjunct to FB increases dysplasia detection; however, WATS as a standalone technique did not detect significantly more HGD or cancer as compared with FBs in an enriched BE population with known dysplasia.

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Competing Interests

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