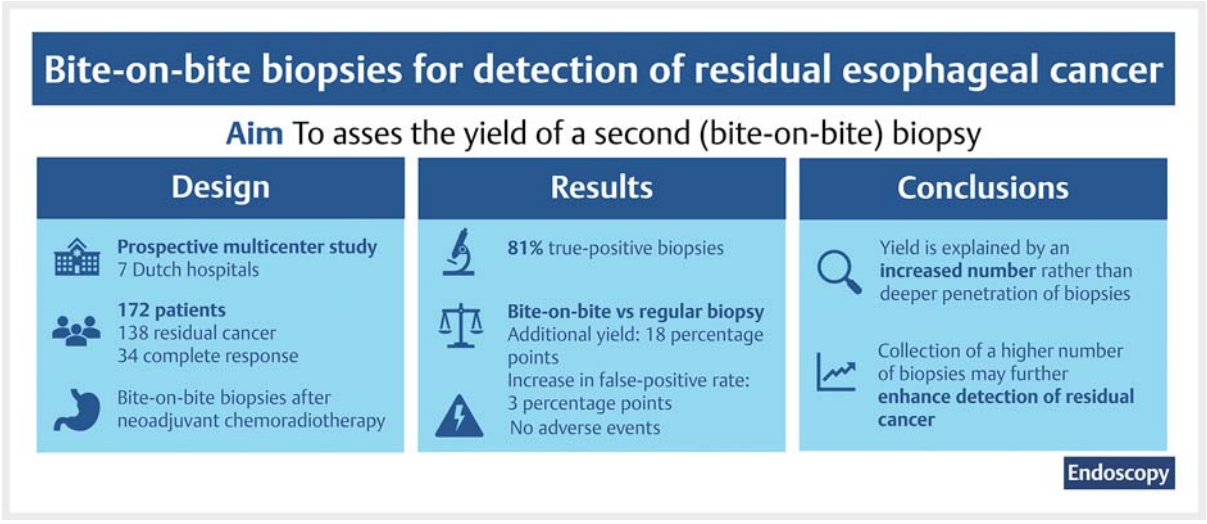
Bite-on-bite biopsies for the detection of residual esophageal cancer after neoadjuvant chemoradiotherapy

Bite-on-bite biopsies for the detection of residual esophageal cancer after neoadjuvant chemoradiotherapy

INFOGRAPHIC



Authors

Ruben D. van der Bogt[1], Berend J. van der Wilk[2], Lindsey Oudijk[3], Erik J. Schoon[4,5], Gesina van Lijnschoten[6], Sietske Corporaal[7], Judith Nieken[8], Peter D. Siersema[9], Tanya M. Bisseling[9], Rachel S. van der Post[10], Rutger Quispel[11], Arjan van Tilburg[12], Liekele E. Oostenbrug[13], Robert G. Riedl[14], Lieke Hol[15], Mike Kliffen[16], Suzan Nikkessen[1], Ben M. Eyck[2], J. Jan B. van Lanschot[2], Michael Doukas[3], Manon C. W. Spaander[1]

Institutions

1 Department of Gastroenterology and Hepatology, Erasmus Cancer Institute, Erasmus MC University Medical Center, Rotterdam, The Netherlands
2 Department of Surgery, Erasmus Cancer Institute, Erasmus MC University Medical Center, Rotterdam, The Netherlands
3 Department of Pathology, Erasmus Cancer Institute, Erasmus MC University Medical Center, Rotterdam, The Netherlands
4 Department of Gastroenterology and Hepatology, Catharina Hospital, Eindhoven, The Netherlands
5 GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands
6 Department of Pathology, Stichting PAMM, Eindhoven, The Netherlands.
7 Department of Gastroenterology and Hepatology, Leeuwarden Medical Center, Leeuwarden, The Netherlands
8 Department of Pathology, Pathology Friesland, Leeuwarden, The Netherlands
9 Department of Gastroenterology and Hepatology, Radboud University Medical Center, Nijmegen, The Netherlands
10 Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands
11 Department of Gastroenterology and Hepatology, Reinier de Graaf Hospital, Delft, The Netherlands
12 Department of Pathology, Reinier de Graaf Hospital, Delft, The Netherlands
13 Department of Gastroenterology and Hepatology, Zuyderland Medical Center, Heerlen, The Netherlands
14 Department of Pathology, Zuyderland Medical Center, Heerlen, The Netherlands
15 Department of Gastroenterology and Hepatology, Maasstad Hospital, Rotterdam, The Netherlands
16 Department of Pathology, Maasstad Hospital, Rotterdam, The Netherlands

submitted 5.12.2021
accepted after revision 6.4.2022
published online 3.6.2022

Bibliography
Endoscopy 2022; 54: 1131–1138
DOI 10.1055/a-1846-1025
ISSN 0013-726X

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Supplementary material
Supplementary material is available under
https://doi.org/10.1055/a-1846-1025

Corresponding author
M. C. W. Spaander, MD, PhD, Erasmus MC University Medical Center, Doctor Molewaterplein 40, 3015 GD, Rotterdam, The Netherlands
v.spaander@erasmusmc.nl

ABSTRACT

Background Active surveillance after neoadjuvant treatment is increasingly implemented. The success of this strategy relies on the accurate detection of residual cancer. This study aimed to assess the diagnostic value of a second (bite-on-bite) biopsy for the detection of residual esophageal cancer and to correlate outcomes to the distribution of residual cancer found in the resection specimen.

Methods A multicenter prospective study of esophageal cancer patients undergoing active surveillance after neoadjuvant chemoradiotherapy was performed. At clinical response evaluations, an upper gastrointestinal (GI) endoscopy was performed with at least four bite-on-bite biopsies of the primary tumor site. First and second biopsies were analyzed separately. Patients with histopathological evidence of residual cancer were included in the primary analysis. Two pathologists blinded for biopsy outcome examined all resection specimens.

Results Between October 2017 and July 2020, 626 upper GI endoscopies were performed in 367 patients. Of 138 patients with residual cancer, 112 patients (81 %) had at least one positive biopsy. In 14 patients (10 %) only the first biopsy was positive and in 25 patients (18 %) only the second biopsy ($P = 0.11$). Remarkably, the rates of patients with tumor-free mucosa and deeper located tumors were higher in patients detected by the first biopsy. The second biopsy increased the false-positive rate by 3 percentage points. No adverse events occurred.

Conclusions A second (bite-on-bite) biopsy improves the detection of residual esophageal cancer by almost 20 percentage points, at the expense of increasing the false-positive rate by 3 percentage points. The higher detection rate is explained by the higher number of biopsies obtained rather than by the penetration depth.

Introduction

With the ongoing success of chemotherapy and radiotherapy, the need for standard surgery following neoadjuvant therapy in the treatment with curative intent of different cancer types has been a topic of debate. Alternatively, the use of active surveillance has been suggested [1–6]. In a so-called "active surveillance strategy," patients will only be operated on if they have proven residual cancer after neoadjuvant therapy, without the presence of distant dissemination. This approach may be beneficial by preventing complete responders to neoadjuvant therapy from undergoing unnecessary surgery. At the same time, the success of this strategy relies on accurate and timely detection of residual cancer, as incomplete responders to neoadjuvant therapy may develop irresectable recurrences during active surveillance. Therefore, the increasing interest in active surveillance provides a new diagnostic field, which comes with great responsibility, as the outcomes of clinical response evaluations (CREs) guide further clinical decision-making.

For active surveillance in esophageal cancer patients, the use of bite-on-bite biopsies, instead of regular biopsies, has been suggested because of their higher detection rate [7]. With the bite-on-bite biopsy technique, a second biopsy is obtained at the exact same place as the first biopsy. It is believed that this may lead to deeper penetration of the gastrointestinal (GI) wall. For this reason, its use has been recommended by current clinical guidelines for classification of subepithelial lesions [8, 9]. In the context of CREs after neoadjuvant chemoradiotherapy (nCRT), the use of bite-on-bite biopsies may facilitate the detection of residual cancer underneath a tumor-free mucosa, which is reported to occur in 7 %–22 % of esophageal cancer patients [10–12]. Insight into the yield of a second (bite-on-bite) biopsy and the reason for this possible increased detection rate are essential for further optimization of the diagnostic strategy used during active surveillance.

This study aimed to assess the yield of a second (bite-on-bite) biopsy for the detection of residual esophageal cancer and to correlate this to the distribution of residual cancer found in the resection specimens.

Methods

This study is a substudy of the phase III multicenter, stepped-wedge cluster randomized controlled SANO trial (details provided in the next section). Patients were eligible for inclusion in this study if they were scheduled to undergo nCRT followed by surgery and underwent CREs after nCRT according to the SANO protocol [1]. Patients who proceeded to surgery and showed histopathological evidence of residual cancer in the resection specimen were included in the final analysis. Before the start of the inclusion period, medical ethical approval was ac-

quired from the medical research ethics committee (MEC-2017–392).

SANO trial

Details of the SANO trial procedures have been published previously [1]. In brief, the SANO trial compares the effectiveness of standard surgery versus active surveillance in clinically complete responders to nCRT for esophageal cancer. After the completion of nCRT, SANO trial participants are scheduled to undergo CREs at 6 and 12 weeks after nCRT. If there is no evidence of residual cancer during the two CREs, a patient is classified as being a clinically complete responder. Clinically complete responders undergo direct surgery or active surveillance based on a stepped-wedge cluster randomization at institutional level. When clinically complete responders are allocated to the standard surgery arm, patients will proceed to surgery if a combined ^{18}F-FDG positron emission tomography and computed tomography (PET/CT) scan shows no evidence of distant dissemination. When allocated to the active surveillance arm, CREs will be repeated at increasing time intervals: every 3 months in the first year, every 4 months in the second year, every 6 months in the third year, and yearly thereafter. In these patients, surgery will only be offered if they have proven or highly suspected residual cancer without distant dissemination.

The first CRE – performed at 6 weeks after completion of nCRT – consists of an upper GI endoscopy with bite-on-bite biopsies. The subsequent CREs consist of a PET/CT scan, upper GI endoscopy with bite-on-bite biopsies, and endoscopic ultrasound with fine-needle aspiration (FNA) of suspicious lymph nodes [7].

Endoscopic biopsies

All endoscopies were performed by dedicated endoscopists. Before nCRT, the proximal and distal border of the tumor were identified and photographic recordings were made for future reference. During CREs, white-light endoscopy was used for residual tumor assessment. At least four sets of bite-on-bite biopsies were obtained, irrespective of the tumor length. The PET/CT outcome was available to aid in the targeting of suspicious lesions.

For this study, bite-on-bite biopsies were collected separately using marked containers. The first biopsy of each set of bite-on-bite biopsies was stored in container 1 and the second biopsy was stored in container 2, eventually leading to at least four biopsies being stored in each container. All upper GI endoscopies were performed with high definition gastroscopes (Fujifilm EG 760/760Z, Olympus GIF-H190, Olympus GIF-H-180J, Pentax EG29-i10, Pentax EC38-i10F2). Bite-on-bite biopsies were obtained using regular-size biopsy forceps (Boston Radial Jaw 4, Cook Captura Biopsy Forceps, FMH Medical Glutton Life, Fujifilm Endobite 2, Olympus EndoJaw). In patients with an endoscopically non-passable stricture where no biopsies were obtained, as per protocol, this was considered an indication for surgery [1].

Histopathological assessment of endoscopic biopsies

Bite-on-bite biopsies were evaluated by a local expert GI pathologist. Biopsies were considered positive if the presence of residual cancer or high grade dysplasia (HGD) was identified [1]. Hematoxylin and eosin staining was used for the initial assessment. If there was no evidence of residual cancer, additional deeper sections and (immuno)histochemical staining (periodic acid–Schiff diastase and [pan]keratin) were performed.

The outcomes of the two containers were reported separately and were used to construct four biopsy groups; "positive first biopsy only," "positive second biopsy only," "both biopsies positive," and "both biopsies negative." This categorization allowed for a direct comparison of the outcomes of the first biopsy (i.e. first bite) and the second biopsy (i.e. second bite).

Furthermore, the combination of constructed biopsy groups allowed for a theoretical comparison of the outcomes of regular biopsies versus bite-on-bite biopsies in the same patient; the combination of the "positive first biopsy only" and "both biopsies positive" group represents the yield of regular biopsies, whereas the combination of the "positive first biopsy only," "positive second biopsy only," and "both biopsies positive" group represents the yield of bite-on-bite biopsies (Table 1s, see online-only Supplementary material). Therefore, the proportion of patients with a "positive second biopsy only" represents the additional yield of the use of bite-on-bite biopsies over regular biopsies.

Histopathological assessment of the resection specimen

Resection specimens were assessed according to the eight TNM classification [13]. The response to nCRT was classified using the modified Tumor Regression Grade (TRG) of Chirieac et al. [14]. This scale considers the rate of residual cancer and fibrosis at the primary tumor site. Histopathologically complete responders are classified as TRG1 (no residual cancer), whereas patients with residual cancer are classified as TRG2 if they have 1%–10% residual cancer, as TRG3 if they have 11%–50% residual cancer, and as TRG4 if they have more than 50% residual cancer.

All resection specimens containing residual cancer were evaluated centrally by two expert GI pathologists (L.O. and M. D.), who were blinded for the outcome of the biopsy containers. Both pathologists independently scored the TRG for each layer of the esophageal wall (mucosa, submucosa, proper muscle layer, adventitia) (Fig. 1s), as well as the most superficial tumor location, defined as the minimum distance between the residual cancer and the esophageal lumen (measured in µm). Any disagreement between the pathologists was resolved by consensus discussion.

Statistical analysis

The primary outcome of this study was the yield of a second (bite-on-bite) biopsy for the detection of residual cancer, defined as the proportion of patients with a positive second biop-

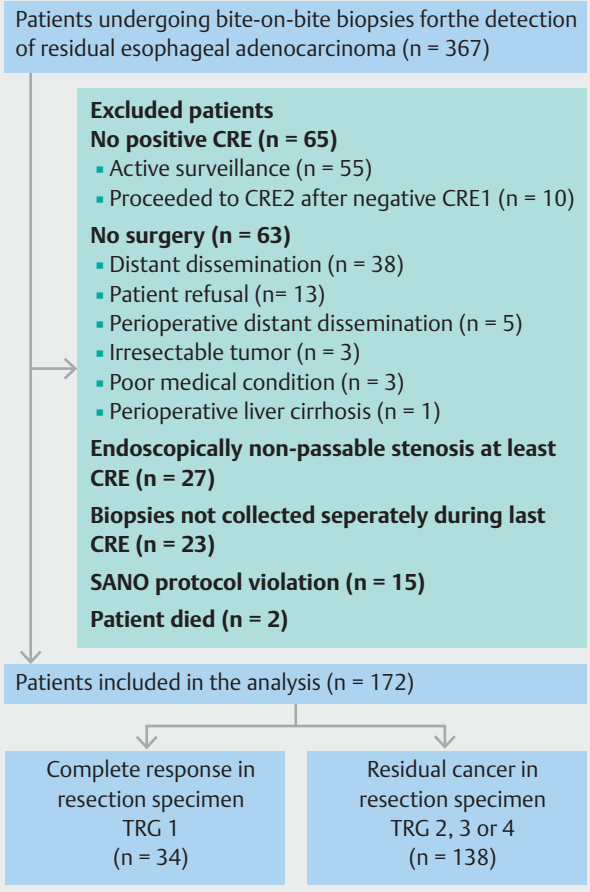
sy only. McNemar's test was used to compare the diagnostic yield of the first and second biopsies. It was assumed that the second biopsy would have a superior diagnostic yield over the first biopsy (see "sample size" section for further details).

The secondary outcomes of this study were safety and the correlation between biopsy outcome and residual cancer distribution among biopsy groups, which was compared using the chi-squared test. Additionally, residual cancer distribution was compared between patients who were detected by bite-on-bite biopsies (i.e. true positives) and patients that remained undetected (i.e. false negatives).

All analyses were performed using SPSS, version 25 (IBM Corp., Armonk, New York, USA). Tests were considered statistically significant if the *P* value was < 0.05 (two-tailed test).

Sample size

We hypothesized that a second (bite-on-bite) biopsy would increase the detection of residual cancer by 18.5 percentage points [7]. Furthermore, it was assumed that 6.2 % of patients (i.e. one-third of 18.5 %) would have a positive first biopsy but negative second biopsy. With the power set at 80 % and an alpha of 0.05, 126 patients with residual esophageal cancer in the resection specimen were needed.



▶ **Fig. 1** Flowchart of initial patient selection and exclusion. CRE, clinical response evaluation; TRG, tumor regression grade.

Results

Between October 2017 and July 2020, the bite-on-bite biopsies from 626 upper GI endoscopies were collected in 367 patients at seven hospitals. A total of 195 patients were excluded for the following reasons: 130 did not undergo surgery, 27 had an endoscopically non-passable stenosis during the last CRE, 23 did not have biopsies taken into separate biopsy containers during the last CRE, and 15 had SANO protocol violations (▶ **Fig. 1**). Of the remaining 172 patients, 34 had a complete response in the resection specimen and 138 had residual cancer in the resection specimen.

▶ **Table 1** summarizes the baseline characteristics of the 138 patients who had residual cancer in their resection specimen. Briefly, most patients were male (88 %), had an adenocarcinoma (92 %), and had a primary tumor located in the distal esophagus or at the gastroesophageal junction (99 %). The median tumor length was 4 cm. The last CRE had been performed at 6 weeks in 67 patients (49 %), at 12 weeks in 56 patients (41 %), at 6 months in 11 patients (8 %), and at ≥ 9 months after nCRT in four patients (3 %: 9 months [n = 1], 12 months [n = 2], 16 months [n = 1]).

A positive biopsy was obtained during CREs in 112 of 138 patients with residual cancer (81 %) (▶ **Table 2**). Fourteen patients (10 %) had a positive first biopsy only, 25 patients (18 %) a posi-

▶ **Table 1** Baseline characteristics of 138 patients with residual cancer in their resection specimen.

Age, median (IQR), years	67 (11)
Sex, n (%)	
▪ Male	122 (88)
▪ Female	16 (12)
Tumor type, n (%)	
▪ Adenocarcinoma	127 (92)
▪ Squamous cell carcinoma	9 (7)
▪ Other*	2 (1)
Clinical T stage, n (%)	
▪ cT1	0
▪ cT2	36 (26)
▪ cT3	100 (72)
▪ cT4	1 (1)
▪ cTx	1 (1)
Tumor distance from incisors, n (%), cm	
▪ < 20	0
▪ 20–30	2 (1)
▪ > 30	136 (99)
Tumor length, median (IQR), cm	4 (3)

IQR, interquartile range.
* Adenosquamous carcinoma (n = 1) and unknown (n = 1).

▶ **Table 2** Outcome of the bite-on-bite biopsies and findings in the resection specimen.

		Resection specimen		Total
		Complete response	Residual cancer	
Bite-on-bite biopsy	Positive	9	112	121
	Negative	25	26	51
Total		34	138	172

tive second biopsy only, and 73 patients (53 %) had both biopsies positive. Therefore, the additional yield of a second (bite-on-bite) biopsy over regular biopsies was 18 percentage points. No statistically significant difference was observed for the diagnostic yield of the first biopsy versus the second biopsy (10 % vs. 18 %; $P = 0.11$). In 40 patients (29 %), the presence of endoscopic residual tumor was reported. A positive biopsy was obtained in 37 of these patients (93 %): six patients (15 %) had a positive second biopsy only and in 31 (78 %) both biopsies were positive.

Of 26 patients with residual cancer that remained undetected by bite-on-bite biopsies (i. e. with a false-negative biopsy outcome; 19 %), 22 (16 %) had a clinically complete response and underwent surgery because they were allocated to the standard surgery arm, three (2 %) had a positive FNA from a suspicious lymph node, and one (1 %) had a high suspicion of residual cancer on the basis of endoscopic and PET/CT findings [15, 16].

Of the 34 patients with a complete response in the resection specimen (i. e. TRG 1), nine (26 %) had a false-positive biopsy: false-positive first biopsy only (n = 3), false-positive second biopsy only (n = 1), and false positive in both biopsies (n = 5). The second biopsy increased the false-positive rate by 3 percentage points. For five of the nine false-positive results, the biopsies showed HGD. Overall, the positive predictive value of the use of bite-on-bite biopsies for the detection of residual esophageal cancer was 93 %.

Tumor distribution

The distribution of residual cancer for each of the biopsy groups is shown in ▶ **Fig. 2**. The observed TRG of the esophageal mucosa and the most superficial tumor location differed statistically significantly among the biopsy groups ($P < 0.001$ and $P = 0.01$, respectively; ▶ **Table 3**). The rate of patients with a tumor-free mucosa was higher in the positive first biopsy only group compared with the positive second biopsy only group (21 % vs. 0 %). Also, patients in the positive first biopsy only group had a less superficial tumor location compared with the positive second biopsy only group, as reflected by the higher rate of patients with residual cancer located 500–1000 µm or > 1000 µm from the esophageal lumen.

A statistically significantly higher rate of patients with a tumor-free mucosa ($P = 0.002$; ▶ **Table 4**) and a less superficial tumor location ($P = 0.03$) were observed in patients with tumors that remained undetected, compared with patients with tumors that were detected by bite-on-bite biopsies. Even so, only two of the 26 patients with undetected tumors had a tumor located > 1000 µm from the esophageal lumen. Patients with tumors that remained undetected by bite-on-bite biopsies tended to have a lower tumor distribution in the mucosa (i. e. TRG 2; 21 % vs. 46 %).

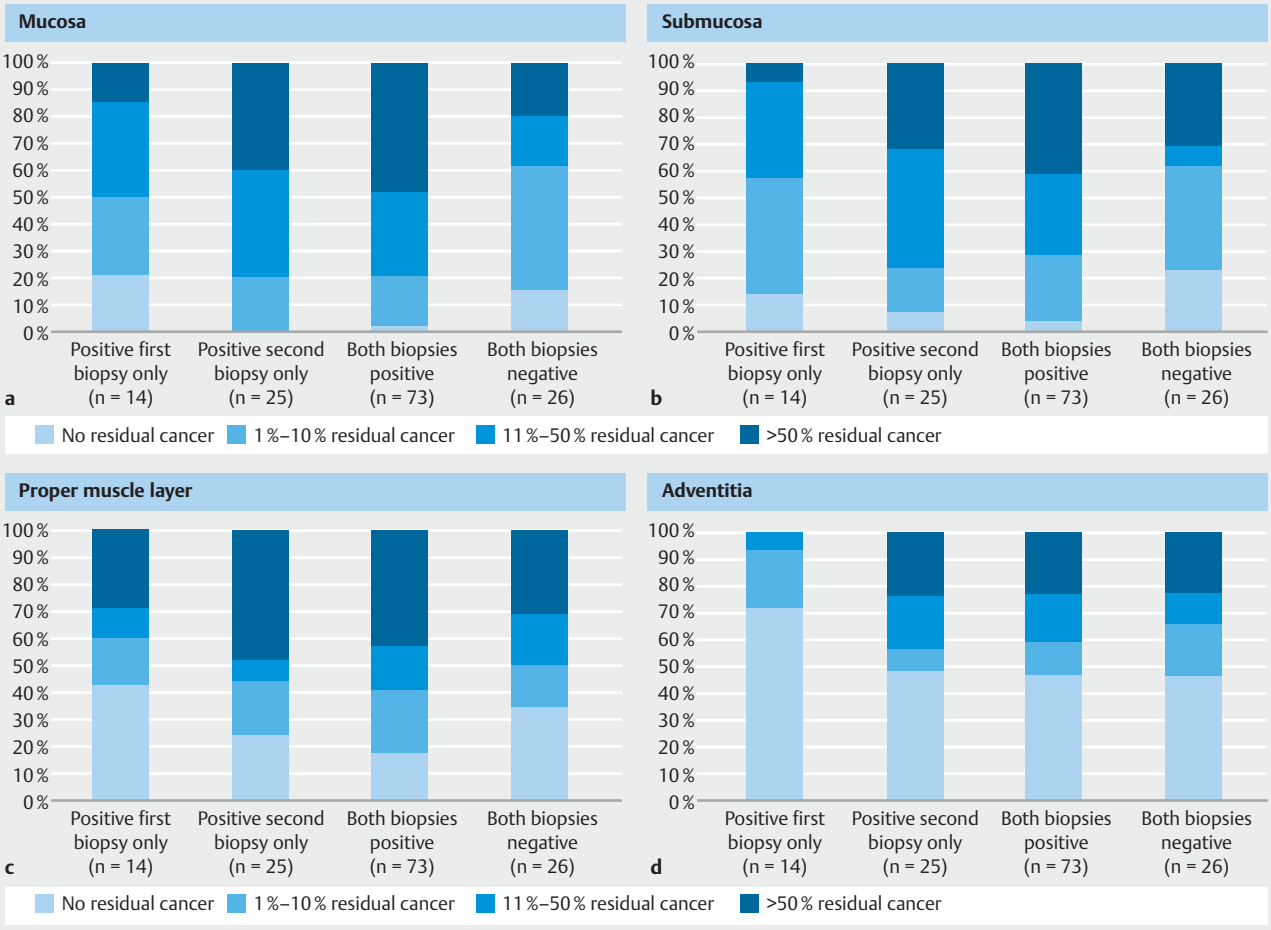
Patients with a tumor-free mucosa

Of eight patients with a tumor-free mucosa in the resection specimen (6 %), four were detected by bite-on-bite biopsies: positive first biopsy only (n = 3) and both biopsies positive (n = 1) (▶ **Table 3**). The most superficial tumor location ranged between 500 and 2450 µm (500, 790, 1070, and 2450 µm, respectively). In the four patients with a tumor-free mucosa who re-

▶ **Table 3** Histopathological assessment of the mucosa and most superficial tumor location in the different biopsy groups.

	Positive first biopsy only (n = 14)	Positive second biopsy only (n = 25)	Both biopsies positive (n = 73)	Both biopsies negative (n = 26)	*P* value
TRG of the mucosa, n (%)					< 0.001
▪ TRG 1	3 (21)	0	1 (1)	4 (15)	
▪ TRG 2	4 (29)	5 (20)	14 (19)	12 (46)	
▪ TRG 3	5 (36)	10 (40)	23 (32)	5 (19)	
▪ TRG 4	2 (14)	10 (40)	35 (48)	5 (19)	
Most superficial tumor location, n (%), µm*					0.01
▪ 0–500	10 (71)	23 (92)	70 (96)	19 (73)	
▪ 500–1000	2 (14)	1 (4)	3 (4)	5 (19)	
▪ > 1000	2 (14)	1 (4)	0	2 (8)	

TRG, tumor regression grade.
* Minimum distance between esophageal lumen and residual cancer.



▶ **Fig. 2** Histograms for the different biopsy groups of the residual tumor distribution within the following layers of the resected esophageal specimen: **a** mucosa; **b** submucosa; **c** proper muscle layer; **d** adventitia.

▶ **Table 4** Histopathological assessment of the mucosa and most superficial tumor location of patients detected by bite-on-bite biopsies and patients that remained undetected.

	Positive bite-on-bite biopsy (n = 112)	Negative bite-on-bite biopsy (n = 26)	*P*-value
TRG of the mucosa, n (%)			0.002
TRG 1	4 (4)	4 (15)	
TRG 2	23 (21)	12 (46)	
TRG 3	38 (34)	5 (19)	
TRG 4	47 (42)	5 (19)	
Most superficial tumor location, n (%), µm			0.03
0–500	103 (92)	19 (73)	
500–1000	6 (5)	5 (19)	
>1000	3 (3)	2 (8)	

TRG, tumor regression grade.

mained undetected, the most superficial tumor location ranged between 500 and 11 900 µm (500, 609, 2090, and 11 900 µm, respectively).

Safety

In the 626 upper GI endoscopies performed for this study, no bite-on-bite biopsy-related adverse events occurred.

Discussion

This is the first study to assess the diagnostic value of a second (bite-on-bite) biopsy for the detection of residual cancer after nCRT. The present study shows that the performance of a second (bite-on-bite) biopsy is safe and increases the detection rate by 18 percentage points, at the expense of increasing the false-positive rate by 3 percentage points. Overall, the use of bite-on-bite biopsies led to a detection rate of 81 % and had a positive predictive value of 93 %. Because of the pragmatic nature of this study, we believe these outcomes reflect daily practice. The additional yield of a second (bite-on-bite) biopsy was not related to the detection of patients with a less superficial tumor location, but can probably be explained by the increased number of biopsies obtained. Based on this outcome, we recommend that for active surveillance strategies at least eight biopsies of the primary tumor site should be obtained for the optimal detection of residual cancer.

Most previous studies evaluating the use of bite-on-bite biopsies have investigated its use for the classification of subepithelial lesions in treatment-naïve patients [8]. Although one study included patients with a previous nonconclusive biopsy outcome, none performed a direct comparison of bite-on-bite biopsies and regular biopsies [17]. In the preSANO trial, an indirect comparison of the outcomes of regular biopsies and bite-on-bite biopsies was performed, by comparing the detection rate of residual esophageal cancer before and after amending the biopsy protocol [7]. The authors suggested that the increased detection rate of bite-on-bite biopsies was related to deeper penetration of the esophageal wall; reaching to the submucosa, leading to detection of residual cancer underneath a tumor-free mucosa.

In this regard, van der Wilk et al. investigated the composition of the bite-on-bite biopsies obtained in the preSANO trial [12]. Only four of the 88 included bite-on-bite biopsy specimens contained histopathological features distinctive for the submucosa, suggesting biopsy penetration of the esophageal wall to be limited. The authors noted, however, that this might be an underestimation, given the low distribution of features distinctive for the submucosa, compromising only 1 %–2 % of the submucosa in the normal esophagus. In other words, it is likely that most biopsies actually reaching the submucosa do not contain these features. Therefore, its absence cannot rule out penetration of the submucosa.

Based on the current findings, no conclusive evidence can be provided as to whether the use of bite-on-bite biopsies leads to deeper penetration of the esophageal wall. Furthermore, although we believe that experienced endoscopists are able to target the same location twice, the performance of bite-on-bite biopsies may be challenging. For instance, bleeding from previous biopsy sites may lead to difficulties while targeting the area of the previous biopsy.

In the present study, insight is provided into the reason for the additional yield of a second (bite-on-bite) biopsy, by allowing for a comparison of residual tumor distribution between patients detected by the first biopsy versus the second biopsy. If related to a deeper biopsy penetration depth, one would expect the rate of patients with a tumor-free mucosa or a less superficial tumor location to be higher in the positive second biopsy only group. Interestingly, the opposite was observed: rates were higher in the positive first biopsy only group. Based on this finding, it seems that the increased number of biopsies collected by bite-on-bite biopsies is a more likely explanation of the additional yield.

An active surveillance strategy is presently being investigated for esophageal cancer, but has already been implemented for rectal cancer [2, 3]. In both esophageal cancer patients and rectal cancer patients, studies have focused on the anatomical location of the residual cancer [10–12, 18]. The present study has further identified that the most superficial tumor location is another interesting parameter to be taken into account when assessing applicability for detection by endoscopic biopsies.

In a setting after neoadjuvant therapy, the anatomical location of residual cancer may be a less reliable marker as neoadjuvant therapy-induced changes, such as atrophy and ulceration, may lead to a more superficial location of structures that would otherwise remain out of reach. Our study shows that, in most patients in whom residual tumor remained undetected, residual cancer was located within 1000 µm of the esophageal lumen, which seems within reach of the available endoscopic biopsy forceps. Furthermore, missed patients tended to have a lower tumor distribution. Therefore, even when the site of residual tumor is adequately identified, the biopsy outcome may be falsely negative as a result of focal tumor distribution. This is strengthened by the finding that some patients with endoscopically identifiable residual tumor present had a negative biopsy outcome or a positive second biopsy only. Increasing the number of biopsies obtained may improve the detection of residual cancer.

Moreover, the current study shows that an increased number of biopsies leads to only a limited increase in the false-positive rate. A false-positive classification among histopathological complete responders was mostly explained by biopsies that showed HGD. To date, it remains unclear whether these patients may be safely treated with endoscopic eradication therapy.

There are some limitations to this study. First, the study design of the SANO trial may have led to an overestimation of the additional yield of bite-on-bite biopsies, as some patients with false-negative biopsies may still undergo active surveillance. Nevertheless, the observed additional yield is comparable to that observed in the preSANO trial, in which patients proceeded to surgery irrespective of the outcomes of CREs [7]. Likewise, the specificity of bite-on-bite biopsies could not be determined, because – in the absence of a false-positive biopsy out-

come – complete responders allocated to the active surveillance arm did not proceed to surgery.

Finally, each biopsy should, ideally, be stored in a separate container to allow for a more thorough comparison of the outcomes of regular biopsies and bite-on-bite biopsies, especially in patients with a positive first biopsy and a positive second biopsy. This might also allow determination of the optimal number of biopsies needed to maximize the detection of residual cancer. Nevertheless, this design is more complicated and prone to confusion, as at each endoscopy at least four bite-on-bite biopsies are obtained. Additionally, it would require vast amounts of resources, because a positive biopsy was collected in only approximately one out of five upper GI endoscopies.

In conclusion, the present study shows that there is an additional yield of almost 20 percentage points to the use of second (bite-on-bite) biopsies for the detection of residual cancer after nCRT. Based on the observed tumor distribution, the additional yield in this setting is most likely explained by the increased number of biopsies obtained, instead of by deeper biopsy penetration of the esophageal wall. The collection of a higher number of biopsies may improve the detection rate of residual cancer during active surveillance and comes at the price of only a limited increase in the false-positive rate.

Acknowledgments

The authors want to thank the Dutch Cancer Society and ZonMW for the funding of this study.

Competing interests

P.D. Siersema has received research support from Pentax, The E-Nose Company, MicroTech, and Motus GI, and consultation fees from Boston Scientific and Motus GI. J.J.B van Lanschot has received research support from the Dutch Cancer Society and ZonMW. The remaining authors declare that they have no conflict of interest.

Funding

KWF Kankerbestrijding | 10825
ZonMw | 843004104

Clinical trial

Netherlands National Trial Register (http://www.trialregister.nl) | Registration number (trial ID): NTR6803 | Type of study: Multicenter study

References

[1] Noordman BJ, Wijnhoven BPL, Lagarde SM et al. Neoadjuvant chemoradiotherapy plus surgery versus active surveillance for oesophageal cancer: a stepped-wedge cluster randomised trial. BMC Cancer 2018; 18: 142

[2] van der Valk MJM, Hilling DE, Bastiaannet E et al. Long-term outcomes of clinical complete responders after neoadjuvant treatment for rectal cancer in the International Watch & Wait Database (IWWD): an international multicentre registry study. Lancet 2018; 391: 2537–2545

[3] Renehan AG, Malcomson L, Emsley R et al. Watch-and-wait approach versus surgical resection after chemoradiotherapy for patients with rectal cancer (the OnCoRe project): a propensity-score matched cohort analysis. Lancet Oncol 2016; 17: 174–183

[4] Klotz L, Zhang L, Lam A et al. Clinical results of long-term follow-up of a large, active surveillance cohort with localized prostate cancer. J Clin Oncol 2010; 28: 126–131

[5] Vokes EE, Kies MS, Haraf DJ et al. Concomitant chemoradiotherapy as primary therapy for locoregionally advanced head and neck cancer. J Clin Oncol 2000; 18: 1652–1661

[6] Rodel C, Weiss C, Sauer R. Trimodality treatment and selective organ preservation for bladder cancer. J Clin Oncol 2006; 24: 5536–5544

[7] Noordman BJ, Spaander MCW, Valkema R et al. Detection of residual disease after neoadjuvant chemoradiotherapy for oesophageal cancer (preSANO): a prospective multicentre, diagnostic cohort study. Lancet Oncol 2018; 19: 965–974

[8] Dumonceau JM, Deprez PH, Jenssen C et al. Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline – Updated January 2017. Endoscopy 2017; 49: 695–714

[9] Faulx AL, Kothari S. Standards of Practice Committee. et al. The role of endoscopy in subepithelial lesions of the GI tract. Gastrointest Endosc 2017; 85: 1117–1132

[10] Shapiro J, ten Kate FJ, van Hagen P et al. Residual esophageal cancer after neoadjuvant chemoradiotherapy frequently involves the mucosa and submucosa. Ann Surg 2013; 258: 678–688 ; discussion 688-679

[11] Chao YK, Chuang WY, Yeh CJ et al. Anatomical distribution of residual cancer in patients with oesophageal squamous cell carcinoma who achieved clinically complete response after neoadjuvant chemoradiotherapy. Eur J Cardiothorac Surg 2018; 53: 201–208

[12] van der Wilk BJ, Eyck BM, Doukas M et al. Residual disease after neoadjuvant chemoradiotherapy for oesophageal cancer: locations undetected by endoscopic biopsies in the preSANO trial. Br J Surg 2020; 107: 1791–1800

[13] Rice TW, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: application to clinical practice. Ann Cardiothorac Surg 2017; 6: 119–130

[14] Chirieac LR, Swisher SG, Ajani JA et al. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. Cancer 2005; 103: 1347–1355

[15] van der Bogt RD, van der Wilk BJ, Nikkessen S et al. Predictive value of endoscopic esophageal findings for residual esophageal cancer after neoadjuvant chemoradiotherapy. Endoscopy 2021; 53: 1098–1104

[16] Valkema MJ, van der Wilk BJ, Eyck BM et al. Surveillance of clinically complete responders using serial (18)F-FDG PET/CT scans in patients with esophageal cancer after neoadjuvant chemoradiotherapy. J Nucl Med 2021; 62: 486–492

[17] Komanduri S, Keefer L, Jakate S. Diagnostic yield of a novel jumbo biopsy "unroofing" technique for tissue acquisition of gastric submucosal masses. Endoscopy 2011; 43: 849–855

[18] Duldulao MP, Lee W, Streja L et al. Distribution of residual cancer cells in the bowel wall after neoadjuvant chemoradiation in patients with rectal cancer. Dis Colon Rectum 2013; 56: 142–149