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Original Article

The SpaTemp cohort: 168 nondysplastic Barrett's esophagus surveillance patients with and without progression to early neoplasia to evaluate the distribution of biomarkers over space and time

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SUMMARY. **The ReBus cohort is a matched nested case–control cohort of patients with nondysplastic (ND) Barrett's esophagus (BE) at baseline who progressed (progressors) or did not progress (nonprogressors) to high-grade dysplasia (HGD) or cancer. This cohort is constructed using the most stringent inclusion criteria to optimize explorative studies on biomarkers predicting malignant progression in NDBE. These explorative studies may benefit from expanding the number of cases and by incorporating samples that allow assessment of the biomarker over space (spatial variability) and over time (temporal variability). To (i) update the ReBus cohort by identifying new progressors and (ii) identify progressors and nonprogressors within the updated ReBus cohort containing spatial and temporal information. The ReBus cohort was updated by identifying Barrett's patients referred for endoscopic work-up of neoplasia at 4 tertiary referral centers. Progressors and nonprogressors with a multilevel (spatial) endoscopy and additional prior (temporal) endoscopies were identified to evaluate biomarkers over space and over time. The original ReBus cohort consisted of 165 progressors and 723 nonprogressors. We identified 65 new progressors meeting the same strict selection criteria, resulting in a total number of 230 progressors and 723 matched nonprogressors in the updated ReBus cohort. Within the updated cohort, 61 progressors and 107 nonprogressors (mean age 61 ± 10 years) with a spatial endoscopy (median level 3 [2–4]) were identified. 33/61 progressors and 50/107 nonprogressors had a median of 3 (2–4) additional temporal endoscopies. Our updated ReBus cohort consists of 230 progressors and 723 matched nonprogressors using the most strict selection criteria. In a subgroup of 168 Barrett's patients (the SpaTemp cohort), multiple levels have been sampled at baseline and during follow-up providing a unique platform to study spatial and temporal distribution of biomarkers in BE.**

KEY WORDS: **Barrett's Esophagus, Esophageal Adenocarcinoma, Risk Stratification, Biomarker, Spatial, Temporal.**

INTRODUCTION

Patients with Barrett's esophagus (BE) undergo endoscopic surveillance to detect esophageal adenocarcinoma (EAC) at an early and curable stage.[1,2] However, endoscopic surveillance has several limitations, since it is subject to biopsy sampling error, histological evaluation of biopsies is subjective and the yearly cumulative risk of progression to EAC of 0.6% is low.[3] Objective biomarkers, which risk stratify

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BE patients into low- and high-risk for progression, may improve current surveillance strategies. Several promising biomarkers were studied in recent years, but none of them has yet been integrated into routine clinical practice.[4–7]

An important reason why translation of biomarkers into clinical practice has been unsuccessful is the variability of biomarker expression across the surface of a Barrett's segment (spatial distribution).[8] If expression of a biomarker is highly variable across a Barrett's segment, external validation of such a biomarker may fail if it is applied on single biopsies or single level biopsies from a longer Barrett's segment.

Additionally, more insight in expression of biomarkers over time (temporal distribution) may be useful in personalizing surveillance intervals. If a biomarker predicts progression early and reliable with little variation over time, patients with a low risk of progression may undergo more lenient surveillance.

More knowledge on spatial and temporal distribution of biomarkers in a Barrett's segment will lead to better risk stratification, lower number of surveillance endoscopies, and consequently decrease overall healthcare costs.[9]

In 2018, the *Predict neoplastic progression in Barrett's esophagUS (ReBus) cohort* was published.[10] The ReBus cohort is a matched case–control cohort of patients with NDBE at baseline with (cases) or without (controls) progression to HGD or cancer. This cohort was constructed using the most stringent inclusion criteria, to prevent inclusion of patients with prevalent neoplasia at baseline, and patients without a confirmed diagnosis of HGD/EAC.

To optimize our ongoing biomarker work to predict malignant progression in NDBE, we attempted to increase the number of progressors and controls while maintaining the strict selection criteria. Therefore, the aims of this project were (i) to update the ReBus cohort by identifying new progressors and (ii) to identify progressors and nonprogressors within the updated ReBus cohort with biopsies obtained at multiple levels and time points to enable testing of SPAtial and TEMPoral variability of biomarkers (SpaTemp cohort).

METHODS

Setting and source population

In 2014, we retrospectively identified all Barrett's patients with early neoplasia (progressors) diagnosed between January 2000 and December 2013 at three tertiary referral centers for BE neoplasia (Academic Medical Center, Amsterdam; Catharina Hospital, Eindhoven; St. Antonius Hospital, Nieuwegein).[10] For the current study, we updated the cohort by screening all additional patients referred for endoscopic work-up of early Barrett's neoplasia between January 2014 and December 2017 at the original three tertiary referral centers. In addition, all patients referred for endoscopic work-up at the Isala hospital Zwolle between January 2006 and December 2017 were identified.

Identification and data collection for progressors

Two researchers (NF, KK) independently performed a chart evaluation of all patients who underwent endoscopic work-up for BE neoplasia using endoscopy lists from the included hospitals. In order to complete surveillance history of these patients, all surveillance endoscopies with biopsies were identified per patient using the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA database), which has nationwide coverage since 1991.[11] All original surveillance endoscopies and pathology reports were retrieved from the referring hospitals. Relevant baseline characteristics (i.e. esophageal landmarks, number and location of biopsy sampling, number and outcome of surveillance endoscopies, worst histological diagnosis, number of endoscopies with HGD/EAC and specimen type on which the progression diagnosis was made) were recorded and entered into a secured, anonymized electronic database. All progressors who were identified in the current study were added to the original ReBus progressor cohort, if they met the following inclusion criteria:

- Nondysplastic BE at baseline, biopsy index ≥0.5 (definition in section below).
- HGD or EAC diagnosed by at least two independent pathologists based on biopsies from two separate endoscopies, or in a single endoscopic or surgical resection specimen.
- Maximum T1 disease at time of progression (including T1a and T1b).[12]

Progressors were excluded in case of:

- A diagnosis of HGD or EAC before baseline.
- Less than 2 years of endoscopic follow-up prior to progression.
- Any (community based and/or expert confirmed) LGD at baseline.

Identification of eligible nonprogressors

In the current study, we did not attempt to identify additional nonprogressors given the availability of 723 nonprogressors in the original ReBus cohort. Nonprogressors were identified as described earlier.[10]

Identification of progressors and nonprogressors with endoscopies with spatial tissue sampling

Within the updated ReBus cohort, we identified all progressors and nonprogressors with a 'spatial' baseline endoscopy (BL), defined as an endoscopy with at least two separate formalin-fixed paraffin-embedded (FFPE) tissue blocks containing intestinal metaplasia (IM) without dysplasia. To exclude any progression after the spatial BL endoscopy, nonprogressors needed an additional high-quality surveillance endoscopy showing no progression to HGD and/or EAC >3 years after the spatial BL endoscopy. The location where biopsies were obtained during endoscopy was extracted from the corresponding histology and/or endoscopy reports.

Identification of progressors and nonprogressors with additional 'temporal' endoscopies prior to baseline

Out of the patients with a spatial BL endoscopy, we identified those with at least one additional (temporal) surveillance endoscopy without dysplasia prior to the spatial baseline endoscopy.

Biopsy index as quality metric for included endoscopies

Recent studies indicate that a high quality of sampling is associated with a higher dysplasia detection rate and thus decreases chance of missed prevalent dysplasia/neoplasia, but no internationally accepted quality metric to assess sampling quality is defined yet.[13,14] We therefore introduced a 'biopsy index' as a quality metric for included endoscopies. The biopsy index was defined as 50% of the biopsies as recommended per 4q2cm Seattle protocol (i.e. 4 biopsies in a 4 cm Barrett = biopsy index of 0.5). Eligible endoscopies needed a biopsy index of ≥0.5 to be included.

Histologic material and legal considerations

All patients were contacted to obtain written informed consent for transferring medical data and tissue obtained during surveillance endoscopies at the referring hospital. The Dutch Medical Research Involving Human Subject act (WMO) does not apply to the ReBus project, and the ethics committee of the AMC has exempted the project from formal review. The ReBus project was approved by the Biobank Review Committee of the AMC in 2014.

Statistical analysis

Categorical data were described using percentages. Continuous variables were described as mean and standard deviation or median and interquartile range. Statistical analysis was performed using SPSS Version 25.

RESULTS

The update of the ReBus cohort and the selection of progressors is summarized in Supplementary Figure Fig. S1 and Figure 1. Limitations of earlier biomarker studies and a summary of the most stringent inclusion criteria applied in all progressors and nonprogressors included in the ReBus cohort are showed in Table 1.

Between 2000 and 2013, a total of 887 BE patients were referred for endoscopic work-up of BE neoplasia to the AMC, St. Antonius hospital and Catharina hospital. A total of 165 out of the 887 BE patients met all inclusion criteria and were eligible as progressors in the ReBus cohort.

Based on a retrospective regional registration database in 10 community hospitals in the Amsterdam region between 2003 and 2013, a total of 2206 Barrett's patients without progression to HGD/EAC were identified. A total of 723/2206 had at least 2 subsequent endoscopies with a minimal interval of 2 years and were therefore included as nonprogressors. Progressors and nonprogressors were matched for age (±5 years), maximal Barrett's length (±2 cm), and sex, resulting in 165 progressors and 723 matched nonprogressors in the original ReBus cohort.

Between 2014 and 2017, a total of 428 BE patients were referred for endoscopic work-up of BE neoplasia to the AMC, St. Antonius hospital and Catharina hospital. A total of 154 patients were diagnosed with HGD/T1 carcinoma. A total of 52/154 patients met inclusion criteria.

Between 2006 and 2017, a total of 138 BE patients were referred for endoscopic work-up of BE neoplasia to the Isala Clinics Zwolle. A total of 51 patients were diagnosed with HGD/T1 carcinoma and 13/51 patients met all inclusion criteria.

The total of 65 newly identified progressors were added to the original ReBus cohort. We did not attempt to expand on the number of nonprogressors for this study. This resulted in 230 Barrett's patients with and 723 without progression to HGD/EAC, amenable for biomarker research and as source population for the SpaTemp cohort.

Progressors SpaTemp cohort

The selection of progressors eligible for the SpaTemp cohort is depicted in Figure 2a, demographics are summarized in Table 2a and b.

'Spatial' endoscopies. A total of 86/230 progressors had a nondysplastic spatial BL endoscopy. In 61/86 progressors, the spatial BL endoscopy was of sufficient quality (median biopsy index 1.0 [IQR 0.7,1.2]) and therefore included in the SpaTemp cohort. First progression (HGD $n = 22$; T1a EAC $n = 31$; T1b EAC $n = 8$) was diagnosed after a mean of 3.7 ± 1.7 years following the spatial BL endoscopy. Spatial BL

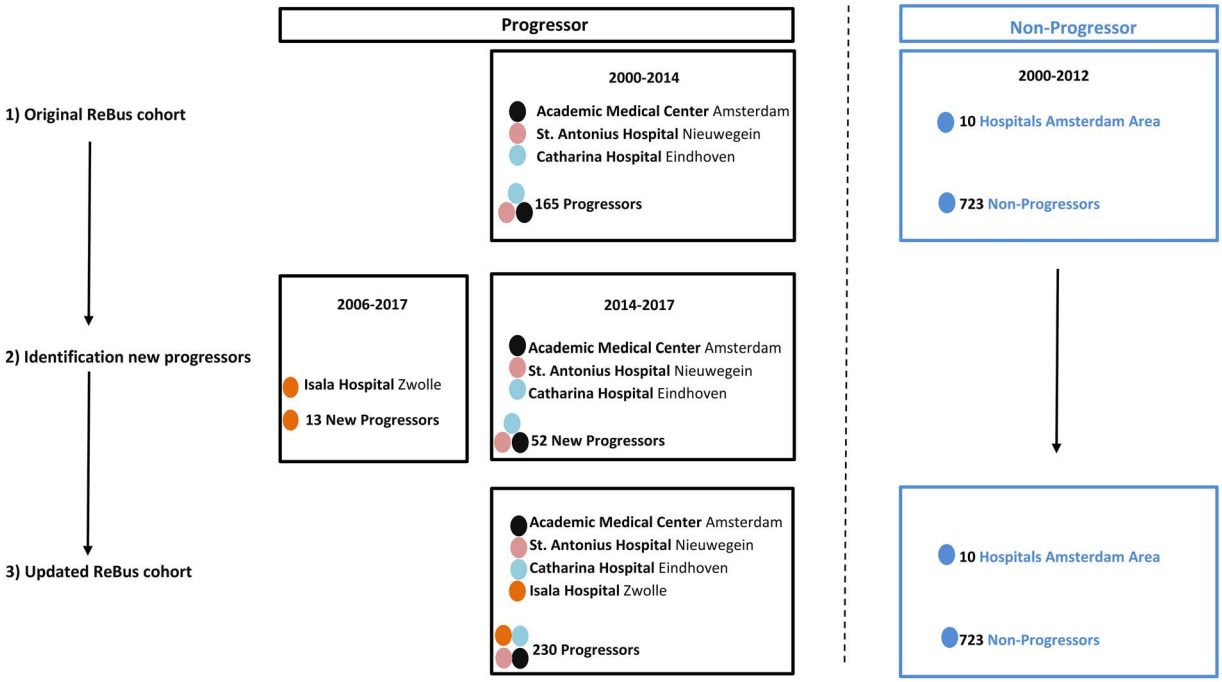

Fig. 1 Illustration of the updated ReBus cohort (adapted by Duits *et al.*), which forms the source population for the derived SpaTemp cohort. (i) The original ReBus cohort consisted of 165 progressors and 723 nonprogressors published by Duits *et al.* (ii) Identification of additional progressors in the newly included Isala hospital Zwolle and updating the three original participating center Amsterdam, Nieuwegein and Eindhoven. (iii) Updated ReBus cohort.

Table 1 Limitations of earlier biomarker studies and interventions/inclusion criteria applied to all progressors and nonprogressors included in the ReBus cohort

	Limitations earlier biomarker studies	Intervention ReBus cohort	Applied inclusion criteria
Progressors	Dilution of progressor cohort by inclusion of BE patients without real progression	Ensure unequivocal progression to HGD/EAC	Expert review of HGD/EAC diagnosis HGD/EAC diagnosed in ER specimen OR esophagectomy specimen OR 2 subsequent biopsies
	Prevalent neoplasia at BL due to: (i) short intervals between BL and progression	Exclude prevalent neoplasia at baseline	≥2 years between BL diagnosis and neoplastic progression
	(ii) inclusion of BE patients with advanced cancers (>T2)	Maximal T1 stage at progression	
	Ensure high-quality BL endoscopy with ≥50% of biopsies as required per Seattle protocol		
	Heterogeneity of included progressors due to inclusion of BL endoscopies with IND and/or LGD	Ensure absence of dysplasia at BL endoscopy to develop/validate biomarker in nondysplastic BE	Exclusion of cases with expert confirmed LGD dysplasia at BL
Nonprogressors	Missed prevalent neoplasia at BL and/or incident progression during follow-up after BL	Ensure absence of progression to HGD/EAC during follow-up after BL	≥2 BE surveillance endoscopies performed
	≥2 years of endoscopic surveillance after BL endoscopy		
	Heterogeneity of included nonprogressors due to inclusion of BL endoscopies with IND and/or LGD	Ensure absence of dysplasia at BL endoscopy to develop/validate biomarker in nondysplastic BE	Exclusion of cases with any LGD dysplasia at BL

BE, Barrett's esophagus; BL, baseline endoscopy; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; IND, indefinite for dysplasia; LGD, low-grade dysplasia.

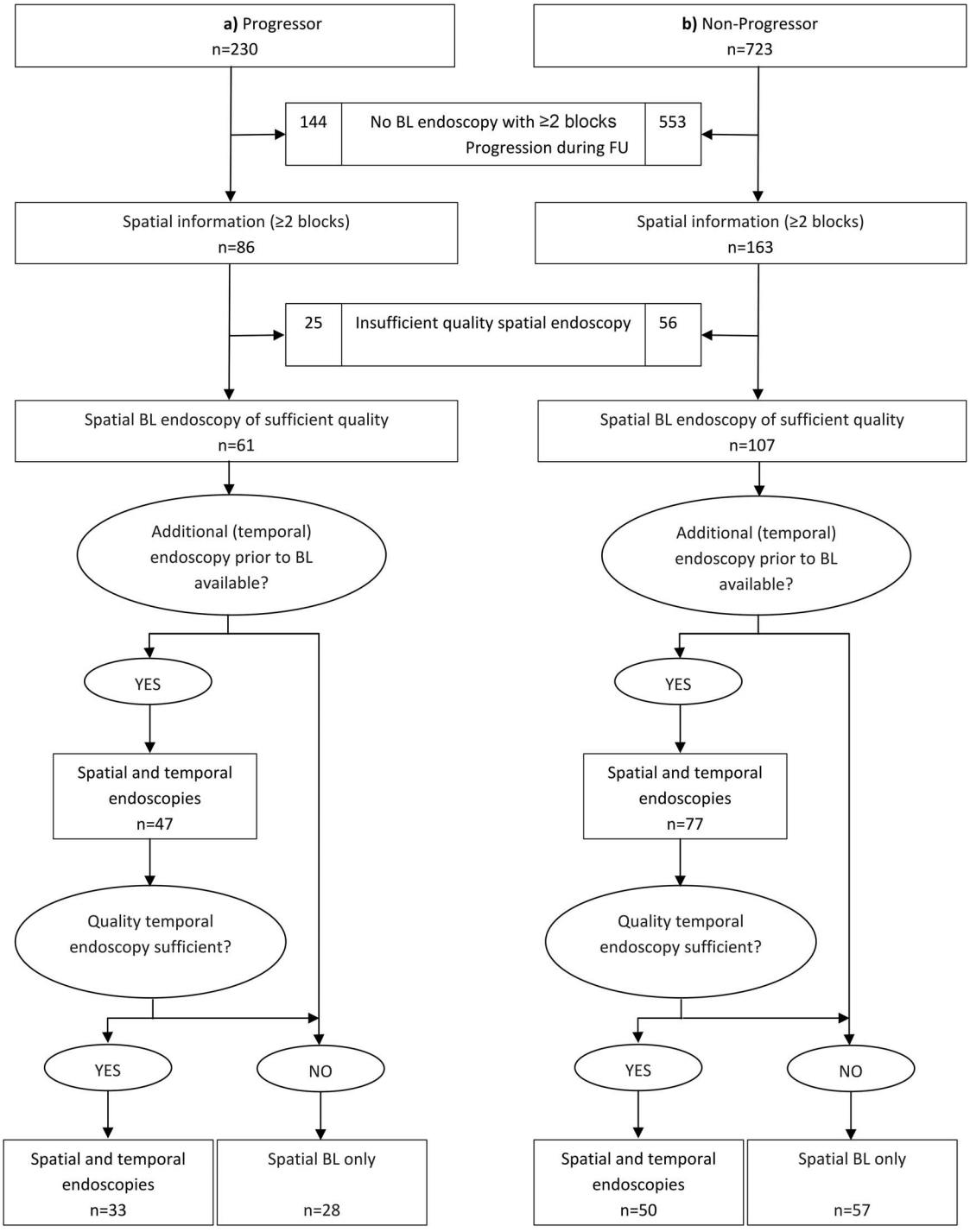

Fig. 2 Flowchart illustrating the selection of (a) progressors (P) and (b) nonprogressors (NP) eligible for biomarker research to evaluate spatial and/or temporal distribution based on the updated ReBus cohort.

endoscopies had a median BE length of 5 (IQR 4,7) and 3 (IQR 2,4) biopsy levels per endoscopy.

'Temporal' endoscopies. In 33/61 progressors with a spatial BL endoscopy, a total of 244 additional temporal endoscopies prior to the baseline endoscopy were available, from which 118 were of sufficient quality. Per patient, a median of 3 (IQR 2,4) temporal endoscopies were of sufficient quality (median biopsy index 0.8 [IQR 0.5, 1.0]) and therefore included in the study cohort.

Nonprogressors SpaTemp cohort

The selection of nonprogressors eligible for the SpaTemp cohort is depicted in Figure 2b, demographics in Table 2a and b.

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Table 2 (a) Baseline characteristics of the 168 patients included in the SpaTemp cohort

	Progressors, $n = 61$	Nonprogressors, $n = 107$
Male, n (%)	47 (77)	74 (69)
Age at BE diagnosis, years ±SD	55 ± 10	55 ± 11
Length of BE segment, cm (IQR)	5 (4,8)	5 (3,6)
Follow-up* after baseline, years ±SD	3.7 ± 1.7	5.1 ± 2.1
Age at HGD/EAC diagnosis, years ±SD	64.9 ± 9.8	67.1 ± 10.0
Progression diagnosis, *n* (%)		
- High-grade dysplasia,	22 (36)	NA
- Intramucosal carcinoma	31 (51)	NA
- Submucosal carcinoma	8 (13)	NA
Diagnosis based on, *n* (%)		
- Endoscopic resection specimen	52 (85)	NA
- Esophagectomy specimen	2 (3)	NA
- Biopsy from 2 separate endoscopies	7 (12)	NA

(b) Baseline characteristics of the (multilevel) spatial baseline endoscopies and temporal endoscopies prior to the baseline

	Progressors, $n = 61$		Nonprogressors, $n = 107$	
	Baseline endoscopy	Temporal endoscopies	Baseline endoscopy	Temporal endoscopies
Levels per endoscopy, *n*, (IQR)	3 (2,4)	2 (2,5)	2 (2,3)	2 (2,4)
Biopsy index, (IQR)	1.0 (0.7,1.2)	0.8 (0.5,1.0)	1.0 (0.6,1.2)	0.8 (0.5,1.0)
Total temporal endoscopies, *n* (IQR)	NA	3 (2,4)	NA	2 (2,5)

*Last follow-up without progression in nonprogressors, first diagnosis of HGD/EAC in progressors.

'Spatial' endoscopies. A total of 164/723 nonprogressors had a nondysplastic spatial BL endoscopy. All patients had one endoscopy >3 years prior the last endoscopic follow-up showing no progression. In 107/164 nonprogressors, the spatial BL endoscopy was of sufficient quality (median biopsy index 1.0 [IQR 0.6,1.2]) and were therefore included in the SpaTemp cohort. Spatial BL endoscopies had a median BE length of 5 cm (IQR 3,6) and 2 (IQR 2,3) biopsy levels per endoscopy.

'Temporal' endoscopies. In 50/107 nonprogressors with a spatial BL endoscopy, a total of 390 additional temporal endoscopies prior to the baseline were available, from which 197 were of sufficient quality. Per patient, a median of 3 (IQR 2,5) temporal endoscopies were of sufficient quality (median biopsy index 0.8 [IQR 0.8,1.0]) and therefore included in the study cohort.

DISCUSSION

Herein we describe an expansion of our original ReBus cohort and the subsequent subselection ('the SpaTemp cohort') for studying biomarkers in Barrett's esophagus. Using the most stringent inclusion criteria, the SpaTemp cohort consists of 168 patients (61 progressors, 107 nonprogressors) with multiple FFPE biopsy levels of the baseline endoscopy and at different moments in time. Since informed consent was obtained to use tissue samples for biomarker studies, this purified and well-described cohort provides a unique platform for studies focusing on expression of biomarkers over space and time.

Three research groups have focused on the evaluation of biomarker variability over space and time (Table 3). The Seattle group has performed 3 studies using fresh-frozen biopsies from a prospective cohort of BE patients at a tertiary referral center.[15–17] Samples from multiple endoscopic levels from two separate time points were used to develop a highly discriminating (AUC = 0.94) prediction model using 29 chromosomal features. However, this study had four important limitations, which diminish the significance of its results. First, only a single biopsy per 2-cm Barrett's length was used instead of the standard four-quadrant sampling in routine practise. Second, fresh-frozen biopsies were used in the analysis, which makes application of the biomarkers in daily practise highly unpractical. In contrast, in the ReBus cohort, FFPE tissue blocks are available, which are currently standard of care in endoscopic surveillance of Barrett's patients. Biomarkers developed in this medium can easily be transferred into clinical practice. Third, prevalent neoplasia cannot be excluded in a majority of the tested progressor samples, since there was no minimal interval between the second time point and progression. Furthermore, although the study protocol states biopsy sampling according to the Seattle protocol with 4-quadrant biopsies every 1–2 cm along the entire Barrett's segment, no quality measurements reinforcing the sampling quality are presented in the results section. We insisted on a minimal interval of 2 years between the nondysplastic sample and the date of progression, with all of them having at least 50% of biopsies as required per Seattle protocol. Last, no minimal 'cancer-free follow-up' is available in nonprogressors. Since the last endoscopy without any additional follow-up was used as second time point, neither prevalent neoplasia nor incident progression shortly thereafter can be excluded. We exclusively included nonprogressors with at least one

Table 3 Summary of published studies testing biomarkers in multiple levels and/or time points

Reference	Study setting and biopsy material	Sample size cases: controls	Investigated biomarker(s)	Spatial evaluation			Temporal evaluation			
				Evaluated levels	Quality sufficient (BI)	Results	TP tested	Quality sufficient (BI)	FU	Results
Li *et al.*[15]	Seattle Barrett's esophagus study Tertiary referral, prospective cohort Fresh frozen biopsies	248 (79:169)	Changes in somatic copy number alterations (SCA)	1q2cm 1 biopsy per level P: mean 3.1 levels NP mean 2.4 levels	No (0.25)	P: Large genomic diversity within 48 months before progression NP: Small genomic diversity	2	No (0.25)	NA	P: Increasing chromosome instability and SCA followed by catastrophic genome doublings <48 months before progression NP: Low and stable levels of SCA over time
Li *et al.*[16]	248 (79:169)	Using 5 types of SCA changes a model with 29 chromosomal features was developed	1q2cm 1 biopsy per level P: mean 3.1 levels NP: mean 2.4 levels	No (0.25)	All levels 1 time point, AUC = 0.94,	2	No (0.25)	NA	All levels 2 time points, AUC = 0.94	
Martinez *et al.*[17]	Seattle Barrett's esophagus study Tertiary referral, prospective cohort Fresh frozen biopsies/surgical resection specimen	8 (4:4)	Changes in SCA, genome doubling	3 levels from a 1q2cm protocol	No (0.25)	P: Increasing genomic divergence toward GEJ NP: Low genomic divergence independent of geographical location in Barrett's segment			NA	
Cotton *et al.*[18]	Posthoc-analysis of two RCT's (SURF/AIM-Dysplasia)	157 patients (n cases and controls unknown)	Low-grade dysplasia (LGD)	SURF trial: 4q2cm AIM trial: 4q1-2 cm	Yes, (1.0)	Increased prevalence of LGD in proximal most quarter (+22.6%) versus most distal quarter			NA	
Nwachokor *et al.*[8]	Tertiary referral, prospective cohort Formalin-fixed paraffin-embedded biopsies	20 (0:20)	DNA aneuploidy, Ki67, Mcm2, Cyclin A, Cyclin D1	3 levels from a 4q1-2 cm protocol	NA	Variability btw 3 levels, —Aneuploidy: 6.8–7.9%—KI67: 25–29%—MCM2: 4.7–11%—Cyclin D1: 20–91.5%—Cyclin A: 8.3–21.9%	2	NA	Yes, mean 6.4 years	Variability btw 3 levels, —Aneuploidy: 7.0–8.1%—KI67: 5.3–77%—MCM2: 54.3–71.7%—Cyclin D1: 11.9–69.7%—Cyclin A: 0–32%
Current study	Population-based nested case–control Formalin-fixed paraffin-embedded biopsies	168 (61:107)	—	>2 levels, 4q2cm protocol	Yes (>0.5)	—	≥2	Yes (>0.5)	Yes, ≥5 years	—

AUC, area under the ROC curve; biopsy index (BI) LGD, low-grade dysplasia; NP, nonprogressors; P, progressors; RCT, randomized controlled trial; SCA, somatic copy number alterations.

high-quality surveillance endoscopy 3 years after the spatial BL endoscopy, proving both endoscopic and histological absence of progression. This avoids irregular high hazard ratios by missed prevalent neoplasia in the tested nonprogressor samples.

Cotton *et al.* evaluated data of the spatial distribution of low-grade dysplasia (LGD) of the baseline endoscopy in progressors and nonprogressors from two large randomized controlled trials.[18–20] An increased prevalence of LGD in the most proximal quarter (+22.6%) compared with the most distal quarter underlined the importance of assessing biomarkers at multiple levels. The strict sampling protocol in both trials guaranteed a sufficient number of biopsies evaluated per endoscopic level, similar to the biopsy index in our collected cohort. However, this study only evaluated the histological diagnosis as provided per level and did not reassess the actual samples nor did the authors have access to the corresponding tissue blocks. This logically renders any additional biomarker testing impossible. In contrast, all formalin-fixed paraffin-embedded (FFPE) tissue blocks included in the SpaTemp cohort have been retrieved from a total of 58 hospitals in the Netherlands and are available for biomarker analysis, with informed consent from all participating patients. These unique circumstances make a head-to-head comparison of different biomarkers on the same patient material possible and allows to determine the biomarker (or a combination of markers) with the best performance.

The group of Nwachokor *et al.* evaluated three levels of biopsies over two serial endoscopies in nondysplastic BE patients without progression to HGD/EAC.[8] Based on both image cytometry and immunohistochemistry, a high variability over space (5–92%) and time (0–77%) was described for the four markers Ki67, Mcm2, cyclin A, and cyclin D1. In contrast, a low variability of 6.8–7.9% over space and 7.0–8.1% over time was observed for aneuploidy. These results are of special interest, since this study evaluated only patients *without* progression to cancer. Logically, it may be assumed that an even more significant variability can be expected in patients *with* progression to cancer. The high number of 61 progressors in the SpaTemp cohort ensures a sufficient number of events to develop and validate models, which accurately risk stratify BE patients into low- and high-risk for progression. The outcomes can subsequently be tested in the remaining 165 progressors and 616 nonprogressors in the ReBus cohort who did not need the strict 'multiple level-multiple endoscopy' selection criteria of the SpaTemp cohort yet who meet all strict selection criteria of the ReBus cohort.

Our study has a number of limitations. First, the retrospective design of the study may have resulted in selection bias. However, it did allow us to apply stringent inclusion criteria.

Additionally, due to the retrospective design biopsy specimens were not always obtained strictly according to the Seattle biopsy protocol. Therefore, we only selected patients in which at least 50% of the biopsies as required per Seattle protocol were available to guarantee adequate sampling quality of the baseline endoscopy.

In conclusion, the ReBus cohort and the SpaTemp subcohort are a unique platform for biomarker studies in Barrett's esophagus. Compared to other studies, the number of progressors, the strict selection criteria, and the use of FFPE samples are important advantages. The availability of all tissue blocks with corresponding informed consent provide the opportunity to evaluate and validate multiple biomarkers on the same samples and to determine the ideal sampling technique and the predictive window by testing samples obtained from multiple levels and at multiple time points.

SUPPLEMENTARY DATA

Supplementary data mentioned in the text are available to subscribers in *DOTESO* online.

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