

High expression of class III β -tubulin in upper gastrointestinal cancer types

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Abstract. Class III β -tubulin (TUBB3) is a component of microtubules of neuronal cells that is upregulated in various cancer entities. To better understand the role of TUBB3 in upper gastrointestinal tract cancer types, the present study assessed TUBB3 expression in tissue microarrays including 189 gastric and 428 esophageal cancer. TUBB3 expression was detected in 62.4% of gastric cancer, 73.8% of esophageal adenocarcinoma and 88.7% of esophageal squamous cell cancer, while control samples of normal esophageal and gastric epithelium were TUBB3-negative. TUBB3 positivity was not associated with the International Union Against Cancer classification, World Health Organization grading, lymph node involvement or distant metastasis in any entity. Of note, TUBB3 expression was associated with tumor localization and prognosis in gastric cancer, with the tumor stage in esophageal adenocarcinoma, and with the resection margin in esophageal squamous cell cancer. In conclusion, the substantial rate of positivity for TUBB3 already in early stages of gastric cancer in combination with the lack of a further increase in frequency with tumor stage, may suggest, that TUBB3 upregulation is rather relevant for cancer development than for cancer progression. TUBB3

might be a suitable prognostic biomarker in gastric cancer types.

Introduction

Upper gastrointestinal cancers are among the leading causes of cancer-associated mortality worldwide. Approximately 1.5 million people are diagnosed with gastric and esophageal cancer each year (1,2). Despite improvements in diagnosis and therapy in the last decades, the outcome for patient with gastric and esophageal cancers remains poor with 5-year survival rates not exceeding 20-30% in Western societies (3-5). The molecular mechanisms underlying carcinogenesis remain largely elusive. Accordingly, molecular markers allowing for prediction of the clinical course of these diseases are currently lacking. Hence, there is a high demand for molecular markers to predict tumor aggressiveness and response to therapy for these cancer types.

Microtubules are multifunctional cytoskeletal proteins involved in numerous cellular processes including maintenance of cell shape, intracellular transport and chromosome segregation during mitosis and meiosis. Microtubules are composed of polymers of α - and β -tubulin heterodimers. Class III β -tubulin (TUBB3) is typically expressed in cells of neuronal origin, where it contributes to the formation of dynamic microtubules essential for neurite formation and maintenance (6). Several lines of evidence suggest that TUBB3 also has an important role in tumor development. In fact, overexpression of TUBB3 has been linked to poor clinical outcome in numerous epithelium-derived tumor types, including non-small cell lung (7), bladder (8), breast (9), ovarian (10) and prostate cancer (11). Several studies analyzing gastric and/or esophageal cancer specimens (n=29-149) have also suggested clinically relevant roles of TUBB3 expression levels in upper gastrointestinal cancer (12-14). Of note, elevated levels of TUBB3 expression have been associated with a reduced response to taxane-based microtubule-targeting cancer therapy (7,10-12,15).

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Abbreviations: TUBB3, Class III β tubulin; TMA, tissue microarray; UICC, International Union Against Cancer

Key words: tubulin, gastric and esophageal cancer, TMA

Here we tested retrospectively TUBB3 expression in upper gastrointestinal cancers from 230 gastric and 594 esophageal cancers on tissue microarrays (TMA) and report the clinical follow up from 189 gastric and 428 esophageal cancers.

Patients and methods

Patients. The 230 patients [mean age (\pm SD), 67 years (\pm 12); female/male-ratio, 0.51] with gastric and 594 patients [mean age (\pm SD), 62 years (\pm 10); female/male-ratio, 0.25] with esophageal cancer received surgical treatment at the Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf (Hamburg, Germany) between June 1994 and October 2006, and between January 1992 and December 2014, respectively. TUBB3 staining and follow-up data was available for 93 patients with gastric cancer with a median time of 13 months and for 393 esophageal cancer patients with a median time of 41 months. Tumors were staged according to the sixth edition of the tumor-nodes-metastasis classification, graded and histologically subtyped according to the recommendations of the International Union Against Cancer (UICC) (16). Data on neoadjuvant or adjuvant cytotoxic therapy regimens or response to treatment were unavailable. The TMA manufacturing was performed as described in previous studies (17,18). Each TMA block contained internal controls of normal esophageal and gastric tissue taken from the same patient cohort.

The Ethics Committee of the Ärztekammer Hamburg approved the present study (no. WF-049/09). According to local laws (HmbKHG §12a), informed consent was not required. Patient records/information were anonymized prior to analysis. All work was performed in compliance with the Helsinki Declaration.

Immunohistochemistry. TUBB3 staining and scoring was performed as described in a previous study (9). The recombinant rabbit monoclonal anti-TUBB3 antibody clone EPR1568Y was used at a dilution 1:150 of (cat. no. ab68193; Abcam, Cambridge, UK). Staining was observed in the cytoplasm of TUBB3-expressing cells and scored as 'negative' (0), 'weak' (1+ in \leq 70% of tumor cells or 2+ in \leq 30% of tumor cells), 'moderate' (1+ in $>$ 70% of tumor cells, or 2+ in 31-70% of tumor cells, or 3+ in \leq 30% of tumor cells) or 'strong' (2+ in $>$ 70% of tumor cells or 3+ in $>$ 30% of tumor cells) (Figs. 1 and 2).

Statistical analysis. JMP 12.0 software (SAS Institute Inc., Carey, NC, USA) was used to calculate contingency tables and P-values with the chi-squared (likelihood) test. Kaplan-Meier curves were drawn and significant differences between groups were assessed by the log-rank method. Cox regression analysis was used to compare hazard ratios in univariate and multivariate models. $P \leq 0.05$ was considered to indicate a statistically significant difference.

Results

TUBB3-staining. The results of the TMA analysis were interpretable for a total of 189/230 (82%) of gastric and 431/594 (73%)

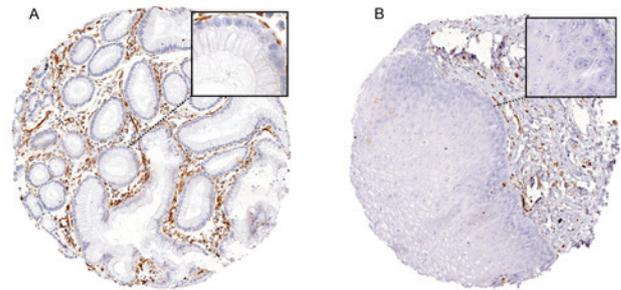


Figure 1. Representative images of 600 μ m-tissue spots at magnification, x100 and x400, respectively, showing normal (A) gastric and (B) esophageal tissue. Note that glandular cells in the gastric and squamous epithelial cells in the esophageal tissue are not stained, while stromal cells are positive in both tissues.

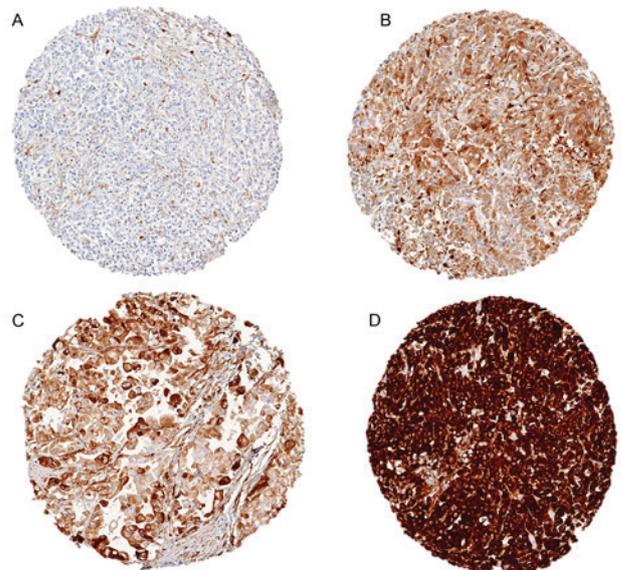


Figure 2. Representative images of 600 μ m-tissue spots showing (A) negative, (B) weak, (C) moderate and (D) strong class III β -tubulin expression in gastric cancer (magnification, x100).

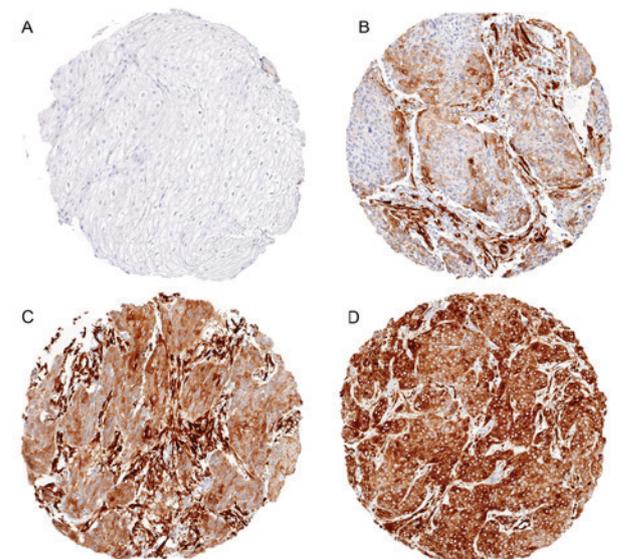


Figure 3. Representative images of 600 μ m-tissue spots showing (A) negative, (B) weak, (C) moderate and (D) strong class III β -tubulin expression in esophageal squamous cell cancer (magnification, x100).

Table I. Association between TUBB3 expression and gastric cancer phenotype.

Parameter	No. evaluable	TUBB3 (%)				P-value
		Negative	Weak	Moderate	Strong	
All cancers	189	37.6	11.1	18.0	33.3	
Tumor stage ^a						
pT1+2	125	36.8	12.0	19.2	32.0	0.7753
pT3+4	62	37.1	9.7	16.1	37.1	
UICC-classification						
I	31	32.3	9.7	22.6	35.5	0.8227
II	28	35.7	21.4	14.3	28.6	
III	86	41.9	8.1	18.6	31.4	
IV	44	34.1	11.4	15.9	38.6	
Laurén classification ^a						
Diffuse	61	52.5	13.1	14.8	19.7	0.0484
Mixed	14	42.9	7.1	21.4	28.6	
Intestinal	109	28.4	11.0	20.2	40.4	
WHO grading ^a						
G1	2	50.0	0.0	0.0	50.0	0.2345
G2	58	25.9	8.6	22.4	43.1	
G3	126	42.1	12.7	15.9	29.4	
Tumor localization ^a						
Antrum	13	23.1	38.5	30.8	7.7	0.0012 ^b
Corpus	7	42.9	0.0	28.6	28.6	
Cardia	47	17.0	19.1	12.8	51.1	
Other/not further specified	93	46.2	7.5	14.0	32.3	
Lymph node metastasis ^a						
N0	53	34.0	17.0	18.9	30.2	0.4896
N1	133	37.6	9.0	18.0	35.3	
Distant metastasis ^a						
M0	129	38.8	10.9	16.3	34.1	0.4076
M1	22	22.7	13.6	13.6	50.0	

^aCategory with some missing data, ^bsignificant result. UICC, International Union Against Cancer; TUBB3, class III β -tubulin; WHO, World Health Organization.

of esophageal tumor samples. In the non-informative TMA spots (18% for gastric cancer and 27% for esophageal cancer), the tissue sample was lacking or no unequivocal cancer tissue was observed. Normal gastric and esophageal tissues exhibited no staining under the selected experimental conditions. Fig. 1 shows representative images of normal gastric and esophageal tissue.

TUBB3-expression in gastric cancer. In gastric cancer, positive staining for TUBB3 was detected in 118 of 189 analyzable spots (62.4%) and was rated weak in 11.1%, moderate in 18% and strong in 33.3% of these samples. Representative images of TUBB3 staining in gastric cancers are given in Fig. 2. TUBB3 expression was unrelated to tumor stage, UICC stage, Lauren classification, WHO grading, and presence of lymph node or distant metastasis ($P > 0.05$ each; Table I). TUBB3

expression varied from 53.8 to 83.0% with the tumor localization ($P = 0.0012$; Table I).

TUBB3-expression in esophageal cancer. In esophageal cancer, cytoplasmic TUBB3 staining was detected in 345 of 428 analyzable tumors (80.7%), including 233 adenocarcinomas and 195 squamous cell cancers. TUBB3 staining in adenocarcinomas (squamous cell cancers) was considered weak in 18.0% (11.8%), moderate in 19.7% (19.0%) and strong in 36.1% (57.9%) of these samples. Representative images of TUBB3 staining in esophageal cancers are given in Fig. 3. In esophageal adenocarcinomas, no association between TUBB3 and UICC stage, WHO grading, or the presence of lymph node or distant metastasis was identified ($P > 0.05$ each; Table II). Only the tumor stage was significantly associated with TUBB3 expression ($P = 0.0289$; Table II). In esophageal squamous cell carcinomas, only the resection margin

Table II. Association between TUBB3 expression and esophageal adenocarcinoma phenotype.

Parameter	No. evaluable	TUBB3 (%)				P-value
		Negative	Weak	Moderate	Strong	
All cancers	233	26.2	18.0	19.7	36.1	
Tumor stage ^a						
pT1a-b	44	29.5	29.5	27.3	13.6	0.0289 ^b
pT2	25	32.0	16.0	24.0	28.0	
pT3	143	23.1	15.4	18.9	42.7	
pT4a-b	17	35.3	17.6	5.9	41.2	
UICC-classification ^a						
I	43	32.6	23.3	25.6	18.6	0.0534
II	26	19.2	11.5	38.5	30.8	
III	134	23.9	19.4	15.7	41.0	
IV	25	36.0	8.0	16.0	40.0	
WHO grading ^a						
G1	9	22.2	22.2	22.2	33.3	0.8693
G2	85	24.7	20.0	21.2	34.1	
G3	130	26.9	16.2	20.0	36.9	
G4	5	40.0	40.0	0.0	20.0	
Resection margin ^a						
0	162	26.5	18.5	23.5	31.5	0.0961
1	63	27.0	17.5	12.7	42.9	
2	3	0.0	0.0	0.0	100.0	
Lymph node metastasis ^a						
pN0	61	29.5	18.0	26.2	26.2	0.4443
pN1	42	16.7	23.8	23.8	35.7	
pN2	57	28.1	15.8	15.8	40.4	
pN3	64	29.7	15.6	14.1	40.6	
Distant metastasis ^a						
0	2	0.0	50.0	0.0	50.0	0.2737
1	26	38.5	7.7	15.4	38.5	

^aCategory with some missing data, ^bsignificant result. UICC, International Union Against Cancer; TUBB3, class III β -tubulin; WHO, World Health Organization.

was significantly associated with TUBB3 ($P < 0.05$; Table III). For the association of TUBB3 with the tumor stage a similar trend as in the adenocarcinomas was observed.

Kaplan-meier analysis. Follow-up data were available from 93 patients with gastric cancer and 393 patients with esophageal cancer (204 adenocarcinomas and 189 squamous cell cancers) with interpretable TUBB3 staining on the TMA. While in gastric cancer TUBB3 expression was associated with shorter overall survival (Fig. 4A and B), TUBB3 expression had no impact on the survival of esophageal cancer patients ($P > 0.05$; Fig. 4C and D).

Multivariate analysis. Hazard ratios for overall survival were calculated. In gastric cancer, TUBB3 expression was an independent risk factor for shorter survival ($P < 0.05$; Table IV).

Discussion

The results of the present study demonstrate that TUBB3 is frequently expressed in upper gastrointestinal cancer types associated with patient prognosis only in gastric cancer, but not in esophageal adenocarcinoma and esophageal squamous cell cancer.

TUBB3 expression was identified in 62.4% of the 189 gastric cancer tissues, in 73.8% of the 233 esophageal adenocarcinoma tissues and 88.7% of the 195 esophageal squamous cell cancer tissues in the present study, but was undetectable in the respective normal tissue samples. In principle, these immunohistochemical results are compatible with earlier studies on these tumor types. This particularly applies to gastric tumors, where two earlier studies on gastric cancer tissues (n=115 and 146) reported comparable data,

Table III. Association between TUBB3 expression and esophageal squamous cell cancer phenotype.

Parameter	No. evaluable	TUBB3 (%)				P-value
		Negative	Weak	Moderate	Strong	
All cancers	195	11.3	11.8	19.0	57.9	
Tumor stage						
pT1a-b	31	19.4	12.9	32.3	35.5	0.1715
pT2	43	11.6	16.3	16.3	55.8	
pT3	109	9.2	11.0	16.5	63.3	
pT4a-b	12	8.3	0.0	16.7	75.0	
UICC-classification ^a						
I	46	13.0	8.7	26.1	52.2	0.5155
II	47	6.4	17.0	17.0	59.6	
III	62	12.9	6.5	19.4	61.3	
IV	39	10.3	17.9	12.8	59.0	
WHO grading						
G1	3	33.3	0.0	0.0	66.7	0.7412
G2	124	10.5	11.3	21.0	57.3	
G3	68	11.8	13.2	16.2	58.8	
Resection margin ^a						
0	148	14.2	11.5	18.2	56.1	0.0461 ^b
1	38	0.0	13.2	23.7	63.2	
2	8	12.5	0.0	12.5	75.0	
Lymph node metastasis ^a						
pN0	91	11.0	9.9	18.7	60.4	0.9046
pN1	41	9.8	14.6	17.1	58.5	
pN2	37	8.1	16.2	24.3	51.4	
pN3	21	14.3	9.5	9.5	66.7	
Distant metastasis ^a						
0	1	100.0	0.0	0.0	0.0	0.1828
1	39	7.7	17.9	12.8	61.5	
1	26	38.5	7.7	15.4	38.5	

^aCategory with some missing data, ^bsignificant result. UICC, International Union Against Cancer; TUBB3, class III β -tubulin; WHO, World Health Organization.

Table IV. Hazard ratio for overall survival of established prognostic parameter and TUBB3 expression in gastric cancer types.

Variable	Univariate analysis	Multivariate analysis
Tumor stage		
pT3+4 vs. pT1+2	2.67 (1.66-4.30) ^c	1.67 (1.00-2.77)
WHO grading		
G3 vs. G1+2	1.65 (1.00-2.83) ^a	2.22 (1.29-3.95) ^a
Lymph node metastasis		
Positive vs. negative	4.43 (2.25-10.1) ^c	3.11 (1.54-7.20) ^b
TUBB3 expression		
Positive vs. negative	2.23 (1.28-4.08) ^a	2.18 (1.22-4.12) ^a

^aP \leq 0.05, ^bP \leq 0.001, ^cP \leq 0.0001. Confidence interval (95%) in brackets. TUBB3, class III β -tubulin; WHO, World Health Organization.

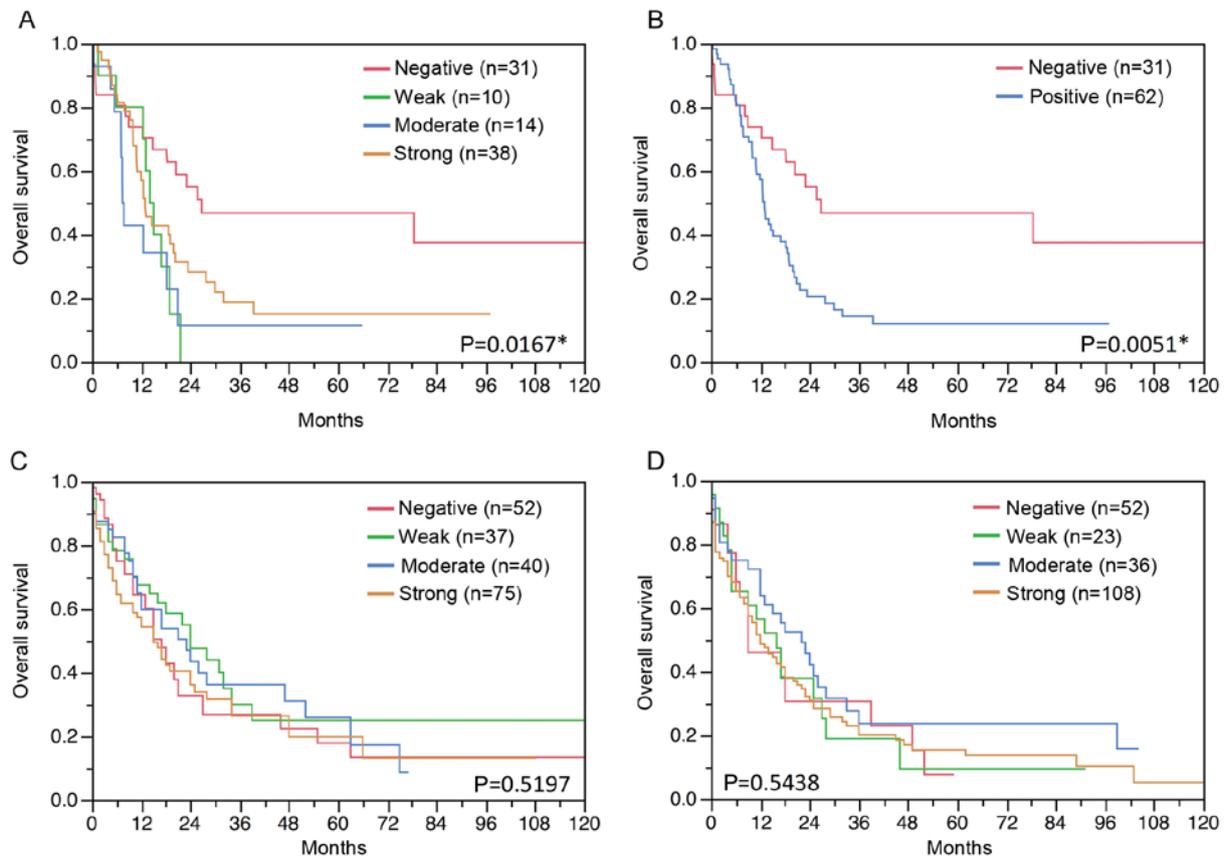


Figure 4. Kaplan-Meier analysis of overall survival and (A) negative, weak, moderate, or strong TUBB3 expression in gastric cancer, (B) negative vs. positive (weak, moderate, or strong) TUBB3 expression in gastric cancer, (C) in esophageal adenocarcinoma, and in (D) esophageal squamous cell cancer. *Significant overall P-value. TUBB3, class III β -tubulin.

namely detectable TUBB3 expression in 36 and 53% of tumor samples (12,19). The results of two earlier studies on esophageal squamous cell cancers were more conflicting, reporting TUBB3-positive rates of 7 and 95%, respectively (14,20). The striking discrepancy of these data is typical for studies using 'homemade' immunohistochemical protocols. It is known, that the use of different antibodies, immunohistochemistry protocols and scoring criteria can result in discrepant data (21).

The important function of TUBB3 in the maintenance of the dynamic plasticity of microtubules (22,23) -a prerequisite for cell motility, invasive growth, mitotic spindle orientation, and cell cycle progression-would be consistent with a significant role for TUBB3 in tumor development and progression. The high frequency of detectable TUBB3 staining in early gastric cancer in combination with the lack of a further elevation in frequency with the tumor stage increasing, may suggest that up regulation of TUBB3 is an event in carcinogenesis of gastric cancer and has a relevance in cancer development rather than cancer progression. Other studies have failed to identify an association between TUBB3 expression and clinico-pathological parameters or patient prognosis in gastric or esophageal carcinomas (12,19,20). In the present study, analysis of a much larger number of tumors did reveal a significant association with patient outcome in gastric cancer providing some arguments for TUBB3 testing. This is in line to the results on the predictive value of TUBB3 in a variety of other cancer types. Using the same staining protocol, another recent study by our group identified the prognostic value of TUBB3 in prostate cancer, which was

independent of established pre- and post-operatively available prognostic features (24). Others studies have reported TUBB3 overexpression is linked to late tumor stage and poor prognosis in breast (25), lung (26,27), colon (28), ovarian (10,29,30), prostate (11,24) and several neurological cancers (28).

The present study was a retrospective study. Thus it remains to be seen whether the prognostic value of TUBB3 expression in gastric cancer can be validated in a prospective study.

In summary, the results of the present study demonstrate that TUBB3 is frequently expressed in upper gastrointestinal cancer types, including esophageal and gastric tumors. For gastric cancer, TUBB3 expression might be a prognostic factor.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

DH, FJ, RS and GS designed the study and drafted the manuscript. EÖ, CS and JRI participated in study design. EN, CG, MCH, CF, KM, MA, MF and AH performed immunohistochemical analysis and scoring. CL, VR, SW and MN participated in pathology data analysis. CH-M, NCB and RS performed statistical analysis. MB, DP, and DSL participated in data interpretation and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of the Ärztekammer Hamburg approved the study protocol (WF-049/09). According to local laws (HmbKHG §12a), patient informed consent was not required. Patient records/information were anonymized and de-identified prior to analysis. All procedures have been performed in compliance with the principles outlined in the Helsinki Declaration.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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