Original article

Loss of SRY-box2 (SOX2) expression and its impact on survival of patients with oesophageal adenocarcinoma

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Background: Oesophageal adenocarcinoma (OAC) is a highly aggressive malignancy with poor survival, which is highly variable amongst patients with comparable conventional prognosticators. Therefore molecular biomarkers are urgently needed to improve the prediction of survival in these patients. SRY (sex determining region Y)-box 2, also known as SOX2, is a transcription factor involved in embryonal development of the gastrointestinal tract as well as in carcinogenesis. The purpose of this study was to see whether SOX2 expression is associated with survival in patients with OAC.

Methods: SOX2 was studied by immunohistochemistry in patients who had undergone potentially curative oesophagectomy for adenocarcinoma. Protein expression of SOX2 was evaluated using tissue microarrays from resection specimens, and results were analysed in relation to the clinical data by Cox regression analysis. SOX2 was evaluated in two independent OAC cohorts (Rotterdam cohort and a multicentre UK cohort).

Results: Loss of SOX2 expression was independently predictive of adverse overall survival in the multivariable analysis, adjusted for known factors influencing survival, in both cohorts (Rotterdam cohort: hazard ratio (HR) 1·42, 95 per cent c.i. 1·07 to 1·89, $P = 0·016$; UK cohort: HR 1·54, 1·08 to 2·19, $P = 0·017$). When combined with clinicopathological staging, loss of SOX2 showed an increased effect in patients with pT1–2 tumours ($P = 0·010$) and node-negative OAC ($P = 0·038$), with an incrementally adverse effect on overall survival for stage I OAC with SOX2 loss (HR 3·18, 1·18 to 8·56; $P = 0·022$).

Conclusion: SOX2 is an independent prognostic factor for long-term survival in OAC, especially in patients with stage I OAC.

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Introduction

Oesophageal adenocarcinoma (OAC) is an aggressive cancer with a steadily increasing incidence[1,2]. The major risk factors for OAC are gastro-oesophageal reflux[3], abdominal obesity[4] and Barrett's oesophagus[5,6]. Patients with non-dysplastic Barrett's oesophagus have a low rate of progression to OAC during surveillance (less than 1 per cent per year)[7], but most patients with OAC exhibit underlying Barrett's oesophagus at the time of OAC diagnosis and are typically diagnosed at an advanced stage[8].

Although the addition of neoadjuvant therapy to primary surgical resection improves overall survival (OS) and disease-specific survival in patients with locally advanced tumours, the prognosis of most patients with advanced OAC, including those treated with curative intent, is dismal, with a 5-year survival rate of 47 per cent at best[9–11]. Postsurgical prognostication is currently based on tumour staging according to the AJCC staging system, supplemented by pathological criteria[12]. However, even after considering all known parameters including resection margin, nodal status, presence of vascular invasion, tumour grade and differentiation grade, the course of the disease remains variable[13–15]. Improving clinical decision-making is essential, especially in early OAC. In these patients numerous treatment modalities are available, depending on tumour

characteristics, and the best treatment modality for the individual patient is still a matter of debate. One method for a better prognostication in early OAC is the use of biomarkers that might improve decision-making to determine the optimal treatment strategy.

Various signalling pathways essential for embryonal development are involved in cancer initiation and progression, including the sex determining region Y (SRY)-box2, also known as SOX2. *SOX2* is a highly conserved gene coded on a single exon that plays a pivotal role in the maintenance of embryonic stem cells[16]. In the gastrointestinal tract it determines the formation and differentiation of oesophageal and gastric epithelium during embryogenesis[17,18]. Besides its role in embryogenesis, SOX2 is involved in various malignancies including squamous cell carcinoma of the oesophagus[19], gastric adenocarcinoma[20], prostate[21] and colorectal[22] cancer. SOX2 functions differ depending on the cell of origin, and both oncogenic and tumour suppressive mechanisms have been described. The *SOX2* gene may be amplified in squamous cell carcinoma of the oesophagus and trachea, and acts as a lineage survival oncogene by promoting cell migration and proliferation[23,24]. Accordingly, upregulation of SOX2 is strongly associated with adverse outcomes in these patients[19]. In contrast, the opposite functions of SOX2 were shown in gastric adenocarcinoma, in which loss of SOX2 expression was correlated with worse prognosis. Phosphatase and tensin homologue (PTEN) has been proposed as a direct target of SOX2[20].

Little is known about the role of SOX2 in established OAC, although it has been shown in association with Barrett's oesophagus[25]. Non-dysplastic Barrett's oesophagus exhibits mixed differentiation and expresses gastric genes including *SOX2* and gastric mucins MUC5A and MUC6, as well as CDX2 as a marker of intestinal differentiation[25]. SOX2 was found in 98 per cent of the biopsies with non-dysplastic Barrett's oesophagus, whereas only 72 per cent of low-grade dysplasia and 29 per cent of OAC samples demonstrated SOX2 expression[26]. Similar observations were detected for markers of intestinal differentiation[27,28]. It was concluded that *SOX2*, in parallel with the gastric mucins and intestinal genes, is gradually lost during progression of Barrett's oesophagus to OAC[26]. SOX2 status has also been shown to be indicative of the pattern of response to neoadjuvant chemoradiotherapy in patients with OAC[29,30], and one small cohort study[31] suggested that SOX2 may have a prognostic effect for disease-free survival (DFS) in surgically treated patients with OAC.

The aim of the present study was to assess the role of SOX2 as a prognostic marker in patients with surgically treated OAC. As *SOX2* is lost during progression of Barrett's oesophagus to OAC, it was hypothesized that this gene would have particular influence in stage I OAC.

Methods

Patient selection

To reduce possible bias of neoadjuvant treatment that might influence SOX2 expression and interfere with OS, two historical OAC cohorts with a high proportion of patients who had surgical resection alone were used. Both the Rotterdam cohort and the UK multicentre cohort from the OCCAMS (Oesophageal Cancer Clinical And Molecular Stratification) study included patients who underwent oesophagectomy with curative intent for pathologically confirmed adenocarcinoma of the oesophagus or gastro-oesophageal junction. Follow-up of all patients was performed in the respective clinical centres and only patients who were alive 1 month after surgery were included in the analysis. The Rotterdam cohort consisted of patients treated at the Department of Surgery at Erasmus Medical Centre, Rotterdam, between 1995 and 2006. The UK cohort comprised patients from six tertiary hospitals who were treated between 1992 and 2000.

Clinical and pathological data for both cohorts were collected, including tumour grade, pathological stage, anatomical location of the tumour divided into three types as described by Siewert *et al.*[32], chemotherapy, age at surgery, co-morbidities and OS. The TNM system according to the UICC seventh edition[12] was used for pathological grading and staging. To ensure reliable classification, all tumours were reviewed by an expert gastrointestinal pathologist.

Tissue microarray

For the construction of a tissue microarray (TMA), formalin-fixed paraffin-embedded tissue from the resection specimens was retrieved from the archives at the Departments of Pathology of the participating institutions. For each tumour, three to six cores from multiple representative areas of OAC, as identified by a pathologist on haematoxylin and eosin-stained slides, were taken from the original paraffin blocks, including the central part and invasive front of the tumour[33,34].

SOX2 immunohistochemistry

The SOX2 immunohistochemical staining technique has been described extensively in previous publications[26,29]. In short, 5-μm sections were cut from the TMA,

Table 1 Clinicopathological characteristics in individual and combined cohorts

	Combined cohort ($n = 756$)	Rotterdam cohort ($n = 336$)	OCCAMS ($n = 420$)	P†
Age at surgery (years)*	65·4 (33–90)	64·7 (33–90)	66·0 (33–88)	0·009‡
Sex ratio (M : F)	602 : 132	293 : 43	309 : 89	0·001
Follow-up (months)*	20·9 (1–199)	25·0 (1–199)	18·0 (1–193)	0·004‡
Siewert classification				< 0·001
Type I	460 (69·7)	190 (57·1)	270 (82·6)	
Type II	168 (25·5)	126 (37·8)	42 (12·8)	
Type III	32 (4·8)	17 (5·1)	15 (4·6)	
Recurrence	182 (54·2)	182 (54·2)	n.a.	
Resection margin status				0·212
pR0	396 (71·0)	245 (72·9)	151 (68·0)	
pR1	162 (29·0)	91 (27·1)	71 (32·0)	
Histological grade				0·007
Well differentiated	52 (7·5)	26 (7·7)	26 (7·3)	
Moderately differentiated	248 (35·7)	139 (41·4)	109 (30·4)	
Poorly differentiated	394 (56·8)	171 (50·9)	223 (62·3)	
pT category				0·001
pT1	79 (11·2)	48 (14·7)	31 (8·2)	
pT2	132 (18·8)	59 (18·0)	73 (19·4)	
pT3	474 (67·3)	218 (66·7)	256 (67·9)	
pT4	19 (2·7)	2 (0·6)	17 (4·5)	
pN category				< 0·001
pN0	245 (35·9)	142 (42·4)	103 (29·6)	
≥ pN1	438 (64·1)	193 (57·6)	245 (70·4)	
(Neo)adjuvant treatment				< 0·001
Yes	214 (31·3)	68 (20·2)	146 (42·1)	
No	469 (68·7)	268 (79·8)	201 (57·9)	
Alive after 60 months				0·752
Yes	234 (31·0)	106 (31·5)	128 (30·5)	
No	522 (69·0)	230 (68·5)	292 (69·5)	
SOX2				< 0·001
Negative	436 (66·1)	181 (57·1)	255 (74·3)	
Positive	224 (33·9)	136 (42·9)	88 (25·7)	

Values in parentheses are percentages unless indicated otherwise; *values are median (range). Data were missing for patients in most categories. OCCAMS, Oesophageal Cancer Clinical And Molecular Stratification; n.a., not available. †χ^2 test, except ‡Student's t test.

deparaffinized and rehydrated. Tissue from squamous cell carcinoma with clear positive staining for SOX2 was placed on each immunohistochemical slide of the TMAs as a positive control. Antigen retrieval was enhanced by heating in a Tris buffer. Endogenous peroxidase activity was blocked by incubating the slides in a solution of 0·3 per cent hydrogen peroxide in phosphate-buffered saline. Primary SOX2 antibody (AF2018, dilution 1 : 800, goat, polyclonal; R&D Systems, Abingdon, UK) was applied for 22 h at 4°C. The secondary antibody was a biotinylated horse antigoat IgG antibody (1 : 150, BA-4000; Vector Laboratories, Peterborough, UK). Visualization was achieved using the horseradish peroxidase avidin–biotin complex method and diaminobenzidine. Slides were counterstained with haematoxylin.

The immunohistochemically stained TMA slides from both cohorts were digitalized and scored independently by two investigators blinded to the clinical and pathological outcome. In case of disagreement, the cores were reviewed by both investigators simultaneously and consensus was achieved.

SOX2 was scored as positive or negative in each of the stained cores. As described previously[29], weak or strong nuclear expression in at least 50 per cent of the tumour cells was defined as positive, whereas nuclear expression in less than 50 per cent of tumour cells as well as cytoplasmic SOX2 expression were defined as negative. Because SOX2 expression might be heterogeneous in OAC, the overall expression in each tumour was calculated from all corresponding cores. Patients with fewer than three cores containing cells representative of the original OAC were excluded from analysis.

The optimal cut-off value of immunohistochemistry with SOX2 to predict survival was calculated by receiver operating characteristic (ROC) curve analysis in the Rotterdam cohort, using the area under the curve (AUC) as the performance measure (*Fig. S1*, supporting information). Based on this evaluation, absence of SOX2 expression was

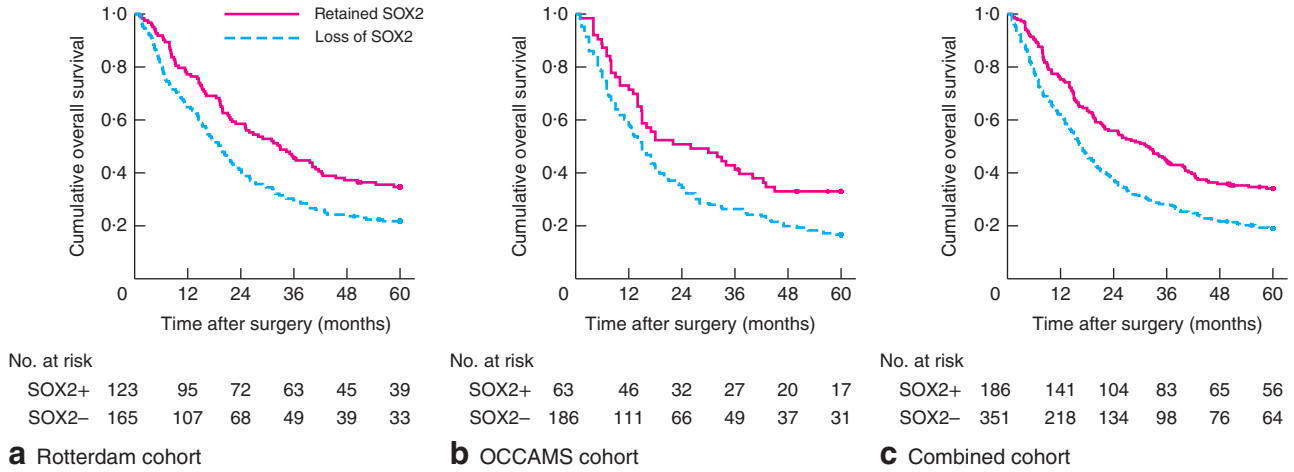


Fig. 1 Kaplan–Meier curves of overall survival of patients with oesophageal adenocarcinoma according to expression of SOX2 in **a** the Rotterdam cohort, **b** the OCCAMS (Oesophageal Cancer Clinical And Molecular Stratification) cohort, and **c** the combined cohort. **a** $P = 0{\cdot}002$, **b** $P = 0{\cdot}008$, **c** $P < 0{\cdot}001$ (log rank test)

defined by negative staining of SOX2 in more than 75 per cent of the cores; otherwise, SOX2 was considered to be present.

Ethics

The investigational protocols for both cohorts were approved by the relevant institutional review boards (MEC-12-469 and LREC 04/Q2006/2).

Statistical analysis

The primary endpoint in this study was 5-year OS, defined as time from surgery until death. Differences between the Rotterdam and UK cohorts were analysed using Student's *t* test for normal distributions and the Mann–Whitney *U* test for non-normal distributions of continuous variables, and χ^2 test for categorical variables. The equality of distribution was tested with Levene's test. Interobserver variation between the two investigators for scoring of SOX2 was calculated using Cohen's κ. Strength of agreement was categorized as follows: 0·00–0·20, poor; 0·21–0·40, fair; 0·41–0·60, moderate; 0·61–0·80, good; and 0·81–1·00, excellent.

Kaplan–Meier curves were used to plot 5-year survival by SOX2 status, and the distribution was analysed using the log rank test. After imputation of missing variables using a linear regression model, univariable and multivariable Cox proportional hazard models were applied to estimate the independent association between SOX2 immunohistochemical expression and survival. In the multivariable analysis, adjustments were made for the clinical and pathological factors that were independently predictive in the univariable analysis. In addition, sensitivity analysis using a multivariable Cox proportional hazards model excluding all patients receiving chemotherapy or chemoradiotherapy with adjustment for clinical and pathological factors was performed to test the role of SOX2 in these patients. A multivariable analysis adjusted for all clinicopathological criteria that were independently predictive in the univariable analysis was performed, to estimate the independent association between SOX2 and survival for each of the stage groupings described in the TNM classification[12]. pN category was dichotomized as pN0 and pN+ (pN1–3) groups for the multivariable analysis. All analyses were performed using SPSS® version 22 software (IBM, Armonk, New York, USA). $P < 0{\cdot}050$ was considered statistically significant.

Results

Patient characteristics

The OAC cohort from Rotterdam consisted of 336 patients, whereas that from the OCCAMS study comprised 420 patients. Clinical characteristics of the patients from both cohorts are shown in *Table 1*. Patients from the OCCAMS cohort were older than those from Rotterdam (median 66·0 *versus* 64·7 years respectively; $P = 0{\cdot}009$) and had a shorter median follow-up (18·0 *versus* 25·0 months; $P = 0{\cdot}004$). A greater proportion of patients in the Rotterdam cohort had a tumour at the oesophagogastric junction (Siewert type II) ($P < 0{\cdot}001$), higher degree of differentiation ($P = 0{\cdot}007$), earlier pT category ($P = 0{\cdot}001$) and a

Table 2 Multivariable analysis of survival for all patients in individual and combined cohorts

	Combined cohort ($n = 402$)		Rotterdam cohort ($n = 287$)		OCCAMS cohort ($n = 115$)	
	Hazard ratio	*P*	Hazard ratio	*P*	Hazard ratio	*P*
Age at surgery (per year increase)	1·02 (1·01, 1·03)	0·002	n.a.	n.a.	n.a.	n.a.
pT category						
pT1	1·00 (reference)		1·00 (reference)		1·00 (reference)	
pT2	1·59 (0·93, 2·72)	0·084	1·12 (0·55, 2·24)	0·759	2·45 (0·99, 6·07)	0·053
pT3–4	2·96 (1·80, 4·91)	< 0·001	2·60 (1·40, 4·84)	0·003	3·58 (1·46, 8·80)	0·005
pN category						
pN0	1·00 (reference)		1·00 (reference)		1·00 (reference)	
≥ pN1	1·68 (1·15, 2·46)	0·011	1·57 (1·14, 2·17)	0·006	1·89 (0·81, 4·45)	0·121
Resection margin status						
pR0	1·00 (reference)		1·00 (reference)		1·00 (reference)	
pR1	1·15 (0·88, 1·50)	0·313	1·27 (0·93, 1·75)	0·133	1·01 (0·66, 1·57)	0·949
Histological grade						
Well/moderately differentiated	1·00 (reference)		1·00 (reference)		1·00 (reference)	
Poorly differentiated	1·57 (1·25, 1·97)	< 0·001	1·52 (1·13, 2·05)	0·006	1·44 (1·04, 2·00)	0·028
(Neo)adjuvant treatment						
Yes	1·00 (reference)		1·00 (reference)		1·00 (reference)	
No	n.a.		1·74 (1·14, 2·67)	0·011	n.a.	
SOX2						
Positive	1·00 (reference)		1·00 (reference)		1·00 (reference)	
Negative	1·42 (1·14, 1·77)	0·002	1·42 (1·07, 1·89)	0·016	1·54 (1·08, 2·19)	0·017

Values in parentheses are 95 per cent confidence intervals. OCCAMS, Oesophageal Cancer Clinical And Molecular Stratification; n.a., not available. For the corresponding univariable analysis, see *Table S2* (supporting information).

Table 3 Multivariable analysis of survival in chemotherapy-naive patients in individual and combined cohorts

	Combined cohort ($n = 297$)		Rotterdam cohort ($n = 241$)		OCCAMS cohort ($n = 56$)	
	Hazard ratio	*P*	Hazard ratio	*P*	Hazard ratio	*P*
Age at surgery (per year increase)	1·02 (1·01, 1·03)	0·002	n.a.		n.a.	
pT category						
pT1	1·00 (reference)		1·00 (reference)		1·00 (reference)	
pT2	1·88 (0·96, 3·68)	0·065	1·40 (0·64, 3·09)	0·400	3·11 (0·77, 12·52)	0·110
pT3–4	3·99 (2·13, 7·48)	< 0·001	3·48 (1·70, 7·09)	0·001	4·61 (1·16, 18·33)	0·030
pN category						
pN0	1·00 (reference)		1·00 (reference)		1·00 (reference)	
≥ pN1	1·61 (1·15, 2·25)	0·006	1·47 (1·04, 2·07)	0·028	2·12 (1·04, 4·29)	0·039
Resection margin status						
pR0	1·00 (reference)		1·00 (reference)		1·00 (reference)	
pR1	1·17 (0·89, 1·54)	0·270	1·27 (0·91, 1·76)	0·162	1·14 (0·67, 1·94)	0·63
Histological grade						
Well/moderately differentiated	1·00 (reference)		1·00 (reference)		1·00 (reference)	
Poorly differentiated	1·51 (1·16, 1·97)	0·003	1·47 (1·07, 2·03)	0·017	n.a.	
SOX2						
Positive	1·00 (reference)		1·00 (reference)		1·00 (reference)	
Negative	1·35 (1·04, 1·75)	0·026	1·40 (1·03, 1·91)	0·030	1·53 (0·95, 2·47)	0·081

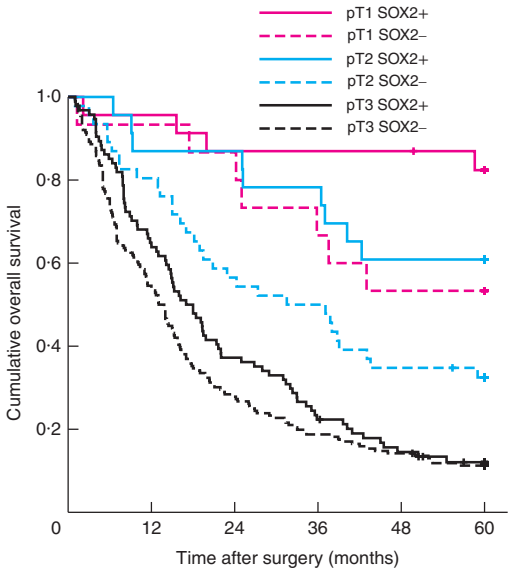
Values in parentheses are 95 per cent confidence intervals. OCCAMS, Oesophageal Cancer Clinical And Molecular Stratification; n.a., not available. For the corresponding univariable analysis, see *Table S4* (supporting information).

greater likelihood of having pN0 disease ($P < 0·001$). Loss of SOX2 expression was more common in the OCCAMS cohort (74·3 per cent *versus* 57·1 per cent in the Rotterdam cohort; $P < 0·001$).

In the Rotterdam cohort, 68 patients (20·2 per cent) received neoadjuvant chemoradiotherapy (29) or chemotherapy (39). In the OCCAMS cohort, 146 patients (42·1 per cent) received neoadjuvant chemotherapy according to UK guidelines (*Table 1*).

Association between SOX2 expression and survival

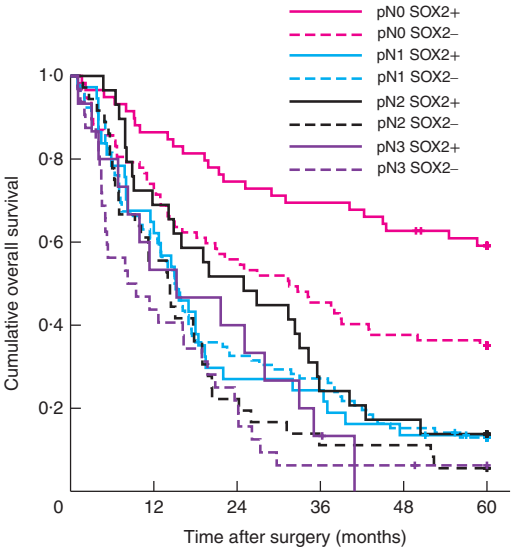
The interobserver agreement for the assessment of SOX2 immunohistochemistry between the two observers was



No. at risk

pT1 SOX2+	23	22	20	20	20	18
pT1 SOX2–	15	14	13	10	8	8
pT2 SOX2+	23	20	20	18	14	14
pT2 SOX2–	46	37	26	23	16	14
pT3 SOX2+	94	61	35	21	13	8
pT3 SOX2–	176	96	49	33	25	19

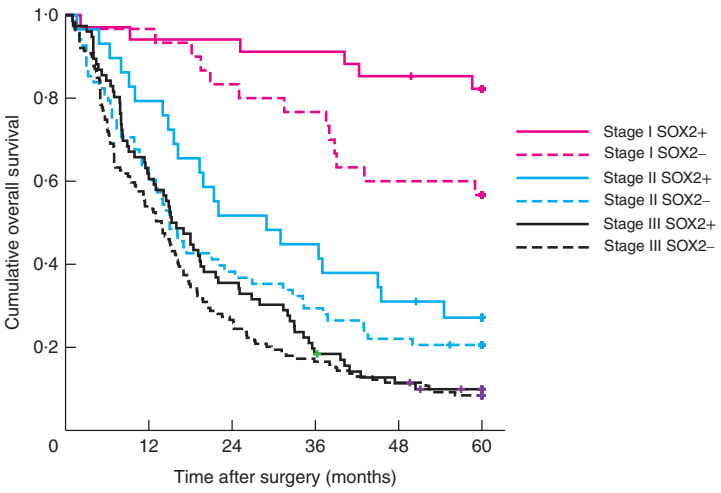
a pT category



No. at risk

pN0 SOX2+	59	51	44	41	36	33
pN0 SOX2–	77	57	43	35	29	27
pN1 SOX2+	37	24	10	9	5	3
pN1 SOX2–	92	56	30	25	14	11
pN2 SOX2+	29	20	15	7	5	4
pN2 SOX2–	36	20	8	4	4	2
pN3 SOX2+	15	8	6	2	0	0
pN3 SOX2–	32	14	7	2	2	1

b pN category



No. at risk

Stage I SOX2+	34	32	32	31	29	27
Stage I SOX2–	30	29	25	23	18	17
Stage II SOX2+	29	23	15	13	9	7
Stage II SOX2–	68	43	26	20	15	13
Stage III SOX2+	76	47	27	16	8	5
Stage III SOX2–	139	75	37	23	16	11

c Stage grouping

Fig. 2 Kaplan–Meier curves of overall survival in chemotherapy-naive patients with oesophageal adenocarcinoma according to SOX2 expression and clinicopathological staging: **a** pT category, **b** pN category and **c** stage grouping

excellent ($\kappa = 0{\cdot}92$, $P < 0{\cdot}001$). After exclusion of patients with fewer than three representative cores available, TMAs from 537 of 756 patients were used in the final analysis of SOX2 immunohistochemistry (288 from the Rotterdam and 249 from the OCCAMS cohort). In total, SOX2 was positive in 186 cancers and negative in 351. Representative examples of SOX2 immunohistochemical expression patterns are shown in *Fig. S2* (supporting information).

In the Rotterdam cohort, negative SOX2 was associated with a shorter median OS compared with positive SOX2 (19·5 *versus* 32·9 months respectively; $P = 0{\cdot}001$). Median survival in the OCCAMS cohort was similar to that in the Rotterdam cohort (15·0 and 26·0 months for negative and positive SOX2 respectively; $P = 0{\cdot}014$) (*Table S1*, supporting information). Corresponding Kaplan–Meier curves for the individual cohorts and the combined group are depicted in *Fig. 1*.

SOX2 expression did not correlate with location of the tumour. In Siewert type I OAC, 32·9 per cent of the tumours showed loss of SOX2, whereas in Siewert type II and III loss of SOX2 was found in 40·3 and 32·3 per cent of tumours respectively ($P = 0{\cdot}260$).

Univariable analysis showed a hazard ratio (HR) for death in patients with SOX2 loss of 1·54 (95 per cent c.i. 1·16 to 2·04; $P = 0{\cdot}003$) for the Rotterdam cohort, 1·58 (1·12 to 2·22; $P = 0{\cdot}009$) for the OCCAMS cohort and 1·55 (1·25 to 1·93; $P < 0{\cdot}001$) for the combined cohort (*Table S2*, supporting information).

Multivariable regression analysis to test the independent value of SOX2 in relation to other clinical parameters showed that SOX2 remained significant for OS in both individual cohorts as well as in the combined cohort (HR 1·42, 95 per cent c.i. 1·14 to 1·77; $P = 0{\cdot}002$) (*Table 2*).

Information on DFS was available only for the Rotterdam cohort; SOX2 was independently predictive of disease recurrence (HR 1·37, 95 per cent c.i. 1·01 to 1·86; $P = 0{\cdot}045$) (*Table S3* and *Fig. S3*, supporting information).

In chemotherapy-naive patients, SOX2 loss was confirmed as a statistically significant prognostic indicator of worse OS in both univariable and multivariable analysis (*Table 3*; *Table S4*, supporting information). When the prognostic value of SOX2 in chemotherapy-naive patients was examined in relation to clinicopathological staging, SOX2 showed separation into prognostic groups for pT1–2 tumours (HR 2·36, 95 per cent c.i. 1·23 to 4·51; $P = 0{\cdot}010$), but not for pT3–4 tumours (*Fig. 2a*; *Table S5* and *S6*, supporting information). Patients with pT1 OAC and loss of SOX2 had a trend towards being pN+ ($P = 0{\cdot}070$) (*Table S7*, supporting information,), whereas for pT2–4 tumours there was no correlation between SOX2 and nodal status.

When combining SOX2 and pN category, a significant separation into prognostic groups was detected for patients with pN0 disease (HR 1·71, 95 per cent c.i. 1·03 to 2·84; $P = 0{\cdot}038$), whereas for pN1–3 no effect of SOX2 was seen (*Fig. 2b*; *Table S8*, supporting information).

Based on the findings for pT and pN status, Kaplan–Meier curves were constructed for the effects of SOX2 for each TNM stage. Only in stage I disease was SOX2 loss associated with an increased HR for death (HR 3·18, 95 per cent c.i. 1·18 to 8·56; $P = 0{\cdot}022$) (*Fig. 2c*; *Table S9*, supporting information).

During follow-up, 289 chemotherapy-naive patients died within 5 years of surgery, of whom 194 showed loss of SOX2. The sensitivity of SOX2 for the prediction of death within 5 years in these patients was 67·1 per cent and the specificity 51·1 per cent. Of the 64 chemotherapy-naive patients with stage I disease, 19 died within 5 years, of whom 13 showed loss of SOX2. The sensitivity of SOX2 for prediction of death in chemotherapy-naive patients with stage I disease was 68 per cent and the specificity 62 per cent. Positive and negative predictive values and AUC for all patients, chemotherapy-naive patients and patients with chemotherapy-naive stage I OAC are shown in *Table S10* (supporting information).

Discussion

SOX2 immunohistochemistry adds prognostic information in patients with OAC. SOX2 loss was predictive of an adverse outcome in two independent cohorts (Rotterdam and OCCAMS), with a significant incremental adverse effect for OS, especially for patients with pN0 and stage I OAC.

Previous studies that attempted to identify clinically applicable predictive biomarkers for treatment response or overall prognosis have often been underpowered[35] or included heterogeneous patient populations with squamous cell carcinoma and adenocarcinoma[36]. Biomarker analysis can also be hampered by different neoadjuvant treatments in advanced OAC, making comparisons between studies difficult[37]. Large collaborative projects using standardized methodology are required to generate a clinically useful approach. Using this strategy, a three-gene immunohistochemical panel was shown to be useful in a previous large multicentre study[38]. Combining TNM staging with this immunohistochemical panel of epidermal growth factor receptor (EGFR), tripartite motif-containing 44 (TRIM44) and sirtuin 2 (SIRT2) allowed segregation of patients with stage II and III disease into distinct prognostic groups, whereas the effect for stage I was minimal[38]. This is different from the SOX2 findings reported here.

Little is yet known about the role of SOX2 in OAC. In Barrett's oesophagus, which exhibits mixed intestinal and gastric differentiation, SOX2 is detected in most patients, whereas during the progression to OAC downregulation of gastric and intestinal gene expression, including SOX2, occurs[26–28]. In advanced OAC, retained expression of SOX2 has previously been related to resistance to neoadjuvant chemoradiotherapy in patients treated according to the CROSS (ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study) regimen[29,30]. An earlier small Dutch study of 94 patients with surgically treated OAC also suggested SOX2 loss to be a predictor of reduced DFS, although it was underpowered to establish the incremental value of SOX2 in OS[31]. The present study focused on surgically treated OAC and not only confirmed the prognostic value of SOX2 for DFS (HR 1·37; $P = 0·045$), but also showed that SOX2 loss predicted adverse OS in patients with OAC. Importantly, SOX2 status was independent of all clinical and histological parameters known to influence survival, including neoadjuvant treatment.

Patients with stage I OAC generally have a good prognosis with 5-year survival rates of 87·7 and 73·3 per cent for stages Ia and Ib respectively[39]. Although patients with pT1a disease can be treated by endoscopic resection or surgery alone, treatment of those with pT1b disease is more controversial owing to the risk of lymph node metastasis. An optimal treatment strategy for these patients is widely debated[40]. The benefits of neoadjuvant therapy, for instance, are unclear[41]. In the present study, a worse OS in chemotherapy-naive patients with stage I OAC was associated with loss of SOX2 (HR 3·18; $P = 0·022$). The results suggest that SOX2 might predict lymph node metastasis in pT1 OAC, although further studies are needed to confirm this.

The role of SOX2 in the pathogenesis of OAC is poorly understood. Significant association of retained SOX2 expression and favourable survival could be explained by *SOX2* function as a tumour suppressor gene, similar to the findings in gastric carcinoma. Lower mitotic rate, increased apoptosis, and reduced invasion and dissemination were detected in patients with gastric cancer with retained SOX2 expression, compared with findings in those with SOX2 loss[42–44]. In line with its tumour suppressive role, several downstream targets of SOX2 were identified in gastric cancer, including cyclin D1 (CCND1), phosphorylated retinoblastoma 1 (pRB1), cyclin-dependent kinase inhibitor 1B (CDKN1B), as well as PTEN and phosphorylated protein kinase B (pAKT)[43–45]. Given the lineage-specific SOX2 function in formation of the stomach and oesophagus during embryogenesis, the role of SOX2 in OAC might be similar to that seen in gastric cancer.

The present study has some limitations, including its retrospective design and the small number of patients with stage I tumours. The expression of SOX2 was assessed in TMAs constructed from resection specimens and not in preoperative biopsies of patients with OAC, which may also be important. Validation of these results in a prospective study, and on pretreatment tumour material as well as resection specimens, still needs to be undertaken. At the same time, SOX2 detection in this study was performed by standardized immunohistochemistry, which is readily reproducible, and although interpretation may be subjective there was excellent interobserver agreement ($\kappa = 0·92$), indicating that accurate classification of SOX2 pattern is possible.

Immunohistochemical detection of SOX2 provided useful prognostic information in patients with OAC, independent of clinical parameters. Use of this marker in addition to current staging systems could be of particular relevance in selected populations of patients with node-negative tumours and those with stage I disease. The precise biological role of SOX2 in OAC requires further elucidation.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Table S1 Overall survival according to SOX2 expression in combined and individual cohorts (Word document)

Table S2 Univariable analysis of survival for all patients in combined and individual cohorts (Word document)

Table S3 Univariable and multivariable analysis of disease-free survival for 288 patients in the Rotterdam cohort (Word document)

Table S4 Univariable analysis of survival in chemotherapy-naive patients in combined and individual cohorts (Word document)

Table S5 Univariable and multivariable analysis of survival in chemotherapy-naive patients with pT1–2 tumours and pT3–4 tumours (Word document)

Table S6 Univariable and multivariable analysis of survival in chemotherapy-naive patients with pT1 and pT2 tumours (Word document)

Table S7 SOX2 in relation to lymph node status in 38 chemotherapy-naive patients with pT1 oesophageal adenocarcinoma (Word document)

Table S8 Univariable and multivariable analysis of survival in chemotherapy-naive patients with pN0 and pN+ tumours (Word document)

Table S9 Univariable and multivariable analysis of survival in chemotherapy-naive patients with stage I, stage II and stage III disease (Word document)

Table S10 Sensitivity, specificity, positive and negative predictive value, prevalence, accuracy and area under the curve of SOX2 loss to predict 5-year survival in all patients, chemotherapy-naive patients and those with stage I oesophageal adenocarcinoma (Word document)

Fig. S1 Receiver operating characteristic (ROC) curves according to percentage SOX2 loss in the Rotterdam cohort (Word document)

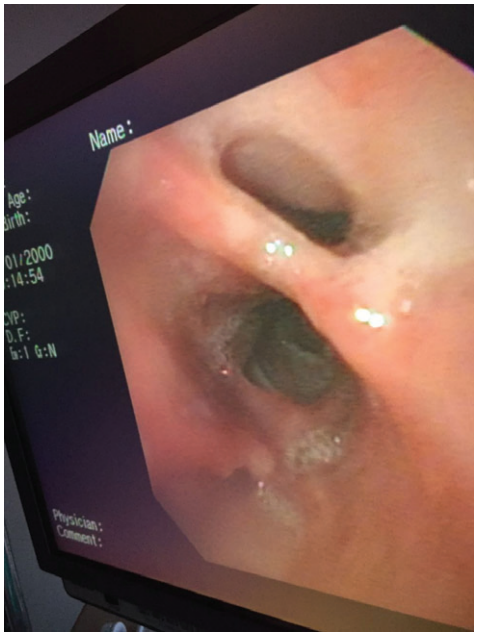
Fig. S2 Representative examples of SOX2 immunohistochemistry (Word document)

Fig. S3 Expression of SOX2 and prognosis of disease-free survival in patients with oesophageal adenocarcinoma in the Rotterdam cohort (Word document)

Snapshot quiz

Snapshot Quiz 17/10

Question: This 45-year-old man underwent gastroscopy with this finding in mid oesophagus. What is the diagnosis?



The answer to the above question is found on p. 1411 of this issue of *BJS*.

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