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COX2 expression in prognosis and in prediction to endocrine therapy in early breast cancer patients

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Abstract

Background In breast cancer, the prognostic impact of COX2 expression varies widely between studies. We examined the prognostic value of COX2 expression in a large cohort of breast cancer patients treated with primary surgery between 1985 and 1994 and explained the variable results of COX2 expression found in the literature.

Methods A tissue microarray was constructed of available tumour material, and ER, PgR, HER2, Ki67 and COX2 were examined by immunohistochemistry.

Results Median follow-up was 19 years. Fifty-five percent (n = 369/677) of patients received no systemic treatment. COX2 was scored using a weighted histoscore. Analysis of COX2 expression in two groups based on the median (148; below vs. above) showed an increased hazard ratio (HR) of 1.35 (95%CI 1.05–1.75; $p = 0.021$) for disease-free survival (DFS) and of 1.39 (95%CI 1.03–1.82; $p = 0.016$) for overall survival (OS). However, COX2 did not remain independent in multivariable analysis. In patients with hormone receptor positive tumours, COX2 expression had a negative influence on outcome (low vs. high: DFS: HR 1.37, 95%CI 1.07–1.76; $p = 0.013$). This effect disappeared when endocrine therapy was administered (low vs. high: DFS: HR 0.93, 95%CI 0.51–1.70; $p = 0.811$) while it remained statistically significant when endocrine therapy was omitted (low vs. high: DFS: HR 1.48, 95%CI 1.12–1.94; $p = 0.005$).

Conclusion Our results show that COX2 plays a role in hormonal pathways. Our results can explain the results found in previously published studies.

Introduction

Breast cancer is the leading cause of death from cancer in women in the western world.[1] Systemic treatment improves disease-free survival (DFS) and overall survival (OS) in patients with early breast cancer.[2] The indication for systemic treatment is based on prognostic and predictive factors.[3] Prognostic factors estimate the patient's risk of relapse in the absence of systemic therapy and include, amongst others, age at diagnosis, tumour grade and lymph node status. Predictive factors estimate the responsiveness of a tumour to a specific treatment, for example, expression of oestrogen receptor (ER) for endocrine therapy and overexpression/amplification of human epidermal growth factor receptor 2 (HER2) for trastuzumab. Prognostic and predictive factors are increasingly important as the relative risk of death from breast cancer is decreased by early diagnosis and improved treatment. Moreover, a substantial proportion of patients with early breast cancer may survive without adjuvant endocrine and/ or chemotherapy. Novel markers are needed to further subcategorize patients for different systemic treatment regimens.

Several epidemiologic and observational studies have examined the relationship between non-steroidal anti-inflammatory drugs (NSAIDS) and early breast cancer. In the prospective Women's Health Initiative Observational Study, regular use of NSAIDS was significantly correlated with a reduction in the incidence of breast cancer.[4] Furthermore, the findings of a meta-analysis of 14 studies suggest that use of NSAIDS may be associated with a small decrease in breast cancer risk.[5] The main target of NSAIDS is cyclooxygenase (COX), which exists in two isoforms: COX1 and COX2, which are regulated independently. COX1 is expressed in normal tissue, whereas COX2 is expressed in various human malignancies, like colon and breast cancer. COX2 catalyzes the conversion of arachidonic acid into prostaglandins and increased levels of prostaglandins are associated with carcinogenesis.[6]

A number of studies have examined the impact of COX2 expression in early breast cancer patients.[7-22] The results of these studies vary widely, which may be partly explained by the fact that most studies did not stratify patients according to systemic therapy. Moreover, in the majority of

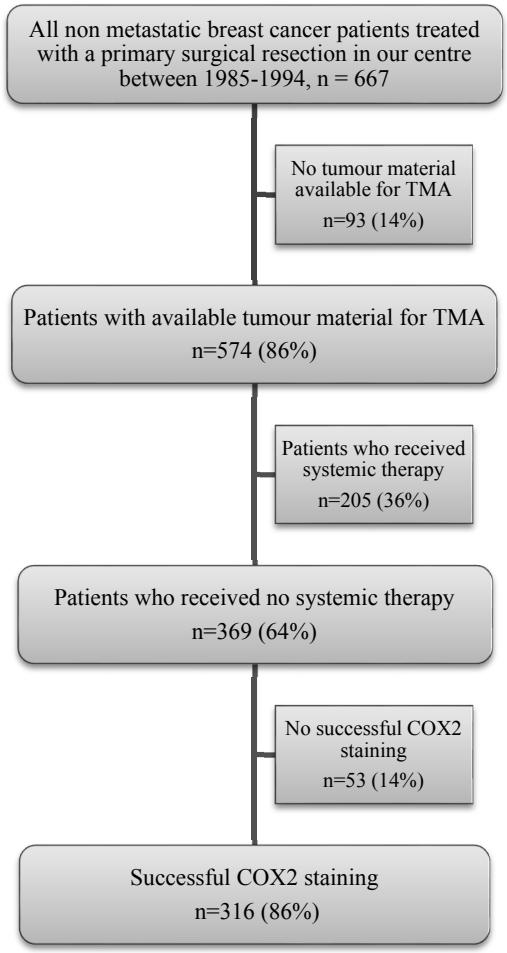


Figure 1 Patient selection illustrated in a diagram.

these studies, patients received some form of systemic therapy so that reliable data on the prognostic impact of COX2 in the absence of systemic therapy is lacking. This might be important, especially for endocrine therapy, as COX2 catalyzes the conversion of arachidonic acids into prostaglandins which stimulates aromatase and thus formation of estrogens.[23]

We examined the prognostic value of COX2 expression in a cohort of operable breast cancer patients in the Leiden University Medical Centre (LUMC) between 1985 and 1994. About one-third of this group was treated with adjuvant endocrine or chemotherapy. We performed stratified analyses to systemic treatment and assessed the relationship between COX2 expression and established prognostic clinicopathological parameters. We used the guidelines for the reporting of tumour marker studies (REMARK).[24]

Patients and methods

Patients

The patient population was a consecutive series of all women with non-metastatic breast cancer who received a primary surgical resection (with or without radiotherapy) in the LUMC between 1985 and 1994. Patients with a prior history of cancer (other than basal cell carcinoma or in situ carcinoma) or bilateral tumours were excluded. Age at diagnosis, tumour grade, morphology, TNM stage, local and systemic therapy, locoregional/distant recurrence, second primaries and death were recorded. All tumours were histologically classified and graded by one pathologist (VS). Approval was obtained from the LUMC Medical Ethics Committee.

Preparation of tumour tissue microarrays

Formalin fixed paraffin-embedded tumour blocks of the primary tumour were collected of the pathology department. All blocks were stored at room temperature. Sections were cut for haematoxylin and eosin staining, and histopathologically representative tumour regions were indicated by a pathologist (MvdV) and used for preparation of tumour tissue microarray (TMA) blocks.[25] From each donor block, three 0.6-mm2 tissue cores were punched from tumour areas and transferred into a recipient paraffin block using a custom-made precision instrument. Four-micrometre thick sections of the TMAs were cut and processed for immunohistochemistry.

Immunohistochemistry

Tissues were dewaxed in xylene and rehydrated in a graded alcohol series. Antigen retrieval was carried out for 5 min in a pressure cooker in 10 mmol/l sodium-citrate buffer (pH 6.0). Slides were immersed in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity and in a blocking solution (DAKO protein-free serum block) for 60 min to block non-specific binding sites. Immunostaining was performed with COX2 (antihuman) monoclonal antibody (160112; Cayman Chemical Co., Ann Arbor, MI) diluted in antibody diluent (DAKO) at a concentration of

1:100 for 30 min at room temperature. One tumour TMA was stained with and without antibody as a positive and negative control, respectively. Sections were treated with envision (DAKO), and visualization was performed using DAB (DAKO). Finally, tissues were counterstained with haematoxylin and dehydrated through graded alcohols and xylene. Immunostaining for ER, progesterone receptor (PgR), Ki67 and HER2 was carried out using established procedures with the following antibodies: NCL-L-ER-6F11 (Novocastra), monoclonal mouse antihuman progesterone receptor Clone PgR 636 (M3569, DAKO), monoclonal mouse antihuman ki67 antigen Clone MIB-1 (M7240, DAKO) and HercepTest™ for DAKO autostainer (K5207 DAKO), respectively.[26]

Evaluation of immunostaining

It has been shown that TMAs with 2 cores from each tumour is a valuable and accurate method for analysis of protein expression in large archival cohorts and correlates highly with whole-section staining.[27,28] COX2 immunohistochemical staining was scored independently and in a blind manner using a weighted histoscore. The proportion of cells with cytoplasmic staining was multiplied by the intensity of staining to provide a score of 0–300. Score = (0 x percentage of cytoplasm not stained) + (1 x percentage weakly stained) + (2 x percentage moderately stained) + (3 x percentage strongly stained).[29] The first investigator (JvN) scored all cores and the second investigator (CF) scored 33% of cores to ensure consistency, both in a blind manner. The interclass correlation coefficient of cores scored was excellent: 0.928.[30] The intra-observer variability analysed using Cohen's kappa coefficient was 0.87. All tumours with discordant scores were re-evaluated by both investigators. The mean score of all cores scored by the first investigator was used for analysis.

Oestrogen receptor and progesterone receptor immunohistochemical staining was scored using a weighted histoscore generated by an automated image analysis machine, the Ariol SL-50 Image Analysis System. The suitability of this system has been reported previously.[31,32] Tumour epithelium was marked for analysis, and the marked areas' quality was assured by a pathologist (DF). The system was trained on the basis of the cellular characteristics of epithelial, stromal and infiltrating non-tumourous cells to produce a trained classifier applicable to all nuclear stains. A positive hormone receptor status was defined as a histoscore of at least 10. Ki67 expression (percentage of positive cells) was also evaluated using the Ariol System. There are different cut points when using Ki67, we used 5. HER2 staining intensity was graded (by DF) in accordance with the HercepTest protocol system as 0, 1+, 2+ or 3+. Samples scored as 0, 1+ or 2+ were considered negative for HER2 overexpression, 3+ was considered to be positive.

Study design

The design comprises a retrospective cohort study (1985–1994). No stratification or matching was used. The end of follow-up period is 1 January 2009 or death or date of lost of follow-up. Objectives of these retrospective analyses are (independent) correlation of COX2 with survival, relation with systemic therapy and relation with established prognostic clinicopathological parameters.

Statistical analysis

All data were analysed using the statistical package SPSS for Windows 15.0 (SPSS Inc, Chicago, IL, USA). Descriptive data are given as mean (±standard deviation, SD) or median (range). DFS was calculated from the date of surgery up to the first date of locoregional or distant recurrence, contralateral breast cancer or death by any cause, whichever came first. OS was defined from the date of surgery up to the date of death from any cause.

In order to examine if COX2 expression was correlated with DFS and OS, univariate Cox analyses were performed. COX2 expression was normally distributed within the population, for statistical analysis samples were divided into two equal groups (high and low) based on the median histoscore, this cut off was selected to maximize statistical power. Multivariable analyses were performed using the Cox proportional hazards model entering COX2 expression with other significant variables (defined as those with $p < 0.1$) on univariate analysis. The relationship between COX2 expression and established prognostic factors was investigated using the chi-square test without Bonferroni correction. All testings were two-tailed with 0.05 as level of significance.[33] Missing data were not used for analyses.

Table 1 Clinicopathological and survival data of all patients with non-metastatic breast cancer treated with a primary surgical resection in our centre between 1985 and 1994.

	All patients		Patients not on TMA[a]		Patients on TMA		On TMA, no systemic Therapy	
	n	%	n	%	n	%	n	%
Age (years)								
Median (range)	57	23-96	52	26-85	57	23-96	59	29-96
Mean (SD)	57	14	52	13	58	14	60	14
<40	66	10	18	19	48	8	22	6
40-50	169	25	24	26	145	25	85	23
51-60	157	24	25	27	132	23	84	23
>60	275	41	26	28	249	43	178	48
Extent of disease								
Early breast cancer	547	82	82	88	465	81	304	82
Locally advanced	120	18	11	12	109	19	65	18
Tumour stage								
pT1	269	40	58	62	211	37	157	43
pT2	295	44	23	25	272	47	165	45
pT3 or T4	78	12	6	7	72	13	38	10
pTmissing	25	4	6	7	19	3	9	2
Grade								
I	87	13	7	8	80	14	50	14
II	298	45	16	17	282	49	194	53
III	209	31	6	7	203	35	117	32
Missing	73	11	64	69	9	2	8	2
Histological type								
Ductal	541	81	28	30	513	89	329	89
Other	54	8	1	1	53	9	33	9
Missing	72	11	64	69	8	1	7	2
Nodal stage								
pN0	365	55	59	63	307	54	265	72
pN+	302	45	34	37	267	47	104	28
Oestrogen receptor								
Negative	Unknown		Unknown		203	35	126	34
Positive	Unknown		Unknown		337	59	221	60
Missing	Unknown		Unknown		34	6	22	6
Progesterone receptor								
Negative	Unknown		Unknown		223	39	138	37
Positive	Unknown		Unknown		313	55	204	55
Missing	Unknown		Unknown		38	7	27	7
HER2								
0+/1+/2+	Unknown		Unknown		387	66	248	67
3+	Unknown		Unknown		44	8	23	6
Missing	Unknown		Unknown		152	27	98	27
Ki67 expression								
<5	Unknown		Unknown		360	63	231	63
>5	Unknown		Unknown		176	31	112	30
Missing	Unknown		Unknown		38	7	26	7
COX2								
Low: <148	Unknown		Unknown		254	44	162	44
High: ≥148	Unknown		Unknown		250	44	154	42
Missing	Unknown		Unknown		70	12	53	14

Table 1 Continued

	All patients		Patients not on TMA[a]		Patients on TMA		On TMA, no systemic Therapy	
Local treatment								
MST without radiotherapy	250	38	27	29	223	39	149	40
MST with radiotherapy	122	18	14	15	108	19	58	16
BCS without radiotherapy	5	1	0	0	5	1	4	1
BCS with radiotherapy	290	44	52	56	238	42	158	43
Systemic treatment								
Chemotherapy alone	134	20	22	24	112	20	0	0
Endocrine therapy alone	83	12	8	9	75	13	0	0
Both	20	3	2	2	18	3	0	0
None	430	65	61	66	369	64	369	100
Survival data								
Local recurrence	73	11	19	20	54	9	38	10
Regional recurrence	22	3	4	4	18	3	9	2
Distant recurrence	274	41	31	33	243	42	148	40
Contralateral breast cancer	54	8	10	11	44	8	33	9
Death	413	62	43	46	370	65	240	65
Total	**667**	**100**	**93**	**100**	**574**	**100**	**369**	**100**

Locally advanced breast cancers are all T3 and T4 cancer or any tumour stage with N2 or N3 disease. Numbers and percentage are calculated on available data, missing data are not shown

[a]If tumour of a patient is not on the TMA means that there was no (not enough) available tumour

BCS breast conserving surgery; *MST* mastectomy; *TMA* tissue micro array.

Results

Patients

A total of 667 patients with non-metastatic breast cancer were treated with a primary surgical resection in the LUMC during the study period. TMAs were made from available paraffin tumour blocks from 574/667 (86%) patients. Of these, 369/574 (64%) patients received no systemic treatment, the remaining 205 patients received a mixture of endocrine and/or chemotherapy (Figure 1). The median follow-up for patients alive was 19 years (range 0–23). Clinicopathological and local treatment characteristics are shown in Table 1.

Distribution of prognostic factors

Oestrogen receptor staining was successful in 94% of tumours (cores were missing, folded or contained no invasive tumour in 6%). Median tumour ER histoscore was 45.77 (range 0–254). PgR staining was successful in 93% of tumours; median tumour PgR histoscore was 25.59 (range 0–300). The interquartile ranges for ER and PgR were 132 and 126, respectively. HER2 expression was successful in 74% of tumours. Overexpression of HER2 was seen in 10% and 90% of tumours had no HER2 overexpression. Ki67 staining was successful in 93%; median tumour Ki67 expression was 2.20 (0–63) and the interquartile range was 6.19.

COX2 expression in tumour tissue

In tumour cells, COX2 staining was observed in the cytoplasm with a granular staining pattern (Figure 2). COX2 staining was successful in 88% of tumours (cores were missing, folded or contained no invasive tumour in 12%). COX2 expression was available for 316/369 (86%) cases with no systemic treatment and for 188/205 (92%) cases receiving systemic treatment (Figure 1), no significant differences were observed between cases with data available for COX2 expression and those for whom data was not available with respect to conventional prognostic markers (data not shown). Median tumour COX2 histoscore was 148.33 (range 3–278), meaning high COX2 had a histoscore >148 and low COX2 a histoscore ≤148.

Prognostic value of COX2 expression

In order to analyse the prognostic value of COX2, we analysed data from the patients who were treated with local therapy only (Table 2; Figure 3). In univariate analysis, high COX2 expression

Table 2 Univariate and multivariable analysis of (A) disease free survival and (B) overall survival.

(A)

			Univariate analysis			Multivariable analysis		
Characteristic		**n**	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	<60	191	1.00		<0.001	1.00		0.002
	≥60	178	1.92	1.50 - 2.45		1.57	1.18 - 2.10	
Tumour stage	pT1	157	1.000		< 0.001	1.00		0.124
	pT2,3,4	203	1.90	1.480 – 2.45		1.23	0.93 – 1.78	
Nodal stage	pN0	265	1.00		< 0.001	1.00		< 0.001
	pN+	104	2.90	2.25 – 3.73		2.25	1.67 - 3.02	
Grade	I/II	244	1.00		0.001	1.00		0.040
	III	117	1.50	1.17 – 1.93		1.34	1.01 - 1.78	
Morphology	Ductal	329	1.00		0.373			
	Other	33	1.20	0.81 – 1.79				
ER	Negative	126	1.00		0.992			
	Positive	221	0.99	0.78 – 1.29				
PgR	Negative	138	1.00		0.300			
	Positive	204	0.88	0.68 – 1.13				
Ki67	<5	231	1.00		0.953			
	>5	112	0.99	0.76 – 1.29				
HER2	Negative	248	1.00		0.988			
	Positive	23	1.00	0.60 – 1.66				
COX2	<148	162	1.00		0.021	1.00		0.633
	≥148	154	1.35	1.05 – 1.75		1.07	0.81 - 1.41	
Surgery	MST	207	1.00		< 0.001	1.00		0.014
	BCS	162	0.51	0.39 – 0.65		0.60	0.40 - 0.90	
Radiotherapy	No	152	1.00		0.010	1.00		0.080
	Yes	217	0.73	0.57 – 0.93		1.39	0.96 - 2.02	

BCS breast conserving surgery; *ER* oestrogen receptor; *HER2* human epidermal growth factor receptor 2; *MST* mastectomy; *PgR* progesterone receptor.

(B)

			Univariate analysis			Multivariable analysis		
Characteristic		**n**	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	<60	191	1.00		<0.001	1.00		<0.001
	≥60	178	2.81	2.16-3.67		2.12	1.57-2.87	
Tumour stage	pT1	157	1.00		<0.001	1.00		0.175
	pT2,3,4	203	2.13	1.62-2.80		1.27	0.90-1.78	
Nodal stage	pN0	265	1.00		<0.001	1.00		<0.001
	pN+	104	2.81	2.16-3.66		2.27	1.68-3.07	
Grade	I/II	244	1.00		0.005	1.00		0.053
	III	117	1.46	1.12-1.90		1.34	1.00-1.79	
Morphology	Ductal	329	1.00		0.310			
	Other	33	1.25	0.82-1.90				
ER	Negative	126	1.00		0.548			
	Positive	221	0.92	0.71-1.20				
PgR	Negative	138	1.00		0.128			
	Positive	204	0.82	0.63-1.06				
Ki67	<5	231	1.00		0.700			
	>5	112	0.95	0.72-1.25				
HER2	Negative	248	1.00		0.769			
	Positive	23	1.08	0.65-1.80				
COX2	<148	162	1.00		0.016	1.00		0.849
	≥148	154	1.39	1.06-1.8		1.03	0.77-1.37	
Surgery	MST	207	1.00		<0.001	1.00		0.016
	BCS	162	0.39	0.29-0.50		0.59	0.39-0.91	
Radiotherapy	No	152	1.00		<0.001	1.00		0.267
	Yes	217	0.58	0.45-0.75		1.24	0.85-1.81	

BCS breast conserving surgery; *ER* oestrogen receptor; *HER2* human epidermal growth factor receptor 2; *MST* mastectomy; *PgR* progesterone receptor.

resulted in a worse DFS (hazard ratio (HR) 1.35, 95%CI 1.05–1.75; p = 0.021) and more deaths (HR 1.39, 95%CI 1.06–1.82; p = 0.016) compared to low COX2 expression. In multivariable analysis, COX2 expression did not provide prognostic information independently of other prognostic variables for DFS (HR 1.07, 95%CI 0.81–1.41; p = 0.633) or OS (HR 1.03, 95%CI 0.77–1.37; p = 0.849). For DFS, only age, nodal status, tumour grade and surgical therapy were statistically significant factors in multivariable analysis. Age, nodal status and surgical therapy were independent prognostic variables for OS.

COX2 expression in the hormonal pathway

In order to investigate the influence of COX2 expression on survival in relation to hormone receptor and endocrine therapy, we first investigated COX2 expression in patients with hormone receptor negative tumours (Figure 4). In these patients, COX2 expression had no influence on overall survival (log-rank p = 0.593). In univariate analyses of patients with hormone receptor positive tumours, however, low COX2 expression was associated with better survival compared to a high COX2 expression (DFS: HR 1.37 95%CI 1.07–1.76; p = 0.013; OS: HR 1.44, 95%CI 1.11–1.86; p = 0.006). Subsequently, within patients with hormone receptor positive tumours, we analysed the effect of endocrine therapy administration in relation to COX2 expression (Figure 5). COX2 expression levels did not differentiate survival in patients treated with endocrine therapy in univariate analyses (low vs. high; DFS: HR 0.93 95%CI 0.51–1.70; p = 0.811; OS: HR 0.91, 95%CI 0.50–1.68; p = 0.767). In contrast, within patients who did not receive endocrine therapy, COX2 expression had an influence on outcome (low vs. high; DFS: HR 1.48, 95%CI 1.12–1.94; p = 0.005; OS: HR 1.58, 95%CI 1.18–2.10; p = 0.002) in univariate analyses. In multivariable analyses, only age, nodal status and surgical therapy were independent prognostic factors. Nevertheless, these results suggest that the clinical impact of COX2 expression in breast cancer patients is closely related to the ER status of the tumour.

COX2 expression and clinicopathological parameters

Increased COX2 expression was observed with increasing grade and stage (p < 0.0001), and tumours from older patients exhibited increased COX2 expression (p < 0.004; Table 3). Both in ER negative and PgR negative tumours exhibited

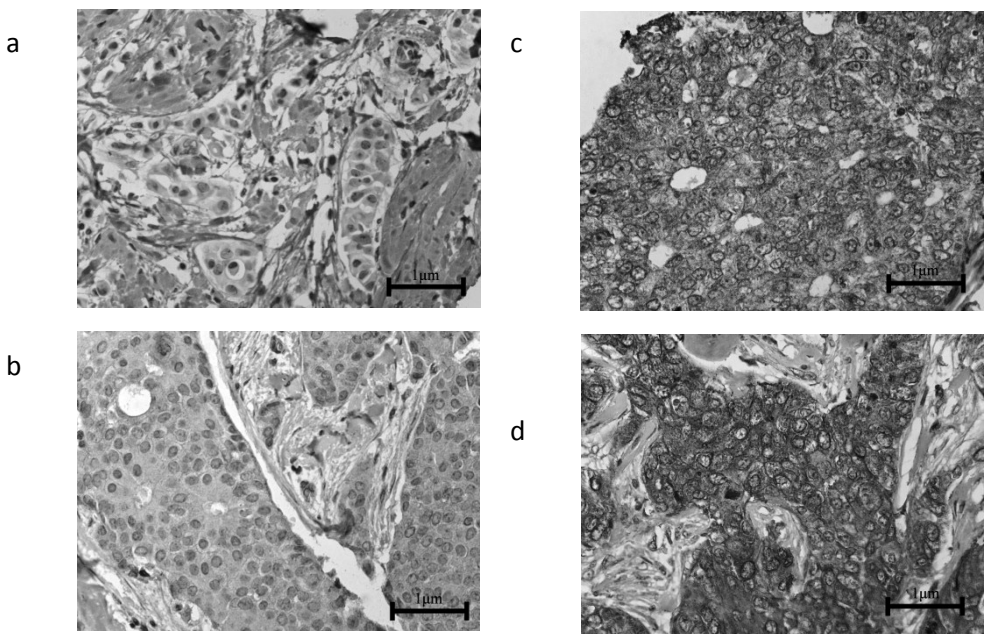
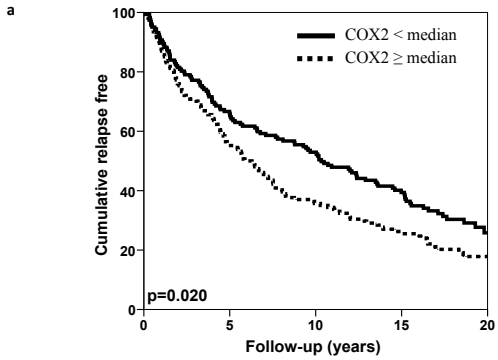
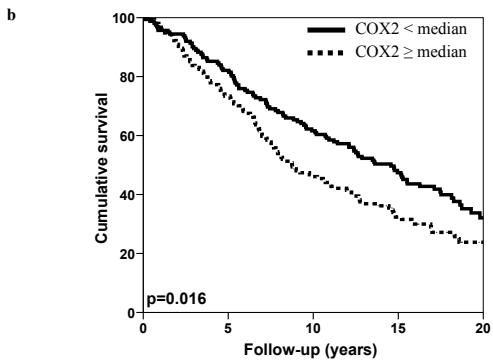


Figure 2 COX2 staining: **a** not stained, **b** largely weakly stained, **c** largely moderately stained and **d** largely strong stained.

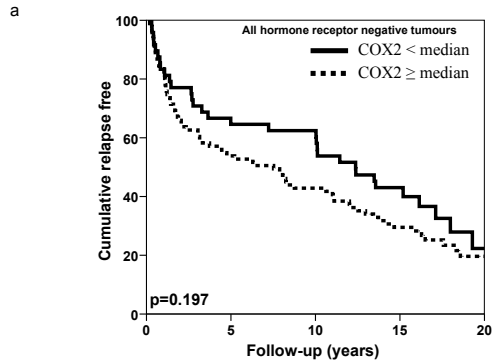


Patients at risk					
COX2<median	162	107	84	53	14
COX2≥median	154	85	54	33	9

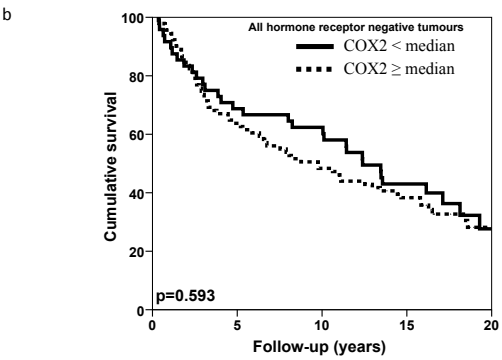


Patients at risk					
COX2<median	162	133	99	63	19
COX2≥median	154	113	70	41	12

Figure 3 Kaplan–Meier for **a** disease-free survival and **b** overall survival. COX2 expression is divided into two groups based on the median histoscore of 148.



Patients at risk					
COX2<median	48	31	29	14	4
COX2≥median	91	49	39	25	5



Patients at risk					
COX2<median	48	39	29	15	6
COX2≥median	91	58	44	31	7

Figure 4 COX2 expression and survival in all patients with hormone receptor negative tumours. Kaplan–Meier **a** and **b** show the disease-free survival and overall survival, respectively. COX2 expression is divided into two groups based on median histoscore of 148.

higher COX2 expression levels than hormone receptor positive tumours (Table 3). There was no significant correlation between Ki67/HER2 expression and COX2 expression.

Discussion

In breast cancer, a number of studies have explored the relationship between COX2 expression and clinical outcome (Table 4).[7-22] However, to the best of our knowledge, this is the first study to investigate and assess the true impact of COX2 expression on prognosis in breast cancer (i.e. outcome in the absence of systemic therapy). In the current study, elevated levels of COX2 expression were associated with an increased risk of relapse and death in the absence of endocrine or chemotherapy in univariate analysis. However, in multivariable analysis, COX2 expression did not provide additional prognostic information over conventional markers like age and lymph node status. Therefore, our data do not support a role for COX2 expression as a prognostic marker independent of conventional clinicopathological criteria. This contradicts much of the published literature (see below). Although the number of patients in the untreated group in the current study is relatively small (369), the hazard ratio for COX2 expression in the multivariable analysis (1.07 for DFS and 1.03 for OS) approaches one. This suggests that

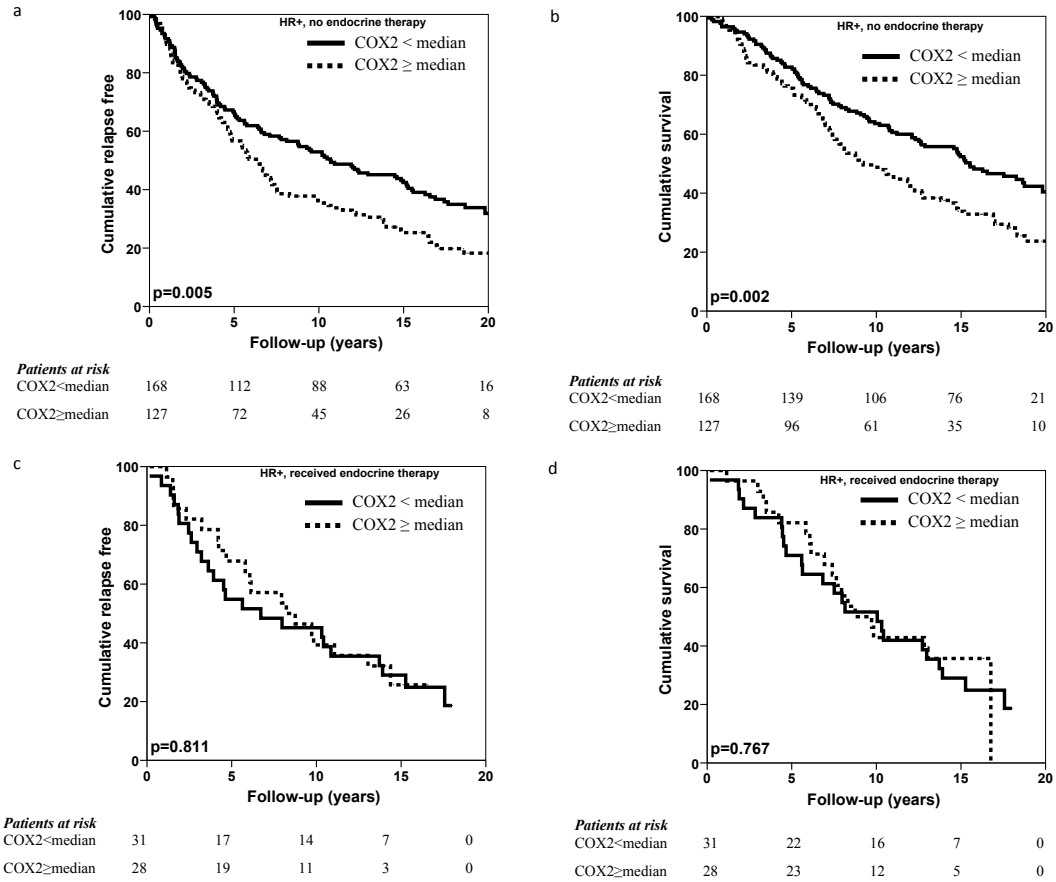


Figure 5 COX2 expression and survival in all patients with hormone receptor positive tumours. Kaplan–Meier **a** and **b** show the disease-free survival and overall survival for patients who did not receive endocrine therapy and **c** and **d** for whom who did. *HR* hormone receptor.

even in a significantly larger patient group, COX2 expression would not provide statistically significant additional prognostic information.

COX2 plays an evident role in the hormonal pathway. We hypothesised that COX2 expression is associated with a worse outcome in patients with hormone receptor positive tumours compared to patients with hormone receptor negative tumours due to the relationship between COX2 and aromatase expression. In addition, we suggested that administration of endocrine therapy would abolish this prognostic effect of COX2 expression within patients with hormone receptor positive tumours. In patients with hormone negative disease, COX2 had no influence on survival. In addition, both Kaplan–Meier survival analyses and Cox regression analyses showed that COX2 expression had no additional effect in patients with hormone receptor positive tumours who received endocrine therapy. However, in the subgroup of patients in which endocrine therapy was not administrated, COX2 expression was of prognostic significance. These findings suggest that COX2 plays a role in the hormonal pathway in breast cancer. Additionally, these findings suggest that endocrine therapy could be omitted in patients who express of low levels of COX2 with hormone sensitive tumours at a low risk according to current risk stratification. However, to prove this, a randomised controlled trial is needed.

We reviewed 16 previous studies examining the immunohistochemical expression of COX2 ex-

Table 3 Correlation between COX2 expressions in groups and well-established prognostic factors using chi-square test.

	COX2 Low		COX2 High		*p*
Age	**n**	**%**	**n**	**%**	**0.004**
<40	25	60	17	41	
40-50	78	63	46	37	
50-60	54	45	65	55	
>60	98	45	122	56	
Tumour stage					**<0.001**
pT1	115	65	63	35	
pT2,3,4	130	42	180	58	
Nodal stage					**0.269**
pN0	139	53	124	47	
pN+	116	48	126	52	
Grade					**<0.001**
I	48	75	16	25	
II	132	53	118	47	
III	71	39	112	61	
Morphology					**0.317**
Ductal	224	50	226	50	
Other	27	57	20	43	
Oestrogen receptor					**0.015**
Negative	82	43	108	57	
Positive	166	54	139	46	
Progesterone receptor					**<0.001**
Negative	82	39	127	61	
Positive	167	58	120	42	
Ki67					**0.321**
<5	162	48	173	52	
>5	85	53	75	47	
Human epidermal growth factor receptor 2					**0.059**
Negative	120	50	121	50	
Positive	7	30	16	70	

COX2 expression was divided into two groups based on the median histoscore; histoscore < 148: low COX2 expression; histoscore ≥ 148: high COX2 expression.

pression in breast tumours (Table 4).[7-22] Cross comparison between studies and our data was not easy since many studies included very small numbers of tumours, or had short median follow-up (2–9 years) and there was a lack of standardization of COX2 analysis. Most studies used the same antibody (Cayman Chemical Co., Ann Arbor, MI), but different concentrations and incubation times. Also, different methods for evaluation of COX2 expression were used; for example, Spizzo et al. examined COX2 expression using the product of the percentage of stained cells and the intensity, whilst Ristimaki et al. compared just the intensity of stained cells.[16,20] We used the weighted histoscore because this combines the intensity as well as the percentage of cells stained. Finally, in published studies, COX2 'positivity' was seen in a range of 5–85% of cases. However, arbitrary distinctions between COX2 positive and COX2 negative cases were used. COX2 expression was defined as any positive staining by Schmitz et al., in contrast to Park et al., where at least 80% of cells needed to be stained before the tumour was classified positive.[15,17] This diversity in methods used for staining, interpretation and analysis of COX2 expression in previous studies complicates comparisons between studies.

In spite of the diversity of methods discussed above, we suggest our results can provide a unifying explanation of the results found in the other studies. Denkert et al. showed a correlation with COX2 expression and DFS in multivariable analysis.[9] However, 63% of tumours in Denkert's study were ER positive (PgR was not shown) and only 23% of patients received endocrine therapy. Therefore, at least 40% of this population was hormone receptor positive and did not receive endocrine therapy. Similar results were seen in the study of Zerkowski et al., where at least 70% of tumours were hormone receptor positive and only 57% of patients received endocrine therapy. The three largest studies included a majority of patients treated prior to 1998.[10,16,22] We regard this as important because results reported by the Early Breast Cancer Trialist' Collaborative Group (following meta-analysis of randomised trials of endocrine therapy in early breast cancer patients) showed a correlation between tumour ER expression and response to endocrine therapy.[34] Prior to this report, endocrine therapy was given on the basis of menopausal status; following the publication of the meta-analysis, endocrine therapy was given based on hormonal status. We believe that the existence of cohorts of ER positive patients who did not received endocrine therapy can explain the results of the earlier studies This may also explain the similarity between our findings and those of Haffty et al. who also showed that COX2 expression was correlated with survival in hormone receptor positive tumours, but not in hormone receptor negative tumours.[10] This hypothesis is readily testable by future studies exploring the impact of COX2 expression in ER

Table 4 Immunohistochemical studies of COX2 expression in invasive non metastatic breast cancer.

Study	Total	Median Follow-up	Kind of patients	Inclusion in	ER+	PgR+	Systemic therapy?	Tissue	COX2 Ab	Score of COX2	Definition COX2 positive	COX2 positive % (overexpr)	Pathological correlates	Clinical correlates
Boland 2004[7]	65	NR	Archival material of a single centre.	NR	60%	NR	NR	Whole section	Santa Cruz Biotechn, San Diego, CA; 1:100 AND Cayman Chemical Co, Ann Arbor, MI	0: no staining 1: weak 2: moderate 3:strong	2 or 3	41 (63%)	ER- Ki67>10% HER2+	NR
Costa 2002[8]	46	Min 2 years	Single centre, all patients treated with mastectomy	NR	61%	NR	NR	Whole section	Santa Cruz Biotechn, San Diego, CA; 1:10	Brown cytoplasmic staining.	Brown	8 (17%)	pN+ Sialyl-Tn expression Microvessel count Apoptotic index	Uni: DFS shorter (only 26 pts used). Not in multivariable
Denkert 2003[9]	221	87 months (2-132)	Archival material, of a single centre, 1991-1996, residents of Berlin	1991-1996	63%	NR	HT: 23%	Whole section	Cayman Chemical Co, Ann Arbor, MI; 1:1000	Product of percentage (5 groups:0-4) and intensity (0-3). Negative: 0-6 points, positive 7-12 points	7-12 points	80 (36%)	pN+ Larger tumour size Poor differentiation Vascular invasion ER- Morphology	Multivariable: DFS (HR 1.90, 95%CI 1.00-3.59; *p*= 0.049) and OS (RR 1.14, 95%CI 0.67-1.93; *p*= 0.64)
Haffty 2008[10]	504	7 years	Single centre, 1975-2003, all breast conserving therapy	1975-2003	53%	50%	CT: 32% HT: 38%	TMA	Cayman Chemical Co, Ann Arbor, MI; 1:50	0: no staining 1: weak 2: moderate 3:strong	2 or 3, if >10% staining	250 (58%)	Younger age Larger tumour size	Multivariable ER+ pts: RFS (RR 2.95, 95%CI 1.20-7.86; *p*=0.03), DR (RR 1.82, 95%CI 0.97-3.44; *p*=0.06) and OS (RR 2.22, 95%CI 1.15-4.26; *p*=0.02). ER-pts: no correlation with survival
Half 2002[11]	57	19 months	Archival material of a single centre.	1995-2002	63%	46%	NR	Whole section	Cayman Chemical Co, Ann Arbor, MI; 1:500	Product of percentage (5 groups: 0-4) and intensity (0-3): low (0-4), medium (5-8), high (9-12)	High	18 (43%)	None found	NR
Hwang 1998[12]	44	NR	NR	NR	NR	NR	NR	Whole section	Homemade anti-COX2, 1:50	NR	NR	2 (5%)	NR	NR
Kelly 2003[13]	106	23 months	Achieval material	NR	NR	NR	NR	Whole section	Cayman Chemical Co, Ann Arbor, MI; 1:500	Product of percentage (5 groups:0-4) and intensity (0-3)	none (0) low (1-4) medium (5-8) high (9-12)	90 (85%) (detected)	None found	None found
O'Connor 2004[14]	23	48 months	Single centre, all patients treated with mastectomy and radiotherapy	1994-2004	65%	NR	CT: 96% HT: NR	Whole section	Alexis Biochemicals, Montreal, Canada; 1:500	Computer-assisted scoring. Percentage of cells expressing COX2 and the level of COX2 staining intensity	>80% intensity >75% expression		Younger age (for percentage, not intensity)	Univariate: DFS and OS

Table 4 Continued

Study	Total	Median Follow-up	Kind of patients	Inclusion in	ER+	PgR+	Systemic therapy?	Tissue	COX2 Ab	Score of COX2	Definition COX2 positive	COX2 positive % (overexpr)	Pathological correlates	Clinical correlates
Park 2006[15]	178	56 months (13-18)	Single canter, all patients are N+ and treated with CT between 1994-1996	1994-1996	58%	NR	CT, FAC: 100% HT: ?	TMA	Cayman Chemical Co, Ann Arbor, MI; 1:200	Absence, weak, moderate or string. Absence for immnoreactivity or weak staining intensity: discharged	>80% of observed cells are positive	39% (70)	Poor differentiation	Multivariable: DFS (RR 1.91, 95%CI 1.24-2.76; p=0.04) and OS (RR 1.73, 95%CI 1.16-2.14; p=0.03)
Ristimaki 2002[16]	1576	Median 6.8 years (5.1-7.8)	All patients diagnosed with breast cancer between 1991-1992 of 50% of the Finnish population.	1991-1992	55%	51%	HT: 57% of patients >50 CT: 37% of patients <50	TMA	Cayman Chemical Co, Ann Arbor, MI; 2.5 µg/ml	0: no staining 1: weak 2: moderate 10-90% 3:strong >90%	Score 2 or 3	589 (37%)	Large tumour size ER-, PgR-, HER2+ High ki67 and/or high p53 expression pN+ Tumour grade and type	Reduced DDFS, not in multivariable-
Schmitz 2006[17]	113	7 years	Single centre, 1989-1996, all pN0	1989-1996	62%	NR	Yes, unknown percentage	Whole section	DCS, Hamburg, Germany; 1:250	Negative (no immunoreactivity) or positive	Positive staining.	43% (49)	Poor differentiation	Multivariable: disease specific OS (RR 3.51, 95%CI 1.10-11.21; p=0.03)
Singh-Ranger 2004[18]	30		Single centre	NR	NR	NR		Whole section	Rabbit IgG antibody to COX2, Oxford Biosciences; 1/NR	Product of percentage (5 groups:0-4) and intensity (0-3)	0: none 1-4: low 5-8: medium 9-12: high	11 (37%)	None	NR
Soslow 2000[19]	17	NR	Randomly, one centre	NR	NR	NR	NR	Whole section	COX-2 polyclonal (PG-27, Lot No. 0599; Oxford Biomedical research, Oxford, MI); 1/500	Product of percentage (5 groups:0-4) and intensity (0-3)	0: none 1-4: low 5-8: medium 9-12: high	7 (42%)	NR	NR
Spizzo 2003[20]	212	11 years	Single centre, 1980-1992	1980-1992	64%	56%	NR	Whole section	Cayman Chemical Co, Ann Arbor, MI; 1:100	Product of percentage (5 groups:0-4) and intensity (0-3)	Score >4	103 (49%)	Age Morfology Ep-CAM	Poor DFS and OS, not in multivariable
Witton 2004[21]	179	6 years (0-15)	Randomly selected, single centre, 1984 - 1993.	1984-1993	59%	NR	All systemic therapy	Whole section	Cayman Chemical Co, Ann Arbor, MI; 0.625 µg/ml	Weighted histoscore: exact percentage * intensity (0-3)	Histoscore > 129.25 based on 10 cases with adjacent normal breast epithelium.	38 (21%)	None significant found	Multivariable: In all pts: reduced DFS (p=0.02) next to ER, pN, tumour size. In ER- reduced disease related survival and time to relapse (both p=0.05) In ER+: no association

Study	Total	Median Follow-up	Kind of patients	Inclusion in	ER+	PgR+	Systemic therapy?	Tissue	COX2 Ab	Score of COX2	Definition COX2 positive	COX2 positive % (overexpr)	Pathological correlates	Clinical correlates
Zerkowski 2007[22]	669	9 years (0-53)	Patients treated in 1953-1983 from a single centre.	1953-1983	70%	55%	Full treatment information unavailable. Most local therapy +/- HT. CT in 10%	TMA	Cayman Chemical Co, Ann Arbor, MI; 1:250 ON incl polyclona AB for AQUA	Using automatic analysis by AQUA	By AQUA. Fluorescent: 19.3	(44%)	Low ER/PgR High grade	Multivariable high COX2 worse OS (RR 1.66, 95%CI 1.12-2.16; p=0.01).
Van Nes (current study)	369 (677)	19 years	Cohort, single institute	1985-1994	64%	60%	0%	TMA	Cayman Chemical Co, Ann Arbor, MI; 1:100	Weighted histoscore: exact percentage * intensity (0-3)	Above median histoscore	162 (51%)	Age Larger tumour size/ stage Poor differentiation ER-, PgR-, HER2+	Reduced DFS and OS, not in multivariable

CT chemotherapy; *DDFS* distant disease-free relapse; *DFS* disease-free survival; *ER* oestrogen receptor; *HT* hormone therapy; *NR* not reported; *PgR* progesterone receptor; *OS* overall survival; *RR* relative risk; *TMA* tissue micro array.

positive patients stratified by treatment with endocrine therapy.

In conclusion, in patients who did not receive systemic treatment, increased COX2 expression was not independently prognostic for an increased risk of relapse and death from breast cancer after correction for current clinicopathological markers. COX2 expression was prognostic in patients with hormone receptor positive breast cancer who did not receive endocrine therapy. Studies measuring the impact of COX2 expression in the context of systemic therapies should be reinterpreted in light of this finding and a randomised controlled trial is needed to prove our results.

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