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The role of cathepsin D and PAI-1 in primary invasive breast cancer as prognosticators and predictors of treatment benefit with adjuvant tamoxifen

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Abstract

In the last few years there has been an increased interest in treatment predictive factors in breast cancer patients. The aim of the study was to analyse the role of cathepsin D and plasminogen activator inhibitor-1 (PAI-1) expression as independent prognosticators and to assess their predictive value with respect to tamoxifen treatment. This study comprises 1851 patients with primary breast cancer diagnosed during 1988–1992. Their median age was 62 years (range: 24–91). The end-point was distant disease recurrence-free interval. Adjuvant treatment with tamoxifen was given to 1136 patients (61%). The median follow-up time was 59 months (range: 39–88). Cathepsin D content was shown to be a significant independent prognosticator in multivariate Cox analysis ($P = 0.02$). The optimal cut-off level was 10 fmol/mg protein, other cut-off levels did not improve the results. The level of cathepsin D also appeared to predict the benefit of tamoxifen among oestrogen receptor (ER)-positive patients although this result did not reach statistical significance ($P = 0.09$). In a multivariate Cox analysis including 497 patients PAI-1 content was shown to be a significant independent prognosticator ($P = 0.003$) but did not appear to predict the benefit of tamoxifen treatment. The optimal cut-off level appeared to be 3 ng/mg protein, which was close to the median value 2.5 ng/mg (range: 0–51). We conclude that cathepsin D is a significant independent prognosticator and may possibly also predict the benefit of tamoxifen amongst ER-positive patients. PAI-1 was also found to be a strong independent prognosticator but no treatment interaction with adjuvant tamoxifen was found.

Keywords: Cathepsin D; PAI-1; Prognosticator; Predictor; Tamoxifen; Breast; Cancer

1. Introduction

In the last few years there has been an increased interest in treatment predictive factors in breast cancer patients. One approach in finding such new markers is to study the way that cancer cells invade surrounding tissue and metastasise. This ability is due to their proteolytic activity [1,2]. Several proteolytic enzymes, regulated by a balance of proteases and their inhibitors, are involved and act by binding to cell surface receptors. Many of these proteases have several functions. Cathepsin D, stimulated by oestrogen, is an acidic lysosomal protease acting directly on the cell membrane, as well as indirectly by activating cathepsin B, which in turn can regulate extracellular collagenases. Plasminogen activator inhibitor-1 (PAI-1) is a serpin protease inhibitor that blocks the activity of urokinase-type plasminogen activator (uPA), which starts a cascade of events leading to the degradation of collagen and of the basement membrane proteins.

Cathepsin D content as a prognosticator in breast cancer patients has been extensively studied [3–9] but the results have been conflicting. The predictive value of cathepsin D for tamoxifen treatment has previously been described only in one of these studies, which was based on a relatively low number of patients [8].

The prognostic impact of PAI-1 content has been demonstrated in multivariate analysis in breast cancer patients [8–11] as well as in the subgroup of node-negative patients [5–7]. In these studies adjuvant tamoxifen was either not given, or only given to few patients. Some results suggest that the strength of PAI-1 content as a prognosticator could vary with time [12].

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The primary objective of this retrospective study which comprises 1851 patients of which 1136 received adjuvant tamoxifen treatment was to analyse the role of cathepsin D and PAI-1 content as independent prognosticators and to assess the predictive value of cathepsin D and PAI-1 content with respect to tamoxifen treatment.

2. Patients and methods

2.1. Patients

This study comprises consecutive 1851 patients with primary breast cancer diagnosed in the Stockholm and Gotland region during 1988–1992. Since there is only one laboratory in this region performing hormone receptor analysis, specimens from 70–80% of all breast cancers in the region were sent to this laboratory. One common reason why tumour specimens were not sent is that the diagnosis was unknown at the time of surgery. Another common reason was screening detected, small cancers provided material that was sufficient only for histopathological examination.

Patients eligible for this study were those with no previous history of cancer, primary radically excised tumours, no sign of distant metastases and sufficient remaining cytosol after hormone receptor analysis to determine cathepsin D content. Their median age was 62 years (range: 24–91). During 1991–1992 PAI-1 content was also determined in the same cytosol as cathepsin D content. Both cathepsin D and PAI-1 content were measured in approximately 30% of all patients. Clinical characteristics are shown in Table 1.

Table 1
Patient characteristics

	Characteristic	*n* (%)
Patients	Total *n*	1851 (100)
	Age (years)	
	< 50	420 (23)
	⩾ 50	1431 (77)
	Median (range)	62 (24–91)
Tumours	Size (pT) (mm)	
	< 20	1067 (58)
	20–50	707 (38)
	⩾ 50	49 (3)
	Data unavailable	28 (1)
	Nodal status (pN)	
	pN0	1182 (64)
	pN1–3	398 (22)
	PN > 3	257 (14)
	Data unavailable	14 (1)
	ER (fmol/μg DNA)	
	< 0.05	358 (19)
	⩾ 0.05	1429 (77)
	Data unavailable	64 (3)
	Median (range)	0.9 (0–28)
	Cathepsin D (fmol/mg protein)	
	< 10	433 (23)
	⩾ 10	1418 (77)
	Median (range)	16 (0–432)
	PAI-1 (ng/mg protein)	
	< 3	316 (17)
	⩾ 3	230 (12)
	Data unavailable	1305 (71)
	Median (range)	2.5 (0–51)
Treatment	Tamoxifen	
	Yes	1136 (61)
	No	635 (34)
	Data unavailable	80 (4)
	Chemotherapy	
	Yes	198 (11)
	No	1567 (85)
	Data unavailable	86 (5)

ER, oestrogen receptor; PAI-1, plasminogen activator inhibitor-1.

2.2. Treatment

Primary treatment was either modified radical mastectomy or breast conserving surgery including lymph node dissection. Patients with positive lymph nodes were treated with radiation therapy of 46 Gy given over a period of 4.5 weeks to the chest wall and regional lymph nodes. Premenopausal women with positive lymph nodes also received 6 months of CMF chemotherapy (cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2 and 5-fluorouracil 600 mg/m^2 intravenously (i.v.) on days 1 and 8, every 4 weeks). Tamoxifen 40 mg orally for 2 or 5 years was given according to the regional treatment practice guidelines for breast cancer patients. All patients who underwent a partial mastectomy received radiation therapy of 50 Gy over a period of 5 weeks to the remaining breast tissue.

2.3. Analysis of hormone receptor content, cathepsin D content and PAI-1 content

Cytosols were prepared according to the method used for oestrogen receptor (ER) [13]. Protein determination was obtained by the Bradford method [14]. The antigen levels of cathepsin D were determined in diluted cytosol fraction by enzyme-linked immunosorbant assay (ELISA) (Cis bio international, Gif-sur-Yvette, France). The PAI-1 antigen was determined in diluted cytosol fraction by ELISA (PAI-1 ELISA Kit # 821, American Diagnostica, Greenwich, CT, USA).

2.4. Distant disease recurrence

Distant disease recurrence was defined either as a cytologically verified lymph node or soft tissue recurrence outside the breast/chest wall, ipsilateral axilla or supraclavicular fossa or radiographical evidence of lung, liver or bone metastases. The endpoint was distant disease recurrence-free interval (DRFI).

2.5. Clinical follow-up

Follow-up visits were scheduled once every 3 months during the first 2 years, every 6 months during the 2–5 year period and yearly thereafter. These visits routinely included a physical examination and a yearly mammogram. Chest X-rays, bone scans, blood tests, etc. were only obtained when signs or symptoms indicated a possible relapse.

Patients were followed to the date of distant disease recurrence or to the closing date of 31 December, 1995. Deaths that were unrelated to breast cancer were treated as censored observations in the survival analyses. The follow-up time ranged from 39 to 88 months with a median of 59 months.

2.6. Statistical methods

The association between cathepsin D content and PAI-1 content with various clinical parameters was examined using the Spearman rank correlation, or with dichotomised parameters with the Chi-square test of independence. Clinical parameters were dichotomised using the following groupings: age, <50 versus $\geqslant 50$ years; lymph node involvement, pN0 versus pN+; tumour size, <20 versus $\geqslant 20$ mm; ER content, <0.05 versus $\geqslant 0.05$ fmol; progesterone receptor content, <0.05 versus $\geqslant 0.05$ fmol; adjuvant tamoxifen, treated versus not treated.

Univariate survival curves were estimated using the method of Kaplan and Meier and differences between curves were tested using the logrank test. Owing to missing or unknown data, 1671 patients were available for the multivariate analysis of cathepsin D and 491 patients in the multivariate analysis of PAI-1 content. Cox's proportional hazards regression model was used to assess the independent prognostic contribution of clinical parameters after adjusting for other factors. Wald Chi-square statistics were used to estimate the significance of each factor. All reported P values refer to two-sided test of significance. Prognostic effects were expressed as hazard rate ratios supplemented with 95% confidence intervals (CI). Test of interaction was performed by including product terms into the multivariate models.

3. Results

3.1. Cut-off levels for cathepsin D and PAI-1 content

Cutpoints analyses were done to find the best separates into two groups for cathepsin D and PAI-1 content. These analyses suggested the cut-off level <10 versus $\geqslant 10$ fmol for cathepsin D content as the best discriminator. Discrimination was almost as good at the median level (often used in other studies) and at a higher level. Different cut-off levels were also tested for PAI-1 content and the optimal level was 3 ng/mg — relatively close to the 2.5 ng/mg which was the median level used in other studies.

3.2. Correlations

Using dichotomised variables the correlation between cathepsin D content, PAI-1 content and other clinical markers is shown in Table 2. Both lymph node involvement and tumour size showed significant positive correlation to cathepsin D content. ER content and cathepsin D content showed a strong significant positive correlation to PAI-1 content. A weaker but also significant correlation to PAI-1 content was found with progesterone receptor content. Lymph node involvement, as well as tumour size were, in this respect, also significant factors.

Using Spearman rank correlation, cathepsin D content also significantly correlated to lymph node involvement as well as tumour size. The strongest correlations with PAI-1 content were with cathepsin D, tumour size and ER content. A weaker (but significant) correlation was also found with lymph node involvement and progesterone receptor content.

3.3. Cathepsin D content as prognosticator for DRFI and predictor in 1671 patients of whom 1136 were treated with tamoxifen

In univariate analysis, cathepsin D content was a strong ($P<0.001$) significant prognosticator. In multivariate analysis, cathepsin D was a weaker ($P=0.020$), but still significant, prognosticator of DRFI independent of lymph node status (Table 3).

In the subgroup analysis of 1072 node-negative patients from the multivariate analysis the distant recurrence rate was 2.7% in patients with low cathepsin D compared with 6.8% in patients with high cathepsin D. This difference was statistically significant ($P=0.031$). The corresponding Kaplan–Meier curves based on all 1182 node-negative patients with cathepsin values, revealed a statistically significant difference ($P=0.01$) and are shown in Fig. 1.

In the subgroup of 599 node-positive patients from the multivariate analysis the distant recurrence rate was 16.7% in patients with low cathepsin D content compared with 21.9% in patients with high cathepsin D content. This difference was not significant. The corresponding Kaplan–Meier curves based on all 655 node-positive patients with cathepsin values did not show any significant difference (Fig. 1).

The benefit of adjuvant tamoxifen treatment was studied in four subgroups by dichotomised ER content and cathepsin D content (Table 4). The level of cathepsin D appeared to predict the benefit with adjuvant tamoxifen

treatment in ER-positive patients although this result did not reach statistical significance ($P = 0.09$). The interaction between cathepsin D level and treatment amongst ER-positive patients was $P = 0.17$. The level of cathepsin D seemingly could not predict treatment benefit with tamoxifen in ER-negative patients.

Table 2
Associations between cathepsin D and PAI-1 content and patient characteristics

	Cathepsin D (fmol/mg protein)			PAI-1 (ng/mg protein)[a]		
Variable	< 10 *n* (%)	⩾10 *n* (%)	*P* value	< 3 *n* (%)	⩾3 *n* (%)	*P* value
Age (years)						
< 50	84 (19)	336 (24)		63 (20)	51 (22)	
⩾50	349 (81)	1082 (76)	0.062	253 (80)	179 (78)	0.525
Nodal status						
N0	332 (77)	850 (60)		217 (69)	131 (57)	
N+	96 (22)	559 (39)	< 0.001	99 (31)	98 (43)	0.006
Unknown	5 (1)	9 (1)			1 (< 1)	
Tumour size (mm)						
< 20	285 (66)	782 (55)		195 (62)	119 (52)	
⩾20	134 (31)	622 (44)	< 0.001	113 (36)	107 (47)	0.013
Unknown	14 (3)	14 (4)		8 (3)	4 (2)	
ER (fmol/mg DNA)						
< 0.05	88 (20)	270 (19)		42 (13)	63 (27)	
⩾0.05	329 (76)	1100 (78)	0.533	269 (85)	165 (72)	< 0.001
Unknown	16 (4)	48 (3)		5 (2)	2 (1)	
PgR (fmol/mg protein)						
< 0.05	134 (31)	417 (29)		100 (32)	102 (44)	
⩾0.05	195 (45)	674 (48)	0.413	200 (63)	117 (51)	0.002
Unknown	104 (24)	327 (23)		16 (5)	11 (5)	
Cathepsin D (fmol/mg protein)						
< 10	–	–		95 (30)	33 (14)	
⩾10	–	–	–	221 (70)	197 (86)	< 0.001

PgR, progesterone receptor; ER, oestrogen receptor.
[a] Not all patients were evaluable.

Table 3
Results of univariate and multivariate analysis on distant recurrence-free interval (DRFI)

			Univariate analysis		Multivariate analysis	
Covariate	No. of events *n* (%)	No. of patients *n* (%)	Hazard ratio (95% confidence interval)	*P* value	Hazard ratio (95% confidence interval)	*P* value
Age (years)						
< 50	56 (30)	363 (22)	1.0		1.0	
⩾50	132 (70)	1308 (78)	0.6 (0.5–0.9)	0.004	0.8 (0.6–1.1)	0.212
Nodal status						
pN0	61 (32)	1072 (64)	1.0		1.0	
pN+	127 (68)	599 (36)	4.2 (3.1–5.6)	< 0.001	3.1 (2.2–4.2)	< 0.001
Tumour size (mm)						
< 20	60 (32)	992 (59)	1.0		1.0	
⩾20	128 (68)	679 (41)	3.3 (2.5–4.6)	< 0.001	2.2 (1.6–3.1)	< 0.001
ER (fmol/mg DNA)						
< 0.05	56 (30)	328 (20)	1.0		1.0	
⩾0.05	132 (70)	1343 (80)	0.5 (0.4–0.7)	< 0.001	0.6 (0.5–0.9)	0.004
Cathepsin D (fmol/mg protein)						
< 10	21 (11)	375 (22)	1.0		1.0	
⩾10	167 (89)	1296 (78)	2.4 (1.5–3.7)	< 0.001	1.7 (1.1–2.7)	0.020
Total	188 (100)	1671 (100)				

ER, oestrogen receptor.

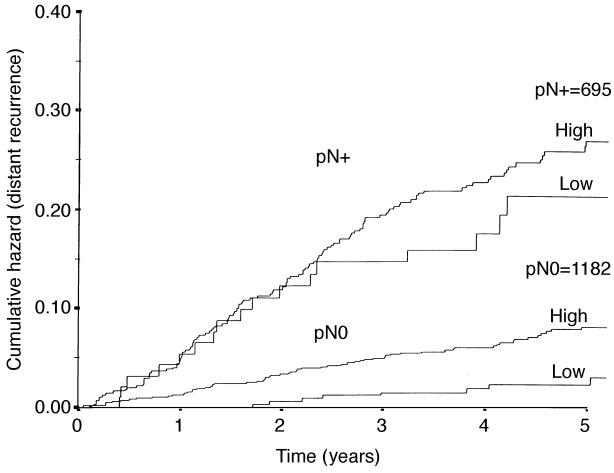

Fig. 1. The risk for a distant recurrence over time according to lymph node status and level of cathepsin D. Low, <10 fmol/mg protein; high, $\geqslant 10$ fmol/mg protein. Number of patients: pN+ (High) = 559, pN+ (Low) = 96, pN0 (High) = 850, pN0 (Low) = 332.

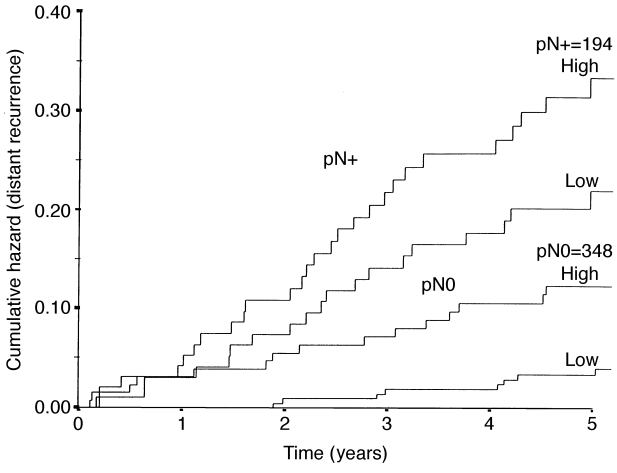

Fig. 2. The risk for a distant recurrence over time according to lymph node status and level of PAI-1. Low, <3 ng/mg protein; High, $\geqslant 3$ ng/mg protein. Number of patients: pN+ (High) = 98, pN+ (Low) = 99, pN0 (High) = 131, pN0 (Low) = 217.

3.4. PAI-1 content as prognosticator for DRFI and predictor in 497 patients of whom 288 received adjuvant treatment with tamoxifen

Results of univariate and multivariate Cox analysis of the effect of different covariates on DRFI are shown in Table 5. In univariate Cox analysis PAI-1 content was a strong significant prognosticator ($P < 0.001$) as well as lymph node involvement. In multivariate Cox analysis, lymph node involvement was the only strong significant prognosticator. Although PAI-1 was weaker in this respect, it was still an independent significant prognosticator ($P = 0.003$).

In the subgroup analysis of 315 node-negative patients from the multivariate analysis the distant recurrence rate was 3.0% in patients with low PAI-1 compared with 12.1% in patients with high PAI-1. This difference was statistically significant ($P = 0.004$). The corresponding Kaplan–Meier curves based on all 348 node-negative patients with PAI-1 values, revealed a statistically significant difference ($P = 0.002$) and are shown in Fig. 2.

In the subgroup of 182 node-positive patients from the multivariate analysis the distant recurrence rate was 17.8% in patients with low PAI-1 content compared with 28.3% in patients with high PAI-1 content. This difference was not significant. The corresponding Kaplan–Meier curves based on all 197 node-positive patients with PAI-1 values did not show any significant difference (Fig. 2). Test of the interaction between nodal status and PAI-1 content revealed a P value of 0.12.

The benefit of adjuvant tamoxifen treatment was studied in four subgroups by dichotomised ER content and PAI-1 content (Table 6). In this investigation the level of PAI-1 content did not appear to predict the benefit of adjuvant tamoxifen.

4. Discussion

Our results showed that the level of cathepsin D content, determined on cytosols using an immunoradiometric assay, is a significant prognosticator of DRFI in a multivariate analysis of 1671 patients. This

Table 4
Effects of adjuvant tamoxifen on distant recurrence-free interval by ER and cathepsin D level

		(Low level) Cathepsin D <10 fmol/mg		(High level) Cathepsin D $\geqslant 10$ fmol/mg	
	Adjuvant tamoxifen	n events/ n patients	Hazard ratio (95% CI)	n events/ n patients	Hazard ratio (95% CI)
Low ER <0.05 fmol/μg DNA	No	4/38	1.0	24/140	1.0
	Yes	5/40	1.3 (0.4–5.2)	23/110	1.3 (0.7–2.3)
High ER $\geqslant 0.05$ fmol/μg DNA	No	2/83	1.0	50/339	1.0
	Yes	10/214	2.4 (0.5–12.3)	70/707	0.7 (0.5–1.1)

ER, oestrogen receptor; CI, confidence interval. Adjusted for age, tumour size and nodal status.

Table 5
Results of univariate and multivariate analysis on distant recurrence-free interval in 497 patients with known PAI-1 values

			Univariate		Multivariate	
Covariate	*n* Events (%)	*n* Patients (%)	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)						
< 50	20 (32)	103 (21)	1.0		1.0	
⩾50	42 (68)	394 (79)	0.5 (0.3–0.9)	0.016	0.7 (0.4–1.2)	0.186
Nodal status						
pN0	20 (32)	315 (63)	1.0		1.0	
pN+	42 (68)	182 (37)	4.0 (2.4–6.9)	< 0.001	3.1 (1.7–5.4)	< 0.001
Tumour size (mm)						
< 20	24 (39)	295 (59)	1.0		1.0	
⩾20	38 (61)	202 (41)	2.4 (1.5–4.1)	0.001	1.6 (0.9–2.7)	0.099
ER (fmol/μg DNA)						
< 0.05	14 (23)	93 (19)	1.0		1.0	
⩾0.05	48 (77)	404 (81)	0.7 (0.4–1.3)	0.299	1.0 (0.5–1.8)	0.887
PAI-1 (ng/mg protein)						
< 3	22 (35)	289 (58)	1.0		1.0	
⩾3	40 (65)	208 (42)	2.7 (1.6–4.6)	< 0.001	2.3 (1.3–3.9)	0.003
Total	62 (100)	497 (100)				

ER, oestrogen receptor; CI, confidence interval. Adding cathepsin D (two levels) and/or adjuvant treatment to the above model did not show any significant difference.

was independent of lymph node status, tumour size and ER content. Our results are in line with smaller previous studies [3,4] even though the endpoints in these studies were different.

In the subgroup analysis of 1072 node-negative patients this statistically significant difference was maintained ($P = 0.031$) but not in the 599 node-positive patients ($P = 0.227$). This result is also in line with previous smaller studies in node-negative patients [5,6] even though the studied endpoints were different.

The level of cathepsin D appeared to predict the benefit with tamoxifen in ER-positive patients although this result did not reach significance ($P = 0.09$). A significant result of cathepsin D as a predictor in lymph node positive patients has been previously suggested [8]. The observed correlation between cathepsin D content and the benefit with adjuvant tamoxifen could potentially be of predictive value but today there is insufficient documentation with regard to this putative correlation to make it reasonable to withhold adjuvant tamoxifen in ER-positive patients on the basis of a cathepsin D assay.

In the multivariate analysis of 497 patients the level of PAI-1 content was shown to be the only significant prognosticator of DRFI independent of lymph node involvement. Cathepsin D level did not, in this subgroup of patients, show any significance as a prognosticator. That cathepsin D loses its independent significance as prognosticator when PAI-1 content is introduced in the multivariate analysis has been demonstrated previously [9,10].

One problem in making comparisons with other published studies is that different methods are used in measuring cathepsin D and PAI-1 content. Cut-off levels also differ between investigators. Methods used are: monoclonal antibodies in tissue sections by immuno-

Table 6
Effects of adjuvant tamoxifen on distant recurrence-free interval by ER and PAI-1 level

		(Low level) PAI-1 < 3 ng/mg protein		(High level) PAI-1 ⩾ 3 ng/mg protein	
	Adjuvant tamoxifen	*n* events/ *n* patients	Hazard ratio (95% CI)	*n* events/ *n* patients	Hazard ratio (95% CI)
Low ER < 0.05 fmol	No	0/23	–	8/36	1.0
	Yes	2/14		4/20	0.7 (0.2–2.7)
High ER ⩾ 0.05 fmol	No	12/103	1.0	12/47	1.0
	Yes	8/149	0.7 (0.3–1.7)	16/105	0.6 (0.3–1.2)

ER, oestrogen receptor; CI, confidence interval. Adjusted for age, tumour size and nodal status.

histochemistry and in tissue extracts by Western blotting, ELISA and IRMA assays. In our study the ELISA method was used on cytosols saved after hormone receptor analysis. Most of the studies have dichotomised the values according to the median value, which enables comparisons of high levels with low levels independently of which methods of measurement are used.

Another problem when comparing with other studies is the chosen endpoint. In this study DRFI and not overall survival was chosen in order to minimise the effect of intercurrent deaths and the effects of treatment due to disease recurrence. Disease-free survival was not used owing to possible interaction with surgical methods and postoperative radiotherapy.

We conclude that cathepsin D is a significant independent prognosticator and may possibly also predict the benefit of tamoxifen amongst ER-positive patients. PAI-1 was also found to be a strong independent prognosticator, whereas no treatment interaction with adjuvant tamoxifen was found.

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