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CLINICAL TRIAL

The prognostic value of Stathmin-1, S100A2, and SYK proteins in ER-positive primary breast cancer patients treated with adjuvant tamoxifen monotherapy: an immunohistochemical study

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Abstract
Introduction We recently found that DNA methylation of *S100A2*, *spleen tyrosine kinase* (*SYK*), and *Stathmin-1* (*STMN1*) correlates with response to tamoxifen therapy in metastatic breast cancer. In this retrospective study, we investigated immunohistochemically whether these three markers are predictors of relapse in early breast cancer (EBC) patients treated with adjuvant tamoxifen alone.
Methods Immunohistochemical staining was performed for S100A2, SYK and STMN1 on a tissue microarray containing ER-positive invasive breast carcinomas from a study cohort of 215 operable breast cancer patients, who underwent radical local therapy and who were treated with adjuvant tamoxifen monotherapy. Cox regression was used to correlate staining intensity of the three markers with main endpoints in our study; disease-free survival (DFS), and disease-specific survival (DSS).
Results In univariate analysis, only *STMN1* staining intensity strongly correlated with DFS ($P = 0.014$) and DSS ($P = 0.002$). In the groups of low and high *STMN1* intensity, DFS was 84% and 63%, and DSS was 89% and 70%. *STMN1* retained its prognostic value for DFS ($P = 0.002$) and DSS (<0.001) in the multivariate model together with lymph node status. We found also a trend to better DFS in patients with low STMN1 intensity in both lymph node-positive ($P = 0.001$) and -negative patients ($P = 0.065$). As the tumour cells did not express S100A2 (except in one case) the potential prognostic value of this marker was not evaluated.
Conclusions Staining intensity of *STMN1*, but not SYK, predicted outcome in our collective of ER- positive tamoxifen treated EBC patients.

Keywords ER · Immunohistochemistry · Primary breast carcinoma · S100A2 · Stathmin-1 · SYK · Tamoxifen

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Introduction

Endocrine therapy is the most common treatment for patients with steroid hormone receptor positive breast cancer, with objective responses seen in about 50% of the patients with recurrent disease. In the adjuvant setting, ER-positive lymph node negative breast cancer patients have a relatively good prognosis and benefit from endocrine therapy either alone or in combination with chemotherapy [1]. A key clinical question in the management of patients with breast cancer concerns accurate assessment of prognosis that supports the choice of an optimal (adjuvant) treatment for each individual patient. With the

conventional drug regimens and the advent of novel therapy approaches targeting specific biological pathways, the dilemma of optimal treatment of primary and recurrent breast cancer is becoming even more complex. To enable and allow tailored therapy concepts that take the individual tumour biology into account, knowledge about novel informative tumour-associated factors is urgently needed.

Aberrant DNA methylation of cytosine phosphoguanine dinucleotides (CpG) within gene regulatory regions leading to gene silencing is a common and early event in cancer and the study of this epigenetic phenomenon is rapidly advancing in the field of breast cancer biology [2–4]. Recent studies by us [5–7] and others [8] have suggested that the methylation status of certain genes may be related to tamoxifen resistance and survival in breast cancer. Analyzing the promoter region of 117 genes, using a microarray-based technology, we found the DNA methylation status of 10 genes to be significantly associated with clinical outcome of tamoxifen therapy in ER-positive recurrent breast cancer [5]. Of the significant genes, hypermethylation of STMN1 and stratifin (SFN) was associated with disease progression after first-line tamoxifen therapy, while the eight remaining genes displayed hypermethylation in tumours that showed remission of the disease after therapy. For the most significant gene, phosphoserine aminotransferase (PSAT), we were able to show that DNA methylation was negatively associated with its messenger RNA expression and that patients with lower PSAT mRNA levels had a longer progression-free survival after first-line tamoxifen therapy suggesting that also the levels of PSAT are predictive for tamoxifen resistance in metastatic breast cancer [5].

One of the clinically important questions in breast cancer is which ER-positive patients do well on adjuvant endocrine therapy with tamoxifen alone and for which of these patients tamoxifen alone must be considered suboptimal. Therefore, we explored which markers, identified by methylation analysis in the metastatic setting, might predict disease recurrence and survival in ER-positive, tamoxifen-treated patients in the adjuvant setting. From a practical point of view, the most rational way of assessing the candidate markers would be to analyse their corresponding proteins by IHC on paraffin-embedded tumour tissue. If successful, this approach would serve as a way to stratify patients by relatively simple and cheap IHC staining of paraffin-embedded tumour tissue. The aim was to analyse all markers identified in the earlier study, however, from only three (*STMN1*, *S100A2* and *SYK)* suitable antibodies were available at the moment we initiated the study. *STMN1* controls cell division by destabilizing microtubules [10], *S100A2* is a calcium binding protein that is involved in cell survival [11], and *SYK* is a tyrosine kinase involved in cell signalling and is considered a tumour suppressor in breast cancer [12]. Thus, in this study we assessed by IHC on TMA of paraffin-embedded specimens the potential of these three methylation markers to predict disease recurrence and survival in ER-positive primary breast cancer patients treated with adjuvant tamoxifen monotherapy. This retrospective study adheres to REMARK criteria [13].

Materials and methods

Patient and tumour characteristics

The study cohort consisted of 215 consecutive operable female breast cancer patients who underwent radical local therapy at the Institute of Oncology, Ljubljana, Slovenia, in the period between 1994 and 1999. All patients had ER-positive invasive breast carcinoma and were treated with adjuvant hormonal therapy, tamoxifen, alone. No patient received neoadjuvant or adjuvant chemotherapy. Patients with bilateral breast cancer were excluded from the study. All conventional clinico-pathological data were evaluated. The median age of the patients at the time of diagnosis was 67.9 years (range, 36–83 years), 207 (96.3%) were postmenopausal and only 8 patients (3.7%) were premenopausal at diagnosis. Lymph node status was negative in 109 (50.7%) patients, positive in 99 (46.0%) patients, and was unknown in seven (3.3%) patients. The median follow-up time of the patients was 84 months (range, 5–133 months). Fifty-four patients (25.1%) experienced recurrence, and 59 (27.4%) died; of the latter 44 deaths were due to breast cancer and 15 deaths were due to other causes. Tumour characteristics: pathological tumour size, tumour histology, tumour grade, and steroid hormone receptor status were determined routinely according to the standard procedure valid at Institute of Oncology Ljubljana at that time and are listed in Table 1. Hormone receptors were determined biochemically in cytosolic extract by the dextran-coated charcoal method, with the values of ER and PR equal to or greater than 10 fmol/mg protein considered positive. The malignancy grade was scored by the method of Bloom–Richardson–Elston [14].

Generation of tissue microarrays (TMA)

The original haematoxylin and eosin (H and E) slides and paraffin-embedded tumour tissues were retrieved from the archives of the Department of Pathology, Institute of Oncology, Ljubljana. H and E stained slides of tumour tissue were reviewed to identify representative tumour regions without necrosis or carcinoma in situ. Three tissue cylinders with a diameter of 0.6 mm were obtained for each corresponding tumour block and arrayed into a

Table 1 Association of Stathmin-1 and SYK with patient and tumour characteristics

Characteristic	N (%)	Stathmin expression		*P*	SYK expression[c]			*P*
		Low N (%)	High N (%)		Weak N (%)	Moderate N (%)	Strong N (%)	
Pathological tumour size				0.702[a]				0.702[a]
pT1 (0–20 mm)	79 (36.7%)	49 (62%)	30 (38%)		9 (11%)	44 (56%)	26 (33%)	
pT2 (21–50 mm)	119 (55.4%)	68 (57%)	51 (43%)		20 (17%)	48 (41%)	50 (42%)	
pT3 (>50 mm)	17 (7.9%)	9 (53%)	8 (47%)		1 (6%)	9 (53%)	7 (41%)	
Histological tumour type				0.346[b]				0.344[b]
Ductal invasive	162 (75.3%)	92 (57%)	70 (43%)		21 (13%)	75 (47%)	65 (40%)	
Other invasive	53 (24.7%)	34 (64%)	19 (36%)		9 (17%)	26 (49%)	18 (34%)	
Malignancy grade:				0.010[a]				0.697[a]
G1	24 (11.2%)	20 (83%)	4 (17%)		5 (21%)	11 (46%)	8 (33%)	
G2	105 (48.8%)	64 (61%)	41 (39%)		15 (14%)	47 (45%)	42 (41%)	
G3	85 (39.5%)	42 (49%)	43 (51%)		10 (12%)	43 (50%)	32 (38%)	
Unknown	1 (0.5%)							
Lymph node status				0.051[b]				0.571[b]
Negative	109 (50.7%)	57 (52%)	52 (48%)		19 (18%)	42 (39%)	47 (43%)	
Positive	99 (46.0%)	65 (66%)	34 (34%)		10 (10%)	56 (57%)	33 (33%)	
Unknown	7 (3.3%)							
Hormone receptor status				0.572[b]				0.177[b]
PR+	166 (77.2%)	99 (60%)	67 (40%)		23 (14%)	73 (44%)	69 (42%)	
PR−	49 (22.8%)	27 (55%)	22 (45%)		7 (14%)	28 (57%)	14 (29%)	

[a] Kruskal–Wallis *H*-test, including a test for trend if appropriate

[b] Mann–Whitney *U*-test

[c] One case is missing due to missing SYK data (214 cases total)

recipient new paraffin block using the tissue chip microarrayer (Beecher Instruments, Silver Spring, MD). Four recipient tissue blocks were constructed. They were subsequently cut into 2–3 μm sections and fixed on silanized glass slides (Knittel Glaeser, Braunschweig, Germany) to support adhesion of the tissue samples for subsequent immunohistochemical staining.

Immunohistochemistry

The monoclonal antibodies used were anti-S100A2 antibody S100A2 Ab-7 (Dako) at a dilution of 1:20, anti-SYK antibody pAb RB-972-PO (Lab Vision, Fremont, CA, USA) at a dilution of 1:300, and anti-Stathmin-1 antibody pAb 1b11296 (Abcam, Cambridge, UK) at a dilution of 1:1200. According to the datasheets, we performed IHC staining using an automated immunostainer (Autostainer Lab Vision) for S100A2, and manually for SYK and Stathmin-1 antibodies. Antigen retrieval was done in citrate buffer (pH 6.0; Dako, Glostrup, Denmark) by incubation for 15 min at 98°C in a water bath. For visualization, we applied a standard DAB Envision (Dako) technique for all three antibodies. Control slides of tonsil and normal breast tissue were reviewed and deemed adequate in all cases.

Tumour heterogeneity in expression was assessed by a single senior pathologist (RG) using a semi quantitative method. As the staining was homogenous with respect to distribution, only intensity was scored. The staining intensity was graded as 0-no staining, 1-weak, 2-moderate, and 3-strong staining.

Statistical analysis

The endpoints in this study were disease-free survival (DFS), and disease-specific survival (DSS). DFS was calculated from the date of the start of primary therapy to the date of breast cancer recurrence (local, loco-regional or distant), the date of death from any cause, or the date of last follow-up; censored observations correspond to patients alive and without evidence of disease recurrence at the time of last follow-up. DSS was calculated from the date of the start of primary therapy to the date of death due to breast cancer; censored observations correspond to patients that are either alive or died of other causes. DFS and DSS

as a function of the markers studied were estimated by the Kaplan-Meier method and the log-rank test was used to test for differences. The Cox uni- and multivariate hazards models were used to calculate the hazard ratios (HR) and their 95% confidence intervals (95% CI) in the analysis of DFS and DSS. The strength of the associations between the marker expression levels and patient and tumour characteristics were tested with the Mann–Whitney *U*-test or the Kruskal–Wallis *H*-test. Computations were done with the use of the SPSS 12 statistical package. All reported p values are two tailed.

Results

Immunohistochemistry

The *STMN1* reactive antibody shows diffuse and strong cytoplasmic staining in both the invasive and in situ component of most carcinoma cells, while stromal cells are clearly negative as illustrated in Fig. 1 representing one case of invasive ductal carcinoma. In normal breast, myoepithelial and luminal cells show variable staining intensity that is localised mostly in myoepithelial and epithelial cells of lobules. Some endothelial cells show cytoplasmic as well as nuclear staining (not shown).

Staining for *SYK* resulted in a strong but diffuse cytoplasmic reaction in all epithelial tumour cells within invasive and non-invasive components. In the majority of cases stromal cells were positive for this marker. One representative case of invasive ductal carcinoma stained with a reactive antibody for *SYK* is presented in Fig. 2. In the normal breast tissue, ductal epithelial and myoepithelial as well as lymphoid and endothelial cells show no or distinct cytoplasmic staining.

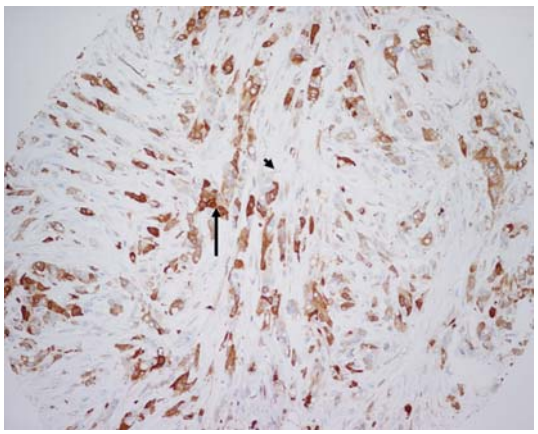


Fig. 1 Stathmin-1 protein expression in invasive ductal breast carcinoma by immunostaining. Most of the cells (*arrow*) show intense cytoplasmic staining in contrast to unreactive stromal cells (*arrowheads*)

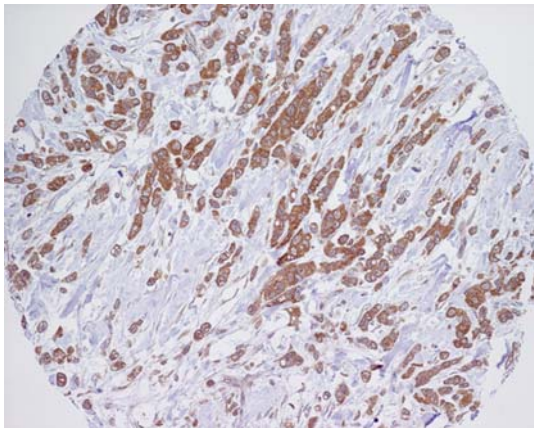


Fig. 2 SYK protein in invasive ductal breast carcinoma determined by immunostaining. Tumour cells show intense diffuse cytoplasmic staining (*arrow*); the adjacent stromal cells are negative (*arrowheads*)

STMN1 and *SYK* were found to be consistently expressed in more than 66% of tumour cells in a large majority of tumours (94% and 100%, respectively). However, the intensity of staining for both antigens varied considerably. Therefore, we grouped our patients for both *SYK* and *STMN1* according to staining intensity and not according to the percentage of positive cells (staining proportion) and consequently did not use H-score (a combination of both) as our primary variable of interest. Weak *SYK* staining was found in 30, moderate *SYK* staining in 101 and strong *SYK* staining in 83 cases; weak *STMN1* staining was found in 13, moderate *STMN1* staining in 113 and strong *STMN1* staining in 89 cases. Since there were only 13 cases of weak *STMN1* staining we decided to merge tumours with weak and moderate staining intensity into one "low expression" group, while tumours with strong STMN1 staining were defined as a "high expression" group.

The antibody to *S100A2* stained predominantly the nucleus and cytoplasm of the myoepithelial layer of normal mammary ducts (not shown) and of ductal carcinoma *in situ* (Fig. 3a). Occasionally, *S100A2* staining of the nucleus and cytoplasm of the luminal epithelial layer was also observed in normal breast lobules. Similar expression of *S100A2* was also exhibited in the cross-striated muscular fibres surrounded with infiltrating carcinoma cells. In our TMA cohort, cells of only one case of carcinoma showed staining for *S100A2*. This metaplastic carcinoma, the only one in the cohort, with spindle cell and chondroid differentiation displayed mild focal *S100A2* staining in only some spindle cells (Fig. 3b). Since the staining of *S100A2* with the antibody used was almost solely confined to myoepithelial layer of non-cancerous ducts we did not evaluate the potential prognostic value of *S100A2* expression in our cohort.

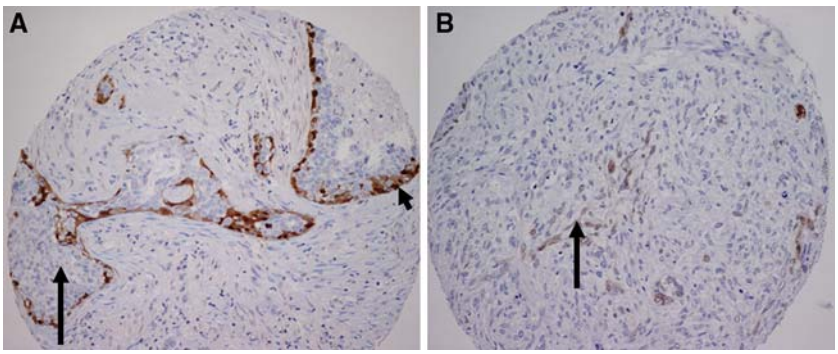


Fig. 3 Ductal carcinoma in situ (*arrow*) component of invasive ductal breast carcinoma with myoepithelial cells (*arrowhead*) expressing S100A2 (**A**), and metaplastic breast carcinoma with some spindle carcinomatous cells (*arrow*) stained for S100A2 (**B**)

Associations

The associations with patient and tumour characteristics were studied for the expression of *STMN1* and *SYK* (Table 1). *SYK* expression as a ternary variable based on the staining intensity alone was not significantly associated with any of the patient or tumour characteristics. *STMN1* expression as a binary variable, was associated positively with tumour grade ($P = 0.010$), and, in this respect surprisingly, weakly inversely with lymph node status ($P = 0.051$). No association between age as a continuous variable and either *STMN1* or *SYK* was found. Although for the purposes of this study, ER was treated as either positive or negative (all included patients were ER positive), we also tested the association between ER concentration as a continuous variable and both, *STMN1* and *SYK*. No association between these variables was found.

Univariate and multivariate analysis

In Cox univariate regression analysis for DFS according to staining intensity of *SYK*, we did not observe statistically significant differences between the three groups ($P = 0.491$) (Fig. 4a). At the medium follow-up time of 84 months, the DFS of patients with weak, moderate, and strong *SYK* intensity was 86.3%, 69.5% and 79.9%, respectively. *SYK* staining intensity was also not significantly associated with DSS (Fig. 4b). Quite the opposite, tumours with a high *STMN1* intensity in univariate analysis for DFS showed a significantly earlier relapse compared with tumours with a low intensity of expression (HR: 1.96, 95% CI 1.15–3.36, $P = 0.014$). At the median follow-up time of 84 months, the DFS of patients with a low *STMN1* staining intensity was 84%, compared with 63% for patients with a high staining intensity in the tumour (Fig. 5a). Moreover, tumours with high *STMN1* intensity also showed a significantly shorter DSS (Fig. 5b) compared with tumours with high intensity of expression (HR: 2.64, 95% CI 1.43–4.87, $P = 0.002$). At the median follow-up time of 84 months, the DSS of patients with a low *STMN1* staining intensity was 89%, compared with 70% for patients with a high staining intensity in the tumour (Fig. 5b).

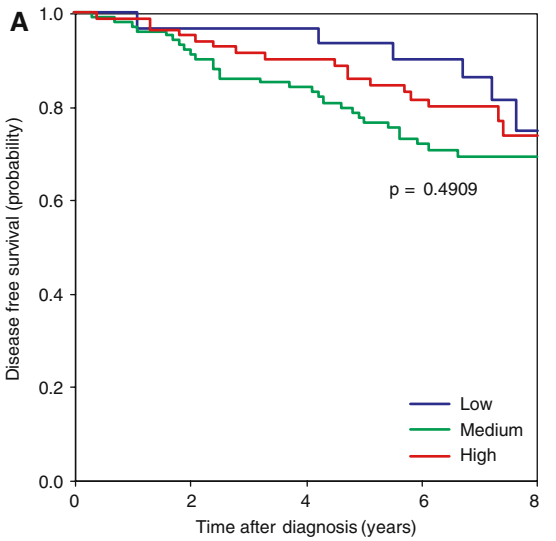
In Cox multivariate analysis for DFS, in addition to lymph node status, *STMN1* expression was the only independent variable that contributes to outcome prediction (Table 2) with a HR of 2.55 (95% CI 1.41–4.61, $P = 0.002$). Tumour size, tumour grade and progesterone receptor status were not significant in the multivariate analysis. Cox multivariate analysis for DSS showed analogous results as for the DFS analysis with, apart from the number of involved lymph nodes, *STMN1* expression as the only other independent prognostic variable with an HR of 3.47 (95% CI 1.75–6.86, $P < 0.001$) (Table 3).

Subgroup analyses

In an exploratory analysis, we studied whether *STMN1* expression was associated with DFS and/or DSS in subgroups of lymph node-negative and lymph node-positive patients. In both cases a high expression of Stathmin-1 was related to a poor DFS. For lymph node-negative patients this association was only of borderline significance (HR: 2.89, 95% CI 0.89–9.38, $P = 0.065$, 13 events) (Fig. 6a). Similarly, in the analysis for DSS in lymph node-negative patients (Fig. 6c), *STMN1* intensity was related with an early death (HR: 2.63, 95% CI: 0.66–10.54), however, this relationship did not reach statistical significance ($P = 0.157$, 9 events). In lymph node-positive patients the association of *STMN1* expression with both DFS (HR: 2.80, 95% CI 1.48–5.31, $P = 0.001$, 41 events) (Fig. 6b) and DSS (HR: 4.30, 95% CI 2.09–8.85, $P < 0.001$, 35 events) (Fig. 6d), was statistically significant.

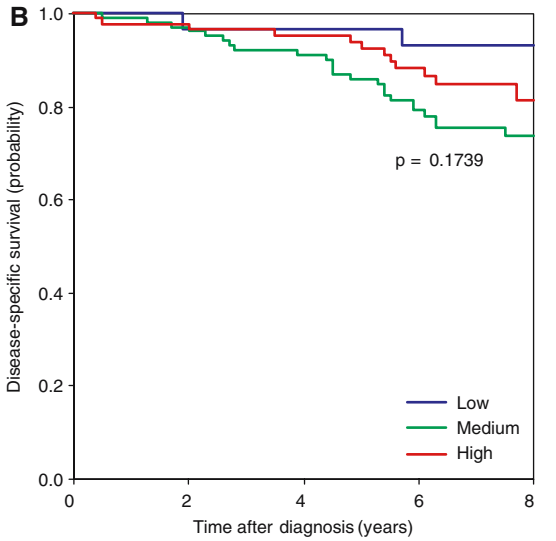
Discussion

In current daily oncology practice, one of the most important questions in planning the treatment of patients with operable breast carcinoma is for which hormone



Numbers at risk

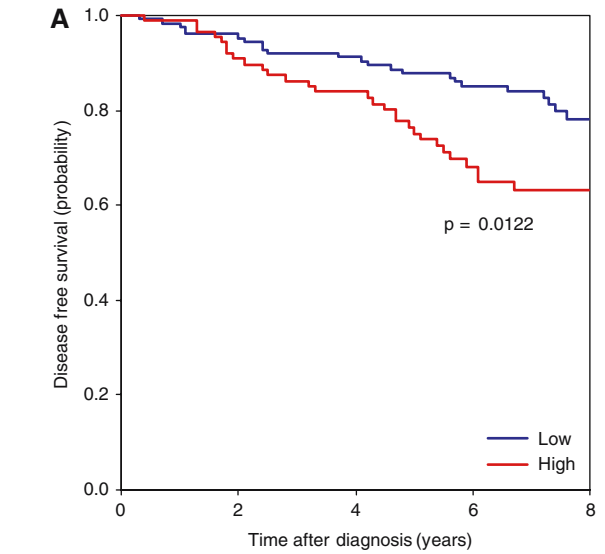
Low	30	29	29	25	11
Medium	101	91	81	61	28
High	83	77	66	51	17



Numbers at risk

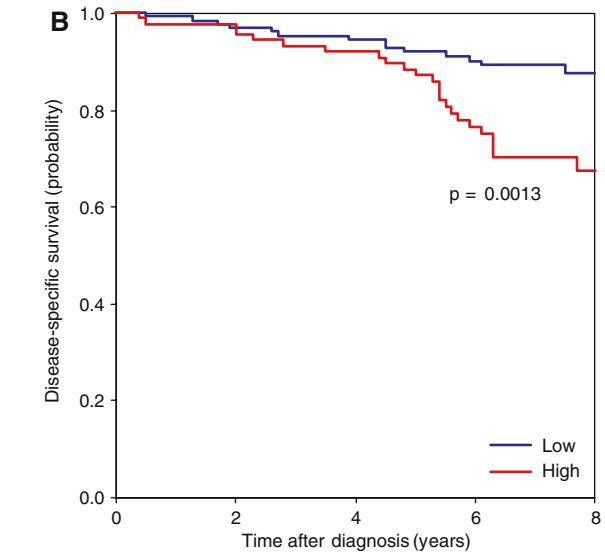
Low	30	29	29	26	14
Medium	101	97	89	68	32
High	83	79	71	59	22

Fig. 4 Disease-free (**A**) and disease-specific survival (**B**) as a function of SYK staining intensity



Numbers at risk

Low	126	119	109	94	40
High	89	79	68	44	16



Numbers at risk

Low	126	122	114	101	48
High	89	85	76	53	20

Fig. 5 Disease-free (**A**) and disease-specific survival (**B**) as a function of Stathmin-1 staining intensity

receptor-positive patients is endocrine therapy with tamoxifen alone sufficient. To identify this subgroup of patients additional molecular markers are urgently needed.

The first part of this retrospective study showed that antibodies against SYK, S100A2 and STMN1, which were identified as predictive markers in previous DNA methylation analyses [5] performed well on TMA of paraffin embedded breast tumours. Tumour cells of the majority of specimens expressed different levels of SYK and STMN1, whereas they did not express S100A2. The latter antigen was expressed only in myoepithelial cells of normal breast ducts and in some tumour cells with mesenchymal characteristics in the only metaplastic breast carcinoma in the cohort. This observation is in concordance with already published data that S100A2 marks a myoepithelial-like breast cancer that tends to be ER negative and more aggressive [15]. Based on our current findings, S100A2 may be a relevant marker for metaplastic breast cancer, a

Table 2 Univariate and multivariate analysis of disease-free survival

Variable	Univariate (*P* value)	HR (95% CI)	Multivariate (*P* value)	HR (95% CI)
Age	ns		ns	
Pathological tumour size	0.054		ns	
pT2 vs pT1	0.143	1.58 (0.86–2.92)		
pT3 vs pT1	0.017	2.98 (1.21–7.33)		
Histological tumour type	ns		ns	
Ductal invasive vs others				
Malignancy grade	ns		ns	
G2 vs G1				
G3 vs G1				
Lymph node status	<0.001		<0.001	
Positive vs negative		4.44 (2.38–8.29)		4.96 (2.55–9.63)
Progesterone receptor status	0.025		ns	
Negative vs positive		1.90 (1.09–3.32)		
Stathmin-1 intensity	0.014		0.002	
High vs low expression		1.96 (1.15–3.36)		2.55 (1.41–4.61)

Table 3 Univariate and multivariate analysis of disease-specific survival

Variable	Univariate (*P* value)	HR (95% CI)	Multivariate (*P* value)	HR (95% CI)
Age	ns		ns	
Pathological tumour size	0.006		ns	
pT2 vs pT1	0.078	1.93 (0.93–4.01)		
pT3 vs pT1	0.001	4.85 (1.83–12.83)		
Histological tumour type	ns		ns	
Ductal invasive vs others				
Malignancy grade:	ns		ns	
G2 vs G1				
G3 vs G1				
Lymph node status	<0.001		<0.001	
Positive vs negative		5.07 (2.43–10.57)		5.68 (2.59–12.47)
Progesterone receptor status	0.039		ns	
Negative vs positive		1.91 (1.03–3.54)		
Stathmin-1 intensity	0.002		<0.001	
High vs low expression		2.64 (1.43–4.87)		3.47 (1.75–6.86)

rare breast cancer subtype but not for ER-positive, luminal-like breast cancer.

The second and most important aim was to determine if one of the three DNA methylation markers was associated with the risk of recurrence after tamoxifen monotherapy in the adjuvant setting. In this respect S100A2 will be of limited clinical use because, as discussed in detail above, ER-positive tumours expressing detectable levels of S100A2 are rare. SYK staining intensity could also not be related with DFS in our patient collective, despite the fact that SYK, in breast cancer xenographs, is a tumour suppressor gene [12]. SYK is also frequently methylated in human breast cancer specimen [17]. Furthermore, reduced SYK mRNA expression has been associated with poor outcome in early breast cancer [16]. Thus, it may be that SYK expression carries useful clinical information, but our work does not support a role for SYK expression for predicting disease recurrence in ER-positive patients who received adjuvant hormonal treatment with tamoxifen.

The third marker, STMN1, separated the breast cancers in two distinctive groups according to the patient's DFS probability. Multivariate Cox regression analysis confirmed, next to nodal involvement, the independent value of STMN1 staining intensity to predict outcome. The exploratory subgroup analysis indicated that STMN1 expression may be a predictor of disease recurrence in both

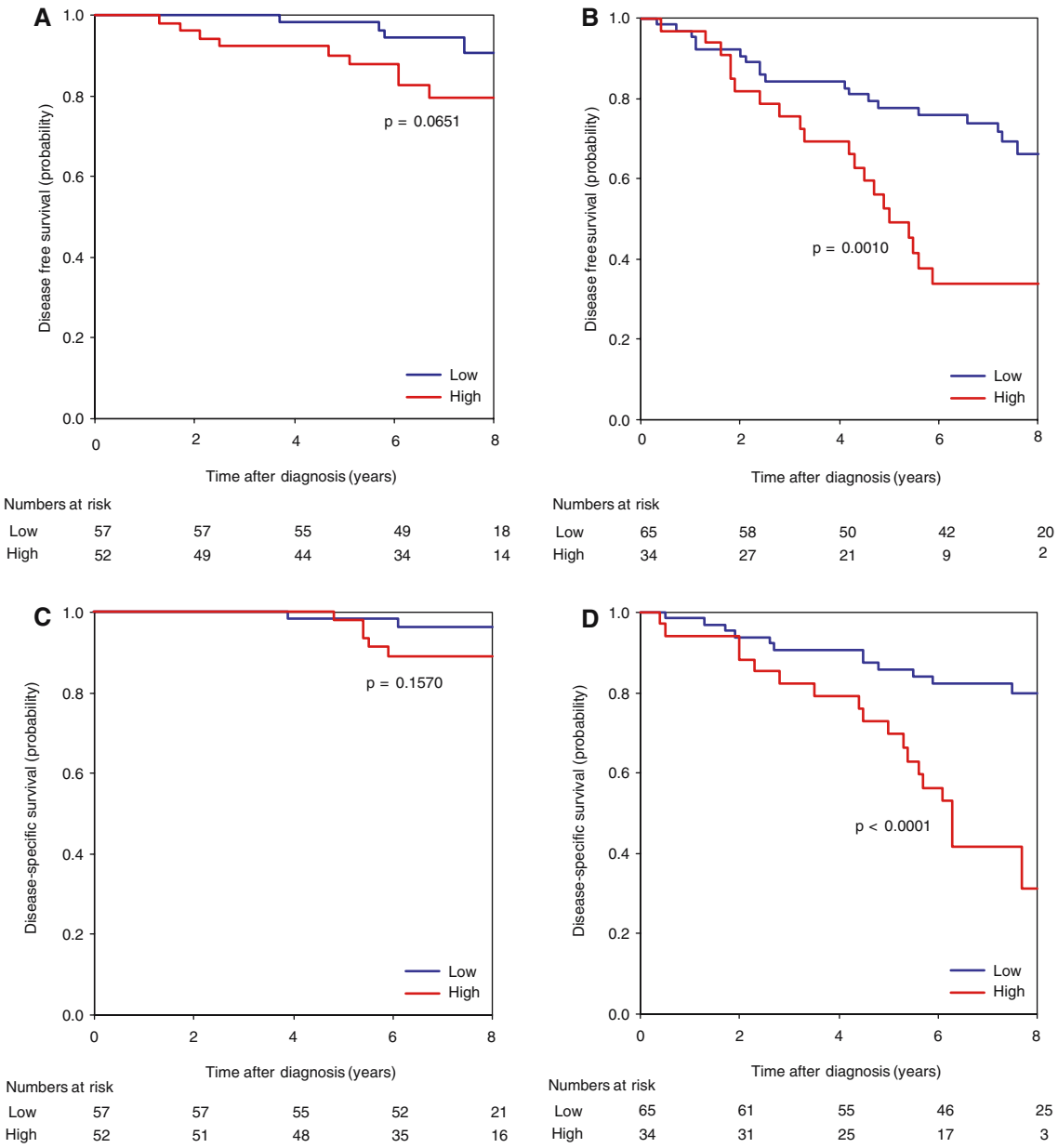


Fig. 6 Disease-free (**A**, **B**) and disease-specific survival (**C**, **D**) as a function of Stathmin-1 staining intensity in node-negative (**A**, **C**) and node-positive patients (**B**, **D**)

lymph node-positive and lymph node-negative subgroups of patients. For lymph node-positive patients this association was highly significant, while the association for lymph node-negative disease was only of borderline significance, however, the latter analysis is hampered by a low number of failures as a consequence of a much better survival of this subgroup. Considering that all patients received adjuvant therapy, STMN1 may also be a marker of tamoxifen resistance. Since the disease-free survival curves begin to split only after year 2 with the separation most visible after year 4, one may speculate that high STMN1 expression predicts for secondary tamoxifen resistance. According to our limited observation the strategy that already yielded positive results in ER-positive EBC, i.e. the switch from tamoxifen to aromatase after two years of tamoxifen therapy, may be of value in subset of patients with high STMN1 expression; however it should be explored and confirmed in a prospective randomized fashion.

Based on our earlier results in metastatic breast cancer [5], where hypermethylation of STMN1 was associated with progressive disease after tamoxifen treatment, the association of high STMN1 staining intensity with early

relapse may sound controversial. However, DNA methylation of particular locus does not necessary lead to silencing of its transcription. For this reason we are currently measuring mRNA levels of STMN1 in our cohort of metastatic breast cancer patient that was used in the earlier study. Another explanation for the controversial role of STMN1 in early and metastatic breast cancer is that predictors of poor response in metastatic breast cancer are for various reasons not necessary also predictors of poor outcome during adjuvant treatment [9]. STMN1 is involved in microtubule dynamics [18] and high expression might expedite assembly and disassembly of microtubules that are needed for mitosis. The necessity of microtubules for breast cancer progression is evident from the effectiveness of microtubules-directed therapeutics [i.e. paclitaxel). The association between STMN1 staining intensity and tumour grade, we have noted, has been observed earlier in studies where STMN1 levels were measured by quantitative RT-PCR and by Western blot [19, 20]. Together these results favour a role for STMN1 in aggressiveness of breast cancer, which would be in line with the earlier recurrence seen in our cohort of hormone receptor positive breast cancers that have high STMN1 levels.

A 21-gene and a 2-gene recurrence predictor that is based on RT-PCR on paraffin-embedded tissue have been reported for a comparable group of patients [21, 22]. We ourselves have identified and validated a DNA methylation marker, PITX2 that also reliably predicts outcome in adjuvant tamoxifen-treated patients [6]. All these markers, however, require further independent validation. Even though all the markers mentioned can be assessed quantitatively on paraffin-embedded material, a shortcoming is, unlike STMN1 staining, that they are not measured by IHC, which is used in the clinical routine for ER, PR, and Her-2 protein determination. Although determination of any IHC marker relies on the subjective evaluation, the IHC procedure in contrast to molecular methods is fast, robust and cheap, which favours the application of STMN1 intensity staining as a marker for disease recurrence prediction in EBC patients eligible for adjuvant tamoxifen monotherapy.

Concluding, our results provide substantial evidence that STMN1 staining intensity defined by IHC on paraffin sections of primary breast tumours could predict outcome in patients on tamoxifen monotherapy, and could identify a low risk group for which 5 years of tamoxifen is sufficient. Lymph node-negative patients with low STMN1 staining activity seem to have an excellent long-term prognosis (an estimated DFS of over 90% at 7 years follow-up). The current finding, however, requires further validation in larger, independent cohorts of early breast cancer patients treated with adjuvant hormonal therapy.

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