St Gallen molecular subtypes in screening-detected and symptomatic breast cancer in a prospective cohort with long-term follow-up

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Background: Diagnosis by screening mammography is considered an independent positive prognostic factor, although the data are not fully in agreement. The aim of the study was to explore whether the mode of detection (screening-detected *versus* symptomatic) adds prognostic information to the St Gallen molecular subtypes of primary breast cancer, in terms of 10-year cumulative breast cancer mortality (BCM).

Methods: A prospective cohort of patients with primary breast cancer, who had regularly been invited to screening mammography, were included. Tissue microarrays were constructed from primary tumours and lymph node metastases, and evaluated by two independent pathologists. Primary tumours and lymph node metastases were classified into St Gallen molecular subtypes. Cause of death was retrieved from the Central Statistics Office.

Results: A total of 434 patients with primary breast cancer were included in the study. Some 370 primary tumours and 111 lymph node metastases were classified into St Gallen molecular subtypes. The luminal A-like subtype was more common among the screening-detected primary tumours ($P = 0.035$) and corresponding lymph node metastases ($P = 0.114$) than among symptomatic cancers. Patients with screening-detected tumours had a lower BCM ($P = 0.017$), and for those diagnosed with luminal A-like tumours the 10-year cumulative BCM was 3 per cent. For patients with luminal A-like lymph node metastases, there was no BCM. In a stepwise multivariable analysis, the prognostic information yielded by screening detection was hampered by stage and tumour biology.

Conclusion: The prognosis was excellent for patients within the screening programme who were diagnosed with a luminal A-like primary tumour and/or lymph node metastases. Stage, molecular pathology and mode of detection help to define patients at low risk of death from breast cancer.

Introduction

Breast cancer prognosis after primary surgery is a major research subject as the disease remains the second leading cause of cancer-related death among women[1]. In Sweden, early diagnosis and access to modern systemic treatment have led to a decrease in breast cancer mortality (BCM), with the 5-year survival rate approaching 90 per cent in 2014[2]. Screening programmes detect breast cancer at earlier stages[3], and screening-detected breast cancer is therefore often associated with improved prognosis compared with symptomatic disease[4]. Moreover, the majority of patients with screening-detected breast cancer show favourable tumour characteristics in the form of small tumours, lymph node-negative disease and hormone receptor-positive tumours of low grade compared with those diagnosed outside a screening programme[5,6].

Symptomatic breast cancer, often associated with a diagnostic delay[7], is usually associated with more aggressive tumour characteristics[8] and therefore a higher mortality rate than screening-detected breast cancer[7]. Indeed, in countries where screening programmes do not exist or do not function properly, breast cancer prognosis at the time of diagnosis is generally poorer[9] than in countries where screening programmes have been established. The mode of detection – screening *versus* symptomatic – has repeatedly been reported to be an independent positive prognostic factor irrespective of diagnosis at an earlier stage, although this finding has been challenged by the introduction of modern molecular pathology[4,10].

Oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER) 2 are biomarkers expressed in breast tumours that carry important prognostic information and are also used as predictive markers for the selection of adjuvant treatment[11]. High expression levels of the proliferation marker Ki-67 are related to poor prognosis[12]. According to the St Gallen 2011 and 2013 guidelines[13,14], a proxy for the molecular subtype classification based on immunohistochemical analysis and *in situ* hybridization (ISH) of ER, PR, Ki-67 and HER2 can be used to divide tumours into four to five main subtypes: luminal A-like, luminal B-like (HER2-positive or HER2-negative), HER2-positive (non-luminal) and triple-negative breast cancer. This classification is important as it provides prognostic information that guides systemic therapy. For example, adjuvant chemotherapy is recommended for breast cancers with high proliferation/high Ki-67[13]. Moreover, the luminal A-like subtype is known to have a favourable prognosis[10], whereas the triple-negative subtype is associated with a poor outcome[15]. The molecular classification of primary breast cancer is being refined continuously, often focusing on patients with poor prognosis, and as an example a novel systematization for apocrine breast cancers has been suggested[16].

Screening-detected tumours often present with the luminal A-like subtype, and the prognostic information conveyed by the mode of detection seems to be restricted to this subgroup[4]. Endocrine systemic therapy is recommended for patients with luminal A-like tumours without lymph node metastases, whereas chemotherapy alone or alongside endocrine therapy and/or HER2-directed therapy is recommended for all other subtypes.

The aim of the present study was to evaluate the prognostic information provided by the mode of detection (screening *versus* symptomatic), with adjustment for the St Gallen molecular subtypes diagnosed by standardized molecular pathology, in terms of 10-year cumulative BCM.

Methods

The study cohort was based on patients included previously in a prospective observational study on the prognostic value of analysing disseminated tumour cells in bone marrow[17]. Briefly, women diagnosed with an unifocal breast cancer between 1999 and 2003 in the Southern Swedish Health Care Region were identified. All patients underwent surgery of the breast and axillary lymph nodes. Clinical examination of axillary lymph nodes was performed before surgery, and patients with no palpable lymph nodes and unifocal tumours smaller than 3 cm had sentinel node biopsy, which was introduced during the study interval. Adjuvant therapy was recommended according to regional guidelines, and included chemotherapy for all premenopausal women with node-positive disease and postmenopausal patients with hormone receptor-negative tumours. Endocrine therapy was recommended to patients with ER-positive tumours. Radiotherapy (50 Gy) to the breast was given to patients who underwent breast-conserving surgery, and locoregional radiotherapy was delivered to those with four or more axillary lymph node metastases. Neoadjuvant endocrine therapy and chemotherapy were administered to less than 1 per cent of the patients, and adjuvant trastuzumab to 1·1 per cent.

Clinical examination and mammography were carried out annually for 5 years, and follow-up data were then extracted from patient charts. The mode of detection (symptomatic or screening) was specified at the time of diagnosis, whereas diagnosis of an interval cancer was not specified. Data on breast cancer-related death were retrieved from the Swedish Register of Causes of Death (Central Statistics Office) after 10 years. Data were retrieved in 2011 and included all recorded events until 31 December 2010.

The present study included patients between 45 and 74 years of age, the inclusion ages for public mammographic screening in Sweden during the study interval. All patients in this age range were regularly invited for public screening mammography. Patients with screening-detected breast cancer had experienced no symptoms before the invitation to screening mammography. The breast cancer was considered symptomatic when diagnostic evaluation, including mammography, was initiated owing to symptoms from the breast either in the interval between screening mammograms or in patients who had not undergone mammographic examination. Data on mammographic history were retrieved from patient charts.

Tumour samples and tissue microarray

Tissue microarray (TMA) and biomarker analyses have been described in detail previously[18]. Briefly, TMAs

were constructed from formalin-fixed, paraffin-embedded archival blocks of study samples retrieved from the Departments of Clinical Pathology in Lund and Helsingborg. Two cores, 1·0 mm in diameter, were punched out from defined areas of invasive tumours, identified from haematoxylin and eosin-stained tissue sections by a pathologist. The cores were mounted on to the recipient block using a tissue array machine in accordance with the manufacturer's instructions (Beecher Instruments, Sun Prairie, Wisconsin, USA). Tissue sections of 4 μm were cut, and glass slides were prepared for microscopy and then scanned (Aperio ScanScope™ CS with Spectrum™ software; Aperio, Vista, California, USA).

Immunohistochemistry and *in situ* hybridization

ER and PR status were assessed using the Ventana Bench-Mark system (Ventana Medical Systems, Tucson, Arizona, USA), with anti-ER clone SP1 and anti-PR clone 1E2 as the primary antibodies[19], at a central clinical laboratory (Skåne University Hospital, Malmö). At least 100 invasive tumour cells were scored visually and the percentage of positive immunostaining was evaluated. Samples with more than 1 per cent stained nuclei were considered positive.

HER2 was evaluated by both immunohistochemistry (IHC) using anti-HER2 clone 4B5, and ISH (Inform HER2 dual ISH DNA, with a silver and chromogen visualization kit; Ventana BenchMark Ultra). All patients with an IHC score of 3 or more and/or an amplified tumour according to silver ISH (ratio at least 2·0 between the *HER2* gene and centromere at chromosome 17) were considered positive.

Ki-67 was assessed using antibody MIB1 (Dako, Glostrup, Denmark), diluted to 1 : 50, incubated for 32 min and visualized with 3,3′-diaminobenzidine. Areas with increased numbers of Ki-67-positive cells within the invasive cancer region (hot spots) were identified, and at least 200 cells were analysed. Cells were scored visually for the percentage of positive immunostaining. The chosen cut-off point for separating high and low proliferation was the one-third of the study population with the highest Ki-67 percentage values, which corresponds to more than 20 per cent positive immunostaining in the present cohort.

All biomarkers were scored independently by two certified pathologists.

Molecular subtype definitions

Classification of molecular subtypes was based on St Gallen recommendations[14] with IHC analysis of ER, PR and Ki-67, and silver ISH analysis of HER2. The molecular subtypes were defined as follows:

luminal A-like (ER-positive and PR more than 20 per cent, low Ki-67 and HER2-negative), luminal B-like HER2-negative (ER-positive, PR 20 per cent or less and/or high Ki-67 and HER2-negative), luminal B-like HER2-positive (ER-positive and/or PR-positive, any Ki-67 and HER2-positive), HER2-positive (non-luminal) (ER-negative, PR-negative, any Ki-67 and HER2-positive) and triple-negative (ER-negative, PR-negative, HER2-negative, any Ki-67).

Statistical analysis

Analyses are based on evaluation by one pathologist because the concordance in evaluations between the two pathologists was close to 100 per cent. Characteristics in symptomatic *versus* screening-detected breast cancer were compared using the χ^2 test, or Fisher's exact test if expected counts in one or more of the cells were below 5. A linear-by-linear test for association was used for ordinal variables with more than two categories, and Mann–Whitney U test for continuous data.

The primary endpoint was cumulative BCM at 10 years' follow-up. Differences in cumulative BCM among subgroups were evaluated by means of Gray's test. The Cox proportional hazards model was used to calculate cause-specific hazard ratios (HRs) for biomarkers and molecular subtypes with and without adjustment for other prognostic factors. Follow-up was censored at date of death for patients who were experiencing competing events (death from causes other than breast cancer). Proportional hazards assumptions were checked graphically. To evaluate the prognostic interaction between the mode of detection and molecular subtypes, multivariable Cox regression was carried out, including screening detection (yes, no), luminal A-like (yes, no) and a term of interaction (screening detection (yes, no) × luminal A-like (yes/no)). $P < 0.050$ was considered significant. Freedman's statistic was applied to calculate the effect of screen detection confounded by stage and biomarkers using the equation $P = 100 (1 - a/b)$, where a is the adjusted logarithm of the HR and b the unadjusted value.

The statistical software packages Stata® 13.1 (StataCorp LP, College Station, Texas, USA) and SPSS® version 19 (IBM Svenska, Stockholm, Sweden) were used for the statistical calculations.

Results

Of 555 women diagnosed with a unifocal breast cancer between 1999 and 2003, 434 patients aged 45–74 years were eligible for inclusion in this study (*Fig. 1*). Two

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                              │     n = 555           │
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          ┌─────────────────────┐        │
          │   Excluded as        │◄───────┤
          │ < 45 or > 74 years   │        │
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                              │  Patients aged       │
                              │  45–74 years         │
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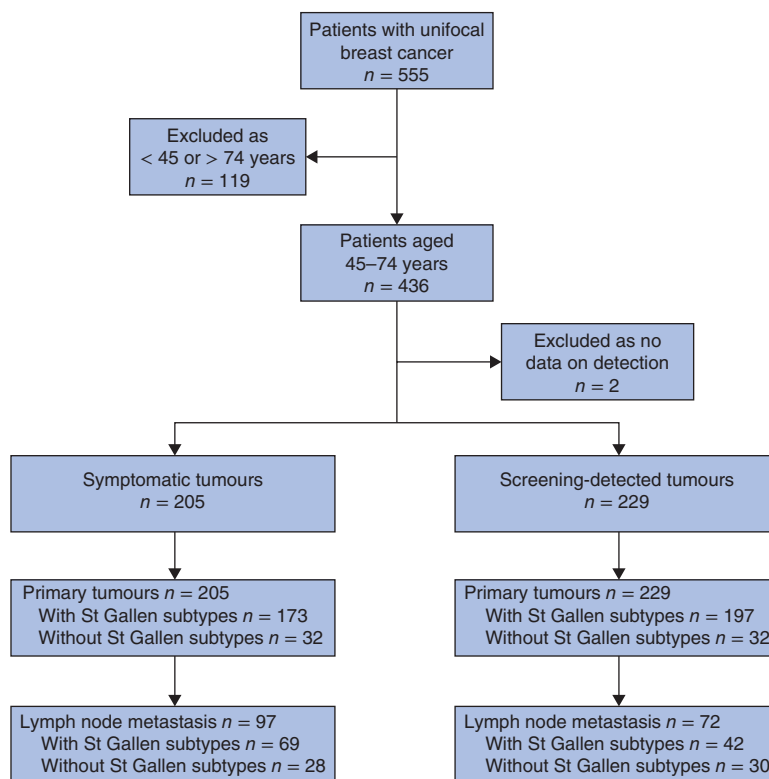| Symptomatic tumours n = 205	Screening-detected tumours n = 229
Primary tumours n = 205 With St Gallen subtypes n = 173 Without St Gallen subtypes n = 32	Primary tumours n = 229 With St Gallen subtypes n = 197 Without St Gallen subtypes n = 32
Lymph node metastasis n = 97 With St Gallen subtypes n = 69 Without St Gallen subtypes n = 28	Lymph node metastasis n = 72 With St Gallen subtypes n = 42 Without St Gallen subtypes n = 30

Fig. 1 Flow chart for the study

patients were excluded because no data were reported on the mode of detection. The final cohort of 434 patients comprised 205 (47·2 per cent) diagnosed with symptomatic breast cancer and 229 (52·8 per cent) who had screening-detected tumours. Patient and tumour characteristics are shown in *Table S1*, supporting information. Tumours diagnosed by screening mammography were detected at a lower stage (T1 and N0) than symptomatic tumours, and were more differentiated, as indicated by a lower Nottingham histological grade (NHG)[20]. There was an even distribution of ductal and lobular carcinomas between symptomatic and screening-detected tumours, whereas medullary cancers were diagnosed only in the symptomatic subgroup; tubular and microinvasive tumours were more common in patients within the screening programme. The fractions of ER- and PR-positive cells were significantly higher in screening-detected tumours, whereas the Ki-67 labelling index was lower, underscoring the predominance of well differentiated tumours detected in a screening programme (*Fig. S1*, supporting information).

The luminal A-like subtype was diagnosed in 46·7 per cent of patients in the screening-detected cohort, whereas luminal B-like HER2-positive and HER2-positive/

non-luminal subtypes were less common. The distribution of molecular subtypes in primary tumours showed a shift towards a favourable subtype in screening-detected tumours ($P = 0.011$), with luminal A-like being more common ($P = 0.035$) (*Table 1*). Some 92 of the 154 luminal A-like tumours were detected at screening, whereas the non-luminal A-like tumours were distributed evenly between the screen-detected and symptomatic groups (105 and 111 of 216 tumours respectively).

The distribution of molecular subgroups in synchronous lymph node metastases was not significantly different between the screening-detected and symptomatic groups ($P = 0.233$) (*Table 3*), although the luminal A-like subtype was diagnosed in 17 (40 per cent) of 42 patients with screening-detected tumours compared with 18 (26 per cent) of 69 with symptomatic disease ($P = 0.114$). Luminal A-like lymph node metastases showed an even distribution according to mode of detection, whereas two-thirds (51 of 76) of the non-luminal A-like lymph node metastases were diagnosed in symptomatic patients and one-third (25 of 76) in patients with screening-detected disease.

As a consequence of lower stage at diagnosis and good prognostic signatures, 71·6 per cent of patients diagnosed within the screening programme had a partial mastectomy

Table 1 Molecular profiles according to mode of detection

	Symptomatic (n = 205)	Screening-detected (n = 229)	P*
Oestrogen receptor status			0·045
Positive (> 1%)	163 (86·7)	193 (92·8)	
Negative (≤ 1%)	25 (13·3)	15 (7·2)	
Unknown	17	21	
Progesterone receptor status			0·399
Positive (> 1%)	134 (77·5)	162 (81·0)	
Negative (≤ 1%)	39 (22·5)	38 (19·0)	
Unknown	32	29	
Ki-67 status (%)			0·004
≤ 20	115 (62·2)	154 (75·5)	
> 20	70 (37·8)	50 (24·5)	
Unknown	20	25	
HER2 status			0·010
Negative	149 (76·0)	184 (86·0)	
Positive	47 (24·0)	30 (14·0)	
Unknown	9	15	
St Gallen subtypes in primary tumours			0·011†
Luminal A-like	62 (35·8)	92 (46·7)	
Luminal B-like HER2−	57 (32·9)	68 (34·5)	
Luminal B-like HER2+	35 (20·2)	23 (11·7)	
HER2+ (non-luminal)	6 (3·5)	4 (2·0)	
TNBC	13 (7·5)	10 (5·1)	
Unknown	32	32	
Luminal A status in primary tumours			0·035
Luminal A-like	62 (35·8)	92 (46·7)	
Non-luminal A-like	111 (64·2)	105 (53·3)	
Unknown	32	32	
St Gallen subtypes in lymph node metastasis			0·233†
Luminal A-like	18 (26)	17 (40)	
Luminal B-like HER2−	23 (33)	14 (33)	
Luminal B-like HER2+	18 (26)	3 (7)	
HER2+ (non-luminal)	4 (6)	6 (14)	
TNBC	6 (9)	2 (5)	
Unknown	28	30	
Luminal A status in lymph node metastasis			0·114
Luminal A-like	18 (26)	17 (40)	
Non-luminal A-like	51 (74)	25 (60)	
Unknown	28	30	

Values in parentheses are percentages. HER, human epidermal growth factor receptor; TNBC, triple-negative breast cancer. *χ^2 test, except †linear-by-linear test for association.

compared with 53·2 per cent of those with symptomatic tumours, and adjuvant systemic therapy (chemotherapy and endocrine therapy) was delivered to more patients in the symptomatic cohort (*Table S1*, supporting information). Sentinel lymph node biopsy was carried out in 45·9 per cent of patients with screening-detected tumours and 31·9 per cent of those with symptomatic disease.

Mode of detection as a prognostic factor in the whole cohort

Screening-detected breast cancer was associated with significantly lower BCM than symptomatic disease (HR 0·53, 95 per cent c.i. 0·31 to 0·90; *P* = 0·023) (*Table 2*; *Fig. S2*, supporting information). Large tumours, lymph node positivity, NHG 3, high Ki-67, ER negativity, PR negativity, HER-2-positive and non-luminal A-like tumour type were negative prognostic factors in the Cox univariable analysis; screening detection was a prognostic factor for favourable outcome (*Table 2*). In the multivariable analysis, node status remained an independent prognostic factor, whereas the mode of detection had no significant impact on BCM after adjustment for other prognostic variables (HR 0·70, 0·38 to 1·30; *P* = 0·240) (*Table 2*). In contrast, the prognostic information yielded by a non-luminal A-like

Table 2 Cox univariable and multivariable regression analysis to identify predictors of breast cancer mortality in all patients

	Univariable analysis		Multivariable analyis	
	Hazard ratio	P	Hazard ratio	P
Mode of detection				
Symptomatic	1·00 (reference)		1·00 (reference)	
Screening	0·53 (0·31, 0·90)	0·023	0·70 (0·38, 1·30)	0·240
Age (per year)	1·01 (0·98, 1·05)	0·466	1·00 (0·97, 1·04)	0·629
Tumour size				
T1	1·00 (reference)		1·00 (reference)	
T2 + T3	2·62 (1·55, 4·42)	<0·001	1·62 (0·88, 2·99)	0·123
Node status				
N0	1·00 (reference)		1·00 (reference)	
N+	3·83 (2·17, 6·77)	<0·001	3·52 (1·80, 6·87)	<0·001
Nottingham histological grade				
1–2	1·00 (reference)		1·00 (reference)	
3	2·22 (1·28, 3·84)	0·005	1·29 (0·62, 2·39)	0·425
Oestrogen receptor status				
Positive (> 1%)	1·00 (reference)			
Negative (≤ 1%)	2·43 (1·22, 4·84)	0·012		
Progesterone receptor status				
Positive (> 1%)	1·00 (reference)			
Negative (≤ 1%)	2·91 (1·62, 5·21)	<0·001		
Ki-67 status (%)				
≤20	1·00 (reference)			
>20	2·15 (1·26, 3·73)	0·006		
HER2-status				
Negative	1·00 (reference)			
Positive	2·05 (1·14, 3·69)	0·017		
St Gallen molecular subtype				
Luminal A-like	1·00 (reference)			
Luminal B-like HER2−	1·84 (0·85, 3·98)	0·123		
Luminal B-like HER2+	2·36 (1·09, 5·11)	0·030		
HER2+ (non-luminal)	2·69 (0·79, 9·13)	0·113		
TNBC	4·31 (1·80, 10·33)	0·001		
St Gallen molecular subtype				
Luminal A-like	1·00 (reference)		1·00 (reference)	
Non-luminal A-like	3·01 (1·50, 6·03)	0·001	2·32 (1·12, 4·80)	0·024

Values in parentheses are 95% c.i. HER, human epidermal growth factor receptor; TNBC, triple-negative breast cancer.

subtype remained significant (HR 2·32, 1·12 to 4·80; $P = 0·024$).

A stepwise multivariable analysis was also performed (*Fig. 2*), indicating that the prognostic information added by mode of detection remained even after adjusting for individual molecular markers and NHG. However, the prognostic significance of diagnostic modality was not recognized when tumour size and node status were introduced. Freedman's statistic showed that ER, PR, Ki-67 and HER2 accounted for 13·8 per cent of the confounding survival benefit related to screening detection and, among these, HER2 status was the most important confounder (*Fig. 2*). In contrast, stage (T and N) conferred almost 50 per cent of the survival advantage related to mammographic screening.

Subgroup analysis

When the cohort was stratified according to St Gallen molecular subtypes in the primary tumour, the mode

of detection added prognostic information in the luminal A-like subtype ($P = 0·053$) but was not significant in the non-luminal A-like subtypes ($P = 0·175$); this was illustrated by an excellent prognosis in patients with screening-detected tumours with the luminal A-like molecular subtype, whose BCM at 10 years was 3 per cent, compared with 21·6 per cent among patients with symptomatic tumours of the non-luminal A-like subtype (*Table 3* and *Fig. 3a,b*). Because screening detection and luminal A-like subtype added prognostic information to each other, an interaction test was performed in a multivariable Cox model. However, no significant prognostic interaction was found between the mode of detection and molecular subtypes (interaction term, $P = 0·222$).

Node status was the most important prognostic factor in both screening-detected and symptomatic groups (*Fig. 3c,d*) and remained significant in multivariable analysis: HR 4·59 (95 per cent c.i. 1·85 to 11·38; $P = 0·001$) and HR 2·97 (1·42 to 6·18; $P = 0·004$) respectively. In contrast, the mode of detection did not modify prognosis for
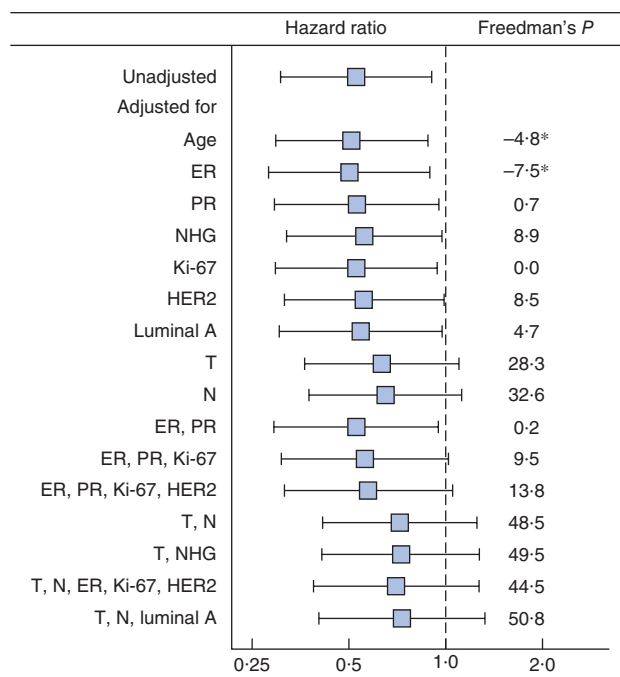
Fig. 2 Forest plot showing hazard ratios and corresponding 95 per cent c.i. for screening-detected *versus* symptomatic cancers before and after adjustment for patient and tumour characteristics. ER, oestrogen receptor; PR, progesterone receptor; NHG, Nottingham histological grade; HER, human epidermal growth factor receptor; T, tumour size; N, node status. *Freedman's statistic; *P* value is negative when the adjusted effect is larger than the unadjusted effect

all patients with node-positive disease (*Table 3*). Similar to the survival findings for primary tumours, in analysis of patients with node-positive disease stratified according

to St Gallen molecular subtypes in lymph node metastases, mode of detection was a significant prognostic factor in patients with luminal A-like lymph node metastases ($P = 0.005$), but not in those with non-luminal A-like lymph node metastases (*Fig. 3e,f* and *Table 3*).

Discussion

The results from this prospective study have confirmed that screening-detected breast cancer is characterized by more favourable tumour biology and is diagnosed at an earlier stage than symptomatic disease. Despite analysis with modern molecular pathology techniques according to validated protocols, stage migration was the most important confounder explaining the beneficial effect of mode of detection on survival. However, a low-risk subset of patients with luminal-A-like tumours in the screening cohort was identified.

Patients diagnosed within the public screening mammography programme had smaller tumours, less lymph node involvement, lower NHG rankings and more favourable histopathological characteristics than those with prevalent disease, resulting in less traumatic surgery in the breast and axilla, and improved survival. These findings are in agreement with published findings[3,19], although the previous results were not adjusted according to St Gallen molecular subtypes in either primary tumours or lymph node metastases, which is one of the strengths of the present study. A number of authors have related molecular profiles to mode of detection, but did not report any data regarding Ki-67 or HER2 status by ISH[21,22], which is a prerequisite for the identification of St Gallen molecular subtypes[3]. Dawson and colleagues[23] reported follow-up after 15 years, but the pathological protocol for St Gallen classification did not

Table 3 Breast cancer mortality at 10 years by St Gallen molecular subtype in primary tumours and lymph node metastases

| | No. of patients | Breast cancer mortality | | *P** |
		Symptomatic (%)	Screening-detected (%)	
All patients aged 45–74 years	434	35 of 205 (17·1)	21 of 229 (9·2)	0·014
St Gallen molecular subtype in primary tumours				
Luminal A-like	154	7 of 62 (11)	3 of 92 (3)	0·047
Non-luminal A-like	216	24 of 111 (21·6)	15 of 105 (14·3)	0·161
Unknown	64			
Node status				
N0	259	10 of 106 (9·4)	7 of 153 (4·6)	0·121
N+	169	25 of 97 (26)	14 of 72 (19)	0·334
Unknown	6			
St Gallen molecular subtype in lymph node metastasis				
Luminal A-like	35	7 of 18 (39)	0 of 17 (0)	0·004
Non-luminal A-like	76	14 of 51 (27)	9 of 25 (36)	0·414
Unknown	58			

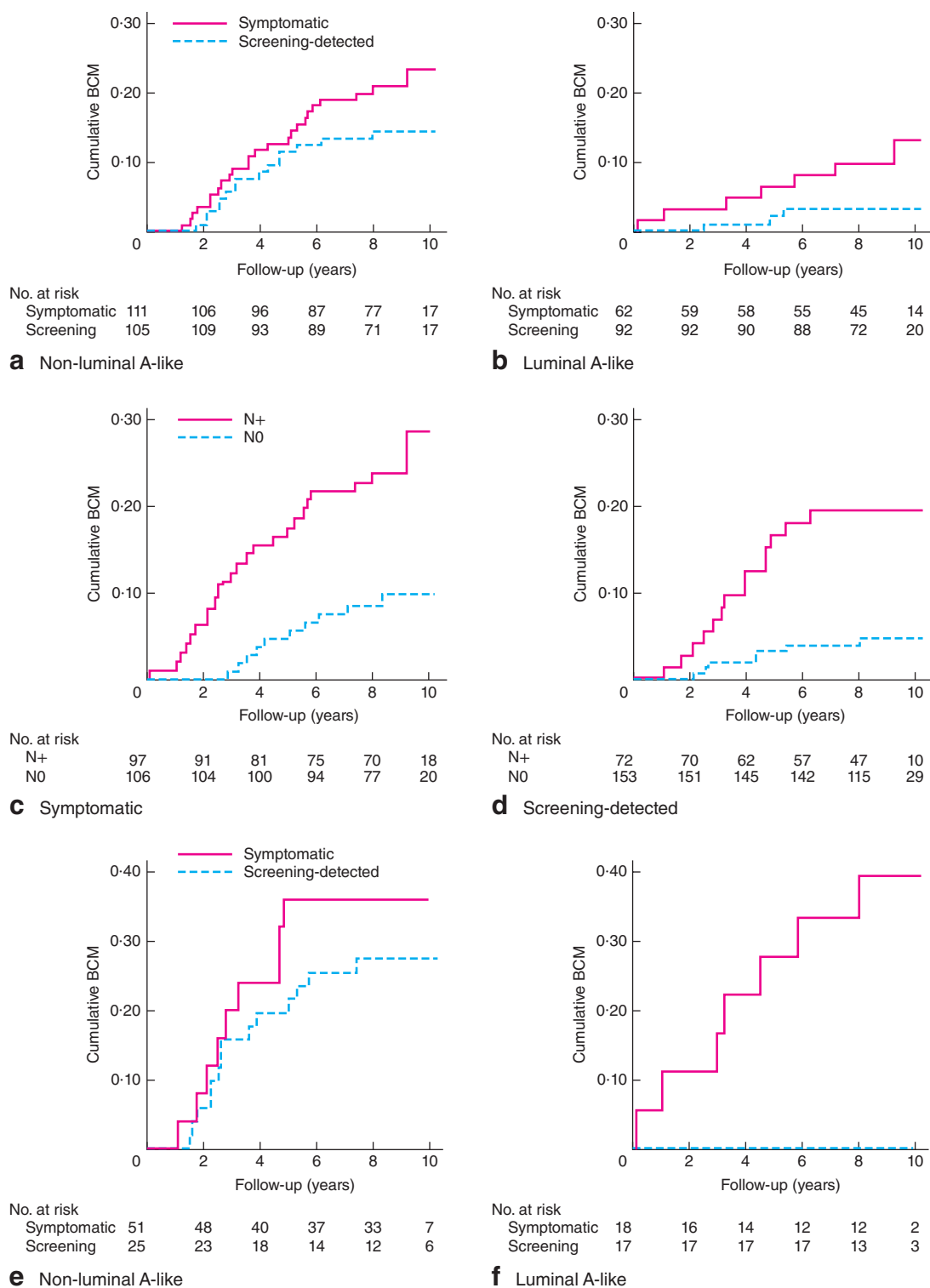Values in parentheses are percentages. *χ^2 test.

Fig. 3 Cumulative breast cancer mortality (BCM) by: **a,b** mode of detection (symptomatic *versus* screening-detected) in primary tumours (**a** non-luminal A-like; **b** luminal A-like); **c,d** lymph node status according to mode of detection (**c** symptomatic; **d** screening-detected); and **e,f** mode of detection (symptomatic *versus* screening-detected) in node-positive tumours (**e** non-luminal A-like; **f** luminal A-like). **a** $P = 0.175$, **b** $P = 0.053$, **c** $P = 0.002$, **d** $P < 0.001$, **e** $P = 0.420$, **f** $P = 0.005$ (Gray's test)

include Ki-67 scoring, and HER2 status was determined by IHC without fluorescence ISH. Kim et al.[4] and Crispo and co-workers[10] provided information on Ki-67 and HER2 status by IHC and fluorescence ISH, but restricted the outcome to 5 years of follow-up.

In Sweden, all inhabitants have a unique ten-digit personal identification code; this enabled retrieval of 10-year follow-up data on cause-specific mortality through the Swedish Register of Causes of Death (Central Statistics Office) for all included patients, ensuring a robust endpoint. In addition, detailed scoring of ER, PR and Ki-67 was available, confirming the association of well differentiated phenotype with hormone-responsive low-proliferating tumours with screening detection. This finding is supported by the results of Drukker et al.[24], who performed molecular profiling by means of a 70-gene signature within the MINDACT (Microarray In Node negative and 1 to 3 node positive lymph node Disease may Avoid ChemoTherapy) trial, showing that 68 per cent of the patients had a low-risk profile.

The distribution of St Gallen molecular subtypes in primary tumours differed significantly according to the mode of detection, with a shift to more non-luminal A-like tumours in symptomatic patients, especially an increase in the luminal B-like HER2-positive subtype. Screening detection added prognostic information in the luminal A-like molecular subgroup of primary tumours, suggesting an excellent prognosis with a BCM of 3 per cent at 10 years. Accordingly, the interaction between screening detection and the luminal A subgroup was analysed, but the term of interaction was not significant and Freedman's statistic showed that less than 5 per cent of the survival benefit in screening cancers was explained by the molecular subtypes (*Fig. 2*). One of the reasons for this may be the limited number of patients included. Kim and colleagues[4] performed survival analysis by individual St Gallen molecular subtypes with similar findings after a shorter follow-up, but did not report any interaction analyses. Several groups[3,22,25] have previously investigated the independent prognostic significance of mode of detection. Wishart et al.[26] reported a small but significant positive impact of screening detection on survival that could not be explained by tumour biology alone. Dawson and colleagues[23] found that more than 30 per cent of the survival benefit in patients diagnosed within a screening programme remained unexplained after adjusting for NHG and stage migration. Although the survival benefit is not explained fully by stage and tumour characteristics, it has been observed that screening-detected tumours indeed have an advantageous profile[24,27]. In the present study, the stepwise multivariable analysis showed a limited impact of mode of detection on survival

benefit after adjustment for individual biomarkers. When stage (tumour size and node status) was added into the model, the evidence for a beneficial effect of screening detection on survival almost disappeared. Calculation of Freedman's statistic revealed that tumour size and node status explained 48·5 per cent of the survival benefit in patients within the screening programme. Individual biomarkers (ER, PR, Ki-67 and HER2) accounted for another 13·8 per cent, whereas NHG explained 8·9 per cent of the survival effect in this cohort.

Although patients diagnosed with breast cancer within the screening-detected cohort generally had a good prognosis, those with screening-detected breast cancer and nodal metastases had an inferior outcome that was independent of other prognostic factors (*Fig. 3c,d*). This illustrates that the screening population is a heterogeneous cohort, and so caution should be exercised when estimating an individual patient's prognosis. In fact, there was no difference in outcome for patients with nodal metastases in relation to the mode of detection, and the distribution according to nodal St Gallen subtypes showed no significant difference between symptomatic and screening-detected tumours (*Table 1*). In the survival analysis of St Gallen classification in lymph node metastases, the beneficial effect of screening detection was restricted to the luminal A-like subtype, similar to the findings for primary tumours. Screening detection and luminal A-like subtype in primary tumours and lymph node metastases identify a low-risk subgroup of patients, but the clinical relevance of this finding in nodal metastases remains to be determined.

A limitation of this paper is lack of identification of the interval cancer population. Cancers that were detected or had developed between two consecutive routine screening sessions were included in the symptomatic group. This might have affected the results, but the number of patients with interval cancers was estimated to be small and may not have influenced the outcome. Data on interval cancer have not always been reported[4], and in Sweden the National Cancer Register for breast cancer reports data on screening detection only, and not on interval cancers.

In the present prospective cohort, screening-detected tumours were associated with St Gallen luminal A-like subtype, and these patients had an excellent prognosis in terms of 10-year BCM, supporting previous findings[4,27]. As low-risk hormone receptor-positive tumours tend to relapse after more than 10 years, longer follow-up is recommended to define the relevance of mode of detection as a prognostic factor for patients with luminal A-like tumours. Despite the analysis of tumour characteristics and scoring of molecular profiles according to current guidelines and by qualified pathologists, the survival benefit related

to screening detection could not be explained by validated molecular characteristics, and stage migration seemed to account for most of the improved survival. Although the screening-detected cohort generally had good prognoses, patients with screening-detected breast cancer and nodal metastases had negative outcomes that were independent of other prognostic factors. Tailoring adjuvant therapy in breast cancer can be improved by considering the mode of detection along with stage and molecular subtypes to avoid overtreatment or undertreatment.

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Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1 Pyramid diagrams showing distribution of oestrogen receptor, progesterone receptor and Ki-67 according to mode of detection (Word document)

Fig. S2 Cumulative breast cancer mortality according to mode of detection: symptomatic *versus* screening-detected breast cancer (Word document)

Table S1 Patient and tumour characteristics according to mode of detection (Word document)