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Altered expression of E-cadherin in breast cancer: patterns, mechanisms and clinical significance

K.S. Ásgeirsson [a], J.G. Jónasson [b], L. Tryggvadóttir [c], K. Ólafsdóttir [b], J.R. Sigurgeirsdóttir [b], S. Ingvarsson [b], H.M. Ögmundsdóttir [a,*]

[a] *Molecular and Cell Biology Laboratory, Icelandic Cancer Society, PO Box 5420, Reykjavík, Iceland*
[b] *Department of Pathology, National Hospital, Reykjavík, Iceland*
[c] *Icelandic Cancer Registry, Icelandic Cancer Society, Reykjavík, Iceland*

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Abstract

Reduced cell adhesion brought about by altered surface expression of E-cadherin has been implicated in invasive and metastatic malignant growth. We investigated the patterns of immunohistochemical E-cadherin expression in 120 breast carcinomas. Furthermore, we analysed DNA from the same samples for loss of heterozygosity (LOH) using three separate microsatellite markers on chromosome 16q22.1. Finally, the clinical outcome was ascertained for 108 patients. 19% (18/97) of infiltrating ductal carcinomas showed complete loss of E-cadherin expression compared with 64% (9/14) of infiltrating lobular carcinomas. LOH was detected in 46% (24/52) of infiltrating ductal carcinomas and 89% (8/9) of infiltrating lobular carcinomas. In the infiltrating lobular carcinomas, LOH was associated with complete loss of cell membrane expression of E-cadherin, although a cytoplasmic expression pattern was evident. In contrast, this association was not seen in the infiltrating ductal carcinomas. In a multivariate analysis, loss of E-cadherin expression was shown to be a significant independent risk factor for a poorer disease-free survival ($P=0.019$), in particular in the node-negative subset of patients ($P=0.029$). Significance was also approached for breast cancer corrected survival ($P=0.056$). We conclude that different mechanisms are involved in the altered E-cadherin expression seen in different subtypes of breast carcinomas. Furthermore, we implicate loss of E-cadherin, regardless of the genetic causes, as an independent prognostic marker for disease recurrence, especially in node-negative breast cancer patients, irrespective of the histological type.

Keywords: Breast cancer; E-cadherin; Prognosis; Node-negative breast cancer

1. Introduction

The development of malignant tumours is in part characterised by alterations in cell–cell adhesion of the tumour cells and their ability to grow invasively into surrounding tissue [1]. *In vitro* studies have shown that the Ca^{2+}-dependent adhesion molecule, E-cadherin, which is expressed on the surface membranes of epithelial cells, plays a crucial anti-invasive role [2]. When E-cadherin antibodies are added to the medium of MDCK epithelial cells, a decrease in intercellular adhesion and an increase in invasiveness becomes evident [3]. Down-regulation of E-cadherin expression of these cells by sarcoma virus transformation results in an invasive phenotype [4]. Furthermore, studies on cell lines derived from various carcinomas have shown that decreased E-cadherin expression is associated with dedifferentiation, invasiveness of epithelial cells and formation of metastases [4–6]. A study in transgenic mice has even suggested that loss of E-cadherin-mediated cell adhesion may play a causal role in the progression from benign adenoma to malignant carcinoma [7]. The implication of E-cadherin in carcinogenesis is supported by the fact that alterations in its expression are frequently seen in various carcinomas including breast cancer [8–12]. The clinical significance of these findings varies between different types of carcinomas, although an association between decreased E-cadherin expression and shortened disease-free survival and poor prognosis has been observed in various malignancies such as renal cell carcinoma, gastric carcinoma and bladder carcinoma [13–16].

* Corresponding author. Tel.: +354-540-1909; fax: +354-540-1905.
E-mail address: helgam@krabb.is (H.M. Ögmundsdóttir).

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For breast cancer, only a few studies have addressed the long-term prognostic effects of altered E-cadherin expression and most of them have indicated an association between altered E-cadherin expression and decreased overall survival [8,17–19]. In one of these studies this association was restricted to axillary node-negative breast cancer patients [18].

The mechanisms leading to alterations in E-cadherin expression are poorly understood. These may include mutations in the E-cadherin gene, post-translational modifications of E-cadherin and abnormal function of the normally expressed E-cadherin molecule because of alterations in its associated proteins [20–23]. *In vitro* studies have shown that when the cytokine IL-6 was added to the medium of three breast cancer cell lines, decreased cell adhesion became evident several days later and this was associated with a decrease in E-cadherin expression of these cells [24]. Furthermore we found that IL-6 serum levels were raised in a significant number of breast cancer patients and that E-cadherin expression of their tumours was often altered, although a direct association could not be established.

In the present study, we examined the pattern of immunohistochemical expression of E-cadherin in paraffin sections from 120 primary breast carcinomas and 37 lymph node metastases. In an attempt to elucidate better the mechanisms by which altered E-cadherin expression occurs we analysed DNA from the same samples for loss of heterozygosity (LOH) of the E-cadherin gene using three microsatellite markers located on chromosome 16q22.1. Furthermore, we did a mutational analysis on four lobular breast carcinomas, two of which showed a cytoplasmic E-cadherin expression pattern on immunohistochemistry and LOH on polymerase chain reaction (PCR). Finally, we evaluated the clinical significance of our findings.

2. Patients and methods

2.1. Tumour specimens and patients

Paraffin sections from 120 non-selected primary breast tumours and 37 lymph node metastases were collected from patients undergoing surgery over a 4-year period, between January 1990 and September 1994. These tumours were kindly supplied by the Dungal's sample collection, Department of Pathology, University of Iceland.

Information on tumour size, lymph node metastases at diagnosis and oestrogen receptor expression was made available from the Department of Pathology, National University Hospital of Iceland and the Icelandic Cancer Registry. Patient registries from the St Joseph's Hospital in Hafnarfjördur, the City Hospital and the National Hospital in Reykjavík were evaluated for information on TNM staging, disease recurrence, metastases development and survival of patients. Additional information on the present disease status was kindly supplied by the patients' oncologists if information in the hospital records was not sufficient.

2.2. Immunohistochemical staining

E-cadherin expression was detected by the 5H9 monoclonal antibody (kindly donated by Professor Walter Birchmeier and Jürgen Behrens, Max-Delbrück-Centrum for Molecular Medicine, Berlin, Germany). Paraffin-embedded 4 μm tumour sections were placed on super frost/plus slides from Menzel-Gläser and dried in an oven at 60°C for 60 min and then at 37°C for 24 h. Thereafter sections were deparaffinised, rehydrated and rinsed in tap water before antigen retrieval by heating in a 0.01 M citric buffer (pH 6.0) twice for 5 min in a Toshiba microwave oven (ER-6E2W) at 850 W. Sections were incubated with 5H9 monoclonal antibody (1:10) overnight at room temperature. Immunohistochemical staining was visualised, using the StreptAB-Complex/horse radish peroxidase (HRP) Duet, Mouse/Rabbit (1:100) (from DAKO, Glostrup, Denmark) according to the manufacturer's instructions.

2.3. Immunohistochemical analysis

E-cadherin expression in a fibroadenoma was used as a positive control. The status of E-cadherin expression was evaluated independently by three of the authors. If any discrepancies between classification of samples arose, they were reviewed and the final result was reached by consensus. Tumours were classified by intensity of staining and the percentage of cells showing loss of E-cadherin expression. These two parameters were then combined into a final classification of staining as follows (Table 1): positive, where staining was similar to the positive control sample; heterogeneous, staining not as intense as the positive control and tumours showing a mixed population of E-cadherin-positive and -negative cells; negative, where most or all tumour cells showed no staining or where cytoplasmic as opposed to membrane staining was observed.

2.4. DNA extraction and analysis

It was possible to extract DNA from 86 breast carcinomas and peripheral blood using conventional phenol/chloroform methods. The DNA was subjected to PCR amplification using DynaZyme polymerase (Finnzymes Oy, Espoo, Finland) in the buffer solution provided by the manufacturer.

The PCR amplification was carried out in 25 μl reaction volumes in 96-well plates (Techne, Cambridge, UK), using 25 ng of genomic DNA, 1.8 pmol of the

Table 1
Classification of tumours according to E-cadherin expression

	% Cells showing loss of E-cadherin expression		
	> 50	5–50	< 5
Intensity of staining			
−	Negative		
+/−	Heterogeneous	Heterogeneous	Heterogeneous
+ +	Heterogeneous	Positive	Positive

forward and reverse primers, 3.3 nmol of each dNTP and 0.67 units of DynaZyme polymerase. A hot start was used by adding the enzyme during the first cycle at approximately 72°C, after pre-incubation at 94°C for 5 min. The samples were amplified in 35 cycles composed of 30 s of denaturation at 94°C, 30 s annealing at 55°C and finally 60 s of extension at 72°C. Three microsatellite markers were used; D16S421 (forward: 5′-ACATGAACCGATTGGACTGA-3′, reverse: 5′-CCGTTCCCTATATTTCCTGG-3′), D16S496 (forward: 5′-GAAAGGCTACT-TCATAGATGGCAAT-3′), reverse: 5′-ATAAGCCACTGCGCCCAT-3′) (both from Research Genetics, Huntsville, AL, USA) and D16S545 (which was kindly donated by Anne-Marie Cleton (Department of Human and Clinical Genetics, Leiden, The Netherlands), forward: 5′-ACTTGAAA-TGCAGAGTCCAGAA-3′, reverse: 5′-TTGCCTGG-AGCACATTAGC-3′).

The primers for SSCP (single-strand conformation polymorphism) and DNA sequencing of genomic DNA by the solid phase method were according to Berx and colleagues [21]. All reverse primers were biotinylated. The PCR products were immobilised on solid support using streptavidin beads (M-280/Dynabeads, Dynal, Oslo, Norway) and denatured with alkaline. The DNA template was sequenced using sequenase and [α^{33}P] dATP (Amersham, Aylesbury, UK). Amplification for the sequencing and SSCP analyses of exon 13 were carried out using the following primers at an annealing temperature of 60°C: forward: 5′-TTTCCTCCCCTG-GTCTCATC-3′; reverse: 5′-TGAGTCACTTGCCAG-CTGGA-3′.

The PCR products were subjected to denaturing gel electrophoresis in 6.5% polyacrylamide, 8 M urea denaturing gel for LOH analysis or denatured in a formamide buffer and subjected to a non-denaturing polyacrylamide gel (MDE (mutation detection enhancement, Rockland, Maine, USA) solution/FMC BioProducts) for SSCP analysis. They were then transferred to a positively-charged nylon membrane, Hybond-N$^+$ (Amersham) and baked for at least 2 h at 80°C. Films were inspected visually by at least two reviewers, comparing the intensity of alleles from normal and tumour DNA. Absence or significant decrease of one allele in the tumour compared with the normal reference sample was considered as LOH.

2.5. Statistical analysis

The Chi square analysis and Fisher's exact test were used for comparison of numbers in each group and the Student's *t*-test was used for comparing mean values. Two primary clinical endpoints were looked at in this study; death from breast cancer and disease recurrence. Follow-up was until the time of the last follow-up visit or the diagnosis of recurrent disease or death, whichever came first. Disease recurrence was considered evident when either local or distal metastases were diagnosed. Data on survival were censored if the patient was still alive and without evidence of recurrent disease at the time of the last follow-up visit or at the time of death from other causes. Survival curves were constructed according to the Kaplan–Meier method [25]. Univariate comparisons of survival for all breast cancer patients and for lymph node-negative patients were conducted on the basis of E-cadherin status with a log-rank analysis. The 5 patients with *in situ* ductal carcinomas, 1 with tubular, 3 with mucinous carcinomas and 1 patient with infiltrating ductal carcinoma, who had evidence of distant metastases at time of diagnosis, were excluded from survival curve evaluation. Information on clinical follow-up for 2 patients with infiltrating ductal carcinomas was lacking. The Cox proportional-hazards model [26] was used in the multivariate analysis to assess the contribution of the following covariates; age of patient, lymph node status, tumour size and oestrogen receptor status. These covariates were retained in the model to illustrate the independent effect of E-cadherin status. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of patients

Table 2 shows the relevant clinical characteristics of the 120 breast cancer patients analysed for E-cadherin expression. They were grouped by immunohistochemical E-cadherin expression into 27 (23%) patients with negative staining and 93 (78%) patients with evident E-cadherin staining of tumours (either positive or heterogeneous). There was no significant difference between age or follow-up of the positive and heterogeneous E-cadherin staining groups on the one hand and the negative staining group on the other. In addition, the two E-cadherin staining groups in Table 2 did not differ significantly with respect to axillary node status, oestrogen receptor expression or tumour size.

3.2. E-cadherin expression in primary breast cancer and lymph node metastases according to histopathology

In all, 120 paraffin sections from primary breast carcinomas and 37 lymph node metastases were stained

Table 2
Characteristics of 120 breast cancer patients and tumours that were evaluated for E-cadherin expression[a]

Characteristics	Total	E-cadherin staining groups	
		Positive and heterogeneous	Negative
Total (%)		93 (78)	27 (23)
Median age at diagnosis, year (range)	58 (29–92)	59.2 (29–92)	57.1 (29–83)
Median follow-up, months (range)	71 (3–108)	71 (3–108)	71 (12–107)
Axillary node status (117 samples informative)			
Node-negative, *n* (%)	49 (42)	37 (41)	12 (46)
Node-positive, *n* (%)	68 (58)	54 (59)	14 (54)
Tumour size (95 samples informative)			
⩽5 cm (%)	82 (86)	62 (85)	20 (91)
>5 cm (%)	13 (14)	11 (15)	2 (9)
Oestrogen receptor expression (105 samples informative)			
Oestrogen-positive, *n* (%)	71 (68)	55 (67)	16 (70)
Oestrogen-negative, *n* (%)	34 (32)	27 (33)	7 (30)

[a] None of the differences between the two E-cadherin groups were statistically significant.

Table 3
E-cadherin staining according to histopathology (number of tumours)

Histopathology of primary breast cancer:	E-cadherin staining groups			Total
	Positive	Heterogeneous	Negative	
	n (%)	*n* (%)	*n* (%)	*n* (%)
In situ ductal carcinoma (non-comedo type)	1 (33)	2 (67)	–	3 (3)
In situ ductal carcinoma (comedo type)		2 (100)	–	2 (2)
Infiltrating ductal carcinoma NOS	24 (25)	55 (57)	18 (19)	97 (81)
Infiltrating lobular carcinoma	1 (7)	4 (29)	9 (64)	14 (12)
Tubular carcinoma	1 (100)	–	–	1 (1)
Mucinous carcinoma	–	3 (100)	–	3 (3)
Total	27 (23)	66 (55)	27 (23)	120 (100)
Lymph node metastases	9 (24)	20 (54)	8 (22)	37 (100)

with monoclonal antibodies to E-cadherin. Table 3 shows E-cadherin expression according to histological type of tumours.

Histologically, 97 (81%) tumours were infiltrating ductal carcinomas, 14 (12%) lobular carcinomas, 3 (3%) mucinous, 1 (1%) tubular, 5 (4%) *in situ* ductal; thereof 2 of the comedo type. In Table 3, E-cadherin staining is grouped according to histopathological type of tumour. As Table 3 shows, 73 out of 97 (75%) of the infiltrating ductal carcinomas show a moderate to a substantial decrease in E-cadherin expression (heterogeneous or negative staining). It should be noted that complete loss of E-cadherin expression was significantly more frequent in lobular carcinoma as 64% (9/14) showed negative staining compared with 19% of the infiltrating ductal carcinomas (18/97) ($P = < 0.001$) although the small numbers in the former group ($n = 14$) compared with the latter group ($n = 97$) should be noted. Furthermore, the lobular carcinomas often showed a staining pattern that was not seen in other types, namely cytoplasmic staining instead of membrane staining. This staining pattern was classified as negative. Fig. 1 shows examples of the staining patterns observed.

In 37 samples (Table 4), the lymph node metastases were available for staining of E-cadherin expression. In 25 (68%), E-cadherin expression was identical to the expression of their primary tumours.

3.3. Association of E-cadherin expression with LOH

Of the 86 samples evaluated for loss of heterozygosity (LOH) with three markers on chromosome 16q close to the E-cadherin gene, 61 samples were informative. LOH

Table 4
Comparison of E-cadherin staining of primary tumour and lymph node metastases ($n = 37$)

	Primary tumour E-cadherin staining groups		
	Positive	Heterogeneous	Negative
Lymph node metastases E-cadherin staining groups			
Positive	5	2	2
Heterogeneous	4	14	2
Negative	0	2	6

was detected in 52% (32/61) of these samples but was clearly more common in the infiltrating lobular carcinomas (89%; 8/9 informative samples) (Table 5). In the infiltrating lobular carcinomas, we observed that LOH was most commonly associated with complete loss of E-cadherin expression (6/8 samples), although the sample group was too small for statistical analysis. Such a tendency was not seen in the infiltrating ductal carcinomas, where LOH was more commonly associated with heterogeneous rather than complete loss of E-cadherin expression (71%, 17/24 samples). This heterogeneous type of E-cadherin expression was also seen in 43% (12/28) of samples without LOH. Nevertheless, 3 out of 24 (13%) infiltrating ductal carcinomas with LOH retained strong positive E-cadherin staining, whereas 5 out of 28 (18%) without LOH were E-cadherin negative.

In the infiltrating lobular carcinomas, a cytoplasmic staining pattern was seen in many cells in all of the six samples that showed both LOH and loss of E-cadherin membrane expression on immunohistochemistry (Fig. 1). In contrast, cytoplasmic staining was never detected in the infiltrating ductal carcinomas (Fig. 1).

Two of the infiltrating lobular carcinomas that showed cytoplasmic staining and LOH, one that showed cytoplasmic staining where PCR was non-informative and one that showed heterogeneous staining without

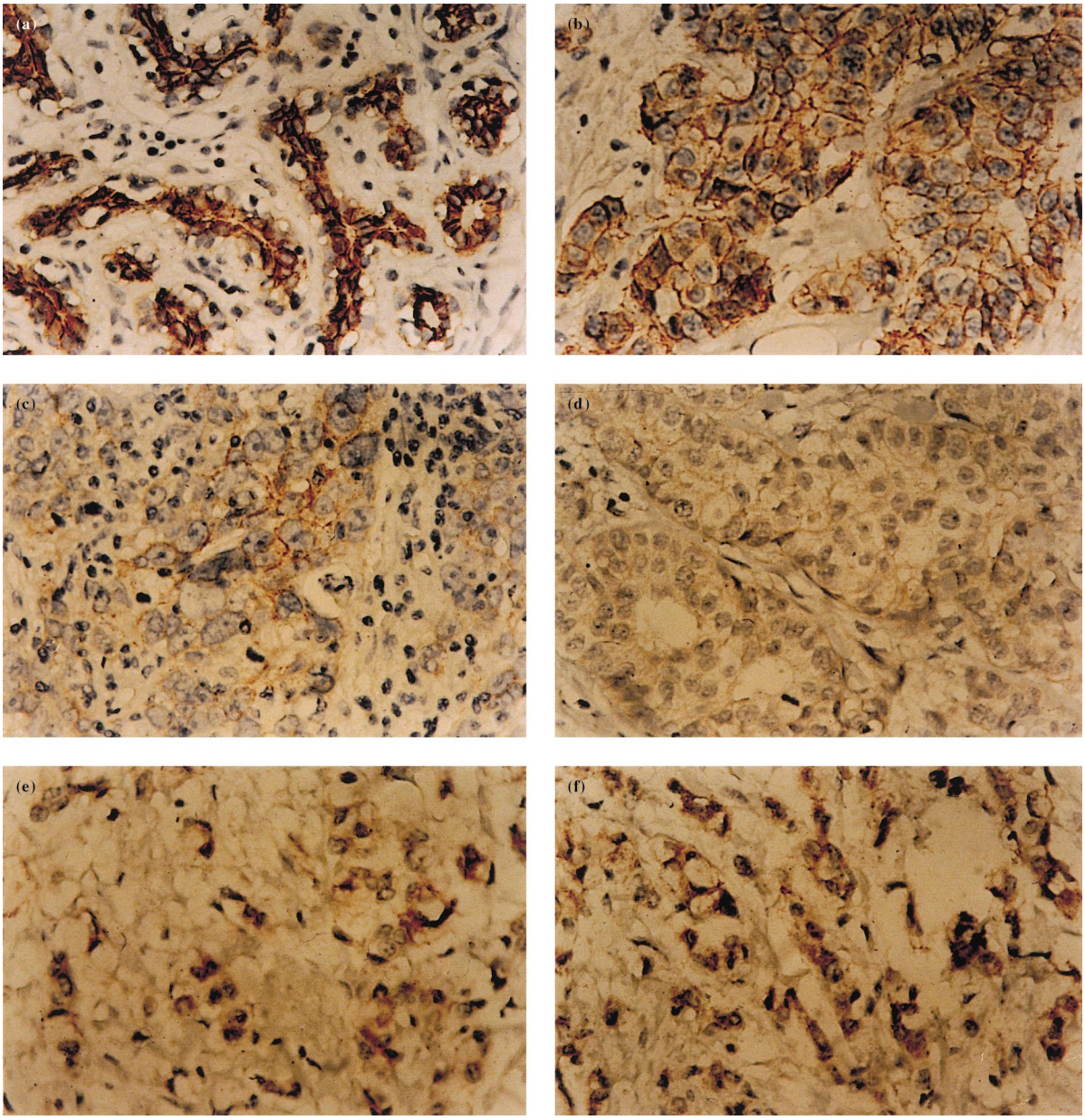

Fig. 1. Patterns of E-cadherin expression in breast carcinoma. (a) Shows a fibroadenoma sample which was used as a positive control; (b) represents an infiltrating ductal breast carcinoma sample showing positive E-cadherin expression; whereas sample (c) shows heterogeneous expression; and sample (d) is negative. Lobular breast carcinomas with cytoplasmic E-cadherin expression are shown in (e) and (f). Original magnification ×600.

Table 5
Association of E-cadherin with LOH ($n=61$ informative)[a]

		E-cadherin staining groups			
		Positive	Heterogeneous	Negative	Total
Infiltrating ductal carcinomas	+LOH	3 (13)	17 (71)	4 (17)	24 (46)
	−LOH	11 (39)	12 (43)	5 (18)	28 (54)
Infiltrating lobular carcinomas	+LOH	0	2 (25)	6 (75)	8 (89)
	−LOH	0	1 (100)	0	1 (11)

[a] LOH, loss of heterozygosity.

LOH, were available for SSCP analysis and DNA sequencing for E-cadherin gene mutations. One tumour with cytoplasmic staining and LOH was found to have a somatic mutation at the exon 13–intron 13 boundary. The mutation was a base substitution in the first nucleotide in intron 13 from G to A.

3.4. Association of E-cadherin expression with clinical parameters, disease recurrence and survival

Patient records on local disease recurrence, development of metastases and breast cancer-corrected survival were available for 108 (90%) of the 120 patients. As shown in Fig. 2a, a trend towards a shorter disease-free survival was seen in patients having tumours with complete loss of E-cadherin expression ($P=0.08$). When axillary node-negative patients were analysed separately, this trend nearly reached significance ($P=0.06$) (Fig. 2b). Of the 11 E-cadherin negative/node-negative patients, 3 were infiltrating lobular- and 8 were infiltrating ductal carcinomas. When known prognostic factors, such as age of patients, lymph node status, tumour size and oestrogen hormone receptor expression were included in the multivariate Cox analysis, loss of E-cadherin expression was shown to be a significant independent risk factor for disease recurrence ($P=0.019$) and approaching significance for breast cancer-corrected survival ($P=0.056$) (Table 6). This prognostic significance was furthermore evident in node-negative breast cancer patients but not in node-positive patients, when these patient groups were analysed separately (Table 6). The effects of loss of E-cadherin expression on breast cancer-corrected survival in the node-negative patients did not reach significance. Loss of E-cadherin expression showed the highest hazard ratio for disease-free survival amongst the factors analysed for the whole group and particularly in node-negative patients and was the only factor reaching statistical significance in this latter subgroup of patients (Table 6). LOH did not have an effect on disease-free survival or breast cancer-corrected survival. In the whole patient group 72% received adjuvant chemo- and/or hormonal therapy, and treatment did not differ widely with respect to E-cadherin expression, i.e. 73% and 67% received treatment in the positive/heterogeneous group and negative group-respectively. However, in the node-negative patient group, the percentage receiving therapy was generally lower (64%) but higher in the E-cadherin-negative patients (57%) than the E-cadherin-positive/heterogeneous patients (40%).

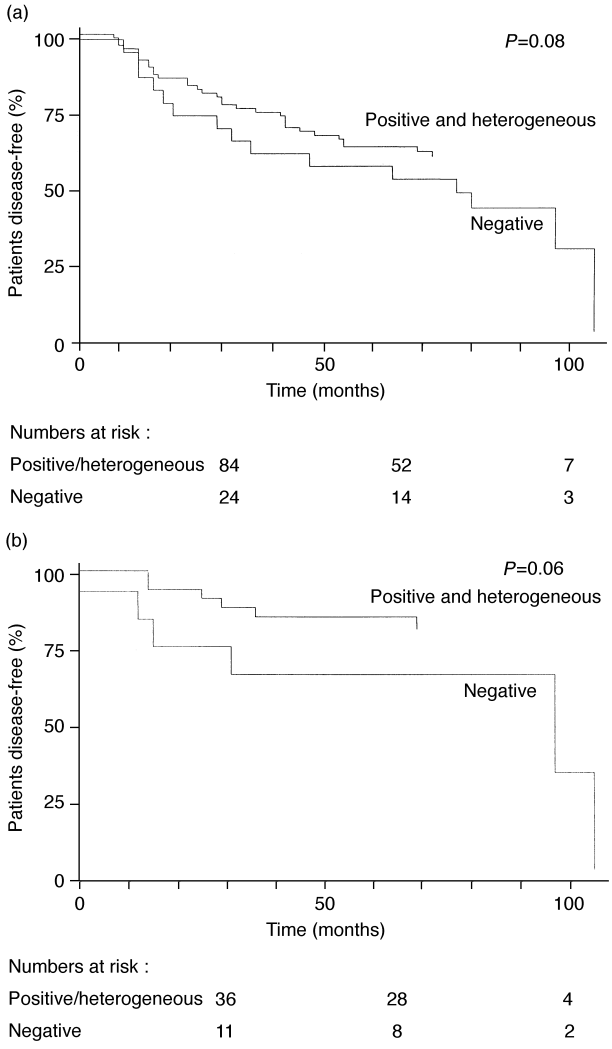

Fig. 2. Disease-free survival in relation to E-cadherin expression. Evaluation was only carried out for infiltrating ductal and lobular carcinoma cases where $n=108$ were available. The number of patients in both E-cadherin expression groups at risk are shown for all patients at 0, 50 and 100 months. (a) All patients; (b) axillary lymph node-negative patients only.

Table 6
Cox analysis of hazard ratio estimates of individual prognostic factors

	Hazard ratio (95% confidence interval)	*P* value
All patients — BCCS		
Axillary lymph nodes	2.00 (0.90–4.43)	0.089
Tumour size	2.36 (1.31–4.26)	0.004
Age of patient	1.00 (0.97–1.02)	0.784
Negative oestrogen receptor expression	0.70 (0.33–1.49)	0.353
Negative E-cadherin expression	2.20 (0.98–4.96)	0.056
All patients — DFS		
Axillary lymph nodes	2.03 (0.96–4.29)	0.064
Tumour size	1.86 (1.51–2.44)	0.029
Age of patient	0.99 (0.96–1.01)	0.264
Negative oestrogen receptor expression	1.11 (1.16–5.14)	0.788
Negative E-cadherin expression	2.44 (1.12–5.14)	0.019
Node-negative patients — DFS		
Tumour size	2.12 (0.79–5.68)	0.133
Age of patient	0.98 (0.94–1.02)	0.311
Negative oestrogen receptor expression	0.55 (0.14–2.10)	0.378
Negative E-cadherin expression	5.03 (1.18–21.41)	0.029
Node-positive patients — DFS		
Tumour size	2.93 (1.23–6.98)	0.016
Age of patient	0.99 (0.95–1.03)	0.621
Negative oestrogen receptor expression	1.71 (0.54–5.44)	0.366
Negative E-cadherin expression	1.82 (0.70–4.73)	0.223

BCCS, breast cancer-corrected survival; DFS, disease-free survival.

4. Discussion

In this study we have shown that decreased E-cadherin expression is frequent in breast cancer and that complete loss of E-cadherin expression is associated with LOH in the infiltrating lobular breast carcinomas but not in the infiltrating ductal carcinomas. We also found that the infiltrating lobular breast carcinomas often show a different immunohistochemical staining pattern for E-cadherin, specifically a cytoplasmic staining pattern, which is unlike the plasma membrane pattern seen in ductal carcinomas. Finally, we found that loss of E-cadherin expression in breast cancer tumours is a significant prognostic factor. To the best of our knowledge, this is the first study to show that loss of E-cadherin expression is a significant independent risk factor for disease recurrence in axillary node-negative breast cancer patients, and in this patient group this seems to be a stronger independent prognostic factor for recurrent disease than the age of the patient, tumour size or negative oestrogen receptor expression although the 95% CIs were fairly wide.

Although many studies have shown that altered E-cadherin expression in various cancer types correlates with tumour dedifferentiation [9–12] and poor prognosis [8,13–19], the mechanisms by which these alterations occur are as yet only partially understood. Somatic mutations affecting expression of the E-cadherin gene, post-translational modifications and abnormal function of the normally expressed molecule because of alterations in its associated proteins, are candidate explanations, supported by both *in vitro* and *in vivo* studies [20–23,27]. Furthermore, germ line mutations in the E-cadherin gene have recently been found to occur in familial gastric cancer [28].

Studies on breast cancer have shown that LOH and mutations in the E-cadherin gene are frequently found in infiltrating lobular carcinomas but not in the infiltrating ductal subtype [29,30]. Our results support these findings. Firstly, we saw LOH on chromosome 16q22.1 in all but one of the nine infiltrating lobular carcinomas and LOH was usually, although not always, associated with complete loss of E-cadherin (6/8). The specific cytoplasmic staining pattern was in one case shown to be associated with a somatic E-cadherin gene mutation in the exon 13–intron 13 boundary. This probably results in loss of the transmembrane and cytoplasmic domains of the protein, thus affecting the correct membrane localisation. In one further sample with confirmed LOH and cytoplasmic E-cadherin expression, no mutation was found, suggesting that loss of one copy of the E-cadherin gene may affect the expression of the remaining allele.

Different characteristics were seen in infiltrating ductal breast carcinomas. LOH was not as common as in the infiltrating lobular subtype, and even when present, immunohistochemical E-cadherin expression was often evident. Furthermore, loss of E-cadherin expression could occur without LOH. It is unlikely that the immunohistochemistry failed to detect E-cadherin when present

in samples without LOH since antigen retrieval and a sensitive secondary detection system were used. The discrepancies between LOH and immunostaining could have several explanations. A gene other than the E-cadherin gene may be the target of the 16q22.1 deletion in ductal carcinomas or the retained E-cadherin gene could possibly be functional. Mechanisms, other than gene deletions can cause loss of E-cadherin expression, including transcriptional defects and CpG methylation [31,32]. *In vitro* studies in our laboratory have shown that the cytokine IL-6 can cause a reversible decrease in E-cadherin expression in breast cancer cell lines [24], and this could provide a mechanism for dislodgement of cells from a primary tumour with re-expression as a metastasis becomes established elsewhere.

One of the drawbacks of immunohistochemical studies is that it is not a functional analysis of the molecule in question. Several studies have shown that normal immunohistochemical expression of E-cadherin can be associated with a compromised functional ability. For instance, *in vitro* studies have shown that defects in catenins (α, β, γ) which link E-cadherin to the submembrane cytoskeletal matrix, can alter the functional ability of E-cadherin [33,34]. Alterations in the expression of these proteins can be seen despite retained E-cadherin expression [23,35]. Several studies on breast cancer cell lines have shown that appropriate gene expression and post-translational modifications of these catenins must be in place for E-cadherin to be fully functional [36,37]. In MDCK cells transformed by v-SRC transfection, the phosphorylation of the E-cadherin–catenin complex led to cell dissociation and invasiveness, although the expression of E-cadherin was retained [22].

The variable clinical outcome of breast cancer patients, has stimulated the search for new prognostic markers that have the ability to predict tumour behaviour. A long list of potential prognostic markers have now been identified [38], although the axillary lymph node status still remains the most important. For instance, the detection of mutations in the *TP53* gene and immunohistochemical overexpression of the c-erbB-2 protein has been correlated with a shorter overall survival of breast cancer patients [39,40]. The value of these new markers in the prognostic evaluation of axillary lymph node-negative breast cancer patients is, however, controversial. A prognostic panel involving the evaluation of tumour size, hormone receptor level and S-phase fraction has been shown to be able to identify those node-negative patients with a sufficiently low risk of disease recurrence so that adjuvant chemotherapy can be avoided [41].

The results of our study show that the clinical importance of E-cadherin in breast cancer depends on whether it is immunohistochemically expressed or not. In the multivariate analysis, loss of E-cadherin expression not only had an effect on breast cancer-corrected survival, but significantly increased the risk of disease recurrence in node-negative breast cancer patients. Several other studies support our findings [8,17–19]. Although they vary with respect to the types of tissue samples used (only Lipponen and colleagues used paraffin-embedded samples, whereas the other authors used frozen sections), the E-cadherin antibodies and the total follow-up, most of them indicate that the overall survival of the breast cancer patients is significantly decreased if their tumours show alterations in E-cadherin expression [17–19]. Only one study has shown a correlation between altered E-cadherin expression and a decreased disease-free survival [8]. The most recent study involving 179 breast cancer patients and a 10-year follow-up shows that the effect on survival seems to be most relevant in axillary node-negative patients [18]. It is unlikely that adjuvant therapy had an impact on our survival analysis since the proportion of node-negative patients receiving therapy was generally lower (64%) than in the group as a whole, although the E-cadherin-negative patients received more therapy (57%) than the E-cadherin-positive patients (40%) in this group.

Therefore, this study suggests that loss of E-cadherin expression, whether it is the result of gene mutations or other mechanisms, is an important prognostic marker, especially for disease recurrence in node-negative breast cancer patients and may even be more informative than tumour size or oestrogen receptor expression. Furthermore, immunohistochemical analysis is more informative, in this regard, than detection of LOH at the E-cadherin gene locus. Further studies are required in order to address the issue of whether the evaluation of E-cadherin expression can help, independently or in addition to conventional biological prognostic markers, to identify those axillary node-negative breast cancer patients who may truly benefit from adjuvant therapy.

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