Virchows Arch (1999) 434:227–234

ORIGINAL ARTICLE

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Myoepithelial cells and basal lamina in poorly differentiated in situ duct carcinoma of the breast

An immunocytochemical study

Received: 29 July 1998 / Accepted: 11 November 1998

Abstract A retrospective study was made of 38 selected brest tumours with a poorly differentiated in situ duct component. These were classified on haematoxylin and eosin (H&E) as ductal carcinoma in situ (DCIS; 10 cases), DCIS with invasion (17 cases) and DCIS with features suggestive of for stromal invasion (11 cases). The last were these lesions composed of neoplastic ducts with irregular outlines and a myoepithelial layer that was not clearly evident or large neoplastic ducts growing close together or surrounded by inflammatory desmoplastic stroma. Cases of DCIS involving areas of sclerosing adenosis were included in this category. Consecutive sections obtained from each case were studied with a panel of antibodies against myoepithelial cells (alpha smooth muscle actin and calponin) and basal lamina (BL) components (laminin and type IV collagen). It was found that in situ lesions showed well-formed basal lamina and/or an evident myoepithelial layer. These features were lacking in the invasive areas. Nine of the 11 cases with suggestive features of stromal invasion were reclassified as invasive duct carcinoma (5 cases)and DCIS (4 cases), according to the absence or presence of a continuous myoepithelial layer and/or basal lamina. In 2 such cases immunohistochemistry yielded equivocal results and the label "suggestive of invasion" was therefore pertinent. Immunohistochemistry facilitates the diagnosis of breast DCIS; myoepithelial and basal lamina markers are useful in differentiating microinvasive from in situ ductal carcinomas of the breast.

Key words Ductal carcinoma in situ · Immunohistochemistry · Myoepithelial cells · Basal lamina · Smooth muscle actin · Calponin

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Introduction

Poorly differentiated in situ carcinomas of the breast (PDCIS) are intraductal proliferations of nonpolarized [24] neoplastic cells growing in a solid, micropapillary or pseudocribriform pattern. The neoplastic cells also show marked nuclear pleomorphism, a high nuclear/cytoplasmic ratio and brisk mitotic count and/or apoptotic bodies [24]. In many instances PDCIS may pose a diagnostic problem, in that it is difficult to establish whether part of these lesions have invaded the stroma, especially when the neoplastic proliferations extend to the lobules or involve areas of sclerosing adenosis [15, 18–20, 30]. Cowen [11] stated that the diagnosis of invasion is confounded in some instances, as invasive carcinoma can simulate DCIS, a view further stressed in a later paper [12]. In situ lesions are invariably surrounded by a continuous layer of myoepithelial cells (MCs) and basal lamina (BL) whereas invasive lesions show discontinuous or fragmented BL, if present at all [2]. BL components and myoepithelial cells can be demonstrated by immunohistochemical methods [5–7, 31], and the most widely used markers in the detection of MCs are antibodies against smooth muscle actin [5–7, 14, 35]. Recently, a novel antibody raised against calponin, a smooth muscle component, has been reported to be useful in revealing MCs [38].

We have evaluated BL and MCs immunohistochemically in a series of poorly differentiated ductal carcino-

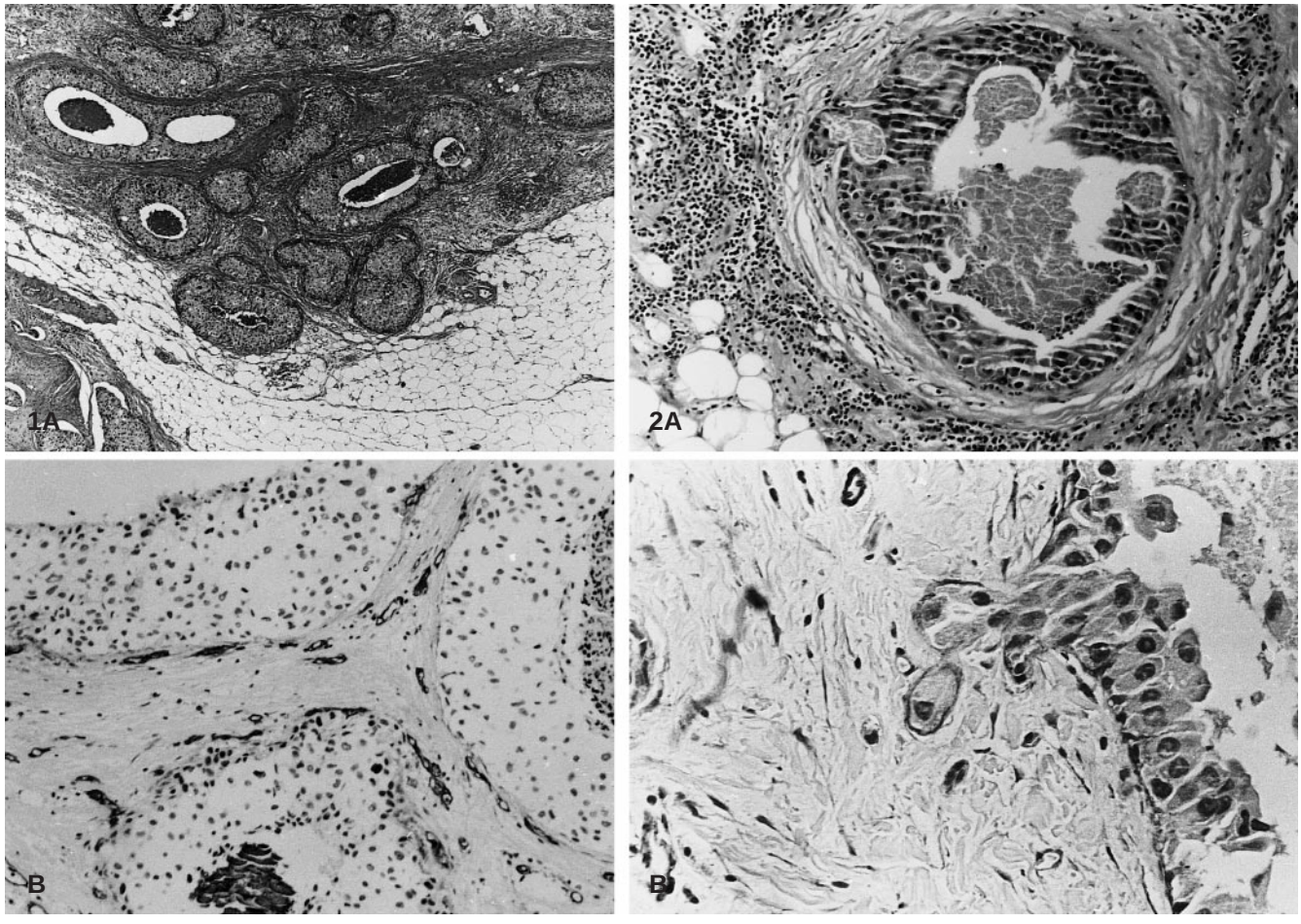


Fig. 1A, B Poorly differentiated DCIS with features of suspicious stromal invasion (case 38). **A** The neoplastic ducts are large and show irregular outlines. H&E, ×125 **B** Collagen IV is not present along the neoplastic ducts; only small vessels are stained. In the same case, laminin and calponin were negative, confirming the invasive nature of the tumour growth. ABC, ×350

Fig. 2A, B Poorly differentiated DCIS with features of suspicious stromal invasion (case 11). **A** This neoplastic duct shows irregular outline and myoepithelial cells are not clearly evident. In addition, a prominent inflammatory infiltrate is also present around the duct. H&E, ×250 **B** Anti-calponin antibody reveals the presence of a continuous layer of myoepithelium, even around the tiny nests of neoplastic cells which simulate stromal invasion. This case was included in the group of the DCIS cases. ABC, ×400

mas, with the aim of establishing more consistent morphological criteria to assess the presence or absence of an invasive phase in problematic cases.

Materials and methods

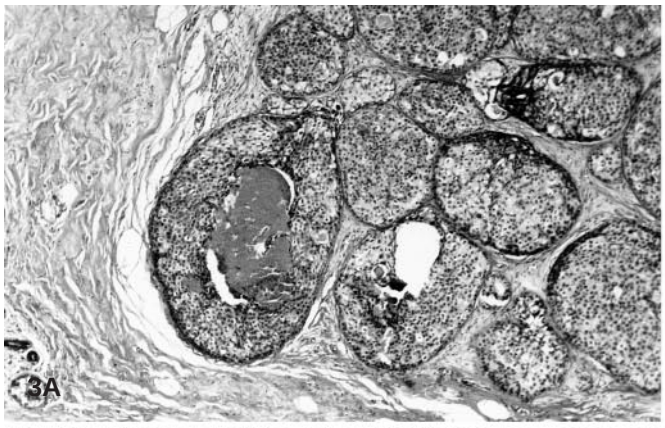
Specimens taken from 38 selected patients with poorly differentiated DCIS of the breast were retrieved from the files of the Institute of Anatomic Pathology of the University of Florence. All cases had been detected mammographically in the period between 1983 and 1991. Information on the clinical presentation of the lesions, treatment of the patients, and follow-up was obtained from the patients' attending physicians and/or from hospital records.

Tissue specimens were formalin fixed and paraffin embedded. Sections 5 µm thick from each lesion were stained with H&E. When several blocks were available the most representative was selected for H&E study and immunohistochemistry. Cases were classified by evaluating the H&E slides before immunohistochemical staining into three categories: DCIS, DCIS with invasion, and DCIS with features suggestive of stromal invasion.

DCIS were considered suggestive of invasion when one of the following features was present: (1) neoplastic ducts with irregular outlines and a myoepithelial layer that was not well defined (Figs. 1A, 2A); (2) large neoplastic ducts growing "back to back" (Fig. 3A); (3) lesions composed of neoplastic nests and cords in-

Fig. 3A–D Poorly differentiated DCIS (case 2). **A** The neoplastic ducts grow back to back, closely packed together. H&E ×100 **B** Collagen IV and **C** laminin are present along the basal lamina which outlines the ducts. ABC, ×100. **D** When the ducts are enlarged by the neoplastic proliferation, the myoepithelial cells are often attenuated and have to be distinguished from the actin-positive elements present around the capillaries adjacent to the expanded ducts. This case was included in the group of the DCIS cases. ABC ×400 ▶

Fig. 4A–D Poorly differentiated DCIS involving sclerosing adenosis (case 21). **A** The neoplastic proliferation is structured in small nests and cords, reminiscent of an invasive carcinoma. H&E, ×75 **B** At higher power there is no continuous layer of myoepithelial cells. H&E, ×125 **C** Collagen IV-positive basal lamina encircles most of the neoplastic nests and cords. ABC, ×200 **D** Anti-actin antibody highlights the attenuated myoepithelial layer in the same case. This case was included in the group of the DCIS cases. ABC, ×400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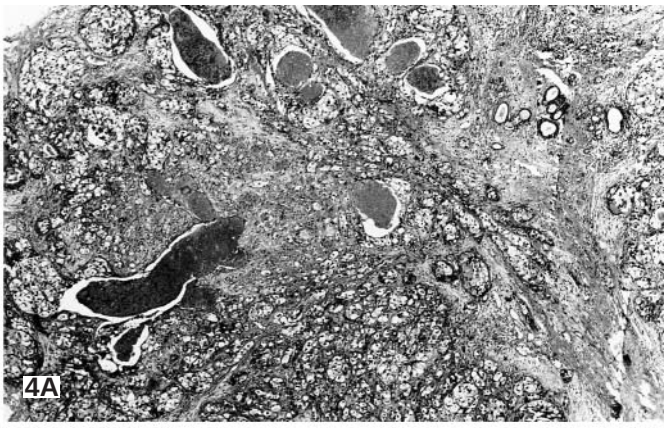
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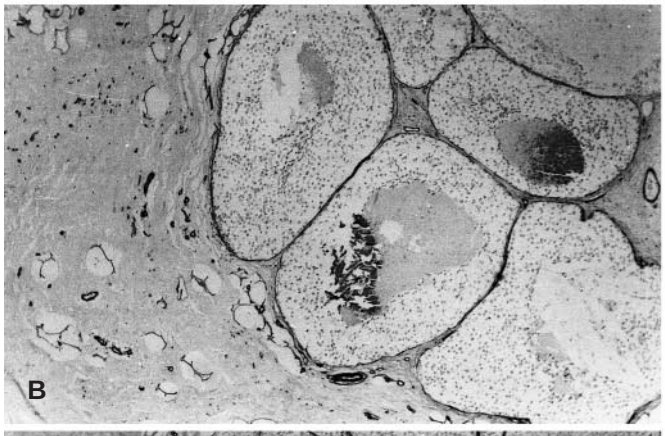
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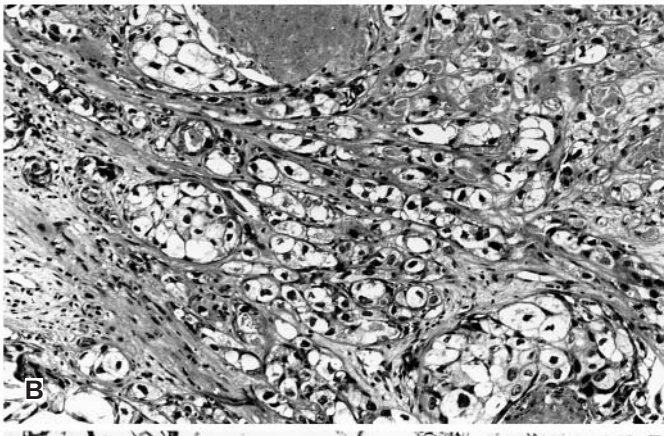
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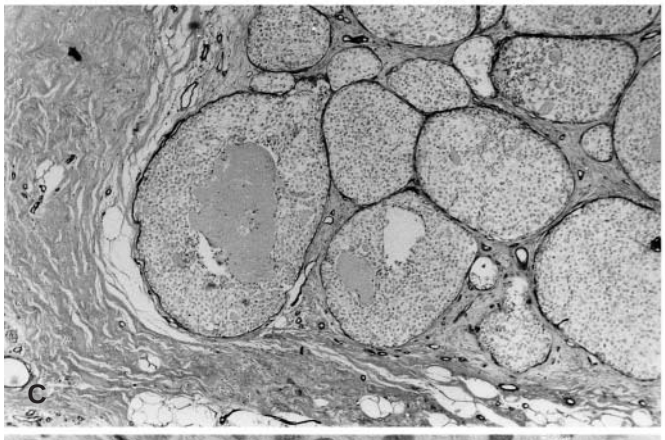
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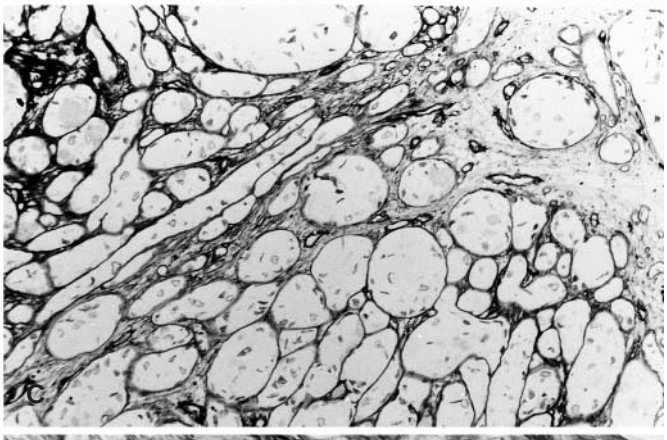
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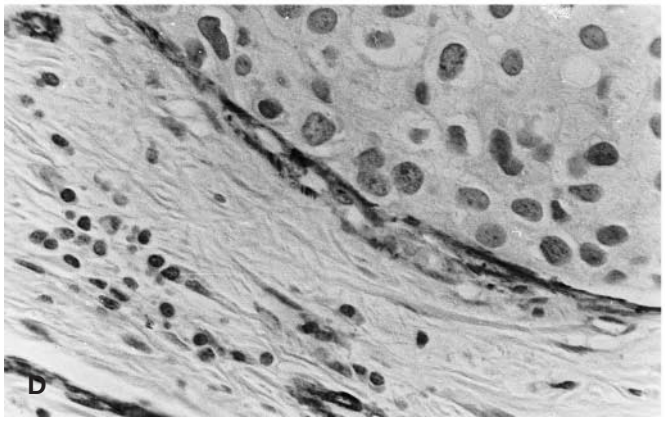
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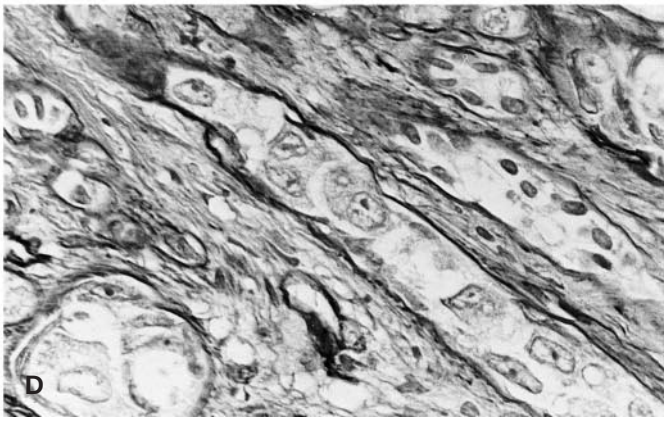
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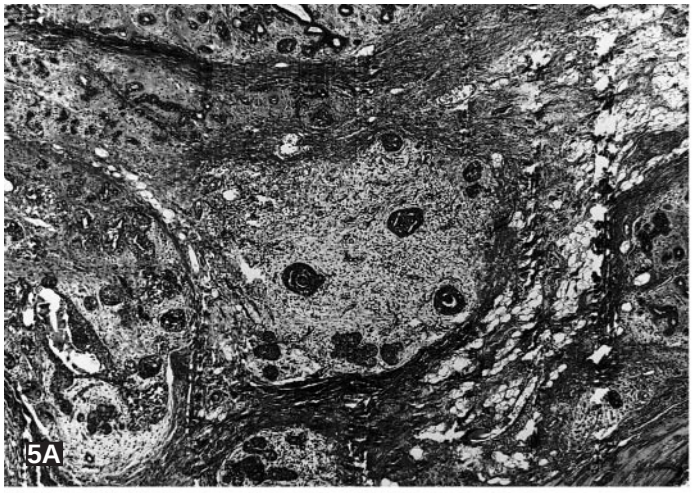

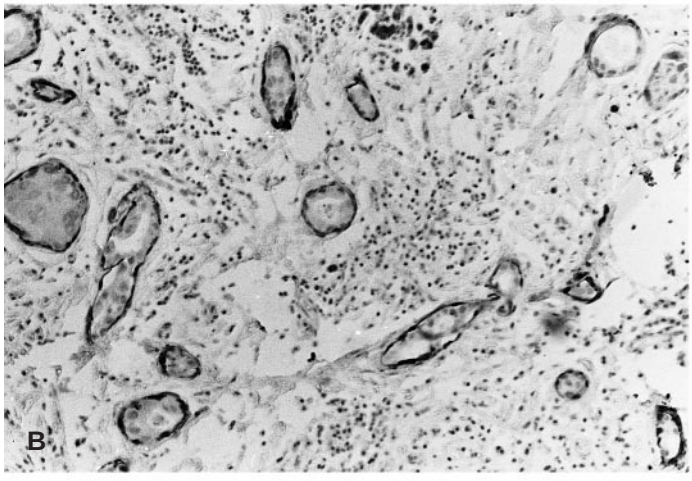

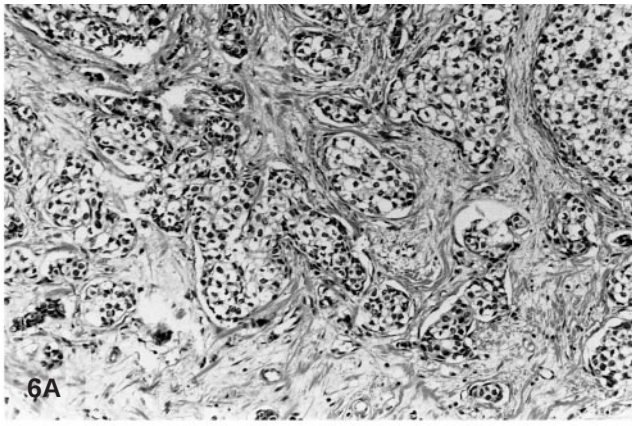

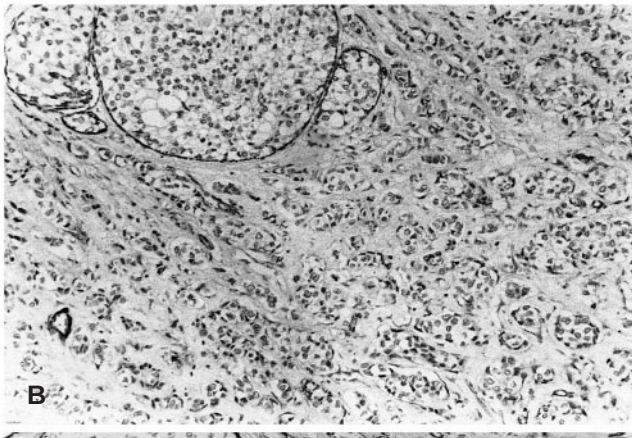

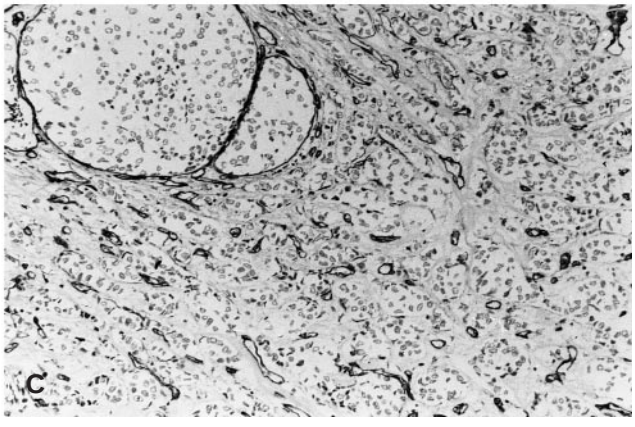

Fig. 5A, B Poorly differentiated DCIS (case 28): **A** Neoplastic ducts are immersed in a loose stroma with dense inflammatory infiltrate and small vessel formation of the granulation tissue-like type. H&E, ×75. **B** Anti-calponin antibody highlights an intact myoepithelial layer around the neoplastic ducts. The stromal fibroblasts are unstained. This case was included in the group of the DCIS cases. ABC, ×125

Fig. 6A–C DCIS with invasive carcinoma (case 7): **A** The neoplastic proliferation shows a non-organoid sclerosing pattern of growth. H&E, ×125. **B** Anti-actin and **C** anti-laminin antisera are both negative in the invasive component of this case. ABC, ×125

volving areas of sclerosing adenosis (Fig. 4A); and (4) neoplastic ducts surrounded by desmoplastic stroma with inflammatory infiltrate and new vessel formation similar in type to that of granulation tissue (Fig. 5A).

Stromal invasion was considered unequivocal at H&E level when there was an indistinct myoepithelial layer and nonorganoid sclerosing growth of cords and single neoplastic cells immersed in a desmoplastic stroma (Fig. 6A). As the definition of microinvasion is yet to be resolved [33], we adopted a practical approach and only selected cases with a single area of invasion no larger than one high-power field (400×).

Slides were evaluated and reviewed independently by two observers (S.D. and V.E.). Any disagreement between observers was settled by reviewing the cases together using a double-headed microscope, thereby reducing the effects of inter- and intraobserver error.

Immunostaining was performed on tissue sections using the avidin–biotin–peroxidase complex method [25]. All cases were tested with the the following monoclonal antisera: alpha smooth muscle actin (Dako, diluted 1:100), laminin (Dako, diluted 1:20) type IV collagen (Dako, diluted 1:100). Cases of DCIS with features suggestive of stromal invasion were also stained with anti-calponin antibody (Biogenex 1:80). This antibody required a heat-induced epitope retrieval accomplished by immersing the slides in 0.01 M citrate buffer at pH 6.0 and placing them in a pressure cooker for 20 min.

The cases were considered to be in situ when there was a linear and continuous distribution of at least one of the BL components around the involved ducts and when, in the same ducts, the MC layer highlighted by anti-actin and/or anti-calponin antisera was uninterrupted. This because small fragments of BL can also be found in invasive carcinomas [10]. The absence of the BL and loss of MCs around agregates of neoplastic cells were taken as evidence of tumour invasion.

Table 1 Ductal carzinoma (DCIS) according to H&E staining (10 cases). Immunocytochemical features (*IHC* immunohistochemistry, *SMA* mooth muscle actin, *COLL IV* collagen IV, *LAM* laminin; + positive, – negative, +/– discontinuous rim of positivity)

Case	Antibodies			IHC diagnosis
	SMA	LAM	COLL IV	
1	+/–	+	+	DCIS
3	+	+	+	DCIS
4	+	+	+	DCIS
5	+	+	+	DCIS
6	+/–	+	+	DCIS
8	+	+/–	+	DCIS
10	+	+	+/–	DCIS
12	+/–	+	+	DCIS
13	+/–	+	+	DCIS
14	+	+	+/–	DCIS

Results

The results of the comparison between the diagnoses based on H&E stained slides and on immunohistochemistry are reported in Tables 1–3 and depicted in Figs. 1–6. Histological diagnose based on H&E-stained sections revealed 10 cases of DCIS (Table 1). Foci of stromal invasion were identified in 17 cases, which are defined as ductal carcinoma in situ (DCIS) with invasion (Table 2). Finally, 11 cases belonged in the category of DCIS suggestive of invasion (Table 3).

The alpha smooth muscle actin antibody stained the cytoplasm of myoepithelial cells around normal ducts and lobules and around DCIS. In some areas in which the ductules were filled and distended by neoplastic cells, myoepithelial cells were flattened and only a thin rim of attenuated positivity for anti-smooth muscle actin antibody was evident (Fig. 3). The anti-calponin antibody displayed a somewhat different staining pattern from that of anti-actin, being more specific but less sensitive: only rare periductal stromal myofibroblasts were stained, in contrast to the pattern obtained with anti-smooth muscle actin antibody (see Figs. 2, 4, 5). The finding of SMA-positive elongated cells around neoplastic ducts, in the absence of positivity with laminin, collagen IV and calponin, was thus interpreted as a myofibroblastic stromal response, rather than as evidence of an intact myoepithelial cell layer (see Table 3, cases 15, 34 and 38).

Around the neoplastic ducts, the staining with anti-calponin was less intense than with anti-actin antibody and, especially when the duct was distended by the neoplastic proliferation, occasional tracts of the flattened myoepithelial layer were focally negative with anti-calponin antibody. Immunostaining with antibodies against laminin and collagen IV was linear, continuous and circumferential around normal glands and in the ducts harbouring DCIS (Fig. 3). In areas where the carcinomatous cells invaded the stroma the staining was discontinuous or lacking, confirming that BL was disrupted (Fig. 6).

When the diagnoses obtained with H&E stains were compared with the immunohistochemical data, in all IDC or DCIS cases the H&E diagnoses correlated with the findings obtained by immunohistochemistry. Among the cases of DCIS suggestive of invasion, in 4 out 11 cases immunohistochemistry revealed the presence of preserved BL with continuous and linear pattern and weakly stained MCs at the periphery of involved ducts. These cases were therefore reclassified as DCIS. They included 2 cases with sclerosing adenosis (cases 11 and 21; Fig. 4) and 1 with lobular involvement (case 28) by the neoplastic proliferation.

Five of the cases (cases 15, 33, 34, 36, 38) of DCIS suggestive of invasion were re-classified as IDC with invasion because the panel of antibodies failed to demonstrate intact BL and MCs (Fig. 1). Finally, 2 cases (cases 16 and 20) remained in the category of "suggestive of invasion"; in both cases the BL markers and anti-smooth

Table 2 DCIS with invasion according to H&E staining (17 cases). Immunocytochemical features on foci of stromal invasion

Case	Antibodies			IHC diagnosis
	SMA	LAM	COLL IV	
7	–	–	–	DCIS with invasion
9	–/+	–	–	DCIS with invasion
17	–	–	–	DCIS with invasion
18	–/+	–	–	DCIS with invasion
19	–	–	–	DCIS with invasion
22	–	–	–/+	DCIS with invasion
23	–	–	–	DCIS with invasion
24	–	–	–	DCIS with invasion
25	–	–	–	DCIS with invasion
26	–	–	–	DCIS with invasion
27	–	–	–	DCIS with invasion
29	–	–	–	DCIS with invasion
30		–	–	DCIS with invasion
31	–	–	–	DCIS with invasion
32	–	–	–	DCIS with invasion
35	–/+	–	–	DCIS with invasion
37	+	–	–	DCIS with invasion

Table 3 DCIS with suggestive features of stromal invasion on H&E staining (11 cases). Immunocytochemical features.

Case	IHC				Revised diagnosis after IHC
	SMA	LAMI-NIN	COLL IV	CAL-PONIN	
2	+/–	+	+	+	DCIS
11	+/–	+	+/–	+	DCIS
15	+	–	–	–	DCIS with invasion
16	–	+/–	+/–	*	Suggestive of invasion
20	+/–	+/–	*	*	Suggestive of invasion
21	+	+	+/–	+	DCIS
28	+	+	–/+	+	DCIS
33	–	+/–	–/+	–	DCIS with invasion
34	+/–	–	–	–	DCIS with invasion
36	–	–	–	–	DCIS with invasion
38	+/–	–	–	–	DCIS with invasion

[a] Sections in which the suggestive foci had disappeared

Table 4 Clinicopathological correlations: ductal carcinoma in situ 14 cases[a]) (*R* recurrence, *M* metastases, *FU* follow-up, *ILC* invasive lobular carcinoma, *LN* lymph-nodes, *NED* no evidence of disease, *DOD* died of disease, *UOQ* upper-outer quadrant, *LIQ* lower-inner quadrant, *LOQ* lower-outer quadrant, *L* left, *R* right, *Q* quadrantectomy, *M* mastectomy,? not known, *N0* no lymph-node metastases, *NX* lymph-node metastases not assessed)

[a] Ten cases originally classified as DCIS and 4 reclassified after IHC (cases 2, 11, 21 and 28)

Case	Age	Site	Therapy	Ln status	Fu years/months/ status	Recurrence/ metastases years/months/R-M
1	67	UIQ-R	M	N0	7/4/NED	–
2	39	LIQ-L	M	N0	7/8/NED	–
3	41	UOQ-R	Q	N0	7/0/NED	–
4	43	LOQ-R	Q	N0	5/1/NED	1/4/R(DCIS)
5	44	LIQ-L	Q	N0	3/8/DOD	1/9/ctrl R(ILC)/M
6	63	UOQ-L	M	N0	LOST	
8	44	UOQ-L	Q	NX	2/4/NED	–
10	56	UOQ-R	Q	N0	5/9/NED	0/6/R(DCIS)
11	55	LOQ-R	Q	N0	LOST	–
12	58	?-L	Q	N0	1/8/NED	–
13	42	UOQ-L	Q	N0	2/4/NED	–
14	44	UOQ-L	Q	NX	9/11/NED	–
21	56	UOQ-L	M	N0	2/9/NED	–
28	48	UOQ-R	M	N0	LOST	–

Table 5 Clinicopathological correlations: ductal carcinomas in situ with invasion (22 cases[a]) (*IDC* invasive ductal carcinoma, *N1* metastases to movable ipsilateral lymph node(s), *AWD* alive with disease)

[a] 17 cases originally classified as DCIS with invasion and 5 cases re-classified after IHC (cases 15, 33, 34, 36 and 38)

Case	Age	Site	Therapy	LN status	FU years/months/ status	Recurrences metastases years/months/R-M
7	56	LIQ-L	Q	N0	4/5/NED	–
9	53	UOQ-R	Q	N0	3/0/NED	–
15	69	UOQ-R	M	N0	1/7/NED	–
17	50	UOQ-L	Q	N0	LOST	–
18	43	Subareolar-R	M	N1	1/2/DOD	M
19	55	UOQ-R	Q	N0	5/7/NED	–
22	39	UOQ-L	Q	N1	5/9/NED	–
23	55	UOQ-R	Q	N0	LOST	–
24	29	UOQ-R	M	N0	LOST	–
25	30	UOQ-L	M	N1	LOST	–
26	60	UOQ-R	Q	N0	5/4/NED	–
27	42	UOQ-L	Q	N0	5/0/NED	–
29	45	UOQ-L	Q	N0	5/0/AWD	3/8/R(IDC) and M
30	71	UOQ-L	M	N0	5/4/NED	–
31	51	UOQ-L	Q	N0	5/4/NED	2/0/R(IDC)
32	42	UOQ-L	Q	N0	LOST	
33	65	Subareolar	M	N1	LOST	
34	55	UOQ-R	M	N0	LOST	
35	60	Subareolar-R	M	N1	1/2/DOD	R(IDC) and M
36	59	LOQ-L	M	N0	1/8/NED	–
37	49	UOQ-L	Q	N0	3/3/DOD	R(IDC) and M
38	61	UOQ-R	Q	N0	4/10/NED	R(IDC)

Table 6 Clinicopathological correlations: DCIS with suggestive of stromal invasion (H&E + IHC diagnoses)

Case	Age	Site	Therapy	LN Status	FU years/months/status	Recurrence / metastases
16	38	UOQ-L	Q	NX	2/0/NED	–
20	46	LOQ-R	M	N0	5/8/NED	–

muscle actin antibody were difficult to interpret as the result of weak staining and, in addition, the lesions had disappeared in the sections available for immunohistochemistry with anti-calponin antibody(see Table 3).

The clinical history of the 38 cases is reported in Tables 4–6. All cases were divided according to the final diagnosis obtained after the immunohistochemical findings. All patients were women. The age of patients with DCIS (including cases 16 and 20) ranged from 38 to 67 years (median 46). Patients with IDC ranged from 29 to 71 years of age (median 60).

There was no significant difference in age, localization, macroscopic feature and initial therapy in either group. All lesions were unifocal and in 27 patients (71%) the upper outer quadrant was involved. All patients underwent surgical treatment; 23 patients had breast-conserving surgical therapy, and 15 patients had mastectomy (in the latter group there were 5 patients with a final diagnosis of DCIS and 10 with a “final” diagnosis of DCIS with invasion). Axillary lymph node clearance was performed in 35 women. Metastases to the axillary lymph nodes were found in 5 patients at the time of diagnosis.

All these patients had been included in the group of DCIS with invasion.

The length of follow-up in the total series varied from 14 to 114 months (average of 55 months). Follow-up information was available for 11 patients with a final diagnosis of DCIS. In 2 of these patients recurrence of DCIS developed. One other patient died of subsequent contralateral invasive lobular carcinoma (see Table 4). Follow-up was possible in 16 patients with IDC. Five patients experienced recurrences or metastases during the postoperative observation period. Three patients of this group died of disease.

Discussion

The clinicopathological features of intraductal carcinoma (DCIS) of the breast have recently been characterized [4, 17] and subdivided into three major types (poorly, intermediately and well differentiated) according to two main morphological features: cytonuclear differentiation and cell polarization [24]. Poorly differentiated DCIS (PDCIS) has the highest rate of concomitant invasion at the time of the first diagnosis [17, 19]. Patchefsky et al. [32] reported that overall, 29% of their patients with DCIS had microinvasion and all of these patients with invasion had a poorly differentiated DCIS.

The ability of malignant cells to cross BL is essential to the process of invasion and metastasis, although the mechanisms of destruction of the BL remains unclear. The fragmentation or disappearance of the BL around invasive tumour foci may be consequent on defective synthesis or secretion of BL components or on activation of proteinases and/or collagenases [1, 3, 5, 6, 8, 10, 29, 31]. Structural defects of BL occurring in association with local invasion have already been studied and reported in various tumours of the uterine cervix [33], oral mucosa [27] and breast [6, 10, 39]. Many studies have also dealt with ultrastructural analysis, mainly of the BL and MCs, in benign and malignant breast tumours [10, 23, 37].

In recent years, immunohistochemistry has appeared to be a reliable method of revealing BL. Its application to tumour tissue has shown that benign and in situ lesions are outlined by intact BL, whereas the majority of invasive carcinomas are characterized by absent or incomplete BL [6, 10, 16, 26]. The same applies to MCs. With the exception of biphasic invasive tumours [21, 22], the presence of a continuous layer of MCs is currently considered to be a sign of an in situ phase [2]. The value of immunohistochemical evaluation of BL as well as MCs in the differential diagnosis between benign glandular proliferations and malignant invasive breast lesions, such as sclerosing adenosis, tubular adenosis, microglandular adenosis and tubular carcinoma, has been recently demonstrated [9, 13–15, 16, 20, 21, 28, 36].

In the present study, in all cases diagnosed as IDC (17 cases) or DCIS (10 cases) at H&E level, the IHC findings with anti-BL and anti-MCs antibodies were consistent with the initial diagnosis. Immunohistochemistry was vital in distinguishing between invasive and pseudoinvasive forms of DCIS in 9 of the cases showing H&E features considered suggestive, but not definitively diagnostic, of stromal invasion. In 5 of these cases, the absence of a continuous layer of BL and MCs, as demonstrated by IHC, lead to the final diagnosis of DCIS with invasion. The opposite was seen in 4 cases, where the presence of a continuous MC layer and BL around the neoplastic ducts confirmed the in situ nature of the lesions. One of these cases showed extension to lobules, and in 2 other cases there were areas of sclerosing adenosis. When DCIS extends into lobular ductules recognition of the in situ nature of the lesion is difficult, as the oedematous specialized stroma of the lobular unit may simulate an early reaction to stromal invasion. Furthermore, overdiagnosis of invasion in cases with DCIS in sclerosing adenosis is a well-known pitfall [15, 18].

All cases with questionable histological features were tested with anti-calponin antibody. This 34-kDa peptide is a recently identified smooth muscle component with contraction-regulatory function. Wang et al. [38] found that calponin staining in breast tissue is specifically restricted to myoepithelial cells, and only a minor proportion of stromal myofibroblasts are usually stained. Similar results were seen in the present cases of PDCIS with questionable features. The staining of MCs with calponin antibody appeared to be less intense but more selective than the staining obtained with anti-actin antibody. Not all the myoepithelial cells were stained by anti-calponin, and the use of this antibody alone may sometimes give a false impression of focal discontinuity of the myoepithelial layer. Nevertheless, anti-smooth muscle actin antibody stained both myoepithelial cells and stromal periductal myofibroblasts. This can render identification of the myoepithelial cell layer difficult, if not impossible. In these cases the anti-calponin antibody appears very useful, and a confident diagnosis of in situ or invasive carcinoma may be reached by means of the comparison between the staining obtained with these two antibodies.

The final diagnoses (H&E and immunohistochemistry) were consistent with the observations recorded at follow-up of the patients. Two cases of DCIS recurred locally. This was probably due to incomplete excision of the lesion, as both these patients had undergone wide resection only. A third patient in the group with DCIS developed a contralateral invasive lobular carcinoma 1 year and 9 months after the DCIS. This led to widespread metastases and death of the patient. Among the patients with a final diagnosis of invasive carcinoma lymph node metastases arose in 5, local recurrence of IDC in 5, and distant metastases in 4 cases. Three patients died of their disease 1–3 years after the initial diagnosis.

Our results indicate that immunohistochemical study of MCs and BL makes evaluation of the in situ phase of poorly differentiated DCIS more objective and leads to more reliable diagnosis, at least in some cases. It appears that in order to avoid inadequate management of the patient, a confident diagnosis of PDCIS needs immunohis-

tochemical assessment of BL and/or MC, at least in cases with equivocal histological features.

Acknowledgements This work was partly financed with grants from MURST 40% and 60%. Dr. Christine M. Betts has to be thanked for reading the manuscript and Mr. A. Busi for microphotographs.

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