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Vimentin expression in 98 breast cancers with medullary features and its prognostic significance

S. Holck[1], L. Pedersen[2], T. Schiødt[3], K. Zedeler[4], H. Mouridsen[2]

[1] Department of Pathology, Frederiksborg County Hospital, Hillerød, Denmark
[2] Department of Oncology ONK, Rigshospitalet, Copenhagen, Denmark
[3] Department of Pathology, Rigshospitalet, Copenhagen, Denmark
[4] Secretariat of the Danish Breast Cancer Cooperative Group, The Finsen Institute, Rigshospitalet, Copenhagen, Denmark

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Abstract. The expression of vimentin, as assessed by immunohistochemistry, has been evaluated in 69 medullary carcinomas of the breast: 28 typical medullary carcinomas (TMC), 41 atypical medullary carcinomas (AMC), and 29 invasive ductal carcinomas with subtle medullary features that, however, did not fulfil the strict criteria of TMC or AMC. Immunoreactivity of at least 10% of the component cells was found in 14 of the medullary carcinomas (5 out of 28 TMC, 9 out of 41 AMC whereas only 1 of the invasive ductal carcinomas was vimentin-positive. The patients were followed for 8-13 years. No difference in recurrence-free survival or overall survival could be documented between vimentin-positive and vimentin-negative carcinomas with medullary features. No biological significance could be established for vimentin labelling in these lesions.

Key words: Vimentin – Medullary carcinoma – Breast

Introduction

The morphological grading of carcinoma of the breast rests on an evaluation of mitotic activity, nuclear pleomorphism and the extent of tubule formation (Bloom and Richardson 1957). The predictive success and reproducibility of this system and its modifications have been emphasized in several studies (Doussal et al. 1989; Elston and Ellis 1991; Fisher et al. 1980; Hopton et al. 1989). Others have failed, however, to reach an acceptable degree of consistency and have observed insufficient prognostic separation (Delides et al. 1982; Gilchrist et al. 1985; Stenkvist et al. 1979). Discouraging results were also obtained from a comparable approach to carcinomas with medullary features in a recent study (Pedersen et al. 1991) where we were unable to identify prognostically significant subgroups amongst breast cancers with medullary features, using histological grading. In order to include other morphological markers, preferably with a minimal element of subjectivity, which could be used to determine the biological behaviour of a given tumour, additional investigative tools should be sought.

Evidence has recently been presented suggesting that vimentin, a cytoskeletal intermediate filament normally characterizing mesenchymal tissue (Leader et al. 1987), may be of value in predicting the behavioural pattern of some epithelial neoplasms (Domagala et al. 1990a; Donhuijsen and Schultz 1989; Raymond and Leong 1989a, b; Upton et al. 1986). Support for this notion was recently provided by Domagala et al. (1990b), who demonstrated aggressivity of vimentin-positive, node-negative ductal carcinomas. Furthermore, a positive correlation between the immunoexpression of vimentin and tumour growth fractions has been documented in a series of carcinomas of the breast (Raymond and Leong 1989b). Their study was, however, hampered by a limited follow-up period, making any definite statement hazardous.

The prevalence of vimentin expression and its potential use in predicting behaviour is analysed in a series of breast cancers with medullary features, well characterized clinically and followed for 8–13 years.

Materials and methods

Carcinomas of the breast, entered as medullary carcinoma in the file of the Danish Breast Cancer Cooperative Group (DBCG) during the 5-year period from 1977 to 1982, were retrieved. The material has previously been described in detail (Pedersen et al. 1989). Based on haematoxylin and eosin- (HE-)stained and van Gieson-stained sections the lesions were evaluated by two of the authors (T.S. and S.H.) and reclassified according to the guidelines devised by Ridolfi et al. (1977) into the following: typical medullary carcinoma (TMC), atypical medullary carcinoma (AMC), and ductal carcinoma with medullary features (NMC). Material was available for this study in 98 cases, of which 80 were protocolled. The clinical data of the latter were collected prospectively nationwide within the framework of the studies conducted by the DBCG (Andersen

Correspondence to: S. Holck, Department of Pathology, Frederiksborg County Hospital, Centralsygehuset, DK-Hillerød 3400, Denmark

and Mouridsen 1988). Reasons for not entering the protocol included distant metastases, medical contraindications, deviation of operative procedure from DBCG's guidelines, and lack of patient agreement to be treated according to the DBCG protocol.

From each tumour two sets of 4-μm-thick sections were cut from formalin-fixed, paraffin-embedded archival tissue. The immunostaining was performed in two laboratories, each preparing one set of slides. The sections were dewaxed and placed in graded series of alcohol. Endogenous peroxidase was blocked by hydrogen peroxide. Non-immune swine serum at a dilution of 1:20 was applied for 5 min to reduce non-specific background staining. Subsequently the sections were incubated for 30 min, at room temperature, with monoclonal mouse anti-human vimentin at a dilution of 1:5 (Dakopatt, Copenhagen, Denmark), followed by incubation for 30 min with the appropriate peroxidase-conjugated secondary antibody. The bound peroxidase activity was localized using 3-amino-9-ethylcarbazol for 10 min as a chromogen. The sections were finally counterstained with haematoxylin and mounted. The presence of positively stained stromal cells provided a built-in positive control for vimentin immunoreactivity. Negative control sections were prepared by replacing the primary antiserum with non-immune serum.

Two of the authors (T.S. and S.H.) each evaluated one set of sections without knowledge of the clinical findings. All parts of each section were scanned and areas with the highest labelling rates were assessed. Tumours were considered to be positive when a prominent granular or diffuse reaction product of the cytoplasm characterized at least 10% of the neoplastic cells. Reaction by desquamated cells in necrotic zones was disregarded. When disagreement on the evaluation occurred, a concensus was reached by joint microscopic review of the slides.

The distribution of immunoreactive tumours according to histological subgroups was then calculated and the prognostic importance [recurrence-free survival (RFS) and overall survival (OS)] of vimentin positivity for the protocolled patients was analysed using Kaplan-Meier plots and log rank tests.

Results

The study was based on 98 breast cancers previously classified into TMC (28 cases), AMC (41 cases) and NMC (29 cases). Among the latter one was grade I, 16 were grade II and 12 were grade III. The low incidence of grade I carcinomas is not surprising, considering that this study is based on material originally classified as medullary carcinoma by the referring pathologists. Some of the cases, though displaying subtle medullary features did not, however, fulfil the strict criteria for medullary carcinoma used by us, and have consequently been reclassified as NMC. After the initial evaluation of vimentin expression, agreement (both positive and negative) was obtained in 89 cases (concordance 91%). Following the subsequent joint review, a concordance of 15 vimentin-positive cases was obtained.

The reaction product was granular or diffuse and localized to the cytoplasm of the neoplastic cells, generally expressed in the entire cell body (Fig. 1). Less commonly, the filaments were largely confined to the ectoplasm. In most cases chromogen-positive tumour cells were scattered diffusely throughout the section examined, constituting at least 10%, and in most cases 50% or more, of the tumour cells. The proportion of positive cells did, however, vary from area to area and in 2 cases, positivity was confined to a limited field of the available material, the majority of the tumour mass being composed of non-reacting cells. A consistent zonal preference within the tumour area was not apparent.

The distribution of immunoreactive tumours accord-

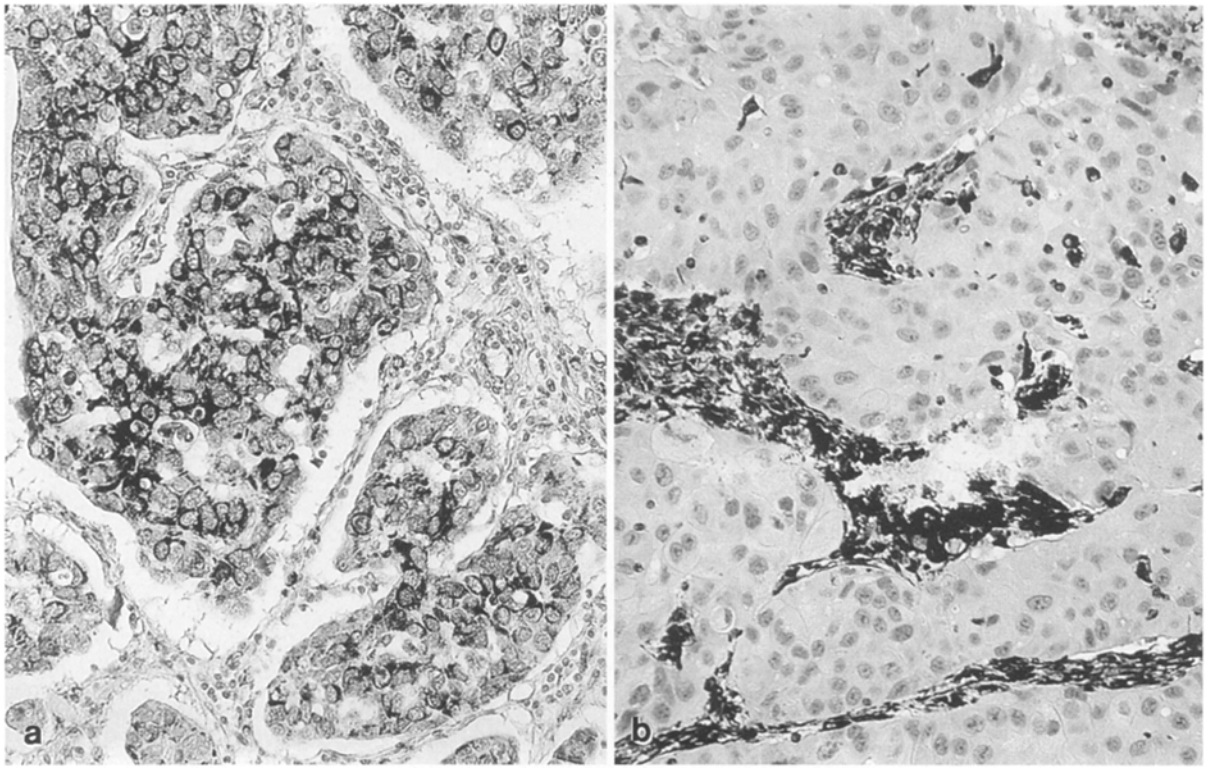

Fig. 1. **a** Portion of a typical medullary carcinoma largely composed of vimentin-immunoreactive cells. ×200. **b** Area of a typical medullary carcinoma void of vimentin-positive tumour cells. The immunoreactive stroma serves as a built-in positive control. ×200

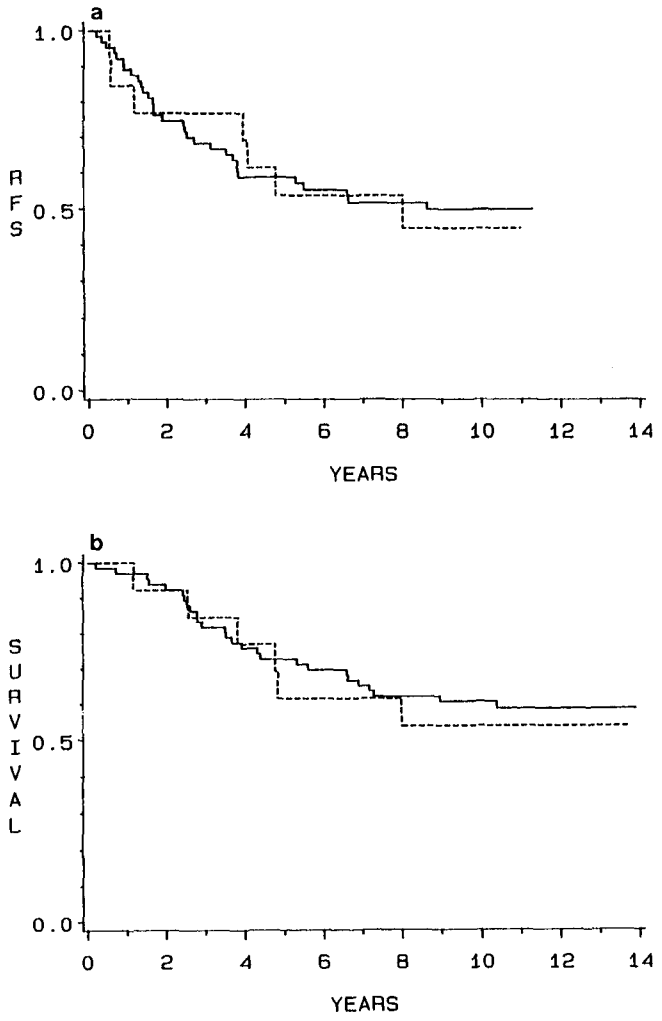

Fig. 2. a, b. Relationship between vimentin status and survival. **a** Recurrence-free survival and **b** overall survival in patients with carcinoma with medullary features. The difference is not statistically significant. (Vimentin-positive, *broken line*; vimentin-negative, *continuous line*

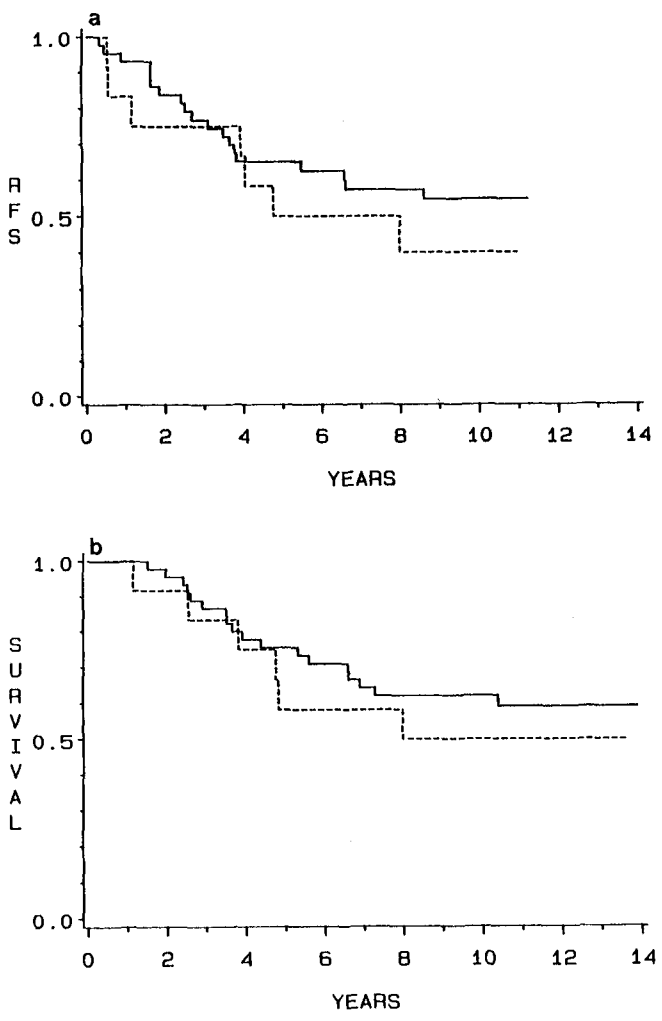

Fig. 3 a, b. Survival curves. **a** Recurrence-free survival and **b** overall survival for patients with medullary carcinoma (typical and atypical) in relation to the vimentin-expression. The difference is not statistically significant. (vimentin-positive, *broken line*; vimentin-negative, *continuous line*)

ing to subgroups was as follows: 5 TMC (18%), 9 AMC (22%) and 1 NMC (3%) expressed vimentin. Among the protocolled subjects 5 TMC (22%), 7 AMC (21%) and 1 NMC (4%) were positive. This difference in immunoreactivity of medullary carcinomas (TMC and AMC) and NMC is of borderline significance ($P=0.09$). Survival studies in relation to vimentin status appear in Figs. 2 and 3. Thus significant differences cannot be established, either for the total group of protocolled patients [$P=0.99$ (RFS); $P=0.71$ (OS); Fig. 2], or for the group of medullary carcinomas [$P=0.48$ (RFS); $P=0.43$ (OS); Fig. 3]. For the 18 non-protocolled patients, which constituted a heterogeneous group, information of recurrent disease was not collected in the DBCG.

Discussion

The predictive success of a test system requires high inter-and intraobserver reproducibility as well as its ability to stratify patients according to the growth rate of the tumour. To be workable in routine diagnostic laboratories it should further not be too labour-intensive nor equipment-expensive. We have previously documented poor observer reproducibility in the traditional histological grading systems applied to carcinomas with medullary features (Pedersen et al. 1991) and correlation between traditional histological grading and biological malignancy seem less apparent for this subtype of carcinoma than for non-medullary carcinomas (Schiødt 1966). For these reasons a need to introduce alternative measures exists.

The immunohistochemical reaction was interpreted as positive for vimentin when a minimum of 10% of the examined tumour cells were reactive. Defined in this way, the overall incidence of vimentin positivity was found to be 15%. The vast majoritiy of these were classified as TMC or AMC, reactivity being distinctly uncommon (roughly 3%) in NMC. Considering that a sizeable proportion of the latter were grade II or III carcinomas,

this figure is lower than that indicated by others. Domagala et al. (1990a) reported that 15 out of 62 (24%) grade II and III were positive and in another study 7 of 24 (29%) grade II and III carcinomas comprised more than 10% vimentin-reactive cells (Raymond and Leong 1989a). The present group of ductal carcinomas may, however, not be wholly comparable with ordinary ductal carcinomas, initially classified as such and a correlation of molecular marker expression between the two groups may not be justified. In this context it is interesting to note that Wargotz and Silverberg (1988) segregated a group of ductal carcinomas, medullary-like though not fulfilling the criteria of TMC or AMC, whose behavioural pattern deviated favorably from that of ordinary ductal carcinomas. It is not inconceivable that the disordant results in vimentin expression observed among different authors are caused, at least in part, by methodological diversities. The present putative false-negative cases, probably reflecting low antigen density, would possibly display reactivity if optimally fixed. Thus, precipitating fixatives, such as ethanol, seem to preserve the antigenicity of larger molecules, such as vimentin, better than formalin (Azumi and Battifora 1987). It should be added, however, that none of our cases displayed complete fixation-induced loss of vimentin antigenicity, as judged by the consistent staining (albeit sometimes weak) of the built-in mesenchymal elements (Fig. 1b). A more restrictive attitude on our part in characterizing a case as positive may also play a role. These considerations suggest that our results should be considered as minimum figures. The use of a more appropriate fixation such as ethanol, coupled with a lower cut-off level, would probably raise the incidence of vimentin detection.

Since the prime aim of the present analysis was to evaluate whether vimentin expression denotes a survival disadvantage, the use of this seemingly less sensitive preparation technique, identifying only tumours with abundant vimentin, should not be considered a drawback. Thus, if immunoreactivity does signify a dismal outcome, the relatively few reactive cases recorded here would most probably pursue such an unfavorable course. Furthermore, the use of a simple technique that is followed in most routine laboratories makes comparative investigations more feasible. In this study all specimens were handled in parallel by two laboratories and the resultant sections interpreted by two of the authors, reaching an acceptable concordance level. Thus, interlaboratory and interobserver differences were slight.

The information on vimentin-reactivity of medullary carcinomas is sparse. Two studies by Domagala et al. (1990a, c) comprise three cases (one of which was vimentin-positive) and 18 cases (14 vimentin-positive), respectively. The single case included in the study by Gould et al. (1990) was non-reacting. The work of Raymond and Leong (1989a, b) did not include this neoplasm. The present study of 69 medullary carcinomas (TMC and AMC) exhibited immunoreactivity in roughly 20%. Whereas the detection rate of vimentin positivity in mammary carcinomas thus proved lower in this study than in the other reported series (Domagala et al. 1990a; Raymond and Leong 1989a), the higher prevalence of vimentin reactivity amongst medullary carcinomas seems a constant observation and was reproduced in the present study. The expression of this filament in AMC has not previously been commmented upon but we find that tumour vimentin is equally common in the two subtypes of medullary carcinomas (TMC and AMC).

Vimentin reactivity of epithelial neoplasms at various sites, including the breast, has been suggested to be a feature with grave prognostic implications (Raymond and Leong 1989b). Support for this view is rendered by the observation that positivity seems prevalent among the poorly differentiated cases (Raymond and Leong 1989a, b), most conspicuously among the spindle cell variant (Ellis et al. 1988). Additionally, a positive correlation between a high growth fraction, demonstrated by immunostaining for the Ki-67, negative oestrogen receptor status and vimentin-positive breast carcinomas has also been documented (Catoretti et al. 1988; Domagala et al. 1990a; Raymond and Leong 1989b). Recently, evidence has been presented suggesting that vimentin expression denotes aggressivity of ductal, node-negative carcinomas, whereas no such statement applies to cases with metastatic deposits.

No other prolonged follow-up study of breast cancer patients related to vimentin analysis has been reported. We find that identification of clinically distinct subgroups among carcinomas with medullary features is not feasible on the basis of vimentin status. The value of this intermediate filament as a prognosticator of medullary carcinomas is dubious though conclusions should be drawn with some caution, considering the relative small size of the subgroups. The apparent insignificance of vimentin positivity and its paradoxically higher incidence in medullary carcinomas parallels several other clinico-morphological studies (DNA aneuploidy, oestrogen receptor negativity) in medullary carcinoma. Vimentin expression may thus be added to the expanding list of markers that generally denote aggression, yet it appears biologically unimportant in medullary carcinomas.

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