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ORIGINAL ARTICLE

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Estimation of the proliferative activity of human breast cancer tissue by means of the Ki-67 and MIB-1 antibodies – comparative studies on frozen and paraffin sections

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Abstract The estimation of the Ki-67 index in human breast cancer tissue has been proven to be a useful prognostic tool. The examination can be performed, however, only on frozen sections (FS). The development of an antibody directed against parts of the Ki-67 antigen (MIB-1) has opened a new route to determine the proliferative activity on paraffin sections (PS). MIB-1 immunohistochemistry is used instead of Ki-67 immunohistochemistry if a tumour is delivered to the pathologist after formalin fixation or if that part of the tissue suspicious for breast cancer must be totally embedded in order to confirm the diagnosis. The present study compares the findings of Ki-67 (FS) and MIB-1 (FS and PS) immunohistochemistry in a total of 544 cases of human breast cancer. The findings confirm a good statistical correlation between the Ki-67 and the MIB-1 findings. The MIB-1 results are 2–2.5 times higher in FS than in PS. Good agreement exists between the Ki-67 indices determined on FS and the MIB-1 indices determined on PS. If the cut-off value for the separation of Ki-67 negative and positive cases is defined as 10%–20%, a MIB-1 index in PS of 10% permits the correct prediction of a negative Ki-67 index in 97% of the cases, and a MIB-1 index of 30% or more correctly predicts a positive Ki-67 index in 90% or more of the cases. Hence, the determination of the MIB-1 index on PS may replace the determination of the Ki-67 index on FS with a high degree of probability.

Key words Breast cancer · Proliferative activity · Ki-67 · MIB-1 · Frozen and paraffin sections

Introduction

The proliferative activity of tumours may be estimated by several methods (mitotic index, ^{3}H autoradiography, AgNOR index, labelling of nuclei with PCNA, and DNA cytophotometry). An important step, particularly with regard to diagnostic practice, has been achieved by the discovery of the Ki-67 antigen in tumour cell nuclei [3, 4, 5]. The Ki-67 antigen may be demonstrated in proliferating and occasionally in non-proliferating cells [10, 11, 12]. Although its demonstration is not unequivocal evidence in favour of proliferative activity in a tumour and although its distribution follows a heterogeneous pattern, the determination of Ki-67 nuclear staining has proven to be a useful tool for the evaluation of proliferative activity, particularly in human breast cancer (for references see [9]).

The Ki-67 index is defined as the percentage of positive nuclei within the total cut surface of a histological slide. Intra-observer agreement is high if the same sections are reexamined after several months by the same investigator [9]. The estimation of the index is restricted to frozen sections (FS), however, but in 1992, J. Gerdes et al. [6] succeeded in developing an antibody against parts of the Ki-67 antigen which could be used on paraffin sections (PS) and which proved specific for native and recombinant Ki-67 protein. The deparaffinized slides must be treated in a microwave oven, or the MIB-1 antibody only labels the cells in mitosis [2].

The literature contains few papers comparing MIB-1 and Ki-67 indices in one and the same tumour. Gerdes et al. [6] mention a close correlation between the Ki-67 findings in FS and the MIB-1 findings in PS. Cattoretti et al. [2] describe an identical staining pattern in MIB-1 stained PS and Ki-67 stained FS. Similar results have been obtained by Weikel et al. [13] while Barbareschi et al. [1] found that nuclear staining with MIB-1 antibody was twice as high as that with the Ki-67 antibody.

The present study was performed to compare the results of Ki-67 staining in FS and of MIB-1 staining in both FS and PS in a large number of human breast cancers. The fundamental question behind this study was to search for a possible correlation between the MIB-1 and the Ki-67 results in those cases where FS are not available. It is known from previous studies in human breast cancer that 10%–20% of labelled nuclei are the cut-off

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point for breast carcinoma with a relatively worse prognosis [8, 14]. Therefore, the question whether the MIB-1 findings might be directly comparable to the Ki-67 findings or whether the MIB-1 findings are usually higher or lower than the Ki-67 findings, is important for the examination of formalin fixed breast carcinomas.

Materials and methods

Five hundred and forty-four cases of human breast cancer were examined from the files of the Department of Pathology, Staedtische Kliniken, Wiesbaden, Germany between 4 January 1990 and 23 March 1994. The patients were between 26- and 92-years-old (average 58.9 years). The number of tumours per histological type was as follows: ductal carcinoma in situ (DCIS; 14), grade 1 invasive ductal carcinoma not otherwise specified (NOS) (85), grade 2 (198), grade 3) (151), invasive ductal carcinoma with predominant DCIS (26), invasive lobular carcinoma (26), mucinous carcinoma (10), medullary carcinoma (25), papillary carcinoma (1), tubular carcinoma (13), others (1). The tumours were delivered to the Department of Pathology as quickly as possible following surgical removal, usually within 15–30 min, cooled with ice pieces from a commercial ice box. Immediately following preparation of sections for the FS diagnosis, additional FS were performed for immunohistochemical estimation of oestrogen and progesterone receptor content and determination of the Ki-67 and MIB-1 index. The residual tumour tissue was fixed in formalin for conventional preparation of the haematoxylin and eosin sections and for MIB-1 staining on PB. If the tumours were large enough, additional native material was frozen in dry ice for biochemical oestrogen and progesterone receptor assay (performed by Bioscientia, Institut fuer Laboruntersuchungen, Ingelheim, Germany or by Prof. K. Pollow, Department of Experimental Endocrinology, University Hospital for Gynecology and Obstetrics, Mainz, Germany). The slides for immunohistochemistry were stored at –70° C until immunohistochemical examination.

The method for Ki-67 immunohistochemistry has been described previously [9]. The primary antibody was obtained from Dakopatts (Hamburg, Germany).

For estimation of the MIB-1 index on FS, the procedure was identical to that used for Ki-67 immunohistochemistry but used the MIB-1 antibody obtained from Dianova (Hamburg, Germany). For the determination of the MIB-1 index on PS, the deparaffinized sections were heated in a microwave oven five times for 5 min each at 750 W as recommended by Dianova. Following rinsing in tap water and TRIS buffered saline (0.05 M, pH 7.6) the sections were treated with normal serum (1:66) and then incubated for 30 min with MIB-1 (diluted 1:50).The secondary antibody was Vectastain Elite ABC mouse IgG-kit (PK 6102) used for 30 min. Incubation with the peroxidase anti-peroxidase reagent from the Vectastain Elite ABC mouse IgG-kit was performed for 30 min.

The number of positively stained nuclei was estimated as follows: 5% = tumours with a maximum of 5% positive nuclei, 10% = tumours between 6%–10% positive nuclei, 20%–90% in 10% steps each. All sections were examined by the same authors (W.R., V.M.). The number of positive nuclei was estimated over the whole slide, and remarkable heterogeneity of nuclear staining was oberved in numerous cases. All nuclei staining light to dark brown were classified as positive. For technical reasons, Ki-67 (FS) and MIB-1 (FS and PS) were not available in each case. Therefore, the results are based upon studies with the following combinations: Ki-67 and MIB-1 (FS) and MIB-1 (PS) 404 cases; Ki-67 (FS) and MIB-1 (PS) 80 cases; Ki-67 and MIB-1 (FS) 55 cases; MIB-1 (FS and PS) 5 cases.

The mean values and the maximum and the minimum values for the MIB-1 index were plotted against the Ki-67 index. Moreover, the regression line and correlation coefficients for the correlation of Ki-67 and MIB-1 values were determined. Sensitivity and specificity of the MIB-1 results for a positive or a negative Ki-67 result were calculated by means of the chi-square test. Cases up to 10% Ki-67 positive nuclei were classified as negative, cases with 20% and more positive nuclei were classified as positive.

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Results

Frozen sections: Ki-67 index vs. MIB-1 index

The MIB-1 indices belonging to a certain Ki-67 index in frozen sections show a wide range of variation (Fig. 1). The maximal values found in low Ki-67 indices overlap the minimal values found in high Ki-67 indices up to Ki-67 indices of 30%. The range decreases with increasing Ki-67 index. While the median MIB-1 values are 2–2.5 times higher than the Ki-67 values in Ki-67 indices between 5% and 30%, they largely agree in Ki-67 indices of 80%–90%. The correlation coefficient is 0.867 and indicates a good correlation of the Ki-67 and MIB-1 values. The overall agreement (total number of truly positive and negative cases) between the Ki-67 and MIB-1 indices is best for MIB-1 indices of 30%–40% (Table 1) and the same is true for sensitivity and specificity. The cut-off value for the separation of Ki-67 negative and positive cases is about twice as high as that for the MIB-1 index.

Frozen sections (Ki-67 index) vs. paraffin sections (MIB 1 index)

The MIB-1 indices in PS for a certain Ki-67 index show as similar range of distribution as the MIB-1 indices in FS (Fig. 2). The overlap of maximal MIB-1 values in low Ki-67 indices and of minimal MIB-1 values in high Ki-67 indices is found over a wider range than observed in the comparison of FS. Only if the Ki-67 index is higher than 70% is the overlap missing. The range is highest in Ki-67 indices of 10%–20%. In contrast with the findings in FS, however, the median Ki-67 and MIB-1 values follow a largely parallel course, and in the highest group

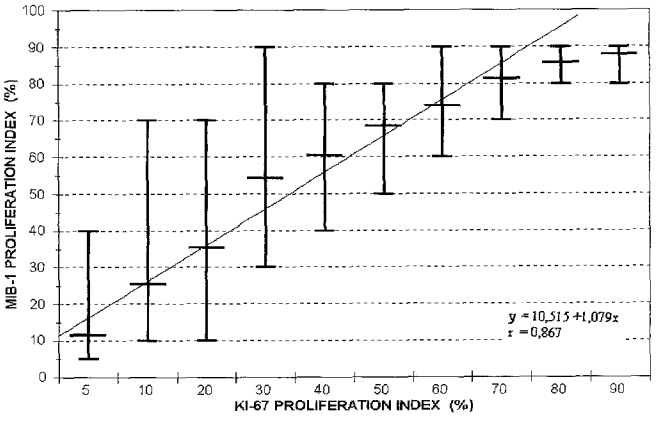

Fig. 1 Correlation between the Ki-67 index (abscissa) and MIB-1 index (ordinate) on frozen sections (FS). Minimum, maximum and mean values, regression line, and correlation coefficient

Table 1 Sensitivity, specificity, predictive value (percentage of truly positive cases) and accuracy of the MIB-1 indices on 459 frozen sections in relation to a Ki-67 cut-off value between 10% and 20%

MIB-1-index (%)	Sensitivity	Specificity	Ki-67 positive MIB-1 truly positive	Ki-67 positive MIB-1 false negative	Ki-67 negative MIB-1 truly negative	Ki-67 negative MIB-1 false positive	Overall agreement
10	100	21	42.2	57.8	100	0	49.9
20	99.4	56	56.6	43.4	99.4	0.6	56.6
30	89.9	84.5	77	23	93.5	6.5	86.5
40	72.6	94.5	88.4	11.6	85.7	14.3	86.5
50	61.3	96.9	92	8	81.3	18.7	83.9
60	49.4	97.6	92.2	7.8	77	23	80
70	35.1	98.6	93.7	6.3	72.5	27.5	75.4
80	22	100	100	0	69	31	71.5
90	9.5	100	100	0	65.7	34.3	66.9

Table 2 Sensitivity, specificity, predictive value and accuracy of the MIB-1 indices on 484 paraffin sections in relation to a Ki-67 cut-off value between 10% and 20%

MIB-1-index (%)	Sensitivity	Specificity	Ki-67 positive MIB-1 truly positive	Ki-67 positive MIB-1 false negative	Ki-67 negative MIB-1 truly negative	Ki-67 negative MIB-1 false positive	Overall agreement
10	97.3	57.2	58.9	41.1	97.1	2.9	72.7
20	85.6	84.5	77.7	22.3	90.3	9.7	84.9
30	57.8	96	90	10	78.3	21.7	81.2
40	39.5	97.6	91.3	8.7	73	28	75.2
50	32.1	99	95.2	4.8	69.8	30.2	73.1
60	20.9	99.3	95.1	4.9	66.6	33.4	69
70	11.8	99.7	95.7	4.4	64.2	35.8	65.7
80	5.3	100	100	0	62.7	37.3	63.4
90	1.6	100	100	0	61.8	38.2	62

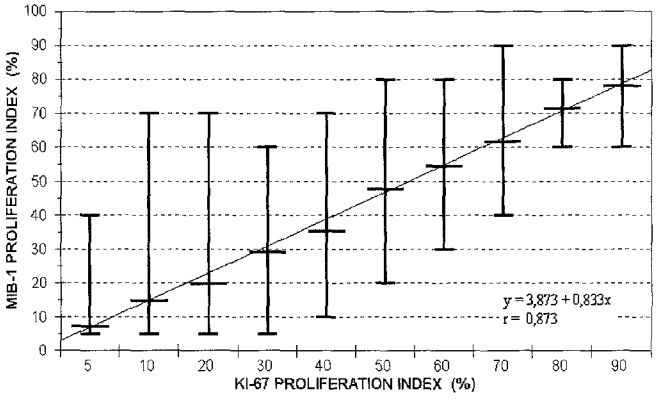

Fig. 2 Correlation between the Ki-67 index (abscissa) and the MIB-1 index (ordinate) on paraffin sections (PS). Minimum, maximum, and mean values, regression line, and correlation coefficient

of 80% and 90%, the MIB-1 indices are even lower than the Ki-67 indices. The correlation coefficient is identical with that for the comparison of Ki-67 and MIB-1 indices on FS (0.873). The overall agreement between the Ki-67 and MIB-1 indices is best for a MIB-1 index of 20%. Sensitivity is highest for a MIB-1 index of 10% and specificity for a MIB-1 index of 80%–90% (Table 2). The cut-off values for the separation of negative and positive tumours are in the range of 20% and, hence, identical with the cut-off value for the Ki-67 index. A positive Ki-67 index may be predicted from the MIB-1 findings with 90%–100% accuracy, beginning with a MIB-1 index of 30% (Table 2).

Two representative cases are depicted in Figure 3.

Discussion

Our findings show higher MIB-1 indices in FS than in PS, the values obtained from FS lying approximately 2–2.5 times higher than the values obtained from PS. They further substantiate the fact that the average MIB-1 values for PS are similar to the average Ki-67 values for FS. These observations are consistent with the previously reported results of Gerdes et al. [6], Cattoretti et al. [2] and McCormick et al. [7]. They do not agree with the results reported by Barbareschi et al. [1], who found higher MIB-1 indices compared with Ki-67 indices both in FS and PS stained for MIB-1. The difference may be due to the small number of cases (n=40) and/or the different methods for determination of the proliferative index (subjective grading in our study, computer-assisted image analysis used by the Italian authors). The question remains open why the MIB-1 index in FS is about twice as high as the Ki-67 index determined on FS. The difference may be caused by a higher avidity and affinity of the MIB-1 antibody for the Ki-67 antigen when compared with the Ki-67 antibody. It is less probable that the MIB-1 antibody might label additional epitopes of the Ki-67 antigen since it is directed against parts of the Ki-67 antigen and not against the whole antigen.

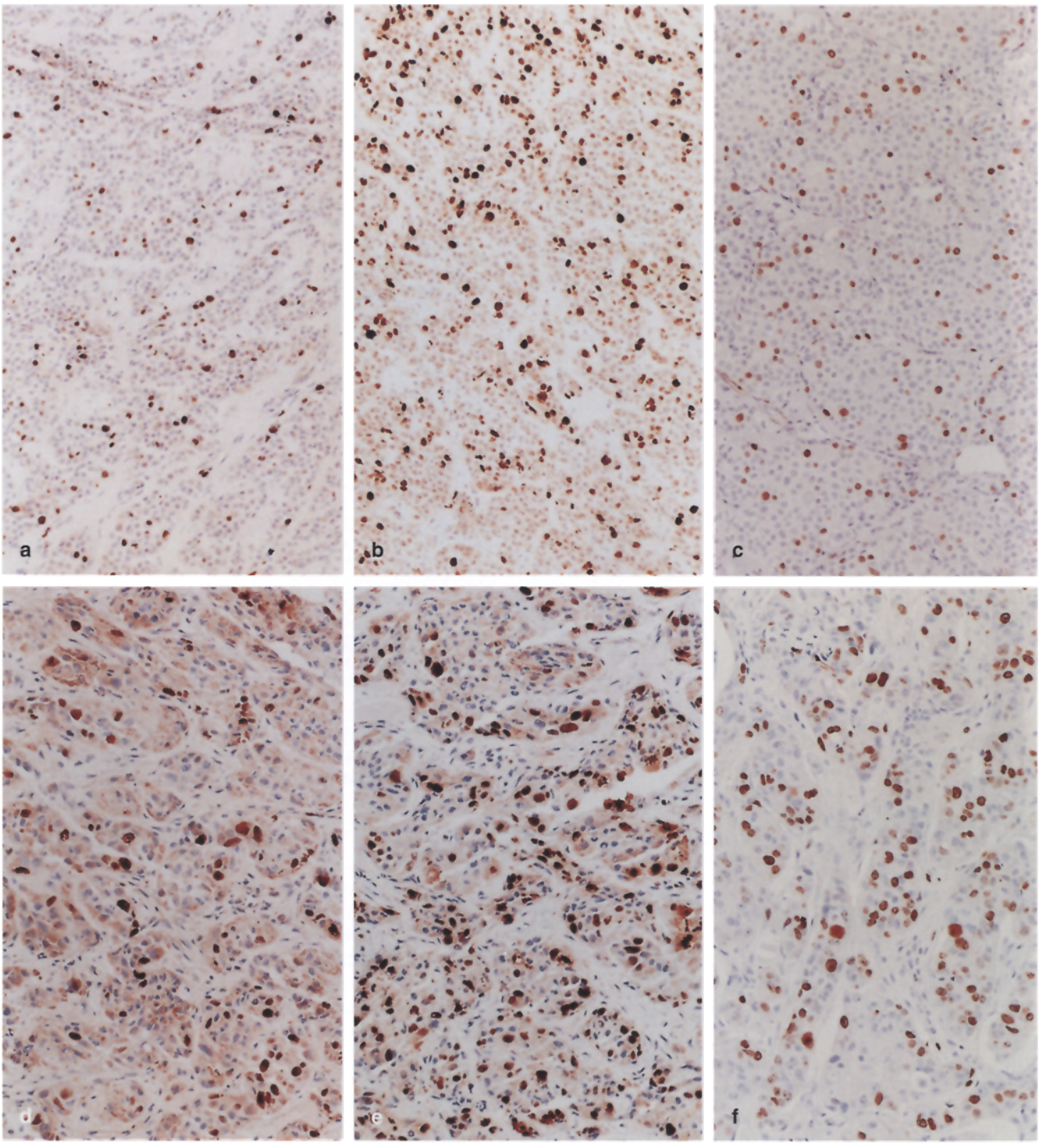

Fig. 3a–f Ki-67 and MIB-1 immunostaining of invasive ductal breast cancer. **a–c** Invasive ductal breast cancer not otherwise specified (NOS) grade 1. (**a**) Ki-67 (FS) 5%, (**b**) MIB-1 (FS) 20%, (**c**) MIB-1 (PS) 5%. **d–f** Invasive ductal breast cancer NOS, grade 2. (**d**) Ki-67 (FS) 10%, (**e**) MIB-1 (FS) 30%, (**f**) MIB-1 (PS) 20%. ×350 each

If our results are evaluated with regard to their practical use, the major problem concerns the usually wide range of MIB-1 values for each Ki-67 index, although there is a satisfactory statistical agreement. This observation rules out a direct comparison of the Ki-67 indices on FS and the MIB-1 indices on PS. If the examination is restricted to the prediction of a negative or positive Ki-67 index on FS (cut-off point between 10% and 20%) from the MIB-1 index on PS, however, a MIB-1 index of 10% correctly predicts a negative Ki-67 index in 97.3% of the

cases, and, vice versa, a MIB-1 index of 30% or more correctly predicts a positive Ki-67 index in 90% increasing to 95% for a MIB-1 index of 50% and to 100% for a MIB-1 index of 80% and 90%, respectively. The close correlation between the Ki-67 findings on FS and the MIB-1 results on PS taken from other parts of the tumour rules out a significant role for heterogeneous distribution of positive nuclei within the tumour tissue for the prediction of Ki-67 from MIB-1 results. It has to be mentioned, however, that both the Ki-67 index and the MIB-1 index have been obtained by subjective grading of the histological sections and that the Ki-67 indices, therefore, cannot be regarded as a "gold standard" with close to a 100% reproducibility.

In summary, the estimation of the MIB-1 index on PS represents a valuable method for the replacement of Ki-67 examination on FS. There is no reason, however, to replace the Ki-67 examination on FS by the MIB-1 examination on FS. This assertion is confirmed by the fact that the examination of the MIB-1 index is at least 20% more expensive than the examination of the Ki-67 index.

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Note added in proof

The number of cases with comparison of Ki-67 and MIB-1 (FS) (Fig. 1) has increased from 459 to 892 cases since the paper was submitted for publication. Statistical analysis largely confirms the results on the 459 tumours depicted in Fig. 1. y has been calculated as 9.4406+1,2082 x and r as 0.84.