

Immunohistochemical factors in patients with the mucinous variant of the breast cancer

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Aim. To analyse the immunohistochemical factors and the prognostic indices assessment, and especially the proliferation rate of MIB-1 and p53 and c-Erb-B2 expression in a group of patients with mucinous breast carcinoma treated at the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Cracow.

Material and methods. Immunohistochemical factors analysis was performed in a group of 81 patients suffering from a mucinous variant of breast cancer. Primary treatment consisted of surgery performed at the Institute of Oncology in Cracow. According to the results of histochemical examination the patients were subdivided into two groups: group 1 consisting of 50 patients with a pure form of mucinous carcinoma (PMC) and group 2 consisting of 31 patients with a mixed form of mucinous carcinoma (MMC). The presence of estrogen and progesterone receptors in the cancerous tissue as well as the expression of p53 protein, c-Erb-B2 and MIB-1 index was determined in all patients. The results were compared in both forms of mucinous carcinoma – pure and mixed.

Results and conclusions. The performed investigations provided the following results: a relatively low proliferation rate of MIB-1, not higher than 14 in more than a half of patients; the presence of the estrogen receptor in over 90% of patients and the presence of the progesterone receptor in over 60% of patients; a low ratio of tumors showing p53 and c-Erb-B2 expression (respectively 12.3% and 9.9%); the adverse influence of p53 expression on 10-year relapse-free survival in all the investigated subjects as well as in the group of patients with the pure form of mucinous carcinoma

Key words: breast cancer, mucinous carcinoma, immunohistochemical factors, estrogen factor, progesterone factor

Aim of the study

Mucinous carcinoma constitutes about 1.5% of all malignant breast cancers [1-6]. The opinion that MC tends to occur more frequently in elderly women, as compared with other microscopic forms of breast cancer, takes precedence [7-10]. The incidence of mucinous carcinoma increases rapidly in subjects over 60 years of age. According to the WHO reports mucinous carcinoma is a tumor which contains a large amount of microscopically, extracellular mucin as well as mucin gathered within cancerous cells [11].

There are two forms of mucinous carcinoma – the pure and the mixed. The pure form is defined as a tumor entirely built of isolated islets of tumor tissue submerged in profuse extracellular mucin. Mixed tumors additionally contain areas of infiltrating carcinoma without extracellular mucin [4, 7]. MC is included to the group of cancers with better prognostic indices, which is

confirmed by a 10-year asymptomatic survival observed in almost 60% of patients [2]. Particularly good prognosis is associated with the pure form of mucinous carcinoma [12, 13] and long survival rates not only reach, but even exceed 90% [14].

An analysis of the immunohistochemical factors as well as that of the prognostic indices assessment focused especially on the proliferation rate of MIB-1 and p53 and c-Erb-B2 expression were performed in the group of patients with mucinous breast carcinoma treated at the Institute of Oncology in Cracow.

Material and methods

Presented retrospective analysis of clinical material was performed on 81 patients with mucinous breast carcinoma. The average age was 62, the youngest patient was 30 years of age and the oldest 84 years of age. All of the patients were primarily treated surgically at the Institute of Oncology in Cracow between the years 1952 and 1993.

The patients were divided into two groups of mucinous carcinoma: pure and mixed – based on the evaluation of histopathological material derived during former surgery. The presence of estrogen and progesterone receptors; p53, c-Erb-B2 expression and MIB-1 index were immunohistochemically determined in all cases. In order to obtain the pathological analysis of all the tumors we used sections routinely stained

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with Hematoxylin and Eosin derived from archival paraffin-embedded tissue blocks stored at the Cancer Pathology Department. Every subject microscopically diagnosed as mucinous carcinoma was assessed for additional histological structure.

Immunohistochemical staining was performed using the same paraffin-embedded blocks that were previously used for obtaining pathological preparations.

Thin sections (4 μm) were sliced, placed on SuperFrost plus microscope slides and heated in the heater for 24 hours at the temperature of 60° Centigrade.

The slides were deparaffinized in xylene twice (30 min each), and further processed in 100% alcohol for 5 minutes, 96% alcohol for 5 minutes and then rinsed in running water. Then the slides were incubated in 3% alcoholic hydrogen peroxide to block endogenous peroxidase.

The sections destined for determining cellular proliferation Ki-67 (MIB-1) antigen were predigested with trypsin solution (Sigma T7168) for 5 min at room temperature. Sections destined for ER, PgR, p53BP and Ki-67 (MIB-1) assessment were placed in citrate buffer and heated in microwave: ER, PgR, p53 twice for 10 min, and Ki-67(MIB-1) twice for 7 min. Buffer loss was replaced during the intervals. After microwave heating the slides were allowed to cool to room temperature.

After the antigen epitopes were recognised the sections were incubated in adequately prepared primary solutions of antibodies.

After incubation the slides were mounted in the chromogen solution (DAB) DAKO nr cat.S3000. The reaction was interrupted by rinsing with water.

After careful rinsing the slides were stained with Harris hematoxylin, dehydrated, illuminated in xylene and enclosed within Canada balm.

The immunohistochemical examination determining the presence of hormonal receptors, p53 protein and Ki-67 (MIB-1) antigen is assumed to be positive within the cellular nuclei. In order to determine the presence of proliferating cells Ki-67 (MIB-1) antigens we defined an index by counting the ratio of positively stained nuclei in 500 cells, where areas of the highest proliferation were chosen.

In case of a positive reaction for the protein product of oncogene c-Erb-B2 presence we assumed a linear reaction localized within the cellular membrane.

The results were compared for both forms of mucinous carcinoma: pure and mixed.

The results of treatment were analyzed basing upon the 10-year survival rate assessment using statistics (Kaplan-Meier method, long-rank test, Cox model); $p \leq 0.05$.

Results

The results of the immunohistochemical investigations of both the pure and the mixed forms of mucinous carcinoma (MIB-1 index, p53 and c-Erb-B2 expression and hormonal receptors) are presented in Table I.

In 43 patients (53.1%) the MIB-1 index was lower or equal 14, and higher than 14 in the remaining 38 patients (46.9%).

The incidence of p53 and c-Erb-B2 expression was rare, and was found only in 12.3% and 9.9% of patients, respectively.

The presence of the estrogen receptor was determined in 74 patients (91.4%), and of the progesterone receptor in 51 patients (63.0%).

When comparing the two groups of patients (i.e. with the pure and the mixed form of mucinous carcinoma) we have found that the presence of the progesterone receptor was slightly higher, however statistically not significant, in patients with the pure form of carcinoma. Among the 31 patients with mixed form of carcinoma 25 tumors showed estrogen receptor presence (80.6%) and among the 50 patients with pure form of carcinoma – 49 tumors (98%). This difference is statistically significant (long-rank test, $p < 0.01$).

The proliferation index MIB-1 was found to exceed 14% in 19 of the 31 cancers of mixed form (61.3%), but in the group of cancers in pure form only it was present only in 38% (19/50 tumors). This difference is statistically significant (log-rank test, $p < 0.05$).

Table I. Immunohistochemical investigation results summary

Indices	Pure form PMC		Mixed form MMC		Together MC	
	Patients nr/percentage		Patients nr/percentage		Patients nr/percentage	
MIB-1 index						
≤ 14	31	62.0	12	38.7	43	53.1
> 14	19	38.0	19	61.3	38	46.9
P53 expression						
yes	6	12.0	4	12.9	10	12.3
no	44	88.0	27	87.1	71	87.7
c-Erb-B2 expression						
yes	4	8.0	4	12.9	8	9.9
no	46	92.0	27	87.1	73	90.1
Estrogen receptor						
present	49	98.0	25	80.6	74	91.4
absent	1	2.0	6	19.4	7	9.6
Progesterone receptor						
present	34	68.0	17	54.8	51	63.0
absent	16	32.0	14	45.2	30	37.0
Together	50	100.0	31	100.0	81	100.0

We have determined the presence of tumors showing positive p53 and c-Erb-B2 expression as equally rare in both groups.

In the evaluated group the predicted 10-year complete and the relapse-free survival rate equals 60.5% (in pure form of mucinous carcinoma – 74% and in mixed form – 39%). The relationship between the results of treatment and the immunohistochemical examinations results have been presented in Table II.

Table II. Treatment effects in correlation with immunohistochemical investigation results

Indices	Number of treated patients	10-year relapse-free survival rate	P (long-rank test)
MIB-1 index			
≤14	43	69.8	
>14	38	60.5	0.0692
P53 expression			
yes	10	20.0	
no	71	70.2	0.0067
c-Erb-B2 expression			
yes	8	60.0	
no	73	59.3	0.5471
Estrogen receptor			
present	74	60.4	
absent	7	45.7	0.5047
Progesterone receptor			
present	51	55.8	
absent	30	60.2	0.4945

Difference statistically significant

When evaluating the treatment results of the 81 patients with mucinous carcinoma we have determined a statistically significant higher probability of 10-year relapse-free survival rate for patients with tumors not showing p53 expression. All other immunohistochemical factors have failed to show statistically significant differences in the course of the evaluation of 10-year survival rates.

The same results were obtained while assessing the group of patients with the pure form of mucinous carcinoma – we have found that patients with non-p53 positive tumours have a statistically higher probability of 10-year relapse-free survival rate. These data are presented in Table III.

There was no statistically significant influence on the relapse-free survival rate of any of the immunohistochemically evaluated factors in the group of 31 patients with mixed form of carcinoma. The data are presented in Table IV.

Table III. Probability of 10-year relapse-free survival rate

Indices	Number of treated patients	10-year relapse-free survival rate	P (long-rank test)
MIB-1 index			
≤14	31	77.4	
>14	19	68.4	0.6542
P53 expression			
yes	6	33.3	
no	44	78.9	0.0257
c-Erb-B2 expression			
yes	4	75.0	
no	46	71.0	0.9393
Estrogen receptor			
present	49	70.2	
absent	1	–	0.2758
Progesterone receptor			
present	34	68.7	
absent	16	67.0	0.7915

Difference statistically significant

Table IV. Probability of 10-year relapse-free survival rate

Indices	Number of treated patients	Percentage of 10-year relapse-free survival rate probability	P (long-rank test)
MIB-1 index			
≤14	12	50.0	
>14	19	31.6	0.2784
P53 expression			
yes	4	25.0	
no	27	65.8	0.1442
c-Erb-B2 expression			
yes	4	37.5	
no	27	35.6	0.5922
Estrogen receptor			
present	25	34.5	
absent	6	31.3	0.7857
Progesterone receptor			
present	17	32.4	
absent	14	55.6	0.5494

Discussion

Immunohistochemical investigations performed in the presented group of patients have revealed the characteristic features of mucinous carcinoma of breast:

- relatively low MIB-1 index, not exceeding 14 in more than a half of the patients (2-46),
- low ratio of tumors showing p53 and c-Erb-B2 expression, respectively 12,3% and 9,9%,
- in more than 90% of patients – the presence of the estrogen receptor and in more than 60% – of the progesterone receptor.

We did not encounter any publications concerning the role of the MIB-1 in patients with mucinous carcinoma.

noma of the breast in available literature. Obviously, there are publications considering the topic of nipple cancer for the whole group of patients [15-18]. Meyer et al., while analyzing another cancerous proliferation marker – the ratio of cells in the S-phase- have stated that it is low in mucinous breast carcinoma [19, 20].

We have failed to find any studies analyzing analyzing the frequency of p53 and c-Erb-B2 expression in mucinous carcinoma of the breast. However, there are other publications considering this issue in the entire group of patients with nipple cancer [15, 21-23].

The data found in literature confirm our observation, that in a vast majority of patients with mucinous carcinoma of the breast the presence of hormonal receptors is revealed [24, 25].

In the evaluated material a comparison between the results of the immunohistochemical investigations in the two groups of patients – with pure and mixed form of carcinoma – was performed. That comparison had revealed that in the group of patients with MMC following statements are statistically significant:

- more patients have a MIB-1 index exceeding 14 (61.3% vs. 38%),
- less patients reveal the presence of the estrogen receptor (80.6% vs. 98%).

In the investigated group cytofluorometric investigations were not performed due to their large number, and in the view of no doubtful data in the literature. There is evidence to prove, that only a small percentage of MC, mostly in its pure form, is aneuploid [2]. It is stressed that aneuploid cancers are mainly present in patients with the mixed form of MC and that aneuploidy correlates positively with metastases to axillary lymph nodes and with the lack of estrogen receptors [2].

Conclusions

The characteristics of mucinous carcinoma of the breast found in immunohistochemical investigations are as follows:

1. Relatively low MIB-1 proliferation index, not exceeding 14 in more than a half of patients
2. Presence of estrogen receptors in more than 90% of patients and progesterone receptors in more than 60% of patients.
3. Low percentage of tumors showing p53 and c-Erb-B2 expression, respectively 12,3% and 9,9%,
4. Presence of p53 expression has an adverse influence on 10-year relapse-free survival rate in whole investigated group as well as in the group with pure form of MC.

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Received: 4 December 2007

Accepted: 12 January 2008