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RELATION BETWEEN PATHOMORPHOLOGICAL RESPONSE IN TUMORS AFTER NEOADJUVANT CHEMOTHERAPY AND CLINICO-MORPHOLOGICAL AND MOLECULAR PROGNOSTIC FACTORS IN PATIENTS WITH BREAST CANCER

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Aim: **To determine the correlation between tumor pathomorphological response (PMR) after neoadjuvant chemotherapy (NACT) and clinico-morphological and molecular prognostic factors in patients with breast cancer (BC), and to determine the possible impact of the PMR and estrogen receptors (ER), progesterone receptors (PR) and Her-2/neu BC status on the disease course.** ***Methods:*** **The data from the medical history of patients on IIB stage (T2N1M0, T3N0M0) (n = 247), who received treatment with NACT, were used. The correlation between the parameters was determined using the Spearman's coefficient. Patient's survival was analyzed by Kaplan – Meier method. The association between PMR grades with the risk of disease relapse was estimated by Cox's regression analysis.** ***Results:*** **PMR grade correlated with tumor differentiation grade ($rho = 0.38$; $p < 0.01$), and is not related to the age of patients ($rho = 0.02$; $p > 0.05$) and BC subtypes ($rho = 0.05$; $p > 0.05$). The patients with the same PMR grades didn't differ by the number of lymph node metastases ($p > 0.05$) and differed by the presence of embolus in tumor vessels ($p < 0.05$). The rates of 3-years disease-free survival (DFS) differed between the groups of patients with different PMR grade ($\chi^2 = 25.5$; $p < 0.0001$). The patients with the grade 2–3 of pR had highest survival ($p < 0.05$). The groups of patients with identical subtype of BC had different survival rates dependent on PMR grades (for basal subtype ($\chi^2 = 15.176$; $p < 0.001$); luminal A subtype ($\chi^2 = 14.9$; $p < 0.001$) and Her-2/neu subtype ($\chi^2 = 2.4$; $p > 0.05$). The risk of disease relapse depended on PMR grade: for grade 2–3 it was significantly decreased (HR = 0.71, 95% CI — 0.25–2.9, $p = 0.0037$), and for grade 4–5 it was the highest (HR = –1.23, 95% CI — 0.24–5.05, $P = 0.0001$), while 0–1 grade had no impact on the risk of disease relapse (HR = 0.22, 95% CI 0.08–0.38; $p = 0.7$).** ***Conclusion:*** **The data of combined clinical, histological and immunohistochemical analysis have shown that PMR grades may serve as the criteria for individualization of adjuvant treatment of the patients with locally advanced BC.**

Key Words: **breast cancer, pathomorphological response, estrogen receptor, progesterone receptor, basal subtype, luminal subtype, Her-2/neu positive subtype.**

Individualization of combined treatment of BC patients is an important component of treatment strategy aimed on improvement of remote results [1]. Evaluation of tumor PMR after NACT can be used for individualization of treatment, serving as an indicator of tumor sensitivity to applied chemotherapy.

The role of PMR grade combined with ER/PR and Her-2/neu status of tumor is not certain [2–5]. Groups of patients with the different PMR grades but with BC stage and undergoing equal neoadjuvant therapy, are heterogeneous by other criteria (grade of differentiation, ER/PR and Her-2/neu status of tumor, age of patient).

We have studied BC cases classified into three subtypes: basal (ER^-, PR^-, Her-2/neu^-), luminal (ER^+ and/or PR^+, Her-2$neu^{+/-}$) and Her-2/neu positive (ER^-, PR^-, Her-2/neu^+), which have a different prognosis, various responsiveness to therapy and require different treatment approaches [4–14].

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Abbreviations used: BC – breast cancer; CAF – 5-fluorouracil (5-FU), doxorubicin and cyclophosphamide); DFS – disease free survival; ER – estrogen receptors; NACT – neoadjuvant chemotherapy; OS – overall survival; PMCR – pathomorphological complete response; PMR – pathomorphological response, PR – progesterone receptors.

The aim of the present research was to determine the correlation between tumor PMR after NACT and clinico-morphological and molecular prognostic factors in BC patients, and to determine the possible impact of PMR and ER, PR and Her-2/neu BC status on the disease course.

MATERIALS AND METHODS

Retrospective study of 247 patients with BC of stage IIB cured in City Oncological Hospital (Kiev, Ukraine) in 2003–2005 has been performed. The age of the patients was from 30 to 70 years (median age of 54.2 ± 10.5 years). Untreated patients with BC stage IIB (T2N1M0, T3N0M0) received two 3-week cycles of 5-fluorouracil (5-FU), doxorubicin and cyclophosphamide (the CAF regimen). All of them underwent surgery after systemic treatment. The morphological variant of BC, tumor differentiation grade, lymph node metastastasis, PMR grade were evaluated by pathomorphological research*.

ER/PR and Her-2/neu expression were evaluated by immunohistochemical analysis. ER and PR expression by less than 5% of tumors cells was considered as expression negative. The Her-2/neu status of tumor was considered positive in cases with strong complete

*The research was performed according to the regulations of institution's Ethical Committee.

membrane Her-2/neu expression (score 3+). All cases with ambiguous expression of Her-2/neu (score 2+) were previously analyzed by FISH analysis as it is described [15, 16]. According to the data of immunohistochemical analysis, we have sudivided BC cases into four groups: basal subtype (ER^-, PR^-, $Her2/neu^-$), luminal A (ER^+ and/or PR^+, $Her2/neu^-$), luminal B (ER^+ and/or PR^+, $Her2/neu^+$), and Her-2/neu positive (ER^-, PR^-, $Her2/neu^+$).

The NACT was identical for all groups of BC patients — two 3-week CAF cycles.

The correlation between the parameters was analyzed using Spearman's coefficient. Patient's survival was analyzed by Kaplan – Meier method. The association between PMR grade with the risk of disease relapse was estimated by Cox regression analysis. Significance of mentioned coefficients was evaluated by χ^2 test. *P* values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Clinico-morphological characteristics of the patients with BC were different. There were 232 (96%) BC patients with defined histological type of tumor: invasive ductal cancer (177 (76.3%) cases), lobular cancer (24 (10.4%) cases), ductal-lobular cancer (10(4.3%) cases), mucous cancer (16 (6.9%) cases), and papillary cancer (5 (2.1%) cases); in 15 (4%) patients with pathomorphological complete response (PMCR) the type of tumor was not defined. Tumor grades were well differentiated (G1) in 29 (13.4%) cases, moderately differentiated (G2) in 173 (79.7%) cases, and poorly differentiated (G3) in 15 (6.9%) cases. There were no nondifferentiated tumors.

In 15 (6%) patients no residual viable tumor cells (invasive or noninvasive) in resected specimens of the breast tumors and lymph nodes were found, that is why morphological and immunohistochemical analysis was not performed.

Lymph node metastases were revealed in 185 (74.9%) patients, and none — in 55 (22.9%) patients.

There were 7 (2.8%) cases with total fibrosis and hyalinosis in lymph nodes, assessed as absence of lymph node metastasis. There were 30 patients, with characteristic for metastasis altered structure of lymphatic nodes, as detected by ultrasound examination. In these cases the metastasis in lymph nodes were not found by histological analysis as well. In 19 patients without lymph node metastasis in the preoperative period, lymph node metastases were found by histological study.

The number of lymph node metastases is a prognostic criterion. In studied BC cases, metastasis in 1–3 lymphatic nodes were found in 78 (32.5%) cases, in 4–6 lymphatic nodes — in 69 (28.8%) cases, in 7–10 lymphatic nodes — in 38 (15.8%) cases. There were 24 (9.7%) patients with embolus in tumors vessels.

The number of lymphatic nodes with metastasis and embolus in tumor vessels characterize metastatic potential of the tumor. We have analyzed the relation between cases with different number of lymph node metastases and cases with embolus in tumor vessels, and have revealed that the patients negative by lymph node metastasis had no embolus in tumor vessels (Fig. 1). The number of lymph node metastases was positively associated with the presence of embolus in tumor vessels ($p < 0.01$).

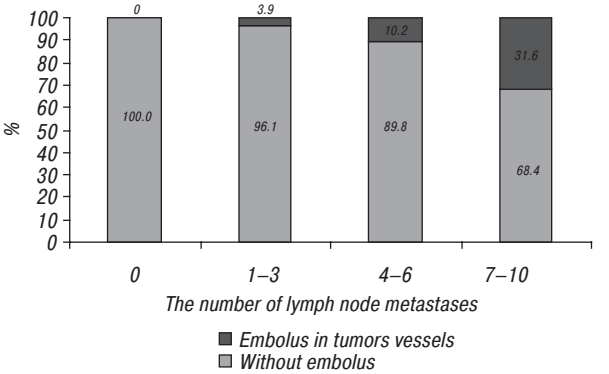

Fig. 1. Relation between embolus in tumors vessels and the number of lymph node metastases

Expression of ER/PR and Her-2/neu were analyzed by immunohistochemical method; in 15 cases with PMCR immunohistochemical analyzes was not available.

We have determined that 80 (34.5%) patients had basal BC subtype, 101 (43.5%) patients — luminal A subtype, 12 (5.2%) — luminal B subtype, and 39 (16.8%) patients — Her-2/neu positive subtype.

There were only 5.2% patients with luminal B subtype of BC. The treatment and the course of the disease in these patients at higher degree depended on hyperexpression of Her-2/neu, than on ER/PR expression. We combined luminal B and Her-2/neu subtypes: this combination looks feasible because both subtypes require similar chemotherapy with anthracyclines and targeted therapy with trastuzumab, and these patients belong to the group with high risk of disease relapse.

Efficacy of performed NACT was evaluated by pathomorphological criteria and PMR grade. Relation between PMR and clinico-morphological and molecular prognostic factors was observed. The patients had different response rates after two identical NACT cycles: PMR grade 0–1 was registered in 107 (43.3%) patients (Fig. 2, *a*), grade 2–3 — in 104 (42.1%) cases (Fig. 2, *b, c*), grade 4–5 — in 36 (14.6%) patients (Fig. 2, *d*).

We have analyzed the factors affecting response rate and PMR grades. Relation between patients age and PMR grade are presented in Table1.

Table 1. The grade of pathological response in BC patients of different age

The PMR	30–39 years		40–49 years		50–59 years		60–70 years	
	n	%	n	%	n	%	n	%
0–1	9	40.9	30	53.6	44	41.9	24	37.5
2–3	5	22.7	16	28.5	50	47.6	33	51.6
4–5	8	36.4	10	17.9	11	10.5	7	10.9
All	22	100	56	100	105	100	64	100

Age less than 40 years was found to be a favorable predictor value for high grade PMR ($p < 0.05$). The morphological structure of tumor, its ER/PR status and proliferative activity depended on age of patients. In senior patients, there are well differentiated ER/PR positive tumors with low level of proliferative activity. The younger patients had poorly differentiated tumors with high level of proliferative activity, and often tumors

were ER/PR-negative or Her-2/neu positive [8, 12, 15]. So, the PMR grade seems not be depended from the age of patients from all age groups ($\chi^2 = 5.84$), and correlation between the age of patients and PMR grade was not found (*rho* = 0.02; $p > 0.05$).

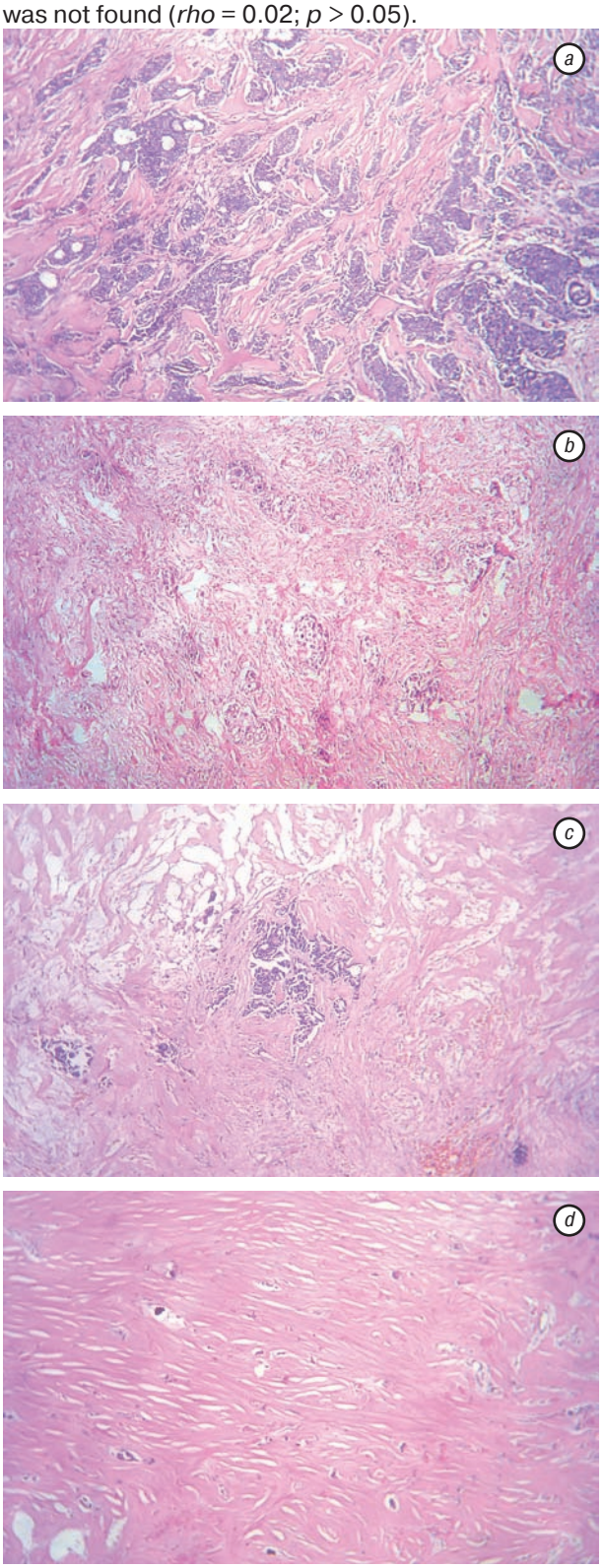

Fig. 2. BC tissue after NACT. *a*, PMR grade I; *b*, PMR grade II; *c*, PMR Grade III; *d*, PMR Grade IV; *e*, PMR Grade IV (hematoxiline-eosin staining, 200X)

We have analyzed the correlation between tumor differentiation grade and PMR grade and determined that well differentited tumors (G1) had minimal response rate (0–1) in 96.6% (30 cases) of patients. Only in one G1 case, PMR grade 2 was found. There were no cases with PMCR in well differentiated tumors. In tumors with differentiation grade G2 we have found PMR grade 0–1 in 44.5% (82 patients), grade 2–3 — in 53.8% (100 patients), grade 4–5 (PMCR) — in 1.7% (3 patients). In tumors with poor differentiation (G3), PMR grade 0–1 was detected in 18.7% (3 patients), grade 2–3 — in 62.6% (10 patients), grade 4–5 — in 18.7% (3 patients). Relation between tumor differentiation grade and PMR grade is presented on Fig. 3. PMR grade is in opposite relation with tumor differentiation grade ($\chi^2 = 31.33$; $p < 0.001$). Well differentiated tumors had poor response rate in 96.6% cases, in 36.6% of them mucous cancer (7 cases) and papillary cancer (5 cases) were found, and these types of cancer belong to the types with favorable prognosis. There were no tumors with simultaneously both features: PMCR and high differentiation grade. So, we have demonstrated that there is a dependence between the tumor differentiation grade and PMR grade ($p < 0.01$), as well as significant correlation (*rho* = 0.38; $p < 0.01$).

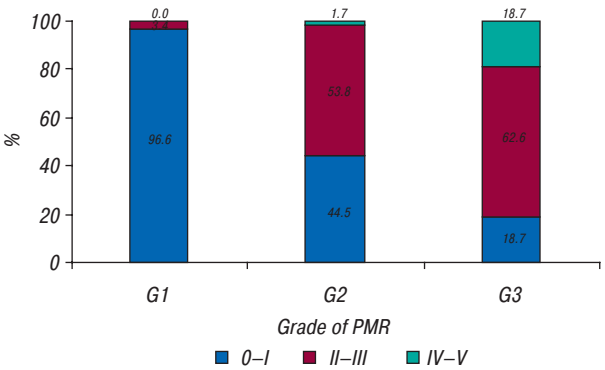

Fig. 3. Relation between PMR grade and tumor differentiation grade

The disease course is dependent on prognostic factors such as the number of lymph node metastases. We have analyzed the relation between tumor PMR grade and the number of lymph node metastases (Table 2).

Table 2. The relation between tumor PMR grade and the number of lymph node metastases

The number of lymph node metastases	PMR grade						All cases	
	0–I		II–III		IV–V			
	n	%	n	%	n	%	n	%
0	26	47.3	26	47.3	3	5.4	55	100
1–3	33	42.3	36	46.2	9	11.5	78	100
4–6	30	43.5	27	39.1	12	17.4	69	100
7–10	18	47.4	15	39.5	5	13.1	38	100

It was found that PMR of grade 4–5 occur more often in the cases with lymph node metastases than in the cases without metastases in lymph nodes with ($p < 0.05$). There was no relation between the number of lymph node metastases, and there was relation between the PMR grade 4–5 and presence or absence of metastases in lymph nodes.

Embolus in tumor vessels were found in 24 (9.7%) of BC cases. We have analyzed a relation between PMR grade and the presence of embolus in tumor vessels. Embolus in tumor vessels were detected in 7 (29.2%) patients with PMR of grade 0–1 (Fig. 4, *a*), 2(8.3%) cases with PMR grade 2–3 (Fig. 4, *b*) and in 15

(62.5%) patients with PMR grade 4 (Fig. 4, *c*). The difference between the groups with PMR grade 0–1 and 4–5 (29.9% *vs*. 62.5%, $p < 0.001$) and between the groups with PMR grade 2–3 and 4 (8.3% *vs*. 62.5%, $p < 0.001$) was statistically significant, as well as that between PMR grade 0–1 and 2–4 (29.9% *vs.* 8.3%) (Fig. 5). Embolus in tumor vessels were more common in PMR grade 4–5 cases than in PMR grade 0–1 and 2–3 cases ($p < 0.01$). Groups of patients with the same PMR grade were different by such pattern as embolus in tumor vessels ($\chi^2 = 17.8$; $p < 0.01$).

The next step was to analyze the results of immunohistochemical research, performed in 232 (96.0%) cases (in 15 (4.0%) from 247 cases there was PMCR, material not available). On the basis of immunohistochemical research on ER/PR and Her-2/neu status, the BC tumors were classified on BC subtypes: basal subtype (80 (34.5%) cases), luminal A subtype (101 (43.5%) cases), and Her-2/neu subtype (51 (22%) cases). We have analyzed the relation between the BC subtypes and PMR grade (Table 3).

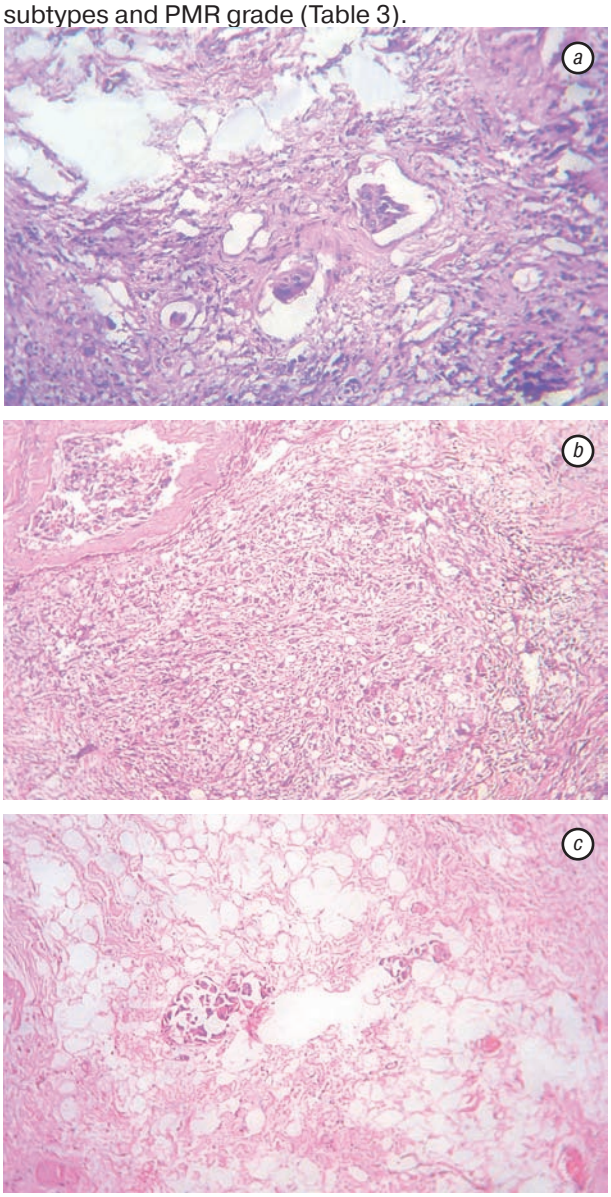

Fig. 4. BC tissue after NACT, embolus in tumors vessels. *a*, PMR grade I; *b*, PMR grade II-III; *c*, PMR Grade IV (hematoxiline-eosin staining, 200X)

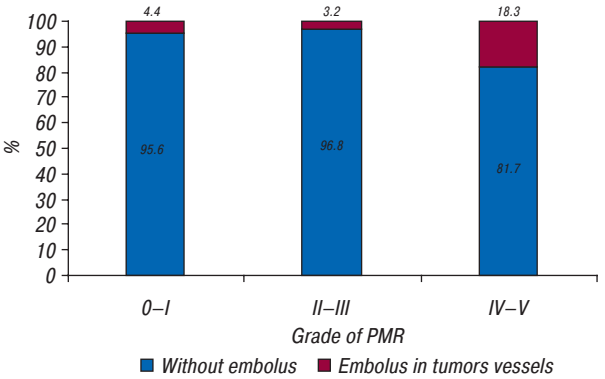

Fig. 5. Relation between PMR grade and embolus in tumors vessels

Table 3. Relation between BC subtypes and PMR grade

BC subtype	PMR grade						All	
	0–I		II–III		IV–V			
	n	%	n	%	n	%	n	%
Basal	37	46.3	34	42.5	9	11.2	80	100
Luminal A	46	45.5	48	47.5	7	7.0	101	100
Her-2/neu$^+$	24	47.1	22	43.1	5	9.8	51	100

There were found no statistically significant difference and no correlation between PMR grade and subtype of BC (*rho* = 0.05; $p > 0.05$).

The 3-years death free survival (DFS) was in 66.8% (165 patients), the 3-years overall survival (OS) — 75.7% (187 patients). During 3-years after the treatment 60 (24.3%) patients died. We defined that 3-years DFS of patients with PMR grade 0–1 was 66.3% (71 patients), with grade 2–3 — 77.9% (81 patients), and with grade 4–5 — 36.1% (13 patients) (Fig. 6).

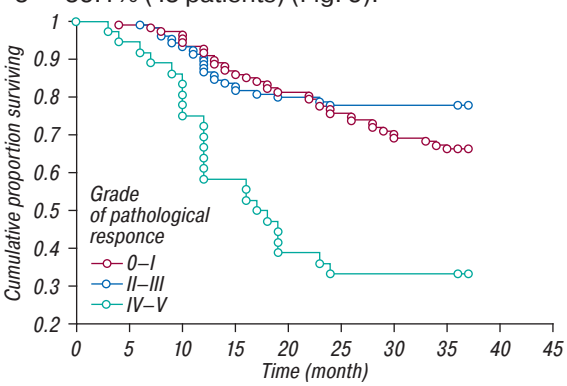

Fig. 6. 3-years DFS of patients with different PMR grade

Analysis of survival curves by the method of Kaplan — Meier in groups of patients with different PMR grades after NACT (Fig. 6) has shown that survival was significantly higher in the group with PMR grade 2–3, lower — in group with PMR grade 0–1, and the lowest — in patients with PMR grade 4–5 ($\chi^2 = 25.5$; $p < 0.0001$). It may be explained by the fact that BC tumors with high mitotic activity and poor differentiation grade without expression of ER/PR are more sensitive to chemotherapy, but such patients have poor prognosis and more aggressive disease course [4, 5].

Then we have analyzed 3-years DFS in the groups of patients with identical BC subtypes dependent on PMR grade (Fig. 7). The rate of 3-years DFS of the patients with basal BC subtype with PMR grade 2–3 (52.9%) was significantly higher than that of the patients with PMR grades 0–1 (28.6%) or 2–3 (22.6%) ($p < 0.05$). The curves of 3-years DFS of patients with

basal BC subtype and different PMR grades are shown on Fig. 8, *a* ($\chi^2 = 15.176$; $p < 0.001$).

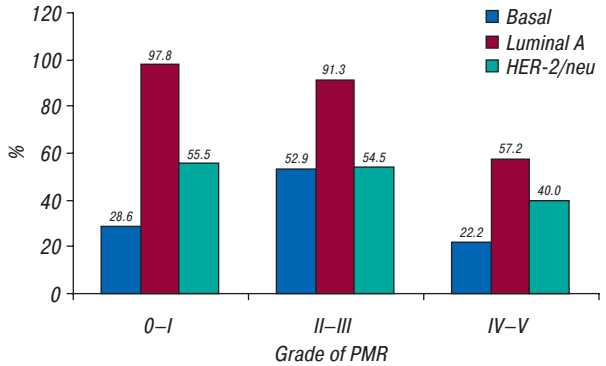

Fig. 7. 3-years DFS of patients with similar BC subtype dependent on PMR

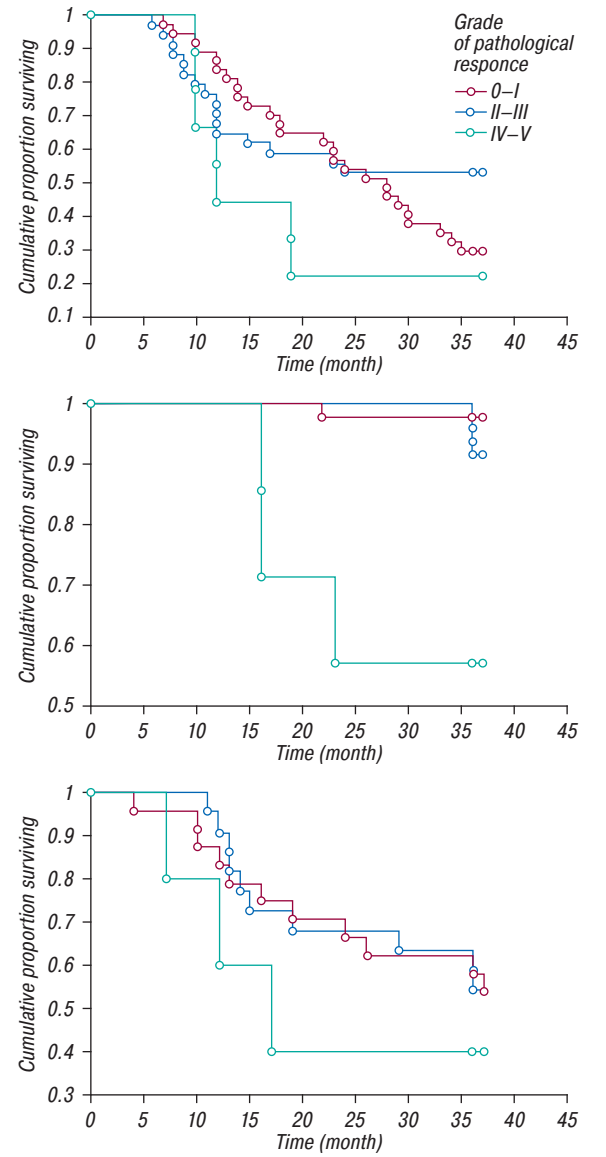

Fig. 8. 3-years DFS of patients with different PMR grade and *a*, basal subtype of BC; *b*, luminal A subtype of BC; *c*, Her-2/neu subtype of BC

The highest 3-years DFS was revealed in the group with PMR grade. The curves of 3-years DFS of the patients with luminal A subtype of BC are shown on Fig. 8, *b* ($\chi^2 = 14.9$; $p < 0.01$). There was no statistically significant difference between the survival of patients with luminal A subtype with PMR grade 0–1 and grade 2–3 (97.8% *vs.* 91.3%, $p > 0.05$). and the value of 3-years DFS of patients with PMR grade 4–5 falls to 57.2% ($p < 0.05$). The curves of 3-years DFS of the patients with Her-2/neu subtypes are presented on Fig. 8, *c* ($\chi^2 = 2.4$; $p > 0.05$). So, we have demonstrated that 3-years DFS rates were dependent on PMR grade.

It was also determined an impact of PMR grade on the risk of disease relapse. The risk of disease relapse was found to depend on PMR grades: significantly decreased risk of relapse (HR = 0.71, 95% CI — 0.25–2.9, $P = 0.0037$) was determined in the cases of PMR grade 2–3, while the highest risk of relapse (HR = –1.23, 95% CI — 0.24–5.05, $P = 0.0001$) — in the patients with PMR grade 4-5, and no dependence between the parameters — in PMR grade 0–1 cases (HR = 0.22, 95% CI; 0.08–0.38; P = 0.7).

The data of combined clinical, histological and immunohistochemical analysis have shown that PMR grades may serve as the criteria for individualization of adjuvant treatment of the patients with locally advanced BC.

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