Metformin in neoadjuvant systemic therapy of breast cancer patients with metabolic syndrome

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SUMMARY

Background: *The aim of this prospective randomized trial was to investigate the influence of metformin on the effectiveness of neoadjuvant systemic therapy in breast cancer patients with metabolic syndrome.* **Patients and methods:** *The study included 72 patients (from 31 to 77 years of age) who received neoadjuvant systemic treatment for stage II-III breast cancer at the National Medical University named after O.O Bogomolets, Municipal City Clinical Oncological Center, Department of Oncology, Kyiv during 2010 - 2014. Metabolic syndrome was diagnosed in all patients according to the International Diabetes Federation criteria. They were divided in two groups: group 1 that included 36 patients with metabolic syndrome and breast cancer who did not intake metformin during neoadjuvant systemic therapy, and group 2 that included 36 patients with metabolic syndrome and breast cancer who received metformin with neoadjuvant systemic therapy.* **Results:** *Complete clinical response was identified in 2 (6%) patients from group 1 and 10 (27.5%) patients from group 2. Overall clinical response rate (cCR + cPR) was achieved in 28 (77.5%) patients treated with metformin compared to 9 (25%) patients from group 1. A stable disease was observed in 19 (53%) patients who were not taking metformin. The rate of pathological complete response was 26.5% (9 patients) in the metformin group and 6% (2 patients) in the non-metformin group.* **Conclusions:** *Combined neoadjuvant systemic anticancer therapy of breast cancer patient with metabolic syndrome with metformin has a higher clinical and pathological overall response rate than treatment without metformin.*

Key words: *breast cancer, metabolic syndrome, neoadjuvant systemic therapy, metformin*

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INTRODUCTION

Breast cancer (BC) is one of the most spread cancers among women worldwide. In 2012 breast cancer incidence was 43.3 per 100 000 in female population, with 1 676 633 new registered cases along with 521 907 deaths from this disease in the world. This accounts for 25.2% of cases and 14.7% of deaths among all cancers in women (1). According to the National Cancer Registry of Ukraine, in 2012 there were 17407 new breast cancer cases and 7727 deaths. This accounts for 19.6% of cases and 20.2% of deaths among all cancers in women in Ukraine. In Ukraine, breast cancer incidence increased from 38.6 cases per 100 000 in female population in 2006 to 41.4 cases in 2011. However, the death rate from breast cancer seems to have a tendency to decrease from 7826 cases in 2006 to 7727 in 2011, or 20.3% (2006) and 20.2% (2011) of the death cases among all cancers in the female population (2, 3).

In 2005, in connection with the overall mortality rate the International Diabetes Federation (IDF) defined metabolic syndrome (MS) as one of the main problems of modern medicine. Prevalence of MS has reached pandemic proportions. In developed countries MS was found in 25-35% of the population and in all age groups. This value increased with age and reached 42-43.5% in the age group above 60 years old (4). A number of epidemiological, experimental and clinical studies proved that the metabolic abnormalities, associated with MS, increase the risk of breast cancer and worsen its prognosis. For example, in MS patients decreased sensitivity of the tumor to systemic anticancer therapy, increased rate of postoperative complications as well as reduced overall and disease-free survival compared to patients without MS was reported (5-8). In addition, some drugs that are used in systemic anticancer therapy of breast cancer increase insulin resistance - the main pathogenetic link of MS. Specifically, dexamethasone, which is commonly used in breast cancer chemotherapy, causes hyperglycemia. In menopausal women with obesity tamoxifen reduced insulin sensitivity by almost seven fold and increased incidence of type 2 diabetes mellitus compared to women who did not intake tamoxifen (9,10).

Experimental studies have found that anti-tumor effect of metformin is associated with activation of AMP-dependent protein kinase (AMPK), which plays a key role in the cell energy balance. Activation of AMPK leads to inhibition of anabolic processes - depression of neoglucogenesis in hepatocytes and lipolysis in adipocytes, protein synthesis reduction by inhibiting mTOR (mammalian target of rapamycin), and launching of catabolic processes in the cell (increased glycolysis and fatty acid oxidation), arrest of the cell cycle in G0/G1-phase and the stimulation of the p53-dependent cell autophagy (11, 12). Furthermore, metformin can directly (without AMPK activation) block protein mTOR, which stimulates the biosynthesis of proteins and promotes cell growth and proliferation, thereby demonstrating antiproliferative activity (11).

The aim of this prospective randomized trial was to investigate the influence of metformin on the effectiveness of neoadjuvant systemic anticancer therapy of breast cancer patients with metabolic syndrome.

MATERIALS AND METHODS

Patients and study design

The study included 72 patients aged from 31 to 77 years (median age, 58±1 years), with stage II or III breast cancer, which received treatment at National Medical University named after O.O Bogomolets, Municipal City Clinical Oncological Center, Department of Oncology, Kyiv, from 2010 to 2014. All patients had clinical examination, chest X-ray, abdominal,

breast and regional lymph node ultrasound, bilateral mammography, and pathological assessment. Node status was assessed through fine needle aspiration of palpable lymph nodes. Metabolic syndrome was diagnosed according to the criteria of the International Diabetes Federation (4). Exclusion criteria were: bilateral breast cancer, male breast cancer and inflammatory breast cancer. Study was approved by the Ethical Committee of National Medical University named after O.O. Bogomolets. All patients signed informed consent before inclusion in this study. Study design is shown in Fig. 1.

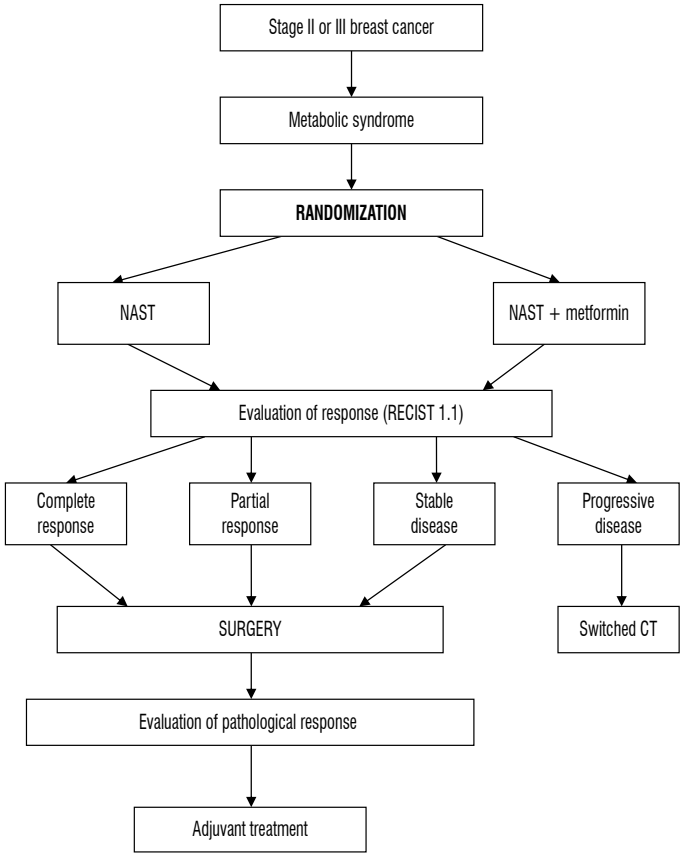


Figure 1. Study design

All breast cancer patients (n = 72) were divided into two groups. The group 1 included 36 breast cancer patients with MS who did not receive metformin during NAST. Group 2 included 36 breast cancer patients with MS who were using metformin concurrently with NAST (according to National breast cancer treatment protocols). Some patients received 4 cycles of anthracycline based chemotherapy in 3 weekly schedules. Postmenopausal patients with Luminal A and Luminal B breast cancer molecular subtypes received hormonotherapy (letrozole 2.5 mg per day) for 16 weeks (13). Concurrently with the NAST patients from group 2 used 500 mg metformin 30 minutes before meal, three times a day. The effectiveness of preoperative systemic therapy was evaluated according to RECIST 1.1 criteria (14). After neoadjuvant chemotherapy, all patients underwent mastectomy or breast conserving surgery with axillary lymph node dissection.

Pathology and Immunohistochemistry

Pathologic response of tumour was graded based on the Miller-Payne system (15), as follows:

- Grade 1: No change or some minor alteration in individual malignant cells, but no reduction in overall cellularity
- Grade 2: A minor loss of tumor cells, but overall high cellularity; up to 30% reduction of cellularity
- Grade 3: Reduction in tumor cellularity between 30% and 90%
- Grade 4: A marked disappearance of more than 90% of tumor cells such that only small clusters or widely dispersed individual cells remain (almost pathological complete response - pCR)
- Grade 5: No invasive malignant cells identifiable in sections from the site of the tumor (pCR)

The cuts of 4-5 μm, by thickness were made out of paraffin blocks, placed on the glass slides and treated with poly-L-lysine. Then, the material was treated using ER-clone 1D5, PgR-clone 636 , and Her-2/neu-clone Cb11 (Dako, Produktionsvej, Glostrup, Denmark) antibodies. Interpretation of the results was done using qualitative assessment of nuclear reaction (negative "–", the weakly positive "+", moderately positive "++" and strongly positive "+++") and quantitative evaluation (% of colored tumor cells).

Assessment of Her-2/neu expression was performed based on color intensity of cytoplasmic basal membrane, as follows: "–" and "+" – absence of hyperexpression and,"+++" – hyperexpression of Her-2/neu. In cases of "++" reaction fluorescent in situ hybridization (FISH) was conducted.

Statistical analysis

Statistical significance of differences between treatment groups was evaluated using ANOVA and analysis of 2xK type contingency tables. Differences were considered statistically significant at a significance level of 5% ($p<0.05$). All statistical calculations were performed using Statistica 6.0 for Windows program.

RESULTS

Depending on the administration of metformin during neoadjuvant systemic anticancer therapy (NAST) all breast cancer patients (n = 72) with metabolic syndrome were divided into two groups. Group 1 included BC patients (n = 36) with MS who did not intake metformin during NAST, and group 2 that included BC patients (n = 36) with MS who simultaneously received metformin with NAST. Patients' characteristics were described in Table 1.

Differences in breast cancer clinical stage, metabolic syndrome components, age at diagnosis, menstrual status, tumor size, lymph node status, tumor grade, histological tumor type, hormone receptor status, Her2/neu expression, Ki-67 value and molecular tumor subtype between the study groups were not statistically significant. This indicates the equal distribution of patients in groups.

Clinical response rates were shown in Table 2. Mammographic data were evaluated before and after treatment.

Overall clinical response rate (cCR+cPR) was achieved in 28 patients (77.5%) in group 2, including 10 patients (27.5%) with complete and 18 patients (50%) with partial response versus 9 patients (25%) in group 1. During inductive systemic therapy 19 patients (53%) from group 1 developed stable disease, 8 patients (22%) from the group 1 and 2 patients (5.5%) from the group 2 developed progression of the disease.

Categorization	Variable	Group 1	Group 2	P value
Clinical stage	Stage II	19 (53%)	16 (44%)	
	Stage III	17 (47%)	20 (56%)	
Metabolic syndrome	Hypertension	19 (53%)	20 (56%)	
	Dyslipidemia	31 (86%)	30 (80%)	
	Class 1 obesity	25 (69.5%)	25 (69.5%)	
	Class 2 obesity	8 (22%)	7 (19.5%)	
	Class 3 obesity	3 (8.5%)	4 (11%)	
Mean age ± SD (years)		58.3±1.3	57.9±1.4	
Menstrual status	Premenopausal	7 (19%)	5 (14%)	
	Postmenopausal	29 (81%)	31 (86%)	
Tumor size	≤ 2 cm	2 (5.5%)	3 (8%)	
	2-5 cm	24 (67%)	20 (56%)	
	> 5 cm	10 (27.5%)	13 (36%)	
Lymph node status	Negative	4 (11%)	5 (14%)	
	Positive	32 (89%)	31 (86%)	
Tumor grade	G1	1 (3%)	1 (3%)	
	G2	32 (89%)	29 (80%)	
	G3,G4	3 (8%)	6 (17%)	
Histological tumor type	Invasive ductal	34 (94%)	31 (86%)	>0.05
	Invasive lobular	2 (6%)	5 (14%)	
Estrogen receptor status	ER-positive	25 (69%)	23 (64%)	
	ER-negative	11 (31%)	13 (36%)	
Progesterone receptor status	PR-positive	16 (44%)	15 (42%)	
	PR-negative	20 (56%)	21 (58%)	
Her2/neu expression	Her2-positive	6 (17%)	5 (14%)	
	Her2-negative	30 (83%)	31 (86%)	
Ki-67 value	≤20%	11 (31%)	12 (33,5%)	
	21-39%	17 (47%)	13 (36%)	
	≥40%	8 (22%)	11 (30,5%)	
Molecular tumor subtype [16]	Luminal A	3 (8%)	4 (11%)	
	Luminal B HER2-negative	22 (61%)	18 (50%)	
	Luminal B HER2-positive	2 (6%)	3 (8%)	
	HER2+	4 (11%)	2 (6%)	
	Basal-like (Triple negative)	5 (14%)	9 (25%)	

Table 1. Clinicopathological characteristics of breast cancer patients

Curative efficacy	Group 1		Group 2		P value
	N	%	N	%	
Clinical response					
Complete response (cCR)	2	6	10	27.5	<0.05
Partial response (cPR)	7	19	18	50	
Stable disease (cSD)	19	53	6	17	
Progressive disease (cPD)	8	22	2	5.5	
Pathological response					
Grade 1	5	15	4	12	>0.05
Grade 2	18	55	10	29	<0.05
Grade 3	6	18	8	23.5	>0.05
Grade 4	2	6	3	9	>0.05
Grade 5	2	6	9	26.5	<0.05

Table 2. Clinical response to NAST

Statistically significant differences between the tumor grade, histological tumor type and Her2/neu expression before and after preoperative systemic therapy, among study groups and within the groups were not found. After NAST, number of patients in the group 1 with ER-negative tumors decreased from 31% (11 patients) to 13% (4 patients), and with PR-negative tumors from 56% (20 patients) to 32% (10 patients). In patients who did not receive metformin (group 1) NAST increased quantity of ER and PR-positive tumors from 69% (25 patients) to 87% (27 patients) and from 44% (16 patients) to 68% (21 patients), respectively. In the group 1 number of PR-negative tumors decreased from 56% to 32% after chemotherapy. In both study groups increased number of tumors with low Ki-67 values (≤20%) was detected, from 33.5% (12 patients) to 60% (15 patients) in metformin group and from 31% (11 patients) to 45% (14 patients) in group 1. Patients from group 2 demonstrated reduction of number of tumors with higher Ki-67 value (≥40%) from 30.5% (11 patients) to 16% (4 patients). After treatment, low Ki-67 values were recorded in 15 patients (60%) from group 2 versus 14 patients (45%) from group 1.

The pathological complete response (pCR) rate, grade 5 according to Miller-Payne grading system was identified in 9 patients (26.5%) from group 2 versus 2 patients (6%) from group 1. In the group 1 grade 2 of tumor regression was identified in 18 patients (55%) versus 10 patients (29%) in metformin arm.

Table 3 shows the changes of morphological and molecular characteristics of the tumor before NAST and after surgery. After surgery, morphological and molecular characteristics of the tumor were not determined in 2 patients (6%) who achieved pCR in the group 1 and in 9 patients (25%) in the group 2. In addition, the above characteristics were not defined in 3 patients (8%) in the group 1 and 2 patients (6%) in the group 2 as disease progressed.

Table 4 shows the type of surgery performed in both groups. In the group 1, 19 patients (52.5%) had contraindications to breast cancer surgery, including 17 patients (47%) - due to the spread of tumor to the skin and 2 patients (5.5%) – due to multicentric breast cancer. In the group 2, 18 patients (50%) were contraindicated to breast cancer surgery, 15 patients (42%) due to the spread of tumor to the skin and 3 patients (8%) due to multicentric tumors.

In the metformin group, in 9 patients (50%) breast cancer surgery was executed versus 4 (23.5%) from group 1. The mastectomy was performed in 13 patients from the group 1 (76.5%) compared to 7 patients from the group 2 (38%).

We evaluated overall and disease free survival. The median follow-up time of the whole population was 39 months (from 8 to 65 months). Fig. 2 and

Morphological and molecular tumor characteristics	Group 1		Group 2	
	Before NAST	After NAST1	Before NAST	After NAST1
Tumor grade				
G1	1 (3%)	1 (3%)	1 (3%)	1 (4%)
G2	32 (89%)	27 (87%)	29 (80%)	21 (84%)
G3,G4	3 (8%)	3 (10%)	6 (17%)	3 (12%)
Histological tumor type				
Invasive ductal	34 (94%)	29 (94%)	31 (86%)	24 (96%)
Invasive lobular	2 (6%)	2 (6%)	5 (14%)	1 (4%)
Estrogen receptor status				
ER-positive	25 (69%)*	27 (87%)*	23 (64%)	19 (76%)
ER-negative	11 (31%)*	4 (13%)*	13 (36%)	6 (24%)
Progesterone receptor status				
PR-positive	16 (44%)*	21 (68%)*[i]	15 (42%)	11 (44%)[i]
PR-negative	20 (56%)*	10 (32%)*[i]	21 (58%)	14 (56%)[i]
Her2/neu expression				
Her2-positive	6 (17%)	5 (16%)	5 (14%)	2 (8%)
Her2-negative	30 (83%)	26 (84%)	31 (86%)	23 (92%)
Ki-67 value				
≤20%	11 (31%)*	14 (45%)*[i]	12 (33.5%)*	15 (60%)*[i]
21-39%	17 (47%)	14 (45%)[i]	13 (36%)	6 (24%)[i]
≥40%	8 (22%)	3 (10%)	11 (30.5%)*	4 (16%)*
Molecular tumor subtype				
Luminal A	3 (8%)*	8 (26%)*	4 (11%)*	8 (32%)*
Luminal B HER2-negative	22 (61%)	15 (48%)	18 (50%)*	9 (36%)*
Luminal B HER2-positive	2 (6%)	3 (10%)	3 (8%)	1 (4%)
HER2+	4 (11%)	2 (6%)	2 (6%)	1 (4%)
Basal-like (Triple negative)	5 (14%)	3 (10%)	9 (25%)	5 (20%)

[1] Morphological and molecular characteristics of the tumor were not determined in 5 (14%) patients who achieved pCR from the group 1 and in 11 (34%) patients from the group 2
* Differences within the group were statistically significant (p<0.05)
[i] Differences between groups were statistically significant (p<0.05)

Table 3. Morphological and molecular tumor characteristics before and after NAST

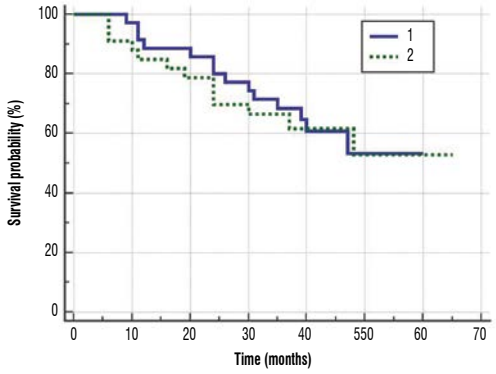
Type of surgery	Group 1	Group 2
Breast-conserving surgery	4 (23,5%)*	9 (50%)*
Radical mastectomy	13 (76,5%)*	7 (38%)*
Skin and nipple sparing mastectomy with breast reconstruction	0	2 (11%)
Total	17 (100%)	18 (100%)

*Differences between groups were statistically significant (p<0.05)

Table 4. Type of surgery after NAST

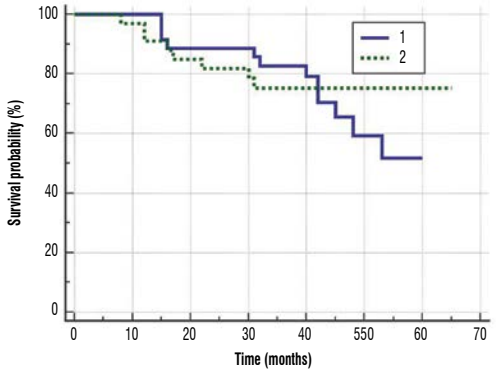
3 show recurrence-free survival (RFS) and overall survival (OS) curves in patients from each study group, respectively. Five-year RFS was similar in both groups (58% in group 1 and 57% in group 2). Five-year overall survival was 76% for patients who received metformin versus 59% for patients from group 1.

Aftrer comparison of survival curves, there was no significant difference between groups in recurrence free survival (Fig. 2) (logrank test, p=0.79) or overall survival (Fig. 3) (logrank test, p=0.67).



Time (months)		12	36	60
Recurrence free survival probability, percent (95% CI)	Group 1	88.6 (72.4–95.5)	68.5 (50.3–81.1)	53.2 (31.5–70.9)
	Group 2	84.8 (66.4–93.4)	66.4 (47.5–79.8)	52.8 (29.4–71.7)

Figure 2. Recurrence-Free Survival.



Time (months)		24	36	60
Recurrence free survival probability, percent (95% CI)	Group 1	88.6 (72.4–95.5)	82.8 (65.6–91.9)	51.7 (28.1–71.0)
	Group 2	81.8 (63.9–91.4)	75.1 (56.3–86.7)	75.1 (56.3–86.7)

Figure 3. Overall Survival.

DISCUSSION

Breast cancer and metabolic syndrome remains one of the most urgent problems of modern medicine worldwide (17).

In current study the effect of metformin on the effectiveness of neoadjuvant systemic antitumor therapy was investigated, as a way to optimize the treatment of patients with breast cancer with metabolic syndrome. The main finding was that patients with breast cancer and metabolic syndrome receiving both metformin and NAST have a higher clinical complete response (27.5% versus 6%) and patological complete response (26.5% versus 6%) rate compared with patients who did not receive metformin.

The anti-tumor effect of metformin is associated with activation of AMPK, inhibition of anabolic processes and launching of catabolic processes in the cell. This arrests cell cycle in G0/G1-phase and stimulates p53-dependent cell autophagy (11, 12). Furthermore, metformin can

directly (without AMPK) block protein mTOR, which stimulates the biosynthesis of proteins and promotes cell growth and proliferation; thereby demonstrating antiproliferative activity (11).

Although our study did not reveal the influence of metformin on overall and disease-free survival, obtained results are linking metformin use with immediate improved effects of NAST. Results of this study may be strong basis for further prospective study of the use of metformin as a supplement drug to the current standard treatment of breast cancer in patients with metabolic syndrome.

CONCLUSION

The aim of this prospective randomized trial was to investigate the influence of metformin on the results of neoadjuvant systemic anticancer therapy, as a way to optimize the treatment of breast cancer patients with metabolic syndrome. According to data obtained in our study administration of metformin in BC patients with MS during NAST lead to:

1. Increased incidence of clinically complete tumor regression by 21.5% and partial regression by 31%
2. Reduction of disease progression rate during neoadjuvant systemic treatment by 15.5%
3. Increased number of overall clinical response rate (cCR + cPR) of the NAST by 52.5%
4. Improved frequency of pathological complete response (grade 5 according to Miller-Payne grading system) by 20.5%
5. Increased frequency of breast-conserving surgery performance after NAST by 26.5%

Although there was no statistically significant difference between the groups in terms of progression-free and overall survival, the numerical trends observed in this prospective pilot study demonstrated potential of metformin as an antitumor agent in breast cancer patients with MS that should be confirmed by further, large-scale studies.

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Declaration of Interests

Authors declare no conflicts of interest.

Ethical Standards Statement

All procedures were in accordance with ethical stadards of responsible institutional Committee on Human Experimentation and Declaration of Helsinki.

Informed Consent

Informed consent was obtained from all patients included in study.

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