The complex evaluation of tumor oxygen state and vasculature during preoperative chemotherapy in patients with breast cancer

M.V. Pavlov[1], P.V. Subochev[3], T.I. Kalganova[2], G.Yu. Golubyatnikov[3], V.I. Plekhanov[3], O.E. Ilyinskaya[1], A.G. Orlova[3], N.M. Shakhova[3] and A.V. Maslennikova[2,4].

[1]Regional Oncological Hospital, Nizhny Novgorod, Russia

[2]State Medical Academy, Nizhny Novgorod, Russia

[3]Institute of Applied Physics RAS, Nizhny Novgorod, Russia

[4]Lobachevsky State University, Nizhny Novgorod, Russia

pavlov.med@mail.ru

ABSTRACT

Effective breast cancer treatment requires the assessment of metabolic changes of tumor tissue during chemo- and hormonotherapy for prediction tumor response. Evaluation of the dynamics of tumor oxygen state (by diffuse optical spectroscopy imaging) and tumor vasculature (by ultrasound investigation in power Doppler mode) was performed before treatment beginning and before the second cycle of chemotherapy in 16 patients who received preoperative chemotherapy. Changes of these indicators were compared then with tumor pathologic response. Breast tumors demonstrated different dynamics of tumor oxygenation depending on the changes of tumor tissue. The increase of the tumor oxygenation after the first cycle of chemotherapy was observed in five of six patients with grade 4 and 5 of pathologic tumor response. Decrease of the oxygenation level was revealed in one patient with the 4th degree of tumor response. Variable changes of the oxygenation level were mentioned in the patients with moderate (the 3d degree) tumor response. Tumor oxygenation decreased or was unchanged in case of 1 or 2 degree of tumor response in five of six cases. The study of the tumor blood vessels didn't reveal any correlation between vasculature changes and tumor response under the performed treatment. The trend of tumor oxygenation in early time after treatment beginning might be a predictive criterion of tumor sensitivity to chemotherapy.

Keywords: breast cancer, oxygen state, tumor vasculature, preoperative chemotherapy, pathologic tumor response, predictive criteria, optical diffuse spectroscopic imaging, ultrasound investigation, Doppler power mode.

INTRODUCTION

Breast cancer is the most common malignant tumor in women and the morbidity continues rising. Standard-of-care of the disease shifted now from mastectomy to neoadjuvant treatment (chemotherapy, hormonotherapy) and consequent organ-sparing surgery. The main objective of neoadjuvant treatment is achieving the complete pathologic tumor response that is a strong predictor of better survival. Unfortunately, not more than 20-30% of patients demonstrate a complete tumor regression after neoadjuvant chemotherapy (NACT) [1]. Considering the higher cost of chemotherapy and the supportive care, the development of predictive criteria of individual breast cancer sensitivity to therapy is of great importance [2]. The objective of our study was to assess the dynamics of biological features of breast tumors during neoadjuvant chemotherapy and propose criteria of tumor sensitivity to treatment. Tumor oxygen state is known to be a powerful factor influencing tumor biological features. Hypoxia is an origin of tumor radioresistance and resistance to chemotherapy. More important, hypoxia is an independent factor of malignant progression due to its effect on various metabolic, genetic and molecular pathological processes, including proliferation, apoptosis, angiogenesis and metastasis. [3]. Standard methods of breast structural imaging – ultrasonic examination and x-ray mammography can't assess oxygenation of the tumor at all. The current methods of functional imaging for study of tumor oxygen state include magnetic resonance imaging and method of nuclear medicine – positron-emission tomography [4,5,6].

Diffuse optical spectroscopic imaging (DOSI) demonstrated its potential for evaluating the dynamics of tumor oxygen state and predicting tumor response to different kinds of therapy [7,8,9]. It is based on the reconstruction of absorption and scattering coefficients of biotissues using information on laser radiation attenuation in by tissue. Concentrations of the principal tissue chromophores: oxyhemoglobin (HbO2), deoxygenated hemoglobin (HHb), water, and lipids are reconstructed by absorption coefficients measured at different wavelengths. This information allows indirect evaluation of

Optical Tomography and Spectroscopy of Tissue XII, edited by Bruce J. Tromberg, Arjun G. Yodh, Eva Marie Sevick-Muraca, Robert R. Alfano, Proc. of SPIE Vol. 10059, 100590P · © 2017 SPIE
CCC code: 1605-7422/17/$18 · doi: 10.1117/12.2251419

the oxygen state of biological tissues.
The other important characteristics of the tumor is the state of its vasculature that can be estimated by ultrasound investigation in power Doppler mode (USI). The combination of USI and DOSI was used for the study of oxygenation and vascularization of large breast tumors [10]. The dynamics of vascularization index in the group of patients with advanced breast cancer who received NACT was studied in [11]. The resistance index, the pulsation index and the maximum systolic blood flow velocity were used to assess the effect of chemotherapy in [12,13].

The study objective was the complex evaluation of the dynamics of tumor oxygen state and tumor vasculature during neoadjuvant chemotherapy in patients with breast cancer in early time after treatment beginning.

MATERIALS AND METHODS

Summary of patients' demographics and clinical characteristics.

Fifty-two patients with stage II-IV of breast cancer were included into the study from November 2015 for June 2016. They ranged in age from 28 to 75 years. The patients received from 4 to 12 cycles of standard chemotherapy before surgery (Table 1). The comparison of DOSI and USI changes and degree of tumor response was performed in 19 patients. Sixteen patients were analyzed, 33 patients continue chemotherapy now.

Study design.

Before start of the treatment patients had a standard examination including mammography, breast USI, core-biopsy of the tumor and study of its receptor status, her2neo status and proliferation index. DOSI and USI of tumor blood flow were performed twice – before the start of treatment and before the second cycle of NACT. After completion the NACT the patients were performed by surgery with the consequent pathological study of the tumor and determination of the tumor response. According to the results of pathology, the patients were referred to the group of complete or partial response or absence of response.

Histopathology.

The degree of tumor response was determined according to Miller and Payne [14]. According to this score system, the first degree of tumor response means slightly noticeable changes of single tumor cells, but without their number decrease. The 5th degree means the absence of detectable invasive sells in the sectional slices from the site of the primary tumor location.

Table 1. The patients' population.

Parameter		Number of patients
Age	<40	2 (10,4%)
	41-50	6 (31,6%)
	51-60	7 (36,9%)
	>61	4 (21,1%)
Stage	IIa	1 (5,3%)
	IIb	2 (10,4%)
	IIIa	6 (31,6%)
	IIIb	6 (31,6%)
	IIIc	3 (15,8%)
	IV	1 (5,3%)
Regional lymph nodes	No metastases	2 (10,4%)
	Metastases	17 (89,6%)
Immunophenotype	Luminal A	1 (5,3%)
	Luminal B, HER2negative.	3 (15,8%)
	Luminal B, HER2positive.	5 (26,3%)
	HER2neu positive	5 (26,3%)
	Triple negative	5 (26,3%)
Total		19 (100%)

Diffuse optical spectroscopy.

The study was performed on the experimental DOSI setup with parallel plane geometry (Institute of Applied Physics RAS, Russia) working at three wavelengths (684 nm, 794 nm, and 850 nm) allowing to reconstruct distribution of different forms of hemoglobin [15].

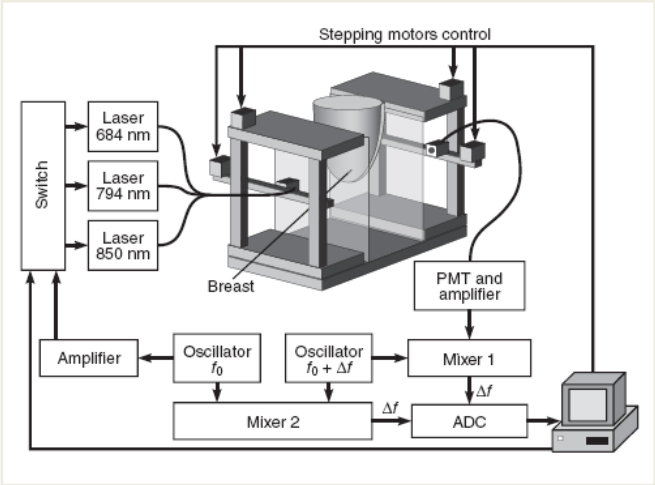

Fig. 1. Scheme of DOSI setup. Specifications: light sources - lasers with wavelengths of 684 nm, 794 nm and 850 nm; amplitude modulation frequency - 140 MHz; Hamamatsu photomultiplier with high sensitivity.

We used high-frequency amplitude modulation, allowing determining absorption coefficients more accurately due to separate determination of scattering and absorption coefficients. Breast images were obtained by synchronous moving the source and the detector located at the opposite sides of the studied subject (Fig.1).

Ultrasound investigation.

Original protocol of ultrasound investigation was developed to determine the dynamics of tumor blood flow during NACT. Scanning of the tumor and its peripheral zone was performed by "Medison Accuvix-V20" using multifrequency linear probe with range of 5.0-13.0 MHz in power Doppler mode with transversal step of 5 mm in mutually perpendicular planes. Using the obtained images we calculated the vessels contribution in tumor and surrounding tissue (a vascularization index). This allowed comparing data on tumor blood supply before treatment and in the course of NACT correctly (Fig.2).

Fig. 2. Tumor in B-mode (a), tumor in power Doppler mode (b), numerical processing in Matlab software (c).

RESULTS

Dynamic of oxygen tumor state.

Dynamics of oxygen state of tumor tissue after the first cycle of NACT was estimated in 16 patients (Fig.3). Breast tumors demonstrated different changes of tumor oxygenation depending on the degree of tumor response. We observed the increase

of tumor oxygenation in five of six patients with grade 4 and 5 of tumor response (right part of the figure). Decrease of the oxygenation level was revealed in one patient with the 4th degree of tumor response. Variable changes of the oxygenation level were mentioned in the patients with moderate (the 3 degree) tumor response. Tumor oxygenation decreased or was unchanged in case of one or two degree of tumor response in five of six patients (left part of the figure). In one patient, tumor oxygenation increased after the first cycle of NACT. This situation demands further study and discussion.

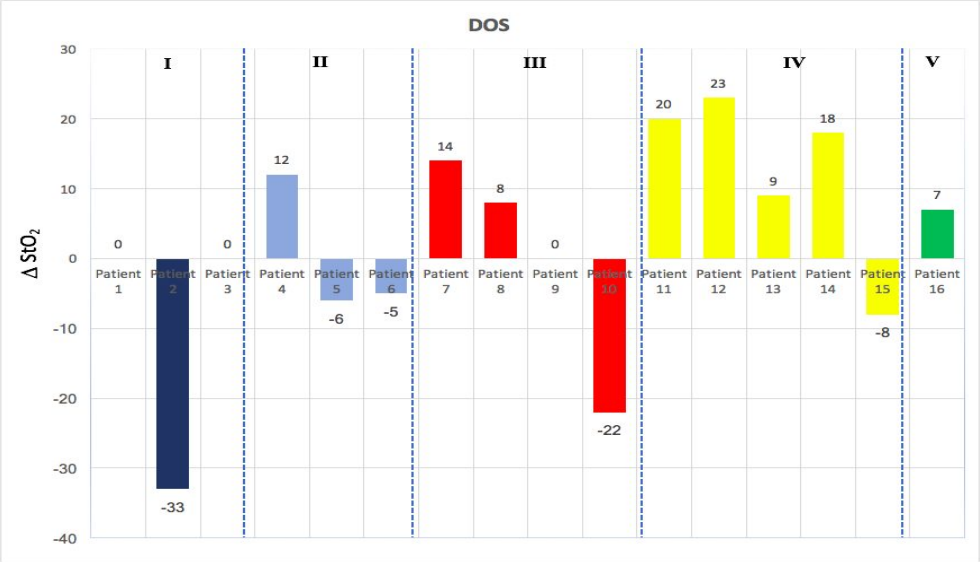

Fig. 3. Dynamic of tumor oxygen state after the 1st cycle of NACT depending on tumor response.

Dynamic of tumor vascularization after the 1st cycle of chemotherapy.

Dynamics of tumor vascularization did not demonstrate any correlation between this indicator and tumor response (Fig.4). We observed as decrease as increase (including significant one) of tumor tissue vascularization independently on the tumor response. In some patients, significant increase of vessels contribution was caused by not only tumor tissue reaction but by the inflammation processes in the peritumoral zone.

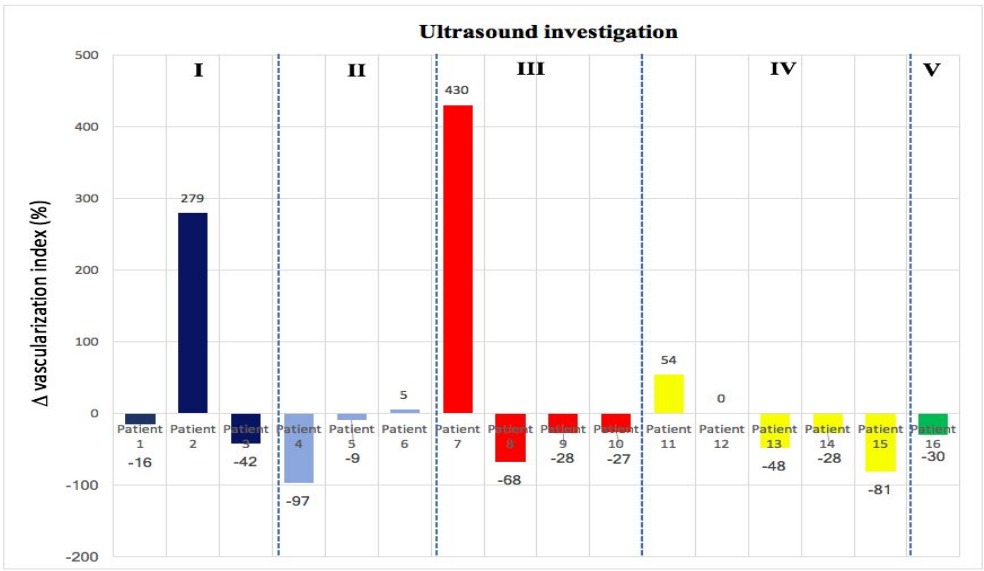

Fig. 4. Dynamic of vessels contribution after the 1st cycle of NACT depending on tumor response.

Clinical examples.

The 50 years old patient with stage IIIa of luminal B Her2neu-negative breast cancer received 6 cycles of preoperative chemotherapy. In three weeks after the first cycle of chemotherapy USI showed the unchanged tumor size and insignificant increase of the blood vessels number. The oxygenation after the first cycle remained unchanged. Pathological study demonstrated the first degree of tumor response (Fig.5).

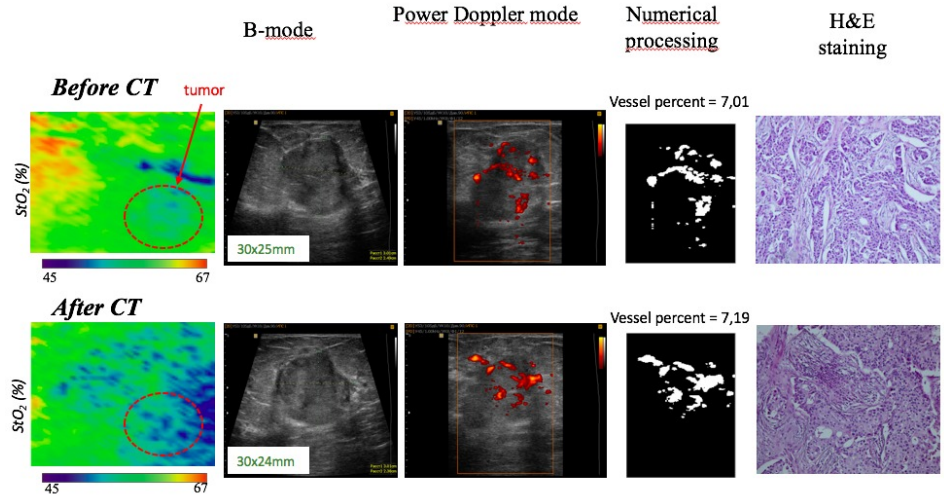

Fig.5. Dynamic of tumor oxygen state, tumor blood flow after the 1st course of NACT and pathological study of patient.

A 45 years old patient who was diagnosed by stage IIb Her2neu-positive breast cancer. The increase of the tumor oxygenation level of 7% and the decrease of number of tumor blood vessels of 30% were detected after the first cycle of NACT. Pathological study after 6 cycles of CT and surgery didn't show viable tumor cells that corresponded to the 5th degree of tumor response (Fig.6).

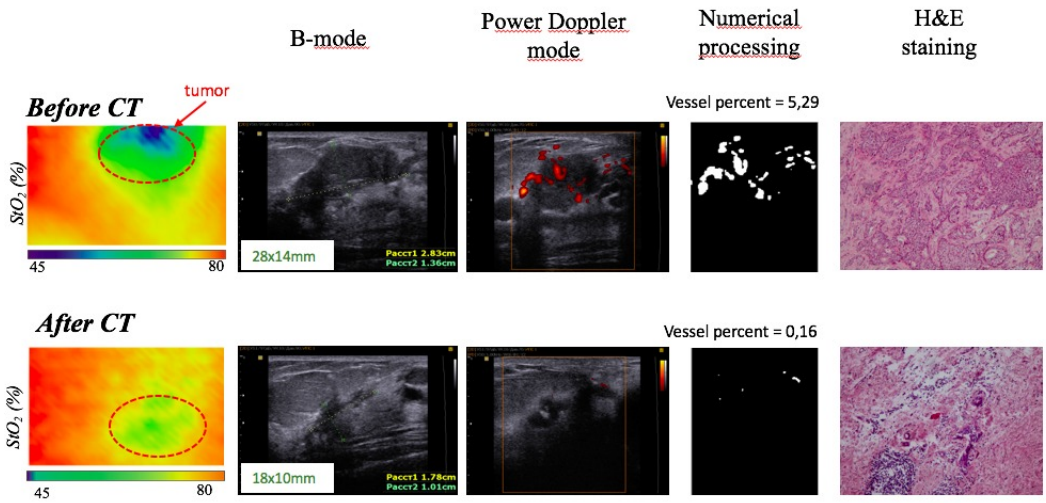

Fig.6. Dynamic of tumor oxygen state, tumor blood flow after the 1st course of NACT and pathological study.

DISCUSSION AND CONCLUSIONS

Numerous studies have demonstrated the great potential of diffuse optical spectroscopy to evaluate the metabolic status of breast tumors [16, 17]. The initial level of total hemoglobin [8] and changes of its concentration in early time after the start of therapy were used for prediction of tumor response to NACT [18]. In our study, the blood oxygen saturation that correctly reflects tissue oxygenation [19] was chosen as the main indicator of tumor response to chemotherapy. DOSI

confirmed that tumor oxygenation after the 1st course of NACT was improved in majority of patients responding to treatment. The results confirmed that the main source of tumor tissue reoxygenation under the influence of antitumor treatment is the decrease of number of viable tumor cells and corresponded decrease of tumor oxygen demand. When evaluating tumor oxygen state, the presence of necrosis in the tumor tissue which exist in the anoxic condition should be taken into account, because it could essentially distort information about integral oxygenation of neoplasm. The reaction of tumor blood flow detected by USI in power Doppler mode seems to play a less role in the changes of oxygen state parameters in early time after chemotherapy beginning.

The study confirmed that tumor oxygenation reflects its metabolic features and its changes in the course of treatment may give additional information about tumor sensitivity to chemotherapy. Those patients who didn't show expected dynamics of tumor oxygenation require additional analysis.

ACKNOWLEDGEMENTS

We are grateful to our colleagues from the Institute of Applied Physics RAS, Nizhny Novgorod State Medical Academy and Regional Oncological Hospital for help.

The Russian Foundation for Basic Research (projects №15-42-02528) for financial support.

REFERENCES

[1] A. M. Thompson, S. L. Moulder-Thompson, "Neoadjuvant treatment of breast cancer," Oxford Journals Medicine. Annals of Oncology 23, 231-236 (2013).

[2] Gunter von Minckwitz et al., "Neoadjuvant Chemotherapy and Bevacizumab for HER2-Negative Breast Cancer," The New England Journal of Medicine 366, 4, 229-309 (2012).

[3] P. Vaupel, "Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis," The Oncologist 13, 21–26 (2008).

[4] D. Angelucci, N. Tinari, A. Grassadonia, et al., "Long-term outcome of neoadjuvant systemic therapy for locally advanced breast cancer in routine clinical practice," J Cancer Res Clin Oncol. 139(2), 269-80 (2013).

[5] R. Warren, C. Hayes, L. Pointon, et al., "A test of performance of breast MRI interpretation in a multicentre screening study," Magnetic resonance imaging 24(7), 917-929 (2006).

[6] I. N. Fleming, F. J. Gilbert, K. A. Miles, D. Cameron, "Opportunities for PET to deliver clinical benefit in cancer: breast cancer as a paradigm." Cancer Imaging 10(1), 144–152 (2010).

[7] D. Roblyer, S. Ueda, A. Cerussi, W. Tanamai, A. Durkin, R. Mehta, D. Hsiang, J.A. Butler, C. McLaren, W.P. Chen, B. Tromberg, "Optical imaging of breast cancer oxyhemoglobin are correlates with neoadjuvant chemotherapy response one day after starting treatment," PNAS 108(35), 14626–14631 (2011).

[8] Q. Zhu, P. A. DeFusco, A. Jr Ricci, E. B. Cronin, P. U. Hegde, M. Kane, B. Tavakoli, Y. Xu, J. Hart, S. H. Tannenbaum, "Breast cancer: assessing response to neoadjuvant chemotherapy by using US-guided near-infrared tomography," Radiology 266, 433-442 (2013).

[9] O. Falou, H. Soliman, A. Sadeghi-Naini, S. Iradji, S. Lemon-Wong, J. Zubovits, J. Spayne, R. Dent, M. Trudeau, J. Francois Boileau, F. C. Wright, M. J. Yaffe, G. J. Czarnota, "Diffuse optical spectroscopy evaluation of treatment response in women with locally advanced breast cancer receiving neoadjuvant chemotherapy," Translational oncology 5(4), 238–246 (2012).

[10] Q. Zhu, S. H. Kurtzman, P. Hegde, "Utilizing optical tomography with ultrasound localization to image heterogeneous hemoglobin distribution in large breast cancers," Neoplasia 7(3), 263–270 (2005).

[11] Wen-Hung Kuo, Chiung-Nien Chen, Fon-Jou Hsieh et al., "Vascularity change and tumor response to neoadjuvant chemotherapy for advanced breast cancer," Ultrasound in medicine & biology 34, 857-866 (2008).

[12] A. Kumar, V. Srivastava, S. Singh, and R. C. Shukla, "Color Doppler ultrasonography for treatment response prediction and evaluation in breast cancer," Future Oncology 6(8), 1265-1278 (2010).

[13] G. Singh, P. Kumar, R. Parshad, et al., "Role of color Doppler indices in predicting disease-free survival of breast cancer patients during neoadjuvant chemotherapy," European Journal of Radiology 75, 158-162 (2010).

[14] I. C. Smith, S. D. Heys, A. W. Hutcheon, I. D. Miller, S. Payne, “Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel,” Journal of clinical oncology 6, 1456-1466 (2002).

[15] A. G. Orlova, I. V. Turchin, V. I. Plehanov, N. M. Shakhova, I. I. Fiks, M. I. Kleshnin, N. Yu. Konuchenko and V. A. Kamensky, “Frequency- domain diffuse optical tomography with single source-detector pair for breast cancer detection,” Laser Physics Letters 5(4), 321-327 (2008).

[16] A. V. Maslennikova, G. Yu. Golubjatnikov, A. G. Orlova, et al., “Non-invasive optical method for evaluating the oxygen status in breast neoplasms,” Tumors of women reproductive system 1, 5-10 (2010).

[17] O. Falou, H. Soliman, A. Sadeghi-Naini, et al., “Diffuse optical spectroscopy evaluation of treatment response in women with locally advanced breast cancer receiving neoadjuvant chemotherapy,” Translational oncology 5, 238–246 (2012).

[18] A. E. Cerussi, V. W. Tanamai, D. Hsiang, J. Butler, R. S. Mehta, B. Tromberg, “Diffuse optical spectroscopic imaging correlates with final pathological response in breast cancer neoadjuvant chemotherapy,” Philosophical transactions of the royal society A 369, 4512-4530 (2011).

[19] A.V. Maslennikova, A.G. Orlova, G.Yu. Golubiatnikov, et al., “Comparative study of tumor hypoxia by diffuse optical spectroscopy and immunohistochemistry in two tumor models,” Journal of Biophotonics 3, 743–751 (2010).