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Efficiency of detection of lymph nodes in breast cancer

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The objective: **improve the quality of sentinel lymph nodes detection in patients with malignant neoplasms of the mammary glands.**
Materials and methods**. At the period from 2009 to 2016, 400 patients with T1-T3N0M0 breast cancer were operated in Odessa Regional Clinical Hospital, using two dyes Patent Blue and ICG.**
The patients who had mastectomy with sentinel lymph node biopsy were diagnosed T2-T3N0M0 breast cancer more frequently. The exceptions were T3-T4 tumors, tumor diameter > 5 cm, invasion into the skin and chest wall, palpable axillary lymph nodes, 3 or more affected lymph nodes during sentinel lymph node biopsy.
100 patients in the first group had sentinel lymph node biopsy. Lymph node staining was performed using Patent Blue dye.
In the patients in the second group, sentinel lymph node biopsy was performed using Patent Blue dye and another fluorescent ICG dye, which was injected intravenously into the arm on the affected side of the mammary gland, along the outflow from the arm to the mammary gland.
Results**. The total five-year survival after axillary lymph node dissection and sentinel lymph node biopsy was 91 % and 92 %, respectively. The five-year recurrence-free survival after axillary lymph node dissection was approximately 82.2 %, and after the sentinel lymph node biopsy – 83.9 %. Regional recurrence in the sentinel lymph nodes on the affected side was determined only in 1.1 %.**
The time of observation of the patients was from 60 to 180 months. The recurrence was registered in 0.2 % patients as isolated metastases into the axillary lymph nodes. Not a single case of lymphostasis of the upper limbs from the side of the biopsy was registered.
Conclusions**. The simplicity of fluorescent dyes usage makes it possible to implement this method in the everyday work of oncologists-surgeons, the advantages of which are the absence of radiation exposure and quick intraoperative detection of lymph nodes.**
Keywords*: lymph nodes, sentinel lymph nodes, breast cancer.*

Ефективність виявлення лімфовузлів при раку грудної залози

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Мета дослідження: **підвищення якості визначення сторожових лімфовузлів у пацієнток зі злоякісними новоутвореннями грудних залоз.**
Матеріали та методи**. У період з 2009 до 2016 року на базі Одеської обласної клінічної лікарні було прооперовано 400 пацієнток із раком грудної залози T1-T3N0M0 з використанням двох барвників Patent Blue та ICG.**
Пацієнтки, яким проведено мастектомію з біопсією сторожових лімфовузлів, частіше мали клінічний діагноз раку грудної залози T2-T3N0M0. При цьому винятками були пухлини T3-T4 діаметром > 5 см або інвазія у шкіру і стінку грудної клітки, а також пахвові лімфовузли, що пальпуються, 3 і більше уражених лімфовузлів при біопсії сторожового лімфовузла.
У першій групі 100 пацієнткам проводили біопсію сторожових лімфовузлів. Фарбування лімфовузлів виконували з використанням барвника Patenet Blue.
У другій групі зроблено біопсію сторожових лімфовузлів з використанням барвника Patenet Blue і другого флуоресцентного барвника ICG, який вводили внутрішньовенно у руку на боці ураженої грудної залози, за ходом відтоку від руки до грудної залози.
Результати**. Загальне п'ятирічне виживання після аксилярної лімфодисекції та біопсії сторожового лімфовузла становило 91 % та 92 % відповідно. Безрецидивне п'ятирічне виживання після аксилярної лімфодисекції становило приблизно 82,2 %, а після виконання біопсії сторожового лімфовузла – 83,9 %. Лише в 1,1 % випадків було виявлено регіонарні рецидиви у сторожові лімфовузли на ураженому боці.**
Час спостереження за хворими становив від 60 до 180 міс. Рецидив був зареєстрований у 0,2 % жінок у формі ізольованих метастазів в аксилярних лімфовузлах. Не зареєстровано жодного випадку лімфостазу верхніх кінцівок із боку біопсії.
Висновки**. Простота використання флуоресцентних барвників дозволяє впровадити даний метод у повсякденну роботу онкологів-хірургів, перевагами якого є відсутність променевого навантаження та швидкий інтраопераційний пошук лімфовузлів.**
Ключові слова: *лімфатичні вузли, сторожові лімфовузли, рак грудної залози.*

The causes of tumors in the human body are still not fully studied and are not entirely clear [2, 8, 16, 27]. It is not possible to fully explain why malignant transformation of cells occurs [1, 7, 18, 35]. At the same time, it becomes clear that the main mechanisms of cellular development are involved in the process of oncogenesis. When a tumor degeneration of cells occurs in one of the organs, they spread (metastasize) throughout the body, which ultimately leads to death [4, 19, 36].

The regularities of cancer cell metastasis are also insufficiently studied [5, 14, 25, 33]. Until recently, it was considered that metastasis of malignant cells in cancer lesions of various organs takes place differently [6, 23, 38]. The main route of cancer cells spreading is their movement by lymphatic vessels, through which cancer cells enter the lymph nodes surrounding the affected organ [21, 29]. Before it was considered that cancer cells can enter different lymph nodes, both of the first, second, and third order, and then metastasize to the liver, lungs, bones, and other organs [5, 10, 26, 39]. So, the main doctrine of the surgical treatment of cancer of any organ was considered to be the complete removal of the affected organ with an obligatory removal of all lymph nodes, both of the first and second, third order, where cancer cells can spread to [9, 13, 20].

The doctrine of human cancers treatment exists to the present time [1, 8, 15, 34]. The better the surgical technique of an operating surgeon-oncologist, the more radically he performs the operation, i. e., in addition to the affected organ, he removes the most of lymph nodes surrounding the affected organ [3, 11, 24, 37]. Modern protocols for the surgical treatment of cancer lesions of various organs require lymph node dissection of at least D2 volume: all lymph nodes of the first and second order must be removed [4, 12, 26, 30]. This requires rather complex and traumatic surgical interventions, after which the quality of patients life is significantly reduced, up to disability [5, 17, 26, 31]. The operated patients often experience a number of complications, postoperative syndromes that do not allow patients to eat, work and live normally [3, 12, 19, 28].

At the same time, the collected data suggest that in early T1/T2 cancer, lymph node lesion takes place in a maximum of 15–20 % of patients [6, 9, 12, 22, 26]. This means that radical removal of lymph nodes was groundless in 80–85 % of patients with early cancer [13, 24, 29, 32]. A thorough study of metastasis mechanisms revealed that in melanoma and breast cancer, certain lymph nodes of the first order are firstly affected, and then, after their defeat, cancer cells spread to other lymph nodes of the first and second order [5, 9, 16, 26]. Such lymph nodes, which are affected first and are a certain barrier to the further spread of cancer cells, are called sentinel lymph nodes. In the 1990s, the leading oncologists formulated the sentinel nodes doctrine [8, 16, 19, 26]. It is considered that if a cancerous lesion is not detected in the sentinel lymph nodes, their detection in the lymph nodes of the second and third order is highly unlikely. This logically led to reduced extent of surgical intervention. If there are no cancer cells in the sentinel lymph nodes, there is no need to perform extended lymph node dissection [1, 6, 19, 35].

The sentinel lymph node doctrine has received brilliant clinical confirmation in breast cancer and melanoma [1, 6, 9, 36]. If before the main method of treating breast cancer was radical mastectomy, including a complete removal of breast tissue with greater and smaller pectoral muscles, with simultaneous radical lymph node dissection of all axillary, subclavian, and in some cases even intrathoracic lymph nodes. Starting from the 1990s the surgical management has been radically changed according to currently existing protocols [10, 19, 26, 32].

In early breast cancer, sentinel lymph nodes are identified and removed with an urgent histological examination [7, 16, 25, 38]. If cancer cells in the sentinel lymph nodes are not detected, the operation is limited to local excision of the tumor, followed by radiation and chemotherapy. In order to avoid deformation of the mammary glands, the organ-preserving plastic surgery are immediately performed, which allow obtaining an excellent cosmetic result. Conducted multicentric studies in different countries confirmed that the new surgical management of breast cancer absolutely equals with the old superradical interventions [1–3, 11, 12, 26, 35].

The main non-invasive methods for detecting the affected regional lymph nodes are ultrasound, CT, MRI, especially in young women with a predominance of the glandular component in the mammary glands [2, 6, 19, 28]. Searching for sentinel lymph nodes is now very actual. Methods for lymph node detection have been significantly innovated [3, 17, 29, 36]. Sentinel lymph node detection solves many problems and gives a surgeon a huge advantage to select and prescribe further treatment and reduce the volume of lymph node dissection, significantly reduce the number of complications and improve the quality of patient's life [26].

Now the assessment of sentinel lymph nodes is used in breast cancer, when the clinical examination (palpation, ultrasound and FNAB) reveals no signs of metastatic lesion of the axillary lymph nodes, and there is a need in sentinel node biopsy — ductal carcinoma in situ, when such patients have a high probability of detecting an invasive component and they are indicated mastectomy [1, 8, 16].

The aim of the study is to improve the quality of sentinel lymph node detection in patients with malignant neoplasms of the mammary glands.

MATERIALS AND METHODS

At the period from 2009 to 2016, 400 patients with T1-T3N0M0 breast cancer were operated on the basis of the Odesa Regional Clinical Hospital, using two Patent Blue and ICG dyes. The age of the patients ranged from 35 to 68 years. All patients were divided into two groups.

The patients who underwent mastectomy with sentinel lymph node biopsy were more likely to have a clinical diagnosis T2-T3N0M0 breast cancer. The exceptions were T3-T4 tumors > 5 cm in diameter, or invasion to the skin and chest wall, as well as palpable axillary lymph nodes, 3 or more affected lymph nodes with sentinel lymph node biopsy.

In the first group, 100 patients underwent sentinel lymph node biopsy. Lymph node staining was performed using Patent Blue dye. In the second group, sentinel lymph node biopsy was performed using Patent Blue dye and fluorescent ICG dye, which was injected intravenously into the

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Fig. 1. Patent Blue dye subdermal injection along the outer edge of the areola

arm on the affected side of the mammary gland, along the outflow from the arm to the mammary gland. The lymph nodes staining method is based on the effect of dye luminescence when irradiated with light of a certain wavelength [19, 20, 26]. After the ICG dye injection, green-stained lymph nodes were detected in 15 minutes. This method was originally developed for laparoscopic operations, after ICG introduction the operating field is illuminated with infrared radiation using the "Karl Storz" endoscopic stand [26].

All the patients with sentinel lymph node involvement had adjuvant therapy in the postoperative period according to ESMO/ NCCN recommendations [5, 18, 24]. All the patients were subdermally injected Patent Blue dye along the outer edge of the areola by 2 ml of diluted dye in order to allow spreading through the lymphatic system (Fig. 1). The time of a standard interval for the stained lymph node appearance was 15–20 minutes. The stained lymph node (lymph nodes) was sent for pathomorphological examination.

Intraoperative cytological examination of stained lymph nodes was performed (Fig. 2, 3), histological assessment by permanent specimens, since the false-positive results were noted.

The pathologist examination was performed using standard hematoxylin-eosin staining, false-negative sentinel nodes were examined using immunohistochemical analysis. The absence of radiation exposure and simplicity of implementation are the advantages of this technology. However, after injection the dye may remain in the tissues for some time. The results of our study have made a breakthrough in the identification and isolation of strategically important lymph nodes in the surgical treatment of breast cancer.

RESULTS AND DISCUSSION

A total five-year survival after axillary lymph node dissection and sentinel node biopsy was 91 % and 92 % respectively.

A 5-year relapse-free survival after axillary lymph node dissection was approximately 82.2 %, and after sentinel node

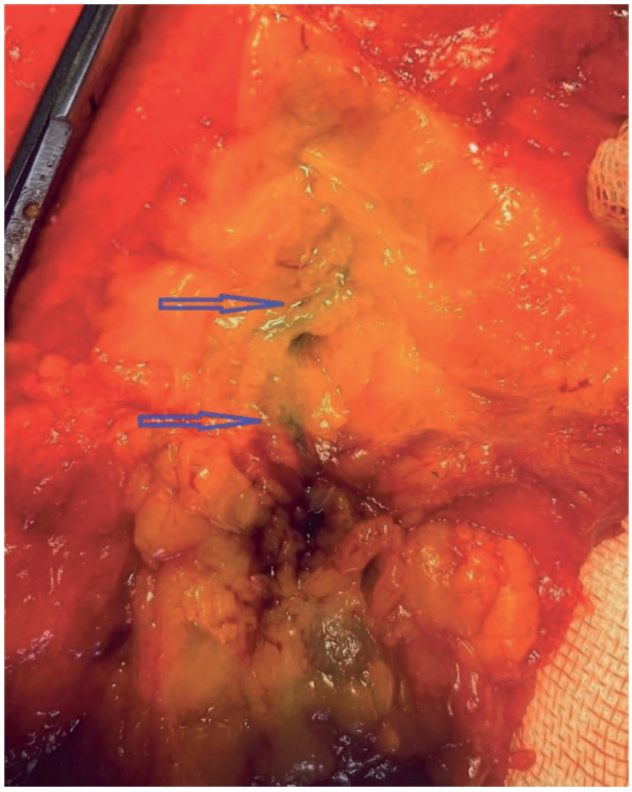

Fig. 2. Patent Blue stained lymph nodes

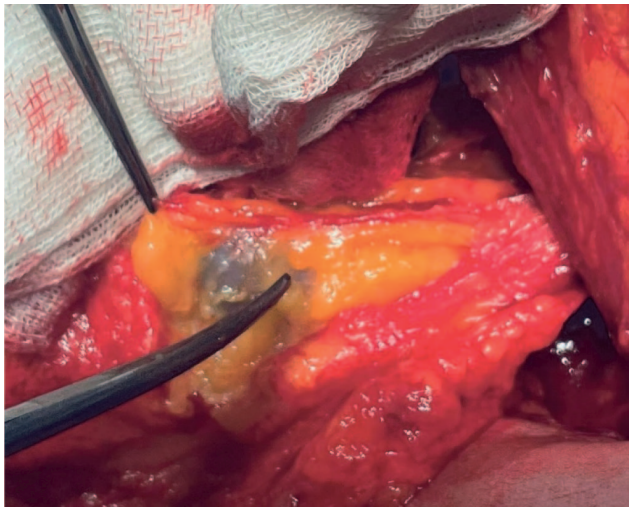

Fig. 3. Patent Blue stained sentinel node

biopsy – 83.9 %. Regional recurrences to the sentinel lymph nodes on the affected side were detected only in 1.1 %. 58 % of patients had intact sentinel lymph nodes, which means that the following lymph node dissection was not performed, and the number of metastatic lymph nodes was 42 %.

The follow-up period ranged from 60 to 180 months. Recurrence was registered in 0.2 % of women as isolated metastases to the axillary lymph nodes. Biopsy revealed no cases of lymphostasis of the upper extremities.

The study revealed no difference in general and relapse-free survival between the groups. Our data confirms the im-

Table 1

Predictive efficiency of sentinel nodes examination method in patients with breast cancer of the control and main groups

Groups	Sensitivity (95 % CI)	Specificity (95 % CI)	Positive result reliability relation (95 % CI)	Negative result reliability relation (95 % CI)
1 group	0.93 (0.71–0.98)	0.93 (0.87–0.96)	14.59 (7.05–30.22)	0.06 (0.01–0.44)
2 group	0.94 (0.71–0.98)	0.94 (0.87–0.97)	16.68 (7.05–39.47)	0.06 (0.01–0.44)
General	0.93 (0.82–0.97)	0.93 (0.89–0.94)	12.74 (8.73–18.60)	0.07 (0.02–0.21)

Table 2

Lymphostasis rate in the control and main groups

Groups of patients	Lymphostasis		χ^2	p
	Yes	No		
1st group (main) (n=35) 1st group (control) (n=35)	1 (2.9 %) 5 (14.4 %)	34 (97.1 %) 30 (8.6 %)	2.92	>0.05*
2nd group (main) (n=35) 2nd group (control) (n=35)	1 (2.9 %) 8 (22.9 %)	34 (97.1 %) 27 (77.1 %)	6.25*	0.03*
3rd group (main) (n=30) 3 rd group (control) (n=30)	0 (0 %) 6 (20.0 %)	30 (100 %) 24 (80.0 %)	6.67*	0.02*

Note. * – Compared with the control group.

provement of approach to breast cancer treatment with less need in major surgical treatment. Our study demonstrates a low recurrence rate to regional lymph nodes, as well as a decrease in such complications as lymphedema and diseases of the upper extremities when performing sentinel lymph node biopsy compared with level 1-2 lymphadenectomy.

The results of our studies highlight that the complete axillary lymph node dissection for staging and prognosis of breast cancer is a thing of the past. The development of new technologies with new approaches to lymph nodes staining becomes necessary in the practice of an oncologist (table 1, 2).

Conducted randomized experiments show that five- and ten-year survival was the same as after radical mastectomy with complete lymph node dissection [5, 9, 16, 28, 34]. At the same time, the results of treatment improved significantly: the quality of life of operated patients improved, such complications as lymphostasis and edema of the upper extremities disappeared, the psychological satisfaction of patients who were able to return to full quality life improved too [13-15].

So, modern technologies make it possible to identify lymph nodes with great accuracy in order to detect their cancerous lesions, which is a predictor of damage to unaffected lymph nodes, and directly affects the extent of surgical intervention, as well as postoperative and intraoperative complications [10, 23, 37]. Now it is a mandatory procedure in breast cancer, which allows to change the extent of surgery [11, 20, 26].

Our studies indicate that the sentinel node concept is correct for different cancer locations [26]. At present, we should improve not only techniques of detecting and removing sentinel nodes but also the methods of their histological examination. The collected data show that staining lymph nodes with hematoxylin eosin gives the false-negative result in 5-10 % of cases. With immunohistochemistry techniques usage the indicator can be reduced to 3 % [5, 20, 34]. The use of genetic biomarkers may significantly increase the accuracy of micrometastasis detection in the future. The laws of the growth and spread of cancer cells in the human body, according to philosophy of life development, should be similar in different tumor locations. So, the work for detection and examination of sentinel lymph nodes is very important [1, 9, 16, 20, 26]. In the future, the sentinel node concept will radically change the surgical approaches to the treatment of different forms of cancer.

CONCLUSIONS

Simplicity of fluorescent dyes use makes it possible to introduce this method into the daily work of surgical oncologists. The disadvantage of this method is the high cost of equipment and dye. However, the advantages are undoubtedly the absence of radiation exposure and the rapid intraoperative detection of lymph nodes. This method of staining sentinel lymph nodes can also be used in other cancers, as the method is 100 % justified and gives an opportunity to improve the quality of surgical treatment of cancer patients with the lowest rate of complications.

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