

Lymphotropic staining of the sentinel lymph nodes in breast cancer - with what, when, how?

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The aim of the study is to define how to choose appropriate dye for marking sentinel lymph nodes in breast cancer. Pre- or intra operatively, dyes, such as Methylen Blue, Drimaren Brilliant Blue, Patent Blue V were applied around the tumor in 135 female patients. To enhance lymphotropism, Gelatin, Alvezin, Haemodex or HAES solutions were used as dye carriers in 92 patients. The volume applied varied from 1.0 to 3.0 ml, as in 25% of the cases Hylase was previously applied to increase absorption. The study also included 29 patients in whom a preoperative chemotherapy with Mitoxantrone was carried out, the cytostatic blue color being used for identification of the first filtrating lymph vessels and nodes. Most frequently, visualization was achieved with Mitoxantrone (80% of cases), Patent Blue V (76%), and the combination of Drimaren with Haemodex (57%).

Key words: breast neoplasms; lymph nodes - anatomy and histology; staining methods

Introduction

The small size of the axillary lymph nodes, their colourlessness, location in the fatty tissue as well as some anatomic-topographic features have necessitated the search for staining methods which would ensure a more precise identification.¹

Until recently, perioperatively use of different dyes, such as Sky Blue, Pontamine Sky Blue etc, injected intramammarily, or the direct colour lymphography with either Lymphotrast or Chromolymphotrast, were used to visualize lymph nodes in order to facilitate their radical treatment.^{1,2}

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Adopting the hypothesis of the succession of axillary metastases from level one to three, and in search for less invasive methods for early breast staging, some reports for identification of the first lymph nodes draining the primary tumor (so called sentinel lymph nodes) by applying various dyes have appeared during the last years.

According to A. Giuliano^{3,4} and A. Barth⁵ after a peritumor application of Isosulfan Blue or Patent Blue V, it is possible to follow up the lymph vessels and the first lymph node (nodes), located on the way of the lymph drainage and after staining them to identify and histologically examine them. Selective biopsy from this "first station" of metastasis and the results obtained from this "strategic side" - negative or positive nodes,

determine the necessity for axillary lymph node dissection.

The wide variety of dyes used, the perspective by carriers with molecular weight over 10 000 to improve the selective transport (lymphotropism),^{6,7} as well as the timing of application: from 18 h before surgery to intraoperative use, grounded our decision regarding the possibilities of lymphotropic marking of sentinel lymph nodes in breast cancer.

Materials and methods

The study included 135 female patients with breast cancer (stages I and II) treated at the University Oncology Center-Pleven during the period January 1995 - April 1997, where pre- or intraoperatively, different lymphotropically dyes were applied around the tumor independently or by carriers (Table 1).

Table 1. Dyes used in patients with breast cancer

Dyes	Producer	Number of cases		
		independently	with carrier	total
Drimaren Brilliant Blue	Fluka - Cat N 44582 1% solution in PBS	10	39	49
Methylen Blau VITIS	Neopharma amp. 1% 5 ml	8	31	39
Patent Blue V	BYK Gulden amp. 2,5% 2ml	25	22	47
Total		43	92	135

To enhance dye lymphotropism, several carriers were applied together with the dye in ratio 1:1 or 1:2 (Table 2).

The quantity of dye applied around the tumor independently or by carriers varied from 1.0 to 3.0 ml, and immediately after application, the site was gently massaged for several minutes.

Based on studies proving the role of Hyaluronidase for increasing the macromolecule transportation from interstitial space to lymph vessels,^{1,7} 39 patients were treated with Hylase (Hylase Dessau, Germed). A total dose of 50-100 U was injected around

the tumor and several minutes later via a needle in the same site the dye with a carrier was introduced.

The study also included 29 females with breast cancer (stages I and II) in whom loco-regional perioperative chemotherapy with Mitoxantrone (Novantrone, Wyeth-Lederle) had been carried out. The cytostatic was used immediately before the introduction of anaesthesia. Two sites around the tumor in the lateral pole of the carcinoma (lateral localization - 18 cases) and around the four poles (central and medial localisation - 11 cases) were injected with 0,5 ml (1mg) Mitoxantrone.

All 164 patients who had a lymphotropic staining done were operated on. A modified radical mastectomy was done in 132 (T>1 cm) patients, and quadrantectomy with axillar lymph dissection - in the rest 32 (T≤1 cm) patients. The intraoperatively found sentinel lymph nodes were biopsied, submitted to his-

tological examination and compared to the results obtained on dissection.

Results

During surgery an intensive staining with a diameter from 2 to 4,5 cm was found in the sites of application and in about a half of the cases dye filled lymphatic tracts were identified at a distance of 2 - 4 cm from the primary site of injection.

Sentinel nodes colored by Mitoxantrone after perioperative locoregional chemothera

Table 2. Carriers applied for enhancement of the selective dye transportation

Carrier	Producer	Characteristics	Number of cases
Haemodex 40	Troyapharm banks x 500 ml	Solution of dextran, mean molecular weight 40,000 in isotonic solution of NaCl	30
Alvezin 40	Berlin-Chemie AG, banks x 1000 ml	L-aminoacid infusion solution with theoretical osmolarity 801,8 mosmol/l.	28
HAES-Steril 10 %	Fresenius AG banks x 1000 ml	10% solution of modified starch with mean molecular weight 200,000 in isotonic NaCl solution	20
Gelatin	Sigma cat N 62625	0,1% PBS solution with molecular weight approximately 80,000	14

Table 3. Distribution of the sentinel lymph nodes according to the dyes and carriers used.

Dyes	Independently	Carriers				Marked sentinel nodes		Number of cases with unstained lymph nodes
		Haemodex	Alvezin	HAES	Gelatin	Number of cases	Number of lymph nodes	
Drimaren	10					3	5	7
Briliant		14				8 (6)	10	6 (2)
Blue			10			5 (3)	7	5 (2)
n=49				9		3 (2)	4	6 (2)
					6	—	—	6 (2)
Methylen	8					2	3	6
Blue		9				3 (1)	4	6 (2)
n=39			11			3 (2)	5	8 (1)
				6		2 (1)	3	4 (1)
					5	2	3	3 (2)
Patent	25					19	28	6
Blue		7				5 (3)	7	2
n=47			7			5 (3)	8	2 (1)
				5		3 (2)	4	2 (1)
					3	1	1	2
Mitoxan								
trone	29		—			23	40	6
n=29								
Total								
n=164	72	30	28	20	14	87 (23)	132	77 (16)

Note: Hylase was used to 39 cases treated with carrier (they are marked in brackets) - in 23 of them stained sentinel lymph nodes were observed and in 16 - unstained.

Table 4. Distribution of the cases with lymphotropically used carrier according to the time of application.

Lymphotropic application of:	Time of application				Total number of marked cased	Number of unstained nodes
	intraoperatively	before surgery				
		1-3h	12h	20h and 1-3h		
stained independently n=43	10(5)	19(12)	5 (1)	9(6)	24	19
stained with carrier n=92	17 (4)	40 (18)	16(4)	19(14)	40	52
Mitoxantrone n=29	4 (1)	12(10)	4(3)	9(9)	23	6
Total n=164	31 (10)	71(40)	25(8)	37(29)	87	77

Note: Patients with marked lymph nodes are enclosed in bracket.

py were the most frequently observed (in 23 out of 29 cases - 80%), followed by the lymphotropic application of Patent Blue V (in 19 out of 25 cases - 76%) and Drimaren with Haemodex (in 5 out of 9 cases - 55.5 %).

The distribution of the sentinel lymph nodes according to the dye used is presented on Table 3.

Patients are divided by the time of dye application. Data are seem in Table 4.

An insignificant local erythema, without subjective complaint was observed in 5 patients: in 3 with used Mitoxantrone and in 2 with Metylen Blau and Alvezin.

Discussion

Our results (identification of sentinel lymph nodes in 87 out of 164 cases with breast cancer) are close to those reported by A. Giuliano³ - 114 out of 259 cases after application of Patent Blue V or Lymphazurin. In 77 of the cases with an identified sentinel lymph node, the latter is located on axillary level I; in seven cases with 2 stained lymph nodes, one is on level I and the other on level II; in three cases the sentinel nodes are on level II.

The highest rate of sentinel lymph nodes detection was achieved when Mitoxantrone was used. Our clinical observation on Mitoxantrone supports the reports of its comparatively good local tolerance,⁸ and its good absoption from the regional lymph nodes draining the injection site.⁹

The results obtained with Patent Blue V supported its good lymphotropism, which rendered it an universal dye in the initial stage of direct contrast lymphography, where it was used for the detection and cannulation of lymphatics.

Additional investigations on a greater number of females are required to confirm the encouraging initial results obtained by the use of Drimaren Brilliant Blue with carrier Haemodex or Alvezin. In the available literature there are no reports on the Drimaren application for sentinel lymph node mapping.

In peritumor injection of Methylen Blau (alone or with carrier) an intensive local staining of the adjacent parenchyma was observed, but a comparatively poor penetration into the draining lymph nodes and vessels was found. These observations are consistent with the experimental study of J. Wong.¹⁰

Insignificant erythema after application, described as a side effect is rare and coincides with the reports of other authors.¹⁰

Conclusions

Lymphotropic staining:

With what?

Our clinical observations point out that Mitoxantrone and Patent Blue V are most appropriate for the identification of the sentinel lymph nodes in breast cancer. An advantage of the chemotherapeutic represents the possibility for locoregional control improvement.

When ?

The most appropriate time for application is: either twofold - 20 h and 1 -3 h before surgery, or single time 1-3 h before surgery. Especially for Patent Blue V only an intraoperative application is relevant.

How?

An injection of Hylase before the application of dye with carrier enhances their resorption from a peritumor depot and improves the sentinel lymph node mapping.

The study continues according to the preliminarily defined program.

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