Chapter 3

Successful sentinel node identification in colon carcinoma using Patent Blue V

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Eur J Surg Oncol 2004; **30**: 633-7

ABSTRACT

Introduction

The aim of this study was to evaluate the use of Patent Blue V for identification of the sentinel node in patients with colon carcinoma.

Patients and methods

From May 2002 35 patients operated for colon carcinoma underwent lymphatic mapping using Patent Blue V as marker. Either directly after resection of the colon or during operation 2 ml of Patent Blue V was injected peritumourally, and the first 1 to 4 blue nodes were marked as sentinel nodes. Pathological evaluation was done on a single HE-stained section of all lymph nodes. Only if all sentinel nodes were negative for metastases, serial sectioning and additional immunohistochemical staining against keratine CK 7/8 was performed to reveal micrometastasis in the sentinel nodes.

Results

In 94% (33/35) of patients at least one sentinel node was identified. In 30% (10/33) the sentinel node was positive for metastases, and in 50% (5/10) this was the only node containing metastases. In one patient a metastasis was found only after immunohistochemical staining. One patient had a false negative sentinel node (accuracy 97%, sensitivity 91%).

Conclusion

This study shows that using Patent Blue V, it is possible to identify the sentinel node in most patients with colon cancer. The results are comparable with other sentinel node studies using Lymphazurin.

INTRODUCTION

Accurate staging of patients with colon carcinoma is important because adjuvant chemotherapy has been shown to improve survival when lymph node involvement is present[1]. In patients without distant metastases, prognosis is highly dependent on lymphatic stage[2]. 27% of patients with Dukes B2 colon carcinoma (no lymph node metastases detected) have recurrence within 5 years[3]. It is suggested that relapse occurs in those patients that have lymph node metastases, but pathological examination fails to identify them. If it were possible to find these 'occult' metastases it might improve staging and identify those patients that would benefit from adjuvant chemotherapy.

Metastatic nodes can be missed when not enough lymph nodes are being examined. The AJCC cancer staging handbook advises a minimum of 7-14 lymph nodes for accurate staging[4]. However, Haboubi suggested that when examining 'only'14 lymph nodes metastases will still be missed[5]. Joseph showed that about 40 lymph nodes need to be examined for a 85% probability that the patient is node negative[6].

Improved staging can be achieved by using immunohistochemical staining[7]or RT-PCR analysis[8]. Both studies described an upstaging with improved survival in the node-negative patients. However, these techniques are time-consuming and expensive.

Recently, various groups developed sentinel node techniques for use in patients with colon carcinoma. The sentinel node concept in colon carcinoma is interesting because it not only indicates which lymph node has the highest probability of containing metastases, but it also makes immunohistochemical staining or RT-PCR analysis more applicable, since only one or a few lymph nodes need to be examined.

Many American groups showed excellent results using Lymphazurin (isosulfan blue) for lymphatic mapping in colon carcinoma. Wong used Lymphazurin in his original feline study on lymphatic drainage[9]. He found lymphazurin to be superior to methylene blue and Cyalume (a fluorescent dye). Lymphazurin is not registered for clinical use in Europe, and studies using Patent Blue V instead did not have comparable good results.

Lymphazurin and Patent Blue V are both dienylidenes. Patent blue V has a calcium-ion instead of a sodium-ion, one extra hydroxy-group and one sulphonate-group in a different position. Although there are studies that have similar detection rates in patients with breast cancer comparing Lymphazurin and methylene blue (which are significantly different molecular structures) [10;11], the only study we know comparing the closely related Lymphazurin and Patent Blue V concerned detection rates in breast cancer, and suggested a higher detection rate for Lymphazurin[12].

We studied the use of Patent Blue V for lymphatic mapping in patient with colon carcinoma.

PATIENTS AND METHODS

Between May 2002 and July 2003, forty-three patients were operated for colon carcinoma in an elective setting. Five patients with distant metastases or gross invasion of other organs, and three patients with clinically obvious lymph node metastases (multiple large and firm nodes) were excluded. This left 35 patients to study. Characteristics are shown in table 1.

Table 1. Pathological stage (T-stage).

		Number	percentage
T-stage	1	2	6%
	2	7	20%
	3	18	51%
	4	8	23%

In the first 22 patients we used an 'ex-vivo' technique, in the following 15 patients we used an 'in-vivo' technique. In the 'ex-vivo' technique, after standard resection of the colon, the specimen was opened at the antimesenteric border and 1 – 2 ml of Patent Blue V (Guerbet laboratories, France) was injected peritumourally in the submucosal layer. In the 'in-vivo' technique Patent Blue V was injected peritumourally in the subserosal layer during operation. In both techniques lymphatics stained blue within a few minutes and the first 1 – 4 blue lymph nodes were categorised as sentinel nodes. Table 2 shows the number of lymph nodes and sentinel nodes found. All lymph nodes (non-sentinel and sentinel nodes) were analysed by conventional hematoxylin-eosin (HE) staining. Lymph nodes smaller than 0.5 cm were embedded as whole, between 0.5 – 1.0 cm they were bisected and when bigger than 1.0 cm they were cut at 0.3 cm intervals. One section of each level was analysed. If the sentinel node was negative for metastases, serial sectioning was performed at 500 μm intervals. Of each level one section was stained with HE and an adjacent section was immunostained with keratine CK 7/8, to reveal micrometastases. The same criteria as in breast cancer were used for describing metastases; tumour cell burdens between 2,0 and 0,2 mm were referred to as micrometastases and smaller than 0,2 mm as isolated tumour cells[4]. Pathological examination was performed by one of the authors (FM).

Table 2. Number of lymph nodes and sentinel nodes found.

	average	range	total
all lymph nodes	9	1 - 23	311
sentinel nodes	1.7	0 - 4	60

RESULTS

Identification of the sentinel node was successful in 94% (33/35) of patients who had lymphatic mapping. In two cases no lymph node was present at the marked site on pathological examination. In one of these the lymph nodes contained metastases.

Table 3 shows the results of successful procedures. In 10 cases the sentinel node contained metastases, of which in 5 this was the only lymph node containing metastases. In two patients metastases were revealed by immunohistochemical staining. In one patient only isolated tumour cells[4] were found, in the other patient, initially false negative on HE-staining, immunohistochemical staining showed metastasis. This metastasis was at revision also recognised in the HE-stained section.

In one patient the sentinel node was false negative, resulting in an accuracy of 97% (32/33), a negative predictive value of 96% (22/23) and a sensitivity of 91% (10/11).

Table 3. Sentinel node results.

	data	percentage
identification rate	33/35	94%
accuracy	32/33	97%
sensitivity	10/11	91%
false negative rate	1/11	9%
positive patients	12/35	34%
only SN pos	5/35	14%
only IHC pos	1/35	3%

DISCUSSION

This study shows that the use of Patent Blue V as marker of the sentinel node in patients with colon carcinoma can be successful. An extensive literature search revealed 30 studies describing the sentinel node technique in colon and/or rectal carcinoma. Joosten[13] and Cserni[14] first described this procedure using Patent Blue V as a marker. However, they had disappointing results with sensitivity rates of 45% and 62%, respectively. Later Wiese and Saha first reported the successful identification of the sentinel node using Lymphazurin[15]. Although they used a slightly different approach with only the first 1 – 4 blue lymph nodes being marked by the surgeon, and categorised as sentinel nodes, other groups did not achieve the same identification and sensitivity rates using Patent Blue V[16].

Table 4 shows all studies using Patent Blue V and the larger studies using Lymphazurin (some groups had several reports)[13-26]. Wong described the 'ex-vivo' technique which we used in the first 22 patients[19]. He showed that this technique is as successful as the 'in-vivo'

Table 4. Literature on sentinel node in colon cancer.

name	no.	tech.[1]	LNN	SN	IHC	identif.	accuracy	sensitivity	LNN pos	SN only pos	upstaging
Joosten[13]	50	PB	2-37 (14)	0-16 (3)	yes	70%	66%	45%	52%	?	4%
Cserni[14]	25	PB	2-34 (15)	0-12 (4)	no	96%	79%	62%	52%	2/25	?
Merrie[17]	26	PB	17 (4-52)	0-8 (3)	no	88%	81%	55%	42%	?	8%
Evangelista[16]	11	PB	11.5 (4-17)	0-3	no	91%	90%	67%	36%	1/11	9%
Gandy[18]	19	PB	16 (7-27)	2-17 (7)	no	63%	92%	83%	47%	3/19	?
Wiese[15]	50	Lymph	17	(1.6)	no	100%	96%	92%	48%	10/50	?
Wong[19]	26	Lymph	18 (8-36)	0-6 (2.8)	yes	92%	96%	94%	62%	5/26	15%
Esser[20]	31	Lymph	15 (4-35)	0-5 (1.7)	no	58%	94%	67%	17%	2/18	?
Feig[21]	48	Lymph	13 (4-46)	0-7 (2.6)	yes	98%	89%	75%	33%	0/48	8%
Saha[22]	203	Lymph	20	0-4 (1.8)	yes	98%	96%	90%	40%	36/203	13%
Fitzgerald[23]	26	Lymph	15	(2.5)	yes	88%	91%	71%	27%	2/26	8%
Paramo[24]	55	Lymph	12	0-4 (1.9)	yes	82%	98%	93%	27%	9/55	11%
Bilchik[25]	100	Lymph	15 (2-28)	0-4 (2)	yes	97%	95%	89%	44%	22/100	19%
Broderick-V.[26]	51	Lymph	8 (1-17)	0-5 (1.5)	yes	92%	79%	50%	39%	1/51	2% ?
present study	35	PB	9 (1-23)	0-4 (1.7)	yes	94%	97%	91%	34%	5/35	3-14%

[1] Technique used; PB referres to Patent Blue V, Lymph to Lymphazurin.

technique. Of all studies using Patent Blue V, only Gandy[18] had comparable sensitivity, but with an inferior identification rate. He used a slightly different technique identifying an average of 7 sentinel nodes in macroscopic slices of the fixed specimen, made by the pathologist. In our opinion, however, examining that many sentinel nodes is inconvenient. One of the advantages of the sentinel node technique is that by identifying only one or a few nodes with the highest probability of containing metastasis, pathological examination will be faster and less expensive, when performing any additional staining.

Upstaging varied from 2% to 19% in these studies, although, upstaging was not always calculated the same way. In our study, the sentinel node was the only site of metastases in 5 patients; since these nodes might have been missed without the use of dye, upstaging could be as high as 14% (5/35). In one patient the sentinel node harboured only isolated tumour cells, indicating that upstaging was at least 3% (1/35).

In Europe Lymphazurin is not registered for clinical use. For the sentinel node technique in melanoma and breast cancer Patent Blue V is used in most European centres. Although this molecule is closely related to Lymphazurin, some reports suggest a possible lower detection rate for Patent Blue V in patients with breast cancer[12]. This study showed that it is possible to identify the sentinel node in patients with colon carcinoma using Patent Blue V, with an equally high identification rate and sensitivity as with Lymphazurin.

Reasons why other studies using Patent Blue V had lower identification rates and sensitivity could be the following: 1) Obstruction of the lymphatics by tumour may alter lymphatic flow. This mechanism is described in patients with breast cancer[27], and is the reason we excluded patients with obvious lymph node metastases. Certainly, these patients have no advantage of the sentinel node technique because metastasis is already clear. 2) In time blue dye may pass the sentinel node, staining other nodes, subsequently falsely called sentinel nodes. It is important to identify the sentinel node within a few minutes of injection. In general there are no more than 4 sentinel nodes, though in time more nodes may stain blue and the sentinel node may even lose its blue stain. When we performed an 'ex-vivo' sentinel node procedure, we always did this directly after excision of the specimen. 3) In depth pathological examination of the sentinel node may reveal metastasis missed with conventional HE-staining. In this study there was one example of this.

Furthermore, the sentinel node procedure used in this study took about five minutes and is easy to learn. Paramo suggested it has a learning curve of 5 patients[24]. In our opinion the 'in-vivo' technique is even easier than the 'ex-vivo' technique because blue nodes can be easily identified in the intact mesocolon in the 'in-vivo' procedure. In some cases the sentinel node may be found outside the planned resection, demanding an extent of the operation in 6% to 8% of cases[22; 25]. In this study such 'ectopic' lymphatic drainage was not seen.

CONCLUSION

This study describes a simple and accurate sentinel node technique using Patent Blue V in patients with colon carcinoma. Patent Blue V is equally suited to be used for lymphatic mapping as Lymphazurin. Furthermore, this procedure can be easily done and may upstage 3–14% of patients with stage I/II colon carcinoma. Other studies will be needed to investigate the role of immunohistochemical staining and/or RT-PCR analysis of the sentinel node.

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