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Should sentinel lymph node mapping be performed for colorectal cancer?

Kolorektal kanserli hastalarda sentinel lenf nodu çalışması yapılmalımıdır?

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Background/aims: *Correct determination of lymphatic nodal statement is essential to stage correctly and to predict survival. As it is vital to make an assessment about the adjacent lymph node(s), this study was designed to compose a sensitive detection on the sentinel lymph nodes (SLN) indicating tumoral lymphatic basin using advanced pathologic examination. Materials and* ***Methods:*** *From June 2002 to June 2003, this prospective study was performed in 41 patients undergoing standard resection for colorectal cancer. In this study we employed the ex-vivo SLN mapping technique.* ***Results:*** *At least one SLN in 37 of 41 patients was identified (90.2%). The lymph nodes (LN) from those patients were studied by hematoxylin and eosin dye (H&E) and multisectioning. Twenty of 37 patients with trace of the metastasis were found. The remaining 17 patients without any metastatic LN by H&E underwent clarification ofmicrometastases (MM) using immunohistochemical (IHC) staining technique. Two patients (11.7%) had MM in the SLN(s). Upstaging was evaluated in those two. The sensitivity of SLNs was obtained as 90%. Two patients with no metastatic SLN had metastasis in the non-sentinel LNs.* ***Conclusions:*** *In the LNs from the basin of tumor, MM exposed by IHC staining was still not obvious to indicate poor prognosis. The need for treatment adjustment in those patients is clear since the upstaging was evident.*

Key words: Colorectal cancer, lymphatic mapping, ultrastaging, immunohistochemical staining

Amaç: *Kolorektal kanserlerde lenfatik tutulumun ortaya konması hem doğru evreleme hemde sağ kalım hakkında karar vermek için gereklidir. Tümör hücrelerinin drene olduğu ilk lenf nodu olan sentinel lenf nod(lar)unun (SLN) belirlenip, ileri patolojik teknik ile değerlendirilmesinin önemli olduğu bilinerek bu çalışma yapıldı.* ***Yöntem:*** *Haziran 2002-2003 arasında prospektif çalışmaya 41 hasta alındı. Kolorektal kanser tanısı olan bütün hastalara standart cerrahi tedavi uygulandıktan sonra "exvivo"yöntem ile SLNharitalaması yapıldı.* ***Bulgular:*** *Hastaların 37'sinde (%90.2) SLN'u bulundu. Lenf nodları hematoksilen-eozin boyası ve çoklu kesit alma işlemleri ile değerlendirildi. Otuzyedi hastanın 20'sinde metastaz bulundu. Geri kalan 17 hastaya immünohistokimyasal boyama uygulanarak metastaz ve I veya Mikrometastaz (MM) araştırıldı. Ik hastada (%11.7) MM bulundu. Bu hastalarda SLN'nda metastaz olmadığı halde tumoral yatak lenf nodlarında metastaz olduğu için evre yükselmesi olarak değerlendirildi. SLN haritalaması % 90 oranında bir duyarlılıkla tumoral yatağın durumunu gösterdi. SLNnda metastaz olmayan 20 hastanın 2 sinde de diğer lenf nodlarında metastaz tespit edildi (yanlış negatiftik %10).* ***Sonuç:*** *Erken ever kolorektal kanserli hastalarda tumor yatağındaki lenf nodlarında MM'ın ortaya konulması kötü prognozu kesin olarak belirtmese bile evre yükselmesi ve buna bağlı olarak kemoterapi tedavisi yapılması gerekliliği açıktır.*

Anahtar kelimeler: Kolorektal kanser lenfatik haritalama, sentinel lenf nodu, immünohistokimya

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world and represents the sixth most frequent cause for cancer deaths in Turkey (1). Surgical resection and anastomosis is selected as the first-step treatment modality. But in rectal tumors neoadjuvant chemo or chemo-radiotherapy is used as the first step treatment with a significant decrease in local recurrences (4-11%) (2,3). The manifestation of invasion of lymphatic basin has vital implications on the prognosis. It was reported that CRC, even in the early stage without lymphatic invasion in the basin, demonstrates a 20-30% rate of recurrence (4). Studies have demonstrated that lymph node micro-metastases (MM) documented by ultra-staging correlate with poor prognosis (5, 6).

Approximately 15-20 lymph nodes are harvested

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by the surgical procedure per case. The pathologist takes one or two sections for each lymph node to stain with hematoxylin and eosin (H&E). It is not feasible to screen all lymph nodes using advanced pathologic techniques. Thus researchers try to find sentinel lymph node(s) (SLN) popularized by Giuliano in melanomas. Bilchik and Saha et al. first used the SLN mapping in CRCs (6,8). The aim of this study was to show that viability of lymphatic mapping in CRC improves staging by advanced pathologic techniques.

MATERIALS AND METHODS

From June 2002 to June 2003, this prospective study was initiated with 46 patients undergoing surgical resection for colorectal cancer. As the first five cases were admitted in the learning period of SLN mapping, they were not included in this study. Forty-one patients, 18 female and 23 male, were enrolled. Mean age of the patients was 63 (31-86) years. Cases with advanced stage CRC according to Astler-Coller classification, presenting mechanical bowel obstruction due to the bulky lesion into lumen, with long distance organ metastases, and those operated in emergency condition for perforation of the bowel were excluded. All patients were instructed to obtain the standardized preoperative assessment including digital rectal exam with abdominal ultrasonography (US), computed tomography (CT), rigid rectosigmoidoscopy and colonoscopy. Preoperative staging of the CRC evaluated by CT was performed for each patient. All of the patients were approached via open surgical procedures such as low anterior resection, abdomino-perineal resection and segmental resection + end-to-end anastomosis (Table 1). It can be seen in that Table that only one patient underwent subtotal colectomy and ileorectal anastomosis due to multiple polyps (less than 10), located in the entire colon. One of the polyps in the descending colon had malignant characteristics. Informed consent form was obtained in the preoperative period from each patient in accordance with the rules of the institutional review board of the local ethics committee of Marmara University (March 2003-0052).

Ex vivo SLN mapping technique

Ex vivo sentinel lymphatic mapping was the main attempt of this study. Our technique was similar to that of Wood et al. (11). After the surgical procedure was completed the specimen was instantly taken to an extra table in the operating room. The procedure was performed just after removal of the specimen. The colonic specimen was incised longitudinally on the antimesenteric side. The rectal specimen was incised on the anterior border across the mesorectum. Lymphatic mapping was engaged on the specimen using 1 ml 1% patent blue V dye (Guerbet Lab, France) subserosally and submucosally around the tumor (peritumoral site was employed) using tuberculin syringe. Massage with little circulatory movements was undertaken on the lesion for a period of 5-7 minutes to move the dye into the lymphatic paths to the SLN(s) in the mesentery. By low level diathermy, sharp dissection of lymphatic path(s) to the SLN(s) was existent under more care. Each SLN was removed from the basin and marked before the specimen was submitted for pathologic appraisal.

Histopathologic procedure

Pathologic analysis entailed routine microscopic examination of the tumor, margins and LNs. Lymph nodes were manually dissected from the mesenteric fat. No chemical clearance method was used. Each identified LN and SLN of more than 5 mm was bisected and embedded in paraffin. Single section was routinely performed. Slices were stained by H&E staining. If the result (after two faces of the LN bigger than 5 mm and only one face for LN smaller than 4 mm was observed) was negative, all SLN's paraffin blocks were sectioned in multiple slices 4 microns thick. Slices separate from each other 200 microns in length were also stained by H&E in the second step of pathological evaluation. When no metastasis was observed in multi-sectioned slices, further analysis via immunohistochemical (IHC) staining was utilized to search for metastasis and/or MM.

Immunohistochemical staining

A single paraffin section was stained with antibodies (Pan-Keratin AE1/3, CAM 5.2®, Beckton-Dickinson, San Jose, California - 35 bH11; prediluted Vantana Medical System Inc., Tuscon, AZ).

Hemotoxylin and eosin and IHC staining and multi-sectioning were employed for LNs harvested as SLN. Isolated tumor cells, MM and any metastatic foci were evaluated in the thinner level of the SLNs. A false negative SLN was described as an SLN containing no tumor cell while one or more LNs in the specimen were positive for tumor. Upstaging was determined as pN1 in the patients with SLN stained by IHC while those LNs had no metastatic deposit using H&E stain.

Table 1. The patients' operational characteristics and tumor localization.

Localization of the lesions	No. of Patients	Type of Operations	SLNs & MM by H&E	Postoperative staging (Astler-Coller) IHC	By	Postoperative staging (TNM) H&Edye	By IHCdye
Ascend-ing colon	3	Hemicolectomy+	2/4	B2	C2	T4N0M0	T4N1M0
		ileotrasversostomy	0/3	B2	B2		
		Segmenter resection+	0/2	Bl	Bl		
Trans-verse colon	4	end-to-end anastomosis	-	Bl	Bl		
		Total colectomy+	0/3	Cl	Cl		
		ileorectal anastomosis	0/4	Bl	Bl		
			0/2	B2	B2		
Descending colon	7		0/2	B2	B2		
			0/3	B2	B2		
			0/6	Cl	Cl		
			0/3	C2	C2		
			0/7	Bl	Bl		
			0/4	B2	B2		
			0/2	B2	B2		
Sigmoid colon	11	Sphincter saving resection+	-	C2	C2		
		anastom.with stapler+	0/3	C2	C2		
		TME Abdomino-Perineal	0/2	Cl	Cl		
		Resection (ARP)	0/1	Bl	Bl		
			0/2	Cl	Cl		
			0/4	B2	B2		
			0/6	Cl	Cl		
			0/2	Cl	Cl		
			0/5	Bl	Bl		
			0/3	Bl	Bl		
				B2	B2		
Rectum Upper 1/3	8	Sphincter saving resection+	0/4	Bl	Bl		
		anastom.with stapler+	0/10	Cl	Cl		
		TME Abdomino-Perineal	2/5	B2	C2	T3NOMO	T3N1MO
		Resection (ARP)	0/4	B2	B2		
			0/4	B2	B2		
			0/4	Bl	Bl		
			0/1	B2	B2		
				B2	B2		
			0/5	Bl	Bl		
Mid 1/3	3		0/4	B2	B2		
			0/1	Cl	Cl		
			0/2	B2	B2		
Lower 1/3	5	APR	0/4	C2	C2		
			0/1	B2	B2		
			0/1	B2	B2		
			0/3	B2	B2		
			0/3	B2	B2		

TME: Total mesorectal excision, SLN: Sentinel lymph node, MM: Micrometastases, IHC: mmunohistochemical, H&E: Hematoxylin & eosin

RESULTS

In 25 patients, primary tumors were localized in the colon (3 in cecum and ascending colon, 4 in transverse colon, 7 in descending colon and 11 in sigmoid colon), while only 16 were found in the rectum (Table 1). From 41 specimens, 351 LNs were harvested during the pathologic clearance. One hundred and twenty-five LNs were defined as SLNs. Three SLN per patient and 5.5 non-SLNs per patient were collected. In 37 of 41 patients, SLN(s) was identified (90.2%). No SLN was harvested in the remaining four patients (9.8%). Most SLNs were found in the first 10 patients. In the first step of pathological staining technique, all SLNs cut out 1-2 slices were colored by H&E stain. Twenty patients had metastases in the SLN(s). The other 17 had no metastasis on SLNs using H&E stain. The SLNs from 17 patients were evaluated by multi-sectioning, and there was no trace of the metastasis on any of them. The last step of pathological staining techniques used in this study was the anti-cytokeratin antibody for IHC staining (Pan-Keratin AE 1/3, CAM 5.2) applied to clarify metastasis and/or MM.

Using IHC staining technique, two patients had MM in the SLN(s) despite the fact that there was

no metastasis or MM after H&E staining and multi-sectioning. The exact stage of CRC in two patients was upstaged (.11.7%). The chemotherapy protocol was changed. In the four patients with no SLN harvested, one had no LNs in the tumoral lymphatic basin. In the remaining three patients, two without SLN had metastasis in the non-SLN (from tumoral basin via H&E stain), which was appraised as a false-negative result (10%) (Table 2).

DISCUSSION

In even early CRC with no LN metastasis, about 20-30% of local recurrence appeared in the first two years after surgery. Several studies have shown that metastatic lesion or tumor cells remained in the tumoral lymphatic basin, which explained why unexpected local recurrences were observed (3, 9, 10).

Joseph et al. in their study stated that about 40 LNs in the tumoral lymphatic basin should be evaluated to make a true staging for T1-T2 CRC. Furthermore it was declared by UICC that 12 LNs must be examined in depth in a specimen's lymphatic basin for this purpose (11). Some authors such as Bilchik, Joseph and Koren have stated that all LNs taken from the basin were not sufficiently evaluated in routine pathologic examination. Because more than 15-20 LNs are with each specimen, detailed examination requires more time and this is not cost effective. In routine pathologic examination about 85-90% of harvested LNs set down were not investigated (6,11,12,13). Joosten et al. in 1997 declared SLN mapping in CRC in the meeting of the society of surgical oncology (14). Authors such as Wood and Feig dealing with the SLN biopsy (SLNB) then suggested SLNB in CRC as a sensitive and analytical process of pathologically staging patients with CRC (15,16). SLNB indicating the basin with 93-95% of accuracy was evaluated using advanced pathologic techniques. Though multiple sectioning and IHC staining are too time-consuming and expensive to examine all LNs, these ultrastaging pathologic techniques could be cost effective for two to four SLNs. But there is current debate regarding both accuracy and significance of SLNB. In CRC, SLNB is used to improve staging, unlike in breast cancer and melanoma in which SLNB is used to evade unnecessary radical lymphatic dissection. The advantages achieved in patients with breast cancer and melanoma, i.e. abolishing the morbidity of regional LN dissection besides improved staging of disease with MM, may not have a significant impact in patients with CRC (14-17). More recent studies in CRC have reported decreasing survival in the patients with MM appraised as pN1, but isolated tumor cell (ITC) was received as pNO as declared by Bilchik's study (3,4,11,18,19). At the same time, authors such as Adell, Broil, Cutait, and Jeffers have stated that the presence of MM had no significant effect on survival. But survival in true node negative stage II CRC could be better than that with MM (20-23).

Pathological assessment of regional LNs provides the most important contribution to decision making regarding adjuvant therapy in CRC, providing a survival benefit for patients with positive LNs, but it is of no benefit to those with negative LNs. SLNB for CRC provides an efficient means of further scrutinizing the regional lymph node basin in patients with CRC, thus providing better evaluation of the stage of the CRC and determining the

Table 2. Overall results in this study

Characteristics of lymph nodes (LNs)		%
No. whole lymph nodes (LN)	351	
No. SLN	125	
Mean SLN	3	
Mean non-SLN	5.5	
Mean SLN with metastasis	2.1	
Pt. with no LNs	1	2.4
SLN and Upstage Characteristics		
Pts. with SLN	37	90.2
No. pts. without SLN harvested	4	9.8
No. SLN with metastasis by H&E	74/125	59.2
No. SLN without metastasis by H&E	51	
No. SLN with MM by IHC	4/51	7.8
No. pts. with cancer stage upgraded after using IHC	2	11.7
No. pts. with SLN without metastasis, but non-SLN with metastasis by IHC (false negativity)	2	10

SLN: Sentinel lymph node, MM: Micrometastases, IHC: Immunohistochemical, H&E: Hematoxylin & eosin stain, Pts.: Patients

division of patients who would benefit from additional therapy (10, 24, 25).

In this study we did perform SLN mapping using blue dye. SLNs were exposed in 37 patients (90.2%). The mean number of SLN was 3 LNs. Upstaging of CRC was revealed in two patients by IHC staining (11.7%). A false negative result was also revealed in two patients (10%) (Table 3). The false negativity may have resulted from the small number of patients in this series, from atypical localization of SLNs, or from whole invasion of SLN by tumor cells preventing staining with the blue dye. In four patients of the first 10 cases in the study, no SLN was exposed. In fact, no LN was assessed in one of four patients, which may have resulted from inattentive surgical technique or erroneous pathological cleansing method. SLNB indicating the basin with 90% accuracy was determined in this study.

SLNB can be performed easily in CRC with a high degree of success, similar to that achieved in breast cancer and melanoma. As is known, SLNB in CRC is not intended to minimize extent or morbidity of dissection as in those diseases. SLNB is currently facilitating concentration on pathologic examination of SLN, more accurately indicating the tumoral basin to detect evidence of MMs which are missed by usual pathologic techniques in a significant percentage of patients with early-staged CRC. Further studies are needed to conclude the prognostic evidence of nodal micrometastatic invasion in CRC.

Table 3. Comparison of the outcomes between several studies and the current study

Study	No. Pts.	Technique	Accuracy of SLN (%)	Mean No. SLN	Up-staged (%)	Sensitivity of SLN (%)	IHC	Multi-secition	RT-PCR
Joosten (1999)	50	Hybrid	70	3	-	66	+	-	-
Saha (2000)	86	In vivo	98.8	1.6	8.2	96	+	+	-
Bilchik (2001)	40	In vivo	100	2	17.5	100	+	+	+
Wong (2001)	26	Ex vivo	92	2.8	16.7	96	+	+	
Wood (2001)	75	Ex vivo							
		In vivo	96	2	17	94	+	+	-
Fitz-gerald (2002)	26	Ex vivo	88	2.5	8.7	91	-	+	-
This study	41	Ex vivo	90.2	3	11.7	90	+	+	-

SLN: Sentinel lymph node, MM: Micrometastases, IHC: Immunohistochemical, H&E: Hematoxylin & eosin stain, Pts.: Patients, RT-PCR: Reverse transcriptase-polymerase chain reaction

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