False-Positive Lymph Nodes by Radioimmunoguided Surgery: Report of a Patient and Analysis of the Problem

Arvil D. Stephens, Usha Punja and Paul H. Sugarbaker

The Cancer Institute and Department of Pathology, Washington Hospital Center, Washington, D.C.

Preoperative administration of radiolabeled monoclonal antibody allows radioimmunoguided surgery with hand-held intraoperative detection devices. From a theoretical perspective, this technology may offer more knowledgable patient management and more complete resection of intra-abdominal cancer. False-positive examinations may seriously jeopardize the widespread application of this apparatus. Our experience with a patient with false-positive lymph nodes following administration of ^{125}I-labeled B72.3 monoclonal antibody is reported. After careful histopathological analysis of five nodes thought to be false-positive for cystadenocarcinoma, one lymph node was found to have a minute nidus of cancer. The cause of false-positive radioimmunoguided tests and their implications for the clinical use of this tool is discussed. We interpreted our data to suggest that tumor antigen-monoclonal antibody complexes processed in reactive lymph nodes, anatomically draining the malignant tissue, may cause false-positive tests.

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Cancer persistence within the abdomen despite radical surgery will result in clinical evidence of recurrent cancer. Surgical extirpation of cancer should be as complete as possible if complications are not increased by increased radicality. Radioimmunoguided surgery (RIGS) can theoretically selectively increase the completeness of the cancer resection and result in only moderate increase in morbidity or mortality (*1–3*). By revealing occult sites of cancer dissemination that cannot be identified by routine dissection and palpation techniques, negative margins of resection may be possible in a larger proportion of patients. An unknown incidence of false-negative examination exists. These diagnostic errors are unfortunate but should not result in direct harm to the patient. The surgical procedure will proceed as if the RIGS had not occurred. False-positive examinations, however, will result in surgical dissections that will not benefit the patient and increase morbidity. False-positive tests should be diligently avoided in the surgical patient.

False-positive increases in normal tissue counts with ^{125}I-labeled B72.3 monoclonal antibody by RIGS have been reported (*1–8,* Martin E, *personal communication*). These false-positive tests are known to occur in the spleen, inflammatory tissue and lymph nodes. False-positive tests in the spleen or inflammatory tissue are not as easily confused as gastrointestinal cancer metastases. This is because the spleen is readily palpated circumferentially in order to rule out the presence of cancer. Unfortunately, false-positive lymph node groups are not easily examined by palpation; confirmation of their positive or negative status for cancer requires a lymph node dissection. Removal of mesenteric lymph nodes often requires sacrifice of the adjacent bowel. Retroperitoneal lymph node dissections may result in hemorrhage, prolonged ileus and cause impaired ejaculation in male patients.

We report on a patient with cystadenocarcinoma of the appendix who showed multiple false-positive lymph nodes by RIGS. The mechanism whereby these false-positive tests in lymph nodes and in the spleen occur is discussed.

CASE REPORT

The patient was a 32-yr-old woman with large volume peritoneal carcinomatosis from a cystadenocarcinoma of a perforated appendix. Prior to her present treatments, she had undergone cytoreductive surgery and therapeutic instillations of intraperitoneal ^{32}P. The patient was evaluated preoperatively by computed tomography of the chest, abdomen and pelvis. The patient was injected with 1.14 mCi of ^{125}I labeled to approximately 0.55 mg of B72.3 monoclonal antibody 22 days prior to surgery. At the time of surgery, a checklist was used to traditionally explore the abdomen and pelvis. The Neoprobe 1000 was next used to obtain 2-sec counts in triplicate at all of the traditionally explored sites. The Neoprobe 1000 system consists of a sensitive gamma ray detector and a microcomputer-based control unit. The detector probe contains a solid-state gamma detector made of cadmium telluride and a preamplifier in a stainless steel tube with an angled tip. The control unit provides

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For correspondence or reprints contact: Paul H. Sugarbaker, MD, FACS, Medical Director, The Cancer Institute, Washington Hospital Center, 110 Irving Street, N.W., Washington, D.C. 20010.

TABLE 1
Radioimmunoguided Versus Gross and Histopathological Study of 36 Intra-abdominal Anatomic Sites

Number	Location	RIGS counts/2 sec		T:N Ratio	Analysis	Totals
		Normal Tissue	Tumor			
20	Rt. abdominal gutter	21	697	33.2	True-Positive	20/36 = 55.6%
	Small bowel surface	29	761	26.2		
	Surface left lobe of liver	24	104	4.3		
	Small bowel mesentery	17	363	21.4		
	Transverse mesocolon	16	145	9.1		
	Greater omentum	10	145	14.5		
	Terminal ileum & right colon	16	245	15.3		
	Lymph node, small bowel mesentery	17	191	11.2		
	Small bowel mesentery	14	476	34.0		
	Small bowel	39	371	9.5		
	Transverse colon	16	143	8.9		
	Greater curvature of stomach	29	137	4.7		
	Left colon and pelvic peritoneum	16	344	21.5		
	Left liver	24	202	8.4		
	Right subdiaphragm	76	203	2.7		
	Morrison's pouch	24	65	2.7		
	Pancreas	9	209	23.3		
	Duodenum	29	449	15.5		
	Cardioesophogeal junction	11	136	12.4		
	Lesser omentum	10	77	7.7		
5	Posterior aspect of abdomen	21	3	0.1	False-Negative	5/36 = 13.8%
	Splenic flexure of colon	16	7	0.4		
	Gallbladder	24	1	0.0		
	Infiltrating right hemidiaphragm	76	68	0.9		
	Spleen	204	242	1.2		
4	Small bowel lymph node	17	182	10.7	False-Positive	4/36 = 11.1%
	Base of right colic artery, lymph node	21	45	2.1		
	Transverse colon, lymph node	16	90	5.6		
	Right colon mesentery, lymph node	16	64	4.0		
7	Midline incision scar	21	11	0.5	True-Negative	7/36 = 19.4%
	Right pelvic wall, lymph node	10	10	1.0		
	Small bowel mesentery, lymph node	17	31	1.8		
	Middle colic artery, lymph node - 1	10	8	0.8		
	Middle colic artery, lymph node - 2	10	13	1.3		
	Peripancreatic lymph node	11	9	0.8		
	Perigastric lymph node	17	19	1.1		

the user interface and translates the gamma pulses received from the probe into understandable displays and sounds.

A positive radioimmunoguided test was defined as twice normal/adjacent tissue counts. All specimens whether positive or negative for tumor by RIGS were examined histopathologically. True-positive, false-positive and accuracy were determined. False-positive specimens were reevaluated histologically by multiple sections and subjected to histochemical and immunohistochemical analysis. Histological stains used included hematoxylin, eosin and mucicarmine. Immunohistochemical analysis included use of monoclonal antibodies to cytokeratins (AE1, AE3 and CAM 5.2).

All specimens were carefully harvested to prevent crushing of tissues. Lymph nodes were harvested intact so that the size could be measured and their general architecture (normoplastic versus hyperplastic) could be determined.

RESULTS

Radioimmunoguided surgery using ^{125}I-labeled B72.3 exhibited sensitivity of 80.0% and specificity of 63.6% in this patient. Thirty-six locations were evaluated intraoperatively and histologically (Table 1). RIGS produced 20 true-positive, 4 false-positive, 5 false-negative and 7 true-negative locations. The greatest tumor-to-normal tissue ratio observed was 34.0:1 for a tumor located on the small bowel mesentery. The average tumor-to-normal tissue ratio for histologically confirmed tumor was 11.6:1.

Radioimmunoguided examination of 11 lymph nodes gave one true-positive and four false-positive and six true-negative nodes (Table 2). Each of the 11 nodes were negative by traditional intraoperative and initial histological examination (Fig. 1). Without exception, false-positive nodes drain areas of the bowel that contain large volumes of cystadenocarcinoma on its surface (Fig. 2). True-negative nodes filtered lymph from anatomic sites that were not tumor-mucin contaminated. These data suggest that false-positive lymph nodes are nodes that actually drain tumor-bearing tissues.

TABLE 2
Radioimmunoguided and Histopathological Study of 11 Intra-abdominal and Pelvic Lymph Nodes

Specimen no.	Site	Size (mm)	Architecture	RIGS node/normal adjacent tissue count ratio	Histological					Analysis
					H&E	Muci-carmine	AE1	AE3	CAM 5.2	
3	Rt common iliac	7	Mild sinus histiocytosis	1.0	–	–	–	–	–	True-Negative
7	Sm bowel mes.	12	Marked reactive follicular	10.7	–	–	–	–	–	False-Positive
8	Rt colic art.	17	Mild reactive hyperplasia	2.1	–	–	–	–	–	False-Positive
12	Trans colon	5	Moderate follicular hyperplasia	5.7	–	–	–	–	–	False-Positive
14	Rt colon mes.	12	Mild follicular hyperplasia	4.1	–	–	–	–	–	False-Positive
17	Sm bowel mes.	13	Two small foci of metastatic adeno ca in the peripheral sinuses	11.2	+	+	+	+	+	True-Positive
18	Sm bowel mes.	7	Mild sinus histiocytosis with pigment laden macrophages	1.8	–	–	–	–	–	True-Negative
21	Mid colic art.	5	No significant abnormality	1.3	–	–	–	–	–	True-Negative
23	Mid colic art.	10	No significant abnormality	1.3	–	–	–	–	–	True-Negative
25	Parapancreatic	10	Mild follicular hyperplasia	0.8	–	–	–	–	–	True-Negative
36	Perigastric	5	Mild sinus histiocytosis	1.1	–	–	–	–	–	True-Negative
35	Normal spleen	130 × 70 × 25	Normal spleen	15.3*	–					False-Negative
	Surface tumor on spleen	70 × 35 × 20	Capsular implant on surface of spleen	1.2	+					False-Negative

*Normal spleen versus mean of all normal tissue counts.

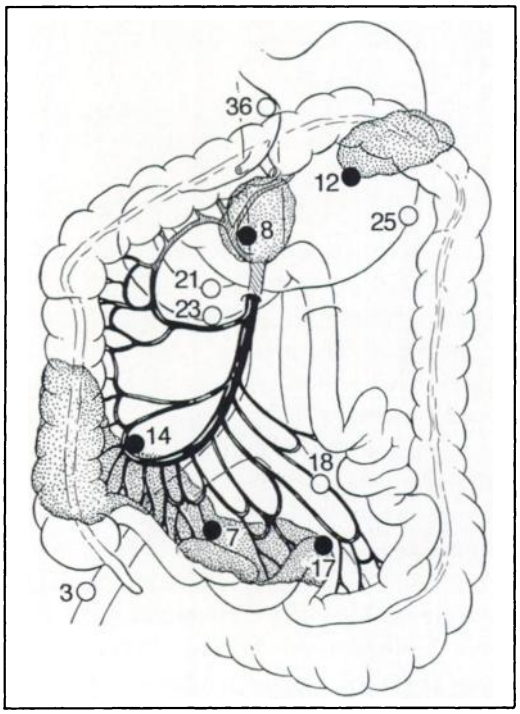

FIGURE 1. Intra-abdominal locations of 11 lymph nodes studied by RIGS and histopathological examination. ○ = histologically normal tissue and ● = histologically confirmed tumor. Number denotes specimen I.D. number.

DISCUSSION

There is no doubt that RIGS detects intra-abdominal cancer. Can this technology accurately detect cancer deposits smaller than those readily palpable by the surgeon or will false-positive and false-negative tests lead to errors in patient management? This important question was not definitively answered by our data. Radioimmunoguided surgery may assist the surgeon in the location of gross tumor deposits at anatomic sites that are not amenable to direct palpation. Also, it is possible that this monoclonal antibody technology may be successfully added to laparoscopic or flexible fiberoptic endoscopy techniques.

The false-positive lymph node examinations seen in this patient may present a serious problem to a more general application of this technology. False-positive tests may lead the surgeon into unnecessary dissections that can result in additional morbidity and mortality. If this situation is intrinsic to the test system and cannot be eliminated, then perhaps the cause of the inaccuracy can be better understood and surgical misadventures avoided.

Several features of lymph node false-positivity seem clear. First, false-positive nodes are only found in those patients whose cancer complexes with the B72.3 monoclonal antibody. This may suggest that a complex of

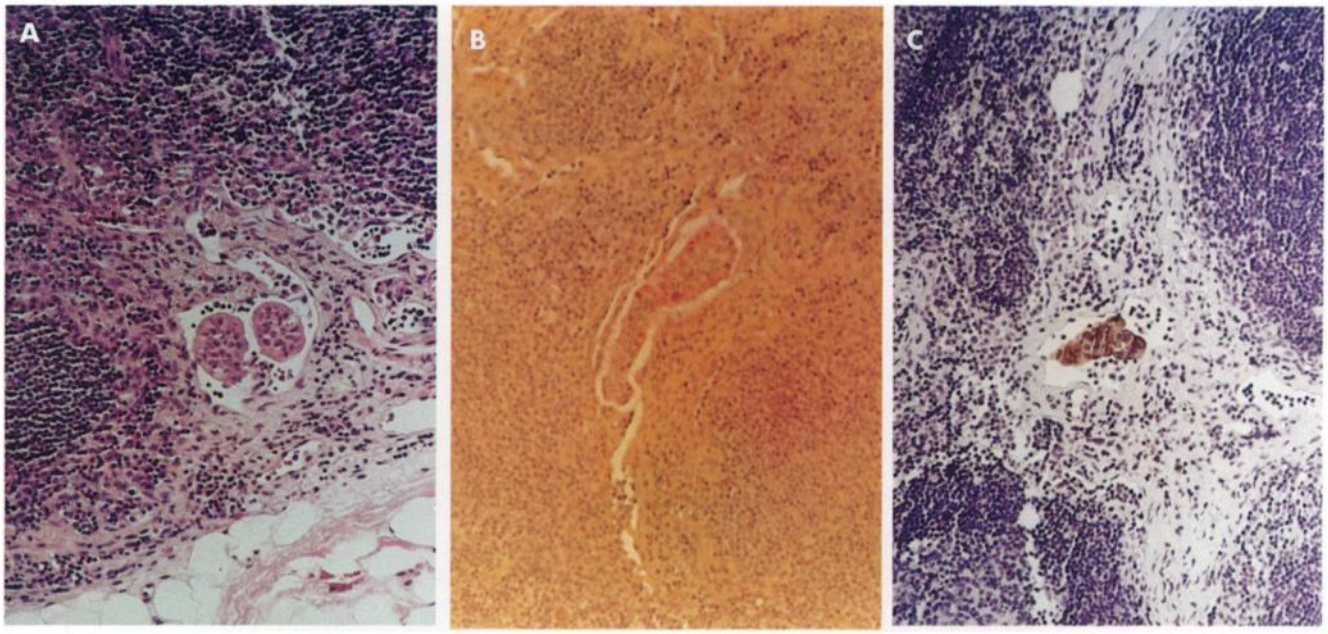

FIGURE 2. Minute focus of cystadenocarcinoma within a false-positive lymph node. (A) Hematoxylin and eosin stain. (B) Mucicarmine stain. (C) Anti-cytokeratin AE1/AE3 stain.

tumor antigen and monoclonal antibody are involved in false-positivity of lymph nodes.

Second, the false-positive nodes are regionally distributed. They drain the tumor tissues that produce the TAG-72 antigen. Our studies did not show false-positive lymph nodes unless there was tumor tissue that could contribute to lymphatic channels directly adjacent to the false-positive nodes.

Third, enlarged and hyperplastic lymph nodes seem to show the highest false-positive counts. Our clinical observations indicate that nodes reacting to an inflammatory stimulus seem to be more involved in this process.

Martin and colleagues have suggested that immunologically mediated tumor cell destruction may occur within lymph nodes showing false-positivity (*6–8,* Martin E, *personal communication*). These RIGS false-positive nodes show CD4-positive T-cells within the node. CD4 cells express the phenotype of killer T-cells and are apparently not present in such great numbers in negative lymph nodes. These investigators suggest that this phenomenon identifies a cell-mediated response to TAG-72 antigen and the destruction of tumor cells by T-cells within the lymphatic system. This interpretation of this phenomenon would be supported by our observations that these nodes were hyperplastic, only within relevant lymphatic arcades, and were only seen in patients reacting to the monoclonal antibody. Also, the antibody-dependent, cell-mediated cytotoxicity phenomenon with antigen-antibody complexes adhering to lymphoid cells within the node is a possibility.

In one of the five RIGS-positive lymph nodes, a minute focus of metastatic cancer was determined when this node was subjected to serial sectioning. It is possible that this cancerous tissue caused the elevated radioactivity within this node. It is also possible that this hyperplastic node showed false-positive counts and an unrelated mi-

TABLE 3
Comparison of Three False-Positive Monoclonal Antibody Test Sites

Feature	Spleen	Inflammatory site	Lymph node
Distribution	Spleen only	Systemic	Nodes draining tumor only
TAG-72-B72.3 complex required	No	No	Yes
Inflammatory response present	No	Yes	±
Kinetics	Early and late	Early	Unknown
Magnitude over background counts	10-20:1	?	2-10:1
Possibly tumor-specific	No	No	Yes
Possible antibody aggregates	Yes	Yes	Yes
Possible antibody	No	No	Yes

croscopic focus of cancer was present. The other four nodes revealed no evidence of cancer. The findings of all lymph nodes were confirmed by immunohistochemistry.

One may wish to compare splenic false-positivity and inflammatory tissue false-positivity to lymph node false-positivity in these patients (Table 3). Splenic false-positive results are seen in all patients, not just those who are TAG-72 positive. Clearly, splenic false-positivity is a systemic rather than regional process and is not limited to tissue directly adjacent to tumor tissue.

Clearly, more investigation of this phenomena is indicated. A hypothesis that may satisfy all the data may be one that relates general spleen and inflammatory tissue false-positivity to regional lymph node positivity. Processed aggregated antibody or antibody-antigen complexes may cause these types of false-positivity. Spleen and inflammatory sites have access to these aggregates by way of the bloodstream. However, lymph nodes can store aggregates only when antibody has access to lymph channels. Antibody that fixes to tumor would, by the invasive nature of the cancer, be allowed access to intestinal fluids within the cancer itself. Therefore, lymph nodes with lymphatic channels supplied by tumor-bearing tissue would contain radioactive aggregates. By this mechanism, only lymph nodes draining tumor sites would be false-positive. These nodes may, as has been demonstrated in this patient, be at special risk for the occurrence of metastatic disease.

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