Immunolymphoscintigraphy and Immunoscintigraphy of Ovarian and Fallopian Tube Cancer Using F(ab')\textsubscript{2} Fragments of Monoclonal Antibody OC 125\textsuperscript{1}

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Abstract

We have used immunolymphoscintigraphy (ILS) alone or in combination with immunoscintigraphy with \textsuperscript{131}I-labeled F(ab')\textsubscript{2}; fragments of monoclonal OC 125 antibodies to improve detection of retroperitoneal lymph node metastases of ovarian and fallopian tube cancer. ILS was carried out with bilateral dorsopedal s.c. injections on nine patients and with bilateral iliopelvic injections into the ischiorectal fossa on two other patients. Radioimaging was performed 2–4 times between 0 and 5 days. An additional dose of labeled antibody fragments was given i.v., and imaging was done 2–3 days later. Conventional immunoscintigraphy without preceding ILS was carried out on another nine patients. Dorsopedal ILS improved detection of pelvic and paraaortic lymph node metastases. Malignant lymph nodes were detectable as early as 3 h after s.c. injection of the tracer. Combined results of ILS and immunoscintigraphy in 16 surgically verified cases indicated a true positive finding in 9 patients, true negative finding in 5, false positive in one, and false negative in 1. Calculated from these figures the sensitivity, specificity, and accuracy of the method were 90, 83, and 88%, respectively. Involved lymph nodes were found more frequently in those patients whose serum CA 125 concentration was elevated demonstrating that an elevated serum CA 125 level does not preclude successful radioimmunodetection.

Introduction

Epithelial ovarian cancer metastasizes early into the paraaortic lymph nodes. In stage I disease paraaortic lymph node metastases have been found in 18.2% of cases, and in stage IV disease their frequency is 66.7% (1). Paraaortic nodes are involved twice as frequently as pelvic lymph nodes. Methods for follow-up and assessment of responses to therapy of ovarian cancer include ultrasonography, CT\textsuperscript{2} (2), and measurement of the serum CA 125 concentration. In the first report, the serum CA 125 level was elevated above 35 units/ml in 82% of patients with primary epithelial ovarian cancer (3). Later studies of stage I ovarian cancer have indicated that the CA 125 levels are elevated in 23% of the cases only (4), and normal levels do not rule out residual or recurrent disease (5, 6).

Radiolabeled antibodies have been used to detect ovarian cancer by immunoscintigraphy (7, 8). These include monoclonal antibodies against placental alkaline phosphatase (9–11), human milk fat globules (12, 13), CA 125 (14–16), and other epitopes (17, 18). Antibodies are usually given i.v. High uptake of radioactivity by tumors has been achieved by i.p. delivery of radiolabeled antibodies (19).

Administration of antibodies into the lymphatics has been studied for diagnosis and treatment of lymph node metastases (20) and for staging of breast cancer (21, 22), lymphoma (23), melanoma (24), and cancer of the prostate (25). Radioimmunodetection using lymphatic delivery of polyclonal anti-carcinoembryonic antigen antibodies has been successful in patients with ovarian cancer (26). Radiocolloids alone have also been used for iliopelvic lymphoscintigraphy (27–29). In theory, immunolymphoscintigraphy should offer advantages over colloidal lymphoscintigraphy and contrast lymphography, because it is thought to be more specific.

We studied the use of bipedal and iliopelvic ILS using radiolabeled F(ab')\textsubscript{2}; fragments of monoclonal OC 125 antibodies for detection of recurrent and metastatic ovarian carcinoma and compared the results with surgical findings. Results by conventional i.v. IS are also presented, and comparison is made between radioimmunodetection and conventional radiological methods.

Patients and Methods

Patients. Twenty women ages 40–75 years (mean, 58 years) gave a written informed consent to the study. Seventeen had an epithelial ovarian cancer and 3 had a fallopian tube cancer. Before radioimmunodetection, ultrasonography of the pelvis and abdomen and computer-assisted tomography (pelvis, abdomen, and thorax) were performed, and the serum CA 125 concentration was measured by radioimmunocassay according to the manufacturer's instructions (Abbott Laboratories, Chicago, IL). ILS was performed on 11 and IS on 9 patients.

Surgical Procedures. Laparotomy was performed 1–3 weeks after radioimmunodetection on 14 patients and 7 months later on one patient. One patient underwent a neck biopsy only. Two had a primary laparotomy and 13 were relaparotomies. Four patients underwent no surgery. Radioactivity of removed tissues was measured by LKB Compu gamma 1282 (Wallac, Turku, Finland), and careful histopathological examination of removed tissues was carried out in each case.

Radiolabeled Antibody. \textsuperscript{131}I-Labeled F(ab')\textsubscript{2}; fragments of monoclonal OC 125 antibody were provided by ORIS (Imacis-2; Gif-sur-Yvette, France). In six different batches the antibody-bound radioactivity was 89–94% as measured by thin layer chromatography (ITLC GS; Gelman Laboratory, Ann Arbor, MI).

Thyroid Blockade. Two days before administration of labeled antibody fragments, 200 mg of KClO\textsubscript{4} and 2 ml of 5% KI solution were given p.o. four times daily for 10 days to prevent accumulation of radioiodine into the thyroid.

Radioimaging Device. Radioimaging was performed using a General Electric Maxi Camera 400 (SPECT/color display), with a resolution capacity of 350 dots, connected to a PDP-11/34 computer equipped with Gamma-11 software.

Immunolymphoscintigraphy. ILS was performed by either (a) bilateral dorsopedal s.c. injections into the first and fourth interdigital spaces or (b) bilateral iliopelvic injections, 4 cm into the ischiorectal fossa. In two cases, i.v. and dorsopedal injections were performed simultaneously. The total injected radioactivity was 1.6–2.7 mCi (66–100 MBq) in 1 mg of antibody.

For dorsopedal delivery the dose was divided into four equal parts, 0.1–0.15 ml each. For iliopelvic injections the administered volume was 2 ml on each side. One half of the radioactive dose was first injected s.c. or iliopelvically. After dorsopedal injections the patients were asked to immediately move their feet. The other half of the dose was given i.v. as a single bolus. At least two imaging studies were performed after dorsopedal or iliopelvic administrations, and one imaging was made 2–3 days after the i.v. injection.

The whole body was scanned by planar spot views including pelvic lateral views in all imaging phases. From 350,000 to 700,000 counts

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\textsuperscript{3} The abbreviations used are: CT, computer-assisted tomography (whole body); ILS, immunolymphoscintigraphy; IS, immunoscintigraphy.
were collected into each scintigram. During the first study the injection sites were also imaged to learn about retention of radioactivity. This was controlled by the region of interest technique. In later images no significant side differences were observed in the rate of lymphatic drainage of radioactivity in the lower extremities.

The scans were independently interpreted by two physicians (K. J. A. K., K. L.). In doubtful cases a third opinion (P. L.) was invited. Sites of radioactive accumulation were controlled by the region of interest technique. The finding was considered positive if the target/nontarget ratio was more than 1.2.

Immunoscintigraphy. Radiolabeled antibody fragments (1 mg) containing 1.9–3.1 mCi (70–115 MBq) of radioactive label were diluted in 100 ml saline and delivered during 30 min as an i.v. infusion.

Radioimageing was performed 2–4 times between days 1 and 7 after infusion of labeled antibody. This included whole body scanning and planar anterior, posterior, and lateral pelvic views. From 350,000 to 1,200,000 counts were collected into each image.

Subtraction Methods. In selected cases, subtraction was used to improve interpretation of the images. In equivocal findings and in order to better delineate the liver and the spleen, 99mTc-labeled tin colloid was given i.v. to five patients. In three patients the disturbing blood pool activity was eliminated by subtraction with pertechnetate (Na99mTcO4) or 99mTc-labeled RBC. The patient was kept in the same position during 131I and 99mTc imagings. The subtraction procedure was done iteratively using scaling factors to suppress radioactivity to near the background level in various organs. The dose of 99mTc tracer varied from 0.8 to 1.7 mCi (30 to 63 MBq). Technetium imaging of the liver and the spleen was started 5–8 min after injection of the tracer, and blood pool subtraction was performed 1–3 min after the injection.

Results

Immunolymphoscintigraphy and immunoscintigraphy were carried out without complications. In ILS the tracer readily entered the lymphatics and disappeared within 3 days in all those cases in which no malignant lymphatic involvement was detected. Fig. 1 shows inguinal lymph node metastases in patient KS/61 as detected by accumulation of radioactivity 4 h after dorsopedal s.c. injections. Another patient (KR/63) had retroperitoneal, pelvic, and paraaortic lymph node involvements visualized in ILS 5 h after injection (Fig. 2A and B). The latter patient also had a pelvic tumor, which became better discernible after the i.v. injection. The imaging times were 52 h (Fig. 2C) and 125 h after the s.c. injections and 72 h after the i.v. injection (Fig. 2D).

Fig. 1. A, anterior view of the thighs of patient KS/61 with mucinous ovarian adenocarcinoma, stage IIb. Malignant involvement is seen in the right inguinal lymph nodes 4 h after dorsopedal s.c. administration of the tracer. B, anterior pelvic view of the same patient. Inguinal and paraaortic involvement is seen bilaterally 4 h after dorsopedal injections.

Fig. 2. Anterior (A) and posterior (B) pelvic views of patient KR/63 with serous ovarian adenocarcinoma, stage III. Bilateral paraaortic, paraaortic, and left inguinal lymph node involvement and pelvic tumor are seen 5 h after dorsopedal s.c. injections. C, anterior pelvic view of patient KR/63 showing higher accumulation in the pelvic tumor. Lymph node radioactivity is disappearing but still clearly discernible 52 h after dorsopedal s.c. injections. D, anterior pelvic view of patient KR/63 125 h after dorsopedal s.c. injections and 72 h after i.v. injection (one-half of the radioantibody was injected i.v.). The tracer has accumulated mainly in the tumor.

Fig. 3. Anterior pelvic view of patient HN/45 with endometrioid ovarian adenocarcinoma, stage Ic. 97 h after bilateral iliopelvic injections. Malignant involvement is seen in the left inguinal lymph node and above the bladder in spite of strong retention of the tracer at the injection sites.
Table 1 Results of 11 patients examined with ILS

<table>
<thead>
<tr>
<th>Patient/age (yr)</th>
<th>Site and type of cancer</th>
<th>Stage*</th>
<th>CT</th>
<th>USb</th>
<th>Pelvic examination</th>
<th>ILS</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>EK/46</td>
<td>Ov e</td>
<td>Ia</td>
<td>−ve</td>
<td>n.d.</td>
<td>−ve</td>
<td>−ve L</td>
<td>NED</td>
</tr>
<tr>
<td>HN/45</td>
<td>Ov e</td>
<td>Ic</td>
<td>+ve</td>
<td>−ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>LE/65</td>
<td>Ov s</td>
<td>IV</td>
<td>−ve</td>
<td>−ve</td>
<td>−ve</td>
<td>+ve</td>
<td>NED</td>
</tr>
<tr>
<td>AH/42</td>
<td>Ov s</td>
<td>III</td>
<td>n.d.</td>
<td>−ve</td>
<td>−ve</td>
<td>n.d.</td>
<td>NED</td>
</tr>
<tr>
<td>KR/63</td>
<td>Ov s</td>
<td>III</td>
<td>n.d.</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
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<tr>
<td>AR/40</td>
<td>Ov e</td>
<td>Ia</td>
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<td>−ve</td>
<td>−ve</td>
<td>+ve</td>
<td>NED</td>
</tr>
<tr>
<td>KS/61</td>
<td>Ov m</td>
<td>IIb</td>
<td>+ve</td>
<td>n.d.</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>RR/75</td>
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<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>IH/51</td>
<td>Ov m</td>
<td>Ic</td>
<td>n.d.</td>
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<td>−ve</td>
<td>+ve</td>
<td>NED</td>
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<tr>
<td>HK/60</td>
<td>Ov s</td>
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<td>n.d.</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>AL/58</td>
<td>Tb</td>
<td>Ia</td>
<td>+ve</td>
<td>−ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

* Federation Internationale des Gynecologistes et Obstetristes classification.

b US, ultrasonography (pelvis and abdomen); L, liver/spleen subtraction (**Tc-Sn colloids); i.d., bipedal interdigital s.c. injections; i.p., bilateral iliopelvic injections; i.v., simultaneous i.v. infusion; Ov, ovarian cancer; Tb, fallopian tube cancer; e, endometrioid; s, serous; m, mucinous; n.d., not done; NED, no evidence of disease; −ve, negative; +ve, positive.

Table 2 Results of nine patients examined with IS

<table>
<thead>
<tr>
<th>Patient/age (yr)</th>
<th>Site and type of cancer</th>
<th>Stage*</th>
<th>CT</th>
<th>USb</th>
<th>Pelvic examination</th>
<th>IS</th>
<th>Surgery</th>
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</thead>
<tbody>
<tr>
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<td>−ve</td>
<td>−ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>RR/50</td>
<td>Tb</td>
<td>III</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>AI/71</td>
<td>Ov e</td>
<td>IV</td>
<td>−ve</td>
<td>n.d.</td>
<td>−ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>BE/65</td>
<td>Ov s</td>
<td>IV</td>
<td>−ve</td>
<td>n.d.</td>
<td>−ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>EH/55</td>
<td>Tb</td>
<td>III</td>
<td>n.d.</td>
<td>−ve</td>
<td>−ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>MH/66</td>
<td>Ov s</td>
<td>III</td>
<td>n.d.</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>TK/62</td>
<td>Ov s</td>
<td>III</td>
<td>+ve</td>
<td>n.d.</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>AL/63</td>
<td>Ov s</td>
<td>III</td>
<td>−ve</td>
<td>−ve</td>
<td>−ve</td>
<td>n.d.</td>
<td>NED</td>
</tr>
<tr>
<td>TM/58</td>
<td>Ov s</td>
<td>IV</td>
<td>+ve</td>
<td>n.d.</td>
<td>+ve</td>
<td>+ve</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* Federation Internationale des Gynecologistes et Obstetristes classification.

b B, blood pool subtraction (**Tc or pertechnetate). For other abbreviations, see Table 1.

c Second look operation 7 months later.

d Biopsy.

Table 3 ILS and IS findings relative to serum CA 125 concentrations

<table>
<thead>
<tr>
<th>Serum CA 125 level</th>
<th>IS/ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;35 units/ml</td>
</tr>
<tr>
<td>Negative (n)</td>
<td>6</td>
</tr>
<tr>
<td>Positive (n)</td>
<td>1</td>
</tr>
</tbody>
</table>

Results of ILS were similar to those obtained by conventional methods in 10 of 11 cases (Table 1). One false positive case (LE/65) was found among nine surgically verified cases. In eight of nine cases the results of IS were compatible with those obtained by conventional methods (Table 2). IS gave one false negative result (SM/60) of seven surgically confirmed cases. Second look laparotomy was performed on this patient 7 months after a negative IS finding, and a 1-cm metastasis was found near the stomach.

In patient AI/71 peritoneal carcinosis was observed by IS but not by CT (Table 2). This was verified by surgery and histological examination. The overall findings by IS were in good agreement with those by CT. There was a patient (HN/45) in whom ILS was more sensitive than CT. Inguinal lymph node involvement was detected by either method (Table 1), whereas pelvic lesions were found by ILS only. However, we probably missed some information in the pelvic area because of strong retention of the tracer at the iliopelvic injection sites. In patient HN/45 radioactivity was still retained at the injection sites 97 h after administration of the tracer (Fig. 3).

In ILS the target/nontarget radioactivity ratio of a surgical specimen containing pelvic lymph node metastases could be as high as 25.8 when studied 152 h after the injection (KR/63).

When the results of ILS and IS were combined, radioimmunodetection gave a true positive finding in 9 of 16 surgically proved cases. Five were considered true negative, one was false positive, and one was false negative (sensitivity, 90%; specificity, 83%; accuracy, 88%). The false positive finding was made by ILS and the false negative was made by IS.

Patients with elevated serum CA 125 levels had more frequently lymph node involvements than those with normal levels, as detected by ILS or IS (Table 3).

Discussion

After the pioneering studies of Goldenberg et al. (30, 31) and Mach et al. (32, 33) radioimmunodetection has gradually been improved and is now gaining acceptable sensitivity and specificity for clinical purposes. Our results show that dorsopedal ILS can improve detection of paraaortic and parailiac lymph node involvement. With this method for obturatoric lymph nodes are still missed, but ovarian cancer rarely sends metastases into this site. Anatomically the best mode of tracer delivery would be the iliopelvic route, but interpretation of subsequent images may pose problems because of nonspecific local retention of the tracer.

A positive correlation was observed between immunoscintigraphic findings and the serum CA 125 concentration. This means that patients with elevated serum CA 125 levels have more frequently affected lymph nodes than those with a normal level. Our results confirm the previous observations (15) indicating that, in the presence of high circulating CA 125 levels, it is possible to detect CA 125-containing lymph node metastases of ovarian cancer by immunolymphoscintigraphy.

Subtraction methods were not considered useful in all patients because of different energies in **Tc and **I. With the
subtraction technique it is possible to delineate the organs of interest and thereby improve the interpretation of equivocal immunoscintigraphic findings. However, satisfactory subtraction of radioactivity from organs with high uptake was not considered reliable for routine clinical purposes. Simultaneous i.v. and dorsopedal injections were used only twice, because it was difficult to distinguish between lymphatic and blood vessel radioactivity.

Immunoscintigraphic methods are now modifying our clinical decisions towards a more radical paraaortic lymph node dissection in early ovarian cancer. More experience is now accumulating to learn whether this more aggressive immunodirected intervention has an effect on the patient survival.

References

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