Br. J. Cancer (1990), **62**, Suppl. X, 18-20

Resection guided by antibodies (REGAJ): a diagnostic procedure during second-look operation in ovarian cancer patients

W. Jäger[1], H. Feistel[2], E.M. Paterok[1], G. Ronay[1], A.H. Tulusan[1], F. Wolf[2] & N. Lang[1]

Departments of [1]Obstetrics and Gynecology and [2]Nuclear Medicine, University of Erlangen-Nürnberg, Erlangen, FRG.

Summary A hand-held probe has been used to localise the ^{131}I-labelled antibody OC125 during second-look operation (SLO) in 22 ovarian cancer patients. In six patients microscopic cancer was detected with the procedure and a further six patients with areas of increased radioactivity during SLO developed recurrent disease within 1-4 months. We suggest examining resection guided by antibodies as a possible means of removing antigen-producing cancerous tissues during surgery.

The aim of postoperative chemotherapy in ovarian cancer patients is the destruction of remaining cancer. Assessment of response to treatment is usually difficult to obtain with clinical examination methods, since tumours are often spread throughout the abdomen. Therefore, a second-look operation (SLO) has been established during which the surgeon gets an impression of the residual disease (Schwartz & Smith, 1980). If no macroscopic tumour can be detected, biopsies are taken at random from different locations in the abdomen. The decision for further treatment is based on the histological findings in these biopsies (Stuart *et al.*, 1982; Dauplat *et al.*, 1986).

However, the evaluation of random biopsies has proved to be unpredictable as nearly 40% of patients who were judged to be tumour-free at SLO developed metastases during further follow-up (Podratz *et al.*, 1988; Rubin *et al.*, 1988). This has led some oncologists to reconsider the role of SLO as a diagnostic procedure (Luesley *et al.*, 1988; Sonnendecker, 1988).

Since it has been demonstrated that most ovarian cancer cells exhibit the cancer antigen-125 (CA-125), the specific binding of the monoclonal antibody OC125 has been used for immunoscintigraphic detection of ovarian cancer (Chatal *et al.*, 1987; Baum *et al.*, 1989). The promising results from these studies led us to determine whether the binding of radiolabelled OC125 to ovarian cancer could also be helpful in detecting these tissues during surgery.

Clinical experience with this procedure, however, demonstrated that it was difficult to localise immunoscintigraphic suspicious areas during surgery. We therefore developed a hand-held probe to detect these tissues by measuring radioactivity in the abdomen - a procedure which we called 'resection guided by antibodies - iodinised' (REGAJ) (Jäger *et al.*, 1987). While in the previous study this method was tested in primary and recurrent ovarian cancer, we have now studied this approach in SLO.

Patients and methods

Patients

Between September 1986 and December 1988 REGAJ has been performed on 22 patients undergoing SLO. All patients had been treated with primary surgery and four treatment cycles of chemotherapy. Patients with progressive cancer or increasing CA-125 serum levels during primary chemotherapy were not submitted to SLO as reported previously (Jäger *et al.*, 1988*a*). The median time interval between primary surgery and REGAJ was 6 months.

Correspondence: W. Jäger.

OC125 injection

One mg of ^{131}I-labelled F(ab')$_2$ fragments of the murine monoclonal antibody OC125 (IMACIS II, ID-CIS, Dreieich, FRG) were infused. The radioactivity was 70-110 MBq mg^{-1} of antibody. The deep frozen antibody solution was thawed on the day of infusion and later infused during a 30 min period. After 3 and 5 days scintigrams were taken by the planar technique applying mean energy collimators (Rotacamera, Siemens, Erlangen, FRG). Thyroid uptake of ^{131}I was blocked by oral administration of perchlorate (3 × 300 mg day^{-1} starting 1 day before injection of antibodies for 7 days).

Contrast imaging of osseous tissues ('anatomical landmarking') was achieved by means of a double nuclide technique (bone marrow scintigraphy).

Probe

A probe was developed using a 1 × 1 inch NaI crystal with a photomultiplier (Sonde 0463-4, Stratec Elektronic, Birkenfeld, FRG). The size of the entrance is 44 mm in diameter. A collimator can be attached with an entrance of 10 mm and a length of 50 mm. The measuring characteristics are shown in Figure 1. Counts per second are shown on a digital ratemeter.

REGAJ procedure

During surgery all patients were catheterised. After abdominal incision, ascites were sucked out and peritoneal washings with saline solution were performed until the washing solu-

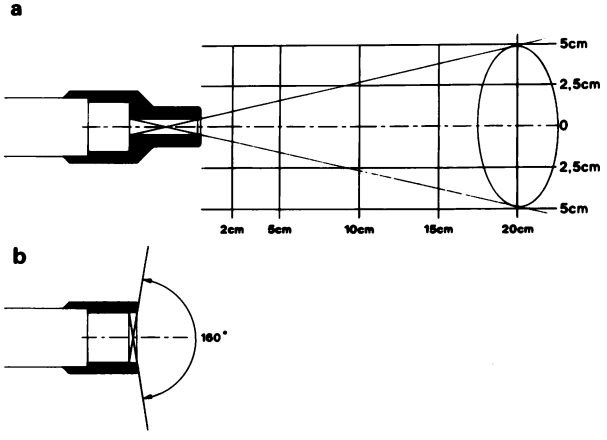

Figure 1 Measuring characteristics of the probe **a** with and **b** without the collimator attached.

tion was clear. Ascites and washing solution were stored until decay of radioactivity. Thereafter 'background' radioactivity was measured above the abdominal aorta by the hand-held probe. Positive countings were arbitrarily defined as at least 50% counts more than the background radioactivity.

To detect areas of higher radioactivity during the first step of the procedure the probe was used without the collimator. The probe was moved along the abdominal walls, the intestine, the liver, the spleen and the pouch of Douglas. Thereafter the same procedure was repeated with the collimator attached to the probe in order to localise more precisely positive countings. Biopsies from areas with positive countings were called REGAJ biopsies. Further tissues were sampled from areas with no increased radioactivity (random biopsies). All tissues were placed in dishes containing Carnoy solution for immunohistochemical preparation. Histological examinations of all tissues removed were performed by one of us (A.H.T.) as well as immunohistochemistry (G.R.). The data from immunohistochemistry will be published elsewhere.

Results

The results of the 22 REGAJ are listed in Table I. REGAJ was usually performed 8 days after the injection of the antibody (range 4–13 days).

In one patient (no. 2) small tumours were spread in the whole abdomen so that no single area with higher radioactivity could be encircled. In one patient (no. 6) some tumours were visible but additional areas with positive countings could be localised where cancer was detected. In two patients (nos. 10 and 11) small tumours could be palpated in the areas of higher radioactivity. In the remaining 18 patients no tumour was visible or palpable. In five of these patients no areas of higher radioactivity could be detected (nos. 1, 4, 5, 8 and 13). All random biopsies from these patients were cancer-free. In the remaining 13 patients areas of higher radioactivity could be localised with the larger probe. These areas could not be further differentiated with the smaller probe. We therefore decided not to take small tissue samples but to remove the whole areas. In six of these 13 patients cancer could be detected (nos. 3, 7, 14, 17, 20 and 21), whereas in the other seven patients no cancer was found at histological examination of the biopsies.

The further follow-up of the 12 patients where no cancer was found during SLO revealed recurrent disease in eight patients. It was interesting to note that six of these eight patients were those who exhibited areas of higher radioactivity during REGAJ, but no cancer was found in the biopsies. They all developed recurrent disease within 4 months after SLO (1, 1, 3, 3, 3, 4 months).

From the five patients where no increased radioactivity was localised, two developed recurrences after 9 months (nos. 4 and 5). One of the patients with positive countings was without recurrent cancer 12 months after REGAJ and three patients with no increased radioactivity were without recurrence for 13, 20 and 31 months (nos. 1, 8 and 13).

Discussion

CA-125 is predominantly produced by ovarian cancer cells, but only in small or undetectable amounts by other tissues (Kabawat *et al.*, 1983). This observation suggests that antibodies to CA-125 such as OC125 may be of use in localising cancerous ovarian tissue (Haisma *et al.*, 1988). We therefore utilised ^{131}I-labelled OC125 to determine whether ovarian tumours could be more easily detected during SLO (Jäger *et al.*, 1988*b*).

We decided to remove those tissues during surgery where increased radioactivity compared with background radioactivity was measured. Background radioactivity was defined as the radioactivity of the blood pool measured above the abdominal aorta.

At the beginning of the study we only wanted to detect the tumours which were suspicious by immunoscintigraphy. It was soon recognised that, besides tumours with circumscript higher radioactivity, we measured increased radioactivity in areas where no tumours could be seen or palpated. It was surprising that the radioactivity in these areas was remarkably higher than background when the larger probe was used, while with the smaller probe the radioactivity was only slightly higher than background.

In those patients in whom radioactivity could be detected, but no cancer was found histologically, recurrent disease occurred within 1–4 months. Since patients with no increased radioactivity during SLO have so far exhibited a longer disease-free survival, we speculated that areas with

Table I Distribution of cancer tissues in random and REGAJ biopsies obtained during second-look operation in 22 ovarian cancer patients. CA-125 serum levels before REGAJ as well as the results of the immunoscintigraphy (Immuno) and computed axial tomography (CT) are given as suspicious (+) or non-suspicious (–).

	Before REGAJ		*Biopsies*						
Patient no.	*CA-125 (units ml^{-1})*	*Immuno/CT*	*No.*	*Malignant*	*Random (total / malignant)*	*REGAJ*	*Only by REGAJ*	*Months until relapse*	*Months without relapse*
1	25	+/–	3	–	3/–	–[a]	–	–	31[c]
2	33	+/+	10	9	10/9	–[a]	–		
3	25	–/–	15	12	3/–	12/12	+		
4	25	+/–	2	–	2/–	–[a]	–	9	
5	25	+/–	5	–	5/–	–[a]	–	9	
6	25	–/+	15	9	9/4	6/5	–		
7	25	–/–	31	1	17/–	14/1	+		
8	25	–/–	21	–	21/–	–[a]	–	–	20[c]
9	25	+/–	9	–	3/–	6/–	–	3	
10	70	+/+	23	17	4/–	19/17	–		
11	15,500	+/+	7	5	2/–	5/5	–		
12	25	–/–	30	–	20/–	10/–	–	1	
13	25	–/–	23	–	23/–	–[a]	–	–	13[c]
14	25	+/–	32	2	19/–	13/2	+		
15	25	+/–	12	–	7/–	5/–	–	3	
16	25	+/–	16	–	11/–	5/–	–	–	12[c]
17	25	+/–	14	3	9/–	5/3	+		
18	25	+/0[b]	14	–	12/–	2/–	–	4	
19	25	–/0[b]	11	–	8/–	3/–	–	3	
20	25	–/0[b]	13	1	12/–	1/1	+		
21	25	–/0[b]	17	3	14/–	3/3	+		
22	25	+/0[b]	11	–	7/–	4/–	–	1	

[a]No differentiation against 'background' possible. [b]Not performed. [c]No relapse at the end of the study.

diffuse higher radioactivity are highly suspicious for microscopic cancer. The technique to sample small biopsies from these areas was probably insufficient in removing the real 'hot spots'. Therefore, greater areas of tissue were removed during the further course of the study.

The histological examination of the resected tissues must be carried out extremely carefully. Some random cuts on tissue blocks may miss cancer cells. In one of the patients only a careful re-evaluation of the resected tissues revealed the cancer cells.

Immunoscintigraphy is limited by the problems of size and distance, since small size of tumours and increasing distance from antibody to camera will diminish the radiation outside the abdomen and therefore the scintigraphic detectability. The use of scintillation detectors during surgery should therefore be advantageous for localising even smaller tumours. The results obtained so far indicate that this procedure could be helpful in detecting cancerous tissues and may possibly define those patients with increased risk of early recurrence.

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