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Comparison of Two CEA Assays in Primary and Recurrent Large Bowel Carcinoma with Different DNA Ploidy Pattern*

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Abstract—*Pre-operative CEA levels were measured in* 100 *patients with large bowel carcinomas with different DNA ploidy pattern and serial post-operative determinations performed in the* 64 *who had been operated for cure. The follow-up period was* 3½–8 *yr. All CEA measurements were performed consecutively with a RIA (Roche), and subsequently repeated in one batch with an EIA (Roche) based on a monoclonal antibody. Both assays showed a similar number of 'false-negative' CEA levels pre-operatively—varying from* 69% *in aneuploid (AN) Dukes' A to* 8% *in AN Dukes' D tumours, and from* 75% *in near diploid (ND) Dukes' A to* 40% *in ND Dukes' D tumours. The sensitivity for detecting recurrence in patients with tumours of either ploidy pattern was slightly better with EIA than with RIA. A difference between the AN and ND group was shown somewhat better with RIA, the sensitivity in the AN group being* 79% *and the median lead time* 7 months *compared to* 13% *and* 2 months *in the ND group. The corresponding figures with EIA were* 71% *and* 7 months *for the AN group and* 63% *and* 1½months *for the ND group. However, all but one of the patients with ND DNA pattern who showed recurrence-associated CEA elevation with EIA also had an elevated level pre-operatively. We conclude that all patients operated for cure should be followed by regular CEA measurements post-operatively if they had an elevated CEA level prior to operation. In addition, patients with AN tumour should be followed with serial CEA measurements even in the absence of pre-operative elevation. The two assays should probably be considered fairly equal from a clinical point of view.*

INTRODUCTION

IT HAS previously been shown that preoperative plasma CEA levels are positively correlated with clinico-pathological stage in patients with aneuploid (AN) large bowel carcinomas, whereas no such correlation is seen in patients with near diploid (ND) tumours [1, 2]. Furthermore, serial plasma CEA measurements are significantly more sensitive as to detection of recurrence of the AN tumours than of the ND ones [2].

These results have been obtained using a commercially available (Roche) CEA-radioimmunoassay (CEA–RIA) (F. Hoffmann–LaRoche, Basel, Switzerland) based on a polyclonal anti-CEA reagent. However, various commercially available CEA assays may differ in their specificity and sensitivity [3, 4]. The purpose of the present investigation, therefore, was to compare the previously reported CEA–RIA results with those obtained in the same samples using a CEA enzyme-immunoassay (CEA–EIA) based on a monoclonal antibody to CEA (Roche).

PATIENTS AND METHODS

Patients

Pre-operative plasma samples from 100 patients operated for large bowel carcinoma were studied, as were serial post-operative samples from those 64 patients who had been operated for cure.

Plasma CEA measurements

Quantitation of CEA was initially carried out with a modified CEA–RIA (Roche) based on a polyclonal anti-CEA reagent. The test was per-

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formed as described by the manufacturer with slight modifications [3], using the dialysis procedure technique as described previously [5]. Internal quality controls with own reference materials were included [6].

The samples were collected over a period of 7 yr and kept at −20° C after being analyzed with the CEA–RIA. At the end of the study all samples were tested with a CEA–EIA (Roche) which is a solid-phase enzyme-immunoassay based on a monoclonal mouse anti-CEA coated on plastic spheres and a polyclonal peroxidase conjugated anti-CEA. Both antibodies were incubated together with patient plasma/serum for 18 hr at 37° C. The monoclonal anti-CEA had been produced against a human colonic carcinoma and showed no specificity against known cross-reacting determinants (NCA1, NCA2 or BGP1). All CEA–EIA kits were of the same batch, and internal quality controls with own reference materials were included.

Diagnostic sensitivity of the CEA-assays were expressed as

$$\frac{\text{No. of patients with elevated CEA} \cdot 100}{\text{Total no. of patients}}$$

In both assays CEA values below 3.5 μg/l were considered normal.

Clinio-pathological staging

Gross and microscopic pathological examinations were performed by the same person throughout the study. The clinico-pathological staging was carried out according to Turnbull *et al.* [5] using their extended Dukes' scheme (Table 1).

DNA flow cytometry

Flow cytometric (FCM) DNA quantification was generally performed on five samples from each tumour specimen. The method has been detailed and discussed previously [1, 8, 9]. The tumour tissue was disaggregated by mechanical and enzymatical methods before being stained according to Göhde and Dittrich [10] with ethidium bromide (Calbiochem, San Diego, CA). Emission measurements were performed in an ICP 11 flow cytometer (PHYWE AG, Göttingen, FRG).

The ploidy pattern of the histograms were determined, and mouse spleen lymphocytes were used as a diploid 2c reference. Peaks < 2.5c were assigned to near-diploid (ND). One or more distinct peaks occurring above that level were regarded as aneuploid (AN).

Statistical methods

Only non-parametric rank methods were applied: distributions were given as medians and observed ranges, and correlation studies were based on the Kendall's τ-test.

RESULTS

In the AN tumour group positive correlations were found between clinico-pathological stage and pre-operative plasma CEA levels with both RIA ($\tau = 0.40$, $P < 0.001$) and EIA ($\tau = 0.42$, $P < 0.001$) (Fig. 1). However, there were 49% 'false negative' CEA values with EIA compared with 43% with RIA.

In patients with ND tumours no correlation between clinico-pathological stage and plasma CEA level was seen with either of the assays (Fig. 1). There were 62% 'false negative' CEA values in this group with EIA and 65% with RIA.

Plasma CEA in recurrent cases

Sixty-four patients who had been operated for cure all had normal post-operative plasma CEA

Table 1. Distribution of clinico-pathological stage (Dukes' stage) and DNA-ploidy patterns*

	Dukes' stage*				
	A	B	C	D	Total
Aneuploid	13	23	15	12	63
Near diploid	8	14	10	5	37
Total	21	38	25	17	100

*Dukes' stage according to Turnbull *et al.* [7]

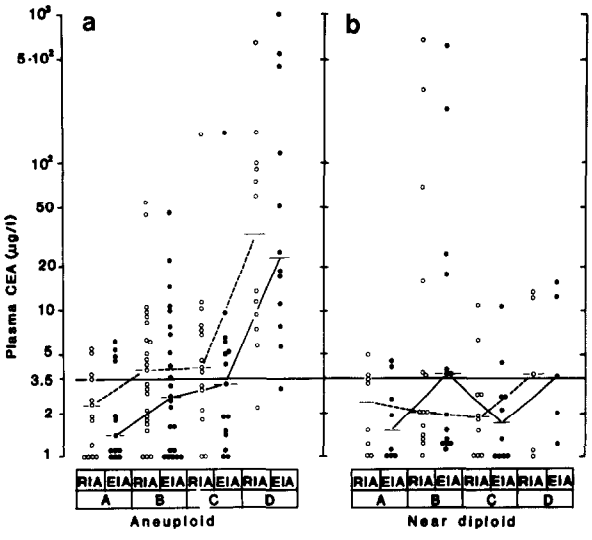

*Fig. 1. Scatter diagram of relation between clinico-pathological stage (A, B, C and D) and pre-operative plasma CEA levels measured by RIA (○) or EIA (●) (medians indicated by horizontal lines and connected by broken or solid lines) in **a** patients with aneuploid and **b** patients with near diploid tumours.*

levels. Thirty-eight of them belonged to the AN group, in whom there have been 15 recurrences. One of the patients was unfortunately not followed up by regular measurements although she had a Dukes' C tumour with pre-operatively elevated CEA level. In the remaining 14 patients, RIA showed CEA elevation for 11 prior to overt clinical recurrence and EIA for 10 (Fig. 2). The median lead time was 6 months (range, 0–24 months) with the former and 7 months (range, 0–26) with the latter assay.

In the 26 patients with ND tumours who had been operated for cure eight recurrences have appeared. With RIA only one patient showed CEA elevation prior to clinically overt recurrence, whereas such elevation was seen in four cases with EIA (Fig. 2). The lead time was 2 months with the former test whereas the latter afforded a median of $1\frac{1}{2}$ months (range, 0–4 months). In the ND group, three of four cases showing recurrence-associated CEA elevation revealed by EIA also had pre-operatively elevated values with this assay (Fig. 2); the corresponding figures in the AN group were five out of 10 (Fig. 2).

Diagnostic sensitivity of the CEA assays

The diagnostic sensitivity of RIA tended to be slightly better than that of EIA in the AN tumour patients, whereas the opposite was true in the ND group (Table 2). In the tumour group as a whole the sensitivity was quite similar, the EIA assay tending to be slightly better for the detection of recurrences (Fig. 2, Table 2).

Table 2. Sensitivity of the two CEA assays in patients with different DNA ploidy pattern and different clinico-pathological stages and cases of recurrence

		Dukes's stage A	B	C	D	Total	Recurrent cases
AN	RIA	31	61	60	92	57	79
	EIA	31	43	47	92	51	71
ND	RIA	25	43	25	60	35	13
	EIA	25	50	25	60	38	63
Total	RIA	29	55	44	82	49	55
	EIA	29	52	36	82	46	64

DISCUSSION

Recent multicentre studies have confirmed the usefulness of serial plasma CEA measurements in the follow-up period after surgical treatment for large bowel carcinoma [11]. However, although only approx. 4% of all large bowel carcinomas lack CEA [12], the problem of 'false-negative' plasma CEA results has hampered the clinical applicability of the test [13]. Different explanations for this phenomenon have been suggested [1, 2, 14, 15].

By using a commercially available CEA–RIA kit (Roche), we have previously shown that CEA

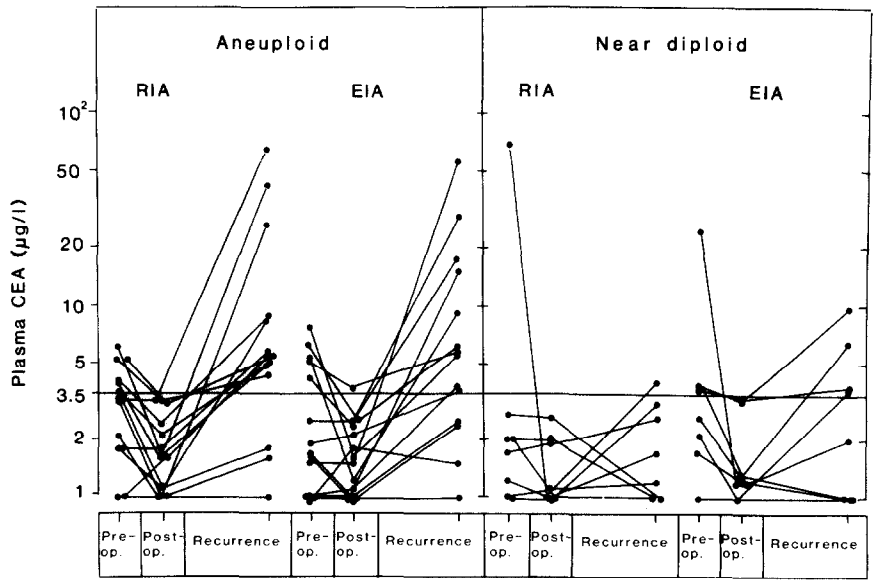

Fig. 2. Plasma CEA profiles obtained by RIA or EIA in 22 patients (14 with aneuploid and 8 with near diploid tumours) with normal post-operative plasma CEA levels who had recurrence after being operated for cure. In the aneuploid group 11 recurrences were preceded by a rise in CEA–RIA values and 10 in CEA–EIA values; the corresponding figures for the ND tumours were one and four, resp.

elevation is a sensitive indicator for recurrence in large bowel carcinoma patients with distinctly ancuploid (AN) tumours, whereas the test cannot predict recurrence in near diploid (ND) cases [2]. In the present study we found that a difference in CEA output between AN and ND tumours was also apparent with a CEA–EIA based on monoclonal antibody (Roche), although not quite as distinct as that revealed with RIA (Fig. 2, Table 2). Approximately half of the recurrences in the ND tumour group were preceded by elevated CEA–EIA levels, compared with one out of eight elevated CEA–RIA levels. However, it is important to recognize that the time between CEA elevation and clinically overt recurrence (lead time) is very short in the ND group, indicating that these tumours seem to have a low CEA output regardless of the CEA assay used.

By combining the information obtained from clinico-pathological staging, the DNA ploidy pattern of the tumour, and the pre-operative plasma CEA level it should be possible to select those patients who may benefit from being monitored by either RIA or EIA for 'second look' surgery. For clinical use we suggest the following guidelines:

1. Pre-operative plasma CEA level should be determined in all patients with large bowel carcinoma.
2. Serial CEA measurement should be performed in all patients operated for cure (i.e. Dukes' stage A, B and C) who show an AN tumour ploidy pattern and also in all those with pre-operative CEA elevation, regardless of ploidy.

By applying these guidelines, it is possible to predict 59% of the recurrences about 4 months before overt clinical presentation when using the Roche CEA–EIA assay; the corresponding figures with the Roche RIA-kit are 55% and $3\frac{1}{2}$ months.

Taking into account that the EIA and RIA measurements were performed in stored and unstored plasma samples, respectively, we feel that the two assays may be considered similar from a clinical point of view. However, the apparent difference between the two assays in relation to tumour DNA ploidy suggests that a distinct relationship between an AN pattern and CEA output may not be equally evident with every CEA assay. Especially for kits based on monoclonal antibody it seems important to be aware of this problem, since such reagents detect different epitopes on the CEA molecules.

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