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# CA-50 as a Tumour Marker for Monitoring Colorectal Cancer: Antigen Rises in Patients Postoperatively Precede Clinical Manifestations of Recurrence

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**Abstract**—*Using a monoclonal antibody-based radioimmunoassay inhibition method we have determined preoperative serum levels of the carcinoma-associated carbohydrate antigen CA-50 in 266 patients with primary colorectal cancer. CA-50 levels exceeding the mean value for blood donor sera by more than 2 standard deviations (≥ 17 U/ml) were found in 47% of these patients, with 15%, 43% and 31% being elevated in patients with Dukes' A, Dukes' B and Dukes' C cancer, respectively, and 63% and 66% being elevated in patients with more advanced localized or disseminated cancer. Only 5% of patients with benign colorectal disease had elevated CA-50 level and these were patients with ulcerative colitis of a duration of more than 10 years. Among patients who had developed a recurrence after operation for a primary Dukes' A–C colorectal cancer 66% had elevated levels, and 25% of resected patients with no clinical evidence of disease at corresponding times after operation also had CA-50 levels above the normal concentrations. From 139 patients operated for a Dukes' A–C colorectal cancer a definitive rise in CA-50 levels from the pre- to a 6–9 month postoperative sample was demonstrated in 12 cases in the absence of any clinical evidence for a recurrence. On prolonged follow-up a clinically manifest recurrence later developed in all of these cases with lead times of CA-50 titre rises ranging from 5 to 40 months. Our findings suggest that a rise in CA-50 levels after resection of a Dukes' A–C primary colorectal cancer is indicative of a recurrence and may precede any clinical evidence of disease by many months or years. Thus CA-50 may be a clinically useful tool for monitoring of patients with colorectal cancer.*

## INTRODUCTION

IN RECENT years, interest in demonstrating tumour-associated antigens has increased markedly with the aim of utilizing these antigens as markers to assist in the diagnosis, prognosis and monitoring of cancer patients [1]. Carcinoembryonic antigen (CEA), the first described colorectal tumour marker antigen [2], has been found to be clinically useful in the follow-up of cancer patients after operation although it is not specific enough to assist in the primary diagnosis of cancer [3]. By making use of the hybridoma technique for producing monoclonal antibodies [4], several groups have recently been able to raise new tumour-specific antibodies against colorectal adenocarcinomas [5–8]. These monoclonal antibodies define different tumour-associated carbohydrate antigens [7–11]. The cell surface carbohydrates, both in glycolipids and in glycoproteins, change appreciably in relation to oncogenic transformation, and this may give rise to novel glycolipids and glycoproteins that might be recognized by the immune system as tumour-associated antigens [12].

CA-50 is one of these new monoclonal-antibody-defined carcinoma-associated carbohydrate antigens, which may occur both as a ganglioside (glycolipid) and as a glycoprotein [10–14]. The CA-50 antigen is present in a high proportion of malignant epithelial tumours including colorectal adenocarcinomas [13]. Shedding of CA-50 antigen into serum has been demonstrated and a simple radioimmunoassay (RIA) inhibition method for the determination of circulating CA-50 has been described [13]. Patients with colorectal adenocarci-

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noma or some other carcinomas often have elevated serum levels of CA-50 compared with healthy controls and benign diseases [13–18]. However, the majority of colorectal cancer patients examined in these studies had advanced, often disseminated, cancer disease and it is known for other tumour markers in serum, e.g. CEA, that they usually reflect the extent and spread of neoplasm [1]. In the present study we have examined the serum level of CA-50 in patients with colorectal carcinoma in different stages of disease, as well as in patients with known recurrences. We have also analysed whether an increase in the CA-50 level of resected colorectal cancer patients during follow-up may predict a recurrence before it is clinically evident.

## SUBJECTS AND METHODS

Two hundred and sixty-six patients with the diagnosis of primary colorectal cancer were studied. Of these patients 139 had a localized tumour in stage A–C according to Dukes' classification system [21], and were subjected to a 'curative' surgical resection of the tumour: 34 patients in stage A, 56 patients in stage B and 49 patients in stage C. The remaining 127 patients had, at the time of diagnosis, a locally advanced disease without known metastases (localized Dukes' D, 56 patients) or disseminated disease (metastatic Dukes' D, 71 patients). A preoperative serum sample was taken and examined from all 266 patients. From the 139 patients operated upon for cure an additional serum sample was taken 6–9 months postoperatively.

From two other groups of patients with colorectal adenocarcinoma a single postoperative serum sample taken 6–36 months after surgical resection for a primary Dukes' A–C colorectal cancer was available for examination, but no preoperative serum sample. One group consisted of 73 patients with a clinically evident local or metastatic recurrence at the time for the serum sampling. The other group comprised 68 patients, who at the time for the follow-up serum sampling had no evidence of disease (NED).

Sera from 100 blood donors and from 74 patients with benign colorectal diseases (ulcerative colitis) were also examined. All sera were stored in aliquots at −80°C until used.

A radioimmunoassay (RIA) inhibition test [13] was used for determination of CA-50 (CanAg CA-50 RIA Inhibition Test; Stena Diagnostics Ltd, Göteborg, Sweden). This test is based on the ability of blood serum containing the CA-50 antigen to inhibit the monoclonal antibody C-50 from binding to plastic-adsorbed purifed CA-50 ganglioside antigen; a $^{125}I$ antimouse immunoglobulin preparation is then used to measure the amount of bound, uninhibited C-50 monoclonal antibody. Each specimen was assayed in duplicate as described by the manufacturer [18], and the serum levels of CA-50 antigen were calculated by comparisons with 'standards' with known CA-50 concentrations.

## RESULTS

### *CA-50 antigen in different stages of primary colorectal cancer disease*

Determination of the CA-50 levels in serum samples from 100 presumed healthy individuals (blood donors) with a CA-50 RIA inhibition test gave a mean value of 7.7 U/ml with a standard deviation of 4.4 U/ml; thus the mean value +2 standard deviations for these blood donors was 16.5 U/ml. Based on similar determinations on blood donor sera a level of 17 U/ml has been suggested as a cut-off level between normal and elevated CA-50 levels [18].

In the 266 patients with colorectal carcinoma examined at the time of the initial diagnosis, elevated CA-50 levels were found in 47% (126 patients) with the RIA. Figure 1 shows that the CA-50 levels were clearly related to the stage of disease. Elevated CA-50 levels were more common in patients with more advanced disease (Dukes' stages C and D), than in those with early stage cancer (Dukes' stages A and B) ($P < 0.01$ by chi-square analysis). Furthermore, the CA-50 positive patients with advanced cancer also more often had markedly elevated levels compared to CA-50 positive cases with less advanced cancer (Fig. 1).

The relationship between the degree of CA-50 'positivity' and disease stage is summarized in Table 1. The table identifies the proportion of patients in different Dukes' stages with CA-50 serum levels elevated above the mean level for blood donors by 2, 3 or 4 standard deviations. The table also shows that very few patients (5% for RIA) with benign colorectal disease had CA-50 levels elevated by 2 standard deviations above the mean level for blood donors, i.e. $\geq$ 17 U/ml. These patients all had ulcerative colitis with more than 10 years duration of their disease [20] (Table 1).

### *CA-50 levels in postoperative monitoring*

In the group of patients from whom a serum sample was taken 6–36 months after curative resection, 62% of the patients with a known recurrence had serum levels of CA-50 exceeding 17 U/ml. In the group with no clinical evidence of a recurrence (NED) 25% had elevated serum levels of CA-50 (Fig. 2).

Among the 139 patients from which we had available both a preoperative serum sample and a serum sample taken 6–9 months after surgical resection of the primary tumour, the large majority had no significant change or a decrease in the CA-50 level from the preoperative to the postoperative

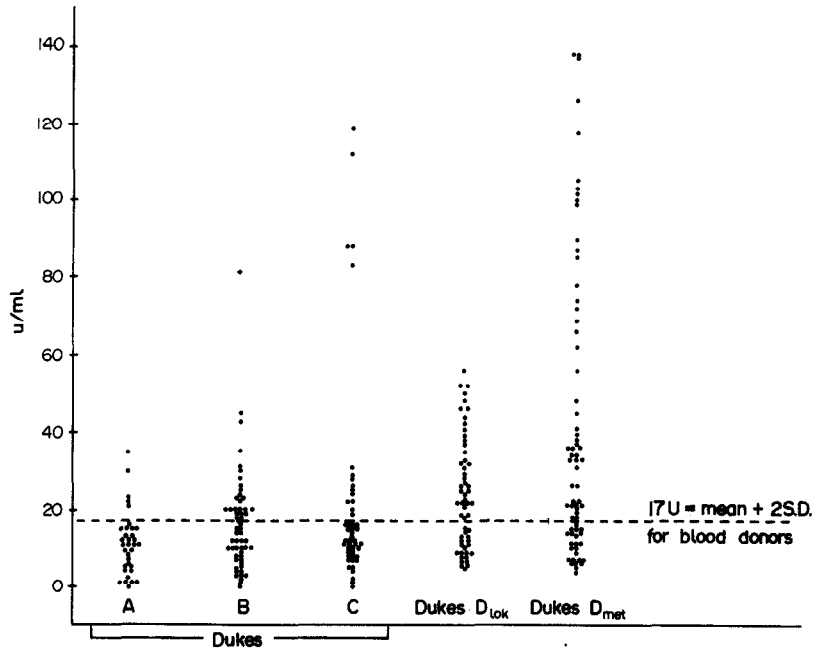


*Fig. 1. Pretreatment levels of CA-50 in 266 patients with primary colorectal carcinoma in different Dukes' classes with percentages in each class having elevated CA-50 levels (≥ 17 U/ml).*

*Table 1. Proportion of patients with colorectal carcinoma in different stages or benign colorectal disease with CA-50 serum levels elevated by 2, 3 or 4 standard deviations above the mean value for healthy blood donors*

| Patient category | Number examined | % with CA-50 levels exceeding the mean normal level by >2 S.D. (≥ 17 U/ml) | ≥ 3 S.D. (≥ 21 U/ml) | ≥ 4 S.D. (≥ 25 U/ml) |
|---|---|---|---|---|
| *RIA* | | | | |
| Colorectal cancer | | | | |
| Dukes' A | 34 | 15 | 12 | 6 |
| Dukes' B | 56 | 43 | 23 | 14 |
| Dukes' C | 49 | 31 | 27 | 18 |
| Localized Dukes' D | 56 | 63 | 55 | 41 |
| Metastatic Dukes' D | 71 | 66 | 55 | 52 |
| Benign disease | 74 | 5 | 1 | 0 |
| Blood donors | 100 | 3 | 0 | 0 |

sample, but in 12 patients there was a clear-cut rise in CA-50 exceeding 15 U/ml, and in nine of these cases the postoperative CA-50 level thereby rose to > 21 U/ml, i.e. clearly elevated (Fig. 3). None of the 12 patients who manifested a postoperative rise in serum CA-50 levels had any clinical evidence of recurrence at the time for the serum sampling. When these patients were followed-up for longer periods of time, a recurrence has later been observed in all cases. The 'lead time' between the serum sample showing elevated and/or a rise in CA-50 levels and the clinically identified recurrence ranged between 5 and 40 months (Fig. 3).

## DISCUSSION

Three of the currently most interesting tumour marker antigens for clinical use in colorectal and other gastrointestinal cancers are CEA, CA 19-9 and CA-50 [1]. CEA is the best established of these tumour marker antigens and has proved to be clinically useful in the monitoring of known cancer patients after operation even though it has limitations in both sensitivity and specificity [3]. The two newer, monoclonal-antibody-defined tumour markers CA 19-9 and CA-50 have greater tumour specificity than CEA, being elevated only rarely in healthy individuals including smokers or in patients

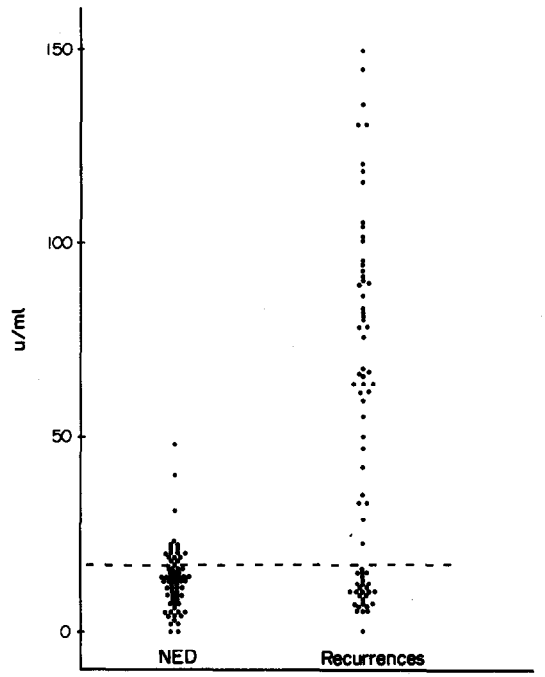


*Fig. 2. Comparison of CA-50 levels in patients with manifest recurrent disease and in patients who on follow-up 6–36 months after colorectal surgery had no clinical evidence of disease (NED).*

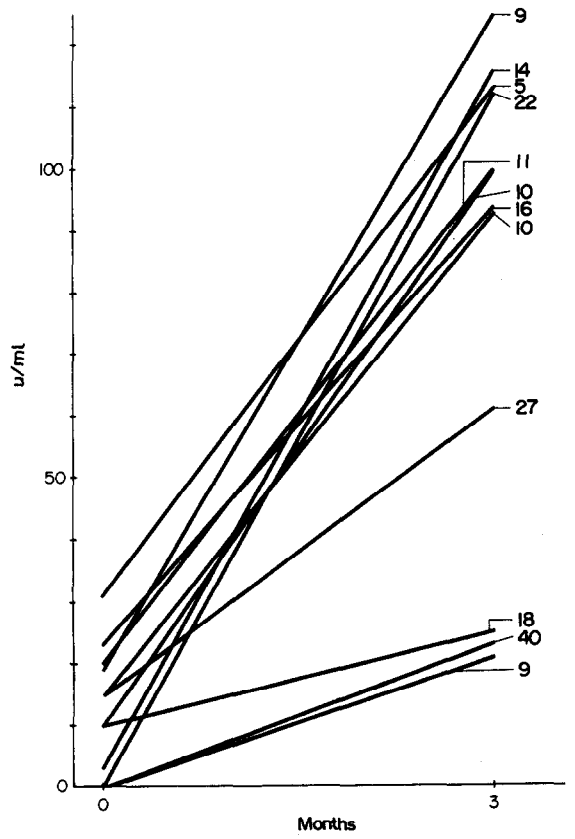


*Fig. 3. Results describing the 12 patients with an elevation of serum level 3 months after operation exceeding ≥ 15 U/ml. The figures at the end of each line tells the lead-time in months until clinical evident tumours has been observed.*

with non-malignant diseases [1, 14–17, 21]. Furthermore, since elevated CA 19–9 or CA-50 levels may occur independently of changes in the CEA level a combination of CEA with CA-50 or 19-9 may increase the sensitivity as well as specificity in cancer monitoring. Thus, Bruhn *et al.* [14] described that in patients with colorectal carcinomas (the majority in advanced stages) CEA was positive in 58%, CA-50 in 62% and the combination of CEA and CA-50 in 81%, and similar findings have been reported by others ([16, 17], Ståhle *et al.*, unpublished).

In this study 47% of all examined cases with manifest primary colorectal cancer had increased serum levels of CA-50 antigen as compared with 5% of patients with benign colorectal disease (ulcerative colitis) and 3% of healthy blood donors using a cut-off level of 17 U/ml, corresponding to the mean value plus 2 standard deviations for Swedish blood donors to discriminate between positive and negative samples. As for other tumour markers, e.g. CEA or 19-9, the proportion of patients with elevated serum levels of CA-50 was lowest in the groups with mucosal carcinomas (Dukes' A) and highest in those with advanced local or metastatic disease (Dukes' D). The few ulcerative colitis patients with CA-50 levels exceeding 17 U/ml all had had their disease for a very long time, and none of the patients with an ulcerative colitis of less than 10 years duration had increased levels [20]. Our findings are in good agreement with the recent results of others. Habib *et al.* [17] described elevated CA-50 levels in 22% of Dukes' A, 29% of Dukes' B, 59% of Dukes' C and 73% of Dukes' D colorectal cancer patients, and did not find elevated serum levels of CA-50 in any of 16 examined patients with various benign colorectal diseases.

These sensitivity and specificity rates for CA-50 may be compared to those reported for 19-9 and CEA. Del Villano *et al.* [21] found 7.4%, 17%, 47% and 58% with elevated levels of CA 19-9 in patients with Dukes' A, B, C and D colorectal cancer disease, respectively, and 3% with elevated levels among patients with benign gastrointestinal diseases. Ritts *et al.* [25], who examined sera obtained from the NCI/Mayo Clinic Serum Bank, found elevated CA 19-9 levels in 0%, 8%, 6% and 29% and elevated CEA levels in 0%, 18%, 14% and 65% of patients with Dukes' A, B, C and D colorectal cancer, respectively, and 100% specificity for CA 19-9 and 92% specificity for CEA at the chosen cut-off levels. The better sensitivity of CA-50 as compared to CA 19-9 may partly be explained by the different cut-off levels used to discriminate between 'normal' and elevated levels for the two tumour markers and it is possible that with many more patients with benign disease the clinically useful reference level for CA-50 may slightly increase. However, the highest sensitivity of CA-50 as compared to CA 19-9 might also reflect the fact that the C-50 monoclonal anti-

body used to identify CA-50 has a broader carbohydrate specificity than the 19-9 monoclonal antibody by reacting both with the carbohydrate chain of sialylated Lewis A blood group glycolipid and with 3′-iso-LM1 which is the corresponding compound lacking fucose [11, 22]. Our recent studies indicate that sera from patients with colorectal cancer may contain glycoprotein expressing both of these epitopes (Holmgren J, unpublished data). Through its ability to react with 3′-iso-LM1, C-50, in contrast to 19-9, may also react with antigen forms present in Lewis blood group negative individuals [23, 24]. However, various demographic factors such as age, sex and genetic make-up of the population tested as well as therapeutic modalities might also influence the assignment of reference values to discriminate elevated from non-elevated samples. Assignment of a particular reference value therefore requires consideration of the clinical context in which an assay will be used.

A main clinical indication for the use of serological tumour marker assays is for monitoring of cancer patients with the purpose to detect recurrences. The proportion (66%) with elevated CA-50 levels among patients with a known recurrence of colorectal carcinoma was similar to that (65%) observed in patients with primary advanced colorectal disease (Dukes' D localized or metastatic cancer). More noteworthy, however, was the fact that as many as 25% of patients with no clinical evidence of disease (NED) at the time for serum sampling 6–36 months after operation for a primary Dukes' A–C coloretal cancer also had slightly elevated CA-50 levels. Habib *et al.* [17] recently described similar findings with 78% CA-50 positive cases among known recurrences and 35% positivity in NED follow-up patients. In these particular groups of patients we could not determine whether the elevated levels in the NED patients represented falsely positive values or whether they indeed reflected true although not yet clinically evident recurrences. The development of a clinically evident recurrence with a lead time of 5–40 months in all 12 cases with CA-50 titre rises exceeding 15 U/ml from the preoperative to the postoperative serum sample, suggests that a clear-cut rise in the serum concentration of CA-50 during the course of follow-up of patients after operation of a primary colorectal cancer is highly predictive of a recurrence. The observation also shows that such rises in CA-50 antigen level may preceed the clinical manifestation of recurrences by several months to years at least as long as non-operative criteria for NED are being used. Even though our study was not truly prospective in that the sera analysed had been stored for up to several years, the design of the study was prospective with regard to serum sampling, analyses and follow-up and patients with postoperative CA-50 rises were identified before their subsequent clinical course was revealed. Our results suggest that CA-50 may be a useful tool for monitorng patients with colorectal cancer after resection. Since rises in CA-50 antigen levels may occur independently of changes in CEA levels and also have a greater cancer specificity the concomitant use of both markers may provide important lead time over current clinical methods to begin a new round of diagnostic and therapeutic procedures. With improvements in the treatment of early recurrences, e.g. surgery on solitary hepatic or pulmonary metastases, and with new more effective treatment modalities based on chemotherapy, the clinical usefulness of monitoring patients operated upon with a colorectal cancer by tumour markers will probably further increase.

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