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Original Paper

Plasma Sialic Acid as a Marker of the Effect of the Treatment on Metastatic Colorectal Cancer

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The concentration of total sialic acid (TSA) is increased in the plasma of patients with many types of cancer. The purpose of this study was to assess the usefulness of the TSA marker in predicting the efficacy of the treatment, and to compare TSA with two common markers, carcinoembryonic antigen (CEA) and the carbohydrate antigen 19–9 (CA 19–9). The study was performed on 44 patients treated for advanced colorectal carcinoma by a weekly 8 h continuous infusion of 5-fluorouracil (1300 mg/m^2) plus bolus injection of L-folinic acid (100 mg/m^2). TSA, CEA and CA 19-9 levels were measured before and after 3 months of treatment and their variations analysed as a function of the response to the treatment. TSA levels of patients with metastatic colorectal carcinoma before treatment (959 ± 265 mg/l) were significantly higher than those of 32 healthy people (584 ± 99 mg/l). The percentage of patients with TSA concentration above the cut-off level (782 mg/l) was 73% before treatment and 23% after. All patients who experienced an objective response to the treatment (complete, partial or minor response) ($n = 29$) had a significant decrease of TSA levels ($t = 5.96$; $P < 0.001$). When the disease was considered as stabilised ($n = 10$), TSA changed slightly, but it increased with progressive disease (4 out of 5 patients). Changes in CEA and CA 19–9 did not correlate as well as TSA to the treatment efficacy. Initial levels of TSA did not permit prediction of the efficacy of the treatment since they were not significantly different between the five response groups. TSA seems to be more likely involved in tumour changes than in tumour volume. Its determination could provide useful information about the spreading and metastatic properties of the tumour. TSA normalisation is an indicator of probable tumour growth arrest and its elevation could be a marker of relapse. © **1997 Elsevier Science Ltd.**

Key words: total sialic acid, carcinoembryonic antigen, metastatic colorectal carcinoma, carbohydrate antigen 19–9, tumour marker
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INTRODUCTION

BIOCHEMICAL TUMOUR markers are non-invasive means most often used for the diagnosis of malignant diseases and relapses than for the evaluation of the efficiency of their treatment. The carcinoembryonic antigen (CEA) and the carbohydrate antigen 19–9 (CA 19–9) are the most often used tumour markers in advanced colorectal cancer, although their usefulness is limited because of their relatively poor sensitivity [1–3]. Previous studies have shown the potential of the measurement of glycoprotein markers, particularly of serum total sialic acid (TSA), for the diagnosis of different types of cancers [4–6]. The purpose of this study was to assess, compared with CEA and CA 19-9, the value of TSA measurement for evaluating the response to treatment of patients with metastatic colorectal cancer.

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PATIENTS AND METHODS

Inclusion criteria

Patients with measurable metastasis of an adenocarcinoma of the colon or of the rectum pathologically confirmed, with or without local recurrence, were included in this study. The

disease had to be measurable in two dimensions by a computed tomographic (CT) scan or an ultrasound. Patients had a performance status of 2 or less, according to the World Health Organisation classification and an adequate haematopoietic function.

Patients with any prior chemotherapy, cerebral metastasis, history of any other malignancy, or aged more than 70 years old were not included in the study. Previous pelvis radiotherapy of the tumour bed or adjuvant chemotherapy were allowed if they had been completed more than 6 months before the diagnosis of the metastatic recurrence. The trial has been submitted to the Regional Ethical Committee before being carried out. Informed consent was obtained from all patients.

Treatment

All patients had long-term venous access established either by means of a catheter or an implantable disc device. 1300 mg/m^2 of 5-fluorouracil (5-FU) diluted in 1 litre of 0.9% normal saline were administered by an 8 h continuous infusion with a battery-operated pump [7]. 200 mg/m^2 intravenous (i.v) bolus L-folinic acid was administered just before and at the fourth hour of 5-FU infusion (H0–H4), up to a weekly total dose of 400 mg/m^2.

Follow-up

The patients had a weekly physical examination. Treatment efficacy was evaluated by comparing tumour measurements before and after 12 weekly cycles or 3 months treatment.

Response criteria

The patients' response to treatment was evaluated at the end of the third month; UICC standard response criteria was used. The number of lesions was noted and all lesions were measured. The largest lesion was considered as an indicator lesion. A complete response (CR) required the disappearance of every lesion. The partial response required at least a 50% reduction in the cross-sectional area of an indicator lesion. Minor responses (MR) were characterised by a reduction of less than 50% but more than 25%. Stabilisation (ST) required modification of less than 25%. For progression (P), any lesion had to increase by more than 25% in the cross-sectional area or a new lesion had to appear. All radiographic documentation was reviewed by the coordination board.

Determination of markers

Blood samples were obtained before initiation of the first chemotherapy session and after 3 months of treatment. Samples were centrifuged within an hour of collection and plasma was stored at −80°C until analysis. TSA was measured using a kit from Boehringer-Mannheim (Meylan, France) based on a sequence of enzymatic reactions leading to the formation of a red dye, whose absorbency was measured at 550 nm with a Cobas Mira® analyser. CEA and CA 19–9 plasma levels were determined by ELSA2-CEA and ELSA-CA 19–9 immunoradiometric assay (Cis-Bio international, Gif-sur-Yvette, France) and radioactivity counted using a 4000 Multi-Well Gamma Counter® (Kontrom analytic).

The normal range of TSA levels was obtained from a control group which consisted of 32 apparently healthy people (16 males and 16 females), mean age 55.9 ± 4.9 years (range 49–71). Values of CEA and CA 19–9 lower than 5 ng/ml and 30 U/ml, respectively, were considered as normal according to literature data [4, 6, 8].

Statistical analysis

TSA sensitivity was calculated as the percentage of patients having a metastatic colorectal carcinoma with TSA values above the defined cut-off level. Student's *t*-test was used to compare the levels of TSA in patients before and after treatment and a Mann–Whitney test to compare TSA levels in healthy men and women.

RESULTS

Patients' characteristics

44 patients (25 males and 19 females) were included. Their mean age was 58.8 ± 9.2 years (range 29–70 years). 34 patients had been treated for colon cancer and 10 for rectal cancer. All had undergone surgery for their primary tumour and had metastases measurable on CT scan or echography. 29 patients had liver metastases, 7 patients had lung metastases, 4 patients had both, 4 patients had peritoneum carcinomatosis, with or without liver metastases. The mean delay between the surgery for the primary tumour and the metastases was 6 months (0–24 months). Of the 10 patients treated for rectal cancer, 3 had received previous pelvic radiotherapy before surgery at a total dose of 45 grays. Of the patients treated for colon cancer, 10 had received adjuvant chemotherapy for their primary tumour, Astler-Coller stage C1 and C2. 8 had received a year's treatment of 5-FU plus levamisole, according to Moertel's regimen [9] and 2 had received 6 months treatment with 5-FU plus L-folinic acid according to Machover's schedule [10]. The delay between prior radiotherapy or adjuvant chemotherapy and the diagnosis of the metastasis was always longer than 6 months. 39 patients fulfilled the conditions for the follow-up of the three tumour markers.

Tumour marker sensitivity

Figure 1 shows the TSA levels of 32 healthy people and 44 patients with metastatic colorectal carcinoma. The mean plasma level of TSA of normal subjects was 584 ± 99 mg/l with a range of 425–895 mg/l and a median value of 563 mg/l.

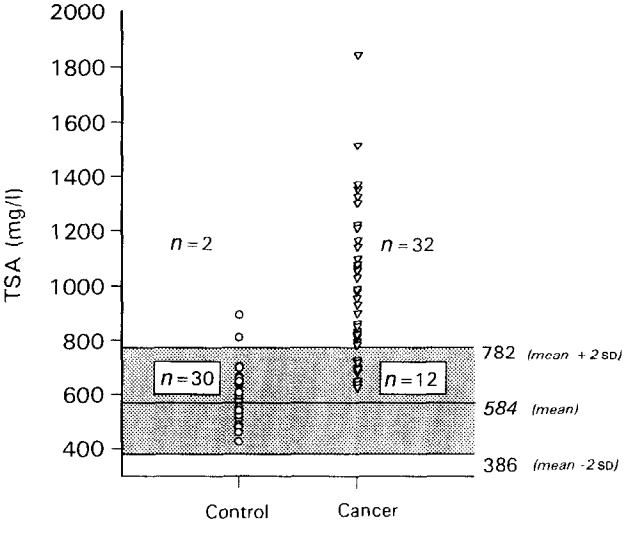

Figure 1. Total sialic acid levels in normal and cancer patients, before treatment.

Table 1. TSA, CEA and CA 19–9 levels in the plasma of colorectal metastatic cancer patients before and after treatment

Tumour markers	*n*	Before treatment: Mean S.D. range	Before treatment: Patients with levels above normal values*	After treatment: Mean S.D. range	After treatment: Patients with levels above normal values*
TSA (mg/l)	44	959.6 265.5 626.6–1842.2	32 (73%)	776.3 203.4 415.0–1610.3	10 (23%)
CEA (ng/ml)	41	86.2 192.1 0.5–1076.0	30 (73%)	35.9 77.3 0.6–420.0	29 (71%)
CA 19–9 (U/ml)	40	430.1 715.2 2.0–2600.0	27 (68%)	478.7 1889.3 1.0–11 600.0	24 (60%)

*≥ 782 mg/l for TSA, ≥ 5 ng/ml for CEA, ≥ 30 U/ml for CA 19–9.
n, number of patients.

The men had slightly higher values than the women (598 ± 118 versus 577 ± 70), but the difference was not statistically significant. A TSA range of normal values from 386 to 782 mg/l, corresponding to mean ± 2 S.D., was defined. This upper limit of TSA does not differ from 800 mg/l, the cut-off level described in previous studies [6, 11]. Only 6% ($n = 2$) of healthy people had TSA values higher than this cut-off level versus 73% ($n = 32$) of patients with metastatic colorectal cancer.

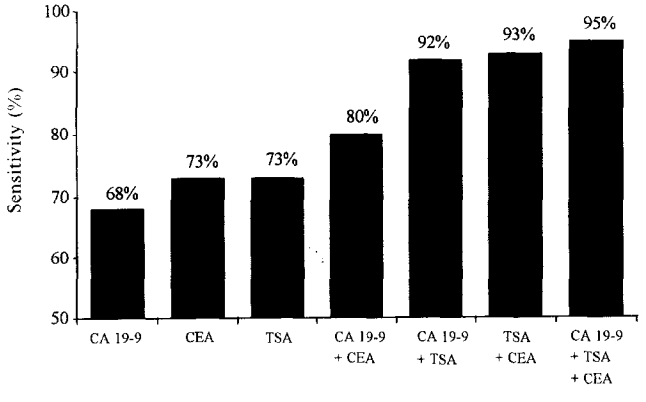

Figure 2. Sensitivity of tumour markers TSA, CEA and CA 19–9, alone or combined, before treatment.

Table 1 shows the levels of TSA, CEA and CA 19-9 and the percentage of patients with levels above the normal range before and after 3 months of treatment. The sensitivities of both CEA and CA 19–9 assay, 73% and 68%, respectively, are closely comparable to that of TSA assay (73%). However, since the same plasma can be positive for one marker and negative for another (Figure 2), sensitivity is increased by using two or three markers and by considering the rise of any one of them as a positive result. The combined assessment of TSA, CEA and CA 19–9 reached a 95% level of sensitivity, but the combination of two markers, TSA-CEA (93%) or TSA-CA 19-9 (92%), was nearly as sensitive as a combination of three. The sensitivity was not much improved by the combination of both CEA and CA 19–9 (80%) compared with either of these two tumour markers used alone.

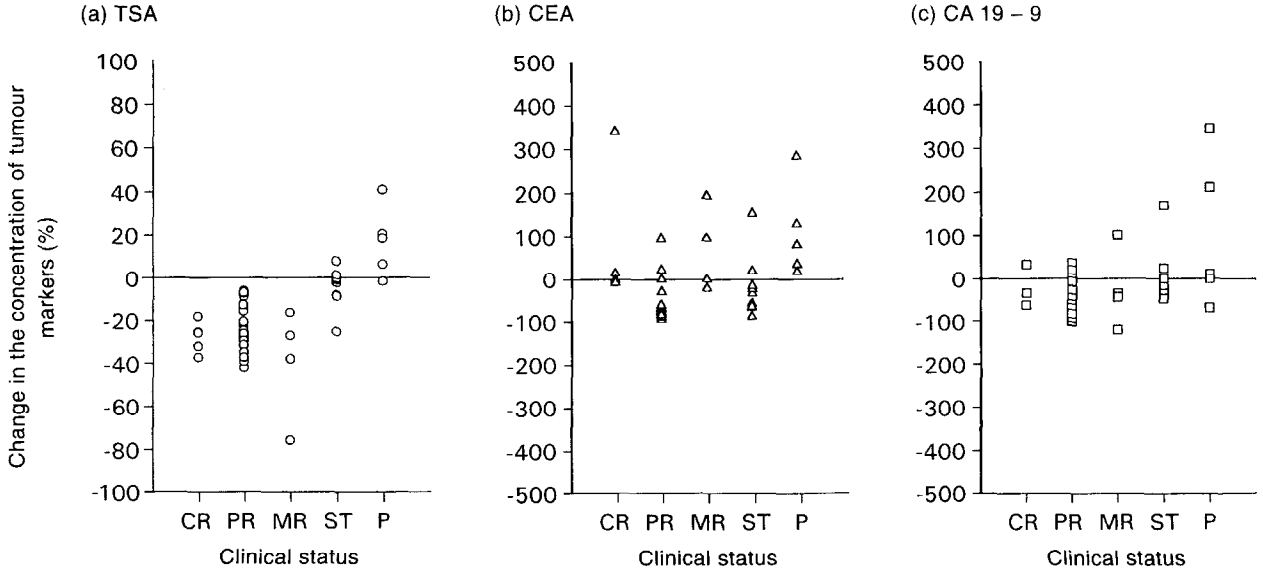

Figure 3. The relationship between clinical status and the change in tumour marker concentration after 3 months treatment (CR, complete response; PR, partial response; MR, minor response; ST, stabilisation; P, progression).

Tumour marker changes in response to therapy

The treatment efficiency was evaluated after 3 months: 5 complete responses, 20 partial responses, 4 minor responses, 10 stabilisations and 5 progressions were observed.

The percentage of patients with TSA levels above the cut-off value fell from 73% to 23%. Among the 29 patients with normal TSA values after 3 months treatment, 25 (86%) belonged to the group who experienced complete or partial response. The influence of the treatment on CEA and CA 19–9 was slight; the percentage of high values remained approximately the same before and after treatment, 73 versus 71% and 68 versus 60%.

Figure 3 shows the changes at 3 months in the plasma levels of the three tumour markers in the five response groups. Compared with the two other tumour markers, TSA was best related to the treatment effect: all patients who had a complete, a partial or a minor response had a significant decrease of their TSA levels ($t = 5.96$; $P < 0.001$), whereas patients considered as stabilised had slight changes and patients with cancer progression had, with one exception, an elevation in the TSA plasma concentration. The changes of CEA and CA 19–9 were not well correlated to the treatment effect since the plasma levels of some patients in objective remission increased.

Initial levels of TSA did not permit prediction of the efficiency of the treatment; indeed, patients who experienced a complete or partial response had initial TSA levels not statistically different from those of patients who had stable or progressive disease. We found no correlation between TSA changes and metastatic sites, but the population studied was not large enough for definitive conclusions.

There was no statistically significant correlation between TSA and CEA changes nor between TSA and CA 19-9 but a positive correlation ($r = 0.778$, $P < 0.05$) was observed between CEA and CA 19–9.

DISCUSSION

TSA includes a small amount of free sialic acid as well as glycoprotein and glycolipid-bound sialic acid. These oligosaccharide chains, with sialic acid on the N-terminal position, are present on the cell surface. Sialic acid induces an electronegative charge [12] because, as a relatively strong acid ($pK_a = 2.6$), it is completely ionised at physiological pH [13]. This ionisation plays a major role because the distribution of cell surface dense anionic sites is correlated, *in vitro*, with tumour cell aggregation [14]. The quantity of glycoconjugates on membranes of neoplastic cells is higher than on membranes of normal cells. This surface sialylation correlates positively with the metastatic potential of cultured murine tumour cell lines [15] and with the *in vivo* tumorigenicity of three human colon carcinoma sublines [16]. It has been hypothesised that tumour cells use their heavily sialylised surface as a mask to avoid recognition by the immune surveillance system and thus facilitate metastatic spreading [17].

The increase of plasma TSA concentration in patients with malignant diseases could be explained by an increased output of protein from the liver as a non-specific secondary reaction and by an intensified output of tumour cells with high contents of sialic acid. This latter explanation is supported by the fact that sialyltransferase levels increased simultaneously with those of sialic acid [17]. Berge and associates, who studied sialyltransferase in the serum of cancer patients, found a significant increase in its activity only when metastases were present [18]. Similarly, Verazin and associates [5] noted that TSA levels were elevated only in patients with metastatic colorectal cancer (Dukes' D stage) and not in patients without metastasis (Dukes' A, B or C).

Our results are in agreement with recent reports which have shown that high CEA, CA 19–9 or TSA values are found in patients with advanced stages (Dukes' D stage) of colorectal cancers [5, 19, 20].

The fact that 6% of apparently healthy people had TSA levels above the normal range, 782 mg/l in our study, could be explained by a lack of specificity. Indeed, high levels of TSA are observed not only in patients with different types of cancer [21–25], but also in patients with cardiovascular diseases [26, 27], rheumatoid arthritis and inflammatory reactions [28]. However, an increase of the plasma TSA level above 900 mg/l is rarely observed in patients with cardiovascular diseases, less than 3% [25, 26], whereas in the present study, it reached 54%. When a high level of TSA is observed, a malignant disease must be suspected.

The sensitivity of CEA observed in our study (73%) is comparable with the sensitivity (77%) reported in a study performed in patients with advanced colorectal cancer [29]. In the same way, the sensitivity of the CA 19–9 test (68%) is similar to that described by Kornek and associates (65%) [30] and the combination of CEA and CA 19–9 (80%) is in the range previously described [1, 3]. The TSA assay alone was no more sensitive than CEA and CA 19–9 assays, but it appears to be independent of them. Thus, its combination with either CEA or CA 19–9 assays improves the sensitivity up to more than 90%. This increase of sensitivity indicates that the factors influencing TSA plasma levels are not strictly the same as those involved in CEA or CA 19-9 variations. The absence of correlation observed in our study between TSA and CEA changes and between TSA and CA 19-9 changes confirms this fact.

Compared with those of CEA or CA 19–9, the changes in TSA levels in patients with metastatic colorectal cancer were well correlated with evolution of the disease. A similar observation has been previously observed by Stringou and associates [11] in patients with bladder, uterus, lung or breast cancer treated by radiotherapy. Silver and associates [21] described the case of one patient with a carcinoma of the ovary whose TSA level fell when she improved after doxorubicin treatment. However, a normalisation of TSA level does not mean that the tumour has disappeared. In our study, 12 patients who had normal TSA levels after treatment experienced partial or minor response. TSA seems better correlated to tumour growth or/and spreading than to the volume of the tumour.

TSA normalisation might indicate that tumour proliferation has stopped. Therefore, TSA could provide useful information on tumour response to treatment and determination of relapse, and merits further investigation.

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