Clinical Value of Serum Glycoprotein Galactosyltransferase Levels in Different Histological Types of Ovarian Carcinoma

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ABSTRACT

Serum glycoprotein galactosyltransferase levels were determined in 28 healthy women and 113 patients with ovarian carcinoma with various histological types, at different clinical stages. Ovomucoid, which possesses terminal N-acetylgalactosaminyl residues, was used as glycoprotein acceptor. Clinical correlations between galactosyltransferase levels and tumor burden were examined, as well as the variations due to histology. Follow-up studies could be done for 60 patients, and correlations with clinical evolution, established. Galactosyltransferase might be a promising marker for the diagnosis and follow-up of ovarian carcinomas.

INTRODUCTION

Important changes in cell surface glycoconjugates have been described in malignant diseases (13, 26, 28). Synthesis of the carbohydrate moieties of those molecules is achieved by glycosyltransferases of various specificities. In general, these are membrane-bound enzymes, but soluble forms have also been found in human physiological fluids (14, 15, 20).

Altered glycosyltransferase activities in malignant cells and tissues (5, 10, 30), as well as in sera of cancer patients (1, 9), have been reported. In earlier publications (16, 29), levels of serum GT* [UDP-galactose:N-acetylgalactosaminyl-glycoprotein GT (EC 2.4.1.38)] in some patients with various types of cancer were elevated compared to those of healthy controls, although in many cases such an increase was not observed (29); on the other hand, other pathologies, especially liver diseases, also led to elevated serum GT levels (16). However, a GT isoenzyme which might be tumor-specific was characterized (22, 23, 25).

Bhattacharya et al. (4) observed that total GT level was significantly elevated in the sera of ovarian cancer patients as compared to normal controls. The levels of the enzyme appeared to correlate well with tumor volume and clinical status of the patient, although discrepancies were observed in a few cases (6–8). Since these papers concerned only small numbers of patients, more data were still needed in order to assess the practical usefulness of this biological marker in ovarian carcinoma. Thus, serum GT levels were tested in 113 patients and 28 healthy women. Correlation between serum levels of this enzyme and clinical status was examined for each histological type. Furthermore, for 60 patients, serum GT levels were serially determined during therapy.

MATERIALS AND METHODS

Materials. UDP-[3H]galactose (13.1 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, England. Unlabeled UDP-galactose, ovomucoid, and sodium cacodylate were obtained from Sigma Chemical Co. Other chemicals were purchased from Merck.

Collection of Serum Samples. Blood was collected by venipuncture in 10-ml B-D Vacutainer tubes without anticoagulant. After centrifugation to remove blood cells, the serum samples were used immediately or stored frozen at −20° until assay. Control subjects were 28 female laboratory workers from our Cancer Institute. Pathological blood samples were drawn from 113 patients with ovarian carcinoma. Some of the patients were in complete clinical remission for 2 to 10 years. Most, however, were included in the study directly after initial surgery, on referral to our Cancer Institute for complementary treatment. Finally, a small number were new patients who had not yet received treatment prior to inclusion in the investigation. In our therapeutic protocol after initial surgery, Stage I patients [according to International Federation of Gynecology and Obstetrics staging (complete surgery for postmenopausal women, including omentectomy and lomboaortic node biopsies)] received no further treatment; Stage II patients received pelvic radiotherapy; and Stages III and IV patients received various monthly courses of chemotherapy. Whenever possible, “second-look” surgery was performed on Stages III and IV patients 6 to 8 months after the first operation. Tumor volume was also evaluated clinically with ultrasonic and radiological examinations when necessary. Choice of treatment was in no way influenced by the results of the serum GT assay, since these were obtained quite some time after the samples had been taken. Histological classification was according to WHO criteria. For 60 patients receiving regular chemotherapy, serial serum GT tests were performed, since blood samples could be taken just before drug infusions.

Assay of GT. Serum GT activity was assayed according to the method described by Podolsky and Weiser (23). However, ovomucoid was used instead of asialoalpha lactofetuin (SGF) as glycoprotein acceptor. Ovomucoid has N-acetylgalactosaminyl residues in a nonreductive terminal position (18). The assay mixture contained 10 μl of serum and 2 mg of ovomucoid in a final volume of 150 μl. Final concentrations of the other chemicals were: 26 mM sodium cacodylate, pH 7.2; 50 mM sodium chloride; 20 mM manganese chloride; and UDP-[3H]galactose, 0.075 mM, with a specific activity of 0.23 Ci/mmol.

An assay mixture without UDP-[3H]galactose was first preincubated at 37° for 30 min. The reaction was then started by adding the UDP-[3H]galactose solution. The reaction was stopped by adding 1 ml of 5% phosphotungstic acid in 2 N HCl. The precipitate was collected on a Whatman GF/A filter. The filter was washed several times with phosphotungstic acid and cold ethanol. Radioactivity precipitated on the filter was counted using 10 ml of Biofluor scintillator (New England Nuclear), with a counting efficiency of 35%. Radioactivity obtained by stopping

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4 The abbreviations used are: GT, galactosyltransferase; SGF, fetuin lacking terminal sialic acid and penultimate galactose residues.
5 Received May 27, 1982; accepted June 10, 1983.
the reaction at 0 min was subtracted from the total radioactivity. The results were expressed as units of GT per ml of serum, whereby one unit of GT is the amount of enzyme which transfers one nmol [3H]-galactose to the acceptor during a 60-min incubation period. Enzyme assays were carried out in duplicate on each serum sample; the mean of those values was used in “Results.” In our experimental conditions, a linear kinetic of incorporation was observed for at least 2 hr. UDP-galactose and acceptor saturation were effective. Virtually no incorporation of galactose occurred when the acceptor was deleted. Manganese ion was an absolute requirement, and an optimal Mn²⁺ concentration was used in the assay.5

RESULTS

GT Activities in Normal and Cancer Subjects

Values of serum GT activities from 28 normal control women ranged from 5.6 to 11.9 units/ml with a mean value at 9 ± 3 units/ml (mean ± 2 S.D.). Thus, a value of over 12 units/ml was considered as abnormal (Chart 1). One of our control subjects, a 23-year-old woman, had an elevated serum GT activity when first assayed; she subsequently received surgery for a benign serous ovarian cyst, and her serum GT returned to normal. Thirty-six of the 113 patients had abnormal serum GT levels at the beginning of the study (Chart 1). The highest values were observed in patients presenting large peritoneal masses, ascitic or pleural effusion, or hepatic metastases. For 4 patients, we observed a dramatic drop of the serum GT level after evacuation of the effusion: in these cases, GT levels in ascitic fluids were very high. On the other hand, almost all of the disease-free patients (49 of 54) had serum GT values within the normal range.

In order to correlate serum GT activities with the estimated tumor volume, patients were first grouped according to histology, and then divided further into 3 subgroups: untreated tumor-bearing patients (no surgery), treated tumor-bearing patients, and clinically or surgically proven disease-free patients. Results of this study are provided in Chart 2. All but 2 of the 15 untreated patients exhibited abnormal values. The values of treated tumor-bearing patients varied. Patients with serious adenocarcinoma frequently had abnormal values, although these were generally lower than those observed for the untreated patients with the same histology. Nine of the 10 patients with mucinous adenocarcinoma were within the normal range, in contrast to the high levels observed for the 3 untreated patients with the same histology. Seven patients were classified as having borderline tumors (3 serous type; 4 mucinous type); all but one had normal GT correlating with the absence of any residual tumor at the time of the assay. Eighteen patients had other histological types of ovarian malignant tumors. Among the 9 patients with germ cell tumors, only one was clinically exhibiting tumor; her GT activity was 18.8 units. The other 8 were clinically free of disease. Two of them had normal GT values; in one case, the assay had been done only 10 days after reductive surgery, and the GT level returned to normal in the subsequent assays. The 4 patients with undifferentiated histology had normal values, although one of them was still bearing tumor. Three of 4 patients with granulosa cell tumors were clinically free of disease; the other had metastasis to the liver, and her GT level was very high. A normal GT value was found for one patient bearing a mesonephroid tumor. Finally, for 5 patients, obvious ovarian malignant tumor was present, although histologies were unknown; 3 of them had high GT values.

Serial Determinations

Patients with Clinical Evidence of Disease (34 Cases). For 11 of the 13 new patients whose initial values had been abnormal, a striking drop of serum GT was observed in correlation with a significant clinical improvement due to the first courses of chemotherapy, as shown in Chart 3 (the other 2 patients died soon after the first treatment).

A good correlation between GT levels and clinical course was observed, during prolonged follow-up studies, for 18 of 34 patients. In 15 cases, the decrease of serum GT correlated with partial or complete clinical remission; when relapse occurred (10 of those patients), serum GT rose simultaneously. For the other 3 patients, high serum GT correlated with permanent clinical evidence of disease. For example, Chart 4 (Patient A. L.) shows a dramatic fall of serum GT after one course of chemotherapy, corresponding to the almost complete disappearance of 2 large pelvic masses. Six months later, there was no clinical evidence of tumor, although small disseminated peritoneal masses were found at second-look laparotomy. Chart 4 shows this same correlation between serum GT alterations and clinical course for 2 cases of papillary serous adenocarcinoma (Patients O. R. and M. I.).

A delayed response was observed for 5 of 34 patients with clinical evidence of disease: the rise in serum GT appeared only after clinical changes had occurred, and at a time when there were no longer any gross clinical changes. For instance, Patient T. L. (Chart 5), who was irradiated 1 year before inclusion in the study, exhibited stable serum GT values despite recurrence of disease; a rise in levels was only observed during the last 4 months prior to death. The same phenomenon was observed for

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GT in Ovarian Carcinoma

Chart 2. GT activity in the sera of cancer patients, according to clinical status and histology: ▲, same as in Chart 1; "other" histologies are: ▲, germ cell; ◆, borderline; △, undifferentiated; ◆, granulosa cell; ◆, mesonephroid; *, unknown. TBP, tumor-bearing patients; CR, complete clinical remissions; UT, untreated; T, under treatment.

Chart 3. Effect of the first chemotherapy courses on serum GT activity. 1, before any treatment; 2, 1 to 2.5 months after the first chemotherapy course. ▲, same as in Chart 1.

Patient D. R. (Chart 6) was in clinical remission from serous adenocarcinoma when she entered the study; this remission was confirmed by second-look laparotomy; however, when local relapse occurred, terminating in massive ascites, no modification of serum GT was observed. When Patient M. J. C. (Chart 6) entered the study, her serum GT level was normal, despite relapsing Stage III serous adenocarcinoma. Serum GT values continued to remain within normal range even after complete remission was obtained under chemotherapy. Patient S. L. (Chart 6), whose recurrent mucinous adenocarcinoma failed to respond to treatment, followed her entire terminal course without exhibiting any change in serum GT levels. Similarly, the Stage III endometrioid adenocarcinoma of Patient M. C. (Chart 6) followed a slow local development, while her serum GT remained slightly subnormal.

Patients in Complete Remission. Twenty-six patients had complete response to therapy and continued to be clinically free of disease during our study. Nineteen of them always had normal GT values. One of the remaining 7 patients, T. L., was in complete clinical remission under chemotherapy, with normal values of GT for 8 months after entering the study. Then she refused the second-look laparotomy and any further treatment. She came back to our institution 3 months later with severe renal insufficiency. At that time, serum GT was 30.7 units/ml. She succumbed to her renal disease shortly thereafter, without any clinical evidence of relapse. Only her autopsy revealed a small cancerous mediastinal node without any other tumor site. Patient G. M. (Chart 7) also suffered from renal insufficiency and hearing problems due to cisplatin toxicity, which improved slightly after cessation of this drug. Second-look surgery did not show any tumor, and she remains well, although her serum GT rose for 5 months, during which time there was no evidence of disease.
DISCUSSION

While there is a general need for tumor markers in the management of malignant disease, this is particularly true for ovarian carcinoma, since the clinical course of this disease is usually occult. Clinicians often have difficulty in assessing response to treatment, and especially in predicting early relapse. Although for other cancer types, in situ assays of tumor markers have been proven to be of great interest in clinical practice, generally, a tumor marker is most useful when present in biological fluids, and when it correlates with tumor burden, allowing a clinical monitoring of the disease.

First described by Bhattacharya et al. (4), serum GT might be just such a marker. We were able to confirm their results by following the serum GT levels of 113 patients with various types of ovarian carcinoma.

These authors used asialoagalactofetuin (SGF) as an acceptor. SGF bears both N-acetylgalcosaminyl- and N-acetylgalactosaminyl-terminal residues (2). Thus, when using SGF as acceptor, at least 2 different enzyme activities are determined simultaneously: UDP-galactose:N-acetylgalactosaminylglycoprotein-β-3-galactosyltransferase ("mucin-type" GT); and UDP-galactose:N-acetylgalactosaminylglycoprotein-β-4-galactosyltransferase. The former generally appears to be low in normal human serum (2, 15); it is present in various tissues; however, in human colon adenocarcinoma extracts (12) and in human lung adenocarcinoma homogenates (11), no significant increase of GT activities were found using mucin as acceptor, as compared with normal tissues. The other enzyme activity, which can be assayed with SGF, can be measured with ovomucoid as acceptor. Saccharidic chains of ovomucoid bear many terminal N-acetylglucosaminyl and no N-acetylgalactosaminyl residues (21) and need no previous desialylation and degalactosylation. In this respect, using ovomucoid as acceptor appears to be more defined, although assaying the other activities might also be clinically useful.

We found that serum GT showed lower Vₘₕ and Kₘ for ovomucoid as compared to SGF. Thus, normal values using ovomucoid and expressed as nmol of galactose transferred/ml/60 min are lower than those obtained by us and others using SGF. This might be due to differences in the structure of the N-glycosaminyl terminal residues.
Serum GT levels satisfactorily reflected the clinical status of the patient for 81 of 113 patients (72%) in the general study. Five false-positives and 27 false-negatives were observed. Prolonged follow-up studies (60 patients) showed, however, that the true clinical usefulness of total serum GT might be lower, since 37 patients would have been correctly classified with this assay (62%) to be compared with 7 false-positives and 16 false-negatives (38% misclassified). Five of these 16 patients had an increase in their serum GT later with disease evolution (delayed response). The increase of serum GT could have been predictive of relapse in 6 of 18 cases. Whether the false-positive patients are in occult evolution, although still in clinical remission, can only be demonstrated by continuing follow-up (becoming predictive rises). Although 14 of 23 patients with serous adenocarcinoma and only 3 of 9 patients with mucinous adenocarcinomas were correctly classified, the number of patients is too low to affirm that the observed difference is significant.

Other diseases might interfere with serum GT determination and explain some of the transient variations we observed in patients in clinical remission. For instance, renal insufficiency, inflammation, or hepatic dysfunction might influence the results either by increasing normal serum GT production by lowering its degradation or possibly reducing elimination, or by modifying the isoenzyme pattern. On the other hand, specific activators or inhibitors, or endogenous acceptors might appear in the serum during these pathologies (24). Thus, further studies are needed, and are in fact underway, to clarify the influence of those different parameters. Obviously, total serum GT activity does not systematically reflect the tumor burden of the patient.

Chatterjee et al. (6) also occasionally observed false-negatives in their studies. Other markers might prove useful in these false-negatives. For almost every tumor-bearing patient with mucinous adenocarcinoma, we observed an abnormal elevation of carcinoembryonic antigen which generally reflected the clinical evolution. In a parallel study, we measured the placenta-like alkaline phosphatase isoenzyme with the specific thermal denaturation technique described by Moss et al. (19). We observed a pathological elevation of this isoenzyme in a few tumor-bearing patients including some with normal GT values. However, a more

* These results will be published elsewhere.
accurate evaluation of placenta-like alkaline phosphatase isoenzyme as an ovarian tumor marker might be soon possible with the recently published more specific and sensitive immunonaoasays (17). Other potential markers have been described recently, such as ovarian isoamylase (27) and β-hexosaminidase (3).

Simultaneous investigations of these various markers, in close correlation with clinical course and histological type, will allow a satisfactory evaluation of each of them and hopefully lead to a choice combination of biochemical markers for the clinical management of ovarian carcinoma.

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