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The Specificity of Serum Uridine 5′–Phosphogalactose: Glycoprotein Galactosyl Transferase Activity in the Diagnosis of Ovarian Cancer

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Abstract—*Serum galactosyl transferase activity was measured in patients with ovarian cancer, non-ovarian cancer, inflammation and infection, miscellaneous control patients and a normal group. Patients with cancer had widely dispersed results, high and low results being recorded. The majority of patients with inflammation and infection had elevated results. Differences in methodology and selection of clinical material make it difficult to compare results from different groups of workers. We conclude that it is not possible at present to decide on the usefulness of serum galactosyl transferase measurements in the investigation of ovarian cancer.*

INTRODUCTION

THE ACTIVITIES of the serum glycosyl transferases are reported to be often raised in malignant disease [1–4]. Of particular interest in the investigation of ovarian cancer is the enzyme uridine 5′-phosphogalactose:glycoprotein galactosyl transferase (galactosyl transferase, GalT; EC 2.4.1.22) which has been proposed as a marker in the diagnosis and monitoring of this group of diseases [5]. Before a tumour-derived antigen or enzyme can be used in diagnosis it is necessary to obtain information regarding its specificity and sensitivity. To this end we have measured GalT in the serum of patients with cancer, patients with infection or inflammation, a miscellaneous control group and normal subjects.

MATERIALS AND METHODS

Clinical material

Normal subjects. Blood was taken from seven male and 12 female subjects with no known disease. The specimens were allowed to clot and the serum separated by centrifugation. Analysis from GalT was undertaken on the same day.

Patients with ovarian cancer. Blood was taken before or during operation from 18 patients who were subsequently diagnosed by surgical and histological criteria to have ovarian cancer. None of them were already undergoing treatment for ovarian cancer or were undergoing a 'second-look' laparotomy. The majority of blood samples were sent directly to the laboratory, although a few were kept at +4°C for up to 24 hr before being taken to the laboratory. Preliminary investigations confirmed the enzyme to be stable under these conditions. On receipt the blood samples were centrifuged and the serum stored at −20°C until analysis.

Non-ovarian cancers. Serum samples from patients with cancer of the breast, gastrointestinal tract, lungs, testes and lymphoid tissues were assayed for GalT. These samples had been submitted to the laboratory for routine analysis and selection was based on availability rather than defined clinical criteria. All patients had biochemical evidence of active disease.

Infection and inflammation. Six patients had respiratory disease, two had acute pancreatitis and one had thromophlebitis. One of the patients with respiratory disease also had septic arthritis.

Miscellaneous control group. Six subjects were under investigation for suspected myocardial infarction, two had undergone heart surgery, one patient had diabetes mellitus and one delirium tremens.

Analytical methods

Analysis. The method used was based on that described by Bhattacharya *et al.* [3]. a–(sialyl-

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galactosyl)fetuin was prepared by the sequential removal of sialic acid and galactose from fetuin [6, 7], freeze-dried in 20-mg aliquots and stored at −20°C. The complete buffered substrate mixture was prepared as follows: 2.0 ml of Tris–maleate buffer 100 mmol/l, pH 6.2, and containing 1% Triton X–100 were added to a vial of a–(sialylgalactosyl)fetuin; after the protein had dissolved, 0.56 ml distilled water, 0.40 ml manganese chloride (200 mmol/l), 0.40 ml ATP (100 mmol/1), 0.20 ml uridine–diphospho–D–galactose sodium (2 mmol/l) and 0.04 ml uridine diphospho-D-[$U^{14}C$] galactose (Amersham International plc, Amersham, U.K.) were added to the vial.

Buffered substrate (100 μl) was pipetted into conical polypropylene tubes and allowed to equilibrate to 25°C, 10 μl of test serum was added and the complete reaction mixture incubated for 4 hr. The reaction was stopped by adding 1.0 ml of phosphotungstic acid (10 g/l in 0.5 mmol/l hydrochloric acid). The precipitate was centrifuged, washed twice with the phosphotungstic acid and dissolved in 0.5 ml sodium hydroxide (10 g/1). The sodium hydroxide solution was transferred to a plastic liquid scintillation counting vial and 10 ml of liquid scintillator (NE 265, Nuclear Enterprises Ltd, Edinburgh, U.K.) added. The radioactivity contained in the redissolved precipitate was measured by liquid scintillation spectrophotometry and disintegrations per min (dpm) calculated by the external standard method of quench correction. Counting efficiency was about 65% and 100 μl of buffered substrate gave approx. 38,000 dpm. Enzyme activity was expressed as pmol/hr/10 μl galactose transferred at 25°C.

The time course of the reaction was linear to 8 hr and 6% substrate utilization. At 24 hr a 19% deviation from linearity associated with 16% consumption of substrate was observed. Between-batch precision, assessed by analysing ten thawed aliquots of a serum sample stored at −20°C, gave a mean value of 315.8 pmol/hr/10 μl, standard deviation 9.2.

Statistical methods

Statistical differences between groups of subjects were assessed by Student's *t*-test.

RESULTS

The mean GalT activity of the normal female group was 133.5 pmol/hr/10 μl, standard deviation (S.D.) 15.5, and of the male group 134.6 pmol/hr/10 μl, S.D. = 12.0. The control group had a mean activity of 142.9 pmol/hr/10 μl S.D. = 18.2. As the results from these three groups did not differ significantly from each other, their results were pooled, a mean of 137.0 pmol/hr/10 μl, S.D. = 15.8, obtained and a reference range of 105.4 – 168.6 pmol/hr/10 μl derived. The results for ovarian cancer, other cancers, infection and inflammatory groups were far more widely dispersed (Fig. 1). Seven of the ovarian cancer patients had results less than 105.4 pmol/hr/10 μl and three greater than 168.6 pmol/hr/10 μl, the lower and upper limits of the reference range. The majority (12/18) of the ovarian cancer patients had advanced (stage III or IV) disease and 4/18 had stage II disease. There was no statistically significant difference in enzyme activities between stages. Even in the group of patients with advanced disease low and elevated results occurred. Similar results were obtained with the non-ovarian cancer group; 15/26 subjects had low and 2/26 high activities. In contrast, the majority (6/9) of patients in the infection and inflammation group had raised activities.

DISCUSSION

A wide variety of serum tumour markers have been proposed in the investigation of ovarian cancer but few have gained widespread acceptance. Amongst those proposed is the enzyme UDP-galactose:glycoprotein galactosyl transferase (GalT), which is present in abnormally high amounts in ovarian cancer tissue extracts and in the serum of patients with ovarian cancer [4]. GalT is one of a family of enzymes involved in the synthesis of glycoproteins. Conventional methods of assay measured the transfer of a sugar from a donor (in the case of GalT, UDP-galactose) to a suitable receptor. We have used a–(sialylgalactosyl)fetuin, as suggested by Bhattacharya *et al.* [3], but other workers have used ovomucoid [5] or a receptor specific for the isoenzyme of GalT produced by malignant tissues [8]. Immunoassays which measure the mass of the enzyme have also been used [9].

The results which we have obtained do not support the use of this enzyme in the diagnosis of ovarian cancer. Rather than being raised, the majority of patients with ovarian cancer had normal or low activities. Most of the non-ovarian cancer group had a majority of patients with low GalT activities, although one patient with lymphoma and two patients with testicular teratoma had elevated results. The high proportion of raised GalT results in patients with infective or inflammatory conditions suggest that raised activities of this enzyme are not diagnostic of malignancy but are part of an inflammatory response.

Although several groups of workers have used this enzyme in the investigation of ovarian cancer, differences in methodology and selection of clinical material have made direct comparison difficult. Our method is very similar to that of Bhattacharya *et al.* [3], but we have used an incubation tempera-

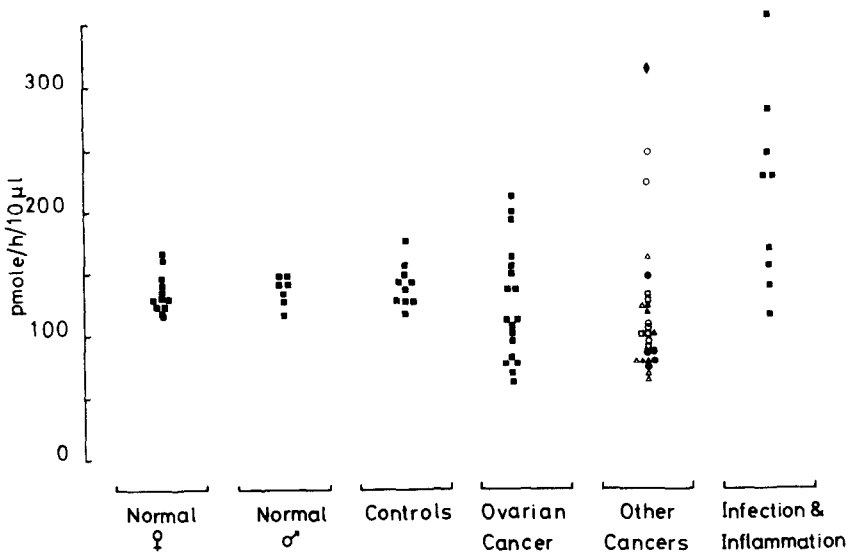

Fig. 1. Serum galactosyl transferase activities in various subjects. The patients with non-ovarian cancers were as follows: cancer of the lung (●), five patients; breast cancer (▲), five patients; cancer of the gastro-intestinal tract (□), five patients; lymphoma (◆), one patient; seminoma of the testis (△), five patients; teratoma of the testis (○), five patients.

ture of 25°C because of reports that the enzyme may be inactivated at high temperatures [10]. The marked differences between our results and those of Chatterjee *et al.* [4] may be due to the selection or nature of the clinical material.

Capel *et al.* [2] investigated several groups of patients, all of which had been treated for varying periods of time. GalT was found to be significantly raised in groups of patients with breast, ovarian, respiratory and gastrointestinal tract cancers. However, patients with breast cancer who eventually died demonstrated a *decline* in GalT activity with advancing disease.

Gauduchon *et al.* [5], who investigated a group of 113 patients at various stages in the progression of their disease, found that about half of their subjects classified as tumour-bearing had raised GalT activities; no low activities were reported. Verdon *et al.* [9] used an immunoassay based on antibodies raised against human milk galactosyl transferase and found raised concentrations of GalT in some patients with ovarian, breast and lung cancers.

The origins of GalT in normal and pathological sera have not yet been determined, although cancer-associated isoenzymes have been described [1, 11]. It may have been expected that the immunoassay of Verdon *et al.* [9] would be more specific for malignancy than conventional enzymatic methods, but GalT in normal human serum, ascitic fluid, a pleural effusion and human liver all exhibited behaviour with a standard curve.

No assays were performed on patients with inflammatory conditions and infections by Chatterjee *et al.* [4], Verdon *et al.* [9] or Gauduchon *et al.* [5].

Our observations on these patients raise the question as to whether the raised GalT activities found in patients with cancer are due to the response of the patient to the malignancy. Differences in methodology, heterogeneity of patients investigated and the possibility of several isoenzymes of GalT being simultaneously assayed make it impossible at present to decide on the usefulness of serum GalT measurements in the investigation of ovarian cancer.

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REFERENCES

1. Wieser MM, Podolsky DK, Isselbacher KJ. Cancer-associated iso-enzyme of serum galactosyl-transferase. *Proc Nat Acad Sci USA* 1976, **73**, 1319–1322.
2. Capel ID, Dorrell HM, Williams DC, Hanheim IWF, Lovitt HM. Serum galactosyl transferase levels in patients with advanced cancer. *Oncology* 1982, **39**, 193–196.

3. Bhattacharya M, Chatterjee SK, Barlow JJ. Uridine 5'diphosphate-galactose:glycoprotein galactosyl transferase activity in the ovarian cancer patients. *Cancer Res* 1976, **36**, 2096–2101.
4. Chatterjee SK, Bhattacharya M, Barlow JJ. Galactosyl-transferase and glycosidase activities in ovarian cancer patients. *Cancer Res* 1979, **39**, 1943–1951.
5. Gauduchon P, Tillier C, Guyonnet C, Heron J-F, Bar-Guilloux E, Le Talaer J-Y. Clinical value of serum glycoprotein galactosyl transferase levels in different histological types of ovarian cancer. *Cancer Res* 1983, **43**, 4491–4496.
6. Spiro RG. Studies of fetuin, a glycoprotein of fetal serum. *J Biol Chem* 1960, **235**, 2860–2869.
7. Spiro RG. Periodate oxidation of the glycoprotein fetuin. *J Biol Chem* 1964, **239**, 567–573.
8. Podolsky DK, Weiser MM. Detection, purification and characterisation of a human cancer-associated galactosyl acceptor. *Biochem J* 1979, **178**, 179–287.
9. Verdon B, Berger EG, Salchli S, Goldhirsch A, Gerber A. An enzyme linked immunosorbent assay for lactose synthase (galatosyl transferase) in serum and its application as a tumour marker in ovarian cancer. *Clin Chem* 1983, **29**, 1928–1933.
10. Kaplan F, Hechtman P. Rat liver Golgi galactosyl transferase. *Biochem J* 1984, **217**, 353–364.
11. Kim YD, Weber GF, Tomita JT, Hiata AA. Galactosyltransferase variant in pleural effusion. *Clin Chem* 1982, **28**, 1133–1136.