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Original Paper

Clinical Characteristics of a Newly Developed Ovarian Tumour Marker, Galactosyltransferase Associated with Tumour (GAT)

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In this study clinical studies were conducted on galactosyltransferase associated with tumour (GAT) as a newly developed marker of ovarian cancers. The positive rates of GAT with a cut-off value of 16 U/ml (which corresponds to the mean + 2 standard deviations (S.D.) for healthy females) were 4.7% for benign ovarian tumours, 4.5% for endometriosis and 45.9% for ovarian cancers. GAT showed a positive rate comparable to that of CA546 or CA72-4 among other tumour markers (CA602, CA125, CA546, CA72-4, STN and SLX) examined in ovarian cancers. However, it showed lower positive rates for benign ovarian diseases and, in particular, it gave the lowest positive rate for endometriosis among the aforementioned tumour markers. Furthermore, the receiver operating characteristic (ROC) analysis for discriminating between ovarian cancer and endometriosis showed a significantly high area under the curve (AUC) for GAT compared with that of the other markers. GAT showed the lowest correlation coefficients with other markers, and the positive rate and the diagnostic efficiency were increased by its combination assay with CA602 and/or CA546. Furthermore, the accuracy of the diagnosis of ovarian cancer improved by examining GAT after screening with CA602 or ultrasonography. These results suggest that GAT is a suitable marker for distinguishing ovarian cancers from benign gynaecological diseases, particularly endometriosis, and is useful for combination assay or secondary screening for ovarian cancers. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: galactosyltransferase, GAT, tumour marker, ovarian cancer, combination assay
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INTRODUCTION

MANY TUMOUR markers have been evaluated and their usefulness in the diagnosis of ovarian cancers has been investigated. However, since the diagnostic reliability of single tumour markers has been found to be limited, combination assays of two or more tumour markers are considered to be one possible way to carry out accurate diagnosis of ovarian cancers. Recently, combinations of tumour markers with different characteristics have been evaluated at several institutions [1] to find combinations more suitable for discriminating between ovarian cancers and benign gynaecological diseases, but optimal combinations were often difficult to obtain from among the known tumour markers. Thus, the development of a new tumour marker for use in combination assays has been a goal of many investigators.

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Many of the recently developed tumour markers are related to aberrant glycosylation of glycoproteins on the cell surface due to malignant transformation [2]. Quantitative or qualitative changes in glycosyltransferases are considered to be a cause of the appearance of such aberrant glycosylation, and some of these changes have been elucidated. Although β (1–4) galactosyltransferase (GalT) in body fluids has been viewed as a potential tumour marker [3, 4], it has not been put into clinical use because of its high false-positive rates for benign diseases [5, 6].

Podolsky and colleagues reported the characterisation of a GalT isoenzyme (GT-II) in the body fluids of cancer patients that showed slow migration on native polyacrylamide gel electrophoresis, and their report prompted various investigations of isoenzymes of GalT related to malignant transformation [7–9]. GT-II was reported to be a highly-glycosylated GalT or a GalT associated with serum proteins, but there has been no consensus as to its identification [10–13].

We recently proposed the existence of a galactosyltransferase associated with tumour (GAT) in the ascites of ovarian cancer patients, an enzyme different from the GalT in the serum of healthy individuals, and we prepared monoclonal antibodies (MAbs) against it [14, 15]. In this study, we tested the usefulness of a GAT assay kit employing these MAbs and using this kit evaluated the serum GAT levels in healthy females and in patients with gynaecological diseases, including ovarian cancers. Further, we assessed the utility of GAT in combination assays.

PATIENTS AND METHODS

Subjects

Blood samples were obtained at the Keio University Hospital from 294 healthy females 10–89 years of age, 46 females whose menstrual cycle was known, 32 pregnant females, 193 patients with benign ovarian tumours, 110 with endometriosis, 40 with cervical cancers, 48 with endometrial cancers and 143 with ovarian cancers. Of the 143 patients with ovarian cancer, 100 had primary ovarian cancer; and the stage of the disease could be determined with certainty in 78. Metastatic tumour was detected in 9 and recurrence was observed in 34. The positive rate was calculated and the clinical usefulness was assessed after exclusion of 34 recurrent cases. These patients with ovarian cancer did not include those with borderline malignancies. Of 100 patients with primary ovarian cancer, the histological type was clear in 83 (cases of unclassifiable adenocarcinomas and squamous cell carcinoma (SCC) were excluded). The sera were separated by centrifugation and stored at -20°C until the assay could be conducted.

GAT assay kit

The serum GAT concentration was determined using a GAT assay kit based on the two-step, double-determinant sandwich enzyme immunoassay (EIA). In this kit, the anti-GAT monoclonal antibody MAb8513 (IgM) was immobilised on beads, and the other anti-GAT monoclonal antibody, MAb8628 (IgG_1), was labelled with horseradish peroxidase (HRP) [14]. The procedure of the assay was as follows: 50 µl of serum sample or standard and 200 µl of buffer were placed in each well. A polystyrene bead coated with MAb8513 was added, and the reaction was allowed to proceed at 45°C for 2 h. Each bead was then washed three times with phosphate buffer, after which 250 µl of HRP-labelled MAb8628 was added to each well. Incubation was then carried out at room temperature for 1 h to form immunocomplexes with the bound GAT. For removal of unreacted labelled antibody, the bead was washed four times with phosphate buffer; and the HRP of the complexes was reacted with H_2O_2–*o*-phenylenediamine as a substrate colour developer. The reaction was stopped with 1 N sulphuric acid, and the absorbance at 492 nm was measured. The serum GAT concentration was determined according to a calibration curve obtained from the values of the standards.

The calibration curve obtained with standards was nearly linear, and the coefficient of variation (CV) of each standard ($n = 10$) was 4% or less. Intra-assay ($n = 10$) and inter-assay (7 runs) reproducibility tests conducted with three samples of different GAT concentrations showed high reproducibility, with CVs of less than 5%. In the dilution test, the values for various dilutions of two sera showed a nearly linear relationship, and the recovery rates in the addition test were 95.0–105.5% (four levels of addition to three sera).

Receiver operating characteristic (ROC) analysis

In the ROC analysis, the area under the curve (AUC) values among the markers were compared using the method of Hanley and McNeil [16].

RESULTS

Determination of cut-off value

The cut-off value was determined using the following method: the GAT values for the 294 healthy females were measured, and their mean value and the standard deviation (S.D.) were 11.5 ± 2.5 U/ml. When the cut-off value was set at 16 U/ml on the basis of the mean + 2S.D. (= 16.5 U/ml), 9 of the 294 healthy females were regarded as positive, i.e. a 3.1% false-positive rate. For each of the subsequent examinations, a cut-off value of 16 U/ml was employed.

GAT levels in healthy females according to age, menstrual cycle and gestational week

The GAT values in healthy females, stratified according to their age, showed no observable differences among the various age groups. The serum GAT levels were determined in 46 females showing normal menstrual cycles, and the values were compared among the phase of the cycle, i.e. menstrual phase, early follicular phase, late follicular phase, ovulatory phase, early luteal phase and late luteal phase. No significant variation in the GAT level was seen throughout the menstrual cycle.

The serum GAT values for 32 normal pregnant females at various gestational weeks are shown in Figure 1. The GAT level increased with the progression of pregnancy.

GAT levels in patients with gynaecological diseases

As shown in Figure 2(a), the positive rates for various gynaecological diseases were as follows: 4.7% for benign ovarian tumours, 4.5% for endometriosis, 20.0% for cervical cancers and 16.7% for endometrial cancers. The positive rate rose to 45.9% for ovarian cancers.

GAT levels in ovarian cancers

Figure 2(b) indicates the positive rates for various histological types of ovarian cancers. The positive rates were 50.0% for clear cell adenocarcinoma and 53.3% for endometrioid adenocarcinoma, which were higher than the 42.9% for serous cystadenocarcinoma and the 29.4% for mucinous

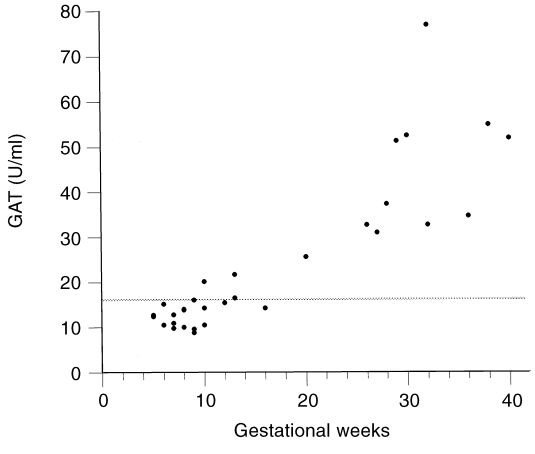

Figure 1. Influence of progression of pregnancy on galactosyltransferase associated with tumour (GAT) value.

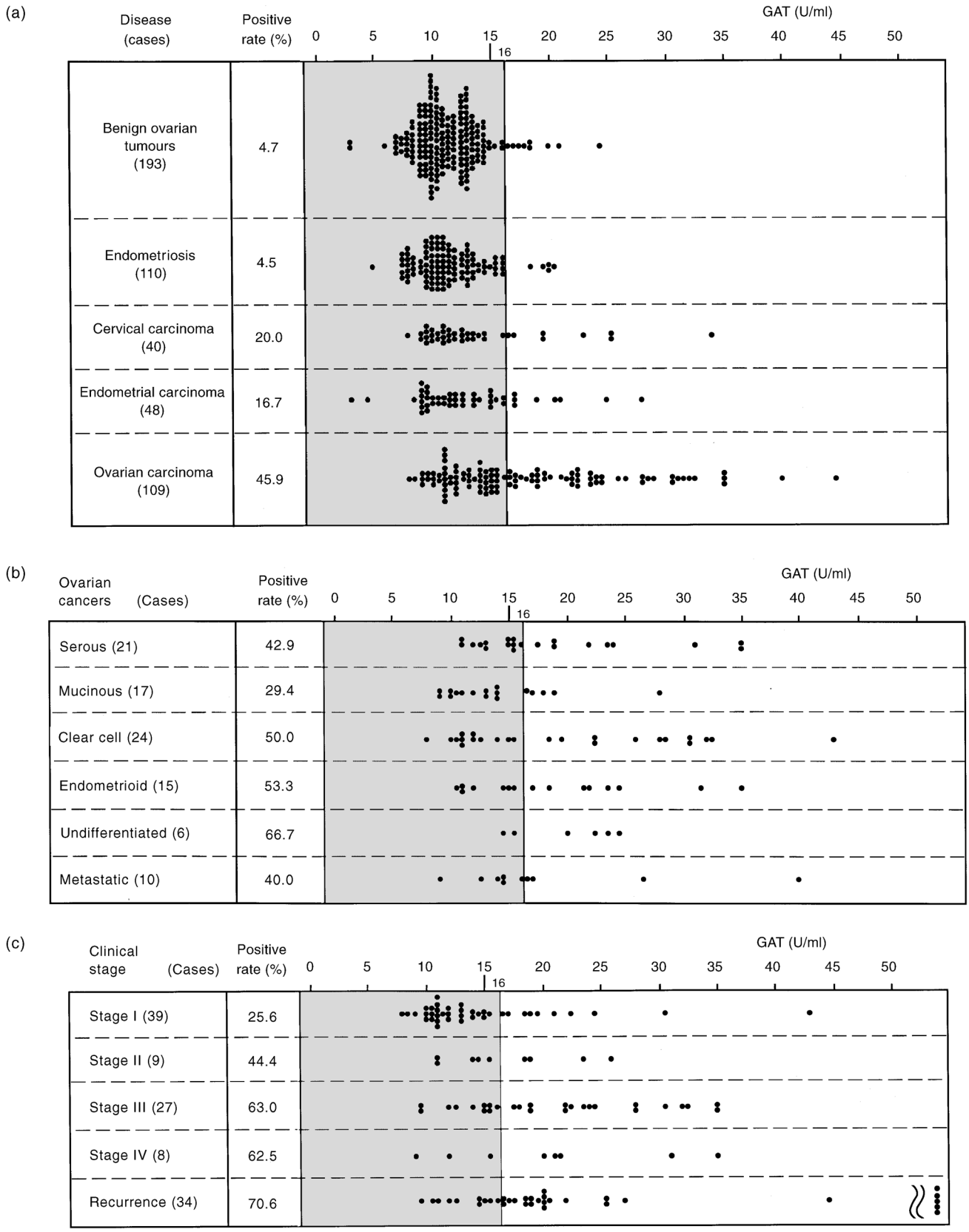

Figure 2. (a) Galactosyltransferase associated with tumour (GAT) levels in various gynaecological diseases. (b) Measurement of GAT in various histological types of ovarian cancers. (c) Measurement of GAT in various stages of ovarian cancers.

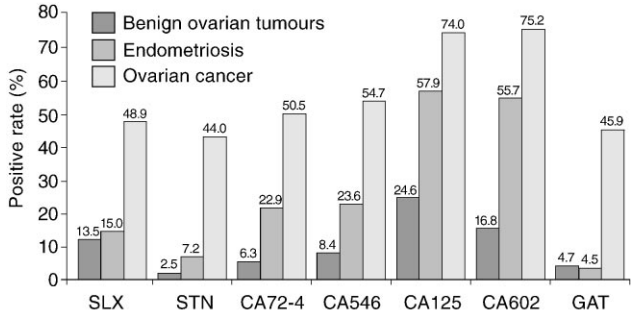

Figure 3. Positive rates of various markers in gynaecological diseases.

cystadenocarcinoma. The GAT values were very high in some patients with clear cell adenocarcinoma or endometrioid adenocarcinoma. The positive rate of ovarian cancer increased slightly with the progression of the clinical stage (Figure 2c).

Positive rates of various markers in gynaecological diseases

Figure 3 shows the positive rates of GAT, CA602 [17], CA125 [18], CA546 [19], CA72-4 (TAG-72) [20], STN (sialyl Tn) [21] and SLX (sialyl Le^x-i) [22] in patients with benign ovarian tumours, endometriosis and ovarian cancers. The cut-off values for these tumour markers were set at 63 U/ml for CA602, 35 U/ml for CA125, 12 U/ml for CA546, 4 U/ml for CA72-4, 45 U/ml for STN and 38 U/ml for SLX, which are the values generally used. The positive rate of GAT for ovarian cancers was 45.9%, which was lower than the rate of 75.2% for CA602, 74.0% for CA125 or 54.0% for CA546 but higher than or comparable with the 50.5% for CA72-4, the 44.0% for STN and the 48.9% for SLX. However, the false-positive rate of GAT for benign ovarian tumours was 4.7%, which was lower than those values for all the other tumour markers except STN; and the false-positive rate of GAT for endometriosis, 4.5%, was the lowest among the tumour markers examined in this study.

ROC analysis

For the discrimination between ovarian cancer and endometriosis, the ROC curve was compared among the markers. As shown in Figure 4, the AUC of GAT (79.1%) was significantly higher ($P < 0.02$) than that of CA602 (66.7%), CA125 (69.0%), CA546 (69.7%), CA72-4 (69.1%), STN (73.5%) or SLX (63.8%). The ROC curve of GAT did not cross that of the other markers.

Combination assays

The effect of GAT in combination assays with six other tumour markers for the diagnosis of ovarian cancers was evaluated according to Spearman's correlation coefficients (Figure 5). The correlation coefficients of GAT with the other tumour markers were the smallest, indicating the usefulness of GAT in combination assays.

Table 1 shows the sensitivity, specificity and diagnostic efficiency of combination assays among GAT, CA546 and CA602 in 136 patients with ovarian cancers and 298 patients with benign ovarian tumours, including endometriosis. The sensitivities and diagnostic efficiencies were increased in all combination assays of two markers compared with single-marker assays, and the diagnostic efficiency was the highest, at 0.617, for the combination of GAT and CA546. The sensitivity for the combination of CA546 and CA602 was 81.6%, the highest among the sets of two markers. The sensitivity was the highest at 84.6% in the combination of the three tumour markers (GAT, CA546 and CA602), and the

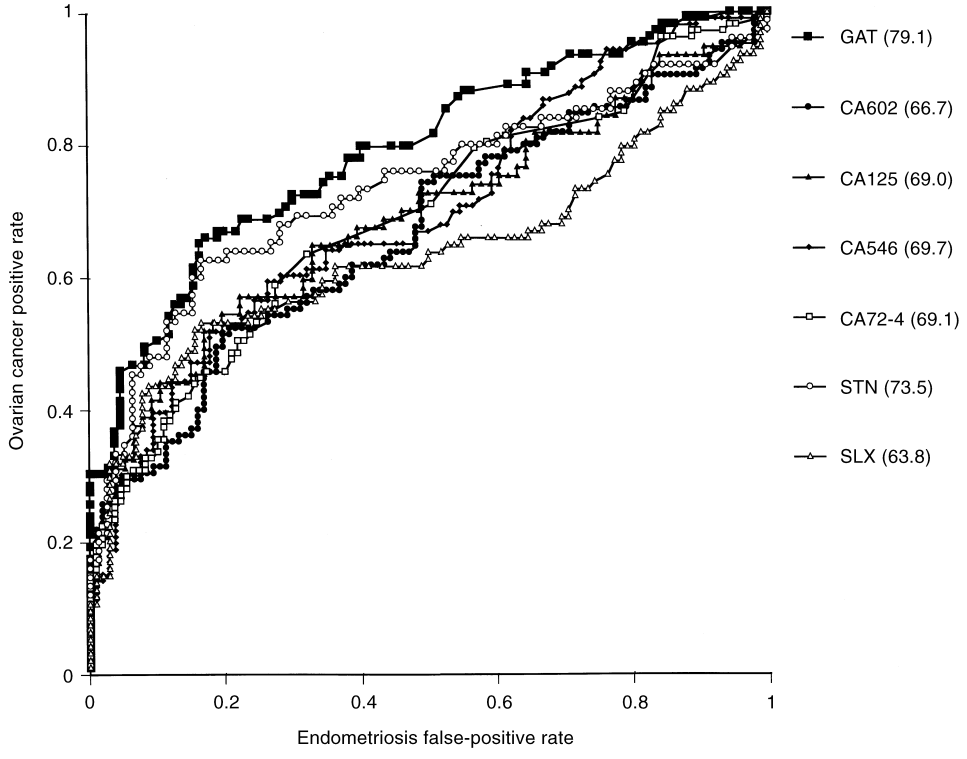

Figure 4. Receiver operating characteristic (ROC) curve of each marker for the discrimination between ovarian cancer and endometrosis. ( ) area under the curve (AUC) value.

CA602	CA125	CA546	CA72-4	STN	SLX	
0.370	0.417	0.202	0.257	0.177	0.291	GAT
	0.941	0.534	0.495	0.450	0.441	CA602
		0.536	0.515	0.378	0.431	CA125
			0.887	0.788	0.371	CA546
				0.782	0.395	CA72-4
					0.326	STN

Figure 5. Spearman's correlation coefficient between galactosyltransferase associated with tumour (GAT) and other markers in ovarian cancers.

diagnostic efficiency for this combination was 0.528, just slightly higher than that for the combination of CA546 and CA602 (0.526).

Monitoring of the course of cancer treatment

GAT values before and after the operation and at the time of recurrence were evaluated in 24 patients with ovarian cancer (Figure 6). After the operation, GAT decreased below the cut-off value and did not show any non-specific increase in the absence of recurrence (including the chemotherapy period). In the 5 patients who did not show an immediate decrease in GAT, the surgery was incomplete. Among these 24 patients with ovarian cancer, recurrence was detected in 5 patients and GAT increased at the time of recurrence in 4 of 5 patients (positive rate was 80%).

DISCUSSION

GAT, a cancer-associated isoenzyme characterised by its readiness to polymerise, is a different molecule from the GalT in normal human serum. GAT is recognised specifically by the monoclonal antibody MAb8513 [14]. The molecular structure of GAT was elucidated by cDNA analysis [15]. GAT is the same genomic product as β(1–4)GalT, namely, GAT is considered to be a GalT molecule released at an abnormal site due to certain changes associated with cancers, when membrane-bound GalT inside a cell is released into the body fluids. GAT has a longer stem region than normal GalT and MAb8513 recognises the protein in this longer stem region (specific to GAT). MAb8628 recognises the portion in the stem region that exists in both GAT and normal GalT [15].

The GAT–EIA kit used in this present study quantifies the GAT level in serum selectively by using the monoclonal antibodies MAb8513 and MAb8628, which recognise two different epitopes on the same molecule [14]. Moreover, this EIA kit can be used safely and easily. Basic studies on this kit showed excellent results for the linearity of the standard curve, intra-assay and interassay reproducibility, addition and recovery test and linear dilution.

Since the GAT values showed no observable difference according to the subject's age, it was possible to determine a uniform cut-off value independent of age. Furthermore, the GAT values showed no observable differences throughout the menstrual cycle, indicating the absence of any influence of sex hormones. The GAT values showed a tendency to increase with the progression of pregnancy. However, the influence on diagnosis can be ignored in early pregnancy as the GAT values were within the normal levels in that period, unlike the case of CA125, which shows high levels in early pregnancy.

Table 1. *Combination assay of galactosyltransferase associated with tumour (GAT), CA*602 *and CA*546

Combined markers	Sensitivity (%)	Specificity (%)	Diagnostic efficiency
GAT	50.0	95.6	0.478
CA602	75.0	69.8	0.523
CA546	57.4	86.9	0.498
GAT/CA602	80.9	67.8	0.548
GAT/CA546	73.5	83.9	0.617
CA602/CA546	81.6	64.4	0.526
GAT/CA602/CA546	84.6	62.4	0.528

In 298 patients with benign gynaecological disease and 136 patients with ovarian cancer, GAT, CA602 and CA546 were measured simultaneously; and the combination assay was performed. Sensitivity, proportion of patients with positive values among those with ovarian cancer; specificity, proportion of patients with negative values among those with benign gynaecological diseases; diagnostic efficiency, sensitivity × specificity.

GAT in the serum of cancer patients may be derived from GAT produced in cancer cells because of the tendency of the serum GAT value to increase with the progression of the clinical stage of ovarian cancers.

In tumour marker examination, there is a trade-off relationship between sensitivity and specificity according to the cut-off value. Therefore, both sensitivity and specificity should be simultaneously evaluated. For a comparison of the usefulness among the markers, the ROC analysis was used in this study [16]. As the ROC analysis for discriminating between ovarian cancer and endometriosis showed a significantly high AUC for GAT compared with that of the

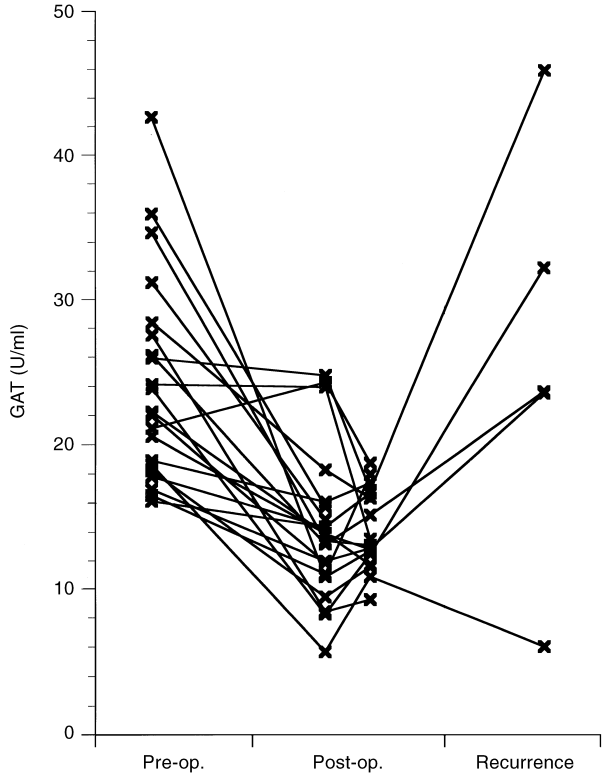

Figure 6. Galactosyltransferase associated with tumour (GAT) values before and after the operation and at the time of recurrence.

other markers, GAT may be the most useful marker for their discrimination. In addition, since the ROC curve of GAT did not cross that of the other markers, GAT is the most appropriate marker even when the cut-off value is changed according to the purpose of the diagnosis.

In this report, a cut-off value of 16 U/ml was used, which was determined as the mean + 2 S.D. in healthy females. However, the maximal diagnostic efficiency (0.595) of ovarian cancers versus benign gynaecological diseases was obtained at a cut-off value of 13.7 U/ml. When a cut-off value of 14 U/ml was used, which was determined as the level maximising the diagnostic efficiency, the positive rate of GAT was 60.6% for ovarian cancers; but 12.2% for healthy females, 11.4% for benign ovarian tumours and 15.5% for endometriosis. However, with a cut-off value of 16 U/ml, the positive rates were 45.9% for ovarian cancers, 3.1% for healthy females, 4.7% for benign ovarian tumours and 4.5% for endometriosis. Such a low false-positive rate of endometriosis seems to be a particular clinical characteristic of GAT. To help this advantage that GAT has over other tumour markers, such as CA125 [1], 16 U/ml was considered to be a suitable cut-off value, in spite of the sacrifice in sensitivity. Compared with the other markers examined in this study, GAT is judged to be a marker with greater specificity rather than sensitivity, and GAT must supplement the known markers because it gives very low false-positive rates in endometriosis with a cut-off value of 16 U/ml. If pelvic examination reveals a mass in the peritoneal cavity, ultrasonography and tumour marker measurement are routinely used to check for ovarian cancer. Some types of benign ovarian tumour can be easily distinguished from malignant ones on ultrasound images. However, endometriosis, which is a benign gynaecological disease most frequently encountered clinically, is often difficult to distinguish from ovarian cancers on ultrasound images. Markers of ovarian cancer have also been reported to have relatively high positive rates in cases of endometriosis. In view of these facts, we can conclude that GAT is a useful tumour marker to assist distinguishing ovarian cancers from endometriosis.

At present, from an economical standpoint, we have to select as few markers as possible for use in combination to obtain high diagnostic efficiency. The six markers examined in this study might all be related to aberrant glycoproteins in cancers, and they can be classified into three groups or categories based on the resemblance of the antigenic determinants recognised by the monoclonal antibodies: core protein-related antigen (CA602, CA125), core chain-related antigen (CA546, CA72-4, STN), and peripheral chain-related antigen (SLX). For a combination assay, it is important to select the markers that complement each other; thus markers from different categories should be employed to obtain high diagnostic efficiency. We have reported that combining detection of core protein-related antigen with that of core chain-related antigen is effective for the diagnosis of ovarian cancers [17, 19].

In this study, CA602 as a core protein-related antigen and CA546 as a core chain-related antigen were selected as partners in a combination assay of GAT. When combination assays of two markers were analysed, they showed higher positive rates and diagnostic efficiency than when the markers were assessed individually. The combination of GAT and CA546 proved to have the highest diagnostic efficiency of all. Therefore, this combination of GAT and CA546 should be viewed as a new approach to distinguishing between benign and malignant ovarian tumours. However, as the combination of CA602 and CA546 provided the highest positive rate, this combination could be suitable to obtain a higher sensitivity of the assay.

To obtain higher positive rates, multiple markers can be combined; but false-positive rates also increase at the same time. Consequently, an increase in diagnostic efficiency cannot be expected in most cases [1]. On the contrary, the sensitivity for the combination assays of three markers (GAT, CA602 and CA546) was increased with only a small decrease in specificity. Thus, this three-marker combination assay demonstrated diagnostic efficiency comparable with the combination of two markers (CA602 and CA546), and was shown to be a suitable combination for obtaining the highest

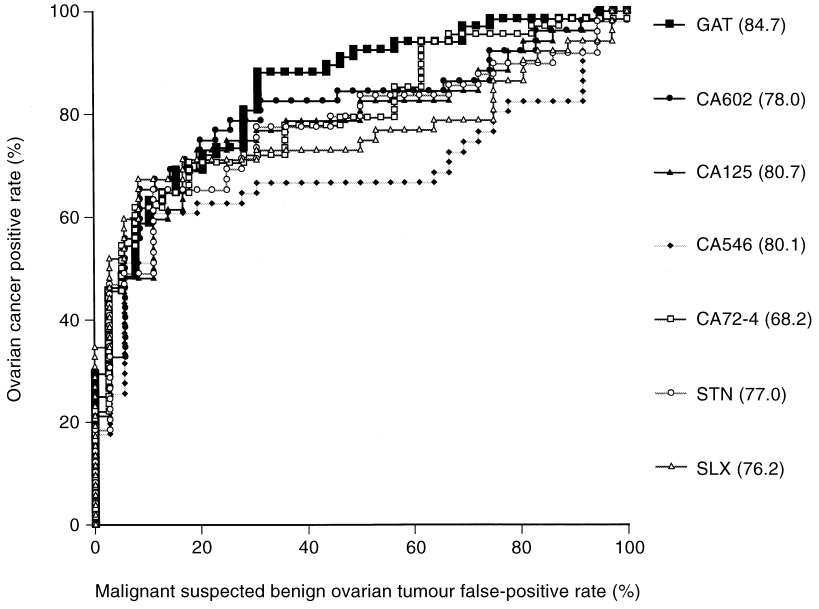

Figure 7. Receiver operating characteristic (ROC) curve of each marker for the discrimination of suspected malignant cases using ultrasonography. ( ) area under the curve (AUC) value.

sensitivity without a decrease in diagnostic efficiency. The combination assay including GAT provides a higher diagnostic efficiency due to the higher specificity of GAT for cancers and the lower correlation with other markers.

CA602 is a tumour marker that recognised the same core protein-related antigen as CA125. Since the correlation coefficients shown in Figure 3 demonstrate that the two markers recognise the same antigen, analysis of one of the two markers was considered to be sufficient. Although CA602, which was measured in a greater number of subjects, was selected in this study, we confirmed that similar results were obtained by the analysis of CA125 (data not shown).

It is, therefore, considered that GAT is a suitable partner for combination assays with markers from the core protein-related (CA602, CA125) and/or core chain-related (CA546, CA72-4, STN) antigen group.

CA602 (and CA125) provides the highest positive rate among existing tumour markers and is considered to be the most appropriate for the screening of ovarian cancer. However, it has the disadvantage of a high false-positive rate in endometriosis. Since many patients showing CA602 levels higher than the cut-off value but in a relatively low range (63–200 U/ml) are false-positive and actually have endometriosis, discrimination between ovarian cancer and endometriosis is considered to be difficult. According to the results of our present study, the accuracy of positive responses for ovarian cancer (positive predictive value [number of ovarian cancer-positive cases/(number of ovarian cancer-positive cases + number of endometriosis-positive cases) × 100]) was only 51% when CA602 was 63–200 U/ml. However, in this range of CA602, the positive predictive value of GAT was very high at 93%, compared with 56% for CA546, 56% for CA72-4, 83% for STN and 75% for SLX. Therefore, the accuracy of the diagnosis of ovarian cancer is considered to be markedly improved by examining GAT after screening with CA602 (or CA125).

Furthermore, we performed the ROC analysis in patients in whom ultrasound diagnosis was difficult, i.e. patients with benign diseases or ovarian cancer noted at screening as 'ovarian cancer suspected' or 'possibility of ovarian cancer not excluded'. In these patients, the AUC value for GAT was higher than those for other markers (Figure 7). At present, ultrasonography is considered to be the most appropriate modality for the screening of ovarian cancer, and these results suggest that GAT is a suitable tumour marker for more exact discrimination of suspected malignant cases using ultrasonography. From these observations, GAT is considered to be useful for secondary screening of ovarian cancer based on the results of existing screening methods.

At present, tumour markers are considered to be the most useful means of monitoring the course of cancer treatment. Indeed, tumour recurrence can be detected by an increase in markers prior to its detection by other examination methods in some patients. Changes in GAT seem to reflect the treatment course. These results suggest the applicability of GAT to the monitoring of the treatment course for ovarian cancer. Furthermore, in the monitoring of therapeutic effects on ovarian cancer, GAT is a more specific indicator of recurrence or reactivation of ovarian cancer than other markers, because it is negligibly affected by inflammation. Thus, GAT was considered to be a useful marker for the diagnosis of ovarian cancers.

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