

## CYFRA 21-1 as a tumor marker used in measuring the serum fragment of cytokeratin subunit 19 by immunoradiometric assay

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Serum levels of cytokeratin subunit 19 (CYFRA 21-1) were measured in 42 healthy volunteers, 104 cases of malignant diseases, 30 patients with chronic renal failure and 13 patients with non-malignant and infectious diseases. The reliability of the method was demonstrated after dilution of serum samples and intra- and inter-assay reproducibility. Serum CYFRA 21-1 concentrations were less than 2.00 ng/ml in all healthy controls and 86% of the malignant cases had high serum CYFRA 21-1 levels. However slightly elevated values of CYFRA 21-1 were observed in most chronic renal failure patients. High correlation was observed between serum CYFRA 21-1 and Tissue Polypeptide Antigen (TPA) values ( $r = 0.90$ ,  $n = 10$ ) but not with serum alpha-feto protein (AFP) concentrations. Furthermore, cross binding tests with the CYFRA 21-1 tracer/CYFRA 21-1 antibody-coated beads and CYFRA 21-1 tracer/TPA antibody-coated beads also gave an almost linear graph. These results indicate that CYFRA 21-1 and TPA share similar type of antigens.

**Key words:** CYFRA 21-1, TPA, tumor marker, cytokeratin 19, chronic renal failure

### INTRODUCTION

AN IDEAL TUMOR MARKER is an abnormality which is specific for a particular type of malignancy. However, there have been no biochemical markers that appear to be absolutely specific for the diagnosis of malignancy. The anaplasia or autonomy of the tumor cells permits the production of molecules in greater than normal amounts or at inappropriate times in the life of the organism, and in this sense the abnormalities may become specific.<sup>1</sup> Cytokeratins are intermediate filament proteins, which are parts of the cytoskeleton, characteristic of epithelial cells, belonging to the family of polypeptides and some of them having 10 cell type specificity.<sup>2</sup> There are about 19 different cytokeratins from 40- to 70-KD and the two main types of them are, acidic and basic. Simple epithelia are characterized by 4 cytokeratins: 7, 8, 18 and 19. Cytokeratin 19 is an acidic (type 1) subunit, expressed on epithelia and in carcinomas which arise from them. A fragment of Cytokeratin subunit 19 can be measured in

the serum by a solid phase sandwich assay with monoclonal antibodies. This cytokeratin 19 fragment is referred to as CYFRA 21-1.

The aims of this study were: (a) to use this tumor marker in diagnosing the malignancy before treatment, (b) to evaluate the reliability of serum CYFRA 21-1 measurement, (c) to investigate the correlation and cross binding studies with other tumor markers, (d) to define the specificity in the healthy population, and (e) to see the effect of impaired renal function on serum CYFRA 21-1 concentrations.

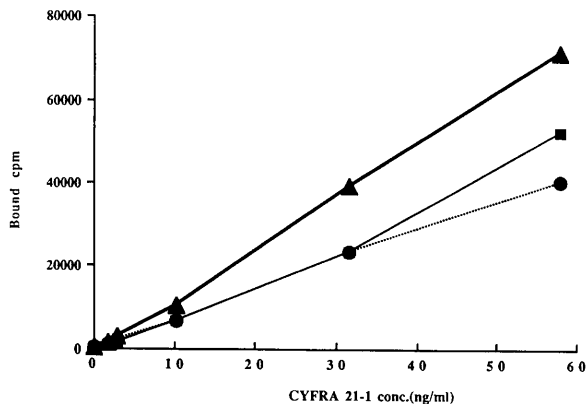
### MATERIALS AND METHODS

#### *Patients and healthy volunteers*

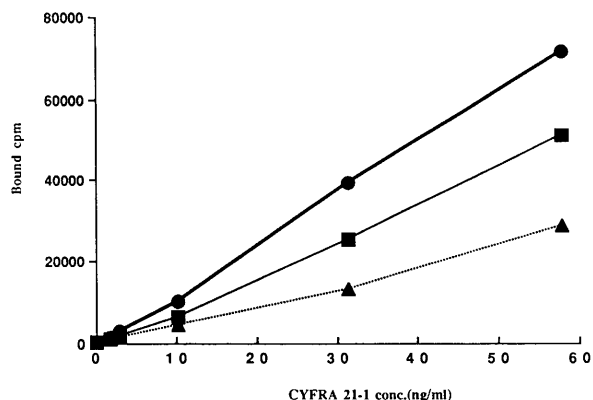
In this study, 147 patients referred to Gunma University Hospital were entered. Out of these, 26 had stomach cancer, 24 colon cancer, 16 lung cancer, 14 hepatoma, 8 breast cancer, 6 ovarian cancer, 5 cancer of the cervix, 3 pheochromocytoma, 2 thyroid cancer and 30 patients of chronic renal failure. The serum CYFRA 21-1 concentration was also measured in 13 other patients with non-malignant, metabolic and infectious diseases. All patients had pathologically confirmed diagnoses. Forty-two people, working in our institute and with no apparent disease

Received February 4, 1994, revision accepted July 5, 1994.

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**Fig. 1** Effect of temperature on the standard curve. Standard solutions were incubated at 4°C (—▲—), room temperature (—■—) and 37°C (—●—) for constant incubation period of 20 hours.



**Fig. 2** Effect of incubation time on the standard curve. Standard solutions were incubated for 4 (—▲—), 8 (—■—) and 20 hours (—●—) at constant temperature of 4°C.

as detected by general physical examination and simple laboratory tests, were included as healthy volunteers in this study.

#### CYFRA 21-1 immunoradiometric assay

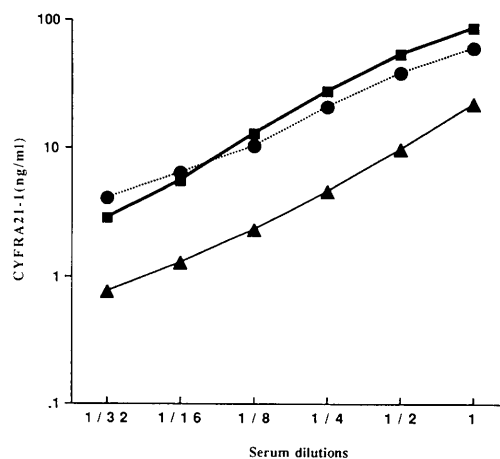
CYFRA 21-1 (CIS Biointernational/Gif Yvette, France) is a solid phase Immunoradiometric assay based on the two site-sandwich method. With this method cytokeratin 19 was recognized by two monoclonal antibodies BM 19-21 and KS 19-1, reactive with two different epitopes on cytokeratin subunit 19, which is referred to as serum CYFRA 21-1. The antibody, KS 19-1 coated on polystyrene beads, 100  $\mu$ l of patient serum, control serum, or standard samples (composed of the following concentrations of cytokeratin 19: 0, 1.68, 2.95, 10.2, 31.5 and 57.9 ng/ml), and 200  $\mu$ l of  $^{125}$ I labeled BM 19-1 antibody, were incubated for 20 hours, at 4°C. Twenty hours later, the solid phase was washed with 3 ml distilled water 3 times. Radioactivity was counted in a well type gamma counter and expressed as cpm. The calculated concentrations of cytokeratin subunit 19 were expressed as ng/ml.

#### Effect of temperature and incubation time

The standard curve was further studied by changing the temperature and incubation time. In this study, standard samples were incubated at different temperatures: 4°C, room temperature and 37°C for 20 hours. In a similar type of study, standard samples were incubated for different time periods: 4, 8 and 20 hours at 4°C.

#### Tests for reliability of the method

The reliability of the measurement was determined by means of the following tests: (a) linearity of the relationship between consecutive dilutions of serum samples of known high cytokeratin levels and results of the titration, (b) assessment of intra-assay reproducibility by measuring the same serum samples 10 times during the same assay, (c) assessment of inter-assay reproducibility by measuring in 10 different assays, and (d) recovery test for



**Fig. 3** Dilution test of sera with high concentrations of CYFRA 21-1.

CYFRA 21-1 with sera from 2 patients.

#### Cross-binding study with other tumor markers

Tissue Polypeptide Antigen (TPA) is known as a part of cytokeratin<sup>3</sup> and the antigenic relation between TPA and CYFRA 21-1 was examined by these methods. Ten serum samples with different CYFRA 21-1 values were incubated for 20 hours at 4°C, with CYFRA 21-1 tracer ( $^{125}$ I-labeled anti CYFRA 21-1 antibody)/CYFRA 21-1 antibody-coated beads and CYFRA 21-1 tracer/TPA antibody-coated beads (Prolifigen TPA Kit "Daiichi" II, Daiichi Radioisotope Lab., Tokyo, Japan). In other studies, the same 10 samples were used with the assay employing TPA tracer ( $^{125}$ I-labeled anti TPA antibody)/TPA antibody-coated beads and TPA tracer/CYFRA 21-1 antibody-coated beads. Moreover  $^{125}$ I-labeled anti AFP antibody (Eiken Chemical Co., Tokyo)/CYFRA 21-1 and TPA antibody-coated beads were also used for the above mentioned time and temperature. Radioactivity was counted with a well-type gamma counter and expressed as cpm.

## RESULTS

Radioactivity bound to beads decreased by increasing the temperature from 4°C to room temperature or to 37°C (Fig. 1). Bound radioactivity also decreased by shortening the incubation time from 20 to 8 or 4 hours (Fig. 2) and the following assay was performed by incubating at 4 °C for 20 hours. According to the data in Figure 3, the dilution test showed a linear relationship between CYFRA 21-1 concentrations and diluted serum samples with high

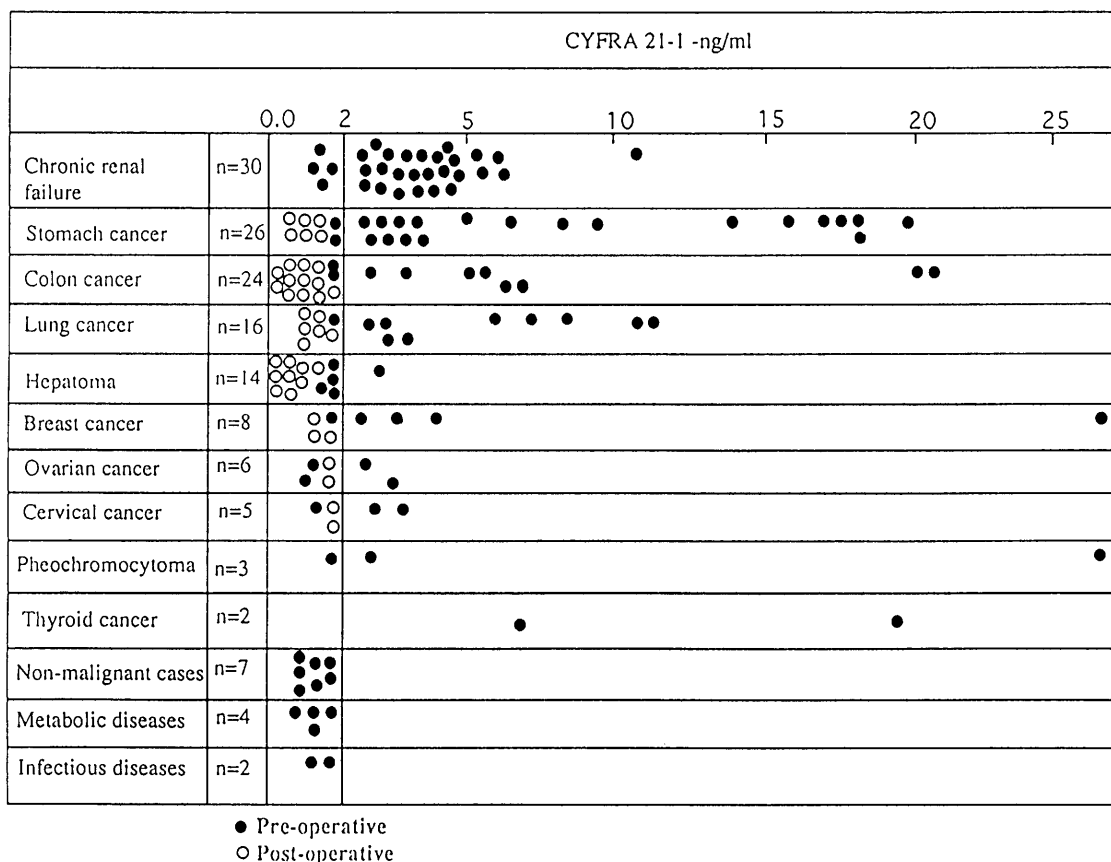
**Table 1** Reproducibility test by inter-assay and intra-assay methods

	Inter-assay	Intra-assay
N	10	10
mean	56.3	57.1
S.D.	2.31	2.22
CV%	4.10	3.38

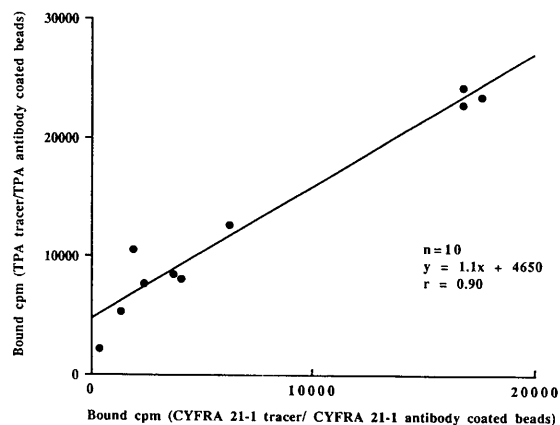
N = No. of subjects, S.D. = standard deviation, CV% = coefficient of variance percentage.

**Table 2** Recovery test of CYFRA 21-1 for the two serum samples

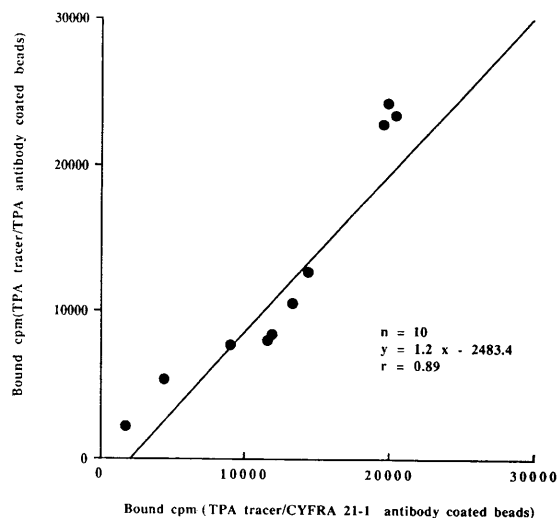
Added conc. of CYFRA 21-1	Measured conc.	Recovered conc.	Recovery	%Recovery mean
ng/ml	ng/ml	ng/ml		
–	1.33			
Sample-A	5.1	4.50	87%	
	15.8	16.6	105%	
	29.0	29.5	101%	98%
–	23.3			
Sample-B	5.1	5.60	109%	
	15.7	17.7	112%	
	29.0	30.5	105%	108%



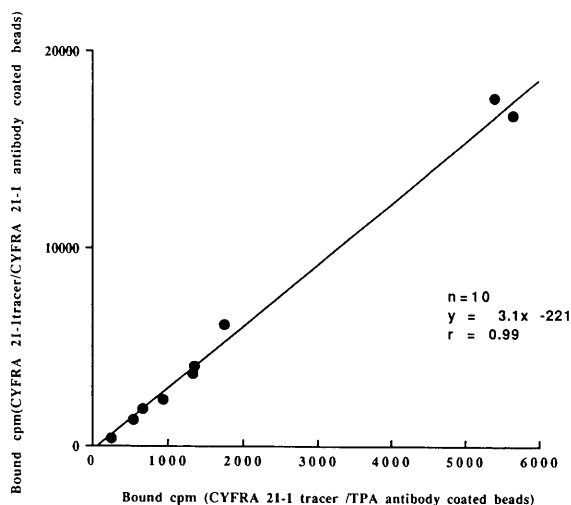
**Fig. 4** Distribution of serum CYFRA 21-1 concentrations in different pathologically confirmed diseases (normal range: less than 2.0 ng/ml).



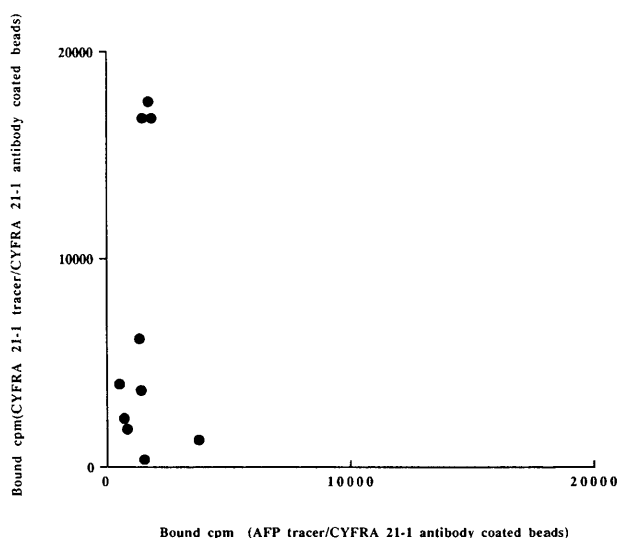
**Fig. 5-A** Correlation of assays using TPA tracer/TPA antibody-coated beads and CYFRA 21-1 tracer/CYFRA 21-1 antibody-coated beads.



**Fig. 5-C** Comparison of assays using TPA tracer/TPA antibody-coated beads and TPA tracer/CYFRA 21-1 antibody-coated beads.



**Fig. 5-B** Comparison of assays using CYFRA 21-1 tracer/CYFRA 21-1 antibody-coated beads and CYFRA 21-1 tracer/TPA antibody-coated beads.



**Fig. 5-D** Comparison of assays using CYFRA 21-1 tracer/CYFRA 21-1 antibody-coated beads and AFP tracer/CYFRA 21-1 antibody-coated beads.

cytokeratin 19 concentrations. As shown in Table 1, the serum sample was measured 10 times during the same assay (intra-assay). The mean of the results of 10 measurements was 57.1 (ng/ml), with an S.D. of 2.22 ng/ml, and the coefficient of variation was calculated to be 3.38%. The same serum was tested on 10 different days with a mean measurement of 56.3 (ng/ml), with an S.D. of 2.31 ng/ml, and the coefficient of variation was 4.10% (Inter-assay). Table 2 shows the recovery test of the CYFRA 21-1 added. Mean recovery of serum samples was 98% and 108%.

A threshold of 2.0 ng/ml was chosen as the upper limit of normal values calculated in the healthy volunteers, since all CYFRA 21-1 values in healthy volunteers were

less than 2.00 ng/ml with a mean of 0.64 ng/ml and an S.D. of 0.13 ng/ml. Higher concentrations of CYFRA 21-1 were seen in sera of patients with lung, colon and stomach cancer (Fig. 4). CYFRA 21-1 values were lower in patients who were operated on when compared with pre-operative patients. Serum samples of the patients with non-malignant diseases metabolic disorders and infectious diseases were found to be within the normal limits for CYFRA 21-1, while patients with chronic renal failure, when measured for CYFRA 21-1, almost all had concentrations above the upper limit of the normal value (Fig. 4). A high correlation was observed between serum CYFRA 21-1 and TPA values as shown in Figure 5-A ( $r = 0.90$ ,  $n = 10$ ). Furthermore, there was a high rate of

binding of CYFRA 21-1 tracer to the antigen, captured on TPA antibody-coated beads and also the TPA tracer to the CYFRA 21-1 antibody-coated beads (Fig. 5 B and C). However there was no binding of AFP tracer, which we employed as an irrelevant antibody, to the antigen, captured on CYFRA 21-1 antibody-coated beads (Fig. 5-D).

## DISCUSSION

Intermediate filaments are parts of the cytoskeleton and provide important information on the cell origin.<sup>4</sup> They are classified into five tissue specific protein filaments. The cytokeratin family is expressed by all epithelial cells<sup>5</sup> including endocrine cells of the dispersed neuroendocrine system<sup>6</sup> and it therefore appears to be a general specific and useful marker of epithelial differentiation. Cytokeratin is a heterotypic tetramer of protofilaments composed of two polypeptides: one acidic type 1 subunit and one basic type 11 subunit. Each type of epithelium and its malignant counterpart expresses a specific cytokeratin polypeptide pattern.<sup>5,7</sup> Selective antibodies raised against simple epithelium type of cytokeratin have been shown to react with lung cancers of all histologies.<sup>8,9</sup> Cytokeratin 19 is also normally present in the basal cell layer of non-cornifying regions of the oral epithelia.<sup>5,10-12</sup> Interestingly, the expression of cytokeratins is not lost by epithelial cells during the malignant transformation,<sup>4,5</sup> a phenomenon in contrast with the well known phenotypic instability of cancer cells.<sup>13</sup> Antibodies raised against some common antigenic determinants of all cytokeratins are therefore reported to be useful in typing malignant tumors with poor histological features of differentiation.<sup>4</sup> It has been found that increased suprabasal expression of cytokeratin 19 is a marker related to preneoplastic situations.<sup>14,15</sup> Some fragments of cytokeratins might be released in the serum owing to cell lysis or tumor necrosis, which gives support to the present research findings on cytokeratin subunit 19 fragment as a tumor marker for lung and gut cancer.

CYFRA 21-1 is an immunoradiometric assay with two monoclonal antibodies BM 19-21 and KS 19-1 as the <sup>125</sup>I-labeled tracer and bead coating. The results of fundamental studies on CYFRA 21-1 measurement, such as the dilution test and intra- and inter-assay reproducibility and recovery tests, were satisfactory and the CYFRA 21-1 values were less than 2.0 ng/ml in all the healthy controls. A high CYFRA 21-1 concentration in about 86% of cancer patients indicated the utility of CYFRA 21-1 as a tumor marker, but slightly increased values were also observed in the sera of 87% chronic renal failure patients who had no apparent malignancies.

TPA is also reported to be a part of cytokeratin<sup>3</sup> and is clinically used as a tumor marker. High correlation was observed between serum CYFRA 21-1 and TPA concentrations (Fig. 5-A). Cross-binding occurs between the CYFRA 21-1 tracer/TPA antibody-coated beads, but not between CYFRA 21-1 and AFP used as an irrelevant

antigen. Furthermore, serum CYFRA 21-1 values increased in patients with chronic renal failure, as we have seen with TPA levels. These values suggested that both tumor markers CYFRA 21-1 and TPA shared the same type of antigens.

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