COMPARISON OF VARIOUS CORRELATION ON IRMA FOR CA19—9 AND CA50 IN THE DIGESTIVE SYSTEM TUMOUR

Mo Qizhong (莫启忠)

(Shanghai College of Traditional Chinese Medicine, Shanghai 200032, China)

R.P.Baum, A.Hertel and G.Hör

(Nuclear Medicine Division of Radiological Centre, Frankfurt University Hospital)

(Received May 1989)

ABSTRACT

This paper compared various correlation of IMRA for CA19- 9 and CA50 in the digestive system tumour antigen, such as linearity, sensitivity, analytical range, precision as well as storage and stability and so on. And it also determined serum level of CA19- 9 and CA50 of patients with different tumours. The results showed it was well correlated to CA19- 9 and CA50 as a marker of digestive system tumour. Particularly high levels of both markers were found in patients with pancreastic colonic cancer. Therefore it is possible to obtain a higher sensitivity and acceptable specificity by combination with CA19- 9 and CA50.

Keywords: Tumour marker CA50 CA19- 9 IRMA Solid phase immunoassay Inter- and intraassay variance

I . INTRODUCTION

Monoclonal antibodies 1116 NS 19- 9 (CA19- 9 Ab) and CA50 (CA50- Ab) were obtained after immunization of mice with human colorectal adenocarcinoma cell lines[1,2]. Both antibodies react with sialosylfucosyllactotetraose, corresponding to sialylated blood group antigen lewisa[3,4], but the CA50- Ab reacts with sialosyll-actotetraose, which lacks the fucose moiety of sialylated[5].

Elevated serum CA19- 9 antigen levels are found in high proportion of patients with gastrointenal malignancies[6,7,9]. In gastrointestinal diseases the expression of the CA50 antigen in serum is quite similar to that of the CA19- 9 antigen [8,9]. Histochemistry and radioimmunoscintigraphy had demonstrated CA19- 9 Ag mainly was located in gastrointestinal tumour cell[10- 12]. However, the CA50 level, but rarely the CA19- 9 level, is elevated also in some patients with non- gastrointestinal tumour[6,8]. The concentration of both antigens is low in serum of healthy individuals. However elevated CA19- 9 and CA50 values have been found in patients with benign diseases, particularly in patients with jaundice[7,9].

For the follow up as well as for the therapy monitoring of patients with gastrointestinal malignancies, we have compared the correlation between CA19- 9 and CA50 in Vitro. Concerning the methodological evaluation we will comment on these topics: Linearity, sensitivity and analytical range; inter- and intra- assay variance; storage and stability; reference and methods.

II. MATERIAL AND METHODS

We determined the CA19- 9 and CA50 serum levels of CA19- 9 and CA50 antigen in 21 patients with rectum cancer, 6 patients with liver metastatic, 6 patients with rectum cancer, 6 patients with pancreas- cancer, 4 patients with sigmoid flexure cancer, 1 patients with carcinoma of prostate and cystocele, colon and biliferous duct, and stamach respectively. Furthermore we have determined the CA19- 9 and CA50 levels of 13 patients with nongastrointestinal system malignancies inclucing breast, ovarian, lung and thyroid gland cancer and 19 patients with undiagnosis disease.

CA50 assay: The serum level of CA50 antigen was measured by a solid phase immunoassay provided by Behring- werke Company associated with Höechst AG, Morburg- Germany. In brief, polypropylene tubes coated with mouse monoclonal antibody to CA50 were incubated with 50 ml of a standard, control serum or test samples and 200 ml of buffer. After incubation at room temperature for 2h, the CA50 present in the specimen was bound to the polypropylene tubes. The unbound material was removed by washing and appropriate anti- CA50 antibody labeled with ^{125}I was then incubated with antigen coated polyproylene tubes. After the unbound ^{125}I antibodies were washed out, the amount of bound radioactive labeled antibody was counted. The mean of duplicate count was used to estimate the content of CA50 in test samples by comparison with the standard curve.

CA19- 9 assay: CA19- 9 levels were determined in duplicate by a solid- phase RIA method based on forward sandwich method, using the CA19- 9 RIA kit manufactured by Centocor Inc. As was done in the CA50 assay, a murine monoclonal antibody CA19- 9 on a solid phase immuno- adsorbant was used to bind CA19- 9 contained in 100 ul portions of serum. After 3h of incubation at 37°C, excess serum was washed from the system, and ^{125}I labeled CA19- 9 was added, following 3 additional hours of incubation, excess radiolabeled material was washed from the system, and the amount of radioactivity associated with immunoadsorbant was measured in a scintillation counter.

Counting instrument is Auto- Gamma 5000 series, models COBRA 5010 (Packard instrument Company).

Specific reference sera was obtained from Bioret. GmbH.

III. RESULTS AND DISCUSSION

1) *Sensivity, Analytical Range, Lineartity and Analytical Recovery* The CA19- 9 and CA50 detection (3 standand deviations of the zero standard) was found at 1.4 and 0.3u/ml respectively. The CA19- 9 analytical range is covered by 5 standards between 6 to 120 u/ml, being linear up to 120 u/ml. The analytical recovery ranged from 98.2 to 102.8%. The CA50 analytical range is covered by 6 standards between 0.1 to 177 u/ml,

being linear up to 177 u/ml. The CA50 analytical recovery ranged from 97% to 101.5%.

2) *Precision* We have compared 4 batches control serum (povided from RIA kit) as well as reference qualitative control serum for CA19- 9 and CA50. As shown in Table 1 intra- assay imprecision revealed a *CV*% between 2.33 and 4.85% different CA19- 9 concentration levels, the corresponding values for inter- assay imprecision proved to be 4.2 to 10.0% ($n=4$ in each concentration).

As shown in Table 2, intra- assay imprecision revealed a *CV*% between 2.9 and 5.25 at 6 different CA50 qualitative control serum levels, the corresponding values for inter- assay imprecision proved to be 3.00 to 19.7%. But for inter- assay on Co1 and Co2 from RIA kit, its *CV*% is lower stable (*CV*% = 3.0% and 6.3%). It suggests that the RIA method is normal.

Table 1

Precision of the centoaor CA19—9

	intra- assay ($n=4$)			inter- assay ($n=4$)		
	X	*SD*	*CV*%	*X*	*SD*	*CV*%
Control (u/ml)	41.36	1.97	4.85	41.36	3.69	8.90
Cose	10.01	0.27	2.78	10.01	0.97	10.00
Co1	21.91	0.52	2.33	21.91	1.22	5.60
QCS (u/ml) Co2	58.44	0.80	0.91	58.44	2.43	4.20
Co3	112.28	3.84	3.95	112.28	6.84	6.10

Table 2

Precision of the behring werke CA50

	intra- assay ($n=4$)			inter- assay ($n=4$)		
	X	*SD*	*CV*%	*X*	*SD*	*CV*%
Control col	17.96	0.91	4.92	17.96	1.13	6.30
(u/ml) co2	113.95	6.08	6.06	113.95	3.40	3.00
cosp	21.31	0.60	2.90	21.31	1.87	8.50
QCS (u/ml) c1	7.99	0.39	4.90	7.99	1.57	19.70
c2	16.48	0.61	3.85	16.48	2.42	14.68
c3	36.43	1.87	5.25	36.43	5.81	16.00

3) *Storage and durability* The assay is performed directly on serum. If the assay is performed within 24 hours the samples should be kept at 2- 4°C, otherwise they should be divided into aliquots and stored frozen(- 20°C).

4) *Reference range* We didn't determin the reference range sera of healthys subjects. But as RIA kit shown that observed range for the CA19- 9 concentration in 260 normal subjects from both sexes is summurized in the below:

No of cases	CA19- 9 (u/ml)	%
171	<10	66
247	<25	95
259	<33	99.6

The CA50 serum levels of healthy subjects ranged from 0.2 to 20.1 u/ml, with a mean value of 6.1±3.7 u/ml. 17.3 u/ml was considered as the upper normal level[13,14]. As with all diagnostic tests, our laboratory should establish own normal range.

5) *Organ-specificity of CA19-9 and CA50* In order to analyse the organ- specificity of CA19- 9 and CA50 we determined two markers in various malignant disease. As Table 3 shown that tumour of the digestive system including colon, liver, pancreas, rectum, sigmoid flexure, stomach, biliterous duct and prostate and cystocele CA19- 9 sera levels more than that 37 u/ml was found from 47.2 to 100% and CA50 sera levels more than that 25 u/ml was found also from 16.7 to 100%. The serum concentration of CA19- 9 was elevated in 83.3% and the CA50 levels in 100% of the patients with pancreatic cancer. High levels of both markers were particularly found in patients with pancreastic, colonic and liver cancer[14].

Table 3

The CA19-9 and CA50 levels in sera of patients with various gastrointestinal malignancies

Histology tumour's name	numbers of patients	CA19-9				CA50			
		No. >200u/ml	%	No >37u/ml	%	No. >13.6u/ml	%	No. >25u/ml	%
Colon	21	16	76.2	10	47.7	16	76.2	13	61.9
Liver	19	16	84.2	14	73.7	17	89.5	16	84.2
Pancreas	6	6	100.0	5	83.3	6	100.0	6	100.0
Rectum	6	6	100.0	3	50.0	4	66.7	1	16.7
Sigmoid- flexure	4	3	75.0	3	75.0	4	100.0	4	100.0
Colon biliferous duct	1	1	100.0	1	100.0	1	100.0	1	100.0
Biliferous duct	1					1	100.0	1	100.0
Prostate	1	1	100.0			1	100.0		
Stomach	1	1	100.0	1	100.0	1	100.0	1	100.0
Ovarian	2	2	100.0	2	100.0	2	100.0	2	100.0
Mamma	6	1	16.7			4	66.7		
Lung	3	3	100.0	3	100.0	2	66.7	2	66.7
Thyroid	2	1	50.0	1	50.0	2	100.0	1	50.0
Uncertain diagnose	19	6	31.6	1	5.3	12	63.2	9	47.4

We have determined correlation between CA19- 9 and CA50 using lineare regression method in 10 patients with colonic tumour and 11 patients with liver malignant disease (the CA19- 9 cut- off levels of 37 u/ml and CA50 cut- off levels of 25 u/ml were used). Their correlation coefficient for colonic and liver cancer were 0.63 and 0.97 respectivly. There are significant correlation for both markers in the colonic and liver tumour (T_r= 2.4 for colonic tumour and T_r=3.7 in liver). This suggest that there are higher specificity and sensitivity by combining different markers. In other words, it is possible to obtain a higher sensitivity and acceptable specificity by the combination with CA19- 9 and CA50.

In 6 patients with mamma cancer CA19- 9 and CA50 were 16.7 and 66.7% respectively, when their concentration of CA19- 9 and CA50 is more than 20 u/ml and

13.6 u/ml respectively.

The CA19-9 and CA50 sera levels are very high in 2 patients of ovarian neoplasm.

Though the CA19- 9 and CA50 sera levels are lower in the nongastrointestinal tumour, the CA50 level was elevated in 63.2 and 47.4% of these uncertain diagnosic patients respectively for CA50 level more than 13.6 and 25 u/ml.

REFERENCES

[1] H.Koprowski et al., *Somat Cell. Genet*, **5** (1979), 957.

[2] Lindholm et al., *Int. Arch. Allergy Appl. Immun.* **71** (1983), 178.

[3] J.L.Magnani et al., *J. Biol. Chem.*, **257** (1982), 14365.

[4] J.E.Mansson et al., *Biochem. Biophy. Acta*, **834** (1985), 110.

[5] O.Nilsson et al., *FEBS Lett.*, **182** (1985), 398.

[6] R.E.Ritts et al., *Int. J. Cancer*, **33** (1984), 339.

[7] C.Haglund et al., *Brit. J., Cancer*, **53** (1986), 197.

[8] J.Holmgren et al., *Brit. Med. J.*, **288** (1984), 1479.

[9] C.Haglund et al., *Int. J. Cancer*, **39** (1987), 477.

[10] C.Haglund et al., *Brit. J. Cancer*, **53** (1986), 189.

[11] C.Haglund et al., *Int. J. Cancer*, **38** (1986), 841.

[12] R.P.Baum et al., Radiolabeled monoclonal antibody for imaging and therapy, Pergamo press, New York, 1988.

[13] R.P.Baum et al., *Int. J. Biol. Marker*, **3** (1988), 177.

[14] A. Lamiquiz Vallejo et al., *Int. J. Biol. Marker*, **3** (1988), 206.