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THE APPLICATION OF TUMOR MARKER RADIOIMMUNOASSAY – PRESENT STATUS IN JAPAN

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In this paper, I will not describe in detail the biochemical, biological, or oncogenetic aspects of tumor markers, but will limit my discussion to the clinical applications of tumor makers, and, in some instances, comment on some specific aspects or methodology.

The biological characteristics of tumor markers can be observed in the differences between normal and cancerous cells. For clinical diagnostics, the important question is whether or not we can detect these changes by in vitro tests. By immunological means, we are able to detect changes on cell surfaces by measuring either differentiated antigens or cell specific antigens.

Table 1 shows a partial list of tumor markers which are most commonly examined in our country. The first tumor marker assays were for α-fetoprotein (AFP) and CEA. After that ferritin and β_2-microglobulin, also increased in some non-cancerous disorders, were studied as tumor markers. Relatively recently, tissue polypeptide antigen (TPA) and CA 19-9 have been introduced and are under evaluation. Some of the results will be shown later. In the right columm the positive response rate is shown for each tumor maker.

Table 2 shows a more extensive list of tumor markers, which have been studied in our country. Some of these are currently practical use, and some are only at the investigational stage. In this table, hormones and related substances, such as calcitonin, HCG or thyroglobulin, are omitted. A brief statement will be made for each tumor marker as an overview. I will then discuss the clinical value of combination measurements (advantages and disadvantages), and speculate on the future.

As in most parts of the world, our initial studies were on α-fetoprotein. It is well-known that this assay has a high diagnostic value for hepatoma, hepatoblastoma, neuroblastoma and the presence of liver metastasis. Serum CEA is high in various cancers, especially colorectal and stomach, which is a high incidence cancer in our country. Plasma ferritin is abnormally increased in cases of malignancies of the digestive organs, as well as pulmonary cancer.

Although β_2-Microglobulin may not be a tumor marker, its plasma level is abnormally high in various lung and gastric carcinomas. Serum TPA, which is considered to be a possible proliferation-associated antigen, is elevated in many kinds of carcinomas. The elevation of CA 19-9 is

Table 1. Tumor Markers (I)

Tumor Markers	High Positive Rate Group
α-fetoprotein (AFP)	hepatocellular ca.
carcinoembryonic antigen (CEA)	colon ca. pancreas ca.
ferritin	hepatocellular ca. pancreas ca.
β_2-microglobulin	multiple myeloma hepatocellular ca.
tissue polypeptide antigen (TPA)	hepatocellular ca. bile duct ca. colon ca.
CA19-9	pancreas ca. colon ca.

Table 2. Tumor Markers (II)

Tumor Markers	High Positive Rate Group
basic fetoprotein (BFP)	hepatocellular ca. stomach ca.
pregnancy specific β_1-glycoprotein (SP_1)	trophoblastic tumor.
pancreatic oncofetal antigen (POA)	pancreas ca. bile duct ca.
pancreatic secretory trypsin inhibitor (PSTI)	pancreas ca. stomach ca. lung ca.
pancreatic cancer-associated antigen (PCAA)	pancreas ca. lung ca. colon ca.
immunosuppressive acid protein (IAP)	colon ca. bile duct ca.
prostatic acid phosphatase (PAP)	stomach ca. prostata ca.
neuron specific enolase (NSE)	lung ca. esophagus ca.
thyroxine binding globulin (TBG)	hepatocellular ca.

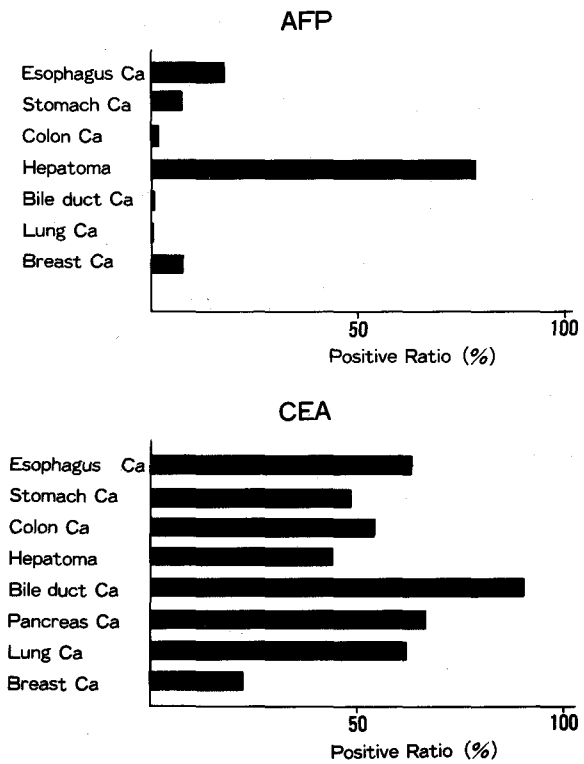

Fig. 1. Positive ratio of serum AFP and CEA in the malignant diseases.

demonstrated in cases of pancreatic, gastric and biliary tract carcinomas. Thyroxine binding globulin (TBG) can be produced in large amounts in some malignancies. We have observed 3 cases of hepatoma, which showed a marked elevation of TBG.

Figure 1 shows the positive frequency of serum AFP and CEA in various types of cancer. As can be seen, AFP has a high diagnostic value for hepatoma, whereas CEA shows elevated values in various types of carcinomas.

Figure 2 shows the positive ratio of serum TPA, CA 19-9 and ferritin. TPA has a low positive ratio in lung cancer, whereas CA 19-9 has less sensitivity for hepatoma, lung and breast cancers. The positive ratio for ferritin is low in colon and breast cancers. From this data, it is apparent that each tumor marker has some degree of tissue specificity.

Figures 3 and 4 show the clinical evaluation of various tumor markers in various malignant diseases. From this data, the highest positive frequency of the markers are as follows: for gastric cancer, CA 19-9; for colon carcinoma, TPA; for the pancreatic cancer, CA 19-9; for hepatoma TPA and AFP; for esophageal cancer, TPA; for bile duct cancer, CEA and TPA; for lung cancer, CEA; and for breast cancer, TPA.

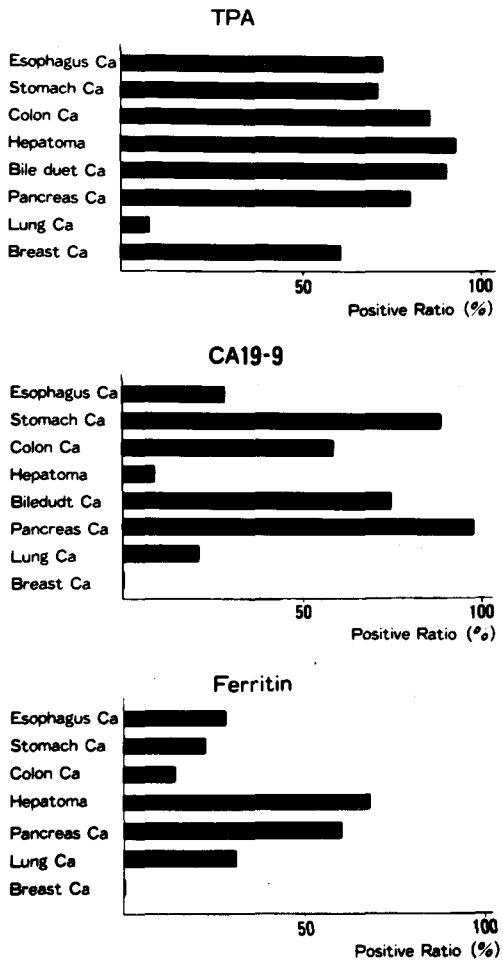

Fig. 2. Positive ratio of serum TPA, CA19-9 and ferritin in the malignant diseases.

Table 3. Measurement of the Quality-Control Sample by Kit Standards (Mode A) and Common Standards (Mode B)

	Name of kit	Mode	Assay values, micro-int. units/mℓ, found by the five institutes				
			1	2	3	4	5
a)	Insulin RIA	A	82.6(5%)[a]	74.0(9%)	65.9(9%)	96.5(5%)	97.8(5%)
	Dainabot	B	46.3(4%)	40.5(4%)	36.2(10%)	48.2(10%)	42.8(8%)
b)	IRI-Pharmacia	A	39.2(16%)	45.1(12%)	55.7(16%)	–	71.4(4%)
	Shionogi	B	54.1(7%)	43.4(9%)	48.7(9%)	–	52.0(6%)
c)	Insulin Eiken	A	49.8(7%)	53.2(10%)	49.5(12%)	–	–
		B	47.8(12%)	49.5(12%)	53.3(11%)	–	–
d)	IRI-Pharmacia	A	53.9(4%)	53.5(6%)	61.4(6%)	54.2(5%)	–
	Daiichi	B	48.2(6%)	45.4(9%)	47.6(9%)	50.2(16%)	–
e)	Insulin-RCC	A	49.8(7%)	74.6(8%)	63.5(9%)	–	–
		B	52.7(17%)	54.6(11%)	46.7(12%)	–	–
f)	Insulin-CIS	A	42.8(21%)	52.6(20%)	41.1(25%)	–	–
		B	48.2(5%)	49.9(10%)	48.8(10%)	–	–

[a]In parentheses: the CV in percent, obtained from precision profile.

Table 4. Results of Analysis of Variance

Varlation	Mode A	Mode B
Between kit	23.0%	6.2%
Within kit	18.2%	7.6%

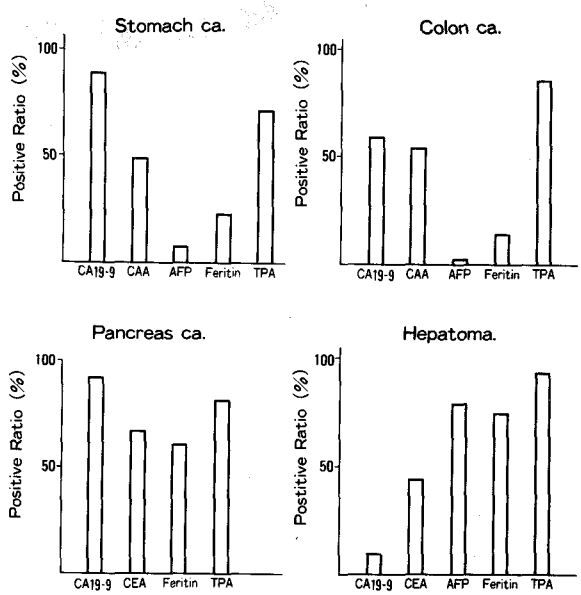

Fig. 3. Clinical evaluation of tumor markers in various malignant diseases.

It is well known that pancreatic carcinoma is one of the most difficult cancers to diagnose. As the stages advance, the concentration of tumor markers increases in serum. Urushizaki et al. (Dept. of Medicine, Sapporo Medical College) studied 13 cases of pancreatic cancer. Positive instances of CA 19-9, ferritin and CEA were 11, 8 and 7, respectively. However, the combinations of CA 19-9/ferritin and CA 19-9/CEA were positive in all 13 cases, suggesting that these

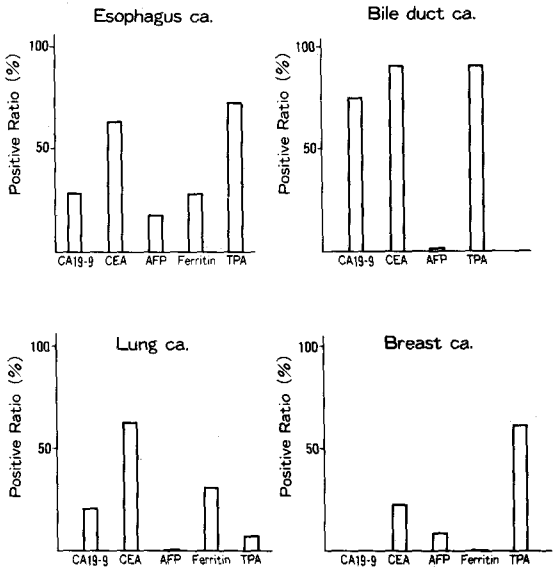

Fig. 4. Clinical evaluation of tumor markers in various malignant diseases.

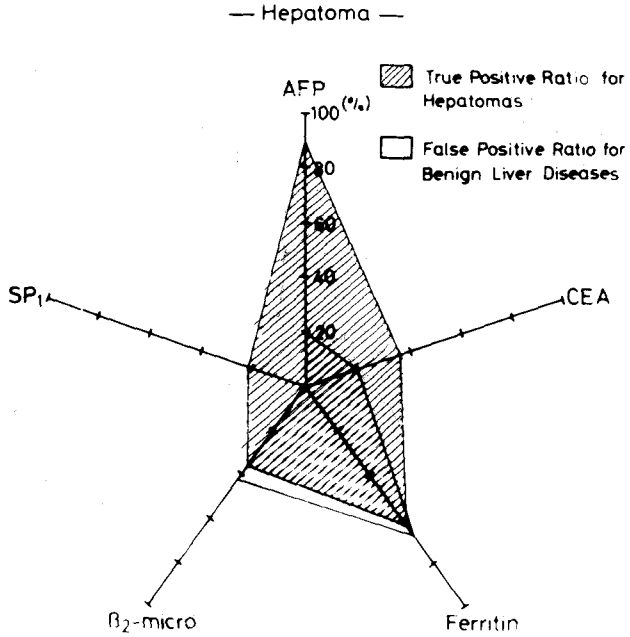

Fig. 5. Comparison of positive ratios among 5 tumor markers.

combinations are useful for diagnosis. In many cases, it has been shown that some (although not all) markers are beneficial in assessing the clinical course of a patient following treatment.

A schema, representing both the true positive ratio and the false positive ratio in benign disorders, is useful in evaluating the clinical situation. Figure 5 and 6 show such schemata in cases of hepatoma and metastatic liver carcinoma. By looking at these figures, it is apparent that AFP and CEA are useful for the diagnosis of hepatoma and metastic liver carcinoma.

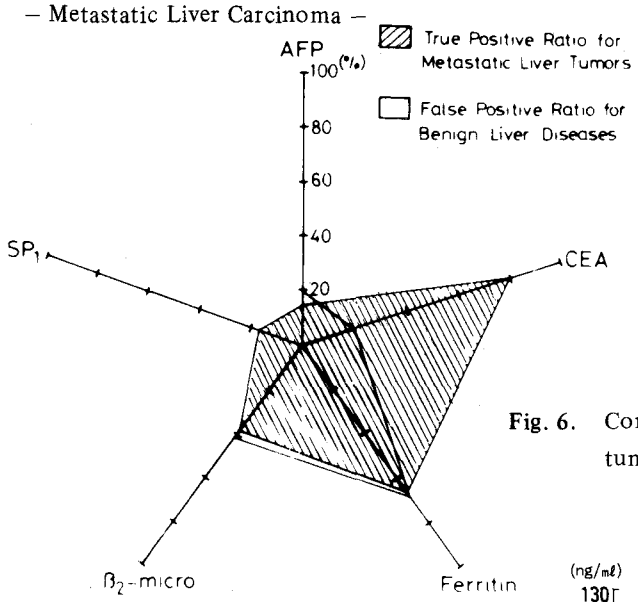

Fig. 6. Comparison of positive ratios among 5 tumor markers.

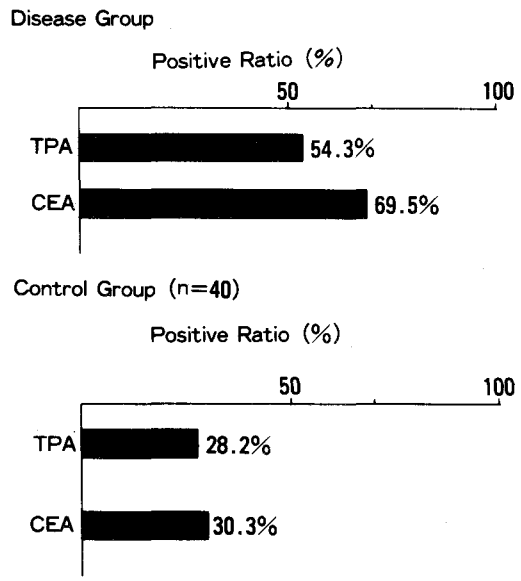

Fig. 7.
Serum TPA and CEA in the patients with cancers of various digestive organs.

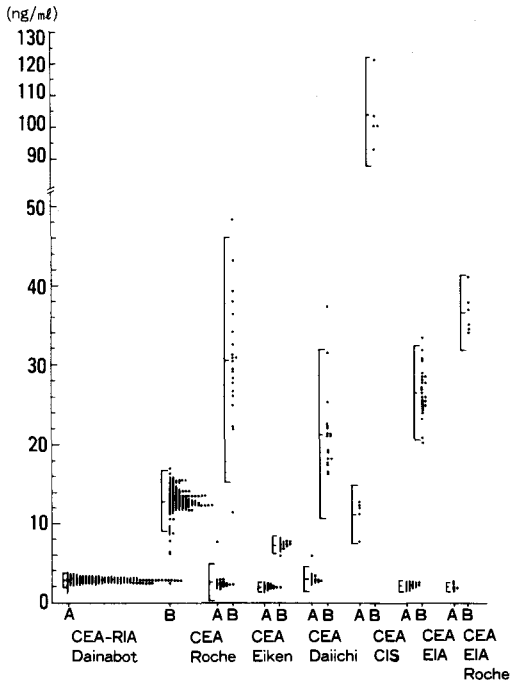

Fig. 8.
Measurement of two samples (A and B) by various CEA kits.

We should keep in mind the fact that subjects with no malignancies can show positive results. Figure 7 shows such results with TPA and CEA; both markers are show approximately a 30% false positive ratio.

I would like to stress some methodological problems in measuring tumor markers, especially on CEA by RIA. As shown in Figure 8, the CEA values obtained with various kits differ largely, perhaps because CEA is a heterogeneous protein. These are the results of a Quality Control Survey by the Japan Radioisotope Society, held yearly for the past 6 years. Similar problems were observed for the assay of insulin. At that time, we evaluated whether we could improve the variation by using common standards or serum in each assay. Table 3 shows the result of this experiment. Mode A and B indicate the results when the kit standard or common standards are used, respectively, as the standard material in the assay. It is clear from this table that Mode B has better results. Table 4 shows the calculated coefficients of variation, both between and within kit; Mode B shows a significant decrease in each variance determination.

A similar experiment was performed to evaluate CEA standardization. Table 5 shows the measurement of CEA-check (a CEA control sera, CEA Products, Scientific Laboratories, Denver, Colorado, USA) and our pooled sera. It can be seen that the variability is very large both between

Table 5. Measurement of the CEA-CHECK Control Plasma and Pooled Sera by Various CEA RIA KITS

	RIA Kit / SAMPLE	LEVEL	A Kit M	A Kit SD	A Kit CV (%)	B Kit M	B Kit SD	B Kit CV (%)	C Kit M	C Kit SD	C Kit CV (%)	D Kit M	D Kit SD	D Kit CV (%)	E Kit M	E Kit SD	E Kit CV (%)
CEA CHECK		1	4.4	1.0	22.3	4.5	0.4	8.4	1.8	0.2	10.9	7.5	2.4	31.5	4.8	0.4	7.8
		2	5.4	0.2	3.4	6.2	0.6	8.9	1.8	0.2	9.4	10.0	0.9	8.5	6.9	0.4	6.4
		3	11.0	0.7	6.3	15.7	1.1	6.8	3.2	0.1	2.7	36.3	3.7	10.1	18.1	0.8	4.5
		4	24.9	1.9	7.8	38.6	1.0	2.7	7.2	0.1	1.3	119.6	2.1	1.7	37.1	2.4	6.3
TOHO UNIV. LAB POOLED SERA		1	1.8	0.2	13.1	2.3	0.2	7.3	2.5	0.3	10.2	11.3	1.6	13.8	4.7	0.3	6.4
		2	3.6	0.5	13.3	4.9	0.4	8.2	3.8	0.5	11.9	22.1	2.3	10.2	7.3	0.2	2.9
		3	42.3	1.5	3.6	60.8	3.8	6.2	18.2	0.5	2.7	293	17.5	6.0	73.3	3.7	5.0
		4	301.9	12.0	4.0	329.4	12.1	3.7	81.9	7.7	9.4				445	2.0	4.7

Table 6. Measurement of Pooled Sera (1-4) by Common Standards (CEA-CHECK control plasma were used as standards)

	RIA Kit / SAMPLE	A Kit M	A Kit SD	A Kit CV (%)	B Kit M	B Kit SD	B Kit CV (%)	C Kit M	C Kit SD	C Kit CV (%)	D Kit M	D Kit SD	D Kit CV (%)	E Kit M	E Kit SD	E Kit CV (%)
TOHO UNIV. LAB POOLED SERA	1	ND			ND			9.2	1.3	15.0	5.9	1.03	17.0	1.3	0.3	26
	2	1.6	1.7	109	3.8	0.6	15.4	15.4	1.8	12.0	10.7	0.5	4.0	4.4	0.3	6.0
	3	45.8	1.5	3.0	56.0	3.9	7.0	85.0	3.6	4.0	32.8	0.9	3.0	70.8	2.2	3.0
	4	142.5	2.9	2.0	250	4.1	2.0	283	11.1	4.0				411	16.5	4.0

and within kits, when using the respective kit standards. However, Table 6 shows the values of our pooled sera when CEA-check sera are used as standards. If we compare the lower part of Table 5 and Table 6, it is clear that the variation is less in Table 6, especially in the values of pooled sera 3 and 4. We plan to determine if the same phenomenon is true for other analytes. If it is so, using common standards many result in more accurate and reproducible assays. The final

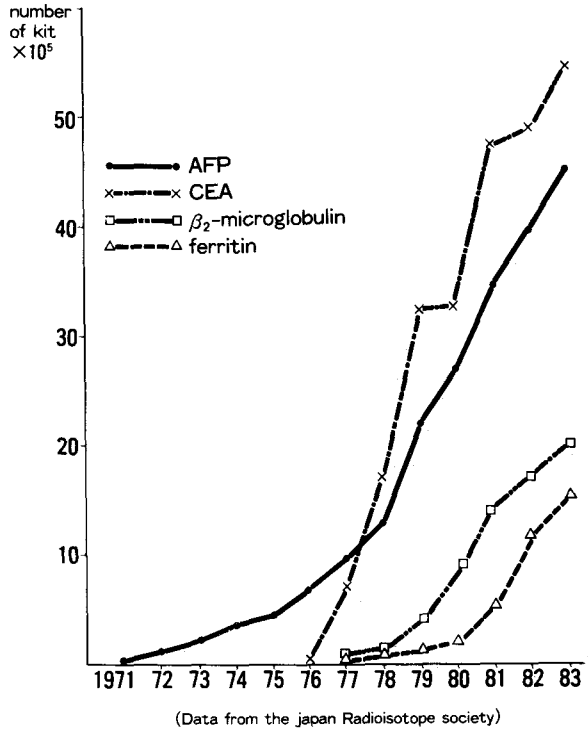

Fig. 9. Change of determination of CEA, AFP, β_2-microglobulin and ferritin, estimated from the number of kit used in Japan (1971-1983).

figure (Figure 9) shows the historical development of the determination of several tumor markers in Japan.

In summary, I have given an overview of the development of tumor markers in Japan. It is obvious that the radioimmunoassay of tumor markers are useful in the diagnosis and follow-up of the treatment of malignant diseases. Analysis of tumor markers in combination is especially important. However, it should be noted that these assays are not yet useful for early diagnosis or for screening at early stages of malignant disease. In addition, some assays have technical problems, which must be solved in future. In the future, the detection of cell or tissue specific oncogenetic substances will be useful in the early diagnosis and localization of malignancies.