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The Serum Levels of Human α-fetoprotein, AFP, Choriogonadotropin, hCG, Placental Lactogen, hPL, and Pregnancy-Specific β_1-Glycoprotein, SP_1, are of no Clinical Significance in Colorectal Carcinoma*

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Abstract—*Four biochemical tumor markers were measured by radioimmunoassays in sera of 233 patients with colorectal carcinoma. Pre-operative serum levels of AFP, hCG, hPL and SP_1 were elevated in 1.3, 5.4, 4.8 and 0.7% of the patients respectively. Post-operative serum levels either increased transiently or were elevated permanently. Even when an elevation of one or more of the markers was fairly stable, it rarely provided useful information, since sensitivity of the test, i.e. ratio of true positives to all patients with recurrence, ranged from 0% for AFP to 15% for hCG. The predictive value of an elevated level of any marker for the diagnosis of recurrence was either misleading or equivocal, which makes each of no value for decision strategy for the individual patient. Therefore, determination in serum of any of these markers in patients with colorectal carcinoma does not seem to provide useful information for pre-operative diagnosis, nor for post-operative monitoring aimed at the detection of operable recurrence.*

INTRODUCTION

ALTHOUGH the CEA plasma level has been considered a valuable biochemical tumor marker in patients with colorectal carcinoma for pre-operative prognosis and post-operative monitoring aimed at early detection of recurrence [1–3], it does not reflect tumor burden in all the patients. Since multiparametric systems have already been proposed for various cancers [4], we decided to evaluate the clinical significance of four other markers, AFP, hCG, hPL and SP_1, chosen because of reported abnormal levels of each in a proportion of patients with colorectal carcinoma [5–18].

To find out whether the frequency of occurrence of elevated serum levels, as well as the extent and stability of the elevation, could have clinical significance, serum levels of each marker were measured concurrently in sera of patients with local or loco-regional disease before and after surgery, or of those with inoperable disease.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 498 individuals. Of 265 healthy subjects, there were 115 men (median age, 29 yr), 75 pre-menopausal women (median age, 24 yr) and 75 post-menopausal women (median age, 59 yr). Sera from post-menopausal women were obtained at random times in the menstrual cycle. A total of

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233 patients, admitted to the Institute of Oncology between January 1976 and November 1979, had histologically-proven colorectal carcinoma (Table 1). Surgical–pathological staging, based on the depth of penetration of carcinoma of the rectum or colon, conformed to the classification of Dukes [19] and Astler and Coller [20] respectively; another category, stage D, as defined by Turnbull *et al.* [21], was used to designate patients with more advanced cancer. The latter stage was subdivided into two substages: unresectable cancer due to extension into adjacent organs, stage D_1, and cancer with distant metastases, stage D_2.

Methods

Blood was collected from each patient on at least one occasion pre-operatively and several times post-operatively. Serum samples from controls and patients were stored at −60°C until assayed.

Serum AFP levels were measured by one-antibody–PEG assay [22, 23] with a sensitivity of 0.5–0.8 ng/ml, using the reagents kindly donated by Dr. E. Ruoslahti (City of Hope National Medical Center, Duarte, CA, U.S.A.) and the First International Standard of AFP 72/225, donated by Dr. P. Sizaret (IARC, Lyon, France). Serum hCG was measured by a double-antibody assay, using an antiserum against the β subunit of hCG [24] with a sensitivity of 0.3–0.6 ng/ml, serum hPL was measured by a double-antibody assay [15] with a sensitivity of 0.5–0.9 ng/ml. Purified hCG (CR-119) and hPL, as well as anti-hCG-β (SB-6) and anti-hPL (CT-3399), were gifts from the NICHHD and NIAMDD (Bethesda, MD, U.S.A.); anti-hCG-β (SB-6, pool 2/72) was a gift from Dr. J. L. Vaitukaitis (Boston University, Boston, MA, U.S.A.). Serum SP_1 was measured by a double-antibody assay [25] with a sensitivity of 0.5–0.7 ng/ml, using antigen donated by Dr. H. Bohn (Behringwerke, Marburg, BRD) and antibody donated by Dr. S. W. Rosen (NIAMDD, Bethesda, MD, U.S.A.). Crude radioimmunoassay data were fitted by a computer program developed in our Institute [26].

The 99th percentiles [27] from the control populations of men, pre- and post-menopausal women were taken as cut-off values (Table 2). The pre-operative results on samples taken at one or more occasions were lumped. The post-operative results were analyzed using standard methods and criteria of clinical decision [28].

During follow-up, the patients had repeated standard clinical, biochemical and endoscopic examinations, complemented, if necessary, by radiographic, scintigraphic and ultrasonographic methods.

RESULTS

Pre-operative serum levels of tumor markers

The results in controls and patients with colorectal carcinoma are shown in Table 2.

The cut-off value for AFP was 12.5 ng/ml for men and 9.5 ng/ml for women. Of the 149 patients with colorectal carcinoma examined before surgery, only two (1.3%) had slightly elevated values: a man with stage C and a woman with stage D_2 disease, who had 14.0 and 12.0 ng/ml respectively.

The cut-off value for hCG was 1.2 ng/ml for men and pre-menopausal women, and 2.9 ng/ml for post-menopausal women. Of the 147 patients, only 8 (5.4%) had slightly elevated values: three pre-menopausal women with stage B, four men with stage C or D_1 and one post-menopausal woman with stage D_2 disease,

Table 1. Localization of the tumor, surgical–pathological stage and type of study as related to the time of surgery

Localization	Type of study	Number of patients: Total	Stage A	B	C	D_1	D_2
Colon	Before and after surgery	45	0	20	12	1	12
	After surgery only	34	1	17	16	0	0
	Subtotal	79	1	37	28	1	12
Rectum	Before and after surgery	106	2	31	41	16	16
	After surgery only	48	3	22	23	0	0
	Subtotal	154	5	53	64	16	16
	Total	233	6	90	92	17	28

Table 2. Median, 95th and 99th sample percentiles in ng/ml *for biochemical tumor markers in controls and patients with colorectal carcinoma before surgery*

Diagnostic category	AFP				hCG				hPL				SP_1			
	n	*M*	95	99	*n*	*M*	95	99	*n*	*M*	95	99	*n*	*M*	95	99
Controls																
Men	115	1.6	7.1	12.4	115	<s	0.9	1.2	115	<s	1.1	1.3	115	1.5	3.5	4.4
Pre-menopausal women	75	1.5	7.7	9.3	75	<s	1.1	1.2	75	<s	1.0	1.3	75	1.4	3.3	4.2
Post-menopausal women	75	1.7	7.2	9.5	75	0.8	1.7	2.9	75	<s	1.2	1.3	75	1.8	3.4	4.5
Colorectal cancer																
Stage B	51	0.7	6.1	8.6	51	<s	1.8	2.5	48	<s	1.4	2.6	49	1.4	3.1	3.3
Stage C	53	1.6	12.0	14.0	53	<s	1.2	1.8	53	<s	2.0	6.6	53	1.3	4.1	12.9
Stage D_1	17	2.6	9.5	9.5	16	<s	1.8	1.8	17	<s	0.7	0.7	17	1.2	1.9	2.8
Stage D_2	28	2.2	9.0	12.0	27	<s	1.7	6.7	28	<s	1.2	1.2	22	1.6	3.0	3.3

Abbreviations: *n*, number of patients; *M*, median; <s, below sensitivity of the assay.

who had the following maximum values: 2.4, 1.8 and 6.7 ng/ml respectively.

The cut-off value for hPL was 1.3 ng/ml for both men and women. Of the 146 patients, only seven (4.8%) had slightly elevated values: two with stage B and five with stage C disease, who had values ranging to 2.6 and 6.6 ng/ml respectively.

The cut-off value for SP_1 was 4.5 ng/ml for both sexes. Of the 141 patients, only one (0.7%) with stage C disease had the level raised to 12.9 ng/ml.

Thus, the sensitivity of AFP, hCG, hPL and SP_1 assays for diagnosis of tumor burden was 1.3, 5.4, 4.8 and 0.7% respectively, which makes each marker of no diagnostic potential for colorectal carcinoma.

Post-operative serum levels of tumor markers

Of a total of 233 patients, 184 were followed-up after curative surgery or palliative resection for a median time of 19 months (the semi-interquartile range was 8 months). Of them, 4 had stage A, 82 stage B, 93 stage C and 5 stage D_2 disease.

Transient elevations of AFP were found in five patients: two weeks after curative surgery the levels increased to 50 ng/ml in two patients with stage B, and from 32 to 59 ng/ml in three others with stage C disease. In all of these patients these values dropped down to the normal range 2–3 weeks later and remained in that range during subsequent examinations.

Elevated levels of hCG were found in 15 patients: in six with stage B and in eight with stage C disease, of whom two and five had recurrences respectively, as well as in one patient with liver metastases. The elevation of hCG in patients with tumor relapse ranged from 1.5 to 6.7 ng/ml, and was found either from 14 to 4 months in advance of, or concomitantly with, clinically evident recurrence. In patients without tumor relapse the elevations ranged from 1.5 to 4.2 ng/ml. The rise was stable in one patient with stage B and in three patients with stage C disease; of the latter, two patients had concomitant cirrhosis of the liver. Only transient elevations were found in the remaining three patients.

Transient elevations only of serum hPL were found in eight patients: in one with stage B disease without apparent tumor relapse, and in seven with stage C, of whom four had recurrence. The elevations ranged from 1.8 to 2.1 ng/ml.

Similarly, transient elevations of serum SP_1 were found in six patients: in two with stage B and in 4 with stage C disease; only one patient in each group had recurrence. The elevations ranged from 4.9 to 13.5 ng/ml.

On the other hand, normal levels of each marker were found in sera of most of the patients with tumor relapse (Table 3). Thus, none of the markers appeared to be useful for post-operative monitoring aimed at diagnosis of recurrence, since sensitivity of each was slender, from 0 to 15%, and the predictive value of a positive test was misleading or equivocal (Table 3).

Concordant elevations. Concordant, though only transient, elevations of hCG to 4.4 ng/ml and of hPL to 1.8 ng/ml were found in two patients with stage C disease (one after curative and the other after palliative surgery). Concordant elevations of hCG to 4.3 ng/ml and of SP_1 to 8.3 ng/ml were found in a patient with

Table 3. Serum levels of biochemical tumor markers; their value for post-operative monitoring (184 patients)

Marker	Truth table* TP	FP	TN	FN	Se	Conventional terms* Sp	PV(+)	PV(−)	Acc
	No. of patients					Percentages			
AFP	0	5	127	52	0	96	0	71	69
hCG	8	7	125	44	15	95	53	74	72
hPL	4	4	128	48	8	97	50	73	72
SP_1	2	4	128	50	4	97	33	72	71

Abbreviations: T, true; F, false; P, positive; N, negative; Se, sensitivity; Sp, specificity; PV, predictive value; (+), of a positive test; (−), of a negative test; Acc, accuracy of all correct outcomes.

*All these parameters are understood as clinical indexes validating the biochemical test[28].

stage B disease, about four months before diagnosis of resectable recurrence; the elevated levels of each marker declined two weeks after surgery. Concordant elevations of hPL to 1.9 ng/ml and of SP_1 to 6.6 ng/ml were found in one patient with stage C disease, apparently without tumor relapse, and elevations of hCG to 1.5–5.0 ng/ml, of hPL to 2.1–6.5 ng/ml and of SP_1 to 4.9–7.0 ng/ml were found after curative surgery in two patients with stage C disease and concomitant liver cirrhosis.

DISCUSSION

The proportion of patients with pre-operatively normal levels of CEA ranges from 75% in stage A to 15% in stage D_2 disease, whereas that of patients with post-operatively normal levels at the time of recurrence is below 10% [3]. Thus, there still exists a need for a marker or a battery of markers which could be useful for diagnosis and prognosis in the majority of patients with less advanced stages, and for the 10% of the patients who relapse without CEA elevation.

The association of elevated levels of AFP with colorectal carcinoma was reported either as case reports [8] or larger studies [5, 7]. To date, serum elevations of AFP have been described in 3–13% of the patients [5, 6], of whom at least 50% had liver metastases. Only Todorov *et al.* [7] described elevated levels of AFP in as many as 60% of 35 patients, of whom four had liver metastases. It is evident from the above reports, excluding the data of Todorov *et al.* [7], and from our study that measurement of serum AFP levels seems to be of no clinical value for diagnosis and monitoring of patients with colorectal carcinoma.

Elevated serum levels of hCG were reported in 0–20% of patients with colorectal carcinoma [9–14]. Our findings of pre-operatively elevated levels in 5.4% of the patients and post-operatively elevated levels in 15% confirm the results of others and provide evidence that hCG seems to be of no value for the diagnosis and monitoring of colorectal carcinoma. The marginal and frequently transient elevations of hCG may be explained by the presence of an hCG-like substance elaborated both in normal tissues [29, 30] and carcinomas [31].

Ectopic production of hPL was reported previously [15, 16]. Our results confirm the previous reports, indicating that raised levels of hPL are usually marginal and occur rarely, and are therefore unlikely to provide useful clinical information.

Marginally raised serum levels of SP_1 were reported by some [17, 18], whereas others [32] could not find elevated levels of SP_1 in patients with gastro-intestinal cancer. Our results support the latter view concerning colorectal carcinoma. The minute elevations of SP_1 found in this study may be due to its production by normal fibroblasts [33]. Therefore, SP_1 is unlikely to provide a guide for diagnosis of tumor burden.

In conclusion, marginally and, for the most part, transiently elevated serum levels of AFP, hCG, hPL and SP_1 are unlikely to make a sensitive guide for pre-operative diagnosis and prognosis, as well as for post-operative monitoring of patients with colorectal carcinoma aimed at early detection of recurrence.

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