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Detection of Carcinomas in an Asymptomatic Chinese Population: Advantage of Screening With Multiple Tumor Markers

Kuo-Chien Tsao,[1] Tsu-Lan Wu,[1] Pi-Yueh Chang,[1] Ji-Hong Hong,[2] and James T. Wu[3,4]*

[1]*Department of Pathology, Chang Gung Memorial Hospital, Taipei, Taiwan*
[2]*Department of Radiation Oncology, Chang Gung Memorial Hospital, Taipei, Taiwan*
[3]*Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah*
[4]*ARUP Laboratories, University of Utah Health Sciences Center, Salt Lake City, Utah*

A total of 73,443 asymptomatic individuals were screened on a voluntary basis for cancer at Chang Gung Memorial Hospital in Taiwan using a panel of tumor markers, including alpha fetoprotein (AFP), CA 125, CA 15-3, CA 19-9, carcinoembryonic antigen (CEA), prostate specific antigen (PSA), chromogranin A (CgA), and squamous cell specific antigen (SCC). The results are derived from data collected from January 1998 to October 2003. A total of 210 cancers (approximately 0.3%) were detected, including cancers of the liver, lung, colon, prostate, stomach, pancreas, breast, cervix, ovary, and bladder. Of the tumor markers monitored, elevated CA 19-9, CEA, and CA 125 were the most frequently detected in a variety of cancers. It was surprising to find that many cancers were not detected by their dominant markers but by the elevation of tumor markers not recommended for monitoring their tumor activity. Screening with multiple circulating tumor markers provides improved sensitivity for cancer detection in asymptomatic individuals before they reach the fatal advanced stage. Screening with multiple tumor markers also allows cancers to be detected in the absence of their dominant markers. If we had not measured the multiple tumor markers, these cancers would have gone undetected. J. Clin. Lab. Anal. 20:42–46, 2006.

Key words: asymptomatic population; Chinese; carcinoma; multiple tumor markers

INTRODUCTION

It is well known that currently employed circulating monoclonal antibody-defined tumor markers do not have sufficient specificity and sensitivity for cancer detection [1]. As a consequence, circulating tumor markers are not recommended for screening asymptomatic individuals for cancer, with the exception of prostate specific antigen (PSA) for prostate cancer [2] and alpha fetoprotein (AFP) for hepatoma in Asian countries.

It is also well recognized that none of the current tumor markers are tumor-specific. Therefore, circulating tumor markers are mainly used to monitor the success of treatment during therapy and detect postsurgical recurrence in cancer patients. It is well known that there is a large time window between the beginning of tumorigenesis and the final advanced stage of cancer development. Multiple mutations have to take place before a tumor develops into cancer and then to the end metastatic stage. Conceivably, detection of cancer at an earlier stage would provide a better opportunity to leading to curative treatment.

Abbreviations: AFP, alpha-fetoprotein; ca, carcinoma; CEA, carcinoembryonic antigen; CgA, chromogranin A; PSA, prostate specific antigen; tPSA, free PSA+PSA-ACT complex; SCC, squamous cell carcinoma antigen; NPC, nasopharyngeal carcinoma.

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*Correspondence to: James T. Wu, Ph.D., ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108. E-mail: wuj@aruplab.com

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TABLE 1. Types and number of cancers detected by screening with multiple tumor markers

Type of cancer	01/1998–10/2001	11/2001–10/2003	Total
Hepatoma	49	13	62
Lung cancer	28	6	34
Colon cancer	25	6	31
Prostate cancer	19	2	21
Gastric cancer	8	2	10
Pancreatic cancer	9	1	10
Breast cancer	9	1	10
Cervical cancer	4	1	5
Ovarian cancer	0	2	2
Bladder cancer	0	2	2
NPC	0	1	1
Esophageal cancer	0	1	1
Others	17	4	21
Total # of cancers detected	168	42	210
# of individuals screened	61,343	12,100	73,443
% of cancer detected	0.274%	0.347%	0.286%

In fact, elevated tumor markers are frequently detectable at the early stage of carcinogenesis. As disease progresses, the levels of tumor markers often increase, and the highest circulating concentration of tumor markers is usually detected at the advanced metastatic stage [1]. In earlier studies we learned that most metastatic malignant diseases are associated with the elevation of not just one but multiple tumor markers [3], especially following metastasis.

In an attempt to detect cancer at an earlier stage, we employed a panel of multiple markers, including AFP, CA 125, CA 15-3, CA 19-9, carcinoembryonic antigen (CEA), PSA, chromogranin A (CgA), and squamous cell specific antigen (SCC), to screen subjects who were apparently healthy and asymptomatic. Individuals participated in the screening were on a voluntary basis. They voluntarily paid the fee for screening; therefore, health insurance was not required. The present report summarizes the results of screening for a period of 5 years, from January 1998 to October 2003 (Table 1). Apparently, various cancers are detectable in asymptomatic individuals if a panel of multiple circulating tumor markers is monitored. Screening with multiple tumor markers greatly improves the sensitivity of cancer detection. We believe that the benefit provided from the simple, inexpensive blood screening outweighs all other factors considered, especially when it is done on a voluntary basis.

MATERIALS AND METHODS

Measurement of Tumor Markers

Serum levels of AFP, CEA, and PSA were determined with the use of Abbott Architect 2000 (Abbott Laboratories, Abbott Park, IL). Serum levels of CA19-9, CA125, CA15-3, and SCC were measured with the Abbott AXSYM instrument. Squamous cell carcinoma antigen (SCC) was determined with the Abbott IMX. CgA was measured with an in-house-developed enzyme-linked immunosorbent assay (ELISA) [4]. The upper cutoffs for the tumor markers are as follows: 15 ng/mL for AFP, 5 ng/mL for CEA, 37 U/mL for CA 19-9, 4 ng/mL for tPSA, 35 U/mL for CA 125, 4 ng/mL for tPSA, 30 U/mL for CA 15-3, 1.9 ng/mL for SCC, and 100 ng/mL for CgA.

TABLE 2. Effect of age and gender on the rate of detection from 1998 to 1999

Age group	#Screened	Cancer detected (%)
20–29	3,602	0
30–39	8,833	0.5
40–49	12,312	0.1
50–59	8,594	0.3
60–69	5,525	0.89
70–80	2,629	0.76

Screening Cohort

The subjects who were screened with multiple tumor markers were all asymptomatic individuals (males and females, 20–80 years old) who visited Chang Gung Memorial Hospital for their regular health checkup (Table 2). The majority of the individuals were 30–59 years old (71.7%). Screening was performed on a voluntary basis. Samples were analyzed within a few days after blood was drawn, and were not frozen.

Screening

The entire screening was conducted from January 1998 to October 2003. A total of 73,443 individuals were screened. There were 38,878 females (53%) and 34,565 males (47%). Individuals detected with elevated tumor markers were invited back to Chang Gung Memorial Hospital and were examined in detail by oncologists. We were not sure how many of these asymptomatic individuals had a history of cancer. It was certain, though, that none of these subjects were actively receiving care by physicians at that time.

Between January 1998 to November 2001 a total of 61,343 individuals participated in the screening. During that period only epithelial-derived tumor markers, such as AFP, CEA, CA 125, CA 15-3, CA 19-9, and PSA, were included in the screening panel. CA 19-9 and PSA were measured only in males. CA 125 and CA 15-3 were measured only in females.

Between December 2002 to October 2003, two cell-specific tumor markers–chromogranin A (CgA) specific for

neuroendocrine cells, and squamous cell specific antigen (SCC) specific for squamous cells–were added to the panel. CA 19-9 was also added to the panel for females.

RESULTS

Types and Numbers of Cancers Detected

Between January 1998 and October 2001 we detected 168 cancers of various types of carcinoma in a total of 61,343 asymptomatic individuals screened (Table 1). We expect that we would have detected more cancers if we had also screened the females with CA 19-9 and males with CA 125 during that period.

Between November 2001 and October 2003, an additional 12,100 asymptomatic individuals were screened and 42 more cancers were detected. The fact that the percentage of cancer detected increased from 0.274% to 0.347% (Table 1) is most likely due to the addition of CA 19-9 for females and the addition of the cell-specific tumor markers CgA and SCC to the panel.

The total number and percentage of cancers detected are startling. Despite the lack of satisfactory specificity and sensitivity of these tumor markers, the panel of multiple tumor markers was able to detect carcinomas at a high percentage in the asymptomatic general public.

Sensitivity of Individual Tumor Markers

We define dominant tumor markers as monoclonal antibody-defined tumor markers with relatively better specificity and sensitivity for a certain type of carcinoma, such as CEA for colorectal carcinoma (ca.), CA 125 for serous ovarian ca., CA 15-3 for breast ca., and CA 19-9 for pancreatic ca. [1]. These individual dominant tumor markers are recommended for monitoring during treatment and detecting recurrence for patients with different types of carcinoma.

The association of the number of individual tumor markers with various types of cancer detected in this study is shown in Table 3. Elevated AFP was largely associated with hepatoma. Elevated CEA and CA 19-9 were also found in most colorectal and pancreatic carcinomas, respectively. PSA was associated mostly with prostate cancer, with high sensitivity. Apparently, CA 15-3, a dominant tumor marker for breast cancer, has poor sensitivity for breast cancer. There was only a 14% detection rate for breast cancer with CA 15-3. In fact, CA 15-3 was seldom found elevated during screening.

Because each cancer detected can be associated with the elevation of more than one tumor marker, any single tumor marker can be elevated in other cancers as well as its individual dominant cancers (Table 3). Both CEA and CA 19-9 appeared to be most sensitive for detecting not only colorectal and pancreatic cancer, respectively, but for other cancers as well (Table 4). CA 15-3 had the lowest sensitivity for detecting not only breast cancer, but also all types of malignant diseases. It was surprising to find such a high percentage of neuroendocrine cell-specific CgA and squamous cell specific SCCs (0.128 and 0.078, respectively) in epithelial cell-derived carcinomas.

It should be noted that because individual cancers were frequently associated with multiple elevated tumor markers, the total number of cancers based on the number of cancers associated with each single tumor marker ($n = 263$) exceeded the total number of cancers detected ($n = 210$). This is because that the number of cancers detected may have been counted more than once.

Effect of Gender and Age

As shown in Table 2, the results of the screening indicate that most of the individuals who tested positive for cancer were 60–69 years old. There was a large increase in the cancer detection rate from the 50–59-year-old group to the 60–69-year-old group. The incidence of cancer in males was almost three times that in females even though an approximately equal number of males and females participated in the screening. In this study no cancer was detected in individuals under the age of 30 years. Our data indicate that a general screening (for carcinomas) should only be conducted for individuals ≥ 40 years of age, for cost-effectiveness.

Advantage of Cancer Detection With Multiple Tumor Markers

We found that most cancers, especially after metastasis, were often associated with elevation of multiple

TABLE 3. Number of cancers associated with individual elevated tumor markers*

Tumor marker	AFP	CEA	CA 19–9	CA 15–3	CA 125	PSA	SCC	CgA
Total ♯ screened	79226	73443	39387	38863	38875	34528	8937	6263
♯ cancer detected	50	83	60	7	25	37	7	8
Detection (%)[a]	0.06	0.113	0.152	0.018	0.064	0.107	0.078	0.128

*Because many cancers are associated with multiple markers, the number of cancers detected was counted more than once based on individual tumor markets. Therefore, the total ♯ of cancers detected here is 263 (should be 210).
[a]% Detection: example for AFP, 50/79226 = 0.06.

TABLE 4. Cancers detected in the absence of dominant tumor markers (D) but with tumor markers not recommended for tumor activities monitoring*

	Liver	Lung	Stomach	Colon-rectal	Ovary	Prostate	Cervix	Pancreas	Breast	Unknown primary
CA 199	10	8	5	6	1			0,D	0	2
CEA	3	24	4	0,D	1			1	0	2
CA 125	2	10	1	2	0,D		1	0	2	1
CA 153		4							0,D	
PSA	1	1		1		0,D				
AFP	0,D	3		1						
CgA	1		1	2						
SCC		2		1						1

*Data for SCC and CgA were only available between 2001–2003. No dominant tumor marker was assigned to lung cancer.
D, dominant marker.

tumor markers. For example, as shown in Table 4, hepatoma was associated not only with the elevation of its dominant tumor marker, AFP, but also with elevation of CA 19-9, CEA, CA 125, and PSA. Colorectal carcinoma was found to be associated with elevation of CEA as well as CA 19-9, CA 125, PSA, AFP, CgA, and SCC.

CA 19-9 and CA 125 have traditionally been recommended as the dominant tumor markers for pancreatic ca. and serous ovarian ca., respectively. CA 19-9 and CA 125 have been recommended to monitor pancreatic tumor activity and ovarian tumor activity, respectively, during therapy. However, as shown in Table 4, we found a surprising number of hepatomas containing elevated CA 19-9, and lung cancers expressing CA 125 and CEA. These data may point out the importance of using multiple tumor markers (not just the dominant marker) for cancer screening.

The use of multiple tumor markers for screening is even more strongly supported by the surprising fact that we found some cancers that did not express their dominant tumor markers. For example, AFP is known to be the dominant tumor marker for hepatoma, but many of the liver cancers detected did not express elevated AFP. As shown in Table 4, elevated CA 19-9, CEA, CA 125, and even PSA and CgA were found in liver cancer in the absence of elevated AFP. Similar situations were found in colorectal carcinoma without elevated CEA (the dominant marker for colon carcinoma), ovarian carcinoma without CA 125 (the dominant marker for serous ovarian cancer), lung cancer without elevated CEA or CA 19-9, breast carcinoma without elevated CA 15-3 (the dominant marker for breast carcinoma), and pancreatic carcinoma without elevated CA 19-9 or CEA. Consequently, the detection of some cancers was attributed to the elevation of tumor markers other than their dominant tumor markers. Conceivably, if we had not screened with a panel of multiple markers, these cancers would have not been detected.

The results in Table 4 also indicate that CA 19-9, CEA, and CA 125 were not only the most sensitive as the dominant markers for detecting pancreatic, colorectal, and ovarian cancer, respectively, they were also the most sensitive tumor markers for detecting a variety of other cancers. Elevation of CA 19-9, CEA, and CA 125 was most frequently detected regardless of the type of cancer. Table 4 shows that the elevation of CA 19-9 was associated with not only cancer of the pancreas, but also cancer of the liver, lung, stomach, colon, and ovary. Similar findings were also obtained for CA 125 and CEA. Apparently the drawback of the nonspecificity of these tumor markers in cancer diagnosis turned out to be an advantage for screening. The fact that many cancers do not express their dominant tumor markers again highlights the importance of screening with multiple tumor markers.

Cell-Specific Tumor Markers

CEA, CA 19-9, CA 125, and CA 15-3 are all monoclonal defined tumor markers for monitoring the tumor activity of epithelial cell-derived carcinomas. However, in this study elevated SCC was also found in carcinomas, which indicates that SCC should be included in the panel of multiple tumor markers for cancer screening. In just 2 years (November 2001–October 2003) we detected three cancers in females (3/4855, 0.06%) and four cancers in males (4/4082, 0.1%) based on the measurement of SCC. Three out of seven cases of elevated SCC were not associated with elevation of other tumor markers. The other four cases of elevated SCC were usually associated with elevated CA 19-9 and CA 125.

CgA, a tumor marker specific for neuroendocrine cells, also appears to be useful for screening carcinoma.

We previously reported the detection of elevated CgA in prostate cancer patients undergoing hormonal therapy [5] and in various carcinomas at the advanced stage [6]. Therefore, it was not surprising to find elevated CgA in this study. However, we did not expect such a high positive rate in carcinomas with this cell-specific tumor marker. In this study elevated CgA was detected in three cancers in females (3/3374, 0.09%) and five cancers in males (5/2889, 0.17%). About half of the elevated CgA cases were not associated with any other elevated tumor markers. The addition of cell-specific tumor markers to the panel in the second period largely accounts for the increased percentage of cancer detection (from 0.274% to 0.347%; Table 1). Part of the increase in the second period was also due to the addition of CA 19-9 for females.

CA 19-9 for Females

CA 19-9 was not included in the screening for females in the first period (1998–2001). It was added for female screening later, from November 2001 to October 2003. Consequently the clinical utility of CA 19-9 has not been tested as extensively as we would like. However, based on the first 2-year screening results, CA 19-9 appears to be useful for detecting cancer in females. Three cancers were detected in 4,856 females screened (3/4856, 0.06%) with CA 19-9. During the same period, approximately three times more cancers were detected in males (9/5456, 0.16%).

DISCUSSION

As pointed out by Schwartz et al. [7], the public is becoming increasingly enthusiastic about cancer screening. The use of multiple markers for screening has also gained increasing acceptance. Although many studies have attempted to use multiple tumor markers for screening, most of these investigations focused on detecting one specific type of cancer. For example, van Haaften-Day et al. [8] focused on detecting early-stage epithelial ovarian carcinoma. They found that the sensitivity of a combination of three serum markers was significantly greater than that of the CA-125-II assay alone in patients with primary ovarian epithelial tumors of different histotypes. Similarly to what we concluded in this study, they also found that the nonspecificity of tumor markers, such as CA 125, was advantageous for screening.

In this study it was surprising to find that this panel of multiple tumor markers was able to detect such a high percentage of various cancers. We selected these tumor markers in a panel for screening because we were interested in detecting only the most frequently occurring carcinomas. The success of this screening leads us to consider expanding the screening program to other malignancies (e.g., hematological and gynecological cancers [9]) and even rare cancers (e.g., pheochromocytoma) in addition to carcinoma in the future. In fact, elevated CA 125 has been detected in many hematological malignancies, and we believe that CA 125 should also be added in the future to the panel for males.

Methods for simultaneously measuring multiple analytes in a given sample were recently developed. These include the multi-analyte profiling (LabMAP^{TM}) system (known as Luminex) developed by the Luminex Corp. (Austin, TX). A different technique was also developed to adapt the ELISA to a multiplexed array format for the development of protein chips [10]. A multiplex ELISA designed to measure PSA, alpha1-antichymotrypsin-bound PSA, and interleukin-6 at the same time has also been described [11]. Efforts should be made in the future to apply these multiplexed array assays to screen for cancer with multiple tumor markers.

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