Clinica Chimica Acta 450 (2015) 273–276

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Cancer screening through a multi-analyte serum biomarker panel during health check-up examinations: Results from a 12-year experience

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ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 26 August 2015
Accepted 1 September 2015
Available online 3 September 2015

Keywords:
Cancer screening
Biomarker
Tumor marker

ABSTRACT

Background: The use of blood-based tumor biomarkers for screening malignancies at early stages has significant advantages, including being convenient, automated, quantitative, objective, and relatively inexpensive compared with histology, endoscopy, and imaging.
Methods: We describe our 12-year experience on the diagnostic usefulness of a biomarker panel consisting of eight molecules (i.e., α-fetoprotein, carcinoembryonic antigen, prostate-specific antigen, CA 19-9, CA125, CA 15-3, squamous cell specific antigen, and cytokeratin 19 fragment) for cancer screening in Taiwanese subjects who underwent a health check-up examination at their own expenses.
Results: The sensitivity of the panel for the detection of specific cancers was higher than that of isolated cancer-specific markers. Specifically, the sensitivity of the panel for identifying the four most commonly diagnosed malignancies (i.e., liver cancer, lung cancer, prostate cancer, and colorectal cancer) was 90.9%, 75.0%, 100%, and 76.9%, respectively. The ability of the panel to detect early-stage (stage 1) hepatocellular carcinoma (HCC) or prostate cancer was similar to that observed for advanced malignancies.
Conclusions: The multi-analyte biomarker panel is clinically useful during health check-up examinations for the screening of different tumors (especially for the early detection of HCC and prostate malignancies).

1. Introduction

Early detection of cancer is paramount for improving treatment strategies and survival outcomes. The use of blood-based tumor biomarkers for screening malignancies at early or preclinical stages has significant advantages, including being convenient, automated, quantitative, objective, and relatively inexpensive compared with histology, endoscopy, and imaging [1]. However, the relative low sensitivity and specificity for early cancer identification has generally limited the widespread use of single biomarkers for screening purposes in a general population setting. A potential strategy to circumvent this issue is the combined use of multiple serum markers into diagnostic biomarker panels.

Previous studies have shown that biomarker panels are diagnostically superior to single markers for the early detection of ovarian cancer [2], gastric cancer [3], and pancreatic cancer [4]. Moreover, Zhang et al. [4] demonstrated that a panel consisting of carbohydrate antigen 19-9 (CA 19-9), albumin, C-reactive protein, and interleukin-28 is not only characterized by a good sensitivity for the specific detection of pancreatic malignancies but also for cancer in general. The possibility of developing combined biomarker panels that could be used for the screening of different malignancies has been previously suggested [5]. In the Department of Laboratory Medicine of the Chang Gung Memorial Hospital (CGMH), we routinely propose to evaluate a cancer biomarker screening panel to all Taiwanese subjects who are willing to undergo a voluntary health check-up examination at their own expenses (without a physician's prescription).

As of January 1998, the cancer biomarker screening panel proposed in the CGMH consists of α-fetoprotein (AFP), carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), CA 19-9 (for men), CA125 (for women), and CA 15-3 (for women). Between December 2001 and May 2002, we introduced the use of CA 19-9 (for women), chromogranin A (CgA, for both genders) and squamous cell-specific antigen (SCC, for both genders). However, the measurement of CgA was discontinued as of May 2002 (because of its low negative predict value) and replaced with cytokeratin 19 fragment (CYFRA 21-1). Previous data at 5 y have shown that the multi-analyte panel has a better sensitivity than single biomarkers for cancer screening [5]. However, the exact sensitivity of the biomarker panel versus that of individual markers for the detection of different malignancies has not been reported. Furthermore, data on the ability of the panel to detect early-stage tumors are not yet available.

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http://dx.doi.org/10.1016/j.cca.2015.09.004
0009-8981/

2. Materials and methods

2.1. Study subjects

Subjects who presented at the CGMH between May 2001 and April 2013 to undergo a health check-up examination at their own expenses (without a physician's prescription) were offered to have a multi-analyte panel of cancer biomarkers measured for screening purposes at the Department of Laboratory Medicine. Blood samples (3 ml) were drawn by venipuncture from all participants. All subjects who had the panel measured and available clinical records at our Outpatient Department within one year of the analytical measurements were included in the study. Subjects with no further examination within one year and presence of malignancies before the analytical measurements were excluded from the study. Of the 71,886 cases included in the original CGMH dataset, a total of 41,516 records (19,998 men and 21,518 women, range 20 to 93 y) were available. The following variables were collected through the connection of our database to the national cancer registry: primary tumor site, date of diagnosis, clinical stage, histological type, and date of death or the last follow-up. The study was approved by the Ethics Committee of the CGMH.

2.2. Serum tumor marker detection

AFP, CA 15-3, CA125, PSA, SCC, CEA (measured until April 12, 2011), and CA19-9 (measured until December 1, 2009) were assayed using kits from Abbott Diagnostics. Assays for CYFRA 21-1 and CA 19-9 (measured as of December 2, 2009) were provided by Roche Diagnostics. CEA (measured as of April 13, 2011) was quantified using a Siemens Healthcare Diagnostics assay. The optimal cut-off values for each biomarker were as follows: 15 ng/ml for AFP, 35 U/ml for CA125, 30 U/ml for CA 15-3, 37 U/ml for CA 19-9, 5 ng/ml for CEA, 3.3 ng/ml for CYFRA 21-1, 4 ng/ml for PSA, 1.5 ng/ml and 2.5 ng/ml for SCC (measured as of November 26, 2008). All of the assays met the requirements for the College of American Pathologists (CAP) Laboratory Accreditation Program and ensured reproducible results. Subjects with at least one of the markers included in the panel showing values above the cut-off point were considered as being positive to the cancer biomarker screening panel.

2.3. Statistical analysis

Data were calculated with Microsoft Office Excel 2007 (Microsoft Inc.). Sensitivity and specificity and negative and positive predictive values were calculated within one year of the analytical measurement.

3. Results

3.1. Sensitivity, specificity, PPV, and NPV

Of the 41,516 study participants, 314 (0.756%) were diagnosed with a malignancy within one year of the assessment of the multi-analyte biomarker screening panel (Table 1). A total of 179 cases had at least one positive biomarker. The sensitivity, specificity, PPV, and NPV of the panel for screening purposes were 57% [179 / (179 + 135)], 88.7% [36,528 / (36,528 + 4674)], 3.7% [179 / (179 + 4674)], and 99.6% [36,528 / (36,528 + 135)], respectively. Specifically, the sensitivity of the panel for the detection of the four most commonly diagnosed malignancies (i.e., liver cancer, lung cancer, prostate cancer, and colorectal cancer) was 90.9%, 75.0%, 100%, and 76.9%, respectively. However, the panel had a poor sensitivity for identifying head and neck cancer (17.6%), breast cancer (37.5%), and cervical cancer (44.4%).

The sensitivity of the cancer biomarker screening panel for the detection of individual malignancies was higher than that of isolated cancer-specific markers (Table 2). The sensitivity of PSA alone for prostate cancer screening was 100%. Although the sensitivity of isolated AFP for the detection of hepatocellular carcinoma (HCC) was only 63.3%, the sensitivity of the panel was as high as 92.3%. Similarly, the sensitivity of the panel for pancreatic cancer screening was 88.9%, a value significantly higher than those observed for individual markers. Compared to the sensitivity of CEA alone for colorectal cancer screening (53.8%), the panel similarly showed an improved sensitivity (76.9%).

Table 1
Types and number of malignancies detected by the multi-analyte biomarker screening panel.

Malignancy (number of identified individuals)	Cancer biomarker screening panel		Sensitivity (%)
	Normal results	Abnormal results[a]	
Liver cancer[b] (33)	3	30	90.9
Lung cancer (36)	9	27	75.0
Prostate cancer (24)	0	24	100
Colorectal cancer (26)	6	20	76.9
Breast cancer (40)	25	15	37.5
Cervical cancer (27)	15	12	44.4
Bladder cancer (14)	5	9	64.3
Pancreatic cancer (9)	1	8	88.9
Gastric cancer (18)	11	7	38.9
Thyroid cancer (23)	17	6	26.1
Other cancers (12)	6	6	50.0
Hematopoietic and lymphoid cancer (11)	6	5	45.5
Head and neck cancer (17)	14	3	17.6
Thymus cancer (2)	0	2	100
Kidney cancer (9)	7	2	22.2
Uterine cancer (1)	0	1	100
Ovarian cancer (3)	2	1	33.3
Skin cancer (9)3	8	1	11.1
Total (314)	135	179	57.0

[a] Subjects with at least one of the markers included in the panel showing values above the cut-off point were considered as being positive to the multi-analyte biomarker screening panel.
[b] Liver cancer included both hepatocellular carcinoma and cholangiocarcinoma.

3.2. Effect of age on cancer detection rates

The prevalence of patients who were diagnosed with a malignancy increased with age, reaching the highest percentage (2.79%) in participants aged between 70 and 79 years (Table 3). Forty-three percent of subjects were >50 years. Notably, the percentage of cases successfully identified using the cancer biomarker screening panel was found to be age-dependent, with almost 80% of cases successfully identified in the 70–79 year age range.

3.3. Relationship between the number of abnormal biomarkers and cancer detection rates

Table 4 shows that 4853 individuals tested positive for 1 or more markers (representing 11.7% of the screened population). This suggests that 89.3% of people screened were negative for all biomarkers employed. Among those testing positive, 4674 did not develop cancer in the 1-year follow-up. This reflects an 11.2% false positive rate. The higher the number of tumor markers showing abnormal values in the screening panel, the greater the likelihood of having a malignancy (Table 4). More than 60% of all subjects with at least 4 abnormal tumor markers actually had a confirmed diagnosis of cancer.

3.4. Results of the panel in relation to the clinical stages of malignancies

The ability of the panel to detect early-stage (stage 1) hepatocellular carcinoma (HCC) or prostate cancer was similar to that observed for advanced malignancies (Table 5). Most of the study participants who were diagnosed with lung cancer or pancreatic cancer were in advanced clinical stages. The ability of the panel to detect colorectal cancer seemed to

Table 2
Sensitivity of the multi-analyte cancer biomarker screening panel and individual tumor markers for each malignancy.

Type of malignancy (number)	PSA (%)	AFP (%)	CEA (%)	CA19-9 (%)	CYFRA 21-1 (%)	CA125 (%)	SCC (%)	CA15-3 (%)	Panel (%)
Prostate cancer (24)	100	0	0	4.8	5.9	–	5.6	–	100
Hepatocellular carcinoma (26)	13.3	63.3	5.6	31.6	10	0	0	0	92.3
Pancreatic cancer (9)	0	0	55.6	62.5	33.3	66.7	0	0	88.9
Colorectal cancer (26)	7.1	5.9	53.8	25.0	38.9	22.2	5.9	12.5	76.9
Lung cancer (36)	9.1	5.7	72.2	12.9	40.9	20.0	8.7	20.0	75.0
Bladder cancer (14)	25.0	0	33.3	69.2	57.1	50.0	60.0	0	64.3
Cervical cancer (27)	–	7.1	20.8	5.0	11.1	30.4	20.8	0	44.4
Gastric cancer (18)	0	6.3	25.0	6.7	41.7	0	9.1	0	38.9
Breast cancer (40)	–	5.4	8.1	9.7	11.1	20.5	3.2	5.4	37.5
Ovarian cancer (3)	–	0	0	50.0	0	0	0	0	33.3
Oral cancer (10)[a]	0	0	0	0	0	0	0	0	0

Data are given as percentages unless otherwise indicated.
Abbreviations: PSA, prostate-specific antigen; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CA, cancer antigen; CYFRA, cytokeratin fragment; SCC, squamous cell-specific antigen.
[a] Oral cancer included malignancies arising in the tongue, oral cavity, and oropharynx.

increase with stage; however, these findings should be interpreted with caution based on the limited number of cases with early-stage cancer. Most of the cases with early-stage breast cancer were not successfully identified by the panel.

4. Discussion

In the current study, the prevalence of cancer in Taiwanese subjects who underwent a voluntary health check-up examination was 0.756%, which is higher than that reported for the general population (0.432%; 2011 Taiwanese data) [6]. Because the CGMH serves as a medical center, we cannot exclude that subjects who voluntarily underwent the screening might have had a higher burden of comorbidities than the general Taiwanese population. The six most common forms of cancer in terms of incidence (i.e., breast cancer, colorectal cancer, liver cancer, lung cancer, cervical cancer, and prostate cancer; 2011 Taiwanese data) accounted for 71.5% (128 out of 179 cases) of all tumors successfully identified by our multi-analyte cancer biomarker screening panel. Notably, most malignancies in our study were detected in subjects aged between 70 and 79 years, a finding in accordance with the Taiwanese 2011 cancer registry annual report. A similar age-related increase in cancer incidence has been reported in the United States [7]. Although >50% of the study participants were aged between 40 and 59 year (Table 3), the results from the current study indicate that cancer biochemical screening programs should be implemented with a special focus on the elderly.

According to previous data obtained between 1998 and 2001 [5], the percentage of screened individuals with a true positive result for cancer diagnosis was 0.274%, and further increased to 0.431% from 2001 through 2013 (Table 6). As of December 2001, the addition to the panel of CA 15-3 (for women), CA 19-9 (for women), SCC, and CYFRA21-1 resulted in an increased screening ability. Consequently, the higher the number of biomarkers included in the panel, the greater its clinical value for the detection of cancer.

Table 3
Effect of age on the number of malignancies detected by the multi-analyte biomarker screening panel between 2001 and 2013.

Age group, years (number of cases)	Number of cases with cancer (%)	Number of detected cases (%)
20–29 (2909)	1 (0.03)	0(0)
30–39 (8283)	17 (0.21)	6 (35.3)
40–49 (12,436)	75 (0.60)	36 (48.0)
50–59 (10,855)	87 (0.80)	43 (49.4)
60–69 (4550)	69 (1.52)	43 (62.3)
70–79 (2042)	57 (2.79)	45 (78.9)
≥80 (441)	8 (1.81)	6 (75.0)
Total (41,516)	314 (0.76)	179 (57.0)

Data are given as counts and percentages.

In this study, we found that 4674 tested positive for one or more markers did not develop cancer in the 1-y follow-up. Through national cancer registry data, further follow-up was done and 15 of them were actually true-positives that developed cancer later. To improve the true positive rate, risk assessment models combining the panel with clinical and other laboratory parameters for each malignancy should be developed [8,9].

The sensitivity of the panel for the detection of HCC was 92.3%, a value significantly higher than those observed for the liver-specific tumor marker (AFP: 63.3%, Table 2). Notably, the sensitivity of the multi-analyte biomarker screening panel for early- and advanced-stage HCC was similar (Table 5). However, a previous nested case–control study demonstrated that the combination of two markers (AFP and descarboxyprothrombin [DCP]) was not superior to either of the biomarkers alone (91%) for the detection of early-stage HCC [10]. Taken together, these data suggest that multiple biochemical markers should be concomitantly measured to improve the detection rates of early-stage HCC.

Although our panel had a 100% sensitivity for identifying prostate cancer (regardless of the clinical stage; Table 5), its clinical usefulness deserves further investigation. The clinical value of measuring PSA alone for the screening of prostate cancer remains controversial [11], especially because of its low sensitivity and specificity at early disease stages [12]. In this scenario, further research on the potential usefulness of our cancer biomarker screening panel for early-stage prostate cancer is warranted.

The sensitivity of the panel for the screening of colorectal cancer was 76.9% (Table 2), a value significantly higher than that previously reported in Taiwan for a single immunochemical fecal occult blood test [iFOBT] (6.98% for all forms of colorectal cancer and 22.1% for advanced colorectal cancer) [13]. Because data from prospective randomized controlled trials demonstrated that annual and biannual guaiac fecal occult blood tests can significantly reduce deaths due to colorectal cancer [14], additional studies are needed to investigate the potential impact of the multi-analyte biomarker screening panel on mortality rates.

The value of our panel for the screening of early-stage lung cancer remains unclear because most (88.9%) of the study participants who received a diagnosis of lung cancer presented with advanced disease

Table 4
Relationship between the number of abnormal biomarkers and cancer detection rates.

Number of abnormal tumor markers	Number of cases	Number of cases with cancer
One	4232	120 (2.8%)
Two	531	32 (6.0%)
Three	67	13 (19.4%)
Four or more	23	14 (60.9%)

Data are given as counts and percentages.

Table 5
Results of the multi-analyte biomarker screening panel in relation to the clinical stages of malignancies.

Type of malignancy	Clinical stage				
	I	II	III	IV	Incomplete
Hepatocellular carcinoma (26)	8	6	7	5	0
Abnormal panel	8	5	6	5	0
Normal panel	0	1	1	0	0
Prostate cancer (24)	2	11	2	1	8
Abnormal panel	2	11	2	1	8
Normal panel	0	0	0	0	0
Colorectal cancer (26)	3	3	5	6	9
Abnormal panel	0	2	5	6	7
Normal panel	3	1	0	0	2
Lung cancer (36)	2	0	7	25	2
Abnormal panel	1	0	2	22	2
Normal panel	1	0	5	3	0
Pancreatic cancer (9)	0	0	3	5	1
Abnormal panel	0	0	3	4	1
Normal panel	0	0	0	1	0
Breast cancer (40)	12	19	2	2	5
Abnormal panel	3	5	1	2	4
Normal panel	9	14	1	0	1

Data are given as counts.

stages (Table 5). Similarly, 85.5% of all cases with lung cancer reported in the annual Taiwanese cancer registry (2011 data) were in advanced clinical stages [6]. Such figures are different from those observed for breast, prostate, and colon malignancies, all of them being characterized by relatively higher proportions of patients with early-stage disease [15]. Low-dose CT scans have been previously shown to reduce mortality in high-risk smokers and former smokers, but their widespread use is limited by the risk of overdiagnosis and the large number of false-positive results [16].

Our multi-analyte biomarker screening panel had poor sensitivity for identifying oral cancer (0.0%) and breast cancer (37.5%), especially in presence of early-stage disease (Table 5). Pilot evidence suggests that the systematic visual examination of all the soft tissues of the mouth may reduce oral cancer mortality rates in patients with risky oral habits [17]. Moreover, mammography remains the mainstay of breast cancer screening with a reported sensitivity of 79.6% in Taiwan [18], although its impact on mortality rates remains a matter of debate [19].

Table 6
Types and number of malignancies detected by the multi-analyte biomarker screening panel: comparison between the 1998–2001 and 2001–2013 periods.

Type of malignancy	January 1998–October 2001	May 2001–April 2013
Liver cancer	49	30
Lung cancer	28	27
Prostate cancer	19	24
Colorectal cancer	25	20
Breast cancer	9	15
Cervical cancer	4	12
Bladder cancer	0	9
Pancreatic cancer	9	8
Gastric cancer	8	7
Nasopharyngeal cancer	0	3
Ovarian cancer	0	1
Other cancers	17	23
Total number of malignancies detected by the panel	168	179
Number of screened subjects	61,343	41,516
Percentage of screened individuals with a true positive result for cancer diagnosis	0.274%	0.431%

Data are given as counts unless otherwise indicated.

5. Conclusions

The results of our study indicate that a multi-analyte biomarker panel is clinically useful during health check-up examinations for the screening of different tumors (especially for the early detection of HCC and prostate malignancies). Future studies are needed to establish whether biomarker-based screening approaches will translate into better patient outcomes compared with screening schemes (usually the current standard implemented by public health systems) without biomarker levels.

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