

Early Detection of Colorectal Cancer Metastasis and Relapse by Recognizing Nonspecific Cross-Reacting Antigen 2 in Commercial Carcinoembryonic Antigen Assays

To the Editor:

Carcinoembryonic antigen (CEA),¹ one of the first known tumor markers, is still the most useful clinical marker to aid in the diagnosis and monitoring of colorectal cancer. Many commercial assays are available for measuring CEA, but their practical use is complicated by the molecular heterogeneity of CEA. CEA is a heavily glycosylated protein of the immunoglobulin gene superfamily and has several related antigens such as nonspecific cross-reacting antigen (NCA), NCA-2, and normal fecal antigen. Previous investigations have indicated that some commercial assays for CEA cross-react with various CEA antigens owing to the epitope group specificity of the monoclonal antibodies (mAbs) employed in the assay (1, 2). NCA-2 is a member of the CEA gene family and is structurally most similar to CEA. It has been reported that CEA might be increased in the serum of some colorectal cancer patients, and the cross-reactivity to NCA-2 might be a beneficial characteristic of CEA assays with respect to their diagnostic sensitivity in colorectal cancer patients (3).

A patient who had colorectal cancer and underwent a transverse colectomy at our hospital in 1996 suffered relapses of the cancer in 2000, 2002, and 2003 and had chemotherapy and surgical resection each time. In 2006, metastasis was detected in the liver, and surgery was performed to resect the liver metas-

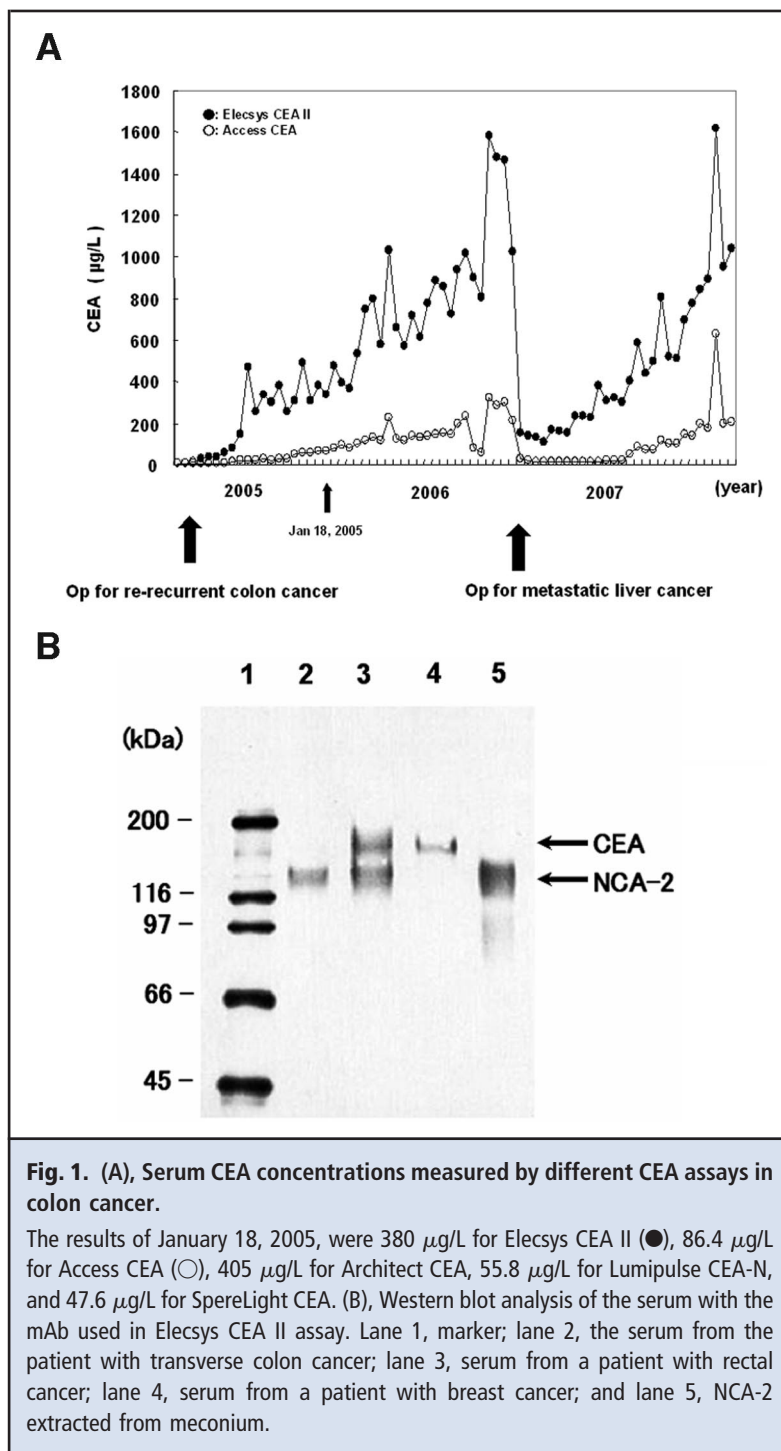


Fig. 1. (A), Serum CEA concentrations measured by different CEA assays in colon cancer.

The results of January 18, 2005, were 380 $\mu\text{g/L}$ for Elecsys CEA II (●), 86.4 $\mu\text{g/L}$ for Access CEA (○), 405 $\mu\text{g/L}$ for Architect CEA, 55.8 $\mu\text{g/L}$ for Lumipulse CEA-N, and 47.6 $\mu\text{g/L}$ for SperaLight CEA. (B), Western blot analysis of the serum with the mAb used in Elecsys CEA II assay. Lane 1, marker; lane 2, the serum from the patient with transverse colon cancer; lane 3, serum from a patient with rectal cancer; lane 4, serum from a patient with breast cancer; and lane 5, NCA-2 extracted from meconium.

¹ Nonstandard abbreviations: CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; mAb, monoclonal antibodies.

tasis. Serum CEA concentrations, measured using the Beckman Access CEA assay (Beckman Coulter) fell into a lower range (near the lower

reference limit) after each surgery, but then increased gradually. We also measured CEA concentrations with the Elecsys CEA II assay (Roche

Diagnostics) starting in December 2003, and the concentrations of CEA increased at a faster rate than with the Access CEA (Fig. 1A).

We subsequently assayed 1 serum sample from the patient on January 18, 2005, with the following 5 commercial CEA assays: Elecsys CEA II, Architect CEA (Abbott Laboratories), Lumipulse CEA-N (Fujirebio), SphereLight CEA (Wako Pure Chemical Industries), and Access CEA (Fig. 1A). Informed consent was obtained from the family of the patient. The results demonstrated that 2 of the 5 assays, the Elecsys CEA II and Architect CEA, yielded higher concentrations of CEA of approximately 400 $\mu\text{g/L}$, whereas the others yielded lower concentrations of CEA <100 $\mu\text{g/L}$. We performed gel-filtration and Western blot experiments with the same patient sample and NCA-2 that was extracted from meconium (3). Gel filtration of the patient's serum revealed that the small amount of antigen was found by Lumipulse CEA-N, SphereLight CEA, and Access CEA, whereas large amounts of antigen were detected by Elecsys CEA II and Architect CEA, and the antigen was eluted at fractions of slightly smaller molecular size than others. NCA-2 showed the same elution profile as the patient specimen detected by the Elecsys CEA II. In addition, Western blot analysis indicated that the mAb of Elecsys CEA II reacted with both CEA and NCA-2 (lanes 4 and 5 in Fig. 1B) and that NCA-2 was clearly detected, yet CEA was barely detected in the patient's serum sample (lane 2), although the mAb usually detects both CEA and NCA-2 in sera from patients with cancer of the colon and rectum (lane 3). According to the manufacturer's documentation and comments, both the Elecsys CEA II and Architect CEA use anti-CEA mAbs cross-reacting with NCA-2, although the other kits use anti-CEA mAbs specific to CEA. This information agrees with the results of our investigations

and strongly suggests that in this case the cross-reactivity with NCA-2 caused the substantially higher CEA concentrations with the Elecsys CEA II and Architect CEA. This finding is clinically important, but the recent National Academy of Clinical Biochemistry guidelines on cancer markers (4) do not address differences between CEA assays or the reports on CEA-related antigens (2).

After the patient underwent resection of liver metastasis in 2006, the CEA concentrations measured with both the Elecsys CEA II and Access CEA decreased rapidly, and the Access CEA results fell within the reference interval and remained unchanged in successive determinations. The Elecsys CEA II, however, remained above the cutoff value and rose in successive determinations (Fig. 1A). The patient suffered metastatic relapse of colorectal cancer, which eventually led to the patient's death. This patient's history reveals that colorectal cancer may mainly express NCA mRNA, as in this case, and thus monitoring NCA-2 concentrations using mAb with cross-reactivity to NCA-2 might be useful in the early detection of metastasis and relapse of colorectal cancer.

Further studies are clearly needed with additional patients with colorectal and other cancers, as well as healthy individuals, to validate the utility of monitoring NCA-2 in clinical practice.

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Highly Sensitive Cardiac Troponin I Assay Leads to Lowered Specificity

To the Editor:

I read with great interest the article of Kavsak et al. (1) on the utility of a next-generation high-sensitivity Beckman Coulter cardiac troponin I (cTnI) assay with a 20% CV at 2.95 ng/L. This approach demonstrated a higher diagnostic sensitivity (81% vs 62%) for myocardial injury with a changing pattern of cTnI values, compared with the AccuTn[®] assay cleared by the US Food and Drug Administration (1). This high-sensitivity assay had an unexpectedly high prevalence (3%) for interfering heterophilic antibodies compared with the 0.05% prevalence reported in the literature for contemporary assays (2). Heterophilic antibodies usually exhibit weak binding and polyspecificity. These antibodies are involved in the development of high-affinity antibodies, self-tolerance, and idiotypic regulatory processes (2). Critical exclusion of individuals with false-positive results has been reported to be feasible during reference-interval studies (1). How the results for patients in this study were affected by false-positive results and how they may therefore have contributed to the increased diagnostic sensitivity of the test is not known. Interferences by heterophilic antibodies in a cTnI test can be reduced markedly, such as with the revised Dimension cTnI assay with a 20% CV at 0.08 µg/L (3). The high-sensitivity cTnI assay reported by Kavsak et al. also deserves such amelioration, if possible. Tests with such a high rate of false positives due to heterophilic antibodies cannot be considered appropriate for routine use because clinicians or laboratory personnel are not always aware of this problem and therefore may not initiate the appropriate follow-up

investigations, such as absorption of such heterophilic antibodies or the application of another test. Criteria with greater stringency are necessary to avoid interference by heterophilic antibodies, and the first step is a strict awareness of this problem. An increased diagnostic sensitivity should be feasible, but care must be taken so that negative consequences of false-positive results do not diminish the potential benefits of high-sensitivity assays.

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25-OH Vitamin D₃ Concentrations in Chinese, Malays, and Indians

To the Editor:

With increasing recognition of the importance of vitamin D deficiency as a risk factor in many common diseases, such as malignancy, diabetes, and cardiovascular disease, there has been growing interest in studies to assess vitamin D concentrations in different populations. Measurement of 25-hydroxyvitamin D (25-OH-D) is accepted as the best estimate of vitamin D status. More than 95% of 25-OH-D is typically 25-OH vitamin D₃ (25-OH-D₃), with 25-OH vitamin D₂ reaching measurable concentrations only in patients taking vitamin D₂ supplements (1). Much of the interest in vitamin D status has concentrated on seasonal variation in populations living at high latitudes, with little work done in populations living closer to the equator, perhaps under the assumption that vitamin D deficiency is unlikely in regions of plentiful sunshine. This study describes the range of 25-OH-D₃ concentrations seen in a multiethnic Asian population living close to the equator.

We measured 25-OH-D₃ concentrations in 240 anonymized leftover fasting venous serum samples from apparently healthy ambulatory outpatients undergoing health screening (40 men and 40

Table 1. Distribution of 25-OH-D₃ concentrations in Chinese, Malay, and Indian female and male groups.^a

25-OH-D ₃ , μg/L	Chinese		Malay		Indian	
	Female	Male	Female	Male	Female	Male
≤10	0 (0)	0 (0)	7.5 (3)	2.5 (1)	12.5 (5)	7.5 (3)
>10 and ≤20	35 (14)	15 (6)	57.5 (23)	37.5 (15)	70 (28)	45 (18)
>20 and ≤30	50 (20)	55 (22)	35 (14)	50 (20)	17.5 (7)	42.5 (17)
>30 and ≤40	15 (6)	30 (12)	0 (0)	10 (4)	0 (0)	5 (2)

^a Data are % (n).

women from each race: Chinese, Malay, and Indian; median age 40 years, range 20–82). This was part of routine reference interval work during evaluation of a new assay and did not require institutional review board approval. The tests were performed on the Roche Cobas e601 immunoassay analyzer using the Roche Elecsys vitamin D₃ assay (Roche Diagnostics). Samples were collected over 6 months, stored at –20 °C until analysis and, after thawing and mixing, randomly allocated to 3 analytical runs over 3 consecutive days. Statistical analysis (median calculations, Mann–Whitney tests, and logistic regression) were performed using Analyze-It for Microsoft Excel and SPSS v12 (SPSS Inc.). A *P* value <0.05 was considered statistically significant.

Table 1 shows the distribution of 25-OH-D₃ concentrations for the Chinese, Malay, and Indian female and male groups. There were no samples with 25-OH-D₃ concentrations >40 μg/L. Median 25-OH-D₃ concentrations in the Malay female (18.3 μg/L) and Indian female (16.9 μg/L) groups were significantly lower than in the Chinese female (22.7 μg/L) group. Similarly, median 25-OH-D₃ concentrations in the Malay male (23.4 μg/L) and Indian male (19.6 μg/L) groups were significantly lower than in the Chinese male (26.3 μg/L) group. Median 25-OH-D₃ concentrations for women were significantly lower than for

men for all 3 races. Logistic regression analysis to assess the independent effects of sex, race, and age on presence of low 25-OH-D₃ concentration (defined here arbitrarily as 25-OH-D₃ <20 μg/L) gave the following significant odds ratios (95% CIs): female (vs male), 3.2 (1.8–5.8); Malay (vs Chinese), 3.5 (1.7–7.3); Indian (vs Chinese), 7.1 (3.4–15.0). Age was not a significant predictor of low 25-OH-D₃ concentration.

This study demonstrates clear differences between sexes and races in 25-OH-D₃ concentrations. Singapore lies 1 degree north of the equator and has uniform temperatures and hours of sunlight throughout the year. It is thus unlikely that environmental factors such as latitude and season contributed significantly to the differences between groups. Personal factors, such as skin pigmentation, food, obesity, clothing, dressing style, and cultural habits, are the likely source of the differences, between both races and sexes. Skin melanin concentrations differ between races, and dark skin requires up to 5 times the exposure time to absorb the same amount of vitamin D (2, 3). Genetic factors may also play a role; some Indians have increased 24-hydroxylase activity that results in lower 25-OH-D concentrations (3). The sex-related differences may reflect negative attitudes and behaviors toward sunlight among Asian women, who may take measures to avoid expo-

sure (4). Different cutoffs ranging from 10 to 30 μg/L have been suggested to define vitamin D deficiency or insufficiency (3, 5). Using these cutoffs, the prevalence of vitamin D deficiency in this study varies from 0–12.5% (≤10 μg/L) to 70%–100% (≤30 μg/L) according to the subgroup examined. At all cutoffs suggested, however, the prevalence is higher in Indians and Malays than in Chinese, and higher in women than in men.

A limitation of this study was the possible inclusion of individuals on vitamin D₂ supplements in the study population. Almost all vitamin D supplements in Singapore are vitamin D₃, however, and this drawback is unlikely to alter the picture of intergroup differences seen here. These findings show the need to consider low 25-OH-D₃ concentration in populations living in even sunny regions and to use sex and race to identify those persons at greatest risk.

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