CA11-19: a tumor marker for the detection of colorectal cancer

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Background and Aim: Colorectal cancer (CRC) remains the second most frequent cause of cancer deaths in the United States. Blood tests using tumor-related antigens aid in diagnosing CRC. However, higher sensitivity and specificity are needed before an acceptable tumor antigen blood test for CRC is clinically useful. This study describes the diagnostic accuracy of an enzyme-linked immunosorbent assay for the CA11-19 serologic tumor antigen for the detection of CRC.

Methods: Serum specimens were obtained from 522 colonoscopy-confirmed subjects in institutional review board–approved studies. Specimens were blind coded. CA11-19 levels were determined by using enzyme-linked immunosorbent assay analysis. The results were tabulated for categories of normal, hyperplastic polyps, benign GI, adenomatous polyps, and CRC based on their final diagnosis.

Results: When a cutoff of 6.4 units/mL for normal is used, the CA11-19 level was elevated in 128 of 131 of CRC subjects, for an observed sensitivity of 98% (95% confidence interval, 93.1%-99.5%). Normal levels were found in 87% of normal subjects (90/103) and 83% of those with benign GI diseases (185/223). When combined, this yields an observed specificity of 84% (95% confidence interval, 80.0%-87.9%).

Conclusion: CA11-19 is a serologic tumor marker for the diagnosis of CRC with a sensitivity of 98% and specificity of 84%. This high sensitivity means that this test will detect 43 of 44 cases of CRC presented. For those older than 50 years of age, it has a positive predictive value of 3.6% and a negative predictive value of 99.98%. Additional prospective studies are needed to further clarify the use of CA11-19 as an aid in the diagnosis of CRC. (Gastrointest Endosc 2016;83:545-51.)

Colorectal cancer (CRC) remains the second most frequent cause of cancer deaths in both sexes in the United States and Europe. It is estimated that in this year alone, 136,830 new cases of CRC will be diagnosed in the United States and that 436,000 new cases of colorectal cancer will be found in Europe. Approximately 50,300 CRC deaths will occur in the United States.[1-3] Early detection of colon cancer is key to improving survival rates because more than 90% of those diagnosed with early-stage CRC live 5 or more years after treatment,[4] and screening procedures are key to an earlier diagnosis. Several different procedures and tests have been used for screening for

Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; CRC, colorectal cancer; ELISA, enzyme-linked immunosorbent assay.

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CRC, including colonoscopy, flexible sigmoidoscopy, the fecal occult blood test, and the fecal immunochemical test. Evidence strongly suggests that screening for CRC has resulted in reduced mortality and morbidity of the disease.[5-7] However, in the United States, only 65% of those older than 50 years of age have undergone any form of CRC screening.[8] The recent rapid decline in CRC deaths has been attributed to the greater adoption of colonoscopy and polypectomy.[9,10] However, colonoscopy is an invasive endoscopic procedure encumbered with colon preparation issues, time requirements, and significant expense. A noninvasive, lower cost, accurate test for CRC would likely improve screening rates.

Blood tests using tumor-specific antigen assays are increasingly being used to monitor and in some cases aid in diagnosing some cancers.[11-15] Carcinoembryonic antigen (CEA) has been used in monitoring the effect of treatments for CRC but, it has not proved to be effective in diagnosing the disease.[11,12] CA19-9 was described initially as a colon cancer antigen but has been used clinically for cancer of the pancreas.[12] Septin 9 methylated DNA, a recently introduced serologic DNA test for CRC, has a sensitivity of 48% (95% confidence interval [CI], 32%-64%) and a specificity of 91.5% (95% CI, 90%-93%).[14,15]

The objective of this study was to assess the diagnostic accuracy of the CA11-19 tumor marker for the detection of CRC.

METHODS

In a research laboratory setting, assays of the tumor marker CA11-19 were conducted on fresh human serum stored at 4° to −8°C that had been obtained from 522 colonoscopy-confirmed individuals enrolled in institutional review board (IRB)–approved studies during the period 2008 to 2014. Two hundred of these samples were collected in 2008 and 2009 from 36 clinics in Texas through Equitech-Bio, Inc (Kerrville, Tex). The remaining 322 samples were collected between 2008 and 2014 from 9 different experienced endoscopists operating out of 1 clinic in Knoxville, Tennessee. The demographics for the study participants are shown in Table 1.

The IRB protocols allowed for stratified sampling. The first stratum contained 391 sequentially enrolled subjects undergoing screening, surveillance, or diagnostic colonoscopies (Western Institutional Review Board [WIRB] #2010-1796). All subjects were accepted for the study except for those with HIV or hepatitis C or those who had previously been diagnosed with cancer. This stratum contained 128 samples from the Texas clinics and 263 samples from the Tennessee clinic. Sixty-nine of these 263 samples came from free colonoscopy screenings.

Because of the low incidence of CRC in the general population, the protocols also allowed a second stratum with as many as 200 serum samples to be obtained before treatment or surgery from subjects known to have CRC. A total of 72 such samples came from the 36 clinics in Texas and 59 came from the 1 clinic in Tennessee (WIRB #2010-1796; Covenant IRB #2012-132).

TABLE 1. Characteristics of 522 participants

Males	245 (47%)
Age range, y	20-87
Mean age, y	56.6
Older than 40 y	501 (96%)
Older than 50 y	412 (79%)

In every instance, the fresh human serum samples from both strata were blind coded and combined into panels of mixed clinical status before being sent to the laboratory for analysis.

Based on the colonoscopic findings, with histopathologic confirmation when appropriate, subjects were assigned to 1 of 5 groups: (1) normal, (2) hyperplasic polyps, (3) other benign GI disease, (4) adenomatous polyps, and (5) CRC. Group 3, the other benign GI disease group, included subjects with hemorrhoids, diverticulosis, GI bleeds, positive fecal occult blood test results, and those who reported a change in bowel habits.

The quantitative measurement of CA11-19 in serum was performed by using a classic sandwich enzyme-linked immunosorbent assay (ELISA). A cocktail of 2 monoclonal antibodies to CA11-19 is coated on the surface of a 96-well microtiter plate as the capture antibody. To each of 2 microtiter wells, 25 μL of ready-to-use calibrators, controls, and patient samples are added to the microtiter plate followed by 75 μL of a diluting buffer (SurModics, Eden Prairie, Minn). During sample incubation, any CA11-19 present in the sample binds to the monoclonal antibodies in the microwells. After incubation, the wells are washed to remove unbound nonspecific components.

Anti–CA11-19 polyclonal antibody conjugated with alkaline phosphatase (AP) enzyme is then added to the wells. During the conjugate incubation, anti–CA11-19 AP-conjugated antibodies bind to any CA11-19 in calibrators, controls, or patient sera bound to monoclonal antibodies on the surface of the well. After conjugate incubation, excess unbound AP-conjugated antibody is removed by washing. The wells are next incubated with a para-nitrophenyl phosphate disodium substrate solution to develop a yellow color in direct proportion to the amount of conjugated antibody present and therefore to the CA11-19 antigen for measurement spectrophotometrically by a microtiter plate reader at 410 or 405 nm.

The intensity of the color formed by the enzyme reaction provides a direct measure of the concentration of CA11-19 in the specimen within the working range of the assay. A standard curve is obtained by plotting the CA11-19 concentration of the CA11-19 calibrators versus the optical density. The CA11-19 concentrations of the