Radioimmunologic Methods for Diagnosis of Malignant Diseases

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The recent availability of the carcinoembryonic antigen (CEA) assay has stimulated great clinical interest in tumor antigens. Carcinoembryonic antigen is not the only specific or tumor-associated antigen currently identified. There are a number of other tumor antigens which have been isolated, some of which are related to CEA and some of which are totally different. There is, for example, a nonspecific cross-reacting antigen (NCA) which has been discovered in certain preparations which were considered originally to be CEA. Nonspecific cross-reacting antigen is a beta globulin (25% carbohydrate) which cross-reacts with antibodies to CEA and may be the nonspecific background element that causes the CEA titer to be elevated in certain nonmalignant diseases. There is also a membrane-associated, low molecular weight tissular autoantigen (MTA) which has been recently identified. However, this material is not antigenetically related to CEA or NCA.

Carcinoembryonic antigen is an antigenetic glycoprotein. The antibody-active site of this antigen is actually in the glucose portion of the molecule rather than the protein portion. Carcinoembryonic antigen has a relatively high molecular weight (200,000) and is soluble in perchloric acid. The perchloric acid solubility of CEA has greatly facilitated extraction of the antigen from tumor tissue and is also responsible for the relative convenience of the current method of performing the radioimmunoassay for detection of this antigen.

Carcinoembryonic antigen is present in the glycocalyx of malignant gastrointestinal cells and is also present in fetal gastrointestinal tissues during the first and second trimesters of fetal life. Gold and Freedman discovered and isolated this material in 1965 (1). A radioimmunoassay for its detection was developed soon after (2). Initial results with this assay indicated that elevated blood CEA level was a specific test for adenocarcinoma of the colon, although occasional patients with pancreatic carcinoma also had elevated titers. However, more extensive clinical studies indicate that circulating CEA is not only present in colonic and pancreatic malignancies but in other malignancies as well, as noted by H. J. Hansen, MD (oral communication, April, 1973). Unfortunately, CEA is also present in certain nonmalignant disorders and some normal patients. The common denominator in most cases of elevated CEA level associated with bowel disease is rapid cellular proliferation with disruption of the basement membrane. The disruption of the basement membrane is important because the antigen must leak into the circulation in order to be detected. The cellular proliferation may be responsible for the cell surface exposure of certain primitive antigens that are not normally found in adult human tissues. The upper limit of normal for the currently available CEA assay is approximately 2.5 ng/ml. However, a finite percentage of normal individuals will have CEA levels above this value. Three percent of healthy, young nonsmoking volunteers and 19% of smokers have CEA levels over 2.5 ng/ml. There is some question as to whether the smokers who do have elevated titers are not in fact candidates for developing carcinoma of the lung, although the evidence for this is currently speculative. Former smokers have a fairly significant CEA titer

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elevation. Thirty percent of patients with nonmalignant disease may have a titer above 2.5 ng/ml, and 10% of these patients have a titer above 5 ng/ml. However, the assay shows more frequent and more marked elevation of the antigen titer in patients with malignancy, especially colorectal carcinomas. Eighty percent of patients with colorectal carcinoma have a titer greater than 2.5 ng/ml. Fifty percent have a titer above 5 ng/ml. Other tumors such as lung carcinoma are also associated with a high incidence of elevated titer.

An early criticism of the CEA assay was the high incidence of false-negative results in patients with early-stage colonic carcinomas. The five-year survival statistics for treated patients with Duke's stage A colonic carcinoma is almost 100%. In the stage B group, the survival is approximately 50% to 60%, and in the stage C group (those patients in whom the tumor has gone through the serosa and actually metastasized to local nodes), the survival drops to only about 25% at five years. Ideally, we would prefer an assay that would detect 100% of patients with colonic carcinoma, Duke's stage A (assuming that most of these tumors do go through a progression from stages A to C). However, using the current CEA assay, only 20% of patients with stage A lesions will have an elevated titer. In other words, 80% of the patients with this type of lesion will have a normal CEA titer. About 40% to 50% of patients with stage B lesions will have positive titers. It is only when the lesion actually breaks through the serosa and is involving local nodes that the probability of an elevated CEA titer approaches 90% to 100%. Therefore, the current CEA immunoassay cannot be used alone as a cancer-screening study. Its value in screening patients, however, should not be totally discounted since there is a significant five-year survival in treated patients even in the stage B and C categories.

Doctor William McCartney, Mrs. Erika Lawrence, and I performed a study at the University of Chicago, comparing the relative value of the CEA titer to the conventional colon examination for the diagnosis of carcinoma of the colon (3). The study included almost 1,000 patients who were referred for radiologic colon examination. Carcinoembryonic antigen titer above 3 ng/ml (a level we arbitrarily selected to divide the normal from abnormal groups) was detected in 15% of patients subsequently diagnosed as normal, or having inactive inflammatory bowel disease. There was a 30% incidence of elevated titers in patients who had cirrhosis and noncolonic malignancies. Less than 10% of the patients with benign colonic polyps in this series had significant titer elevation.

By comparison, the conventional radiologic colon examination was positive, that is, showed evidence for malignancy in only 10 out of 850 patients who were subsequently established not to have colonic malignancy. The colon examination was, therefore, much more specific for excluding the diagnosis of carcinoma of the colon in patients subsequently proven not to have colonic malignancy.

Forty-eight of the patients in this series were subsequently proven to have carcinoma of the colon, first diagnosed at the time of the study. The CEA titer was normal in 16 of these patients and was abnormal in only 67%, whereas the conventional radiologic colon examination was abnormal in 90% of these patients. The radiologic colon examination was, therefore, clearly superior to the CEA test for detection of new carcinomas of the colon. However, there were three patients with carcinoma of the colon in this study in whom the radiologic colon examination was originally considered to be normal or show signs representing benign disease, who did have elevated CEA titers. The CEA titer was potentially helpful in these cases in raising the suspicion of colonic carcinoma. If the results of the two tests are combined, the accuracy of the diagnosis is 96%, which represents some improvement over the radiologic colon examination alone.

In active ulcerative colitis, the CEA level is frequently elevated and the level itself is not a useful clue to detect early development of malignancy. The patients with quiescent bowel disease usually have normal titers. In a patient with quiescent bowel disease, a rising CEA titer should be treated with great suspicion for neoplasm.

Although the radiologic colon examination is clearly superior for the initial diagnosis of colonic malignancy, the CEA titer is equally superior for the diagnosis of recurrence of colonic carcinoma. In our studies of patients with recurrent colonic carcinoma, only 2 of 15 patients with recurrence had a normal CEA titer. The barium enema indicated recurrence in only 3 of these 15 patients. The barium enema did detect the two cases of recurrence with normal CEA titers. Frequently, the recurrence of colonic carcinoma is not in the area of the primary tumor but rather in the liver or elsewhere in the body. It is also very difficult to distinguish postoperative changes in the bowel from early recurrence of tumor on a conventional radiologic colon examination. These factors probably account for the poor detection rate of

recurrence seen with the conventional radiologic colon examination.

In summary, the CEA titer is not as sensitive as the radiologic colon examination for the diagnosis of primary carcinoma, but it is very useful in following patients for recurrent carcinoma.

Another area of potential use for the CEA radioimmunoassay is in the detection of metastatic malignancy of noncolonic origin. The test is extremely useful when interpreted in conjunction with other tests for metastatic disease. Doctor William McCartney, Erika Lawrence, and I studied 368 patients who had both liver scans and CEA immunoassay (4). The liver scan is a useful test for the detection of hepatic metastasis for many primary malignancies; however, it has a 30% incidence of false-negative results and a 10% to 20% incidence of false-positive results. The false-positive cases are primarily patients with other disorders such as cirrhosis, who show lesions on the liver scan which closely mimic metastatic tumor. In this study, we chose a level of 9 ng/ml as a dividing line between a positive result for metastatic tumor and negative evidence for metastatic tumor. It should be noted that this level is considerably higher than the level usually selected for detecting carcinoma of the colon.

Neither the liver scan nor the CEA assay was perfect in detecting hepatic metastasis. However, when the results of both tests were positive, the probability that the patient had metastasis was almost 100%, and when both tests were negative, the probability of hepatic metastasis dropped to 1%. If the liver scan only was positive, the probability of metastasis dropped to 60%, and if the CEA titer only was positive, that is, above 9 ng/ml, the probability of hepatic metastasis was only 30%. These results suggest an important role of the CEA titer in evaluating the patients with many types of tumors other than colonic malignancy.

The continuing clinical use of the CEA titer will undoubtedly reveal many other situations in which the study is valuable. It is certainly not a diagnostic panacea, and the results must be interpreted with considerable caution. However, I believe we can look forward to an era of continued expansion in this type of tumor radioimmunoassay. More specific assays for colonic tumor are being developed and will undoubtedly become available in the future. I am equally confident that similar assay techniques will be developed for other types of malignancies.

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