Current Status of Carcinoembryonic Antigen (CEA) and CEA-S Assays in the Evaluation of Neoplasm of the Gastrointestinal Tract*

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ABSTRACT

Carcinoembryonic antigen (CEA) is heterogeneous and may represent a set of closely related glycoproteins which have been referred to as isomeric species of CEA. The existence of other CEA-related glycoproteins such as non-specific cross reacting antigen (NCA) has been recognized. Recently a highly purified homogeneous isomeric species of CEA described as CEA-S has shown differences in diagnostic results upon analysis of clinical sera as well as quantitative immunochemical differences.

In a blind study of 308 sera, the CEA and CEA-S assays were compared. A significant difference in false positive results was observed between the CEA and CEA-S assay results. In contrast to the low but significant evidence of elevated CEA in sera of random normal persons and patients with liver disease or inflammatory disease of the gastrointestinal tract or lung, none of the sera had elevated concentrations of CEA-S. Among patients with tumor of the gastrointestinal tract that were considered surgically resectable, 46 percent were elevated using the CEA-S assay and only 34.7 percent were elevated above 5 nanograms per ml by the CEA assay. The CEA assays detect CEA-related molecules produced by lung, breast and other tumors; the CEA-S assay appears equally sensitive to CEA of gastrointestinal origin but detects only a small subgroup of breast, lung and, rarely, other types of tumors.

Introduction

Carcinoembryonic antigen (CEA) refers to a glycoprotein set bearing an-...
nature of the antigenic determinants in CEA is not entirely known. Reported studies have demonstrated that preparations of CEA exhibit heterogeneity with respect to a number of analytical parameters.\textsuperscript{1, 4, 25, 35, 37, 40} The isoelectric profile of CEA extracted from tumors has been shown to differ from that extracted from fetal or embryonic tissue and six or more molecular species of CEA have been observed.\textsuperscript{35} Heterogeneity of CEA has also been demonstrated with respect to expression of blood group antigens while some preparations of CEA appear to be essentially devoid of A-like blood group determinants.\textsuperscript{10, 30}

A variety of glycoproteins which may bear some relationship to CEA have been identified in human tissues, normal human tissues and tumors. The most highly characterized non-CEA glycoprotein is non-specific cross reacting antigen (NCA)\textsuperscript{27, 39} and also is described as normal glycoprotein (NGP).\textsuperscript{22} NCA is smaller than CEA and has been distinguished on the basis of size as well as the use of specific antisera. A glycoprotein similar or identical to NCA which shares antigenic determinants with conventional preparations of CEA has been described by other investigators as CEX,\textsuperscript{6} CCA-3\textsuperscript{28} and CCEA-2.\textsuperscript{38} Tissue glycoproteins other than CEA and NCA have been described and are of interest as potential markers of disease and also because of structural and immunochemical features related to CEA. They have included NCA-2 recovered from feces,\textsuperscript{3, 27} breast cancer glycoprotein (BCGP),\textsuperscript{18} other membrane-associated CEA-related molecules\textsuperscript{34} and alpha\textsubscript{1} antichymotrypsin.\textsuperscript{29}

**Methods**

Most clinical studies of CEA have been concerned with correlation between CEA concentration and the clinical status of the patient. The diagnostic usefulness of plasma CEA has been limited since elevated concentrations have been observed in a significant number of normal persons and in a wide variety of non-neoplastic diseases such as liver diseases, inflammatory bowel diseases and chronic renal diseases.\textsuperscript{15, 24, 37} Most conventional CEA assays detect CEA-related molecules produced by lung,\textsuperscript{5, 15, 19, 24} breast,\textsuperscript{2, 15, 19, 36} gynecologic malignancies\textsuperscript{8} and other types of cancer.\textsuperscript{15, 33}

Quantitative studies in normal subjects with various diseases on conventional CEA assay may be summarized as follows:

1. About 10 percent of normal people have elevated CEA levels although this percentage shifts depending on values chosen as the upper limits of normal.\textsuperscript{15}

2. CEA is elevated in carcinoma of the gastrointestinal tract, but it is also elevated in other endodermal and non-endodermal malignancies.\textsuperscript{15, 24, 33, 37}

3. Fifty to 80 percent of patients with established bowel cancer limited to the bowel wall had normal CEA values.\textsuperscript{7, 19, 20, 21}

4. Eighty-three percent of patients with primary breast cancer without metastases had normal levels of CEA.\textsuperscript{36}

5. Seventeen percent of patients suspected of malignancy where no lesions could be detected had concentrations of CEA above normal.\textsuperscript{37}

It is generally agreed that conventional CEA assays are impractical as a general population screen. The major reason for this is about 10 percent of the population had elevated CEA concentrations. There is no valid proof at the present time that a conventional CEA test is useful as a diagnostic adjunct when cancer is suspected, and there is no reported study demonstrating that the accuracy of the differential diagnosis of cancer is improved by adding the CEA assay to established diagnostic procedures.\textsuperscript{37} Several studies have shown a good correlation between effectiveness of therapy and
CEA serum concentration\textsuperscript{7,15,19,23,24,32,37} Continued elevation of CEA after therapy is presumptive evidence that therapy was inadequate.

Edgington, Plow and co-workers have isolated and characterized a homogeneous species of glycoprotein from adenocarcinoma of the colon which is called CEA-S.\textsuperscript{9,50} CEA-S is a homogeneous glycoprotein from adenocarcinoma of the colon. It is a specific isomeric species of carcinoembryonic antigen which is found predominantly in tumor tissues but can be detected in fetal and normal body fluids. In contrast to the described heterogeneity of conventional CEA preparations, CEA-S is homogeneous by reference to size, charge and density and is devoid of ABO blood group antigens.

**CEA Properties**

The physicochemical properties of CEA and CEA-S are outlined in table I. The sedimentation velocity ($S_{20,w}$) for CEA appears slightly higher for CEA-S (6.8 compared to 6.6).\textsuperscript{11} The isoelectric distribution of CEA was heterogeneous and extending from less than 3.0 to approximately 5.2 as compared to the homogeneous isoelectric point of approximately 4.5 for CEA-S observed by analysis on ampholyte columns.\textsuperscript{11}

<table>
<thead>
<tr>
<th>Physical Constant</th>
<th>CEA</th>
<th>CEA-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>heterogeneous</td>
<td>4.5</td>
</tr>
<tr>
<td>$S_{20,w}$</td>
<td>6.8</td>
<td>6.6</td>
</tr>
<tr>
<td>$K_d$ (G-2000)</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>$D_{20,w}$</td>
<td>$3.05 \times 10^{-7}$ cm$^2$/s</td>
<td>$3.05 \times 10^{-7}$ cm$^2$/s</td>
</tr>
<tr>
<td>Stokes radius</td>
<td>65 Å</td>
<td>65 Å</td>
</tr>
<tr>
<td>Buoyant density</td>
<td>1.37 g/ml</td>
<td>1.41 g/ml</td>
</tr>
<tr>
<td>Estimated molecular weight*</td>
<td>201,000</td>
<td>181,000</td>
</tr>
</tbody>
</table>

*From Svedberg equation.

Studies on the amino acid composition of CEA and CEA-S isolated from the same tumor showed similarity except for a 10 percent relative increase of lysine and a 5 percent decrease of threonine in CEA-S.\textsuperscript{11} Immunochemical characterization studies have demonstrated differences between CEA and CEA-S isolated from the same tumor suggesting differences may result from intrinsic heterogeneity of glycoproteins within a given tumor.

A radioimmunoassay for CEA-S has been developed and is performed directly on serum.\textsuperscript{9,11,30} The assay uses a $^{125}$I-CEA-S as ligand and the antisera is produced by immunization of rabbits with either crude perchloric acid soluble glycoprotein from colonic adenocarcinoma or conventional CEA. The antisera has broad specificity and the antibody has been extensively absorbed with normal erythrocyte stroma of each ABO blood group, soluble blood group substance, lyophilized spleen, liver, colon, lung and normal serum. The assay is a competitive inhibition assay of the equilibrium type using a second antibody for phase separation. A high conductance borate buffer (14 mmhos) is utilized to diminish weak immunochemical cross reaction and permit direct assay of serum.

An arbitrary standard prepared from a homogenate of an adenocarcinoma of colon has been used to the CEA-S assay procedure because of differences in specific activity of preparations of CEA-S and CEA.\textsuperscript{8,10,30} The serum levels of CEA-S are reported in arbitrary units and recent assay of the CEA-S tumor standard by the zirconyl phosphate radioimmunoassay for CEA has yielded a conversion factor of 0.16 units of CEA-S standard per ng CEA. The observed threshold of 14 U CEA-S per ml may be considered equivalent to approximately 2.2 ng CEA per ml.
CEA-S in Sera

A thorough study of CEA-S levels in sera of 909 individuals was completed and the results are summarized in tables II, III and IV.

In the analysis of 435 random patients without evidence of neoplasia, 0.23 percent had values greater than 14.0 units CEA-S per ml. Therefore, normal values are considered to be from 0 to 14.0 units CEA-S per ml and elevated values are considered to be greater than 14.0 units CEA-S per ml.

In study, one of 43 patients with liver disease and one of 110 patients with inflammatory diseases of the GI tract had elevated levels of CEA-S. Elevated levels of CEA-S were not found in association with chronic renal disease.

In cancer patients, levels of CEA-S greater than 14.0 units per ml were found in 84 percent of patients with adenocarcinoma of the colon, 55 percent of patients with rectal carcinoma, six of nine patients with gastric carcinoma and 12 of 13 patients with carcinoma of the pancreas. The concentration of CEA-S in serum was higher in patients with metastatic adenocarcinoma of the colon and rectum than in patients without clinically established metastases suggesting a relationship to tumor mass. Elevated levels of CEA-S were demonstrated in only 14 percent of patients with carcinoma of the lung, 15 percent of patients with carcinoma of the breast and 3.5 percent of patients with other tumors.

These results suggest moderate but not absolute specificity of CEA-S for tumors of the gastrointestinal tract. There appears to be a relationship between the mass of tumor and concentration of serum CEA-S as suggested by the study shown in table IV.

When patients are followed with serial assays, values tend to rise progressively with the growth of the tumor and incom-

### TABLE II

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number</th>
<th>Elevated Serum CEA-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Random</td>
<td>435</td>
<td>0.23</td>
</tr>
</tbody>
</table>
| II. Inflammatory disease of gastrointestinal tract | 110 | 0.91
| Chronic ulcerative colitis | 35 | 2.90
| Acute pancreatitis | 4 | 0
| III. Liver disease | 43 | 2.30
| IV. Advanced renal disease | 32 | 0
| V. Other diseases | 42 | 0

*Serum CEA-S > 14.0 units per ml.

A subsequent blind study was conducted on 308 sera to compare the relative diagnostic applicability of the CEA-S and CEA assays. The CEA assays were performed directly on serum in the labo-

### TABLE III

<table>
<thead>
<tr>
<th>Tumor Patient Group</th>
<th>Number</th>
<th>Elevated Serum CEA-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. G-I tract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>82</td>
<td>69 84</td>
</tr>
<tr>
<td>Rectum</td>
<td>22</td>
<td>12 55</td>
</tr>
<tr>
<td>Stomach</td>
<td>9</td>
<td>6 67</td>
</tr>
<tr>
<td>II. Pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exocrine</td>
<td>13</td>
<td>12 92</td>
</tr>
<tr>
<td>Endocrine</td>
<td>2</td>
<td>0 0</td>
</tr>
<tr>
<td>III. Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exocrine</td>
<td>22</td>
<td>3 14</td>
</tr>
<tr>
<td>IV. Breast</td>
<td>40</td>
<td>6 15</td>
</tr>
<tr>
<td>V. Other</td>
<td>57</td>
<td>2 3.5</td>
</tr>
</tbody>
</table>

*Serum CEA-S > 14.0 units per ml.
TABLE IV

Relationship Between Clinical Evidence of Metastasis of Adenocarcinomas of the Colon and Rectum and Levels of CEA-S in Serum

<table>
<thead>
<tr>
<th>Tumor Patient Group</th>
<th>Number</th>
<th>Percent Elevated Concentration Serum CEA-S*</th>
<th>Mean Concentration (Units CEA-S/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastases Established</td>
<td>49</td>
<td>91.8</td>
<td>135.0</td>
</tr>
<tr>
<td>Not established</td>
<td>41</td>
<td>75.6</td>
<td>37.7</td>
</tr>
</tbody>
</table>

*Serum CEA-S > 14.0 units per ml.

TABLE V

Incidence of Elevated CEA-S and CEA in a Blind Study of 308 Sera from Patients with Neoplastic and Non-neoplastic Diseases

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number</th>
<th>CEA Threshold 5 ng/ml</th>
<th>CEA-S Threshold 14 U/ml</th>
<th>Significance (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>75</td>
<td>4.0</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>Benign pulmonary disease</td>
<td>44</td>
<td>13.6</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Benign GI disease</td>
<td>92</td>
<td>9.8</td>
<td>0</td>
<td>0.008</td>
</tr>
<tr>
<td>Carcinoma of lung</td>
<td>48</td>
<td>31.2</td>
<td>10.4</td>
<td>0.001</td>
</tr>
<tr>
<td>GI tumors†</td>
<td>49</td>
<td>34.7</td>
<td>46</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Chi-square analysis.
†Most surgically resectable tumors.

In summary, CEA-S represents an isomeric or subspecies of CEA. The CEA-S assay has demonstrated an im-
proved specificity for gastrointestinal neoplasms and the incidence of false positive results was very low (0.41 percent) in non-neoplastic diseases. The CEA-S assay should facilitate current improvements in the detection and differential diagnoses, as well as management, of the gastrointestinal cancers.

References


