Carcinoembryonic Antigen (CEA) In Cervical Neoplasia (Review)

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By OBGYN.net Staff [3]

One of the important problems in the clinical management of cancer is the early detection of its occurrence. The demonstration of immunoreactive substances, which are specific or associated with cancer and measurable in serum, provides an interesting and promising approach to cancer detection.

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Introduction
One of the important problems in the clinical management of cancer is the early detection of its occurrence. The demonstration of immunoreactive substances, which are specific or associated with cancer and measurable in serum, provides an interesting and promising approach to cancer detection (1).

The measurement of tumour-associated antigens as tumour markers in the serum is useful for early diagnosis, differential diagnosis, and the determination of remission after therapy in cases of malignancy. However, it is important to be aware that each tumour-associated antigen reveals only one biologic characteristic of the tumour cells, and that a tumour mass consists of various types of cells. It must also be understood that a tumour-associated antigen is not always present in all the cells forming the tumour and may also be detected in other tumours or normal organs (2).

One of the important advantages of measuring secreted tumour markers in contrast to physical methods of assessment of tumour masses lies in their representative of viable tumour burden, an important consideration when apparent mass constitutes a viable tumour (3).

The application of monoclonal antibody technology to development of cancer in humans has created an increasing number of biologic tumour markers. However, their use in clinical medicine remains limited because the required specificity has not been achieved. To date, no tumour associated proteins unique or clearly specific to any neoplastic disease have been found. Because these proteins are also produced by normal cells, interpretation of assay results depends on the quantitative value reported and not merely on the presence or absence of the antigen. Tumour markers it is unlikely that tumour associated proteins will ever be useful for population screening when present in abnormal quantities, however, tumour markers have proved valuable in diagnosis and in following disease activity or response to treatment (4).

In practical terms, Van Nagell et al. (1981) (5), considered a tumour marker as a substance which is selectively produced by tumour cells and then released into the blood stream in sufficient quantities to be measured by conventional techniques. Ideally, such a marker should be useful in tumour diagnosis and serve as a biochemical monitor of disease status following therapy. Disappearance of the marker should indicate eradication of the tumour, whereas an increase in marker concentration should indicate tumour growth (5).

Coombes and Powles (1982) (6), have suggested the following list for the potential clinical applications of circulating tumour markers. They may:

- Facilitate the primary detection of cancer.
- Assist in monitoring the earlier detection of recurrence.
- Assist in predicting response to treatment.
- Assist in selection of patients for adjuvant chemotherapy.
- Help in the localization of metastatic tumour: e.g. by using a specific biochemical parameter which originates from a particular organ (e.g. liver enzymes).

The oncofetal antigens comprise one particular group of markers produced by human neoplasms, these antigens have been detected in the sera of patients with gynaecological cancer. The practical use of such markers in the diagnosis and follow-up has been limited by the sensitivity and specificity of their tests (7). Carcinoembryonic antigen (CEA) is one of the first known tumour markers. Since then, many more have been described, but CEA, determined alone or in combination with others, is still one of the most used. CEA is not organ specific and abnormal values may be found in a wide range of carcinomas (8).

The Discovery of CEA

The two best-characterized oncofetal markers are Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) in 1963, Abelev et al. (9) using immunodiffusion, discovered for the first time the Alpha-fetoprotein (AFP). This discovery started the modern era of tumour markers. CEA which was first described as a gastrointestinal system-specific tumour antigen is still the most widely used and studied member of this group. It should be noted, however, that CEA, AFP and other oncofetal antigens such as foetal sulphoglucoprotein associated with carcinoma of the stomach, pancreatic...
oncofoetal antigens, and oncofoetal antigens associated with carcinomas of the colon, lung, breast, liver and kidney do not normally elicit an immune response in the patient. They are this not truly “antigenic” in the host and the term marker is more appropriate. Anti-CEA monoclonal antibodies have been used in attempts to define cancer-specific epitopes of CEA, in evaluating CEA by immunohistology and as agents for radiomunooimaging. Similarly, anti-AFP monoclonal antibodies have been used to develop sensitive radio-immunoassay, to explore radiomunooimaging/immunotherapy and to study the control of AFP gene expression (10).

The idea for the work on CEA devolved from two lectures. The first described the attempts and failures to detect either components or functions unique to cancer cells and not found in any normal tissues (11). The second lecture dealt with the then relatively recent description of Acquired Immunological Tolerance (11) -- until 1953 only a theoretical concept -- and the new perspectives that this phenomenon was creating in the understanding of the immune system and in various areas of clinical medicine. Hence, the experiments subsequently undertaken were predicated on the somewhat naive hope that the burgeoning tools of immunology, such as immunologic tolerance, would provide both the specificity and sensitivity that had been lacking in the previous efforts to find one or more specific tumor constituents in human cancer tissues (11).

In the 1950s, after perhaps a century of study in a variety of disciplines and using numerous technologies, the paradigm in the field was very simply that "tumor-specific materials" did not exist in cancer tissue and, moreover, that these would not be found (12). Nevertheless, by the early 1960s, studies of the rejection of well-defined transplantable tumors between highly inbred, or syngeneic, animals had clearly demonstrated the existence of tumor-specific transplantation antigens (TSTAs) in these animal models (13). Conventional wisdom of the day maintained that such artificially-generated could hardly be applicable to outbreed, "wild-type" humans. Notwithstanding this, a sizable field of investigation grew up around the question of how TSTA-bearing animal tumors escape the immune surveillance system, a question that has yet to be answered satisfactorily. It is to a field of tumor immunology in this state of flux that CEA research traces its origin (11).

In 1965, Gold and Freedman (14) described for the first time common specific antigens or antigenic determinants in adenocarcinoma of the human colon. Components identifiable with the colonic tumour-specific antigens were found in carcinomata originating in other digestive system organs. Cancers of the lower bowel (colon and rectum) contained higher concentrations of tumour-specific antigens than those of the upper gastrointestinal tract (oesophagus and stomach). These tumour-specific antigens could not be detected in malignant tumours of the uppermost portion of the digestive system; i.e., in cancers of the palate and anterior portion of the floor of the...
the liver, for example, were devoid of such activity. Hence, the parameter that determined expression of the antigenic activity described was the site of tumor origin, and not the site of growth.(11)(14).
A good deal of interest was generated by the "embryologic Confinement" of the tumor antigenic activity in question. The substance appeared to be related to cancers that arose from tissue lying between the embryologic stomatodeum, above, to the proctodeum, below. Thus, further investigations were done with human embryo- and fetus-derived tissues. Since this was done at a time well before abortion-on-demand, it took a fair amount of time to collect tissues from spontaneous abortuses at various points in pregnancy. Nevertheless, it was found that gut-derived tissue in the first two trimesters of gestation expressed the antigenic moiety of interest but that comparable tissue in the third trimester did not. Hence, the material was named the carcinoembryonic antigen(s) of the human digestive system, subsequently abbreviated to CEA (14). Table (1): shows the milestone in the development of tumour markers and antigens.(9)

<table>
<thead>
<tr>
<th>Year</th>
<th>Author(s)</th>
<th>Discovery/Event</th>
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<tbody>
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<td>Bence – Jones</td>
<td>Bence – Jones</td>
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<tr>
<td>1911</td>
<td>Rous</td>
<td>Sarcoma transmission by cell-free extracts</td>
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<tr>
<td>1928</td>
<td>Brown</td>
<td>Extopic hormone syndrome</td>
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<td>1929</td>
<td>Woglom</td>
<td>Concept of immunotherapy</td>
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<td>1929</td>
<td>Witebsky, Firzelfeld</td>
<td>Intestinal cancer – specific antigens</td>
</tr>
<tr>
<td>1930</td>
<td>Zondek</td>
<td>Human chorionic gonadotropin (HCG)</td>
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<tr>
<td>1932</td>
<td>Cushing</td>
<td>Adrenocorticotrophic hormone</td>
</tr>
<tr>
<td>1933</td>
<td>Gutmann &amp; Gutmann</td>
<td>Prostatic acid phosphatase</td>
</tr>
<tr>
<td>1940s</td>
<td>Groes, Foley, Lewis, et al</td>
<td>Inbred (syngeneic) strains of mice and rats</td>
</tr>
<tr>
<td>1950s</td>
<td>Baldwin, Prehn &amp; Main, Foley Lewis &amp; Aptekman</td>
<td>Polymorphism of chemically induced tumours. Absence of cross-reactions</td>
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<td>1949</td>
<td>Oh – Uti</td>
<td>Deletion of blood-group antigens</td>
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<tr>
<td>1959</td>
<td>Markert</td>
<td>Isozymes</td>
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<td>1960</td>
<td>Newell</td>
<td>Philadelphia chromosome</td>
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<td>1963</td>
<td>Abelev</td>
<td>Alpha-Fetoprotein</td>
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<td>1965</td>
<td>Gold &amp; Freedman</td>
<td>Carcinoembryonic antigen</td>
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<tr>
<td>1969</td>
<td>Heubner &amp; Todaro</td>
<td>Oncogenes</td>
</tr>
<tr>
<td>1960s</td>
<td>Potter</td>
<td>Induction of myelomas in mice</td>
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<tr>
<td>1965+</td>
<td>Sachs, Horibata, Lennox, Cohn</td>
<td>Growth of myelomas in culture</td>
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<tr>
<td>1975</td>
<td>Kohler &amp; Milestein</td>
<td>Monoclonal antibodies of predefined specificity</td>
</tr>
<tr>
<td>1980</td>
<td>Cooper, Weinberg, Bishop, et al</td>
<td>Transfection, oncogene probes</td>
</tr>
<tr>
<td>1981</td>
<td>Yunis</td>
<td>Fragile sites</td>
</tr>
<tr>
<td>1984</td>
<td>Drebin, Weinberg Green. et al</td>
<td>Activated c-onc products identified with monoclonal antibodies</td>
</tr>
</tbody>
</table>

(Daar and Lennox , 1987)(10)

**Ideal Marker**
Coombes and Neville (1978)(16), have suggested specific criteria of an ideal marker, in that it should:

- Be easy and inexpensive to measure.
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Be specific to the tumour studied and commonly associated with it.

Have a relationship between plasma level of the marker and tumour cell burden.

Have an abnormal plasma and/or urine level in the presence of micrometastases, i.e. at a stage when no clinical or presently available diagnostic methods reveal their presence.

Have plasma and/or urine levels that are stable i.e. not subject to wild fluctuations.

Exist normally in a much lower concentration than that found in association with all stages of cancer.

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The Biology of CEA

The extensions of the two initial series of studies can primarily be divided between those focused on the basic biology (localization, purification, chemical definition, functional activity and genetic control) of the CEA molecule, and those directed at answering clinical questions (11). As concerns the biology of CEA, early investigations involved the purification and characterization of the molecule, utilizing anti-CEA antiserum as a guide to the recovery and concentration of CEA at each stage (17)(18). These early studies would, years later, lead to the cloning of the CEA gene and to the present understanding of the functional biology of the CEA molecule and other members of its molecular family (19)(20)(21)(22)(23).

Hypothetical Explanation of CEA Production in Malignant Cells

In the very early stages of human embryonic cellular division, each primitive cell is endowed with the totality of inherited genetic information. This genetic potential which is passed on to all subsequent generations of somatic cells, could theoretically allow each cell to specialize into any human cellular form. As anatomical orientation is initiated under the influence of certain “organizers” distinct organ systems begin to develop. Systems of “regulator gene – repressor – operator” act first to suppress the areas of the unclear genome not required for differentiation of these cells. However, the genetic potentialities necessary to allow the cells to undergo their particular type of specialization continue to be expressed (14)(15).

Fig. (1): proposed model for regulation of protein synthesis in somatic cells. (24)
Combination of repressor with the operator inhibits the production of mRNA on the structural genes. The introduction of a substrate – inducer, into the cytoplasm, which combines with the repressor leads to initiation of mRNA production on the structural genes and thereby to the synthesis of specific molecule in the cytoplasm (24).

Upon adequate differentiation of the digestive system epithelium prior to birth, certain of the cellular components needed during the course of specialization appear to be no longer required. At this stage further repression of genetic information occurs and the production of “primitive” components ceases. The disappearance of the carcinoembryonic antigens from foetal gut, liver and pancreas in the Third trimester of gestation and their absence from the corresponding normal tissues of the adult is compatible with this hypothetical sequence of events.(14)(24)

During the process of carcinogenesis in the gastrointestinal tract and the pancreas, depression of previously repressed genetic information may occur leading to the recommencement of cytoplasmic production of carcinoembryonic antigens. Depression may be due to alteration of regulator or operator genes by mutagenic carcinogens, or inactivation of certain cytoplasmic repressors by non mutagenic carcinogens. Carcinoembryonic components seem to function in maintaining the viability of the foetal and cancer cells of the gastrointestinal tract in their incompletely differentiated state, this may explain the system specificity of the production of these antigens. When differentiation is completed in the foetal cells to the point where the Carcinoembryonic antigens disappear; other mechanisms may become operative in order to maintain the function of the specialized cellular units. While in cancer cells, the carcinoembryonic constituents have to be produced continuously in order to maintain the life of the malignant cells (14)(15).

Isolation and Characterization of Carcinoembryonic Antigen [CEA]

Krupey et al. (1967)(17), attempted to purify and characterize the chemical composition of carcinoembryonic antigen (CEA). Using paper chromatography for quantities carbohydrate analysis, they revealed the presence of galactose, , mannose, focus and glucoseamine. Sialic acid was also demonstrated in purified CEA samples. The amino acid analysis of CEA showed the presence of eight amino acids. Tyrosine and lysins were found in relatively high proportions than the other six amino acid residues namely: aspartic acid, threonine, serine, glutamine, glycine and alanine.(17)

Krupey et al. (1968)(18), mentioned that the degree of purity of CEA could be identified by finding a single peak in the ultracentrifuge and a single band against untreated rabbit anti-tumour antiserum on immunoelectrophoresis. However, there were evidences that the purified preparations contained other components than carcinoembryonic antigen.
Coligan et al. (1972)(25), showed that tumours even of the same tissue type, can vary considerably in their CEA content and CEA of two molecular sizes can exist in the same tumour as proved by gel filtration, electrophoresis and ultra centrifugation. They believed that while CEA might exist in more than one form, these forms represented definable chemical entities susceptible to complete chemical characterization.

Martin and Martin (1970)(26), demonstrated the presence of a material immunologically identical to CEA in crude percholic acid extracts of non cancerous colon mucosa.

Lo Gerfo and Herter (1972)(27), showed by immunoelectrophoresis that CEA was present in benign polyps of the colon and in non-malignant rectal mucosa obtained after haemorrhoidectomy.

Pusztaszeri and Mach (1973)(28), Sizaret and Martin (1973)(29), and Lo Gerfo et al. (1972)(30), demonstrated substances immunologically identical to CEA in bronchial carcinoma and normal lung.

Fritsche and Mach (1977)(31), were able to purify CEA from normal colon mucosa. It was indistinguishable by immunological and physico-chemical criteria from reference colon carcinoma CEA. They noted that the concentration of CEA-reactive material in normal colon mucosa was about 10-40 times lower than in primary large bowel carcinomas and 50-300 times lower than in met static colon or rectum carcinomas (31)

Martin et al. (1976)(32), found an increasing data supporting the premise that CEA was elevated in many benign conditions and in non entodermally derived tumours. Significant CEA positively was detected in tumours of the , lung head and neck, reproductive organs and breast.(32)

Bale et al. (1980)(33), considered CEA to be a normal organ-specific antigen expressed to a greater extent on tumours arising in that organ.

**Physicochemical Properties of Carcinoembryonic Antigen**

CEA is a glycoprotein with a molecular weight of approximately 200.000 Daltons (18), it migrates with in the beta globulin region on electrophoresis. At neutral pH, the molecules appear in the electron microscope as twisted rod-shaped particles

At pH4, which is near their average isoelectric points, the molecules tend to be more globular. At pH9 they become more elongated presumably because the negative charges distributed throughout the molecule repel each other.

The CEA molecule is soluble in water, in percholic acid and in half saturated ammonium sulfate. The properties of solubility in percholic acid and half saturated ammonium sulfate form the basis of many of the radioimmunoassays for serum CEA (34)(35). While there is a consensus between several laboratories about the molecular weight of CEA corresponding to 180+20 kd Grunert et al (1985)(36), found two distinguishable CEA molecules in this molecular weight range (160 kd and 180 kd) which showed a peptide composition differing mainly quantitatively (36).

Pletsch and Goldenberg (1974)(37), noted that the molecular size of CEA in the serum of patients with cancer colon, cervix, ovary and bronchus was larger than the 200.0000 - Dalton CEA described initially. It was 370.000 + 4000 Daltons but it was immunologically identical to the 200.000 Dalton CEA. They put the following possibilities to explain the differences in the molecular size of the circulating antigen:

1-The CEA in the plasma of patients with certain malignant disorders might be a dimer, or perhaps a mucin - CEA complex.
2-The plasma antigen might be chemically unrelated to CEA except that it shared with it the immunoreactive site measured in radioimmunoassay.
3-The 370.000 - Dalton CEA might represent a more native form of the CEA molecule which
had not undergone enzymatic degradation to a smaller size. (37)

The most striking difference in the physicochemical properties of CEA is in the presence of widely variable percentage of carbohydrates ranging from 40% up to 82%. However, Terry et al (1974)(38) found that the percent of carbohydrate varied over a narrower range (45%-57%). The carbohydrate protein ratio of CEA was usually of the order of 3/1, a ratio as high as 5/1 in purified CEA of gastric cancer origin, and as low as 1/1 material obtained from colonic cancer tissue (39).

The principal carbohydrate constituent of purified CEA preparations is N-acety1 glucosamine which usually constitutes approximately 25% of the total weight of each preparation of purified material. In contrast N-acetyl galactosamine is usually either or present only in very low concentrations in the CEA molecule. Some degree of variation is usually either or present only in very low concentrations in the CEA molecule. Some degree of variation is usually noted in fucose, galactose mannose and sialic acid content between different preparations of CEA. The sialic acid moiety is usually the most variable of the carbohydrate constituents, up to 5 fold difference in sialic acid content has been found (39).

The carbohydrate moieties were distributed throughout the molecule, 30-36% of the carbohydrate portion of CEA was N-acetyl glucosamine, 21-27% galactose, 13-20% mannose and 13-20% fucose. Sialic acid comprised from 1-10% of the weight and was the most variable component. Traces of N-acety1 galactosamine were found (34).

The amino acid composition of the CEA preparations from all sources was constant within experimental error (Table 2).(39)(40)(41)(42)

<table>
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<td>Aspartic Acid</td>
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<tr>
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<td>11.4</td>
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<td>3.8</td>
<td>3.9</td>
<td>3.43</td>
</tr>
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</table>

ND: not done (Hirai, 1977)(43)

The amino acid sequence suggested by Terry et al (1972)(40) were confirmed by Kuroki et al (1982)(41).

Koga et al (1985)(42), presented the following amino acid sequence of CEA, NCA-2 and NFA-2. (Table 3)

<table>
<thead>
<tr>
<th>Table (3): Amino acid sequence of CEA, NCA-2 and NFA-2</th>
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<tr>
<td>Lys Leu Thr Ile Glu Ser Thr Pro Phe Asn Val Ala Glu Gly Lys Glu Val Leu Leu Leu Val His Asn Leu</td>
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</table>
Intracellular Localization of CEA

Studies with tissue cultured cells of colon cancer origin showed that CEA is a constituent of the tumour cell surface. This observation was confirmed by immunofluorescence microscopy and immunoferritin technique using electron microscopy (44). Martin et al (1976)(32), found that at least a portion of the CEA is situated in the glycocalyx of the tumour cells immediately adjacent to the surface membrane (32). CEA is located in the glycocalyx of the plasma membranes of colonic tumour cells, and in particular in that part of the membrane bordering the lumen of the anaplastic acini.(25)(45)

The studies of the metabolism of the glycocalyx showed that the carbohydrate layer can be replaced within a half life of about one day. This would account for the occurrence of CEA in plasma, if it was true for CEA also (45). The presence of CEA on the luminal surface of the tumour cells and the demonstration of CEA in considerable amounts in the mucous of hypersecretory well differentiated mucoid carcinomas proved that CEA is secreted by the tumour cells (46).

Immunological Criteria of Carcinoembryonic Antigen

Since the discovery of CEA, the definition of this antigen has been based on fulfilling certain physicochemical, immuno-chemical and even biological criteria. The identification of this substance was solely dependant upon the ability of an antiserum to recognize a specific immunodominant grouping as that detected by the original antiserum of Gold and Freedman (1965)(14). It has been subsequently shown that CEA exhibits extensive heterogeneity in its physicochemical and immunological properties.

Conventional antisera raised against CEA characteristically contain antibodies that react with a group of substances closely related to CEA. At present the major members of this family of CEA related antigens consist of the NCA which shares a similar tissue distribution with CEA, the NCA-2 and NFA-2 found in meconium and adult faeces and the biliary glycoprotein - I present in normal bile (47).

Recent studies have demonstrated the immunological heterogeneity of CEA, polyclonal antisera and monoclonal antibodies raised against CEA also react with CEA related antigens present in normal adult tissues such as spleen, lung, colon polymorphonuclear leukocytes, lymphocytes and RBCs. Thus CEA has been further defined as a family of "isoantigens" which have multiple antigenic determinants, some of them may be common to all the isoantigens, and others may be antigenically distinct for each of the members of CEA related antigens (48)(49).

Monoclonal antibodies provide a new powerful tool for the investigation of the immunologic relationship between the different CEA related antigens, and may provide a better distinction between tumour associated and normally occurring CEA related antigenic determinants (50).

However, Rogers et al (1981)(51) and Primus et al (1983)(47) anticipated that cross reactivity with CEA related antigens will be encountered with monoclonal antibodies to CEA analogous to their conventional counterpart.
The Antigenic Structure of Carcinoembryonic Antigen

Chism et al (1977)(52) considered that at least 4 different antigenic determinants existed on CEA molecule. They defined them as follows:

- A determinant specific for colon cancer CEA
- An antigenic Determinant specific for breast and ovarian CEA
- A determinant present on breast and ovarian CEA and also on colon carcinoma antigen III 
- A determinant shared by all these molecules

Primus et al (1983)(47) using monoclonal antibodies developed against colonic tumour CEA and focusing on their relative bonding to CEA, NCA (non specific cross reacting antigen) and MA (meconium antigen), were able to differentiate 3 general classes of antigenic determinants.

- Class I, contained epitopes (antigenic sites) shared among all the 3 antigens and recognized by the monoclonal antibody (NP-1)
- Class II, included antigenic sites shared between CEA and MA and identified by monoclonal antibodies (NP-2, NP-3)
- Class III, involved only antigenic determinants unique to CEA Molecule and recognized by monoclonal antibody (NP-4)

Depending on these finding they concluded that at least 4 antigenic sites (epitomes) could be differentiated on colonic tumour CEA.

One was shared by CEA, NCA and MA. Two others shared by CEA and MA, and a fourth that appeared specific for CEA. Additional CEA determinants were expected to be identified within all the 3 classes. (figure 2)(47)

**Fig. (2):**

Wagener et al. (1983)(50), using monoclonal antibodies recognized 5 different epitomes on CEA. All of the epitomes resided on the moiety of the molecule. By the use of these monoclonal antibodies, they were able to distinguish CEA from related antigens in liver and granulocytes.

Haskell et al. (1983)(53), also identified 5 different antigenic determinants on CEA with monoclonal antibodies. But they did not define whether these determinants were part of the protein or the carbohydrate components of the CEA molecule. However, they found that the binding of some monoclonal antibodies to antigens, unlike polyclonal antisera, might be quite sensitive to the ionic strength of the reaction mixture. Their study confirmed considerable Variability in the binding of CEA by monoclonal antibodies as function of the ionic strength of the medium.(53)
Imai et al. (1984)(54) established 4 distinct monoclonal antibodies, which recognized 4 different antigenic determinants. Two of the recognized determinants were of peptide nature, while the others were of carbohydrate nature. None of the monoclonal antibodies reacted with NCA or NCA-2.(54)

Yachi et al. (1984)(55), also detected 4 antigenic determinants on CEA molecules using monoclonal antibodies. The nature of only two of these determinants were recognized, one was carbohydrate and the other a peptide one.

Muraro et al (1985)(48), generated fifteen monoclonal antibodies (col 1-15), which recognized at least 5 epitopes on the CEA molecule and discriminated primary and metastatic colon carcinomas from adult tissues. None of the col-monoclonal antibodies were reactive with the surface of polymorphonuclear leukocytes. A major CEA related antigen termed NCA-1 (non specific cross reacting antigen-1) was found to be expressed by normal polymorphonuclear leukocytes. This antigen might explain the high levels of CEA commonly detected in the sera of patients with and also in smokers. At least of the col-monoclonal antibodies (col-4 and col-12) were shown to react with distal metastases.(48)

Kurokli et al (1984)(56), using polyclonal antisera in comparative studies among CEA and 5 different cross reacting substances, suggested that the antigenic structure of CEA could be divided at least into the following 4 antigenic parts (fig. 3):

1. NCA (non specific cross reacting antigen) common part: Shared with NCA, NCA-2 (in meconium), NFCA and NFA-2.
2. NFCA (normal faecal cross reacting antigen) common part: Retained on NFCA, NFA-2 and NCA-2
3. NFA-1 (normal faecal antigen-1) common part: Shared with NFA-1, NFA-2 and NCA-2.
4. CEA distinctive part. (56)

When monoclonal antibodies against CEA and related antigen were used, Kurokli et al.(1984)(56), were able to separate these antibodies into 4 distinct groups according to their reactivity’s with the studied antigens. (figure 3)

**Group 1:**
Reacted with the epitopes on NCA common part present in CEA, NFA-2, NFCA, NCA but not with NFA-1.

**Group II:**
Showed reactivity with the epitomes on NFCA common part found in CEA, NFA, NCA-2 and NFCA but not with NFA-1 and NCA.

**Group III:**
The antibodies in this group were reactive with the antigenic determinants on NFA-1 common part. Located in CEA, NFA-2, NCA-2 and NFA-1 but not NCA.

**Group IV:**
Bound by CEA.

**Fig (3):**
The results of Kuroki et al. (1984) (56), revealed that at least eight epitomes could be identified on the CEA molecule; two in group I, one in group II, 3 in group III and 2 in group IV. The antigenic sites defined by antibodies in-group IV were referred to as CEA allotypic part. The epitomes in this part were closely related and they might be one of the factors that gave rise to the various immunology forms of CEA. All the eight epitomes identified in their study were in protein moiety of the CEA molecule. Although previously it had been suggested by several investigators (57)(58) that the CEA related antigens in carcinomas outside the colon and rectum e.g. breast, ovarian and lung carcinomas, were cross reactive antigens rather than CEA. The use of monoclonal anti-CEA antibodies proved that the antigens present in most of the vital tumour tissues of different human carcinomas studied were very similar to CEA as they contain all the antigenic determinants recognized in purified CEA preparations. So it seems that in human carcinomas, the production of cross-reacting antigen without a concomitant production of CEA is a rare event excluding necrotic tissue areas where binding heterogeneity is sometimes observed (57)(58)(59).

The epitomes reactivate of 52 well-characterized monoclonal antibodies against CEA from 11 different research groups were studied using competitive solid phase immunoassays. The monoclonal antibodies against carcinoembryonic antigen appear to react with only a limited number of different antigenic areas of CEA.

C.P.: Common Part
All. P: Allotypic part
NFCA: Normal faecal cross reacting antigen.
NFA-1: Normal faecal antigen -1.
NFA-2: Normal faecal antigen -2.
NCA: Non specific cross reacting antigen.
NCA-2: Non specific cross reacting antigen -2.
Almost all (41/52) of the studied monoclonal antibodies are directed against carbohydrate epitomes and two gave inconclusive results. About 83% of those monoclonal antibodies could be classified into one of five non-interacting epitomes groups containing between 4 and 15 monoclonal antibodies each (61).

**CEA Cross Reacting Substances:**

Clarification of the chemical, antigenic, and development inter-relationships between CEA and CEA-like antigens is needed. They may have role tumour markers themselves or contribute to the measurement of CEA levels in view of their strong immunological cross-reactivity with this antigen (47).

The major members of the family of CEA related antigens could be categorized as follows:

- **NCA** (non specific cross reacting antigen)
- **BGP – 1 biliary glycoprotein – 1)**
- **NFA in faeces, NCA –2 in meconium and faeces, CEA – like substances in gastric juice, NCW and BGP II in bile. These were found in the alimentary canal and were reported to be very similar to CEA**
- **CEA – No in normal colon mucosa, identical to CEA in tumour tissue (47)(50)**

The exact size of the CEA family is unknown. To date 13 most likely different CEA – related macromolecules have been identified biochemical or by molecular biology techniques, they are:

- **NFA II (56)**
- **NCA – 160 “NCA of molecular weight 160.000 (62)**
- **NCA – II meconium antigen (62)**
- **CEA low “CEA of molecular weight 128.000” (63)**
- **NCA – 55 “NCA of molecular Weight 55.000” (38)**
- **NCA – 95 “NCA of molecular weight 95.000” (64)**
- **NFA – 1 (58)**
  - Normal plasma antigen of molecular weight 114.000 (63)
  - NCA species of molecular weight 75.000 (38)
  - CEA related meconium antigen (63)
  - Two new forms of granulocyte NCA of molecular weight 90.000 and 160.000 (65)
  - CEA related foetal liver antigen (66)

These antigens show variable degrees of immunological cross – reactivity with CEA (61).

**Non-specific Cross-Reacting [NCA]:**

Von Kleist et al (1974)(67), were the first to describe this antigen and to give it the name of non specific cross reacting antigen. NCA is a B- globulin that remains present in the percholic acid extracts of colonic carcinoma. It has a molecular weight of approximately 50.000 Daltons. It contains 25% carbohydrates and its location in the colonic cell is quite similar to that of CEA (i.e. it is situated at the surface of cells and in intraglandular deposits).(67)

NCA can be ground normally in many argons such as lung, spleen, stomach and colonic mucosa. It is also present in cancerous tissues especially of the digestive tract (32)

Several CEA like substances that had been described before were probably NCA based on immuno – chemical similarities. Among these substances were: Normal glycoprotein, CEA associated , colonic CEA-2, Colonic carcinoma antigen III, tumour associated antigen and the CEX antigen (57)(68)

As regards the faecal NCA no differences were observed between it and NCA in lungs or spleens. The molecular weight of faecal NCA is about 50.000 to 90.000 it has at least 2 parts of antigenic determinants, one unique for NCA itself and other common to CEA, NFA-2 and NFCA (57).
CEA and NCA contain homologous regions, 24 amino acids in length at the N-terminus of the polypeptide chain. The exact molecular relationship to CEA is not clearly understood and is still subject to controversy. Most difficulties in the identification of the molecules arise from the fact that CEA and NCA have different molecular weights.

For CEA, 2 molecular species of 160 and 180 kd (70) or 180 and 200 kd (56) were reported. While for NCA, molecular species of 60-80 and 100-120 kd had been reported (59)(71)(72)(73).

**Normal Faecal Antigens [NFA]:**

Although CEA related antigens in faeces other than faecal NCA have been inclusively NFA, it was found that there were 3 other molecular species of CEA related antigens in faeces, designated as: Normal faecal antigen-1 (NFA-1, NFA-2), and normal faecal cross reacting antigen (NFCA) respectively. Among these antigens NFA-1, NFA-2 and faecal NCA were isolated in pure form. NFCA has not yet been obtained in pure form but was identified as an antigen separate from the other three antigens (57).

The origin and production mechanism of faecal antigens are still unclear, but it seems most probable that NFA-1 and NFCA are produced from NFA-2 through degradation by bacterial or host enzymes in the alimentary canal. Aseptic conditions and probable lack of proteolysis enzymes in the foetal digestive tract may result in the absence of antigens in meconium corresponding to NFA-1 and NFCA (62).

**Normal Faecal Antigen – 1 [NFA-1]:**

It is antigenically unrelated to NCA, has a molecular weight of 20.000 to 30.000 and seems to be the smallest CEA related antigen reported thus far. The antigenic determinants corresponding to NFA-1 (NFA-1 determinant) appears to be the most dominant immunogenic group among several antigenic moieties on the CEA molecule because the amount of antibodies reactive with the NFA-1 is usually the largest in conventional rabbit anti-CEA antisera (57).

**Normal Faecal Antigen – 2 (NFA-2):**

It is the largest cross-reacting antigen in faeces with a molecular weight (160,000 to 170,000) similar to that of the CEA molecule from tumour tissues. Amino acid and carbohydrate compositions of NFA-2 are similar to those of CEA. Antigenically as far as the reactivity with rabbit or goat antisera is concerned, NFA-2 is indistinguishable from CEA. However, both CEA and NFA-2 has its own unique determinant residing in different parts of the molecule with different chemical characteristics. The origin of NFA – 2 in the alimentary canal is still unclear.

Fritsche and Mach (1977)(31), reported the presence of small amounts of CEA (CEA-No) in normal colon mucosa that was identical to CEA in tumour tissues. The CEA-No might be a progenitor molecule of the NFA-2 in normal faeces. The antigenic differences between NFA-2 and CEA form tumour tissues could be an expression of molecular changes of CEA-No resulting from exposure to enzymes and/or bacteria in the digestive tract. Another similar antigen described was NCW in normal colon washings. At present it is unclear whether NCW is identical to CEA-No or NFA-2 (74) CEA-like substances in gastric juice and BGP III in bile must be taken into consideration, they seem to be very similar NFA-2 (57).

**Normal Faecal Cross-Reacting Antigen (NFCA):**

The antigenic determinants of this antigen can be divided into at least 2 moieties, one common to NCA and the other common to CEA or NFA-2 but unrelated to NFA-1. This antigen is not yet separated in a pure form (57).

**Non-Specific Cross-Reacting Antigen-2 (NCA-2):**

In 1973, Burtin at al.(75), reported a cross-reactive antigen in meconium and faeces, which seemed to be similar to NFA. They named it NCA-2 to distinguish it from NCA. NCA-2 was detected in meconium and stools but it immunological and physiological characterization...
were performed only on antigen isolated from meconium. Two molecular species of CEA related antigen (NCA and NCA-2) exist in meconium in contrast to 4 molecular species (FA-1, NFA-2, NFCA and NCA) in adult faeces. NFA-2 and NCA-2 are considered the counterparts of CEA in adult faeces and meconium respectively. The molecular weight and the content of carbohydrate and amino acids among these substances are indistinguishable from those of CEA. However, antigenically each of them has a unique determinant.

These findings lead to a suggestion that NCA-2 can be a protype of CEA glycoprotein family and that its place will be taken by NFA-2 in adult tissues and by CEA in tumour tissues. The presence of small amount of molecules possessing the NFA-2 distinctive determinant in NCA-2 preparation may reflect a differentiation phase at the neonatal period during which most molecules remained as protype (NCA-2) but a small portion of them were differentiated into the adult type NFA-2 (62)

The molecular weight of purified NCA-2 was estimated to be 150,000 to 170,000 Daltons. Although NCA-2, NFA-2 and CEA from tumour tissues were indistinguishable from each other with conventional anti-CEA antiserum, the CEA distinctive determinant was absent in NCA-2 and the NFA-2 distinctive determinant was detectable in only 20% of NCA-2 molecules (76).

NCA-2 is the CEA gene family member that is structurally most similar to CEA. There are evidences that this antigen is increased in the serum of many cancer patients (77). Table (4) shows Carbohydrate composition of CEA and CEA-related antigens. (62) Table (5) shows Amino acid composition of CEA and CEA-related antigens. (62)

**Table (4): Carbohydrate composition of CEA and CEA-related antigens:**

<table>
<thead>
<tr>
<th>MONOSACCHARIDE</th>
<th>CEA</th>
<th>NFA-2</th>
<th>NCA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucose</td>
<td>10.2</td>
<td>7.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Mannose</td>
<td>8.9</td>
<td>6.7</td>
<td>5</td>
</tr>
<tr>
<td>Galactose</td>
<td>9.3</td>
<td>11.2</td>
<td>11.9</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>3.4</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>N-acetyl glucosamine</td>
<td>19.3</td>
<td>16.6</td>
<td>17.6</td>
</tr>
<tr>
<td>N-acetyl galactosamine</td>
<td>1.6</td>
<td>2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

(Matsouka et al 1982)(62)

**Table (5): Amino acid composition of CEA and CEA-related antigens:**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>CEA</th>
<th>NFA-2</th>
<th>NCA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>147</td>
<td>142.5</td>
<td>140</td>
</tr>
<tr>
<td>Threonine</td>
<td>80.6</td>
<td>88.1</td>
<td>89.2</td>
</tr>
<tr>
<td>Serine</td>
<td>85.9</td>
<td>94.5</td>
<td>90.2</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>99.7</td>
<td>106.1</td>
<td>113.1</td>
</tr>
<tr>
<td>Glycine</td>
<td>52.8</td>
<td>52</td>
<td>53.4</td>
</tr>
<tr>
<td>Alanine</td>
<td>55</td>
<td>55</td>
<td>55.5</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>2.1</td>
<td>13</td>
<td>10.6</td>
</tr>
<tr>
<td>Valine</td>
<td>65.2</td>
<td>68.4</td>
<td>73.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.9</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>48.6</td>
<td>50.1</td>
<td>51.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>79.3</td>
<td>88.1</td>
<td>92.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>39.1</td>
<td>45.2</td>
<td>42.1</td>
</tr>
<tr>
<td>Phenyl alanine</td>
<td>23.7</td>
<td>25.1</td>
<td>28.7</td>
</tr>
<tr>
<td>Lysine</td>
<td>23.8</td>
<td>241</td>
<td>24.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>15.3</td>
<td>15.6</td>
<td>20.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>35</td>
<td>37.3</td>
<td>43.2</td>
</tr>
<tr>
<td>Proline</td>
<td>72.6</td>
<td>78.1</td>
<td>75.6</td>
</tr>
</tbody>
</table>

Tryptophan was not determined (Matsouka et al 1982)(62)
Meconium Antigen (MA):

Primus et al. (1983)(47), identified 2 NCA unrelated, CEA cross-reactive substances in meconium. One of them was termed meconium antigen (MA). The relationship or identity of MA with NCA-2, and NFA family in adult faeces, particularly NFA-2 was unclear. Preliminary characterization of this antigen showed it to be a glycoprotein migrating as an alpha globulin and having a molecular weight similar to that of CEA (185,000 – 200,000 Daltons). Meconium antigen shared at least 3 epitopes with CEA. However, the observation that MA appeared at about 6 fold higher concentration than CEA in meconium could indicate that this antigen was an earlier marker of differentiation than CEA. Hedin et al. (1983)(78), considered that NCA-2 was the meconium antigen and was unable to distinguish it from CEA using a monoclonal antibody enzyme immunoassay for serum CEA.

Two meconium antigens of molecular weight 165 KD and 105 KD were isolated, their amino acid sequence was similar to that of CEA.(63)(79).

Zoubir et al. (1990)(80), considered that the molecular weight of meconium antigen was 160 KD and termed it (NCA-160).

Blood Group Determinants:

Blood group determinants have been found in CEA preparations. These finding are consistent with the idea that incomplete or unexpected glycosylation patterns occur in glycoproteins produced by tumour cells. Since antibodies directed against blood group substances cross-react with carbohydrate determinants on CEA, clinical determination of CEA or anti-CEA levels in serum may be adversely affected. The reported occurrence of natural antibodies to CEA may be an artifact caused by the binding of anti-blood group immunoglobulins form one patient to the radio labeled CEA of another. The blood group antigens that cross-react with CEA include A1,B,H,Lea,Leb and MN blood group determinants (81)(82).

Carcinoembryonic antigen in clinical practice

CEA survived the early over enthusiasm of commercial promoters to become the most widely used of the so-called tumour markers. Although its failure as a screening test for colorectal cancer was resounding, today CEA plays a major role in staging, and particularly the monitoring of a variety of malignant processes. The value of CEA as a tumour marker lies in the fact that when it is expressed its level in serum usually accurately mirrors the state of the malignant process. A clinical response to treatment weather surgical intervention, chemotherapy, or radiation therapy, is usually matched by a decline in the serum CEA level. Total remission is signalled by a fall in the CEA level to within the normal range. Use of serum CEA levels to correlate with tumour burden or to stage malignancies on less form ground (35).

Carcinoembryonic antigen in serum: Immunoassay for CEA in Serum

Thomson et al. (1969)(83), developed a radioimmunoassay technique, which could detect 2.5 mg CEA/ml. Egan et al. (1972)(84), interested in the CEA concept developed other techniques. Commercially available immunoassays for CEA include tests with radio-labelled antigens and with enzyme-labelled antibodies. The radio immunoassays are competitive binding tests in which radio labelled CEA. Competes with endogenous CEA for binding sites on antibodies to CEA. After separation of the bound form the free label, the bound portion is counted with a gamma counter, and unknown specimens are measured against a standard curve to obtain results.(84)

The enzyme immunoassays employ a sandwich procedure in which two antibodies to different sites on the CEA molecule are incubated with serum or fluids suspected to contain CEA. One antibody is bound to a solid phase; the other is labelled with an enzyme, usually horseradish peroxides. After the CEA sandwich has been formed and extraneous material has been washed away from the solid phase, a containing a chromo graphic substrate for the enzyme label is added and the color generated is proportional to the amount of the enzyme and thus to the level of CEA present.(84)
The antibodies used in these assays are raised in different ways. Some are guinea pig or goat antibodies generated using CEA isolated from tumour material; others are monoclonal, raised using CEA form tumour cells grown in tissue culture. It is inevitable that a variety of immunoassays will show differing specificities and avidities for antigenic sites on glycoprotein molecule that may not itself are homogeneous (84).

Serum CEA Levels in Normal Individuals

Martin et al. (1976)(32), found that plasma CEA levels in normal individuals were less than 2.5 mg/ml in 97% of the studied group. The remaining 3% with elevated CEA levels were heavy smokers. Among pregnant females also 3% had elevated CEA values.

Some investigators considered 2.5 mg/ml as the upper limit of normal because they noted that about 90% of normal adults had CEA levels below this limit. No differences in CEA levels related to sex, but the levels were higher over 40 years of age.(43)(85)(86)

Others considered the normal range of serum CEA from “0” to less than 5 ng/ml.(87)(88). Tholander et al. (1990)(89), reported that the upper limit of normal for 95% of the non smokers (of their control group) was 4.5 ug/litre, and for the smokers it was 7.5 mg/litre.

Meier et al. (1990)(90) and Leminen (1990)(91), considered that normal serum levels for CEA were below 3 ng/litre. While, Lundqvist et al. (1992)(94), took the value of 5.2 ng/ml as the upper limit of the normal range of serum CEA values.

Cut-Off Level for Serum CEA

Elevated levels of CEA do occur in benign and malignant disease. Most of the benign conditions that produce slight elevation in CEA antigenicity rarely exceed 10 ng/ml and most of these are between 2.5 and 5 ng/ml. Liver cirrhosis may give slightly higher results (32). The level of 2.5 ng/ml is stated to be the upper limit of the normal for plasma CEA levels.

Values greater than 2.5 ng/ml may be found in association with cancer, in particular those of gastrointestinal tract, pancreas, ovary, lung and breast.

Similarly raised CEA levels may, however, be detected in cigarette smokers, in patients with benign neoplasm, and in 15% to 20% of subjects with inflammatory disorders such as, Chron’s disease, pancreatitis, liver disease and pulmonary infections. Thus raised plasma CEA values are not specific for cancer, although very high levels (e.g. > 20 mg/ml) are highly suggestive of malignancy. It is important that serial assays of CEA are used in reaching a clinical judgment, and not any single determination. Each laboratory doing CEA assays should establish its own “normal range”. The recommended upper level of normal (2.5 mg/ml) in the population requires additional evaluation (93)(94). The level of CEA defined as the upper limit of normal markedly affects the results obtained. Raising this limit to 5 mg/ml reduced the number of elevated values in patients with benign disease to only 4%. Also, 59% if patients with intraepithelial cancer and 43% of patients with invasive cancer had been excluded on taking this level. Using the limit of 2.5 mg/ml permitted the inclusion of these patients in the positive group. On the other hand, 11% of normal healthy volunteers and 18% of patients with benign gynecologic disease had plasma CEA concentration above 2.5 mg/ml. This lack of specificity makes CEA unreliable as sole screening method for patients with gynecologic malignancy (95).

Seppala et al. (1975)(96), considered the serum CEA level 5 n/ml as a discriminating one between malignant and non-malignant states. By setting this higher threshold greater specificity for cancer was achieved, but at the cost of missing some cases, the cut-off level of 5 mg/ml was also recommended (88)(97)(98).

Serum CEA in Gynaecological Malignancies:
Lo Gerfo et al. (1971)(30), were the first to report elevated serum CEA in patients with gynaecological malignancies.

Estimation of plasma CEA levels doesn’t appear to be a worthwhile screening procedure, because of the detection of CEA in non-cancerous conditions. However, it is valuable in the diagnosis of some cancers (45)(99).

The most significant applications of CEA to gynecologic malignancy would be in the diagnosis and management of patients with ovarian cancer. Carcinoembryonic antigen from the plasma and cyst fluid of patients with ovarian cystadenocarcinoma had been shown to have immunologic cancer. Elevated CEA level correlated guide well with malignancy. Elevated CEA level correlated guide well with malignancy. In those patients with ovarian cancer who had elevated CEA levels prior to therapy, serial CEA determinations following treatment had been reported to be of value in diagnosing non-palpable recurrent disease(95).

Disaia et al.(1977)(100), studied a group of patients with invasive squamous cell carcinoma of the cervix and found that there was a progressive increase in the percentage of patients with positive CEA values with the advance of the stage from 26% in stage I to 88% in stage II. Eighty five percent (85%) of the recurrent cases showed a positive CEA values.

Difficulty of marking diagnosis of recurrence is encountered particularly in patients who had received radiotherapy, the presence of radiation fibrosis often makes the detection of tumours impossible. In these patients, the CEA assay could be of considerable value. (99)(100).

Three significant patterns of change in serum CEA levels have been shown. The first is the pattern of free disease, when there is a rapid disappearance of CEA in serum after treatment. The second is residual cancer, if CEA is persistently detected in serum often at high levels. The third is clinical recurrence that is detected during close follow up of patients when CEA reappears in the serum before clinical detection (99).

It appears that the greatest value of CEA assay lies in monitoring the results of primary treatment and in the follow of patients whose tumour or plasma contain high antigen concentrations before therapy. Characteristically, plasma CEA concentrations decrease to normal levels within 4-8 weeks following complete excision of cervical or ovarian tumours.

In contrast, the decline of plasma CEA to normal often takes as long as 12 weeks after completion of radiation therapy. This is presumably due to the prolonged release of antigen form the cell membrane of radiation damaged tumour cells. The progressive increase in plasma CEA levels, during the follow up patients with the cervical and ovarian cancers, accurately predicted tumour recurrence in over 80% of patients whose tumour strained immunohistochemically for CEA. Rising plasma CEA values preceded clinical detection of recurrence by up to 6 months cases (101).

Parente and Greston (1981)(102), found elevated serum CEA levels in 60% of the patients with malignant lesions involving the female reproductive tract. They considered that CEA assay was not suitable for screening of malignant tumours because of the large number of patients with benign having false-positive results.

Schwartz et al.(1987)(103), reported an elevated levels of CEA in the serum of 30-65% of ovarian, 43-69% of cervical, 32-57% of vulvar cancer.

The use of CEA in the diagnosis and follow up of patients has been hampered by conflicting reports in the literature. The proportion of ovarian carcinoma patients having an abnormal serum levels (.5 mg/ml) during the course of the disease ranges form 0 to 70%, and a variable relationship of elevated CEA to histologic cell type was observed (104). The differences in clinical correlations might be due to effects of antigens cross-reacting with CEA, (e.g.: NCA, NCA-2 and BGP (105). The selection of patients for serial plasma CEA evaluation could probably be improved by selection of patients with CEA detected in tumour tissue. Unfortunately, tissue distribution studies have shown marked divergence as to the proportion of the cases and the type of epithelial ovarian cancer associated with CEA (106). Although carcinoembryonic antigen initially stimulated great interest as a possible diagnosis marker for common epithelial malignant tumours, including gynecologic malignancies in only a small percentage of gynaecologic cancer patients and also in some patients with benign disease. Thus, limiting its value as a clinically useful tumour marker (107).
Carcinoembryonic Antigen in Tumour Tissues: Immunocytochemical Detection of CEA

ImmunocytoLOGY has provided significant contributions to biological and science since its inception by coons et al. (1942)(108), because its unique ability to correlate the location of antigen cellular constituents with anatomical structures. Although immunofluorescence remains the most widely used immunocytological technique, the development of immunoenzyme procedures in which the site of immunologicaL reactivity is detected by histochemical reaction has permitted the localization of numerous substance of biological and medical interest. Most studies have used immune-peroxides technique.

Unlike immunofluorescence, specimens stained by immunoperoxidase are permanent, can be counterstained with routine histology stains allowing direct comparison of immunologic reaction with morphologic details, and don’t require special tissue manipulations if antigen activity is preserved following formalin fixation and paraffin embedding (109).

After the discovery of CEA attention was focused primarily on detection of CEA in the blood of suspected or proven cancer patients. It seems reasonable to determine first if the antigen is indeed present in the original malignant lesion before extensive and repeated monitoring is undertaken. Positive immunocytochemical reacting for CEA in the primary tumour predicts well, which cases will have a blood CEA value reflecting disease activity. A negative CEA immunocytochemical reaction doesn’t mean tat the tumour is devoid of CEA, but only its CEA concentration is below the threshold of the staining reaction (110).

CEA producing tumours and their metastases could be localized using photoscan detection of radiolabeled anti – CEA antibodies even when these tumours were undetectable by other conventional diagnostic procedures.(111)

Immunocytochemical detection of CEA in metastatic tumour tissue may be helpful in suggesting the site of the primary tumour (105).

Techniques of Immunoperoxidase Staining for CEA: Three-Step Unlabeled Antibody Technique

The initial demonstration of CEA in conventional tissue

Sections with immunoperoxidase were done (112) using the 3-step, unlabeled, antibody technique. This method starts with reacting the primary goat CEA antiserum with the antigen containing tissue section. After incubation, excess antiserum is removed and the tissue is treated with the “bridging” secondary antiserum, rabbit anti-goat IgG, which attaches to the primary CEA antibody with one of its two combining sites. The remaining combining site is then free to react with the last antiserum addition, goat-antiperoxidase antibody. Both of the combining sites of the later antibody are free to react with horseradish peroxides and the sites of antigen localization are detected by a brown reaction with diaminobenzidine and hydrogen peroxide. Prior to dehydration and mounting, the tissue sections can be treated with a nuclear or cytoplasmic stain although non-counter stained tissue section may be required in specimens showing weak CEA reactivity.(112).

A variation of the 3-step procedure is peroxidase-antiperoxidase method (PAP method) of Sternberger (1974)(113). This method simplifies the 3-step procedure since performed complexes of peroxidase-antiperoxidase are added to tissue sections previously treated with the primary and secondary antibodies. Thus, eliminating the incubation step with free peroxides (figure 4)(114).

Fig (4): Simplified Schematic Representation of Immunoperoxidase Reactions:

- 3- step, unlabeled antibody (A).
- 3- step unlabeled antibody PAP, (B).
- 2- step, unlabeled antibody (c)
- 1- step, unlabeled antibody (D)
(Primus et al 1978)(114)
Two Step Labelled Antibody Techniques:

The most commonly used immunohistochemical method, in which the tracer peroxides, is chemically conjugated to the second antibody (rabbit anti-goat IgG). This shortens the time required to perform the staining procedure and also obviates some of the complications associated with antibody dilutions encountered in the 3-step methods (114).

One Step Technique:

For immunoelectron microscopy, the direct or one-step method is used, because of the difficulties in antibody penetration, tissue manipulation procedures and structural preservation met within other methods (114).

Threshold CEA Tissue Staining Reaction

The 3-step unlabeled antibody immunoperoxidase reaction for CEA has been found to detect CEA concentrations above 0.7 mg/g tissues in frozen, ethanol fixed specimens, and above 3-5 mg/g in formalin-fixed paraffin embedded tissues (115)(116).
Tumour Tissue Susceptibility for CEA Staining:

There is a proclivity for CEA staining in certain tumour type, and also among different populations of tumour cells within a lesion.

- First, tumours of epithelial character are particularly prone to CEA staining.
- Secondly, of epithelial tumours adenocarcinoma and squamous cell carcinoma express CEA. In adenocarcinoma CEA is most closely associated with the apical border of the cells lining the lumen of the malignant glands or cysts.
- Finally, in cystadenocarcinoma of the ovary, tumours of the mucinous variety stain for CEA more frequently than those of the serous types.

Carcinoembryonic antigen staining was most commonly seen in the more keratinized differentiated areas of the squamous carcinomas. The localization of tissue CEA in more differentiated areas, and the tendency of elevated serum levels to occur predominantly in poorly differentiated cases is controversial and can not readily be explained (117)(118).

In lesions with more undifferentiated cell forms, tissue CEA may be masked and therefore difficult to be demonstrated immunohistochemically, since antibody molecules may not combine with antigen material latent molecules may not combine with antigen material latent in tissue sections. Pre-treatment of tissues with trypsin unmasks latent cellular antigens and renders them readily demonstrable (119).

Ogawa et al (1992)(120), found faint expression of CEA in a few superficial and intermediate cells of normal cervical squamous epithelium. However, CEA was more strongly expressed in cancer tissues of the large cell and keratinized types.

Light and electron microscopic findings in immunostained tumours for CEA

The majority of the light microscopic studies have shown a linear labelling of the apical poles of adenocarcinoma cells lining the glandular lumen and staining of intraglandular deposits. A diffuse or granular cytoplasmic label is specially evident in anaplastic tumour cells. Immunoelectron microscopy studies with ferritin or peroxidase – labelled antibodies revealed an intimate association of CEA with the glycop concentrating. However, other evidences suggest that CEA is also a structural glycoprotein of the plasma membrane (114).

Carcinoembryonic Antigen in Cervical Neoplasms

Tissue CEA in neoplasms of the cervix:

- Carcinoembryonic antigen has been demonstrated in invasive squamous cell carcinoma and endocervical carcinoma of the cervix (121).
- CEA was present in all adenosquamous carcinomas, but only in 77% of adenocarcinomas.(122)
- CEA content is related to cell type in primary and metastatic cervical cancer and may vary widely between patients. In general keratinizing squamous cell carcinomas and adenocarcinomas contain the highest antigen levels and antigen distribution is quite uniform throughout the tumours. Conversely, non keratinizing squamous cell tumours contain little antigen and the distribution is uneven CEA content and distribution are similar in primary cervical cancers and in their lymph node metastases.

Therefore, immunohistochemical staining of the primary tumour for CEA can help identify those patients who benefit most from lymphoscintigraphy (101). Different degrees of positively to CEA were found according to the histological types of cervical Aden carcinomas. All cases of endometriosis – type Aden carcinomas were strongly positive with anti-CEA, while in the end cervical type Aden carcinomas the less differentiated cases were strongly positive with anti-CEA than the well differentiated ones. Immunohistochemical reactions with anti-CEA were detected in the lesions of Aden carcinoma in situ. However, Aden carcinoma in situ of the cervix has 2 histological types; common or endometriosis and end cervical. The endometrioses type
adenocarcinoma in situ exhibited a more positive reaction with anti-CEA than the endocervical type (123).

**Tissue CEA in Rare Cervical Neoplasma:**

- True mesonephric adenocarcinoma of the cervix appears to be a genuine, rare histopathologic entity.
- Immunohistochemical staining for CEA doesn't appear to be helpful in distinguishing this variant of cervical adenocarcinoma from the more common type, as mesonephroid adenocarcinomas of the endocervix are CEA negative (124)(125).
- Although CEA is rarely demonstrable in benign lesions of the cervix, it was found in a very rare case of villous adenoma of the uterine cervix. This finding is consistent with the positive staining of colonic villous adenomas mentioned in literature. Carcinoembryonic antigen is consequently not useful in distinguishing villous adenomas from exophytic papillary cervical adenocarcinomas (126).
- Adenoma malignum of the cervix is an extremely rare variant of adenocarcinoma. It is a potentially malignant tumour histologically characterized by adenomatous proliferation with structural abnormality in the shape and arrangement of the glands, and budding invasive pattern in the stroma. The glands filled with mucous substances often ballooned and ruptured with leakage of mucous into the stroma. Its diagnosis is rendered very difficult by its strong similarity to benign endocervical hyperplasia.
- Immunohistochemical staining for CEA has proved to be of great help in the differentiated diagnosis (127)(128).
  - CEA antigen present in the columnar cell of adenoma malignum, and absent in the normal endocervical glands.(129).
- The finding of positive CEA staining in the cytoplasm of adenoma malignum cells led to further study to detect CEA in the cervical mucous in careening for this tumour (128).

**Patterns of Immunohistochemical Staining for CEA in Different Histological Types of Cervical Malignancy:**

Immunoperoxidase histochemical techniques allow the visualization by light microscopy of tumour CEA when more than 3-5 mg/g are present. Tumour concentration of CEA correlates well with immunoperoxidase staining. Plasma CEA titre doesn't necessarily reflect tumour CEA content but rather total tumour burden (i.e. tumour CEA concentration x tumour mass). Over 30% of cervical epidermoid carcinomas show positive CEA immunoperoxidase staining, whereas non of the cases of carcinoma in situ or dysplasia is positive(133). Wahlstrom et al (1979)(130), found immunoreactivity to CEA in 80% of cases of cervical adenocarcinoma (131).

**Squamous Cell Carcinoma:**

Staining for CEA was detected in the atypical epithelium on or about cell membranes with some cytoplasmic localization. Normal cervical epithelium, as well as areas of reactive basal hyperplasia and squamous metaplasia, did not show any appreciable staining. In carcinoma in situ involving endocervical, CEA was found with maximum intensity near the lumen and was negative near the basal layer. Grading of non invasive lesions did not have a significant impact on the degree of the positive reaction except that mild dysplasia did not show a strong positivity (132).

CEA was demonstrated in all studied cases of cervical intraepithelial neoplasia and in 70.6% of invasive squamous cell carcinomas. A heterogeneous pattern of staining was observed in different cases and also within the tumour itself.(133)

CEA was not expressed in normal squamous epithelium except for a faint expression in a few superficial and intermediate cells.(120)

CEA is an oncogenic antigen of cervical squamous cell carcinoma due to its strong expression in these tumours. No significant difference in immunoreactivity was observed between primary and metastatic lesions of carcinoma or between primary lesions with and without metastasis. Eighty eight percent (88%) of invasive squamous carcinomas showed positive staining. They exhibited greater variability in the number and topography of CEA positive cells than invasive lesions. More than half (57%) of invasive squamous carcinomas showed positively stained cell in the layers
adjacent to stroma, a feature designated as “CEA marginal effect”. This emerging pattern of CEA tissue distribution may be used to differentiate between non invasive and invasive squamous lesions. The degree of positive staining for CEA depends to some extent on the cellular types of invasive squamous carcinomas. Small cell carcinomas frequently lack stainable CEA while large cell non keratinizing carcinomas show a stronger reaction than their keratinizing counterparts.(120). Metastatic lesions show the same CEA pattern as the corresponding primary tumour including the marginal effect. Goldenberg et al (1978)(110), utilized this phenomenon , proposed a photo scanning technique for the detection of metastatic spread of cervical carcinoma using radiolabeled of metastatic spread of cervical carcinoma using radiolabeled anti - CEA antibodies (110)(132).

Dallenbach and Lang (1991)(134), depending on histological and immunohistochemical examination subdivided invasive carcinomas and preinvasive lesions of the uterine cervix into the squamous (large cell, ectocervical) type and the reserve cell (small, large of clear cell, endocervical) type. Immunohistochemical all of the invasive and preinvasive squamous cell carcinomas were negative with anti -CEA. Whereas most of the invasive and preinvasive reserve cell carcinomas acquired expression of CEA.

Toki and Yajima (1991)(135), investigated the relationship of the positive pattern of CEA staining in squamous cell carcinomas of the cervix, to the prognosis of the patients with the neoplasm. Four patterns of localization of CEA were observed depending on the location of CEA - positive areas in cancer nests: central type, surrounding type, diffuse type and focal type. The prognosis of patients was most excellent in the disease of central type, diffuse type and focal type.(135).

The associations of CEA, tissue polypeptide antigen (TPA) and squamous cell carcinoma antigen (SCC-Ag) for detection of cervical cancers with type of cancer, clinical stage and lymph node metastasis were studied. The sensitivities of CEA, TPA, and SCC-Ag were 15.2%,33.6%,and 49.3%.The specificities were 89.3%,96.2%,and 95.1% respectively. The positive rate of CEA was found significantly higher among patients with adenocarcinoma than those with squamous cell carcinoma. The positive rate of CEA and SCC-Ag increased with advance of the clinical stages. There was no significant in the rate of each tumour marker between patients with lymph node metastasis and those without metastasis.(136).

A total of 120 patients with squamous cell carcinoma of the cervix were prospectively monitored with simultaneous serum level estimations of squamous cell carcinoma antigen (SCC), CEA, and tumour-associated trypsin inhibitor (TATI) in different stages and phases of their disease. Positive values were observed for SCC in 72 patients(60%),for CEA in 47(39%).and for TATI in12(10%).In addition,28 patients, who had a renewed progression after a complete remission with normal marker levels, showed an increase in SCC in 26 cases(92%) and CEA in 7(25%).(137).

IN other study SCC was raised in 65% of squamous cell carcinoma of the cervix ,CEA was raised in less than 25%.,(138).Pretreatment of SCC in conjunction with CEA is a valuable tumour marker to predict the prognosis of squamous cell carcinoma of the uterine cervix and to foresee a clinical response to subsequent neoadjuvant chemotherapy.(139).

Adenocarcinoma:

Carcinoembryonic antigen immunohistochemistry may be a valuable adjunctive tool for the diagnosis of endocervical adenocarcinoma, anything more than focal positively clearly signifying malignancy. Negative staining strongly suggests malignancy especially if the tissue sample is large. Sixty four percent (64%) of adenocarcinomas are positive for CEA. The staining is often locally intense near the apex of the columnar cells in well - differentiated lesions, but is more diffuse in the more poorly differentiated specimen. Not all the cells are positive in any given tumour, and the extent of differentiation of the cells.

Luminal staining representing sloughed cells and/or secretion products is occasionally intense. Most of the cases (95%) of microglandular hyperplasia are negative for CEA, few only show focal positively (140).

A review of the literature concerning tumour markers used in adenocarcinoma of the cervix shows that squamous cell carcinoma antigen, tissue polypeptide antigen, CEA, CA 125,and a few others such as CA15.3,TAG72,trypsin inhibitor have been studied(140)(141)(142).There is, however, no consensus on how useful tumour markers in adenocarcinoma of the cervix, nor is there any clear agreement on which one to use. Ngan et al 1998(144), reported that CEA was raised in 26% of patients with adenocarcinoma of the cervix, SCC was raised in 25%,tissue polypeptide antigen was
Carcinoembryonic Antigen (CEA) In Cervical Neoplasia (Review)
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raised in 35%, and CA 125 was raised in 27% of these patients. Kudo et al. (1990) reported that 50% of in situ cervical adenocarcinomas and 55% of invasive adenocarcinomas had positive staining reaction for CEA. Whereas 67% of the adenosquamous carcinomas showed a similar reaction. They also mentioned that CEA was less effective in identifying poorly differentiated lesions.

Distinction Between Endocervical and Endometrial Adenocarcinoma Using Tissue Staining for CEA

The distribution of CEA as determined by immunohistochemistry has been suggested to be useful in the distinction between endocervical and endometrial adenocarcinomas. Eighty percent (80%) of cervical adenocarcinomas and 8% of endometrial adenocarcinomas are CEA positive. The commonest exceptions are endocervical mesonephroid adenocarcinomas (which are CEA negative) and endometrial adenosquamous carcinoma (which are CEA positive) (130).

Maes et al (1988), using well absorbed anti-CEA serum or monospecific monoclonal anti-CEA antibody, found no difference in the expression of CEA between endocervical and endometrial adenocarcinoma.

The majority of endocervical adenocarcinomas were showing abundant diffusely distributed intracellular CEA and mucin, and luminal secretions of acidic mucosubstances. Only half of the endometrial adenocarcinomas contained CEA, usually focally distributed in small amounts particularly at the apical cell surface, with focally distributed mucin in luminal secretions.

96% of endocervical and 70% of endometrial adenocarcinomas revealed positive CEA immunostain. The extent of staining did not seem to correlate with the degree of differentiation in either kind of cancer. Among the positive cases, CEA staining was located over whole cytoplasm in 80% of positive endocervical adenocarcinomas and on apical surface in 75% of positive endometrial adenocarcinomas. The pattern of CEA distribution in endocervical adenocarcinoma of endometrioid type was not similar to that observed for primary endometrial adenocarcinoma.

Uedal et al. (1983), using immunoperoxidase stain for CEA, realized that all endocervical adenocarcinomas revealed the presence of CEA, while a mesonephroid adenocarcinoma did not. The antigen was not observed in the glandular structures of endometrial adenocarcinomas, although CEA was detected in all foci of squamous metaplasia within these tumours. CEA was also located in endocervical type of glandular epithelium within a special endometrial adenocarcinoma containing predominantly this type of epithelium. The staining pattern for CEA in the endocervical adenocarcinoma was related to the degree of histological differentiation of tumours, in the well differentiated glandular structure CEA was located on the luminal surface, while it was detected in the whole cytoplasm of tumour cells within the moderately and poorly differentiated areas. Finally, they concluded that the immunoperoxidase stain for CEA would be useful for estimating malignancy of glandular structures within the uterus, distinguishing endocervical adenocarcinoma from endometrial one, and grading of histological differentiation of endocervical adenocarcinoma.

Endometrial adenocarcinomas did not show reactivity with CEA immunostain, while all sections from endocervical adenocarcinomas were positive for CEA. CEA was considered as a useful tumour marker in differentiating endocervical and endometrial adenocarcinomas.

The discrepancy of the results of different may possible be attributed to variations in the specificity of polyclonal anti-CEA sera and the influence of cross-reactivity of these antisera with normal tissue antigens such as NCA 1, NCA2, and BGP 1.

Serum CEA in Cervical Neoplasms

Plasma CEA levels are significantly elevated > 2.5 mg/ml in 48% of patients with cervical cancer prior to therapy as opposed to 11% plasma CEA elevations in healthy volunteers. Carcinoembryonic antigen values greater than 5 mg/ml are obtained in 24% of patients with cervical malignancy as opposed to 1% in healthy volunteers (146).
The incidence of elevated plasma CEA is directly related to the stage or extent of the disease. Forty-two percent (42%) of patients with stage I cervical cancer had increased plasma CEA concentrations, whereas 60% of patients with advanced stage disease had elevated antigenaemia. Plasma CEA levels are more consistently elevated in patients with endocervical adenocarcinoma that in patients with squamous cell carcinomas, CEA is more often increased in patients with large cell tumours than those with small cell lesions. There is a trend toward increasing frequency of abnormal plasma CEA values with progressive tumour dedifferentiation. Only 36% of well differentiated cervical tumours had elevated plasma CEA levels as compared to 58% of poorly differentiated lesions (146).

Cut off Level for Carcinoembryonic Antigen in Serum

When a level of 2.5 mg/ml is taken as the cut-off point, an acceptably high false positive rate is detected in normal controls e.g. 11% (97), 10% (102), and 18% (111). Ito et al (1978)(147), found that the mean CEA level for normal controls was 2.03 mg/ml with a standard deviation of 0.49. Hence, they considered 3.01 mg/ml to be the upper limit of normal for serum CEA levels.

When a CEA level of 5 mg/ml is taken as a cut-off point, the average proportion of patients with high levels varies from 63% in cancer of the ovary to 36% with cervical cancer compared to 0% in controls (148)(149)(150).

A cut-off value of 5 mg/ml is more appropriate than 2.5 mg/ml although such a high cut-off level will limit the possible value of CEA as a monitoring parameter post-treatment. However, if a lower cut-off value is used, e.g. 2.5 mg/ml, many cases with non specific elevations will be included, which will make interpretation of the test difficult and further discredit the use of CEA determination for practical purposes (97)(151).

Cauchi et al (1981)(152), found that 38% of controls had high values, compared to 54% of cancer cervix patient when a cut-off value of 2.5 ug/1 was used. But with a 10 ug/1 cut-off point, none of the controls had a high value, compared to 8% of cancer patients.

Hansen et al (1974)(153), and Franchimont et al (1976)(156), also emphasized the importance of taking CEA > 10 ug/1 as the cut-off level.

Braun et al (1981)(155), suggested a cut-off level of 12 mg/ml as this was the highest level of CEA encountered in a group of healthy volunteers in their study.

Lemenen (1990)(91), Meier et al (1990)(90) and Lemenen et al (1992)(156), took the value of 3 mg/ml as the cut-off level for serum CEA in cases of cervical carcinomas. While Lundqvist et al (1992)(92), considered 5.2 mg/ml as the suitable cut-off point.

Pre - Treatment Serum CEA in Cancer Cervix

Elevated serum levels of carcinoembryonic antigen have been regarded as an unfavourable prognostic sign in malignant disease. It appears to correlate with the total tumour burden, but it is not known whether CEA positive tumour cells are more aggressive than those that do not express CEA (157).

Pre-treatment serum levels of CEA exceeding the cut-off limits were the highest in advanced disease stages and in highly differentiated forms of cervical cancer (158). TeVelde et al. (1982)(159), indicated that a pre-treatment level exceeding the individual upper limit of normal was associated with a very poor prognosis as it was encountered in about 40% of all patients who developed recurrence.

However, Braun et al (1981)(160), could not establish a relation between the pre-treatment plasma CEA levels and the probability of recurrence after treatment of cervical cancer.

Yokoyama et al (1987)(161), found elevated pre-treatment plasma CEA level in only 19% of patients with cervical cancer.
Effect of Histological Type of Cervical Carcinoma on Pre-Treatment Serum CEA Levels

It is important to differentiate between tumours of different histological types. Adenocarcinomas of the cervix are basically different from squamous cell carcinomas in their ability to produce high levels of plasma CEA. So a high pre-treatment level in patients with adenocarcinoma doesn’t necessarily correspond to the high recurrence rate found in patients with squamous cell carcinoma with grossly elevated values, because these 2 tumours are biologically different and may respond differently to treatment. In approximately 50% of all patients with squamous cell cancer of the cervix, CEA will be of predictive value. Pre-treatment levels over 5 ug/l are highly suggestive of metastatic disease as they are associated with metastases in pelvic or para-aortic lymph nodes in 50% of patients with stage IB disease. Also in advanced disease such as stage III and IV 48% of patients had a pre-therapy value exceeding 5 ug/L (151)(162).

Plasma CEA is elevated in 48% of patients with invasive carcinoma of the cervix uteri. The percentage of patients with positive CEA values rises appreciably in those with advanced squamous cell carcinoma of the cervix uteri. Positive CEA values were detected in 92% of patients with recurrent squamous cell carcinoma of the cervix (163).

Carcinoembryonic antigen is a reliable tumour marker in patients with adenocarcinoma of the cervix. An excellent correlation has been noted between pre-treatment plasma levels and extent of the disease, high pre-treatment values were associated with bad prognosis. Contrary to squamous cell carcinomas, a low value is equivocal, meaning either that the tumour volume is small or that the tumour releases little no CEA. The presence of lymph node metastases has more pronounced effect on the CEA values than the size of the primary tumour. It is unlikely that metastases have different capacity for CEA production than the primary tumour.

The reason for the lack of antigen release from some primary tumours initially and the later appearance of CEA in serum with recurrent disease remains unknown. These tumours are more probably non-releasers than non-producers (151)(164).

Meier et al (1990)(90), found an elevated serum CEA level (> 3 ng/ml) in 54% of the cases of squamous cell carcinoma of the cervix and in all cases of adenocarcinoma and adenosquamous carcinomas.

Leminen (1990)(91), reported an elevated pre-treatment serum CEA level (>3 ng/ml) in 36% and 33% of the cases of adenocarcinoma and adenosquamous carcinoma respectively. They noted an increase in the incidence of cases with positive serum CEA levels with the increase of grade of malignancy being 33% in grade I, and grades II & III. They recommended the determination of pre-treatment levels of serum CEA and its subsequent use for monitoring of treatment even if the initial level was negative, because during follow up the tumours may become marker positive.

Effect of Clinical Stage of the Disease on Pre-Treatment Serum CEA Levels

Fritsche et al (1982)(97), using a cut-off value of 4.5 mg/ml found that the incident of elevated serum CEA in patients with squamous cell carcinoma of the uterine cervix may range from zero – 10% for in situ carcinoma, 13-30% for stage I disease, 25-40% for stage II, 40-60% for stage III and 30-60% for stage IV disease. Elevated plasma CEA levels (over 2.5 ng/ml) are reported in 36-80% of invasive carcinomas of the cervix (both epidermoid and adenocarcinomas). The frequency of elevated CEA values increases with advancing stage of the disease reflecting tumour burden. In stage I carcinoma of the cervix 21-63% of patients have elevated levels, while in stage IV disease 75-100% are plasma CEA positive frequencies of plasma CEA elevations are lower in cervical dysplasias, with 23% positivity in moderate dysplasia and 28% positivity in severe dysplasia as compared with 33% positivity in insitu carcinomas. Recurrent cervical tumour result in elevated plasma CEA levels in 85% of patients (97)(131).

Braun and Leyendecker (1983)(165), taking 12 mg/ml as the upper limit of normal for CEA, found that only 14% of patients with stage I and 29% of those with stage II carcinoma of the cervix had
elevated serum levels. Similar results were also observed in 45% and 73% of the patients with stage III and IV respectively.

Aaran et al (1988)(166), noted in a prolonged study that CEA levels of cervical carcinoma patients were higher than those of normal population years before the diagnosis of cervical carcinoma was made.

Leminen (1990)(91), found and elevated serum CEA level (>3 ng/ml) in 32% of the cases of cervical adenocarcinoma (stage I, II) and in 50% of the patients with stages III and IV.

Serum CEA levels were significantly higher among patients with adenocarcinoma than those with squamous cell carcinoma. These levels increased with advance of clinical stages.(144)(167).

Ito et al (1978) (147), after studying serum CEA in patients with carcinoma of the cervix reached the following conclusions:

1. Determination of serum CEA levels cannot serve as a test for the early diagnosis of cervical cancer or pre-cancerous lesions.
2. In patients with clinical stage I or II cervical cancer, an elevated serum level indicates a greater likelihood or parametral invasion or lymph node metastases.
3. In patients with treated carcinoma of the cervix, continued normal levels of serum CEA affirm continued absence of the disease, whereas development of an elevated level is highly suspicious of occult residual or recurrent cancer.

Van Nagell et al (1978)(168), presented the relation between plasma CEA and stage of cervical malignancy, cell type and histological differentiation in the following 2 tables (table 6&7)

| Table (6): Plasma Carcinoembryonic Antigen Related to Stage |
|-----------------|-------------|-------------|-----------|----------|
| **Stage**       | **< 2.5**   | **2.5-4.9** | **5-10**  | **>10**  |
| Ia              | 41%         | 36%         | 23%       | 0%       |
| Ib              | 63%         | 27%         | 9%        | 1%       |
| Ila             | 44%         | 32%         | 12%       | 12%      |
| Iib             | 57%         | 18%         | 16%       | 9%       |
| IIIa            | 50%         | 25%         | 25%       | 0%       |
| IIIb            | 44%         | 19%         | 31%       | 6%       |
| Iva             | 44%         | 32%         | 12%       | 12%      |
| Ivb             | 33%         | 33%         | 1%        | 33%      |
| **Total**       | 52%         | 24%         | 17%       | 7%       |

(Van Nagell et al 1978) (168)

| Table (7): Plasma carcinoembryonic antigen related to cell type and histological differentiation |
|-----------------------------------------------|-------------|-------------|-----------|----------|
| **Cell type**                                | **< 2.5**   | **2.5-4.9** | **5-10**  | **>10**  |
| Keratinizing squamous Cell                   | 52%         | 32%         | 12%       | 4%       |
| Small cell                                   | 82%         | 6%          | 6%        | 6%       |
| Large cell non Keratinizing                  | 52%         | 23%         | 20%       | 5%       |
| Adenocarcinoma                               | 40%         | 20%         | 20%       | 20%      |

| **Histological differentiation**             | **< 2.5**   | **2.5-4.9** | **5-10**  | **>10**  |
| Well                                         | 64%         | 21%         | 14%       | 1%       |
| Moderately                                   | 51%         | 26%         | 15%       | 8%       |
Post - Treatment Serum CEA in Cancer Cervix

Patterns of Change in Serum CEA Levels following Treatment

Plasma CEA levels returned to normal in over 90% of patients within 4 weeks following complete tumour excision. This was in marked contrast to the patterns of decrease in CEA values following radiation therapy, in which plasma antigen levels often did not return to normal until 16 weeks following completion of therapy. This difference has been attributed to the protracted release of membrane associated antigen after radiation – induced cell damage, compared to the abrupt removal of the antigen source by surgery (168)(169).

Kjorstad and Orjasaester (1982)(151), found that the lowest post radiotherapy level was not reached until many months after termination after termination of treatment, and in some patients the values continued to fall for as long as 18 months. However, the CEA level measured immediately after radiation therapy in patients with initially elevated CEA is indicative of the prognosis of the respective patients.

The median survival time of the patients with persistently elevated CEA levels was significantly shorter than that of patients in whom initially elevated CEA levels declined into the normal range immediately after therapy (155)(160).

Chemotherapy is often the only available treatment for advanced cervical carcinoma or recurrent disease, even though the results of treatment in squamous cell carcinoma remain poor. The course of CEA level can help in early decision, whether or not patient would profit from a continuation of therapy (170).

A group of patients with cervical carcinoma treated with cytotoxic drugs were followed up for years. In more than 90 % of the patients CEA was elevated before the treatment, so that the course of the tumour marker levels fell rapidly to normal after one or two cycles. Non of these patients showed tumour progression while the tumour marker levels were within the normal range. Clinical remission was not obtained in those patients with levels, which remained high or rose again following an initial decrease.(170).In cases of primary progression of cervical carcinomas, serum CEA levels decrease for a short time initially following chemotherapy, but never approach normal values. In all cases, the levels increase steeply after 2-3 months. In all these cases, chemotherapy should be discounted, even if the clinical and radiological examinations have not yet demonstrated the progression. The course of CEA can help to decide early whether patients will benefit from continuation of treatment or not. If the levels are not within the normal range after, at least, three cycles of treatment, the chemotherapy should be stopped if the tumour marker levels are normal. It should be restarted only when the tumour marker levels begin to increase again (170).

Leminen et al (1992)(156), studied the clinical and tumour markers response among the cases of cervical carcinoma which received chemotherapy as an initial treatment. The overall clinical response rate to initial chemotherapy was about 78% (80% in early and 78% in advanced disease), according to WHO recommendations (171), a complete response (disappearance of all clinical signs of malignancy) was achieved in 13% of the patients, and partial response (decrease in tumour size of 50% or more for at least 4 weeks) in 65% of the cases. A decreasing level of CEA had partial response, and 25% had no response to treatment. So they concluded that CEA often respond to initial chemotherapy and a decrease in its level was often associated with clinical response.(171).

Post-Treatment Follow Up with Serum CEA

CEA measurement can be used for follow up. In patients with initially elevated CEA levels. The pattern of serially determined CEA in serum correlated closely with the course of the disease. A decline in CEA serum levels during treatment concurred with a clinical regression of the tumour. While increasing or persistently elevated CEA levels provided the first indication of recurrent disease.
and metastases. Also in some patients with initially normal or undetectable levels of CEA, recurrence of the disease was preceded or accompanied by a rise in CEA levels (155). Hiura et al (1990)(172), found a good correlation between CEA concentration and the outcome of treatment. They considered that effective serial plasma determinations of CEA, in patients with cervical carcinoma following therapy might often be useful in the evaluation of therapy as well as in the earlier detection of recurrence.

Absolute plasma CEA concentration is related to total tumour burden, and antigen release. Likewise CEA metabolism by the host undoubtedly affects the level of circulating CEA. Several investigators have emphasized the importance of hepatic function in the metabolism of CEA in both animals and human subjects. Nevertheless 97% of patients with progressively increasing plasma CEA concentrations following therapy developed recurrent cancer. In nearly one half of the cases, this progressive of recurrence by an average of 6 months. One third of the patients experiencing a rapid increase in plasma CEA concentration did not have levels above 2.5 mg/ml prior to therapy (101)(118).

Donaldson et al 1976)(169), found that biochemical evidence of recurrence preceded clinical detection of disease by 1-4 months only. Duke et al (1989)(173), noted biochemical indication of recurrence 3 months following therapy. Serial plasma CEA values did not correlate with disease states when the primary tumour contained little or no antigen.

Kjorstad and Orjasaester (1984)(164), reported that all the patients with pre-treatment levels over 15 ug/l died during the follow up. In the range between 5 – 15 ug/l, two third developed recurrence. So they suggested that for patients with levels over 5 ug/l surgery and/or radiotherapy were inadequate. Because of the very unfavourable prognosis such patients may be candidates for chemotherapy and sequential determination of CEA can be used for monitoring such treatment. In patients with initial values under 5 ug/l serial determinations as a help in the follow up seemed to be very useful.

There was no difference in the survival rates between CEA – positive and CEA – negative patients at any time during a 10 year follow up period following treatment with radical surgery or radiotherapy for cervical cancer(157).

Causes of the Discrepancy Between the Results of Serum CEA in Different Investigations

TeVelde et al (1982)(159), attributed the differences in the published to the following factors:

- The lack of adequate information both in the frequency of the follow up visits and the clinical methods applied routinely during follow up of the patients.
- In view of the relatively slow growth rate of the tumour a prolonged follow up period is a prerequisite for the correct assessment of a tumour marker in invasive cervical cancer, to avoid considering occult tumour growth incorrectly as non recurrence cases.
- The methodology used for establishment of recurrence has not always been described. If histological confirmation cannot be established, the clinical criteria applied to the diagnosis of recurrence should be stated.

The failure to drive an appropriate cut-off which cut-off level which clearly delineates the normal from the abnormal range.(159).

Braun et al (1981)(160), also stressed on the importance of the upper limit of normal for CEA and the relative distribution of the different clinical stages of the disease within the groups of patients studied as important causes of variation of results.

The specificity of anti-CEA antibodies varied depending on the source, most commercial anti-CEA antibodies cross - reacted with structurally similar glycoproteins, including non-specific cross-reacting antigens (NCA) (123).

The use of monoclonal antibodies for CEA seemed to increase the positive rate for CEA in invasive cervical carcinoma than the polyclonal ones. However, Rogers et al (1981)(51) and Primus et al...
anticipated the appearance of potential problems in the correlation between blood levels of CEA measured with monoclonal antibodies and disease activity due to the existence of tissue CEA determinant heterogeneity.

Hurlimann and Gloor (1984)(122), considered that the differences in results were due to the dilutions of antisera able to detect concentrations of CEA higher or lower than those expected.

Magon et al (1981)(174), gathered the factors resulting in the discrepancy between the results of plasma CEA in different investigations in the following:

1. Assay variability.
2. Patient population selected for study.
3. Tumour types, i.e. not all ovarian and cervical tumours produce CEA.
4. The level of CEA regarded as positive (cut-off) level.

Carcinoembryonic Antigen in Cervical Intraepithelial Neoplasia

Intraepithelial neoplasia has been considered by many investigators as an intermediate step in the biologic progression toward invasive malignancy, particularly in the uterine cervix. The incidence of elevation of serum CEA increased with the progression of the histological epithelial. Abnormalities from moderate dysplasia to carcinoma in situ. The association of increased plasma CEA levels with the production of keratin by the cervical epithelium CEA expression and chronic cervical inflammation. Both lymphoplasmacytic infiltration and benign adenomatoid hyperplasia of the endocervical glands are unrelated to the level of CEA (175).

There is little difference in tissue localization of CEA between severe dysplasia and in situ carcinoma. A few moderate dysplasias show positive reaction in isolated groups of cell, they may represent a transitional phase to severe dysplasia. No positive cellular staining is detected in mild dysplasia. Although CEA has been demonstrated in the parakeratotic surface of mild dysplasia, this staining pattern can not be differentiated from non specific absorption of anti-CEA. The negative results for CEA in chronic cervicitis and reserve cell hyperplasia suggest that the CEA in dysplastic epithelium is not merely a reflection of increased cell proliferation but that it is associated with definite transformation in the epithelial cell (119).

In CIN-1 and 2 lesions the staining reaction to CEA tended to be concentrated in the superficial and intermediate layers while in the sections of CIN-3 the positive stain became diffuse (176). The aggressive lesions of cervical intraepithelial neoplasia could be found among the cases of mild dysplasia that expressed CEA. CEA was found in 24% of mild dysplasias, in 37% of severe dysplasias, and in 60% of cases classified as carcinoma in situ (157)(177).

Complete excision of intraepithelial cancer is associated with a return to normal plasma CEA titre within 8 weeks in over 75% of the cases. The use of CEA as an aid in determining residual in situ carcinoma of the cervix following cervical conation is potentially important. A persistently elevated plasma CEA (> 5 mg/ml) following conation is uniformly associated with in situ carcinoma of the cervix (175).

Carcinoembryonic Antigen in Cervical Mucous and Cervico - Vaginal Washout Fluid

Carcinoembryonic antigen or carcinoembryonic antigen like substances are found in cervical mucous and cervico-vaginal washout fluid in benign, pre-malignant and malignant cervical epithelial lesions. The level of CEA was below 300 mg/ml in 71% of benign cases, while in severe dysplasia and carcinoma in situ 83% had CEA levels over 300 mg/ml (178).

The study of CEA in cervical secretions and the demonstration of CEA in cytology specimens, is useful objective analysis complementary to cytomorphological examination of cytology specimens.
and biopsies. This is specially valuable in follow up of CEA positive dysplasias treated by cone biopsy (119).

The estimation of CEA in cervico-vaginal irrigation fluid considered a more diagnostic tool for CIN-1 and II than serum CEA estimation(179).

The degree of positivity for CEA in cervical smears increased from pre-malignant lesions to invasive cervical cancer(180).

The cervical mucous samples from the control and leiomyoma bearing - women contain low levels of CEA. Whereas the mucous samples from squamous cell carcinoma exhibit slightly higher levels of CEA than those of the former two groups with significant differences. In contrast, the CEA level in the cervical mucous from cervical adenocarcinomas is extremely high with significant differences from that of squamous cell carcinomas. Characteristically endometrioid - type adenocarcinomas of the cervix show extremely high levels of CEA. These results suggest that certain types of cervical adenocarcinomas may synthesize and secrete CEA into cervical mucous (181).

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