Tumor Associated Markers in Clinical Diagnosis

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ABSTRACT

Simple means for detecting the presence of tumors when they are still small and localized would be of major clinical benefit. Various tumor markers have been found and methods are available for measuring them in the blood. The results of studies suggest that the tumor markers of promise include onco-fetal antigens, placental antigens, polypeptide hormones and exocrine secretion products. Some, such as alpha-fetoprotein, are elevated early in the disease, frequently prior to detection by all other diagnostic aids. Others, notably carcinoembryonic antigen (CEA), are frequently only elevated when the tumor has progressed. The present-day practical application of tumor markers is in monitoring the progression of the tumor and its response to therapy; under these conditions they are frequently more sensitive than other indices including scanning. Human chorionic gonadotrophin has proved to be an excellent marker for following patients with trophoblastic neoplasms; its sensitivity as a marker can be improved by determining the levels of its alpha and beta subunits. The benefits of measuring ectopic hormone production including their precursors and partial products require further study; in some instances a hormonal syndrome is the first clinical manifestation of the presence of malignancy. Certain markers, although not useful in aiding the diagnosis of cancers, may assist in predicting a response to specific therapy; these markers include the estrogen and progesterone receptors and lactalbumin. None of the known tumor markers when used alone have proved to be of sufficient value in population screening; nevertheless, when the levels are interpreted in conjunction with other clinical and laboratory data, they frequently aid a diagnosis.

Introduction

The importance of the early diagnosis of cancer cannot be over-emphasized. At present more than half of all cancers develop life-threatening metastases before they are first detected. If the presence of tumors could be established while they were still small and localized, some scientists believe that up to 90 percent of patients with cancer might be cured. It is therefore not surprising that great efforts
are being made in seeking simple laboratory tests that can detect the presence of occult primary tumors, which by reason of their size or deep-seated position escape clinical diagnosis.

There has been a growing awareness during the past 15 years of the ability of neoplastic tissue to synthesize and release into the circulation substances that are either absent or are produced in extremely limited amounts by the original normal parent cells. These substances, often referred to as "tumor markers," have been found to be either polypeptides or glyco-proteins. Since they are not readily detected in non-tumor tissue, it was originally considered that such markers were foreign and might result in an immune response; hence the term "cancer antigens."

Tumor markers can be categorized into at least three groups. The first group includes fetal and embryonic antigens,— substances that are present in the tissues and serum of the fetus and which either disappear or are markedly reduced in concentration at the end of gestation or in the first week of extra-uterine life. At present, two onco-fetal antigens are receiving the most attention; alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA). Very sensitive assays to detect these substances have been developed and currently are in clinical use. They will be discussed in particular detail.

The second group of substances synthesized by the cancer cell which has the potentiality of being tumor markers is the placental antigen. These are normally present only in the sera of pregnant women and include hormones, such as human placental lactogen, and enzymes, such as placental alkaline phosphatase.

The third group of antigens concerns polypeptide hormones. It has now been amply demonstrated that various tumors are capable of secreting all of the polypeptide hormones, including adrenocorticotrophin, parathormone calcitonin and growth hormone.

A fourth group that may become of importance consists of exocrine secretion products, i.e., casein and lactoferrin.

These groups are by no means all-inclusive and other constituents such as polyamines and enzymes may also be produced in large excess by certain cancer cells; whether or not they could serve as tumor markers is as yet unknown.

ONCO-FETAL ANTIGENS

Alpha-fetoprotein (AFP). AFP, a normal component of the serum proteins of newborn mice, was found in the serum of adult mice bearing a transplantable hepatoma. This finding was followed by the discovery that AFP could also be elevated in patients with primary carcinoma of the liver; AFP is a normal serum component of the human fetus.

Subsequent and repeated large studies have shown that 85 to 90 percent of patients with primary hepatocarcinoma show marked elevations in serum AFP (> 1 μg per ml); nevertheless, 5 to 10 percent of cases have values within normal limits (< 20 ng per ml). No significant correlation has been found between serum AFP and clinical signs or biochemical data in hepatocellular carcinoma patients. Several investigators claim a correlation between tumor AFP content and the extent of dedifferentiation with the more anaplastic tumors showing the highest tumor AFP. However, others have described highly anaplastic hepatocellular carcinomata with little or no AFP. A correlation has been claimed between the content of AFP in tumor and circulating AFP but not between AFP serum concentration and tumor size, growth rate, severity of the disease or grade of malignancy. Because of these varying reports, the actual level of the circulating AFP should not be ac-
cepted as an indicator of the histopathology or biology of the tumor.

An elevated AFP is a sensitive indicator for hepatocellular carcinoma. The evidence comes from experiments on baboons and rodents, when serum AFP levels have been found elevated before the presence of such tumors could be identified by other diagnostic criteria. There has been one reported case involving a patient with an elevated serum AFP that was detected 7 to 12 months before a hepatic tumor was discovered. When considering AFP serum determinations in reference to other measures of diagnosis, it appears that the use of the AFP test is as reliable as, and certainly safer than, percutaneous liver biopsy. Further, serial determinations of AFP are useful in monitoring therapy; thus, a fall to normal values is an indication of complete tumor regression. A rise of a decreased AFP concentration is a sign of recurrence, and no response with an elevated AFP or only a small response after therapy is indicative of incomplete removal of tumor or the presence of metastases.

From the clinical standpoint it is important to appreciate that elevated serum AFP values have been found in association with liver metastases for carcinomas arising in the stomach, esophagus, bronchus, colon and pancreas. Also, several non-malignant hepatic diseases frequently also show elevations in AFP values, but usually are less than 500 ng per ml.

Elevated serum AFP has also been found in association with germ cell neoplasms of the gonads and extragonadal sites. This elevation seems to reflect the presence of endodermal sinus tumor (yolk sac tumor). Patients in remission have normal serum AFP values and a gradual rise in antigen level occurs with recurrence of disease. Several determinations of serum AFP are, therefore, of value in assessing whether or not the disease has been eradicated, for early detection of metastases and for assessment of efficacy of therapy. AFP is thus a good and reliable tumor marker with germ cell neoplasms containing endodermal sinus tumor.

Carcinoembryonic Antigen (CEA). The findings with CEA are in contrast to the relative organ specificity of AFP. Although when originally described, CEA was found only in association with endodermal tissue, further studies have found it to be associated with tumors of mesodermal and ectodermal origin.

The early studies on CEA raised great hopes. Its importance in diagnosis, the monitoring of response to therapy and perhaps the early diagnosis of cancer was shown by many studies, thus giving this antigen enormous value. However, the problems and apparent inconsistencies have, at the same time, clouded the originally promising horizons. Numerous studies, in some cases positive, in others negative, should allow a definition of the practical application of this test and its role in clinical medicine.

CEA is increasingly being widely used in the management of patients with colorectal cancer. Blood CEA levels vary with the stage of the cancers. Fewer than 50 percent of patients with localized cancer (Duke's stage A) may have elevated levels, compared to as many as 100 percent of those with liver metastases. A normal value, however, does not include early cancer. The higher the level, the poorer the prognosis; however, a normal pre-operative serum CEA determination does not guarantee localized tumor, but a markedly elevated pre-operative CEA determination is consistent with metastases.

Pre-operatively elevated levels fall to normal after complete resection of colonic cancer. Elevated or rising serial CEA determinations following resection correlate with residual or metastatic cancer and usually precede clinical evidence of recurrence by two to ten months in asymptomatic patients. Non-CEA pro-
Reducing cancers (10 to 15 percent of all colo-rectal cancers) cannot be followed reliably by CEA determinations. Second-look surgery performed on patients on the basis of rising CEA levels following apparently curative surgery confirmed the presence of cancer in over 80 percent of cases.33

Elevations in circulating CEA are less frequently observed in gastric than in colonic cancer.31 Nevertheless, in 40 percent of patients with gastric carcinoma, serial CEA determination provides a useful index of the extent of disease and for following patients receiving chemotherapy.15

Although elevations in circulating CEA levels are also less frequently found (45 to 50 percent of cases)30 in patients with breast cancer, when it is elevated it has been found useful in monitoring a response to treatment. In these cases, a rising titer indicates recurrence or metastases.50

Fifty percent of patients with thyroid cancer have an elevated CEA.42 In some studies this elevation has been found only in association with medullary carcinoma26; in others, when normal CEA levels were obtained, they were mainly in patients with a history of irradiation exposure during childhood.42

Patients with cancer of the cervix, corpus uteri and ovary, frequently (60 to 75 percent of cases) also show an elevation in circulating CEA. The incidence seems to be influenced by the clinical stage and by the degree of tumor differentiation. In the monitoring of disease, serial CEA levels have provided a helpful index to the adequacy of surgical excision and to the management of ovarian cancer during cytotoxic chemotherapy. More importantly, it has been shown that CEA, when serially measured, has assisted in the prompt recognition of recurrence during long term follow-up.28

More than any other common benign disease, liver disease27 (especially severe alcoholic cirrhosis) may give moderately elevated CEA levels (70 percent of cases). Some of the elevated CEA levels in patients with inflammatory bowel disease may be due, in part, to co-existing disease. Elevated levels commonly accompany pancreatitis (50 to 55 percent of cases) and may be due to the contributing factors of smoking and/or liver disease. It is now well established that up to 20 percent of smokers have an elevation in CEA.4 Recent studies show that this interference can be minimized by subjects not smoking for one week prior to testing for CEA.

How sensitive is CEA as a screening procedure? A four-year follow-up on a large unselected elderly population found a significantly greater proportion of cancers (18 percent) in persons positive for CEA49 whereas 2 percent of the persons negative for CEA developed CEA associated cancers. In another study12 where patients were undergoing barium enema, 78 percent of those found to have a cancer also had an elevated CEA; in addition, 18 percent of "false" positive CEA was found although half of these patients suffered from gastrointestinal disorders. Repeat sampling in a third of the "false" CEA positive group produced normal results in most cases.

These studies suggest that CEA could be helpful in screening. However, caution is advised against interpreting a single elevated CEA value as synonymous with cancer. To be more useful, the CEA assay requires greater specificity.

Major limiting factors in the further evaluation of the CEA test is the lack of a homogeneous standard and the presence of antigens cross-reactive with CEA. CEA from different sources, although possessing similar amino-acid composition, demonstrates considerable differences in carbohydrate,11,27 the portion of the molecule conferring antigenicity in the radio-immunoassay system. It appears as if the CEA measured in the blood may not be the same as that in the tumor, and the con-
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tributing role of antigens cross-reacting with CEA, such as NCA, NCA₂, CEA₉ₒw and CEA-III, remains to be defined.⁵³

Pancreatic onco-fetal antigen. Cancer of the pancreas is difficult to diagnose, especially in its early stages. Therefore the recent finding of a pancreatic onco-fetal antigen⁷ is of interest. In cancer of the pancreas, this antigen is as frequently elevated as CEA but has the advantages of being less frequently elevated in malignant and inflammatory diseases of the liver and gastrointestinal tract.¹⁹

PLACENTAL PROTEINS AND THEIR SUBUNITS

Elevated levels of human placental lactogen (HPL) by non-trophoblastic neoplasms have been detected in 5 percent of 295 cancer sera but in none of 281 controls.⁴³ HPL seems to be a specific tumor marker when detected in a man or a non-pregnant woman. Tumors that secrete HPL are not limited only to those of trophoblastic origin⁵⁷ but include a wide variety of other neoplasms of various cell types with no apparent clinical features.⁴³

Human chorionic gonadotrophin (HCG) has long been an important tumor marker in the diagnosis of all cases of trophoblastic neoplasms and in the monitoring of their treatment. In addition, HCG has also been identified in 12.2 percent of 1,319 patients with a variety of other neoplasms. The highest incidence of positive sera was found with gonadal tumors,—42 percent of patients with adenocarcinoma of the ovary and 51 percent with testicular tumors. In patients with non-gonadal tumors, the highest incidence was found in association with carcinoma of the pancreas (33 percent), gastric adenocarcinoma (22 percent) and lung cancer (9 percent).¹⁰

HCG has become established and widely employed as a reliable index of tumor activity for choriocarcinoma and hydatidiform mole. However, when HCG levels are low, the interpretation of results becomes difficult because of immunologic cross-reactivity with human luteinizing hormone (HLH).⁴⁶,⁵⁶ The ability to assay for the hormone-specific but biologically inactive β-HCG subunit, which recognizes intact HCG and its β-subunit but not HLH, has allowed for monitoring even when HCG levels are within the range of HLH. The possibility of the production of free α and β subunits by the tumors exists; if so, the measurement of the β-subunit becomes of even greater importance. Radioreceptor assay for HCG has also been studied. This shows a good correlation with the radioimmunoassay for HCG and has the advantage of greater biospecificity. In addition, interference by the subunit is minimal. In some patients with choriocarcinoma who eventually develop cerebral metastases, elevated levels of the alpha-HCG subunit have been demonstrated during periods when the concentration of HCG and its β-subunit were declining or were not detectable.¹³ This suggests that the monitoring of trophoblastic tumors can be improved by assaying also for both of the subunits of HCG.

Clinical interest in human placental alkaline phosphatase (EC 3.1.3.1) was stimulated by the findings of Fishman et al.¹⁶ that a lung cancer patient produced ectopic alkaline phosphatase (Regan isoenzyme) in his cancer cells and that this isoenzyme was also detectable in the serum. In two detailed studies placental alkaline phosphatase was found elevated in the sera of 10 to 12 percent of patients with a variety of neoplastic diseases.¹⁷,⁵⁵ As with other reports, this was especially so for ovarian and other gynecological cancers. Perhaps of greater interest is the finding of elevated serum isoenzyme levels in patients with cervical squamous cell carcinoma in situ, suggesting that elevations in this isoenzyme may assist in the detection of early cancer. Much depends on increasing the sensitivity of the assay, studying patients with localized tumors and monitoring the effects of
therapy. One aspect of biological interest in regard to this enzyme is whether or not it contributes to the hyperphosphatemia described with certain patients suffering from malignancy.

ECTOPIC HORMONES

That tumors arising from "non-endocrine" tissues may synthesize hormones ("ectopic" hormones) was first established in the early 1960's by the demonstration of high plasma and tumor ACTH levels in patients with extrapituitary tumors. At that time, ectopic hormone secretion was considered a rarity, though of obvious clinical interest. In the last decade, however, the ectopic secretion of most polypeptide hormones has been recognized. Although ectopic hormone production has been described in specific types of tumor, such as oat cell carcinoma of the lung, further studies are needed to define whether or not it is so limited. This ectopic hormone production is not confined to the normal hormone, so that precursor, subunit and degradative products have also been found. Whereas these may have relatively little biological activity, they may retain full immunological reactivity. These latter observations have particular relevance to the possibilities of their use as tumor markers.

The clinical effects of ectopic hormone secretion may be dramatic, and prognosis may be more related to control of the deranged metabolism than to the natural history of the tumor itself. In rare instances, the development of a recognizable clinical syndrome may be the first indication of a neoplasm and precede tumor diagnosis by months.

The three most commonly recognized ectopic hormonal syndromes are those due to secretion of ACTH (Cushing's syndrome), ADH (syndrome of inappropriate ADH secretion) and hypercalcemic agents (non-metastatic hypercalcemia). Estimates of the presence of these entities differ widely, depending on whether clinical or biochemical criteria are used. One study reported that 8.5 percent of patients with lung cancer have overt endocrine disturbance attributable to the tumor. In other studies, plasma cortisol and ACTH levels have been found in 22 percent of lung cancer patients and the hypercalcemia syndrome in 16 percent.

In patients without clinical manifestations of ectopic hormone production, the value of hormonal measurements has not yet been adequately assessed.

SECRETION AND RECEPTOR PROTEINS AS MARKERS

Studies of secretory products, especially from the breast, are being actively pursued as an aid to the diagnosis of breast cancer. In one study, serum Kappa-casein was found elevated in 15 percent of patients at the beginning of the clinical course, i.e., prior to treatment and when no metastases were detected by scan examination. In patients with metastases, 44 percent were positive and after chemotherapy or radiotherapy, the positive incidence decreased to 16 percent. This suggests that this phosphoprotein could be used in monitoring the response to therapy. However, further studies are required to assess its role in medical oncology since serum Kappa-casein is also elevated in other malignancies, such as lung cancer.

There are certain markers which, although not useful in aiding the diagnosis of cancer, do play a role in predicting a response to specific therapy. The measurement of estrogen receptors for predicting a hormonal response to breast cancer is the most well explored of these entities. Others, such as the progesterone receptor and, more recently, lactalbumin, seem promising but require further study.

The previous discussions on tumor markers emphasized only those which
have been the subject of extensive clinical investigation. Numerous new antigens associated with tumors are continually being reported. Since these are mainly isolated reports, the role (if any) of these putative tumor markers cannot as yet be assessed. Some of these markers have been found to be system or organ specific whereas others seem to have no such specificity.

Conclusion

In the vast majority of cancers, it is apparent that alone none of the tumor markers that are reviewed fulfill all the requirements for early diagnosis and as a monitoring system. The use of multiple simple tests is a well-known approach to ascribing a patient to the appropriate group in a classification of disease. It may well be that at this stage, and perhaps also in the future, reliance on multiple tumor markers together with already well-established laboratory assays may yield the best correlations in diagnosis and monitoring for cancer.

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