HUMAN TUMOR MARKERS

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Proceedings of the International Symposium on Human Tumor Markers, held in Taipei, Taiwan, 3–5 September 1988

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1989

EXCERPTA MEDICA, Amsterdam - New York - Oxford

International Congress Series No. 807
ISBN 0 444 81065 X

Published by:
Elsevier Science Publishers B.V.
(Biomedical Division)
P.O. Box 211
1000 AE Amsterdam
The Netherlands

Sole distributors for the USA and Canada:
Elsevier Science Publishing Company Inc.
655 Avenue of the Americas
New York, NY 10010
USA

Printed in The Netherlands

PREFACE

The 'International Symposium on Human Tumor Markers' was organized by the Chinese Association for Clinical Biochemistry, Taipei, Taiwan.
The purpose of this symposium was to bring together leading investigators from the international scientific community in the field of tumor markers to exchange knowledge and experience in this rapidly advancing research field.

The topics dealt with at the symposium were research areas which are very popular at present. Up-to-date research on tumor markers which may help early detection and monitoring the progress of human neoplastic disease were presented. The refinement of existing techniques and development of potentially important new methods reported here, undoubtly will help us to control cancer in the future. This book includes papers representing several major fields of tumor marker research, such as: clinical biochemistry; genetics; virology; immunology; patient surveillance; clinical application; molecular biology; biotechnology and immunodiagnostic methods. I believe these proceedings will provide scientists and clinicians with a concise compilation of current knowledge and future aspects of research in tumor markers. These aspects are of immediate importance in the clinical diagnosis and treatment of neoplastic disease.

Acknowledgement is due to the contributing authors, the contributors, Dr. Morton K. Schwartz and my colleagues, especially Professor Jui-San Chen, Dr. Kwang-Jen Hsiao and Dr. Chen-Kung Chou, as well as Ms. Jen Hsien Sun who assisted in the preparation of this book.

Taipei, September 1988

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PLENARY LECTURE

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TUMOR MARKERS IN DIAGNOSIS AND SCREENING

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INTRODUCTION

For almost 150 years the clinical laboratory has provided assay results which have been used by physicians in the management of their patients with cancer. As early as 1848 Bence Jones protein was described as a marker of multiple myeloma and a few years later urinary amylase measurements were described in patients with pancreatic cancer. More than 50 years ago alkaline phosphatase was successfully utilized in diagnosis of osteogenic sarcoma and other bone cancers and shortly afterwards total acid phosphatase and the tartrate inhibited fraction were used to monitor patients with prostate cancer. During the same period chorionic gonadotropin became a standard test in initial diagnosis and then in monitoring choriocarcinoma and vanillylmandelic acid and catecholamines were established as absolute markers of neuroblastoma and pheochromocytoma. Glycolytic enzymes such as phosphohexose isomerase also became popular, particularly as indicators of liver metastases as did 5'-nucleotidase. However it was not until the invention of the radioimmunoassay and then of other truly quantitative immunoassay methods that the term tumor marker was introduced and following the excitement about CEA became a part of our every day laboratory vocabulary.

DEFINITIONS

Tumor markers are defined as substances which can be measured quantitatively by biochemical or immunochemical means in tissue or body fluids to identify the presence of a cancer, possibly the organ where it resides, to establish the extent of tumor burden before treatment as well as to monitor the response to therapy. With this definition, a wide variety of substances can be identified as tumor markers. These include tumor associated antigens, enzymes, specific proteins and metabolites. Measurement of these constituents has been suggested as useful in screening both total populations and high risk groups. They are used in diagnosis either as aides in staging or confirmation of histopathology. Finally tumor markers are used in therapy where the marker can assist in predicting drug response, in predicting prognosis and perhaps most importantly in following the course of the disease. In addition, studies of tumor markers yield a great deal of information about the natural history

and epidemiology of the cancer. An understanding of the epidemiological definition of sensitivity, specificity and prevalence as well as the analytical interpretation of sensitivity and specificity are required before the clinical utility of tumor markers can be understood. From the epidemiological point of view specificity is an indicator of false positives and sensitivity of false negatives. Prevalence is the expected cancer cases in a population. From the analytical point of view sensitivity is the lowest amount of the analyte which can be detected and specificity is the interference by other materials. The analytical precision of the test is an essential consideration particularly if the test is to be used to monitor the disease.

SCREENING

The pot of gold at the end of the rainbow is to use tumor markers in screening. As pointed out earlier screening can be used in total populations or in high risk groups. High risk groups range from genetic populations with a high incidence of cancer such as individuals with familial polyposis to more general groups such as all individuals over 40 who smoke. Cervical and other forms of cytology as well as fecal occult blood and mammography are universally accepted in cancer screening. However these techniques do not meet our definition of a tumor marker since they are all qualitative and the question is whether constituents in blood or other body fluids which can be measured quantitatively by biochemical or immunochemical means can be used as a screening tool.

Screening has been defined by the United States Commission on Chronic Illness as "presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures that can be applied rapidly and can be carried out in the general population or in individuals at high risk."(1) I would have added cheaply to this definition. Screening clearly is not equivalent to diagnosis. Rather, it hints that further testing and closer examination are needed for diagnosis. Screening also differs from case finding, defined as "utilization of a special procedure to identify existing, but, up until then, unsuspected disease, which may be unrelated to their chief complaints, when someone attends a physician in the context of seeking medical care."(2) A laboratory test that could detect early cancer would be extremely useful in both screening and case finding.

The question is whether a circulating tumor marker can be used in screening. The following conditions should be met before a screening program is even considered: the disease must be common and have a substantial

mortality and morbidity rate; we must have knowledge of the natural history of the cancer; it must be known at which stage in the progression of the disease death can be prevented; effective treatment must be available which will lead to reduction in mortality; the test must be acceptable to both physicians and patients and must be safe and relatively inexpensive to perform.

There are several accepted but relatively limited uses of tumor markers as screening tools. In Africa and Southeast Asia, alpha-fetoprotein is measured to screen for liver cancer: In these areas of the world, hepatitis B is endemic and there is a high incidence of hepatocellular carcinoma. In an early report of the Chinese experience 394,000 individuals were tested and there were 143 positive values. Eighteen of these were shown to be false positives. There were 53 cases of clinically documented hepatocellular carcinoma. Of these 33 would not have been found without the alpha feto protein assay.(3)

In Japan, the urinary vanillylmandelic acid (VMA) of newborns is assayed in screening for neuroblastoma. In 1985 the sequence of events in the neuroblastoma screening program was reported.(4) In Nagoya City, 20,503 infants were screened with a qualitative paper strip method. Five hundred and fifty positive results were obtained. These were then tested with thin layer chromatography. Of these, 38 were positive. These were then retested by high-performance liquid chromatography, and 20 were positive. Finally, when a precise, quantitative procedure was employed, results for five of the infants were positive. The cost of detecting each of the five cases of neuroblastoma in the population of 20,503 was $7,628. The Japanese concluded that this was an acceptable expenditure because the identified children would presumably receive curative treatment and be spared a lifetime of expensive treatment or institutionalization. The sequence of testing in the Japanese example illustrates that screening is not diagnostic but, rather, leads to one or more expensive--but more definitive-tests.

In a follow-up report, there have been 38 positive patients in 682,470 children tested or about 1 in every 18,000.(5) Twenty-five patients who were treated, based on the screening test have been followed for at least 20 months. Of these 92% or 23 are without disease. An interesting comment is that neuroblastoma is a rare cancer, there are only 200 cases a year in Japan. It has been established that the screening procedure will detect 135-146 of these. In the United States there are 600 cases per year. We must consider whether any of the currently available circulating tumor markers can be used in screening for the cancers which occur in greater numbers in western society. These include lung, colon-rectal, breast and prostate cancers and

ovarian-uterine cancer.

GERM CELL TUMORS

I have mentioned the use of chorionic gonadotropin in choriocarcinoma and alpha-fetoprotein in hepatocellular cancer. Together these markers in germ cell tumors, particularly embryonal testicular cancer, represent perhaps the greatest tumor marker success. They facilitate initial diagnosis, and successful treatment requires frequent measurement of both the beta subunit of chorionic gonadotropin and alpha-fetoprotein. A return to a normal value of both is considered a successful treatment response and elevations are signals to resume therapy.(6)

In most patients chorionic gonadotropin and alpha-fetoprotein rise and fall reflecting the course of disease. However, in some patients in whom both markers are elevated before therapy, there will be a simultaneous fall reflecting successful therapy, but when there is recurrence only one will become elevated. This clearly indicates that these markers are synthesized by different clones of cells and that the chemotherapy will kill one set but not the other. Both markers must be measured if therapy is to be successful and if therapy is to be initiated sufficiently early to have a significant effect. Another important role of markers in germ cell tumors is in predicting the success or failure of a drug regimen. In multi-variate analysis of a large number of factors it has been found that chorionic gonadotropin, lactic dehydrogenase and the total number of metastatic sites predict whether or not the drug will work. Alpha-fetoprotein and CEA do not enter into the calculation. Of 121 patients predicted to respond 114 or 94% did so. However, only 28 of 50 (56%) of patients not predicted to respond did not respond to treatment.(7)

In pure seminoma neither chorionic gonadotropin or alpha feto protein is elevated and there has been a need for a marker to fill the void. Placental alkaline phosphatase which is elevated in a large percentage of these patients has been proposed as a marker to confirm and monitor patients with seminoma. In one study placental alkaline phosphatase was elevated in 9 of 21 men at primary diagnosis and 9/12 men with metastatic disease.(8) The enzyme also rises during metastases and falls following successful therapy. This enzyme is also elevated in almost 30% of patients with ovarian cancer, particularly those with serous cyst adenocarcinoma and may be a useful adjunct to CA-125 and CEA in ovarian cancer. CA 125 is an exciting new marker recommended for the management of patients with ovarian cancer.

OVARIAN CANCER

In the initial study of CA 125 a cutoff value of 35 U/ml (the mean value in normal women plus 3 standard deviations) was used.(9) Elevations were seen in 82% of 105 women with ovarian cancer, 1% of 888 normal women and 6% of 42 women with benign disease. Elevations were also seen in 59% of 27 patients with pancreatic cancer, 32% of 25 patients with lung cancer, 21% of individuals with colorectal cancer and 12% of 25 women with breast cancer. Most of these patients had advanced disease. It is not clear how early in the course of the disease CA-125 is elevated. If an attempt is made to eliminate the "noise" from other cancers or benign disease by raising the normal-abnormal cutoff the following is observed.

If the cutoff level is raised to 65 U/ml, or 7.6 standard deviations above the mean, elevations are observed in only 0.2% of normals and 2% of benign disease patients. The percentage pickup of ovarian cancer is reduced to 73% but the positive values in the other cancers are still significant. At 200 U/ml, there are no abnormal values in normal individuals or those with benign disease, but the pick up of individuals with cancer of the ovary is reduced to 62% and there are still some patients with other cancers who have elevations. Even at a level of 600 U/ml, where the pick up of ovarian cancer is reduced to 31%, there are still some patients with other cancers who show elevations.

The dilemma is in picking the correct cutoff value to achieve the purpose of the test. In this case if a value somewhere between 65 U/ml to 220 U/ml was chosen, about 10% of women with ovarian cancer who are detected with the 35 U/ml value would be missed, but other problems of positive values in women without cancer would be eliminated. In a study by Ricolleau at a cutoff level of 100 U/ml there were no positives in the reference group or those with benign disease, but elevations were seen in 10/14 patients with cirrhosis.(10) Elevations were seen in 25/27 women with serous ovarian cancer and 8/11 with non serous cancer. Elevations were also observed in 13% of women with breast cancer and 20% of patients with gastrointestinal cancer.

Once the cancer is detected and the patient is being monitored, the lower cutoff level would be preferable and an individual's own base line value would be used. When CA-125 is used to monitor patients, rising and falling levels correlate well with progression or regression of disease. In Ricolleau's study progression was indicated by a rising CA 125 in 11/12 patients and regression in 7/8 was accompanied by a fall in CA 125. In another study each of 20 patients in whom the disease regressed, demonstrated a fall in the CA 125. In 17 of 19 patients in whom the disease progressed, the CA 125 was

increased. In the other two patients the CA 125 remained unchanged. In five of six patients the disease remained unchanged as did the CA 125. In one patient the CA 125 concentration decreased.

Recently it has been reported that urinary assays of chorionic gonadotropin may be a sensitive marker of ovarian cancer and other gynecological tumors. O'Connor and his associate developed a series of monoclonal and polyclonal antibodies for two-site immunoassays in urine of total chorionic gonadotropin the β-subunit and a β-subunit fragment.(11) They also proposed a simultaneious assay for all three. These assays had little cross-reactivity and the interassay precision was sufficiently good to permit clinical trials. The analytical sensitivity was between 0.01 ng/ml and 0.06 ng/ml. In a clinical study of chorionic gonadotropin fragments, the urinary values were normalized to the excretion of creatinine. Age did not effect the values, nor did urinary tract infections. Only 3% of women without cancer had elevated values whereas 73% of patients with ovarian cancer demonstrated elevated urinary concentrations. In addition elevations were seen in 70% of women with cervical cancer, 77% of the patients with endometrial cancer and 80% of women with miscellaneous gynecological cancers. Regardless of the kind of gynecological cancer, elevations wree seen in 50% of women with Stage 1 disease, 62% with Stage 2, 75% with Stage 3 and 86% with stage 4 gynecological cancer. Elevations were also observed in each of 14 women who experienced a recurrence. These percentages of elevations in ovarian cancer are about the same as those reported for CA 125 and much higher than those observed with other markers such as CEA.

COLON-RECTAL CANCER

In any discussion of tumor markers it is necessary to include CEA. We have now had more than 20 years of experience with CEA. It is a well known fact that CEA is elevated in the serum of patients with solid tumors. Elevations will be seen in more than 30% of patients with cancer of the lung, liver, pancreas, breast, colon, rectum, head and neck, bladder, cervix and prostate. Elevations are also observed, but to a lesser extent, in patients with leukemia, lymphoma, Hodgkin's disease, multiple myeloma, and malignant melanoma.(12)

In patients with colon rectal cancer, elevations are found in 4% of individuals with Dukes' A cancer, 25% of patients with Dukes' B cancer, 45% of patients with Dukes' C lesions and 65% of patients with extensive metastatic carcinoma.

CEA cannot be used for initial diagnosis or screening but in some cancers,

particularly lung, breast, and colon, it is a useful tool in evaluating prognosis and in monitoring the progress of the cancer during therapy. In colon rectal cancer prognosis is much better in patients with preoperative values less than 10 ng/ml than in those with higher values.

Declining concentrations of CEA are indicative of effective therapy and rising levels indicate disease activity, though the increased CEA may precede clinical symptoms of recurrence by months. Elevations of CEA do not occur in all patients who experience recurrence. Two patients with equally fulminating liver metastases can present completely different patterns. In one there may be a crescendo-like rise in CEA and in the other there may be no rise at all. Immunostaining techniques have shown that CEA is not produced by poorly differentiated tumors. Thus, the individual with the non-rising CEA level undoubtedly has a poorly differentiated tumor.(12)

A review of the literature has revealed that from 61% to 94% of patients with recurrent colorectal cancer have elevations of CEA.(13) This means that from 6% to 39% of patients would have a value considered a "false negative." When all of the reported patients were combined, elevations were observed in 172 of 225 patients or 76%. Hence, the overall false negative rate was 24%. The important point to be remembered is that a positive value is very meaningful, but a negative CEA concentration should not give a false sense of security that the disease has not recurred.

There has been some disagreement about how CEA values should be evaluated when monitoring therapy. According to one investigator, fluctuations exceeding 20% are significant in assessing breast cancer, whereas in another report of 70 patients two successive rises in CEA greater than 12% sometimes were accompanied by a positive clinical response, sometimes by progression of disease.

In colon cancer, CEA changes greater than 25% of the baseline value have been considered significant and the slope of the rise has been suggested as an indicator of the extent of the metastatic spread. In many patients these rises will precede clinical evidence of recurrence by many months.

A number of investigators have proposed that "second look" colon surgery should be considered when there is a rising CEA titre and no other clinical or laboratory indications of recurrence.(13) A group at Ohio State University has had the most experience with CEA-related "second look" surgery in colorectal cancer. Their criterion for surgery was a CEA value that exceeded the baseline by more than two standard deviations. In a retrospective study, 19 of 22 patients or 86% of patients subjected to surgery were found to have a recurrence, but only 6 or 27% had a resectable tumor. The results were much

better in a prospective study; 17 of 18 patients or 94% had recurrent cancer and 13 of these or 72% had a resectable tumor.(13)

Other investigators have observed similar percentages of recurrence (78% to 100%) during "second look" operations, but the number of resectable cases was much poorer, varying from 7% to 43%. This may be related to the time that elapsed between the rise in CEA and the surgery. Further studies are required to determine the effect of "second look" surgery on the survival of these patients and the quality of their lives. Second look surgery related to CEA has not been performed in other forms of cancer.

Before leaving CEA, it is important to repeat the conclusion of the National Cancer Institute consensus panel that "CEA is the best presently available non-invasive technique for postoperative surveillance of patients to detect disseminated recurrence of colorectal cancer."

There have been several antigens which have similar properties to CEA and attempts have been made to use them in the evaluation of gastrointestinal disease either alone or in association with CEA. CA 19-9 is such a marker.(14) In clinical trials it has been clearly shown that although CA 19-9 is an antigen found in a colon cancer cell line, it is not found in human serum to the same extent as CEA in patients with colon rectal cancer. Whereas CEA was elevated in 125 of 174 or 72% of patients, CA 19-9 was elevated in 74 or 42% of these individuals. However, CA 19-9 was elevated in 13/15 or 85% of patients with pancreatic cancer and CEA was elevated in 10 of these (67%). In a larger study of pancreatic cancer CA 19-9 was elevated in 32/37 (87%) of patients with cancer of the pancreas and CEA in only 15/31 (48%) of these individuals. A combination of the two was not useful. If a cutoff of 75 U/ml was used, elevations were observed in only 3/48 (6%) of persons with benign pancreatic disease and none with renal failure or malabsorption. However 11/58 (19%) of patients with benign jaundice had elevations. Clearly CA 19-9 is a better indicator of pancreatic disease than is CEA and it has now achieved a world-wide acceptance as perhaps the best available marker for confirmative and monitoring of patients with pancreatic cancer.

Another marker which is similar to CA 19-9 is CA 195. There is a significant correlation between CA 195 and CEA.(15) In our study of CA 195, CEA was elevated in 55 of 72 (77%) of patients with metastatic colon cancer and CA 195 was elevated in only 42 (58%) of these patients. In combination the two markers detected 60 of the 72 patients or 83%. In pancreatic cancer CA 195 was elevated in 16 of 23 (73%) of patients and CEA in 8 or 36%. The combination of CA 195 and CEA increased the yield by only 1 patient or 17/22 (77%) CEA was also elevated to a greater extent than CA 195 in breast cancer