Br. J. Cancer (1980) **41**, Suppl. IV, 186

MARKER PROTEINS AS INDICATORS OF TUMOUR RESPONSE TO THERAPY

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Summary.—Serological markers which are secreted products of tumour cells have the potential to provide an indicator of viable tumour mass. In practice their ability to do this depends on a variety of factors which include specificity, mode of metabolism and concentration in relation to viable tumour bulk and assay sensitivity. In general, eutopic products have proved more useful than ectopic products which with few exceptions tend to be produced in only small concentrations even in advanced disease states. Human chorionic gonadotrophin produced by choriocarcinoma remains the most effective marker for any human tumour. For more than 20 years this marker has provided a reliable guide to the course of the disease and the response to therapy There is a clear distinction between therapy which is ineffective and that which is effective in terms of marker concentration. The limitations of this substance and other tumour markers as indicators of response to therapy will be discussed.

FOR MEASUREMENTS of tumour response to be useful in the clinical context a number of requirements have to be fulfilled: the procedure needs to be practical, readily repeatable and to provide relevant and reliable information quickly.

In contrast to many of the methods reviewed at this Conference, the measurement of tumour markers in serum can be undertaken on a scale appropriate to clinical problems. For many clinical purposes measurements must be made at least weekly and less frequent measurements can give misleading results. In practical terms this means measurements being made at a rate of hundreds per day in a clinical cancer department and methods which are not capable of scaling up in this way, at low cost, are irrelevant to routine clinical application. Tumour markers in serum generally lend themselves to such techniques as radioimmunoassay (RIA) and this in turn can be automated (Bagshawe, 1975). Moreover, information which is not readily assimilated by the clinician in a busy clinic is unlikely to prove widely applicable.

Inevitably, all measurements of tumour response have their limitations but I suggest that the most fundamental index is the total number of clonogenic tumour cells and that this is often quite inadequately reflected by volume measurements.

The tumour marker field is already very large. More than 80 substances have been proposed although the number that have found routine clinical application is quite small. The problem is, of course, the great variety of morphological and perhaps what we should call "chemological" types of cancer. Thus a marker which is relevant and useful in say 15% of cases with a common cancer tends to be unused whereas one which is present in all cases of a rare cancer is routinely used.

I propose to restrict myself in this presentation to tumour markers which are secreted tumour products. These can of course be classified in different ways. Ectopic or inappropriate tumour products have aroused relatively more interest than the appropriate, eutopic variety although in practice eutopic products have, so far, proved the more important.

The nature of secreted tumour products need not be considered here except in relation to the limits of detection. Clearly, substances which are present in physiological body fluids in amounts detectable by the measurement system have a limit of detection or sensitivity imposed by the physiological concentration. In theory, substances produced by the foetus or placenta may be absent from adult body fluids. So far as I am aware, all known foetal substances are in fact detectable in the adult but their concentration tends to be very low and this is highly advantageous.

This problem is closely linked with that of specificity since there may be closely related substances of non-tumour origin which may also impose limits of sensitivity through cross-reactions.

A further consideration is the dynamic state represented by the concentration of a substance in a body fluid. Clearly, the concentration is a function of the rate of secretion into body fluids on the one hand and the rates of metabolism and excretion on the other. Secretion rates of tumour products can be determined by equilibrium studies but only as a research procedure.

I shall illustrate the potential of tumour markers largely on the basis of human chorionic gonadotrophin in gestational choriocarcinoma. This was the first tumour marker to be identified and it has been the subject of fairly intensive study. Even here, however, there are still many gaps in our knowledge and although comparable information could be derived from other markers for other more common tumours the considerable effort to achieve this has not yet been brought to fruition.

It is evident that we can follow the course of gestational trophoblastic tumours by measuring serum concentrations or urinary excretion rates of HCG and that the observations made are consistent with our knowledge of the disease obtained by other means. In our series of 600 cases of trophoblastic disease we have seen no patient with clinically detectable disease who did not have detectable HCG. We have seen no patient whose HCG has fallen to undetectable levels who has died of choriocarcinoma and no patient with progressively rising HCG has failed to die of the disease. The HCG concentration at the start of treatment shows a reasonably good correlation with the total body burden of tumour, as far as this can be judged radiologically.

It follows that HCG provides a measure of the magnitude of response to a particular course of therapy and therefore provides a remarkably reliable, sensitive and prompt guide to the development of drug resistance. The magnitude of the response to a course of treatment can be defined as log change in HCG concentration between the beginning of a course of treatment and the earliest time at which it would be safe to start the subsequent course. Where the response is profound and toxicity slight it may be necessary to take 2 or 3 similar courses in sequence and obtain a mean value.

It is possible to localize tumours by selective venous sampling for the sites of production of HCG but this is a laborious and invasive technique which demands an assay of very high precision over the particular range of concentration being studied. The detection and monitoring of brain metastases is a form of tissue localization which has been possible by comparing the simultaneous concentrations of HCG in serum and spinal fluid (Bagshawe & Harland, 1976).

There are two important limitations. First, present RIA techniques allow us to measure concentrations of about 1 iu/l ($\simeq$1 μg/l) and this is insufficient to detect the minimal viable mass of tumour. Extraction methods can be used to further enhance "sensitivity" but cost prevents this in routine practice. The second limitation is biological variability between different tumours and to a lesser extent within the same patient in the course of time.

How does one interpret the values of HCG concentration? Can this be trans-

lated into cell numbers? One has first to recognize that trophoblastic tumours show a characteristic form of tissue differentiation with a dual population of cells. The cytotrophoblastic cells which are or contain the clonogenic population are less active in synthesizing HCG than the multinucleate cells. Syncytotrophoblastic cells, formed from the cytotrophoblast, probably only have a life of a few days. Clearly the concept of cell numbers has to imply a very arbitrary concept of the mean cell. Moreover when trophoblast is grown in culture the cell types that emerge are difficult to relate to the *in vivo* population although their production of HCG and other trophoblastic markers confirms their origin. Various studies in my laboratory and elsewhere have attempted to relate *in vitro* rates of HCG production to cell numbers and on this basis we have taken a value of 10^{-5} iu HCG/cell/day as a working number. Some corroboration of this is obtained by excising minimal detectable tumour masses associated with serum HCG concentrations of about 10–100 iu/l by HCG-β assay. These are usually masses in which viable tumour is only identifiable microscopically and in volume terms are 1 mm^3 or less. This method has provided a figure of the same order of magnitude for the mean cell production rate but, not surprisingly, there is considerable variation.

If we use such a conversion figure it can be argued that in the individual patient it is speculative but, on the other hand, it is useful to know that a patient with resistant choriocarcinoma manifested by a serum HCG value of say 100 iu/l has something of the order of 1–10 mm^3 of viable tumour and not some cubic centimetres of tumour.

The correlation of HCG values with tumour volume measurements is evident over the whole series, in that patients with clinically and radiologically bulky disease have high HCG values whereas clinically undetectable disease is associated with lower values. However, one may see values as high as 5000 iu/l or more without being able to locate the tumour(s) by any radiological procedure including whole body computerized tomography. Conversely, persisting tumour masses may be readily apparent but misleading in that they have been "sterilized" by preceding therapy. Thus persisting lesions may be excised and no viable tumour found or, on the basis of persisting normal HCG values, be left alone and no further treatment given. Resolution of some such lesions has taken up to 2 years to complete. The difficulty is, of course, that a lesion of a few centimetres diameter has to contain only a few clonogenic cells whose production of HCG is below the limit of detection for tumour regrowth to occur.

Further validation of the HCG concentration/tumour cell number relationship is seen from the prognostic value of the initial HCG concentration. The higher the HCG the worse the prognosis in general, although there may be rare biological variants which fall outside the general pattern (Bagshawe, 1976).

The limit of detection of the assay system has to be appreciated in relation to the duration of therapy. If treatment is discontinued as soon as, or soon after, HCG has become undetectable then, since this corresponds with a tumour mass of the order of 10^5 cells, tumour recurrence is inevitable. Some idea of the duration of therapy required may be obtained by extrapolating to zero cells. The limitations of such an exercise are obvious but it is not without value (Bagshawe & Searle, 1977).

In the trophoblastic tumour field we also have perhaps the only situation where screening for cancer by biochemical means can be undertaken usefully. In the U.K. a National Screening Service for choriocarcinoma following hydatidiform mole has been in operation since 1973 and over 3500 patients have been followed up systematically. Less than 10% of these patients are admitted for treatment and the need for treatment is largely defined on the basis of the HCG values.

In addition HCG is a good marker for

some cases of dysgerminoma, gonablastoma and trophoblastic teratoma. As an ectopic product it can also be useful but the range of values tends to be much narrower in most cases.

Consideration of the malignant teratomas, however, leads us to consider α-foetoprotein. This marker is to hepatoma and to pure endodermal serous tumours as HCG is to gestational choriocarcinoma, but many of the malignant teratomas are "mixed" and contain both endodermal serous elements and trophoblastic elements. In addition there may be malignant undifferentiated elements which produce no known marker and differentiated elements with a low order of malignancy which also produce no marker.

The complex nature of malignant teratomas therefore makes them perhaps the least suitable of tumours for monitoring by measurement of tumour products. Despite this grave limitation we have long advocated the careful use of HCG and AFP measurements in the management of these patients and there can be little doubt that they add substantially to the information provided by the physical diagnostic methods. In essence, positive results indicate tumour activity and serial values provide a means to monitor the response of one component of the tumour. Negative results do not exclude tumour activity.

HCG and AFP values at the start of treatment appear to show a correlation with prognosis which is at least as good as those provided by other indicators. HCG values > 100,000 iu/l or AFP values > 1000 ng/l are associated with a poor prognosis.

The serum clearance rate of AFP (5·5 days) is rather slower than HCG (44 h) and therefore their respective rates of change in response to therapy are widely different. We have studied the logistics of the decline of these substances for many years but feel the complexity of the factors involved inhibits easy interpretation. Prognosis both in gestational choriocarcinoma and in malignant teratoma is largely determined by the propensity of a tumour to become drug resistant and clearly, resistance may emerge from a very small number of cells. Against a background of many millions of cells the failure of a small subset of cells to respond cannot be detected. Moreover the rate of destruction of a tumour population is likely to be influenced by its growth kinetics and therefore the rate of decline of a serum marker's concentration may reflect this, rather than the potential for resistance. Also, in our experience there is individual variation in the rate of clearance of substances such as AFP and HCG. In general a slow decline in marker concentration is less favourable than a rapid decline which approaches the limits of physiological clearance, but this is only an indirect measure of the magnitude of the total change following therapeutic procedures. Certainly one may see a decline in marker concentration at a rate approaching the clearance rate and values falling to a very low level only to be followed by a progressive increase as a resistant clone emerges. Within the histopathological entity we call choriocarcinoma, marker measurements reveal a seemingly infinite variety of response patterns. This variety emphasizes the difficulty of treating patients as a group rather than on an individual basis.

A transient rise in HCG concentration sometimes occurs when a tumour is exposed to chemotherapy and particularly on its first exposure. It is possible that this results from increased synthesis such as has been observed in culture studies or it could conceivably be due to tumour cell dissolution with release of antigenically active marker or fragments. This phenomenon is sometimes mistaken for drug resistance but it is a process which does not last more than 4–5 days.

Of the 80 or so tumour markers already described (Bagshawe & Searle, 1977) only a small number are in regular clinical use. Undoubtedly, carcinoembryonic antigen is the one with the widest, though not most precise, application. In the clinical context it is evident that a tumour

marker only tends to be used properly when it is clear that it provides additional information that is useful and when there are therapeutic possibilities. A good marker cannot make ineffective therapy effective but it can help to ensure that therapy is better used. Few, if any, of the available markers have been studied as extensively as is necessary to extract all the information they can provide.

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