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CHORIOCARCINOMA

A model for tumour markers

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Human chorionic gonadotrophin (hCG) was the first major tumour marker to be identified and in gestational choriocarcinoma remains the closest we have to the ideal indicator of tumour activity. What is measured in assays for this substance is however a complex subject which has to be understood if misinterpretation of the data is to be avoided. It is also necessary to understand the pathology and natural history of the spectrum of hCG producing tumours. The distinction between luteinising hormone (LH) and hCG only became possible with the production of antisera predominantly directed at the β subunit of hCG. Since then monoclonal antibodies directed at restricted epitopes have revealed that a range of hCG fragments contribute to what is measured. Within the spectrum of hCG producing lesions are those that are self-terminating whilst others are premalignant, malignant but responsive to chemotherapy, or refractory to all present agents. Awareness of this complexity is essential for interpretation of values. For patients with hydatidiform mole hCG measurements form the basis of identifying progressive lesions and thus constituting a still unique biochemical screening programme for cancer. Its roles in diagnosis, prognostication, monitoring the course of the disease and in follow-up for detection of recurrence are unique in many respects. Although hCG measurements provide information critical to the management of each of these lesions that information can only be properly understood through an understanding of the pathological entities involved and the pharmacokinetics of hCG metabolism and excretion.

Key words: Choriocarcinoma, human chorionic gonadotrophin (hCG), clinical application, review.

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In the last 25 years, tumour markers have found important applications in many areas of oncology but gestational choriocarcinoma remains the archetypal tumour and human chorionic gonadotrophin (hCG) the model marker. hCG plays a valuable role in diagnosis, prognostication, monitoring response, detecting relapse, immunohistochemistry and targeting for tumour localisation and therapeutic purposes. Yet hCG is synthesised, not by the cytotrophoblastic stem cells but by the terminally differentiated syncytiotrophoblast.

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Both choriocarcinoma and hCG are highly complex entities which need to be put into context if the tumour and its relationship to the marker is to be defined.

One area of complexity lies in the relationship of choriocarcinoma to other forms of trophoblastic disease. It is usually undesirable and unnecessary to perform hysterectomy for choriocarcinoma and it is therefore the only cancer for which a tissue diagnosis is not mandatory, but choriocarcinoma has to be distinguished from the two genetically distinct forms of hydatidiform mole (complete and partial), from invasive mole, from placental site trophoblastic tumour and from other tumours which produce hCG or hCG like substance.

A second area of complexity lies in what we call 'hCG'. There is not only intact hCG but also free α and free β

subunits, all of which contribute variably to what is measured in an 'hCG' assay. Moreover, not all antibodies used to assay hCG distinguish it cleanly from luteinising hormone.

Pathology and natural history of trophoblastic tumours

Choriocarcinoma may arise from any pregnancy but in European populations it occurs in less than 1 : 30 000 normal term pregnancies whereas it probably occurs in 3–4% of patients who have hydatidiform mole (HM) which itself accounts for about 1 : 1 000 pregnancies. Since some invasive moles also require chemotherapy a total of about 8% of patients require chemotherapy after hydatidiform mole (1). The histological distinction between choriocarcinoma and invasive mole can usually be made if hysterectomy is performed but since both lesions respond to similar chemotherapy conservation of reproductive function takes precedence. Choriocarcinoma has not been confirmed to have developed from partial hydatidiform mole but about 0.5% of these women require chemotherapy for persisting trophoblastic lesions, probably invasive mole (2).

The distinction between complete and partial hydatidiform mole is genetic and morphologic as well as prognostic. Complete hydatidiform mole (CHM) derives its entire nuclear genome from the male and is therefore described as androgenetic (3, 4). Partial hydatidiform mole (PHM) is triploid with two paternal and one maternal contributions (5, 6). Since choriocarcinoma can follow normal pregnancy or CHM present evidence indicates that gestational choriocarcinoma arises from two genetically distinct cell types.

Placental site trophoblastic tumour (PSTT) is a malignant trophoblastic tumour of even greater rarity than choriocarcinoma. Whereas choriocarcinoma arises from villous trophoblast of placenta or HM, PSTT arises from placental bed trophoblast and this tumour more commonly follows a term pregnancy than a hydatidiform mole (7, 8). Histologically as its name suggests the cells resemble those of placental bed trophoblast. In contrast to other trophoblastic tumours there is rather little syncytiotrophoblast. Since it is largely the syncytial cells that synthesise hCG the levels of this marker are characteristically much lower than is associated with comparable body burdens or choriocarcinoma and with extensive disease may only be in the hundreds or low thousands of international unit (IU)/l serum. The distinction between PSTT and choriocarcinoma is of the utmost importance since the response of PSTT to chemotherapy is often poor and incomplete and hysterectomy may be required if the disease is localised to the uterus. A relatively low serum level of hCG in a patient with a trophoblastic tumour is therefore an important feature which should arouse suspicion of PSTT.

hCG and its assay

Although 60 years have passed since Zondek's discovery of hCG we are only now beginning to appreciate the full complexity of this glycoprotein. Fortunately, some, but not all, assays using antibodies or antisera directed at the β subunit provide most of the information required for clinical management but assays with more defined specificities may provide important supplementary data. It has long been known that hCG is not a uniform product. During the course of pregnancy the mean number of sialic acid moieties per molecule of hCG changes and this affects its rate of clearance and hence its biological activity (9). Asialo-hCG is rapidly removed from plasma by hepatic galactose receptors.

The 92 amino acid α subunit is almost identical to that of luteinising hormone (HLH) but the 145 amino acid β subunit has only 80% homology with HLH (10). Free α and free β subunit measurements require specific antibodies to be measured separately although the subunits may contribute to the 'hCG' measured in β subunit assays depending on the antibody/antisera used in the assay. Specific antibodies now exist for the measurement of intact hCG (11). In recent years it has also been recognised that a substance consisting of the amino acids 6–40 linked to 46–92 of β subunit and known as β core fragment can be detected in urine by specific assay and it may also be detected in hCG assays using antibodies directed at the β subunit (12). A still further variant is clipped or nicked hCG in which the peptide linkages between B44–45 and 47–48 are missing (13) resulting in an opening out of the molecule with loss of biological and immunological activity. There is also clipped free β hCG.

It is therefore unlikely that any two assays for 'hCG' using different antibodies/antisera or different reference standards will yield identical results and what is detected is influenced by the relative proportions of the contributory peptides in the sample.

Present evidence suggests that an estimate of the ratio of free β subunit to intact hCG may be an important prognostic indicator in patients with choriocarcinoma (14), but more work needs to be done to determine at what point in the natural history of trophoblastic tumours this indication becomes clinically important. β core fragment is associated in urine with other peptides and has been found by immunohistochemistry in more than 90% of one series of malignancies (15, 16) and in the urine in increased amounts of many patients with a variety of cancers. It has only been found with difficulty in serum, perhaps because it is masked by association with other peptides (17). It has been found to be relatively abundant in the urine of a small series of patients with PSTT but again more experience is needed before definitive assessments can be made. It is also possible that β core fragment accounts for many cases of so called ectopic hCG production (18–21).

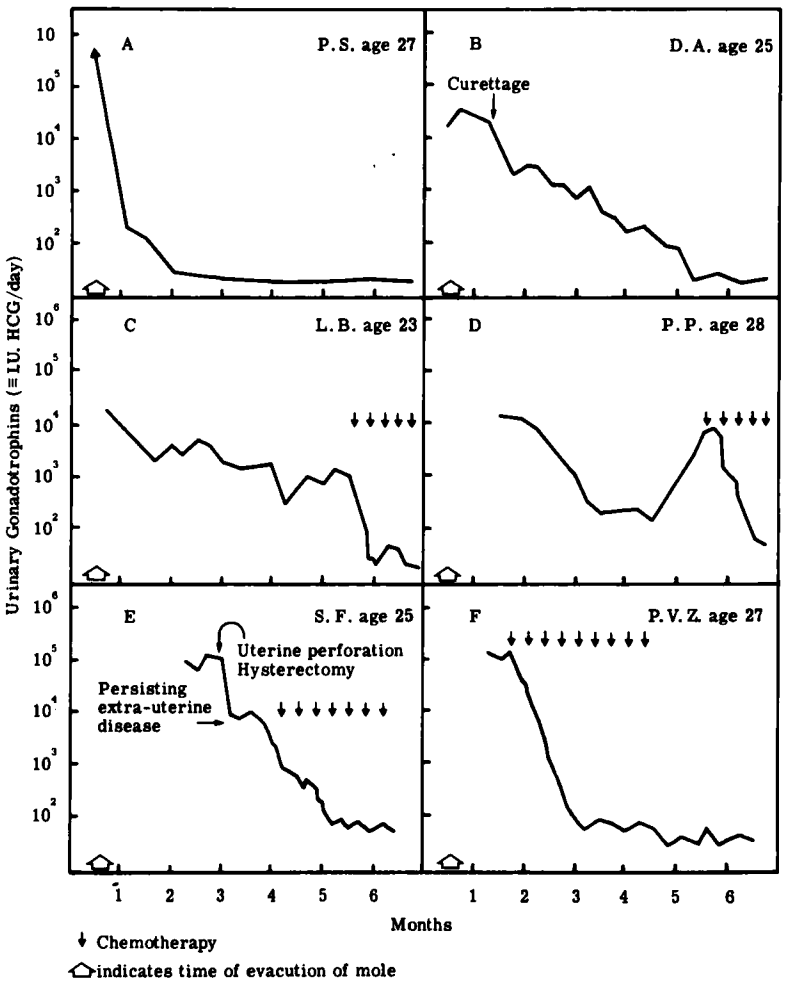

Fig. 1. hCG values illustrating different patterns after evacuation of hydatidiform mole. These data were based on 24 h urinary excretion rates but serum hCG values in IU/l or mIU/ml are similar. a) Common pattern of rapid trophoblastic regression; b) Slow progressive regression; c) Activity persisting 4–5 months post evacuation and requiring chemotherapy; d) Recrudescence of hCG production indicating need for therapy. e) Persisting high level of hCG associated with uterine perforation. f) Similar to e) but with early therapeutic intervention.

Clinical applications of hCG measurements

hCG and hydatidiform mole

Having outlined some of the complexities of trophoblastic tumours and of hCG itself we can now examine the applications and implications of hCG measurements.

The role of hCG in the primary diagnosis of HM hardly extends beyond the confirmation of pregnancy. Although values may be substantially higher in CHM than in normal singleton pregnancies of similar gestational age, this is by no means invariable and the primary diagnosis of HM now rests largely on clinical suspicion and on ultrasound examination, but of course a negative result excludes HM.

hCG measurements have, however, a central position in the management of patients after HM has been evacuated. Typically there is then a rapid fall in serum levels and urine excretion rates for several days (22–24). The mean renal clearance rate of immunoreactive hCG is around 1 ml/min (0.4 ml/min/m^2) and the metabolic clearance is about 1.9 ml/min/m^2 (25). Since hCG molecules have a distribution volume much greater than plasma volume, it takes around 21 days for hCG values to fall to the limit of detection after the end of a normal pregnancy or miscarriage. Thus, after the complete evacuation of HM, where hCG values are higher than at term delivery, hCG may continue to be detectable for more than 21 days without implying persistent trophoblastic activity.

The rapid initial fall in serum and urine levels of hCG following evacuation of HM is in turn followed by a period in which patterns of hCG level divide into several forms (Fig. 1). Most commonly there is a progressive fall to the limits of detection but this may take from 3 weeks to 6 months to achieve and a temporary plateau occurs in some patients. Where hCG becomes undetectable within 56 days of evacuation complete regression of the HM can be inferred since the risk of recurrence is then less than 1 : 3 000 (1). In these cases follow-up at Charing Cross Hospital is continued to 6 months post evacuation. Where

hCG becomes undetectable more than 56 days post evacuation follow-up for 2 years remains necessary since the risk of recrudescence is then about 1 : 200.

If hCG values remain high (> 20 000 IU/l) more than 4 weeks post evacuation and are then not falling rapidly urgent chemotherapy is usually indicated. These patients are at risk of fatal uterine rupture due to an aggressive invasive mole of choriocarcinoma.

In other patients hCG values may fall for a time and then start to increase again. If values continue to increase for 3–4 weeks intervention is indicated. Similarly, if there is any level of hCG detected at more than 4–5 months post evacuation intervention is required (22).

In the USA great emphasis is put on plateau values so that if 2–3 samples at weekly intervals remain roughly comparable in value, chemotherapy is given promptly. This appears to result in 20–30% or more of all HM patients being given chemotherapy (26). The justification usually given for this policy, which results in much unnecessary chemotherapy, is the risk of litigation, resulting from delayed intervention. Unnecessary intervention appears not to carry risks of litigation and may be financially rewarding.

The policy of giving chemotherapy to a high proportion of patients with HM may account for differing evidence relating to the use of oral contraceptives (OC). In the UK it has been found that about 1 : 4 women who take OC when hCG values are still raised require chemotherapy, whilst taking OC after hCG has reached normal levels does not carry any additional risk (27). This was not confirmed in some US studies (28). Recent analyses of the UK data confirm the original observation but the lower oestrogen dosage used in recent years may be associated with less increase in risk.

Diagnostic applications of hCG measurements

A history of hydatidiform mole or of an abortion in which HM was not excluded, in a woman with uterine mass or uterine bleeding, pulmonary, hepatic or renal masses or an intracranial lesion should raise the possible diagnosis of choriocarcinoma.

The same manifestation may result from choriocarcinoma following a normal pregnancy and occasional patients may not have been sure of a recent pregnancy. The absence of a recent history of pregnancy should not allay suspicion since the time interval between a pregnancy and choriocarcinoma can be 4–5 years and sometimes is even longer although mostly it is much shorter. Although a quantitative assay for hCG is usually desirable the diagnosis of choriocarcinoma in a woman with an appropriate history can often be effectively and cheaply confirmed, though not excluded, by a simple pregnancy test. The possibility of a non-trophoblastic tumour in a pregnant woman has also to be considered.

hCG and tumor burden

hCG values in patients with established choriocarcinoma or invasive mole vary substantially between patients but there is a broad correlation with tumour burden. Values tend to be in the range of 5 000–1 000 000 IU/l but may fall outside these limits. Within the individual patient changes in hCG values appear to reflect changes in viable cell mass. Since a high proportion of choriocarcinoma is necrotic tissue or blood clot, hCG values reflect viable tumour burden more truly than radiological opacities.

Quantitative studies based on in vitro growth and studies in patients with isolated lesions that were surgically removed and tissue culture studies indicated that a total body burden of $10^4 - 10^5$ cells produce a serum level of about 1 IU/ml—effectively the present limit of detection (29, 30). This is equivalent to about 1 mm^3 of viable tumour.

Where hCG values seem low relative to the clinical and radiologically detectable tumour burden, placental site trophoblastic tumour (PSTT), a malignant teratoma of ovary or other site, or ectopic production by a non-trophoblastic tumour should be considered, although both malignant teratoma and ectopic hCG producing tumours can also be associated with very high hCG values (7).

Prognostic factors

In patients with invasive mole or choriocarcinoma hCG values constitute one of several prognostic indicators. In the context of these tumours prognostic or risk factors are used to define the potential of the tumour to become drug resistant and to define the appropriate form of chemotherapy. In general the higher the hCG value the greater the body burden of tumour and the worse the prognosis. The exception to this generalisation is PSTT with low hCG and high risk of drug resistance.

CNS metastases

Although CT and NMR scans are now the primary method for detecting CNS metastases the ratio of serum/spinal fluid has been useful in many instances (31–33). The spinal fluid values are influenced by the concentration of hCG in systemic body fluids but also by direct secretion. Lesions deep in the brain substance are less likely to be detectable than those that are close to the cortex or ventricles. Serum/CSF ratios below 60 : 1 are strongly suggestive of CNS disease. Reversed ratios (CSF > serum) may occur where the CNS is the principal site of hCG production.

Choriocarcinoma in malignant teratoma (germ cell tumour)

Malignant teratoma arising in the testis, ovary, mediastinum, pineal gland and at mid line sites is well-known to contain choriocarcinomatous elements in about 80% of

cases. Distinction from gestational choriocarcinoma in the female is often aided by the presence of high levels of alpha feto protein (αFP) and lactate dehydrogenase (LDH). However, 'pure' choriocarcinomatous lesions do occur with hCG as the only marker. Where hCG is the only marker similar considerations to those in gestational choriocarcinoma apply. But in mixed tumours hCG is a marker for only part of the tumour and therefore of more restricted value.

Monitoring the response to therapy

During the course of therapy, hCG values reliably reflect excision of tumour and response or non-response to chemotherapy. It is usual to plot hCG values on a logarithmic scale against time. There may be an initial increase in hCG values with the first course of chemotherapy and 7–10 days may elapse before they start to fall. The initial rise may result from release of hCG like molecules on cell death or from the differentiation of cytotrophoblast into syncytiotrophoblast (34). When plotted graphically, as log hCG against time, the high rate of fall which occurs early in treatment tends to be reduced as treatment proceeds but there should be a progressive fall to normal. When there is no satisfactory fall between the start of one cycle of therapy and the start of the succeeding cycle, resistance to those drugs is evident. A 'satisfactory' fall depends on many factors—including hCG value and the stage of treatment, but in general a one-log fall may be expected with each treatment cycle early in treatment but a much smaller net fall is frequent towards the latter stages of treatment. In patients being treated for postmole tumours hCG values fall progressively and deviation in the direction of slowing indicates the need to change chemotherapy in about 25% of patients.

Even though hCG for choriocarcinoma is the most sensitive detection system we have for any cancer it is evident that the smallest viable cell mass that can be detected ($10^4 - 10^5$ cells) is quite sufficient for regrowth of the tumour. The implication is that if treatment is stopped as soon as hCG reaches the limit of detection then regrowth will almost inevitably recur. Treatment must therefore continue. Theoretical extrapolation to the zero cell level illustrates the problem (Fig. 2). In practice it is usual to give 3 further cycles of therapy in the low-risk patients and 6 cycles or more in the high-risk patients. (Figs 3 and 4). Thus, although hCG in patients with choriocarcinoma still constitutes the most sensitive detection system we have for any cancer it has limitations.

Follow-up after treatment

hCG is the most sensitive marker for detecting recurrence of choriocarcinoma. It therefore largely replaces dependence on clinical and radiological examinations for

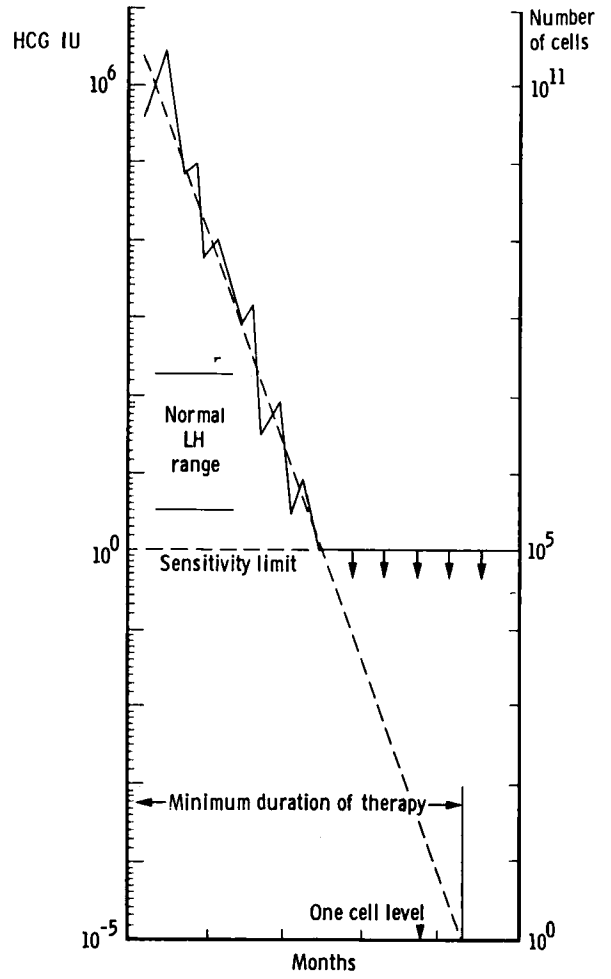

Fig. 2. Diagramatic illustration of consistent rate of log cell kill and implication of extrapolation to one cell level for duration of chemotherapy.

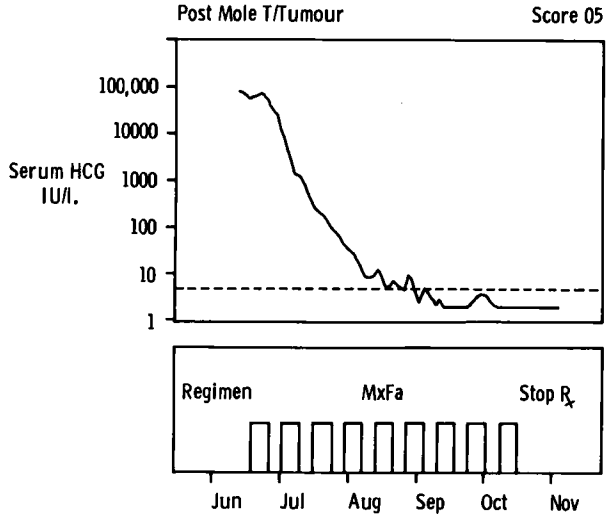

Fig. 3. hCG values during treatment of patient with relatively low risk post-mole tumour and treated with methotrexate and folinic acid. (MX.FA). Three courses of treatment were given after hCG reached the 'normal' level.

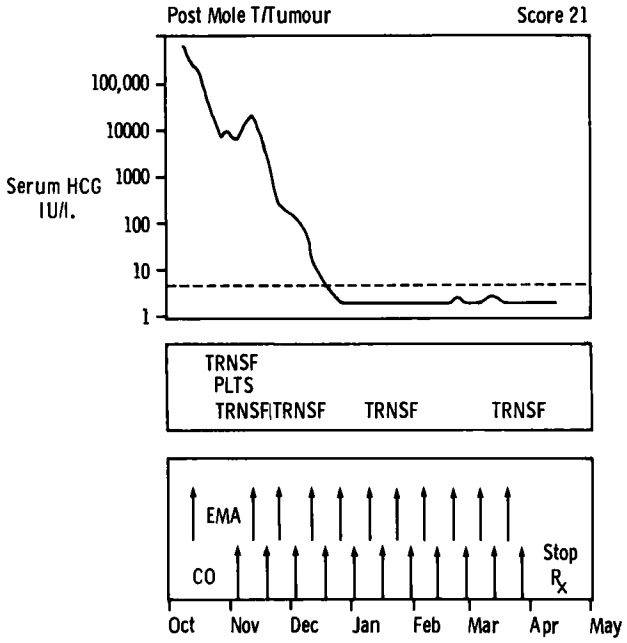

Fig. 4. hCG values during treatment of patient with high-risk post mole choriocarcinoma and treated by multi-drug regimen (EMA/CO). The rise in hCG values following the first course of treatment occurred because further treatment had to be delayed and reflects recrudescence of the disease during the interval. Trnsf = blood transfusion; Plts = platelet infusions; EMA = Etoposide, methotrexate, actinomycin D; CO = Cyclophosphamide, vincristine (Oncovin).

detection of recurrence although there may be other clinical reasons for continuing these. Relapse is infrequent (<4% in the Charing Cross series) and is most likely to occur in the first year (Fig. 5) but has occurred as late as 7 years. One patient who had hysterectomy at the age of 17 for benign hydatidiform mole was referred to us at the age of 34 with a massive pelvic choriocarcinoma; she had been followed up only with pregnancy tests.

The problem of low hCG production by placental site trophoblastic tumour has to be borne in mind; although hPL is detectable in the tumour by immunohistochemical examination it is not secreted in significant amounts. It is possible that β core fragment in urine may prove the best marker for this tumour.

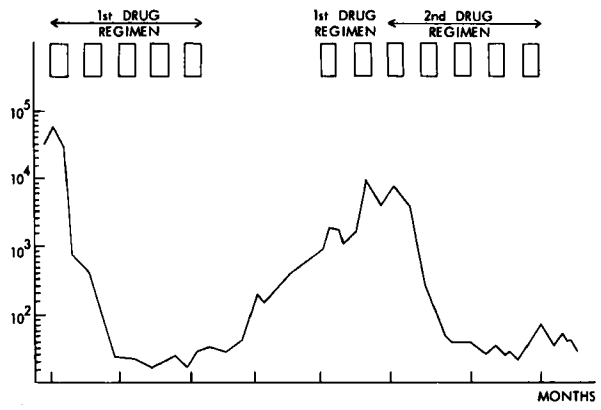

Fig. 5. This case illustrates initial and apparently complete response but relapse followed and tumour was then resistant to initial protocol but sensitive to second protocol.

The frequency of conducting follow-up tests for hCG has to take into account the rapidity of growth of choriocarcinoma; it may be obvious that those that recur early tend to grow more rapidly than those that recur after a long disease-free interval.

Where a woman has not undergone hysterectomy there is always the possibility that a recurrent choriocarcinoma is a new tumour resulting from an intervening pregnancy which may not have been recognised. One or more pregnancies may also intervene between a hydatidiform mole and a choriocarcinoma arising from it. hCG may be undetectable with standard methods during intervening periods. For these reasons it is desirable to maintain follow-ups for at least 10 years on patients who have required therapy.

hCG tests in the follow-up after treatment should therefore be frequent in the early stages and based on serum samples. Later it may be convenient and helpful in patient compliance to use urine samples even though there is more variation at the base line level. A routine follow-up procedure for Charing Cross patients is shown in the Table.

Immunohistochemistry

Antibodies directed at tumour markers have found an increasing role in the practice of cancer pathology both as an aid to detecting isolated micrometastases in tissue specimens and in helping to distinguish particular subclasses of tumour. hCG antibodies have a well-established role in this field but the demonstration of hCG-like activity may be due to hCG, to βhCG or βcore fragment depending on the specificity of the antibody used.

Table

Follow-up for gestational trophoblastic disease

After discharge from hospital

1. Weekly serum and urine for 6 weeks (clinical check-up at 6/52)
2. Serum and urine every 2 weeks until 6 months post therapy
3. Urine only every 2 weeks until 1 year post therapy.

Then

Year 2	Urine sample every 4 weeks
Year 3	Urine sample every 8 weeks
Year 4	Urine sample every 12 weeks
Year 5	Urine sample every 16 weeks

and subsequently every 6 months indefinitely

Any equivocal urine result is followed by an immediate request for a serum sample. A rise in hCG values may result from a new pregnancy which will need to be confirmed by ultrasound or serial hCG measurements.

Moreover, hCG-like activity is not restricted to gestational choriocarcinoma but occurs also in germ cell tumours and as an 'ectopic' product in a wide variety of epithelial cancers which may demonstrate varying degrees of trophoblastic differentiation (21, 35, 36). In the case of placental site trophoblastic tumour, an important point of distinction from choriocarcinoma is the relatively few cells staining for hCG and the greater abundance of cells staining for human placental lactogen. hCG or hCG-like substances are also synthesised in various normal tissues (37–39).

In malignant teratoma αFP staining may be used to identify yolk sac elements but their absence does not preculde a non-gestational origin.

Targeting

Antigens with some degree of specificity for tumours have also been used as the targets for antibodies in both diagnostic and therapeutic roles. There has been much discussion about the suitability of secreted antigens such as hCG for this purpose since it may be expected that the 'smoke screen' of hCG in serum and body fluids would prevent antibodies directed at hCG from localising effectively at tumour sites. However, it has been shown that effective localisation is obtained with hCG values 1 000–500 000 IU/l (40). Below 100 IU/l the tumour is likely to be too small for resolution by present gamma camera technology although tumours have been detected when computerised tomography has failed to identify them. With hCG values >500 000 IU/l localisation is probably impeded.

In some patients who are resistant to all known chemotherapy radioimmunolocalisation with ^{131}I anti-hCG has therefore proved to be an important adjunct to other methods of tumour localisation. Surgical removal of residual resistant metastases, particularly in the lungs, has proved curative in several patients.

Recently it has been shown that a new approach to cancer therapy was effective against drug resistant choriocarcinoma xenografts in nude mice. In this approach, monoclonal $F(ab')_2$ anti-hCG antibodies were conjugated to a bacterial enzyme carboxypeptidase G2 and injected into tumour bearing mice. After the antibody enzyme conjugate had cleared from plasma and other tissues a prodrug was injected (41). The prodrug was a relatively inert agent but on contact with the enzyme located in the tumour it was converted into a potent alkylating agent, resulting in eradication of the choriocarcinoma xenografts. A small scale clinical trial with this approach is being undertaken.

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