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The Growth Rate of Metastatic Non-seminomatous Germ Cell Testicular Tumours Measured by Marker Production Doubling Time—I. Theoretical Basis and Practical Application

P. Price, S.J. Hogan and A. Horwich

Changes in serum tumour marker levels in non-seminomatous germ cell testicular tumours (NSGCTT) are used to monitor tumour growth and response to treatment. A novel method of calculating the actual tumour marker production (TMP) per day is reported; estimation of the rate of change of TMP is a measure of the tumour growth or regression rate. TMP is calculated mathematically from the rate of increase in serum marker level and its natural half life. TMP is assumed to be proportional to the number of marker producing cells in the tumour. TMP was calculated over the time between orchidectomy and the start of chemotherapy. The rate of increase in TMP with time is expressed as the marker production doubling time (MPDT) and is a measure of the growth rate. In a group of 51 patients with metastatic NSGCTT, TMP varied from 0.012 to 5985 iu/l/day (AFP) and 0.08–5404 iu/l/day (HCG). MPDT varied from 0.5 to > 80 days (45 cases) for AFP +ve patients and from 1.8 to > 80 days (34 cases) for HCG +ve patients; > 80% of cases had a MPDT ≤ 32 days. In 45/51 (88%) patients, there was no discrepancy in MPDT between markers. The use of changes in serum marker level to follow tumour progression and regression is simple, but the calculation of actual TMP provides clearer information about the change in number of marker producing cells and can be used as non-invasive method for measuring the tumour growth rate of metastatic disease and response to treatment.
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INTRODUCTION

THE INCREASE in serum tumour marker level with time in patients with non-seminomatous germ cell testicular tumours (NSGCTT) has usually been assumed to reflect the growth rate of the tumour and has been extensively used to assess response to treatment. However, the serum tumour marker level at any one time is determined by the production of marker by the tumour and clearance from the plasma. Clearance is governed by first order kinetics and so is directly related to the amount of serum in the plasma at that time. The relationship between the change in serum marker level and the change in the amount produced by the tumour at any one time is therefore more complex than has been assumed. It is the amount of tumour marker produced which is proportional to the number of marker producing cells and so it is the change in tumour marker production with time which provides a measure of growth rate.

This paper reports a novel method of calculating tumour marker production, TMP (iu/l/day). The rate of TMP increase with time, marker production doubling time, MPDT (days), reflects the increase in the number of marker producing cells and is a measure of the tumour growth rate.

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METHOD

Calculation of tumour marker production

Tumour marker production in patients with NSGCTT was calculated from serum levels of alpha foetoprotein (AFP), human chorionic gonadotrophin (HGG), or both markers, as described in the Appendix. Briefly, the serum marker level at any one time is dependent on the rate of production of the marker from tumour cells and the clearance of the marker from the plasma. Thus the amount of marker produced by the tumour cells each day can be calculated mathematically from the rate of increase in serum tumour marker level and its known natural half-life:

$$\mathrm{TMP} = C(t_2) - C(t_1)\exp\left[-\frac{\log_e 2}{t_{1/2}}(t_2 - t_1)\right]$$

where C is the plasma marker level at time t_1 and t_2 and $t_{1/2}$ is the natural half life of the marker. This rate of production in an individual patient at a specific time is assumed to be proportional to the number of marker producing cells in the tumour.

Calculation of marker production doubling time

If the tumour marker production per day is calculated at a series of time points, the rate of increase reflects the increase in the number of marker producing cells and therefore will be a measure of the tumour growth rate. This growth rate is expressed as the marker production doubling time (MPDT) and can be calculated for the period following orchidectomy and before the start of chemotherapy to provide a measure of the growth rate of metastatic disease.

Evaluable patients

One hundred and thirty-two patients were treated in the Royal Marsden Hospital Testicular Tumour Unit between 1979 and 1986 using BEP combination chemotherapy (bleomycin, etoposide and cisplatin) following orchidectomy as first line treatment for metastatic disease [1]. Ninety-six (73%) of the 132 patients were marker positive and 58 of these (44% of the total) had three consecutive serum marker levels measured between orchidectomy and the start of chemotherapy from which the change in rate of tumour marker production (TMP) per day by the tumour could be determined. Of these 58 evaluable patients, 20 were AFF +ve only, nine were HGG +ve only and 29 patients expressed both markers. TMP values were therefore able to be calculated in 49 AFP +ve cases and in 38 HCG +ve cases. A rise in TMP with time occurred in 51 patients (45 AFP +ve patients and 34 HCG +ve patients), from whom calculation of MPDT was possible. In seven patients (four with AFP measurements and four with HCG measurements), TMP values decreased with time and therefore no MPDT calculation was possible. As this group of patients had clinically progressive disease, it was assumed that cells producing markers were no longer contributing to tumour growth, and so this group were excluded from the analysis.

Serum markers were measured at the Department of Medical Oncology, Charing Cross Hospital, London using radioimmunoassay. Units were expressed as international units/litre (iu/l).

RESULTS

Of the 132 patients, TMP and MPDT was able to be calculated in 51. Reasons for inability to calculate these in certain patients were: marker negative disease (36), less than three serum marker levels recorded between orchidectomy and the start of chemotherapy (38 patients), or no increase in calculated TMP with time (seven patients).

TMP in the 51 patients ranged from 0.012 to 5985 iu/l/day (AFP) and 0.08–5404 iu/l/day (HCG). The rise in TMP appeared to be uniformly exponential in 36/45 (80%) AFP cases, and in 27/34 (79%) HCG cases. More than two TMP values were available in 20/45 (44%) AFP cases and 17/34 (50%) HCG cases, only two TMP values being available in the rest. MPDT was calculated from this exponential rise. Two AFP cases and one HCG case showed two distinct exponential rises in TMP, the faster rise being just prior to chemotherapy. In these cases the MPDT was taken as the rate of rise of TMP just prior to chemotherapy. In seven AFP cases and six HCG cases an increase in TMP with time was barely detectable and in these cases the MPDT was recorded as > 80 days. The range of MPDT were for AFP, 0.5 to > 80 days and for HCG 1.8 to > 80 days. MPDT tended to cluster around the lower end of the range: in 80% of AFP cases and 82% of HCG cases, the MPDT was ≤ 32 days.

Figure 1 demonstrates the relationship between doubling times in the group of 28 patients where a MPDT was available for each tumour marker. A horizontal line indicates consistency across the two markers. In 6/51 (12%) patients there is a large discrepancy between doubling times with apparently slow growth calculated from one marker but a faster growth calculated from the other.

Figure 2 demonstrates the disparity between the MPDTs and the time taken for the serum tumour marker level to double. The lack of direct relationship is explained by the relationship given in equation (A2) and demonstrates why the rise in serum marker level alone does not reveal information about the amount of marker produced by the tumour.

DISCUSSION

Proliferation rates in NSGC tumours have generally been difficult to measure. Volume doubling time in metastases of 10–30 days have been calculated [2]. However, clinical volume measurements can underestimate the cellular doubling time because of heterogeneity of tissue—for example the varying proportions of necrotic tissue and cysts. Flow cytometric estimation of the S phase fraction [3] is also limited by tumour heterogeneity; in most cases the G2 + M peak from diploid tumour subpopulations appears within the S phase region of the non-diploid tumour cell population making the S phase calculation inaccurate. Calculation of a proliferative index from flow cytometrically derived DNA histograms [4] attempts to overcome this problem and is arguably more accurate a measure of proliferative activity when applied to archival material. However, no absolute measure of proliferation rate can be obtained with this method. Also, both flow cytometric methods have only been used to measure proliferative activity in the excised primary tumour, which may be different from that of the metastatic disease present in the patient. We report a novel method of calculating the proliferation rate of metastatic NSGC tumours which is non-invasive and can be used retrospectively to analyse patients with marker positive tumours when there have been at least three serum marker levels measured between orchidectomy and the start of chemotherapy.

This method relies upon a number of assumptions. First, that all marker producing tumour cells produce marker at a constant rate. *In vitro*, mostly using gestational choriocarcinoma cell lines, this has been shown not always to be the case. In tissue

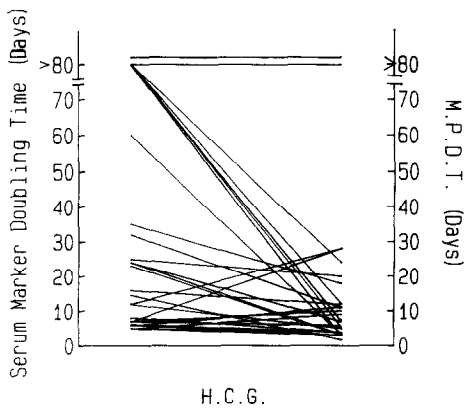

Fig. 1. Relationship between AFP and HCG MPDTs.

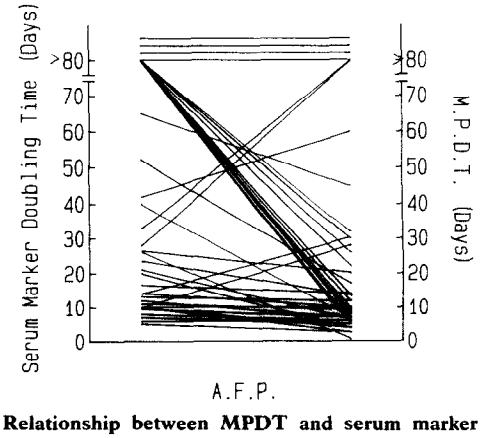

Fig. 2. Relationship between MPDT and serum marker DT for HCG.

culture, the amount of HCG synthesized per cell can vary with cell density [5], and it has been reported that the rate of tumour antigen production from cell *in vitro* varies with the kinetics of the population [6]. Also, chemotherapeutic agents can increase cellular HCG production in cell culture [7–9]. Despite these reservations, however, there is no clinical evidence that HCG production per unit mass of tumour varies greatly with time. Also, analysis of MPDT is based on the assumption that increase in TMP with time is exponential. In 80% of cases this was so, and in most of the other cases, increase in TMP was barely detectable. Secondly, the amount of tumour marker produced by the tumour cells must be reliably reflected in the serum marker level. In support of this, in human tumour teratoma xenografts, it has been demonstrated that tumour marker concentrations (AFP) do correlate with plasma tumour marker levels [10]. Thirdly, clearance of the tumour marker from the plasma must not be impaired so that clearance is according to the natural plasma half life. No cases of impaired clearance of HCG or AFP in patients with NSGCTT have been reported; in clinical practice if clearance is delayed after treatment, or the serum marker level does not decline exponentially according to the curve of its natural half life, this is taken to indicate residual tumour—the concept of apparent of half life [11]. Also, in a group of 20 patients with stage 1, AFP positive disease, treated at this institute, decrease in serum marker level after orchidectomy was used to assess variations in natural plasma half life. The range was 3.5–8 days, median 5.25 days, with 85% of patients having a natural half life between 4 and 6.25 days. In this study a plasma half life of 5 days (AFP) and 1.5 days (HCG) was used for the calculation. In those few individuals in whom plasma half life may be different, the effect on the MPDT is small due to the logging of $t_{1/2}$ in the mathematical calculation; thus the degree of error in the MPDT is minimized.

Finally, MPDT is only a measure of the proliferation rate of the marker producing cells within the tumour. Sub-populations of cells that are marker negative will not be measured by this method. Germ cell tumours histologically and functionally have a mixed cell population and may be derived from each of the three germinal layers, HCG and AFP are found in and synthesized by different cell types [12]. It is well recognised that tumour marker levels may vary independently (discordance) [13] and this explains why treatment may sometimes affect one cell population and not the other. This has also been clarified *in vitro* [10] in one xenograft line illustrating the evolution of marker negative tumour cells from a patient who was initially AFP positive. Despite this recognised heterogeneity, there is a good clinical correlation between serum marker level and bulk of disease, and between serum marker level and outcome after chemotherapy [14]. There is probably a spectrum of marker production in germ cell tumours. For instance in gestational choriocarcinomas, all tumour cells may produce HCG, and attempts have even been made to estimate HCG production per unit mass of tumour [15].

Other germ cell tumours may be composed of both marker-negative and marker-positive cells. A dominant cell population may emerge, possibly because of more rapid proliferation of a subgroup of cells. This latter consideration may explain the seven patients in the present study in whom TMP was available but decreased with time, despite clinically progressive tumour, and why some tumours had longer MPDTs than would be clinically expected, e.g. > 80 days.

This novel method of estimating tumour marker production from the serum tumour marker level and the known natural half life clarifies the use of serum tumour marker levels in describing tumour growth. Increase in serum tumour marker level alone does not provide absolute or as meaningful information. Calculation of actual TMP provides information about the change in number of marker producing cells and can be used as a non-invasive method of measuring tumour growth rate.

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Further details of the mathematical method outlined in the Appendix are available on request.

APPENDIX

The essence of the mathematical analysis is a technique for calculating a more representative TMP. The observed concentration levels of serum marker are the result of two competing processes. The continuous marker clearance by the kidney is assumed to occur at a known rate a given by

$$a = \frac{\log_e 2}{t_{1/2}} \qquad \text{(A1)}$$

where $t_{1/2}$ is the half life of the relevant marker. We take $t_{1/2}$ (AFP) = 5 days and $t_{1/2}$ (HCG) = $1\frac{1}{2}$ days.

The other process is of course TMP itself. It is here that our method takes a novel approach. We could have assumed a simple half life production rate and measured the rate difference between production and excretion. But this has drawbacks in that TMP varies intermittently. It makes sense to try to get an average TMP rather than fit a production half life to concentration levels which may be the result of a high or low production phase.

We proceed as follows. We have readings of concentration levels at three successive times t_1, t_2 and t_3. In the first time interval (t_2-t_1), we estimate the average daily TMP. We then do the same for the second time interval (t_3-t_2). This procedure gives two values for the average daily TMP which *we then assume to behave exponentially*, with a half life β and a marker production doubling time (MPDT) given by

$$T = \frac{\log_e 2}{\beta}. \tag{A2}$$

The calculation is straightforward. In the *absence* of TMP, the serum marker concentration level $C(t_1)$ at time t_1 would be

$$C(t_1)\exp[-\alpha(t_2-t_1)] \tag{A3}$$

at the later time t_2. But in fact we measure $C(t_2)$. Therefore TMP accounts for the difference, viz

$$C(t_2) - C(t_1)\exp[-\alpha(t_2-t_1)] \tag{A4}$$

and this occurs at an average daily rate

$$Q(t_2, t_1) = \frac{C(t_2) - C(t_1)\exp[-\alpha(t_2-t_1)]}{(t_2-t_1)} \tag{A5}$$

during this period. Similarly in the later period t_2 to t_3 the average daily rate is given by

$$Q(t_3, t_2) = \frac{C(t_3) - C(t_2)\exp[-\alpha(t_3-t_2)]}{(t_3-t_2)}. \tag{A6}$$

Then we assume that Q itself behaves exponentially by taking

$$Q = qe^{\beta t} \tag{A7}$$

at the two times t_2 and t_3. Therefore

$$\beta = \frac{1}{(t_3-t_2)}\log_e \frac{Q(t_3, t_2)}{Q(t_2, t_1)}. \tag{A8}$$

From a computational standpoint we know t_1,t_2 and t_3 together with $C(t_1)$, $C(t_2)$ and $C(t_3)$. We calculate $Q(t_2, t_1)$ by equation (A5) and $Q(t_3, t_2)$ by equation (A6). Then we find β from equation (A8) and finally the MPDT from equation (A2).

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The Growth Rate of Metastatic Non-seminomatous Germ Cell Testicular Tumours Measured by Marker Production Doubling Time—II. Prognostic Significance in Patients Treated by Chemotherapy

P. Price, S.J. Hogan, J.M. Bliss and A. Horwich

Tumour growth rates have been measured in metastatic non-seminomatous germ cell testicular tumours (NSGCTT) by estimating the rate of rise of tumour marker production (TMP). TMP was calculated for the time between orchidectomy and the start of chemotherapy in a group of 58 patients with metastatic NSGCTT treated with BEP combination chemotherapy (bleomycin, etoposide and cisplatin). Calculation of TMP (iu/l/day) took account of the continuing clearance of marker from the serum. TMP increased with time in 51 patients and this rise generally appeared to be exponential. The rate of this increase was expressed as the marker production doubling time (MPDT) and is a measure of the tumour growth rate. MPDT varied from 0.5 to > 80 days (45 cases) for AFP +ve patients and from 1.8 to > 80 days (34 cases) for HCG +ve patients. Patients who failed BEP first line therapy had shorter MPDTs than those who responded (AFP $P = 0.08$, HCG $P = 0.003$). It was found that patients with a MPDT ≤ 4 days were more likely to fail treatment than those who had a MPDT > 4 days (AFP $P = 0.009$, HCG $P = 0.005$). MPDTs were independent of initial serum marker concentration. Patients with small volume disease had longer MPDTs than patients with large volume disease (AFP $P = 0.02$, HCG $P = 0.04$). Rapid tumour growth rate reflected by short MPDT carries a poor prognosis in patients with NSGCTT treated by BEP chemotherapy.

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