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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INTRODUCTION

ALTHOUGH the chemotherapy of metastatic non-seminomatous germ cell testicular tumours (NSGCTT) is highly successful, 30–50% of patients fail first line chemotherapy [1]. Three adverse prognostic features of patients with metastatic NSGCTT have already been identified: volume and extent of disease, and serum concentration of tumour marker [2]. The development of less intensive chemotherapeutic regimens makes it important to judge prognosis accurately. There is a need to identify individual features which may further define prognostic groups.

There is good clinical evidence that NSGCTT grow rapidly. Volume doubling times of metastases tend to be short (10–30 days) [3], relapse after primary treatment, when it occurs, tends to present within the first year [4], and uncontrolled tumour usually results in the rapid demise of the patient.

Proliferative activity in NSGCTT has been measured using flow cytometric analysis of the S phase fraction. In a prospective study of 20 patients using fresh frozen tissue obtained at orchidectomy, accurate assessment from analysable histograms was available in eight cases and the S phase fraction ranged from 6 to 51%, suggesting very fast proliferation in some cases [5].

There is evidence that the proliferative activity of NSGCTT may be clinically important. NSGCTT are more likely to fail treatment if chemotherapy is prolonged by delay between courses [6, 7] suggesting that rapidly proliferating tumours carry a worse prognosis. In a study of 50 patients using archival paraffin-embedded, formalin-fixed tumour from the primary pre-chemotherapy lesions, Sledge and colleagues measured the proliferative index from flow cytometrically derived DNA histograms [8]. Multivariate analysis suggested that proliferative activity was correlated significantly with survival: patients with a proliferative index greater than the mean had a significantly worse survival.

This paper uses a novel method of calculating tumour growth rate in patients with metastatic NSGCTT using the change in rate of tumour marker production per day [9]. The relationship between this measure of tumour growth rate and prognosis has been assessed in those patients receiving bleomycin, etoposide and cisplatin as first line treatment for metastatic NSGC testicular tumours.

METHOD

Calculation of tumour proliferation rates

Tumour proliferation rates were calculated from serum tumour marker levels either, AFP, HCG or both, as described elsewhere in this issue [9]. In summary, the serum marker level at any one time is dependent on the rate of production of the marker from the 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. 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. If this 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 has been calculated for the period following orchidectomy and before the start of chemotherapy.

Evaluable patients

One hundred and thirty-two patients treated at the Royal Marsden Hospital Testicular Teratoma Unit between 1979 and 1986 with bleomycin, etoposide and cisplatin (BEP) [10] as first line treatment for metastatic disease are included in the study. Ninety-six (73%) of the 132 patients were marker positive and 58 of these (44% of 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 AFP +ve only, nine were HCG +ve only and 29 patients expressed both markers. TMP values could be calculated in 49 AFP +ve cases and 38 HCG +ve cases. A rise in TMP with time occured 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 was excluded from the analysis.

Seven out of the 51 evaluable patients (14%) were considered as being in an unfavourable group, on the basis of established prognostic factors [10], i.e. by having one or more of the following features: bulky abdominal (Stage C) disease with high markers (see Table 1 legend), extensive lung (L_3) disease, or involvement of liver, bone or brain. Follow up was available in all patients with a median of 51 months (range 14–84 months). Four out of the 51 patients (8%) have died of disease and one relapsed but was salvaged with a similar but more intensive weekly regimen [11]. The four patients who died of disease all had poor prognostic features: large volume disease and high initial serum marker levels. The patient who failed BEP but was salvaged, originally had stage 1 m (marker only) disease, with low initial serum marker levels, and received four complete courses of BEP without any delay between courses. One of the 51 patients died of bleomycin induced lung toxicity and at *post mortem* was found to be in complete remission. For the purpose of analysis he was included in the non-relapsed group.

Staging and investigations

Patients were staged according to Royal Marsden Hospital classification [12] with measurement of serum AFP and HCG, and CT scans of thorax and abdomen. Serum markers were measured at the Department of Medical Oncology, Charing Cross Hospital, London using radioimmunoassay. Units were expressed in iu/l.

Statistical analysis

AFP and HCG MPDTs were analysed separately. Statistical analysis was performed using the nonparametric Mann–Whitney *U* Test and Fisher's exact test. Multivariate analysis to assess the prognostic independence of MPDT was not possible due to the small number of events observed.

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Table 1. Comparison of those patients in whom MPDT was available and patients in whom calculation of MPDT was not possible

	No. of Patients	Volume of disease		Initial marker level		Outcome of BEP first line chemotherapy	
		Small	Large	Low	High	Responded	Failed
Evaluable Patients:	51	42 (82%)	9 (18%)	36 (71%)	15 (29%)	46 (90%)	5 (10%)
Unevaluable Patients:							
1. Marker negative	36	31 (86%)	5 (14%)			33 (92%)	3 (8%)
2. < 3 serum marker levels	38	18 (47%)	20 (53%)	23 (60%)	15 (40%)	25 (66%)	13 (34%)
3. TMP did not ↑ with time	7	4 (57%)	3 (43%)	3 (43%)	4 (57%)	6 (86%)	1 (14%)

Volume of disease: small = lm, 2A/B, 3A/B, 4A/BL1/2; large = 2c, 3c, 4cL1/2/3.
Initial marker level: high = AFP > 50 kU/l, HCG > 1000 iu/l.

RESULTS

Table 1 shows the characteristics of the 51 evaluable patients and the 81 patients in whom it was not possible to calculate a MPDT. Reasons for inability to calculate MPDT in certain patients were: marker negative disease (36), less than three serum marker levels recorded between orchidectomy and the start of chemotherapy (38), or no increase in calculated TMP with time (7). More patients with poor prognostic features were in the group with less than three marker levels recorded after orchidectomy and before chemotherapy.

The 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 the 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 immediately 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 ranges 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% 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 six patients there is a large discrepancy between doubling times with apparently slow growth calculated from one marker but faster growth calculated from the other.

Figure 2 demonstrates the relationship between MPDTs and prognosis. A difference was observed between growth rates of those patients who failed BEP and those who did not (Mann–Whitney U test: AFP, $P = 0.08$, HCG, $P = 0.003$). No inconsistency between the marker readings was observed: of those patients who failed BEP, three had comparable MPDTs calculated for both AFP and HCG (MPDTs of 2.5 and 3, 2 and

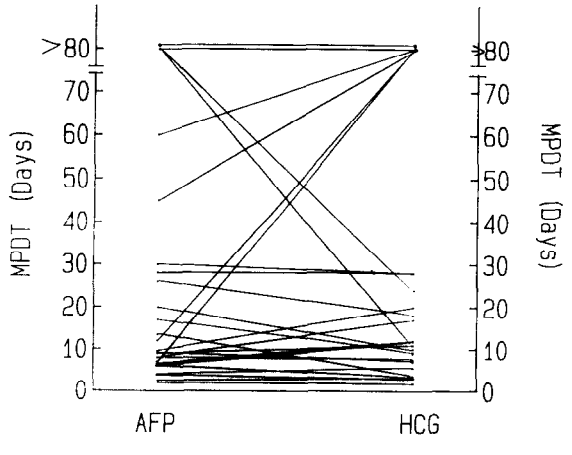

Fig. 1. Relationship between AFP and HCG MPDTs.

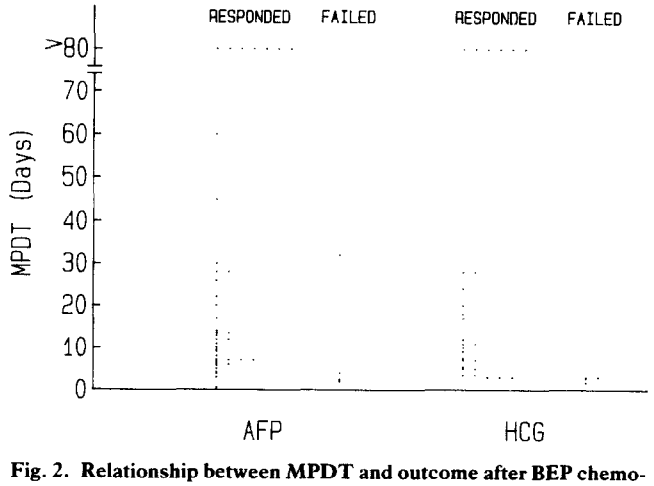

Fig. 2. Relationship between MPDT and outcome after BEP chemotherapy.

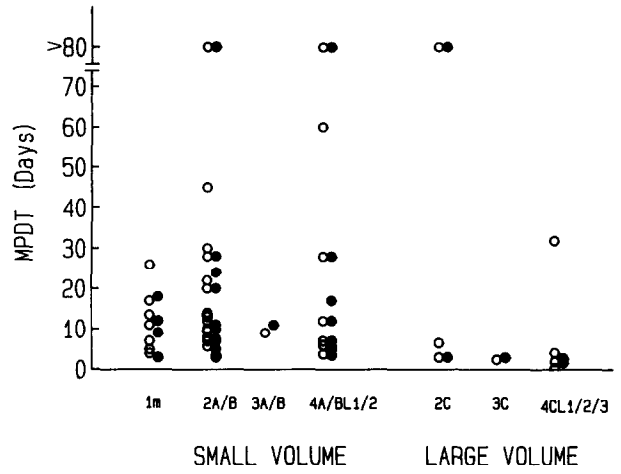

Fig. 3. Relationship between MPDT and tumour stage and bulk of disease. AFP MPDT (○) and HCG MPDT (●). Occasionally a symbol may represent more than one patient with the same MPDT.

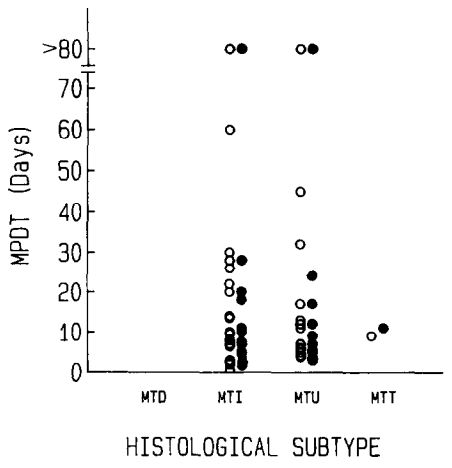

Fig. 5. Relationship between MPDT and histological subtype of disease. AFT MPDT (○) and HCG MPDT (●). Occasionally a symbol may represent more than one patient with the same MPDT.

1.7, 4 and 3 days for AFP and HCG respectively). The two remaining patients who failed BEP had the MPDT calculated from one marker only.

Taking a cut off of 4 days (using a data-derived dichotomous hypothesis), it emerges that those patients with a MPDT $\leq$ 4 days were significantly more likely to fail BEP chemotherapy than those with a MPDT of $>$ 4 days (AFP $P = 0.009$ and HCG $P = 0.005$). The single patient who failed BEP with a MPDT of $>$ 4 days (AFP), subsequently relapsed with and died of marker negative disease.

Figures 3 and 4 show the relationship between MPDTs and the three recognised poor prognostic features: volume and extent of metastatic disease and initial serum concentration of tumour marker. Patients with small volume disease had significantly longer MPDTs than those with large volume disease (AFP $P = 0.02$, HCG $P = 0.04$). MPDT appeared to be independent of initial tumour marker level (AFP $P > 0.50$, HCG $P = 0.20$).

Figure 5 shows that the MPDT was independent of histological subtype of disease (AFP $P > 0.50$, HCG $P > 0.50$). Using the cut-off of 4 days, MPDTs were also found to be independent of length of clinical history (AFP $P = 0.97$, HCG $P = 0.47$).

Twenty-three of the evaluable patients had been initially assessed as having Stage 1 disease with no adverse histological features and had been entered into a surveillance study. Patients received regular follow up and were only offered combination chemotherapy on relapse. They had relapsed with metastatic disease at intervals of between 1 and 44 months. MPDT was assessed at relapse before the start of chemotherapy, and 22/23 (96%) patients had both, or their only, MPDTs $>$ 4 days. Fourteen patients had at least one MPDT $\leq$ 4 days, of whom 4 (29%) failed BEP chemotherapy. Five of the seven patients (71%) considered already as being in an unfavourable group on the basis of established prognostic factors had both, or their only, MPDT $\leq$ 4 days. All three of the seven poor prognostic group patients who died had both or their only MPDT $\leq$ 4 days.

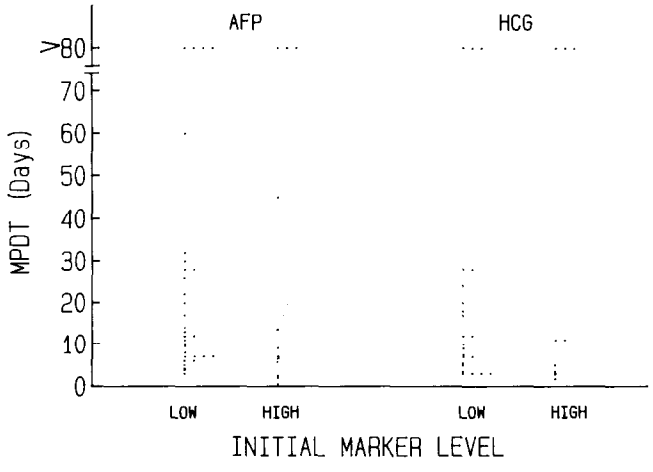

Fig. 4. Relationship between MPDT and initial serum marker level.

DISCUSSION

The results of this study show that rapid tumour growth rate reflected by short MPDT is associated with a poor prognosis in patients with NSGCTT treated by bleomycin, etoposide and cisplatin.

The small numbers of evaluable patients and patients who failed chemotherapy posed difficulties for the analysis and a larger experience may better define the relationship between MPDT and known prognostic features. The significant correlation of patients with small volume disease having longer MPDT than patients with large volume disease is biologically plausible.

The discrepancy between AFP and HCG MPDTs in six patients suggests that if there was a more universally available measure of tumour proliferation rate, in particular for marker negative cells, it might be possible to define more accurately the high risk group of patients with fast growth rates.

Results reported here would agree with the suggestion made by Sledge and colleagues [8], measuring a flow cytometrically derived proliferation index obtained from the primary lesion, that patients with a proliferation index greater than the mean had a significantly worse prognosis. Possible explanations are that: a rapid proliferation rate is simply a reflection of the extent to which a tumour has escaped normal growth controls and so is a measure of biological aggressiveness; or that rapidly growing tumours are able to proliferate significantly between courses of treatment and due to the fractional cell killing of chemotherapy,

a continually regenerating tumour cell population will not be eradicated. If the latter explanation is correct, pretreatment tumour proliferation rate is providing a guide to the regenerative capacity of the tumour cells. If proliferation does occur between cycles of chemotherapy, this would explain why NSGCTT are more likely to fail treatment if chemotherapy is protracted by delays between cycles.

Volume and extent of disease, and serum concentration of tumour marker are established poor prognostic features in patients with metastatic NSGCTT. MPDT may define individuals within the poor prognostic group who are more likely to fail BEP chemotherapy. Our study suggests that a MPDT of 4 days may be a clinically useful cut off point, but this may not be optimal and a larger investigation to test this hypothesis is needed. Our evaluable group included seven patients who would be considered, by established criteria, to be in a poor risk group. The three patients in this group who died, all had MPDT of $\leq$ 4 days. With such a measure of tumour growth rate, it may be possible to predict those poor risk patients who are most likely to fail conventional chemotherapy. A rational modification might be to deliver chemotherapy more rapidly for these poor risk patients with fast proliferation rates. It is of interest that a more intensive chemotherapy regimen employing weekly bleomycin, vincristine and cisplatin [11] was effective salvage therapy in a patient with a short MPDT who failed conventional BEP chemotherapy.

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Blood Transfusion and Prognosis in Dukes' B and C Colorectal Cancer

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To evaluate the prognostic influence of blood transfusion in cancer patients, transfusion data were reviewed on 468 radically operated patients (260 Dukes' B and 208 Dukes' C) with carcinoma of the rectum and the rectosigmoid. Data on whole blood and packed red blood cell transfusions were recorded together with a number of clinical, pathological and histochemical characteristics. The endpoint used was death with cancer. All patients were followed for 2–7 years or until time of death.

Univariate statistical methods revealed a highly significant trend towards worsened prognosis with increasing volume of transfusion blood. However, this effect was insignificant when multivariate statistical methods were employed: patients receiving whole blood or packed red blood cell transfusions did no worse than expected from their clinico-pathological characteristics.

It is concluded that in this series the observed association between transfusion status and prognosis is adequately explained by a multivariate prognostic model including well-established prognostic factors.
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