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Serum Tumour Marker Regression Rate Following Chemotherapy for Malignant Teratoma*

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Abstract—*The rate of fall of the serum tumour markers alphafetoprotein (AFP) and the beta sub-unit of human chorionic gonadotrophin (HCG) was analysed following platinum-based chemotherapy for metastatic non-seminomatous germ cell tumours. Of* 90 *evaluable patients* 81% *were alive and disease-free* 1.5–4 yr *(median* 28 months) *from the start of chemotherapy and* 69 (77%) *had remained continuously disease free. All three patients with an initial AFP half-life greater than* 9 days *relapsed; however, a further eight relapsing patients had an initial regression rate of serum AFP within the same range as patients remaining in remission (half-life* 6–9 days). *The HCG regression rate did not discriminate between patients remaining well or those who relapsed after chemotherapy. In* 11 *examples of a pattern of late slowing of the rate of marker fall (i.e. increasing half-life), five relapses were seen* (45%), *though this pattern was also observed in the context of large residual differentiated teratoma masses.*

INTRODUCTION

THE SERUM tumour markers alphafetoprotein (AFP) and human chorionic gonadotrophin (HCG) are well-established to be useful in the diagnosis and management of non-seminomatous germ-cell tumours, one or both of these markers being elevated in approximately 75% of patients with metastatic disease [1–10]. Alphafetoprotein has a molecular weight of 70,000 and is the major serum protein of the human foetus. It has been found in the serum of patients with hepatocellular carcinoma [11, 12] and subsequently in a high percentage of patients with testicular teratomas [13]. Additionally, it is elevated in the serum of a small proportion of patients with other tumours [14].

Human chorionic gonadotrophin is a hormone of molecular weight 45,000 secreted by the syncytiotrophoblastic cells of the normal placenta. It contains two dissimilar sub-units designated alpha and beta. The amino acid sequence of the alpha sub-unit is very similar to that of some other human glycoprotein hormones, such as luteinizing hormone. However, differences in the amino acid sequences of the beta chains confer specificity [15]. A radioimmunoassay was developed which was specific for the beta chain of HCG [16].

Serum elevations of this marker occur in gestational trophoblastic tumours, a high proportion of germ-cell tumours and occasionally in non-germ-cell tumours, especially cancers of the stomach and pancreas [17–19].

The rate of clearance of markers from the serum of patients can be expressed in terms of the apparent half-life (AHL) [10, 20]. Metabolic studies, supported by marker analysis following orchidectomy in stage I disease, have demonstrated an AHL of 4–6 days for AFP and 1–2 days for HCG [21–23]. Slower rates of fall may reflect a balance between marker clearance and marker production by viable tumour cells, and thus the AHL following chemotherapy may be an index of the success of treatment. This study analyses the prognostic significance in metastatic non-seminomatous germ-cell testicular tumours of the initial rate and subsequent pattern of fall of the serum tumour markers AFP and HCG following chemotherapy.

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MATERIALS AND METHODS

The patients studied were 91 men with metastatic non-seminomatous germ tumours, referred between 1979 and 1981 to the testicular tumour unit at the Royal Marsden Hospital. They represented all such patients who had received no treatment other than orchidectomy for this disease. Staging investigations included lymphography, abdominal sonography and CT scanning of the thorax and abdomen in all cases, and included an assessment of tumour bulk, as well as extent of disease (Table 1) [24]. All were treated with first-line chemotherapy protocols under investigation at the Royal Marsden Hospital, using *cis*-platinum, vinblastine and bleomycin (PVB); bleomycin, etoposide and *cis*-platinum (BEP); or bleomycin, etoposide, vinblastine and *cis*-platinum (BEVIP) [25, 26], as shown in Table 2. Ninety evaluable patients have been followed for 18–48 months (median 28 months) from start of this chemotherapy.

Serum levels of AFP and of HCG were assayed just prior to commencement of chemotherapy and were repeated at minimum intervals of 21 days. Seventy-four patients (82%) had elevated levels of at least one of the markers prior to chemotherapy (i.e. AFP >20 μg/l or HCG >5 iu/l). In many patients, following chemotherapy, the marker had fallen to within normal limits before a second assay had been performed and the regression rate could not be assessed. In the remainder the marker level was plotted against time from start of chemotherapy, using a semilog plot. Where there were more than two points, the graph was plotted and analysed using the SCIPLOT and CURFIT software programs (designed by Paul K. Warner and obtained from Interactive Microware Inc., P.O. Box 771, State College, PA 16801, U.S.A.) in conjunction with an Apple II microcomputer.

Table 1. The Royal Marsden Hospital staging system for malignant teratoma

I	no evidence of disease outside the testis.
IMk	no evidence of disease outside the testis, but rising tumour, markers post-orchidectomy
II	infradiaphragmatic node involvement
IIA	maximum diameter of metastases <2 cm
IIB	maximum diameter of metastases 2–5 cm
IIC	maximum diameter of metastases >5 cm
III	supra and infradiaphramatic lymph node involvement; abdominal nodes: A, B, C as for stage II; mediastinal nodes noted M+; neck nodes noted N+ 0 = no abdominal node metastases
IV	extension of tumour to extralymphatic sites O, A, B, C, for abdominal nodes as for stages II and III; mediastinal nodes noted M+; neck nodes noted N+; lung substage: L_1 metastases ≤3 in number; L_2 metastases >3 in number and <2 cm maximum diameter; L_3 metastases >3 in number and >2 cm maximum diameter; H+ hepatic involvement; other sites, e.g. bone and brain, are specified

Table 2. Chemotherapy treatment protocols

	Chemotherapy schedules	Dose	Treatment (days)	Cycle
PVB	*cis*-platinum	20 mg/m²/day	1–5	
	vinblastine	0.15 mg/kg/day	2, 3	3 weeks
	bleomycin	30 mg	2, 9, 16	
BEP	bleomycin	30 mg	2, 9, 16	
	etoposide	120 mg/m²/day	1–3*	3 weeks
	cis-platinum	20 mg/m²/day	1–5	
BEVIP	bleomycin	30 mg	2	
	etoposide	120 mg/m²/day	1–3	4 weeks
	vinblastine	0.15 mg/kg/day	2, 3	
	cis-platinum	20 mg/m²/day	1–5	

*In the initial phase of the study some patients were treated with a 5-day etoposide schedule.

The program fitted a linear regression line using least-squares analysis and presents the accuracy of fit by enumerating the coefficients of determination and of correlation [27]. The gradient (G) of the linear regression can be converted to give the apparent half-life (AHL) of the marker level by the calculation:

$$\text{AHL} = \frac{\ln 0.5}{G}, \text{ i.e. } = \frac{0.693}{G}.$$

The programmes were used to distinguish a linear fall of marker from a pattern of increasing AHL (Fig. 1a, b), and regression patterns accepted by the eye as linear all had calculated least-squares regression lines with coefficients of correlation >0.985 and coefficients of determination >0.975.

Alternatively, where only two points were available regression was also expressed as an apparent half-life by

$$\text{AHL} = \frac{0.3T_1}{\log_{10}\dfrac{(\text{conc. } T_1)}{(\text{conc. } T_0)}},$$

[28] where conc. T_0 is the original marker level and conc. T_1 is the level to which the marker has fallen after T_1 days.

Half-lives following chemotherapy were obtained for AFP in 38 patients and HCG in 29 patients. To assess the value of the initial fall of tumour marker, i.e. the response to the first course of chemotherapy, the rate of regression was calculated between days 1 and 21, namely the interval between the first day of cycles one and two. If prognostically important, this value might identify a sub-group of patients where modified chemotherapy might be indicated to pre-empt overt drug resistance. The pattern of fall of marker level was followed either to normal levels or to clear-cut relapse in all patients in order to assess the prognostic relevance of the shape of the curve, and the shape was expressed in terms of either a linear plot or an increasing half-life plot. Additionally, a number of patients had several sequential samples assayed within the first 10 days following the start of chemotherapy to identify transient elevations of marker levels above the initial values. This was termed a 'surge phenomenon' [1, 29] (see Fig. 2).

Of the 91 patients who were eligible for this retrospective analysis one was lost to follow-up after one course of chemotherapy and was not evaluable. Of the 90 evaluable patients 13 (14%) died of progressive teratoma, three died of treatment-related complications but with no evidence of malignancy, six patients (7%) relapsed on or after chemotherapy (of whom four

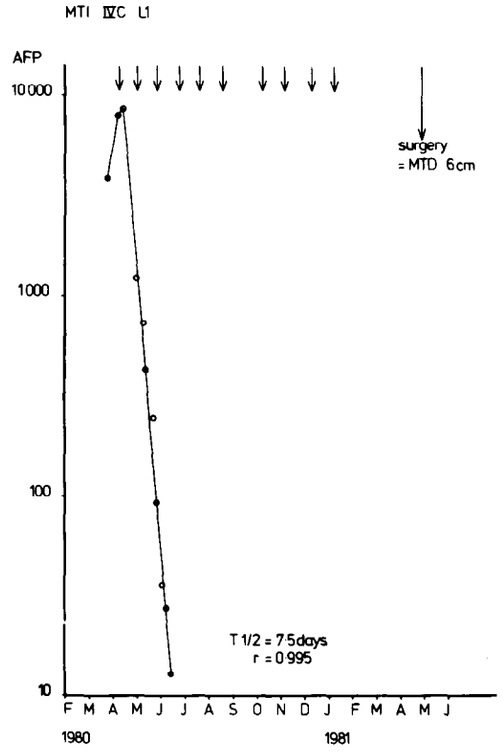

Fig. 1a. The pattern of fall of serum AFP after chemotherapy for stage IV malignant teratoma presenting with a large abdominal mass and small volume lung deposits. The pattern is exponential with a half-life of 7.5 days and a high correlation coefficient. After chemotherapy a 6-cm residuum of mature teratoma was excised (AFP in μg/l).

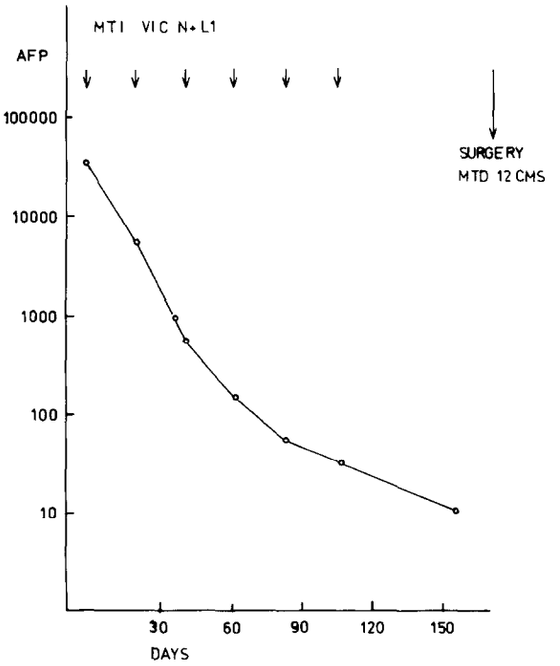

Fig. 1b. A pattern of fall of serum AFP with increasing apparent half-life (AHL). After chemotherapy a 12-cm mass of mature teratoma was excised (AFP in μg/l).

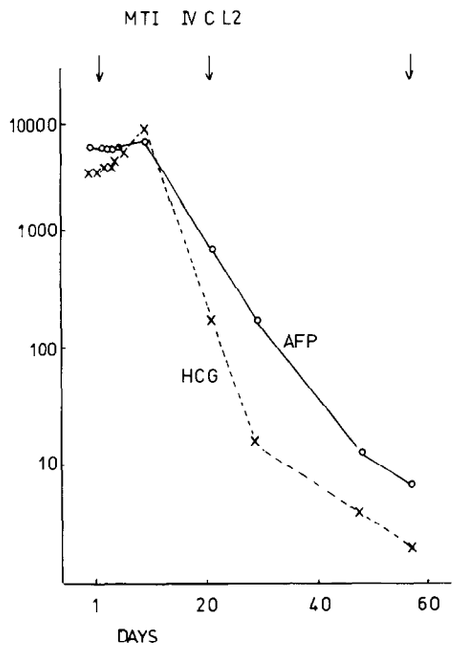

Fig. 2. Transient elevation of serum HCG after chemotherapy in a patient successfully treated for teratoma metastatic to abdominal nodes and lung fields (the marker 'surge' phenomenon) (HCG in iu/l).

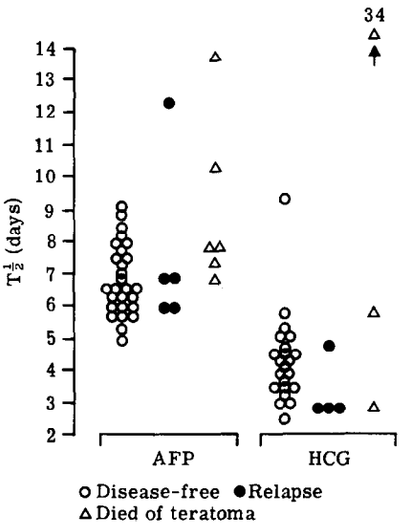

Fig. 3. The apparent initial half-life of marker fall after chemotherapy in patients who subsequently either remained disease-free or relapsed. The half-life was calculated from a comparison of the pre-treatment (day 1) serum level and the level on day 21 (see text).

are disease-free after further treatment) and 69 patients have remained continuously disease-free 1.5–4 yr (median 28 months) from start of chemotherapy. Thus 73 out of 90 (81%) of patients are alive and disease-free.

Serum AFP levels were assayed by radioimmunoassay at the Protein Reference Laboratory, Putney Hospital, London [30]. Serum beta HCG levels were assayed by radio immunoassay at the Department of Medical Oncology, Charing Cross Hospital, London [31].

RESULTS

The rate of fall of serum tumour marker in response to the first course of chemotherapy was assessed by comparing the level on day 21 with the level before chemotherapy on day 1, and expressing the rate of fall in terms of the apparent half-life in days.

Figure 3 shows the AHL of groups with differing prognoses, namely those who remained disease-free after treatment, those who relapsed but remained alive and those who relapsed and died. For AFP there was a narrow range of AHL of patients remaining disease-free. The 27 well patients had AHL of AFP between 5 and 9 days (mean 6.7 days). Those who relapsed and are alive (five) and those who died with teratoma (six) had AFP AHL ranging from 6 to 14 days (mean 8.8 days), and it is notable that eight of the 11 values in patients who did badly are in the same range of AHL as patients who did well. However, all three patients with AHL greater than 9 days relapsed.

The HCG AHL in 22 patients who subsequently remained relapse-free range from 2.5 to 9 days (mean 4.4 days) and of the seven patients who did badly on chemotherapy the initial rate of fall was well within this range. The patient with prolonged HCG AHL of 34 days had advanced trophoblastic teratoma with extensive lung, brain and liver deposits. Although his subsequent marker fall was more rapid he never achieved clinical or marker remission and died 6 months after starting chemotherapy.

The later pattern of fall of serum markers was investigated in 34 patients with initial levels of one or both markers sufficiently high to permit a number of points to be plotted. An exponential fall (linear on semilog plot) of AFP (Fig. 1) was seen in 19 patients, of whom 16 (84%) remained continuously disease-free, two died of teratoma and one relapsed after initial chemotherapy but is now disease-free. An increasing AFP half-life (Fig. 1b) was seen in five patients (Table 3), of whom only two remained continuously disease-free, two having died of teratoma and one relapsing after initial chemotherapy (Table 4).

The rapid clearance of HCG from the serum following chemotherapy meant that the overall pattern was analysed only rarely (and predominantly in those presenting with high marker levels). Six patients had linear (exponential) falls of marker levels, of whom five remained disease-free and one relapsed but is now disease-free. A patern of increasing AHL of HCG was seen in six patients (Table 3), of whom one died of teratoma,

Table 3. The prognosis of patients with the pattern of increasing half-life of fall of tumour marker following chemotherapy at the Royal Marsden Hospital

Marker	Histology	Stage*	Initial marker level (u/l)	Initial half-life (days)	Well	Relapsed after initial therapy	Died of teratoma
AFP	MTI	IVB H+	27,000	6.5			×
AFP	MT†	IVC L3	3200	5.5		×	
AFP	MTI	IVC L2	5000	7		×	
AFP	MTI	IVC N+ L1	35,000	7	×		
AFP	MTI	11C	410	10	×		
HCG	MTT	Med‡ L3	24,000	3			×
HCG	MTI	IVC L2	3000	2.5		×	
HCG	MTI	11C	400	5	×		
HCG	MTT	IVA L3 H+	309,000	4.5	×		
HCG	MTT	IVC L3	471,000	3	×		
HCG	MTU	IVB L2	2900	3	×		

*See Table 1.
†On cytology.
‡Mediastinal primary.

one relapsed but is now disease-free and four remained well after chemotherapy (Table 4). Clinical details on patients with an increasing half-life of tumour markers are shown in Table 3. In no case did the change in half-life coincide in time with an impairment of renal or liver function. For AFP the pattern of increased half-life was associated with non-resolution of the teratoma in three cases and with massive residual differentiated teratoma in the other two. With HCG of six patients showing a pattern of increasing AHL, but normalization of the marker level, four have remained continuously relapse-free. This result is not significantly different from those patients showing an exponential fall. In the patient who died, the increasing AHL represented the transition between falling and rising marker levels. The patient who relapsed but who is now disease-free did not relapse with rising HCG and thus the significance of the increase in AHL is uncertain. In three of the other patients there was residual bulky differentiated teratoma persisting at the end of the chemotherapy and this has either been excised or, in the case of multiple lung deposits in two patients, has remained completely stable since the end of the treatment.

Table 4. Pattern of fall of serum markers in relation to outcome of chemotherapy in advanced testicular non-seminoma

Pattern of serum marker fall	Serum marker	
	Alphafetoprotein	Beta-HCG
Exponential	16/19†	5/6†
Increasing half-life	2/5†	4/6†

*Defined in text and illustrated in Fig. 1 (a and b).
†Continuously disease free/total patients treated

In summary, of 11 examples of the pattern of increasing AHL, five had massive residual differentiated teratoma and two had non-resolution of malignant teratoma.

A marker surge pattern was seen in 12 of 14 first courses of chemotherapy when the serum marker level was repeated within 2 weeks of start of chemotherapy. Although all of these patients have remained relapse-free following chemotherapy, the prognostic significance of the surge phenomenon has yet to be determined.

DISCUSSION

Modern chemotherapy regimens employing combinations of *cis*-platinum, vinblastine and bleomycin [32] and/or etoposide [25] have dramatically transformed the prognosis of patients with advanced testicular non-seminoma. In spite of these advances treatment failures still occur, particularly in patients with bulky extensive metastatic tumour. Clinical experience suggests that drug resistance emerges rapidly in testicular germ-cell tumours and it will clearly be advantageous to discriminate between patients with a high chance of treatment failure earlier in their management so that alternative chemotherapy can be employed. For this reason we have investigated marker clearance rate as a possibly useful index of the initial effect of chemotherapy in order to predict the eventual outcome of treatment. A useful concept in expressing this rate of fall is the apparent half-life [28]. The maximum rate of fall of markers is an AHL of 4–6 days for AFP and AHL of 24–36 hr for HCG. These clearance rates would correspond to cessation of all *in vivo* marker production and in theory after chemotherapy might be considered to correspond to the destruction of all the tumour.

Major reservations in the application of this concept are:

1. Teratomas are usually heterogeneous with respect to marker production and a drug-resistant cell clone may be marker-negative or may form only a minor component of the initial cell population. Also, marker production may occur from non-clonogenic cells.
2. The rates of fall of serum levels in tumour markers may be influenced by physiological factors such as protein metabolism or renal clearance, or by delay within a particular tissue or fluid compartment such as might occur, for example, in a large and poorly vascularized tumour.

Previous reports have suggested that a slow initial rate of marker after treatment might be useful in individual patients, perhaps providing an indication for change of chemotherapy [20, 8]. The present analysis, restricted entirely to highly effective chemotherapy combinations and to previously untreated patients, does not support the prognostic usefulness of the initial marker AHL since in the majority of cases it would fail to distinguish patients destined to relapse from those remaining free of disease. A small proportion (3/36 or 8.5%, in this study) of patients demonstrated AHLs clearly longer than the other patients and did in fact relapse but the usefulness of this observation is weakened by the finding of 14 relapsing patients with AHL well within the range of the AHLs of relapse-free patients. The range of AHL in patients successfully treated was not very wide and the likelihood is that even in patients not successfully treated, the initial chemotherapy course killed the vast majority of marker-producing cells.

Lange *et al.* [10] have demonstrated prolonged AHL in patients who had a poor chemotherapy response. These observations related to what would now be considered relatively ineffective chemotherapy regimes (2/18 complete responses) and therefore are not incompatible with our data; indeed, a long average AHL in a series of patients treated with a new chemotherapy regimen might be an early indicator of a relatively ineffective treatment.

The model whereby the emergence of a chemoresistant cell clone is reflected in a slowly increasing AHL (e.g. Fig. 1b) was examined by reviewing the 11 examples of this pattern in our series. Overall this indicated a poorer prognosis since almost half of the patients subsequently relapsed (usually with a rise in the same marker that had shown the increase in AHL). However, it is noteworthy that in the other patients this pattern was consistent with resolution of their malignant teratoma with no alteration in chemotherapy regime, implying that the change in half-life did not reflect the emergence of a drug-resistant clone. These results are substantially in agreement with a report from Willemse *et al.* [33], who also noted an association between the pattern of increasing marker half-life and the finding of mature teratoma masses on subsequent surgical exploration.

The detection of the surge effect, wherein there is a transient rise in tumour marker following chemotherapy, requires frequent marker assays and in our series was not sought systematically. Where multiple assays were performed the phenomenon was commonly found and, in contrast to the report by Thompson and Haddow [8], was detected with AFP as well as with HCG. Possible explanations would include the effects of cell cycle perturbations [34] or of cell density changes [35], or of inducing differentiation to cells producing higher levels of marker, as has been suggested following studies of an HCG-producing tumour cell line *in vitro* [36, 37]. Our limited data would not indicate that the phenomenon has major prognostic significance when occurring following the initial chemotherapy course; a prospective study is being addressed to this question.

In summary, our data would indicate that with known effective chemotherapy regimes and previously untreated patients the initial rate of tumour marker fall only rarely has prognostic significance. An increasing marker AHL was an early indicator of relapse in only 5/11 instances where this pattern was seen.

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