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Prognostic Implications of Tumour Marker Analysis in Non-seminomatous Germ Cell Tumours with Poor Prognosis Metastatic Disease

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86 unselected patients with poor risk metastatic non-seminomatous germ cell tumours (NSGCT) treated from 1979 to 1990 at a single institution were reviewed with regard to the prognostic relevance of tumour marker analysis. The number of elevated tumour markers was not able to distinguish patients into prognostic subgroups. Pretreatment levels of human chorionic gonadotropin (HCG), alpha-fetoprotein (AFP) and lactate dehydrogenase (LDH) did not have a significant influence on clinical outcome. HCG and AFP half-life analysis during the first chemotherapy cycles also failed to define prognostic subgroups. If early deaths within 90 days after the onset of chemotherapy were excluded, patients with a half-life of HCG decline greater than 3.5 days tended to have a poorer prognosis which did not reach significance.
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INTRODUCTION

CISPLATIN-BASED chemotherapy dramatically improved clinical outcome of patients with disseminated non-seminomatous germ cell tumours (NSGCT). However, reported complete remission (CR) rates greater than 90% for good risk patients obscure the fact that 20–30% of all patients ultimately die of their disease [1].

Some prognostic factors have been defined by different authors to subdivide patients into good and poor risk groups. Age of patient [2, 3], primary extragonadal origin [4], histology [2, 4, 5], number and location of metastatic sites [1, 2, 6, 7], performance status [8], baseline human chorionic gonadotropin (HCG) [2–7, 9, 10], baseline alpha-fetoprotein (AFP) [2, 3, 5, 7, 10], lactate dehydrogenase (LDH) [6, 11, 12], lactate dehydrogenase isoenzyme I [13] and the rates of tumour marker decline during chemotherapy [9, 14] have been identified to influence prognosis. Since some of these variables are interrelated, multivariate analyses were performed to determine their relative prognostic importance [1, 2, 5–7].

A staging system developed at Indiana University allows identification of a group of patients with poor prognosis metastatic disease who are at a high risk for treatment failure [15]. This staging system only considers distribution and bulk of disease and does not include tumour markers as prognostic variables. To further characterise the patient group with poor risk metastatic disease we used complementary studies of baseline tumour markers and marker changes during chemotherapy.

PATIENTS AND METHODS

86 consecutive patients with poor prognosis metastatic NSGCT were treated between May 1979 and June 1990 at our institution. The median age of patients was 27 years with a range from 17 to 65 years. Poor risk metastatic disease was consistent with advanced status of the Indiana staging system except for a minor modification: abdominal bulky disease was defined as a palpable mass or a mass greater than 10 cm at computerised tomography (CT) (Table 1). The records, X-rays and CT scans were reviewed by one investigator, and all patients treated at

Table 1. Definition of poor-prognosis metastatic disease

Primary mediastinal non-seminomatous germ cell tumour or >10 pulmonary metastases per lung field or multiple lung metastases >3 cm
Palpable abdominal mass (or >10 cm at CT scan) with supradiaphragmatic disease
Liver, bone or brain metastases

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our institution in the above mentioned time frame and assigned poor risk metastatic disease status were included in this study. The histological diagnosis was made according to the British classification [16]. All patients underwent chest X-ray and abdominal CT scan prior to chemotherapy. A thoracic CT scan was performed in the majority of patients. Serum tumour markers HCG and AFP were determined in all patients before chemotherapy by radioimmunoassays described elsewhere [17–19]. Tumour marker analysis was standardised and was performed by the same investigators throughout the period of study. All values for HCG > 5 U/l and AFP > 15 U/ml were regarded as elevated. 85 patients were evaluable for baseline HCG and AFP studies. Lactate dehydrogenase was measured in 77 patients (normal range < 240 U/l); these patients were subdivided into four groups according to the number of elevated markers. Patient characteristics pertained to status immediately prior to chemotherapy (Table 2).

None of the patients had received prior radiation or chemotherapy. In 2 patients with primary mediastinal tumours, non-cisplatin-containing regimens were initially administered due to false histological diagnoses, but chemotherapy schedules were changed immediately after revision of histology. 1 patient did not receive any chemotherapy because of an uncontrolled haemorrhage due to metastatic infiltration of the duodenum; he was excluded from marker analyses. Between 1979 and 1983 the majority of patients was treated according to the PVB protocol, which consisted of cisplatin 20 mg/m^2 for 5 days, vinblastine 0.2 mg/kg for 2 days and bleomycin 30 mg on days 1, 8 and 15 [15]. In the following years chemotherapy was mainly administered according to the ECBC schedule (Table 3) [20, 21], which included etoposide 120 mg/m^2, cisplatin 30 mg/m^2, bleomycin 12 mg/m^2 (24 h infusion) and cyclophosphamide 300 mg/m^2 for 4 days and an additional 15 mg bleomycin bolus injection on day 1. The patient groups treated by PVB and ECBC did not vary with regard to disease distribution or pretreatment tumour marker levels.

In the majority of patients tumour marker levels were frequently (3–10 times) determined during the first 21 days of chemotherapy cycles. All patients at least had marker measurements prior to each chemotherapy cycle. Marker values were plotted semilogarithmically (log concentration vs. linear time), and the half-lives of marker regression were calculated using a computer spreadsheet program; calculation was based on two consecutive values [22] or on three values using regression analysis, if the regression coefficient was not lower than 0.95. The first values measured more than 7 days after the beginning of chemotherapy were used for calculations, since the peak of initial marker increase was mainly observed within the first week. A transient marker elevation following chemotherapy was found in 50–70% of patients and was deemed a marker release due to tumour cell death [14, 23]. Since some patients also experienced a surge in the serum markers in the second and third cycle of chemotherapy, marker half-life analysis was based on measurements prior to these treatment courses. A late inflexion of marker fall was seen frequently, especially in patients with high pretreatment levels. Half-life calculation used only values measured prior to this inflexion. In all cases, the time span of marker half-life studies did not exceed 90 days after the start of chemotherapy. Patients whose first or second marker value measured more than 7 days after the start of chemotherapy was already within the normal range were not considered for half-life analysis. Considering these exclusion criteria, 57 of 65 HCG-positive, and 50 of 58 AFP-positive patients were evaluable for marker half-life analysis. Since early deaths were often not definitely attributable to disease- or treatment-related complications, patients who died within 90 days after the start of chemotherapy were excluded from marker analysis in a second step. The cut-offs of half-lives of marker decline were chosen at 3.5 days for HCG and 7 days for AFP.

Follow-up examinations of patients were performed as

Table 2. Survival rate according to patients' characteristics univariate comparisons using log-rank test

Prognostic Variables	No.	Alive	%	P
Age (years)				
≤35	70	37	53	
>35	16	5	31	0.169
Primary site				
Testis	65	32	49	
Extragonadal	21	10	45	0.828
Histological diagnosis				
MTU	29	14	48	
MTI	17	6	35	
MTT	14	6	42	
MTD	3	1	33	
Mixed GCT	22	14	64	
Pure endodermal sinus tumour	1	1	100	0.632
No. of metastatic/extragonadal sites*				
1	16	9	56	
2	39	20	51	
3	21	12	57	
4 or more	10	1	10	0.001
Sites of metastases/extragonadal manifestations				
Retroperitoneum				
No involvement	20	11	55	
Moderate disease	11	5	45	
Bulky disease (>10 cm at CT or palpable mass)	55	26	47	0.618
Lung				
No involvement	16	7	44	
Moderate volume†	16	9	56	
Large volume†	35	21	60	
Very large volume†	19	5	26	0.031
Mediastinum				
No involvement	63	30	48	
Mass < 10 cm	9	4	44	
Mass > 10 cm	14	8	57	0.613
Liver				
No involvement	68	36	53	
Involvement	18	6	33	0.018
Brain				
No involvement	77	41	53	
Involvement	9	1	11	0.003
Bone				
No involvement	83	41	49	
Involvement	3	1	33	0.687
No. of elevated markers				
0	3	3	100	
1	8	3	38	
2	25	10	40	
3	41	23	56	0.301

*Cervical lymph nodes are not considered.
†Moderate volume EQ pulmonary metastases < 10 per lung field, each metastasis < 3 cm; large volume = pulmonary metastases > 10 per lung field or metastasis (-es) > 3 cm; very large volume = pulmonary metastases > 10 per lung field and metastasis (-es) > 3 cm.

Table 3. Chemotherapy regimens and treatment results

	No.	%
Chemotherapy regimens		
PVB	36	42
ECBC	35	41
Other cisplatin-based regimens	9	10
No/less than 1 complete cycle	6	7
Treatment results/patient status		
Alive	42	49
No evidence of disease	33	38
Stable residual disease	4	5
Alive with relapse	3	3
Alive, unknown status	2	2
Dead due to disease	39	45
Therapy-related deaths	5	6
Early deaths (< 90 days after start of chemotherapy)	10	12

described elsewhere [24]. The beginning of survival and the endpoint were defined as the start of chemotherapy and the date of last follow-up or death of the patient, respectively. Survival distributions were estimated by the Kaplan–Meier method [25]. Univariate comparisons were made using the log-rank test [26]. Multivariate comparisons were performed according to Cox regression analysis using forward stepwise procedure.

RESULTS

The overall survival rate was 49% at the end of the study in March 1992 (Table 3). 44% of patients treated according to the PVB protocol and 66% of the ECBC group survived. The disease-free survival rates were 33 and 54%, respectively. Minimal follow-up was 21 months from the end of treatment. The median follow-up was 102 months for the PVB group and 44 months for the ECBC group.

In univariate comparisons significant risk factors identifying groups with a lower proportion of survival were the number of metastatic sites, very large volume pulmonary dissemination and the presence of liver or brain metastases (Table 2). The prognostic relevance of liver and brain metastases was confirmed by multivariate analysis (P-values 0.001 and 0.004, respectively).

There was no significant difference with regard to survival rates for the four patient groups defined by the number of elevated markers (Table 2). Pretreatment HCG > 10000 U/l, AFP > 1000 U/ml and LDH > 1000 U/l were also not significant risk factors (Figs 1–3).

63 patients were evaluable for marker half-life analysis. In 57 patients HCG half-lives, in 50 patients AFP half-lives and in 29 patients both HCG and AFP half-lives could be calculated. The medians of half-lives were 3.0 days for HCG and 6.2 days for AFP in the patient group as a whole. The median half-lives of marker decline were longer in patients treated by PVB (median half-life for HCG 3.9 days, range 1.8–17.8, median half-life for AFP 7.8 days, range 3.9–65.4) than in the ECBC group (median half-life for HCG 2.4 days, range 0.9–16.7, median half-life for AFP 5.8 days, range 2.5–10.2). There was no difference in survival rates for patient groups with HCG half-lives up to 3.5 days and longer than 3.5 days. If patients dying within 90 days after the onset of chemotherapy were excluded, there was a non-significant tendency to better outcome in the group with lower half-lives (Fig. 4). This discrepancy is explained by the fact that 5 patients who died within 1 month due to disease- or therapy-related complications had HCG half-lives of less than 3.5 days. AFP half-life analysis using a cut-off of 7 days was not able to separate two different prognostic groups (Fig. 5), even if patients with early deaths were not considered (P-value 0.599, not shown). The proportion of patients with half-life data for both HCG and AFP was too small to define subgroups with equivalent and different behaviour of marker decline.

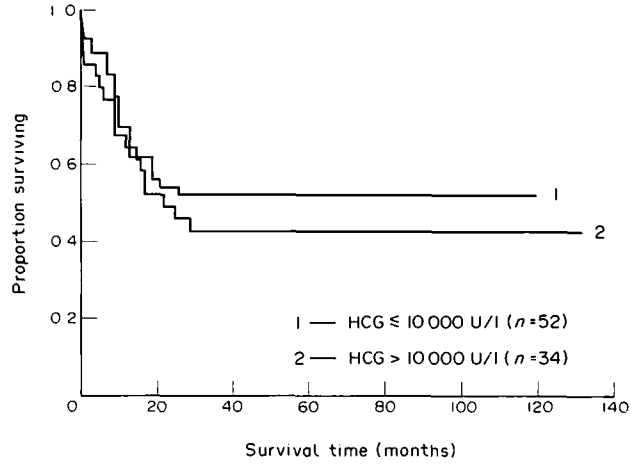

Fig. 1. Kaplan–Meier plots of survival distribution from date of the start of chemotherapy for patients with pretreatment HCG below and above 10 000 U/l. The difference in survival distributions is not significant (P = 0.399).

DISCUSSION

The goal of present studies is to minimise toxicity in good risk patients and to treat poor risk patients more effectively with tolerable toxicity. Different staging systems were developed using extent of disease and tumour markers as prognostic variables. The Indiana staging system, which considers only size and distribution of disease, allows division of patients into three prognostic groups with minimal, moderate and advanced disease [15]. Whereas patients with minimal or moderate disease achieve a complete remission in a high proportion (99 and 90%, respectively), favourable responses to treatment were observed

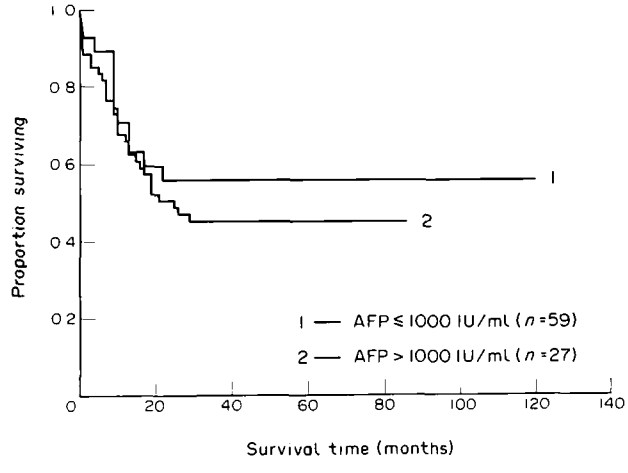

Fig. 2. Kaplan–Meier plots of survival distribution from date of the start of chemotherapy for patients with pretreatment AFP below and above 1000 U/ml. There is no significant difference in survival distributions (P = 0.401).

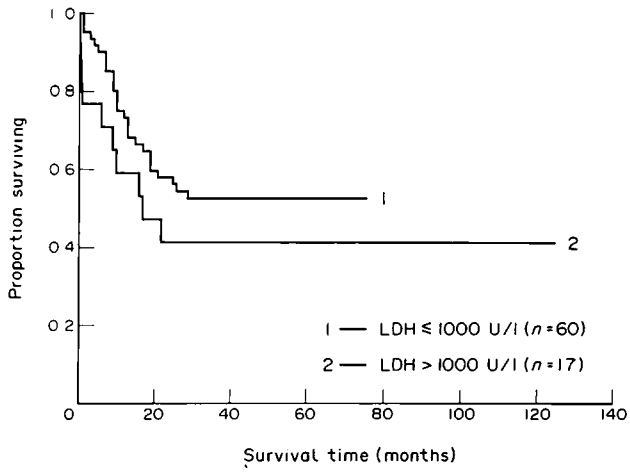

Fig. 3. Kaplan–Meier plots of survival distribution from date of the start of chemotherapy for patients with pretreatment LDH below and above 1000 U/l. The difference in survival distributions is not significant ($P = 0.270$).

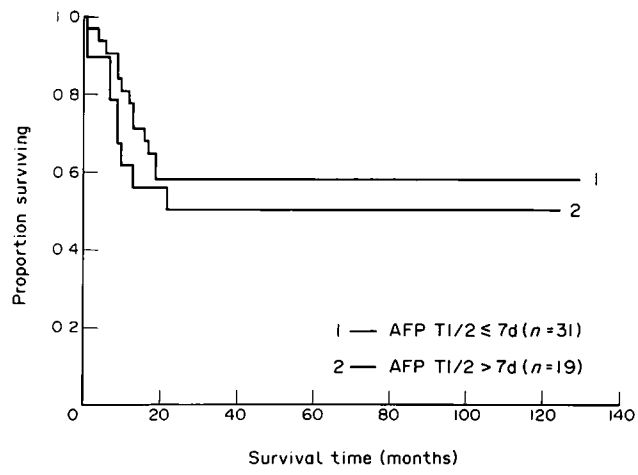

Fig. 5. Kaplan–Meier plots of survival distributions from date of the start of chemotherapy for patients with AFP half-lives ≤ 7 days and >7 days following chemotherapy. The difference in survival distributions is not significant ($P = 0.479$).

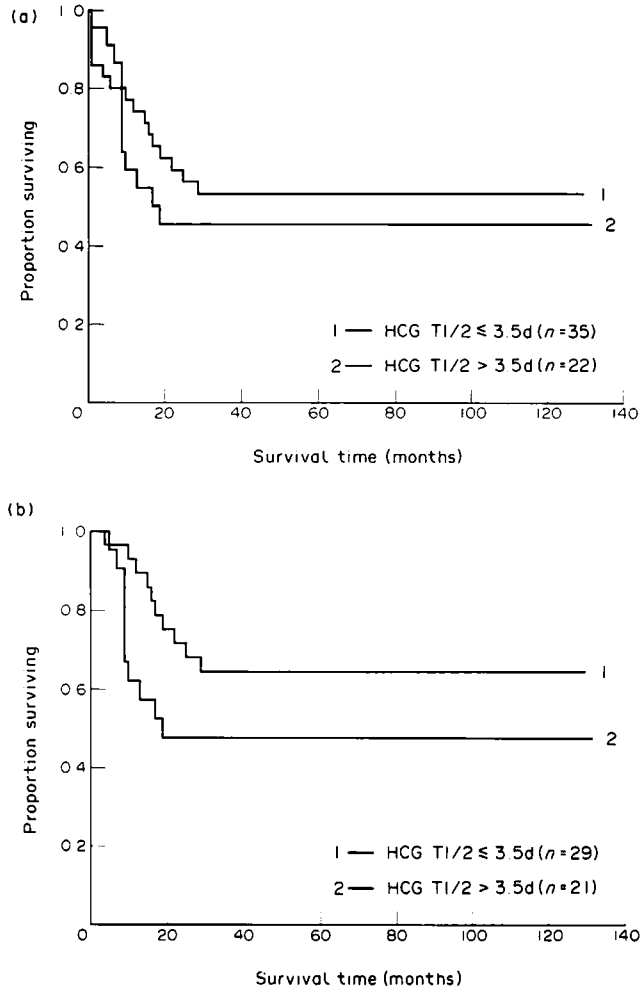

Fig. 4. (a)Kaplan–Meier plots of survival distribution from date of the start of chemotherapy for patients with HCG half-lives ≤3.5 days and >3.5 days following chemotherapy. The difference in survival distributions is not significant ($P = 0.524$). (b)Survival distribution for patients with HCG half-lives ≤3.5 days and >3.5 days excluding patients with early deaths (< 90 days from the start of chemotherapy). There is a tendency for a poorer outcome for patients with HCG >3.5 days, but the difference also does not reach significance ($P = 0.127$).

only in 58% of patients with advanced disease [1]. Excluding patients with early deaths (10 patients) the survival rate of patients with "advanced" NSGCT treated at our institution was 58% in the end of the study and was thereby in agreement with the data of the literature [1].

The prognostic relevance of tumour marker analysis has been reported by several investigators [2, 4–7, 9–12]. However, little information is available about the predictive value of marker analysis in a poor prognostic group defined by a large tumour volume and widespread dissemination. Whereas in the aforementioned report [1] patients with advanced NSGCT were subdivided into three prognostic subgroups defined only by the number of elevated tumour markers, in our study this criterion failed to gain prognostic importance. The prognostic relevance of HCG, AFP and LDH in pretreatment risk stratification has been documented by several authors [2–7, 9, 10]. In contrast, these parameters had no significant impact on clinical outcome in our study of poor prognosis metastatic disease.

There are only a few previous reports which studied tumour marker regression rates during initial chemotherapy as prognostic variables in NSGCT [9, 14, 27]. Whereas Picozzi *et al.* [9] examined only HCG decline and did not consider possible initial marker elevations following chemotherapy, Toner *et al.* [14] studied both HCG and AFP regression rates and took the marker surge phenomenon into account [23]. Patients dying within 90 days after the onset of chemotherapy were excluded by Toner *et al.* Both investigators found that studies of tumour marker decline were able to complement pretreatment risk stratification and to distinguish between patient groups highly likely and unlikely to achieve a CR. As a consequence, Motzer *et al.* used an inappropriately slow decline of tumour markers after two chemotherapy cycles as an eligibility criterion for high-dose chemotherapy with autologous bone marrow rescue [28]. In our study, there was only a non-significant tendency to poorer outcome for patients with a longer HCG half-life, if early deaths, in whom response to chemotherapy could not be estimated sufficiently, were excluded. AFP half-life was not able to define different prognostic groups. This discrepancy between our and other investigators' results may be due to the selection of poor risk patients who entered our study, whereas the other studies included patients with low and large volume disease.

It must be taken into account that tumour marker half-life analysis following chemotherapy only incompletely reflects tumour cell death. The stroma and vascularisation of the tumour as well as the metabolism and excretion of tumour markers may also influence serum marker levels. In addition, the molecular heterogeneity of HCG has to be considered [17]. A reduced metabolic clearance was found for acidic variants of HCG [29]. Since the proportion of acidic variants of HCG increases during chemotherapy, a prolonged half-life of HCG decline does not necessarily indicate drug resistance. An alternative to the calculation of marker half-life following chemotherapy may be the determination of marker production doubling time between orchiectomy and the start of chemotherapy. Rapid tumour growth rate reflected by short marker production doubling time carries a poor prognosis [30].

We conclude that pretreatment HCG, AFP and LDH, as well as marker half-life analysis following chemotherapy, do not significantly add to risk stratification in poor prognosis metastatic NSGCT. Only the presence of hepatic or cerebral metastases was confirmed to have a significant impact on clinical outcome.

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