Pergamon

European Journal of Cancer Vol. 30A, No. 8, pp. 1052–1058, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)E0126-O

Papers

Clinico-pathological Criteria with Prognostic Relevance in Hepatoblastoma

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We investigated clinical data and histological specimens of 46 patients with a hepatoblastoma (HB) for prognostic criteria. Disease-free survival (DFS) of 23 patients treated in the German Cooperative Study HB-89 (1988–1990) was 83%, in contrast to 40% in 10 children with other chemotherapy regimes (1977–1987) and 38% in 13 with only a tumour resection ($P = 0.005$). Tumour residence after resection (R category) correlated significantly with probability of DFS ($P = 0.0001$). This was also the case for pT status, according to the pTNM classification for liver carcinoma ($P = 0.0007$), involvement of one or both liver lobes ($P = 0.004$), multiplicity of tumour nodes ($P = 0.001$), vascular invasion ($P = 0.0006$) and expression of nucleolar organiser regions as an indicator for proliferation activity of tumour cells ($P = 0.05$). Patients' age and histopathological subtypes could only indicate outcome, while tumour size and serum α-fetoprotein values were not significantly related to prognosis. In multivariate analysis, pT status and R categories remained significant. These should be applied in all cooperative trials on HB.

Key words: hepatoblastoma, prognosis, pTNM classification, R categories, histological subtype, nuclear organiser regions

Eur J Cancer, Vol. 30A, No. 8, pp. 1052–1058, 1994

INTRODUCTION

HEPATOBLASTOMA (HB) COMPRISES only 1% of all childhood malignancies, yet it is the most common liver tumour in children older than 6 months and, therefore, of clinical importance [1]. Only during recent years has the prognosis of patients with this tumour improved as different cooperative studies applying effective chemotherapy, and advanced surgical strategies have been introduced [2–5]. However, even now, the prognosis of an individual patient remains unpredictable. In the context of cooperative studies, only few analyses were undertaken to find prognostic criteria of HB. Two separate investigations [6, 7] studied the significance of histological subtypes of HB for patients' prognosis, and found that pure fetal histology correlated with a favourable outcome, while all other histological subtypes could not predict patients' prognosis. A Japanese study [8] showed that young age is associated with a better chance of cure. In the attempt to find other objective criteria for patients' prognosis, we recently performed a study on the significance of DNA ploidy and DNA proliferation index as prognostic markers in HB [9]. In agreement with the results of two other investigations [7, 10], we found that patients with diploid HB, measured by DNA flow cytometry, have a better chance of tumour-free survival than those with an aneuploid tumour, and that the DNA proliferation index is also of prognostic value in HB. In the present study, we investigated clinical data and tumour specimens of the same patients to evaluate whether clinico-pathological criteria, that is, patients age, serum α-fetoprotein (AFP), chemotherapy modality, residual tumour after surgery (R category) [11], pTNM status [11], vascular invasion, tumour size and localisation, histological subtype [12] and the expression of nucleolar organiser regions (NORs) as a proliferation marker [13], can be significant factors for prediction of prognosis in this tumour. In order to identify independent prognostic factors, we performed a multivariate analysis with the most important of the above-mentioned criteria. It will be shown that the results of this study can be integrated into a staging system on the basis of the pTNM system as proposed by the International Union Against Cancer for liver carcinoma [11], which is independant of treatment modalities, and has a high prognostic relevance in HB.

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Revised 18 Jan. 1994; accepted 2 Mar. 1994.

PATIENTS AND METHODS

Clinical data and tumour specimens of 48 children with an HB were available for investigation. 2 patients, who died of early treatment complications, were not included in this study. One of these did not survive initial laparotomy because of massive haemorrhage and the other died of toxic liver failure during chemotherapy with vincristine, actinomycin D and cyclophosphamide 5 days after presentation. Of the remaining 46 patients, 23 were treated in our hospital during the years 1977 to 1987. They underwent an initial laparotomy with resection of the tumour. Of these, 10 patients were additionally treated with chemotherapy: 2 patients received vincristine (1.5 mg/m^2 days 1, 15, 29), actinomycin D (15 μg/kg days 1–5) and cyclophosphamide (200 mg/m^2 days 1–5) in 6-week courses (Dr. P.A Voûte, Emma Kinderziekenhuis, Amsterdam). 8 patients were treated with vincristine (1.5 mg/m^2 days 1 and 22), cyclophosphamide (500 mg/m^2 days 2 and 23), doxorubicin (25 mg/m^2 days 1, 2 and 3) and 5-fluorouracil (500 mg/m^2 days 24 and 31). 2 patients of this group were additionally treated with bleomycin (15 mg/m^2 as continuous infusion days 22, 23 and 24) and cisplatin (90 mg/m^2 day 25) in 6-week courses according to the combined protocol of the CCSG and POG (CCG 881/POG 8301) [14]. 13 patients did not receive any adjuvant chemotherapy. 23 patients were treated from 1988 to 1990, according to the protocol of the Cooperative German Pediatric Liver Tumour Study HB-89 [15]. It demands an initial laparotomy in all children with a primary liver tumour, except in infants with a highly elevated serum AFP and distant metastasis. At operation, tumours confined to one lobe of the liver are resected. In case of tumour extension into both lobes, only a biopsy is taken, to be followed by chemotherapy. After two or three courses of chemotherapy, a second look operation is performed with the aim to resect all tumour tissue, if necessary even with extended resection techniques. The chemotherapy consists of a combination of ifosfamide (0.5 g/m^2 bolus and 3.0 g/m^2 over 72 h, days 1–3), cisplatin (20 mg/m^2, days 4–8) and doxorubicin (60 mg over 48 h, days 9 and 10) in 3-week courses (IPA) [4]. If a tumour responds to this regimen, but regression is insufficient, high-dose cisplatin (90 mg/m^2 over 4 h, day 1) and doxorubicin (80 mg/m^2 over 90 h, days 2–5) are given as continuous infusion (PA-cont) similar to the schedule of the CCG study 823F [2].

Clinical data were evaluated including age, sex, AFP serum levels, chemotherapy regimes and surgical procedures (Table 1). Serum AFP of all children was determined by radioimmunoassay during routine laboratory investigations at initial examination, and in 27 patients also after tumour resection, after each course of chemotherapy and regularly during follow-up. For determination of long-term outcome of patients, the results of clinical and sonographic examinations, thoracic X-ray and serum AFP during follow-up were evaluated. Mortality from progressive or recurrent tumour was registered, and complete remission at latest examination was defined as no evidence of disease (NED).

Table 1. Clinico-pathological factors of 46 children with hepatoblastoma, which were correlated with patients' long-term disease-free survival

Patients	Tumour
Age	Tumour volume
Sex	Involvement of liver lobes
Serum α-fetoprotein	Multiplicity of tumour nodes
Surgical procedure	Macroscopic/microscopic vascular invasion
Chemotherapy regime	Residual tumour after resection
	Histological subtype
	Expression of nuclear organiser regions

The following characteristics of the tumours were registered: tumour size measured intra-operatively and/or by sonography, the volume calculated with the formula: $1/2 \times a \times b \times c$ (cm^3), involvement of one or both lobes of the liver, multiplicity of tumour nodes, macroscopic tumour invasion of hepatic or portal veins, and residual tumour after resection.

All tumours were staged after initial surgery and histological examination, applying the pTNM system according to the guidelines set by the International Union Against Cancer (UICC) for hepatocellular carcinoma [11]. It contains the following elements: tumour size ($\leq$ or $>$ 2 cm), vascular invasion, involvement of one or both liver lobes, multiplicity of tumour nodes and occurrence of lymph nodes or distant metastasis. This staging system is objective and independent of treatment modalities. Therefore, differences in surgical treatment and chemotherapy of patients in this study do not influence classification of tumours. Residual tumour was documented using the R categories with R0 for no remaining tumour, R1 for microscopic and R2 for macroscopic residue.

Representative tissue of 37 not pretreated HBs was available for histological examination. In 9 patients, tumour specimens were obtained only after chemotherapy. Slides were prepared from formalin-fixed and paraffin-embedded tissue, and stained with haematoxylin-eosin (H&E), Giemsa, periodic acid-Schiff (PAS), Bielschofsky's reticulin and Goldner stains. We determined the histological subtype and microscopic vascular invasion of tumour cells. Concerning histological subtypes, tumours were categorised as: (1) pure epithelial, mixed epithelial and mesenchymal HB; and (2) according to their epithelial differentiation as pure fetal, fetal and embryonal, and embryonal hepatoblastomas [12]. Six representative slides of each tumour were independently seen by three pathologists in order to obtain a uniform classification.

Parallel sections of paraffin-embedded tissue of 33 not pretreated HBs were also stained for nucleolar organiser regions (NOR) as previously described [13]. Briefly, 3-μm thick paraffin sections were deparaffinised in xylene, rehydrated in ethanol and rinsed in aqua dest. The slides were then incubated for 30 min with the staining solution consisting of 0.02 g gelatine (Merck) in 1 ml formic acid (1%) (Merck) and 1 g of silver nitrate dissolved in 2 ml aqua dest. After rinsing, the slides were dehydrated with ethanol and prepared for microscopical examination. NORs appeared as small dark dots in tumour cells' nuclei. Numbers of NORs per nucleus and their size were registered with a computerised image analyser. The quotient of these parameters is the equivalent of the cells NOR content, and reflects rDNA transcriptional activity [16]. It is, therefore, an indicator for cellular proliferation activity. For analysis of correlation between NOR expression and patients' prognosis, we divided the tumours into groups with NOR quotients $>$ 12 and $\leq$ 12, respectively.

All data were correlated with patients' long-term disease-free survival (DFS). For estimation of probability of DFS, we used the method of Kaplan and Meier [17]. Statistical analysis was performed with the log-rank test and for comparison of groups with the Fisher's exact test [18]. Significance was assumed in cases of $P \leq 0.05$.

Finally, we performed a multivariate analysis, utilising the

proportional hazards regression model of Cox [18], with which we examined significance of the effect of chemotherapy regimens, completeness of resection (R categories), pT status, histological subtypes and NOR expression of 22 patients, for whom complete sets of data were available. Patients' age and AFP values were not included, since there was no relationship between these two criteria to prognosis. The same was so for tumour size, involvement of liver lobes, multiplicity of tumour nodes and vascular invasion, which are elements of pT status and, therefore, not independent factors.

Informed consent for evaluation of clinical data and central review of tumour specimens was obtained from the patients' parents in all cases.

RESULTS

Patients' characteristics

Of the 46 patients, 20 were female and 26 male, with no difference in outcome of disease. Patients' ages ranged from 4 months to 7.5 years (91 months), with a mean age of 24.4 months. At time of evaluation, 28 of the 46 patients (61%) were alive and well, 18 had died of progressive or recurrent tumour. Follow-up was 2 to 10.5 years (median 3.5 years). Patients under 1 year of age had a 92.3% probability of DFS, compared with 50.0% of those between 1 and 3 years, and 57.1% above 3 years. These differences were non-significant ($P = 0.06$).

Serum AFP

Initial AFP serum levels ranged from not detectable to 5 093 000 ng/ml. There was no correlation between initial values and outcome of disease. In 27 evaluable patients, AFP levels decreased more or less quickly under chemotherapy and/or tumour resection. Those patients in whom the AFP level never decreased to normal (9 patients), or in whom a renewed rise during therapy (3 patients) or first remission (10 patients) was observed, had an unfavourable outcome. However, statistical analysis of these data was not feasible.

Chemotherapy regimen

In order to evaluate the influence of therapy modalities on prognosis, we divided the 46 patients into three groups: the first (group 1) comprised 13 patients who did not receive any chemotherapy in addition to tumour resection. 2 of these patients had a pT2, 4 a pT3, 6 a pT4 tumour and 1 was unknown (Table 2). The second group (group 2) comprised 10 patients treated with surgery and chemotherapy from 1977 to 1987 as described above: 2 received chemotherapy with vincristine, actinomycin D and cyclophosphamide, 1 child two and 1 four courses. Chemotherapy, according to the CCG-881/POG-8301 study, was given in a total of two courses to 3, five courses to 1 and six courses to 4 children. The tumours were pT2 in 2, pT3 in 3 and pT4 in 5 patients. The third group (group 3) included the 23 patients treated according to the protocol of the study HB-89 as described above: the tumours of 9 patients were pT2, 7 pT3, 6 pT4 and 1 unknown.

DFS was 38% for patients of group 1 with only 5/13 patients being without tumour, 1 of these after a primary liver transplantation and 1 after complete resection of late lung metastasis. Four children of group 2 remained in permanent remission, 2 after resection of lung metastasis. DFS was 40% in this group. In contrast DFS was 83% for the patients of group 3 with 19 surviving with NED, one after a liver transplantation. The other 4 died from recurrent tumour after 6, 7, 10 and 22 months, respectively (Table 2). The differences of probability of DFS between groups 1 or 2, respectively, and group 3 were significant ($P = 0.005$). However, no patient in group 3 had primary lymph node or distant metastasis, and the rate of advanced tumours was higher in groups 1 and 2, since in former years, most patients were referred to our institution especially because of advanced tumour. Considering this fact, the above-mentioned difference loses significance.

Surgical procedures and R categories

In 4 patients, the tumour could be resected with a wedge resection, in 13 a hemihepatectomy, and in 21 a trisegmentectomy was necessary. A liver transplantation was performed in 3 cases. In 5 children the tumour was only biopsied because of advanced disease. DFS was 100% after wedge resection, 83% after a hemihepatectomy, 60% after a trisegmentectomy and 67% after a liver transplantation. All patients without a tumour resection died. In 33 patients, the tumour could be completely removed (category R0), and 26 (79%) of these survived without tumour. In contrast, only 2 of 4 patients with microscopic residue (category R1) survived, while all children with gross residual tumour (category R2) died ($n = 9$). The overall rate of DFS of patients with residual tumour (R1 and R2) was 15% (2 of 13 patients). The correlation of R categories with probability of DFS was highly significant ($P = 0.0001$) (Figure 1).

pTNM status

None of the evaluable 44 HBs were pT1, 13 were T2, 14 were pT3 and 17 were pT4. 2 patients had metastasis of the hilar

Table 2. Relationship of pT status, therapy regimens and patients' long-term outcome in hepatoblastoma. The correlations between patients with NED and pT status (P = 0.0007) *and therapy regimen* (P = 0.005), *respectively, were significant*

	No chemotherapy $n = 13$	Regimens 1977–1987 $n = 10$	Study HB-89 $n = 23$	NED (pT status)
pT2 ($n = 13$)	2	2	9	13 (100%)
pT3 ($n = 14$)	4	3	7	9 (64%)
pT4 ($n = 17$)	6	5	6	5 (29%)
Unknown ($n = 2$)	1		1	
NED (therapy)	5 (38%)	4 (40%)	19 (83%)	

NED, no evidence of disease.

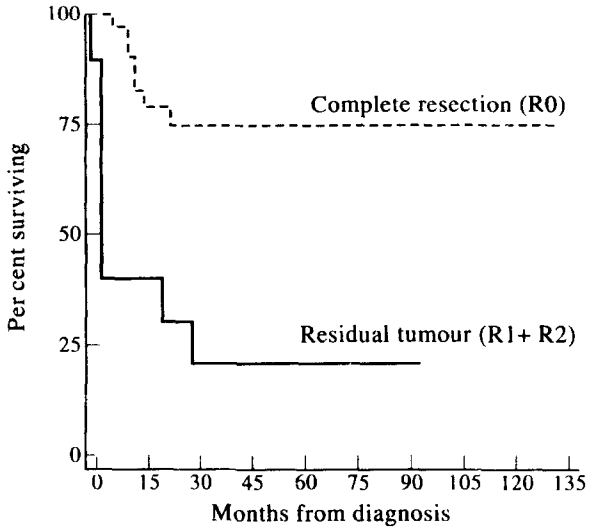

Figure 1. Correlation of results of surgical resection of hepatoblastomas (R categories) with Kaplan–Meier probability of disease-free survival. The difference between the curves for patients with complete tumour resection (R0) and residual tumour (R1 and R2) was significant ($P = 0.0001$), $n = 46$.

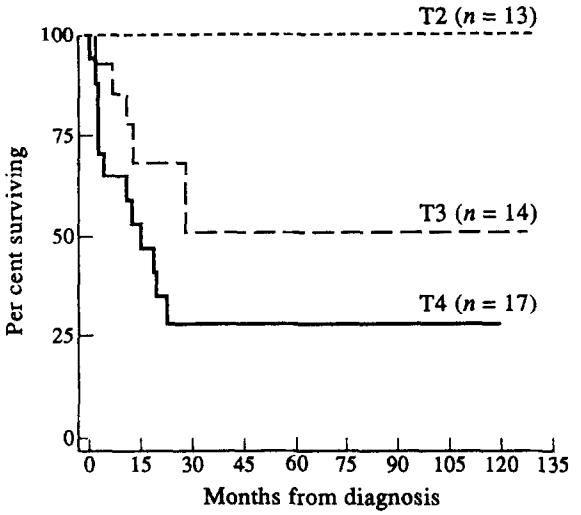

Figure 2. Kaplan–Meier probability of disease-free survival of hepatoblastoma patients relative to pT status of tumours as proposed by the International Union Against Cancer (UICC) for hepatocellular carcinoma. The differences between the curves 1 or 2 and 3 were significant ($P = 0.0007$), $n = 46$.

lymph nodes (N1) and 4 had distant metastasis (M1) at diagnosis; these 6 had a pT4 tumour. All children with a pT2 HB survived NED, while prognosis was worse in patients with a pT3 tumour (64% DFS), and poor in those with a pT4 tumour (29% DFS) (Table 2, Figure 2). These differences were highly significant ($P = 0.0007$). Median patient survival was 34 months (range 15–78) with pT2 tumours, 26 months (range 2–119) in pT3, and 10 months (range 0.5–129) with pT4 HBs.

Examination of the four tumour characteristics integrated into the T status for their prognostic value revealed the following results:

Tumour sizes. The size of 26 evaluable tumours varied from 64 to 1209 cm^3 with an average of 454.4 cm^3. Mean size of the tumours of 18 long-term survivors was 378.7 cm^3 (range 65–1209), in contrast to a mean size of 499.1 cm^3 (range 151–904) of the tumours of the 8 patients who died. The correlation between tumour size and outcome of disease was non-significant ($P > 0.05$).

Tumour involvement of liver lobes. Twelve HBs were confined to the right lobe of the liver, four to the left and 30 extended into both lobes. Three of the latter were located in the liver hilus. 2 of 16 patients with a tumour confined to one lobe had a poor outcome, while 16 of 30 patients with tumour extension over both lobes died, including 2 patients with tumour in the liver hilus. This difference was significant ($P = 0.004$).

Multiplicity of tumour nodes. Thirty-seven HBs were unifocal, with only one tumour node in the liver, 9 appeared with multiple, separated nodes disseminated throughout the liver. Patients with an unifocal tumour had a significantly better prognosis (27 NED, 10 died) than those with a multifocal HB (one NED, 8 died) ($P = 0.001$).

Tumour invasion of vessels. In 22 of 33 evaluable HBs, vascular invasion by the tumour was found at histological examination and in 8 cases also macroscopically at operation. Vessel invasion indicated a poor prognosis, with a DFS of 25% compared with 100% in patients without this finding. The difference was highly significant ($P = 0.0006$).

Histology

Pre-treatment tumour specimens from 37 patients were available for histological review: 29 HBs were epithelial and 8 mixed epithelial and mesenchymal, with no prognostic difference between these groups. Epithelial areas were composed purely of fetal cells in 10 tumours and of embryonal cells in 3. In 24 cases, both cell types were found in different growth patterns. Anaplastic histology was not encountered. There was no significant correlation with the pTNM status ($P = 0.08$, Table 3).

Table 3. Relationship of histological subtypes of untreated hepatoblastomas with NOR quotient, pTNM status and patients' outcome

Subtype	NOR quotient	pTNM status T2	T3	T4	N1	M1	NED
Pure fetal ($n = 10$)	9.47		3	1			9 (90%)
Fetal and embryonal ($n = 24$)	12.61	6	10	10	2	4	13 (54%)
Predominant embryonal ($n = 3$)	20.81	4	1	2			1 (33%)

NOR, nuclear organiser region; NED, no evidence of disease.

DFS was 90% for children with a pure fetal, 54% for those with a fetal and embryonal, and 33% for those with an embryonal tumour (Table 3). These differences were non-significant ($P = 0.09$). Of the nine HBs examined after chemotherapy, five were epithelial and four were mixed epithelial and mesenchymal. There was no correlation of tumour cell differentiation and outcome in these tumours: 2 of 3 patients with a pure fetal, and 3 of 6 with a fetal and embryonal tumour survived disease-free.

NOR staining

The analysis of staining for NORs in 33 not pretreated HBs revealed a mean NOR size of 0.38 μm^2 (range 0.22–0.64 μm^2) and a mean number of 4.38 NORs per nucleus (range 3.08–6.60). We found a linear inverse correlation between NOR number and size (correlation coefficient −0.56, $P < 0.001$), which allowed the application of the NOR quotient for further analysis. The mean NOR quotient for all tumours was 12.59 (range 5.63–24.69). There was a significant difference ($P = 0.0009$) between pure fetal HBs ($n = 8$), with a low mean NOR quotient (9.47), and embryonal HBs ($n = 3$; mean NOR quotient 20.81). Fetal and embryonal tumours ($n = 22$) had a mean value of 12.61 ($P = 0.004$, Table 3). We also found a correlation of pT status and NOR expression: pT2 tumours ($n = 8$) had a mean NOR quotient of 10.15, while this was 12.67 for pT3 ($n = 13$) and 14.67 for pT4 ($n = 12$) ($P = 0.03$). HBs with a NOR quotient ≤ 12 ($n = 18$) were associated with a significantly ($P = 0.05$) better chance of DFS (72% NED) than those with a quotient > 12 ($n = 15$, 47% NED; Figure 3).

Multivariate analysis

In multivariate analysis of 22 cases, we determined R categories and pT status to be independent prognostic criteria with significance ($P < 0.005$), while this was not the case for chemotherapy modality, histological subtype and NOR quotient. The results of statistical analyses of the investigated prognostic criteria are summarised in Table 4.

DISCUSSION

Hepatoblastoma (HB) is the most important liver tumour in young children. The prognosis of these patients has been dismal because, in institutions without experience in the treatment of this rare tumour, it often caused surgical problems, and efficient chemotherapy had not been introduced. Recently, therapy results improved, when more efficient chemotherapy regimens and surgical strategies were introduced in different cooperative studies. The results of these studies are, in part, still preliminary [2–5], and do not allow the accurate prediction of the clinical course of an individual patient. There have been only a few attempts to determine prognostic factors in HB. We, therefore, searched for objective clinical and histological criteria, which could serve as prognostic factors for an individual patient, and also be the basis for a widely accepted staging system, which should be independent of therapy regimens.

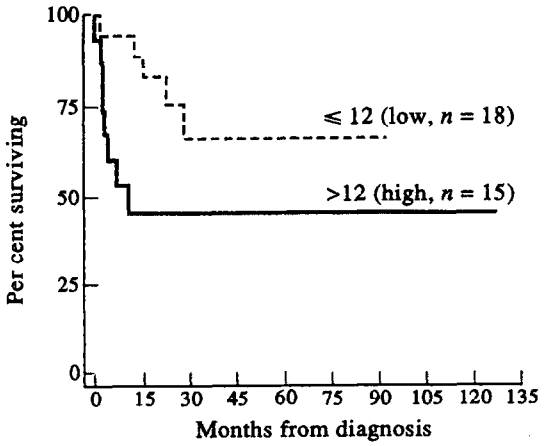

Figure 3. Correlation between the expression of nuclear organiser regions (NOR quotient) in hepatoblastoma and Kaplan–Meier probability of patients' disease-free survival. The difference between tumours with an NOR quotient ≤ 12 (low) and > 12 (high) was significant ($P = 0.05$), $n = 33$.

Table 4. Prognostic significance of clinical and histological criteria in hepatoblastoma

Criterium	Analysis	
	Univariate	Multivariate
Patients' age (K-M)	$P > 0.05$	Not tested
Serum α-fetoprotein (F)	$P > 0.05$	Not tested
Chemotherapy (K-M)	$P = 0.005$	$P > 0.05$
R category (K-M)	$P = 0.0001$	$P = 0.04$
pT status (K-M)	$P = 0.0007$	$P = 0.001$
Tumour size (F)	$P > 0.05$	Not tested
Involvements of lobes (F)	$P = 0.04$	Not tested
Multiplicity of nodes (F)	$P = 0.01$	Not tested
Vascular invasion (K-M)	$P = 0.0006$	Not tested
Histological subtype (K-M)	$P > 0.05$	$P > 0.05$
NOR expression (K-M)	$P = 0.05$	$P > 0.05$

K-M, probability of disease-free survival (Kaplan and Meier [18]); F, determination with Fisher's exact test; NOR, nuclear organiser regions.

In a retrospective study on the data of the Japanese National Pediatric Tumour Registry, Hata [8] found that young children with an HB have a better prognosis than older ones. We observed the same relationship in our patients, with the greatest difference between children younger and older than 15 months, yet this was statistically non-significant. We could not find a correlation between patients' age and a predominant histological subtype or tumour stage.

AFP is well known to be produced by many HBs [1]. Therefore it is a valuable tumour marker for monitoring the course of an individual disease [4, 19]. However, in our series, the initial level of this marker did not correlate with the extent of tumour nor with patients' prognosis. Children with undifferentiated, anaplastic HB have a poor prognosis, despite normal serum AFP [20]. Nevertheless, if the AFP is initially elevated, the rate of decrease and the return to normal values is an indicator for the effect of therapy [2–5, 8, 19].

Treatment results of patients with an HB are, to date, satisfying in the German Cooperative Pediatric Liver Tumour Study HB-89, with an overall remission rate of 81% [4]. This includes the 23 patients in our study treated according to the HB-89 protocol. They had a better outcome in comparison with the patients treated in former years (Table 2), although this was non-significant when the initial extension of tumours was taken into consideration. Calculation of DFS of the three patient groups suggested that chemotherapy in former years did not contribute very much to cure rates. However, the drugs used in the above-described therapy regimes (vincristine, cyclophosphamide, actinomycin D, doxorubicin, 5-fluorouracil and bleomycin) are all known to be effective in HB [5, 14]. It has to be emphasised that, before 1988, we used them only for adjuvant

therapy after tumour resections [15]. In the study HB-89, a modified surgery strategy and chemotherapy with ifosfamide, cisplatin and doxorubicin (IPA) was introduced [4, 15]. Comparison of the two regimes indicates that chemotherapy has its greatest benefit when administered before surgery, and is less effective if given exclusively after liver resection [2, 3, 5].

It is noteworthy in this context that patients with an incomplete tumour resection had a significantly worse outcome than those with a microscopically complete excision of all tumour (Figure 1). Children with gross residual tumour do not seem to have a chance of final cure [15]. This is especially true for children with an insufficient tumour resection because of multifocal nodes [4]. In these cases, a liver transplantation can be of benefit as long as the disease is restricted to the liver [21]. Therefore, the R categories, as they are specified in the TNM classification [11] are suitably applicable to HB. As shown in multivariate analysis, they have an independent prognostic significance and should be documented postoperatively.

In an effort to find a staging system for HB, which is objective and independent of any treatment modality, we investigated the prognostic relevance of the pTNM system, as it is proposed by the International Union Against Cancer (UICC) for liver carcinoma [11]. It contains elements, which themselves promised to have a prognostic value. Our results showed that the pT status correlated with prognosis in HB (Table 2, Figure 2), and retained its significance in multivariate analysis (Table 4). This also applied for the N and M status, since no patient with primary lymph node or distant metastases survived. In order to determine the value of the single elements, which amount to the pT status, we correlated these with the patients' prognosis. The extension of tumour, especially whether it affects one or both liver lobes, has a significant influence on patients' outcome because of surgical reasons [4]. In contrast, the size of an HB (in cm^3) does not have prognostic relevance. Large tumours growing in the periphery of the liver might be easily removable, while small tumours in the hilar region or at the venous confluence are often unresectable and, therefore, associated with a poor prognosis. Multiplicity of tumour nodes has a significant prognostic relevance in HB. Multifocal HB is almost always associated with an unfavourable outcome [4]. We also found vascular invasion to be correlated with prognosis. This is true for macroscopic invasion, which also means T4 status. It can often be documented pre-operatively by sonography or angiography [22], and should be noticed during laparotomy. Microscopic vascular invasion also results in a reduced chance of cure and, therefore, has to be searched for at histological examination [23]. In conclusion, the pTNM system seems applicable for childhood HB, although tumour size could be omitted as an element of the T status. The proposal of the Japanese National Cancer Society to score the number of affected liver segments [24] does not seem feasible. In our patients, this was not a prognostic factor (data not shown), since only the involvement of central or peripheral segments was important for resectability.

Investigations on the prognostic significance of histological subtypes in HB [12] on large collectives of patients [6, 7, 23] showed that low stage HBs with a pure fetal histology have a good prognosis. Our analysis confirmed the relationship between epithelial differentiation of HB and prognosis (Table 3). However, as in the former studies, this was statistically non-significant. Nevertheless, it seems appropriate to integrate tumour differentiation into the pTNM classification. This is possible by assigning differentiation of the predominant epithelial component to G grades of the histopathological grading system [11]. Pure fetal histology should be categorised as G1 (well differentiated), fetal and embryonal as G2 (moderately differentiated), embryonal as G3 (poorly differentiated) and anaplastic as G4 (undifferentiated).

The expression of NORs, as measured by the quotient of their number per nucleus and their mean size [16], indicates the proliferation activity of tumour cells in HB. The NOR quotient was clearly related to differentiation of tumour cells (Table 3). Recently this was also shown for other malignancies [25, 26]. It is remarkable that the expression of NORs in HB is significantly correlated with prognosis (Figure 3), regardless of initial tumour extension or treatment modality, as was also found in neuroblastoma [27] and carcinomas of the sigmoid and rectum [26]. Staining for NORs is easy to perform and more precise than counting mitosis on conventional histological slides [23]. It can, therefore, contribute to prediction of prognosis of an individual HB patient. The NOR quotient can also be integrated into the histological G grading of the pTNM system [11], by defining groups with different values corresponding with grades G1 to G4.

Our results show that there indeed exist relevant prognostic clinical and pathological criteria in HB. They can sufficiently be integrated into the pTNM classification system, including R categories and a G grading. This classification is objective and independent of treatment modalities, and applies for many other malignancies. Therefore, it is ideal for comparison of treatment results of different cooperative studies on HB. It is superior to the usual postsurgical staging [2, 4, 5] and to the grouping system of the International Pediatric Liver Tumour Study of the SIOP (SIOPEL-1) [28], particularly since the latter has not been investigated on its prognostic reliability. We propose the application of the pTNM classification in future cooperative trials on HB.

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Acknowledgements—We gratefully acknowledge the kind assistance of Dr H. Hecker (Department of Biometrics, Medical School Hannover) in statistical analysis, and deeply thank the following colleagues for providing clinical data of their patients: Dr U. Bode (Bonn); Dr H. Breu (Dortmund); Dr R. Dyckerhoff (St. Augustin); Dr U. Göbel (Düsseldorf); Dr P. Gutjahr (Mainz); Dr W. Havers (Essen); Dr A. Jobke (Nürnberg); Dr J. Kühl (Würzburg); Dr R. Ludwig (Heidelberg); Dr P. Neuhaus (Berlin); Dr J. Ritter (Münster); Dr M. Rose (Minden); Dr W. Schröter (Göttingen); Dr W Sternschulte (Köln); Dr C. Tautz (Herdecke); Dr J.-H. Thaben (Coburg); Dr P. Weinel (Hannover); Dr M. Wright (Kassel) and Dr G.F. Wündisch (Bayreuth).

Pergamon

European Journal of Cancer Vol. 30A, No. 8, pp. 1058–1060, 1994
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00+0.00

0959-8049(93)00198-7

Activity of Docetaxel (Taxotere) in Small Cell Lung Cancer

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Docetaxel (Taxotere) is a new cytotoxic compound with a broad spectrum of activity in preclinical studies. This paper reports a phase II trial in patients with previously-treated small cell carcinoma of the lung. 34 patients received 100 mg/m² of docetaxel in an intravenous infusion given over 1 h every 21 days. Seven partial responses were reported (25% of 28 evaluable patients). Duration of response was 3.5–12.6 months. Toxicities were predominantly neutropenia, alopecia and asthenia. Docetaxel is a new compound with activity in previously-treated patients with small cell lung cancer, and is suitable for evaluation in combination with other cytotoxic drugs active in this disease.

Key words: phase II trial, small cell lung cancer, docetaxel
Eur J Cancer, Vol. 30A, No. 8, pp. 1058–1060, 1994

INTRODUCTION

DESPITE THE proven sensitivity of small cell carcinoma of the bronchus to a wide range of cytotoxic drugs and to radiation, progress in long-term control remains elusive, and there is an obvious requirement for new systemic treatments. Docetaxel is a new hemi-synthetic taxoid prepared from the needles of *Taxus baccata*, the European yew tree [1]. Docetaxel's mechanism of cytotoxic activity is thought to be due to enhanced tubulin assembly into microtubules and inhibition of the depolymerisation of microtubules [1, 2]. This leads to cell cycle arrest in