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Hepatoblastoma with a low serum alpha-fetoprotein level at diagnosis: The SIOPEL group experience

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ABSTRACT

Aim of the study: To investigate the characteristics of patients with hepatoblastoma and low serum alpha-fetoprotein (AFP) at diagnosis.
Patients and methods: Inclusion of all 21 patients accrued onto SIOPEL trials, whose serum AFP was <100 ng/ml at diagnosis. Slides of all 15 patients with available histological material were centrally reviewed.
Results: Median age: 10 months. Disease extension at diagnosis: PRETEXT group: II (3 patients), III (10 patients) and IV (8 patients). Extra-hepatic extension: 8 patients. Multifocal tumour: 8 patients. Histology at review: wholly epithelial subtype: 11/15 patients including nine with a small-cell undifferentiated histology. Outcome: only 9 patients achieved a partial response and 16 died. Median survival: 4.4 months. Two-year overall survival: 24% (confidence interval 10–45%).
Conclusion: This study clearly identifies patients with hepatoblastoma and low serum AFP at diagnosis as a high-risk subgroup with extensive disease at diagnosis, poor response to chemotherapy and a poor outcome.

1. Introduction

Hepatoblastoma (HB) is the most common primary liver tumour of childhood but accounts for less than 1% of all paediatric tumours.[1]

The introduction of cisplatin into treatment in the early 1980s dramatically improved the prognosis for most children with HB and survival rates have risen to at least 70% in most recent large series.[2–5] The prognosis mainly depends on disease extension at the time of diagnosis and the completeness

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of surgical resection. However, the histological subtype and serum alpha-fetoprotein (AFP) level at diagnosis have also been shown to impact the outcome.[6,7] The serum AFP concentration is markedly elevated in most patients at diagnosis, but unexpectedly low or even normal AFP values are reported in around 5–10% of cases. Due to the rarity of HBs with a low AFP level at diagnosis, very little is known about the biological, histological and clinical profile of such HB.

Von Schweinitz et al. were the first to point out that a serum AFP level below 100 ng/ml could be a negative prognostic factor in children suffering from HB.[2,6] A retrospective analysis of patients enrolled in the first two trials run by the International Childhood Liver Tumour Study Group – the SIOPEL group – (SIOPEL trials 1 and 2) seemed to support this finding.[7] This resulted in an addendum to the SIOPEL 3 protocol recommending that future patients with HB and a low level of AFP at diagnosis should be categorised as high-risk regardless to the extent of the disease at diagnosis, and should be treated with more aggressive chemotherapy. The aim of this work was to investigate the clinical characteristics, histology and outcome of patients with HB and a low AFP level who have been accrued onto SIOPEL 1, 2 and 3.

2. Patients and methods

The study population consisted of patients enrolled onto the SIOPEL 1, 2 and 3 trials, whose serum AFP concentration was <100 ng/ml at diagnosis. This cut-off value for serum AFP was based on Van Tornout's[8] and Von Schweinitz's criteria.[2] The SIOPEL 1 trial opened in June 1990 and the high-risk SIOPEL 3 trial closed to patient accrual in December 2004. This series therefore spans a 14-year period. Twenty-four (2.7%) of 833 patients enrolled onto one of the three trials were reported, by the treating centre, to have an AFP level equal to or less than 100 ng/ml at diagnosis. Three patients were excluded because they had secondary elevation of serum AFP during therapy, indicating a potential false negative initial value. The 21 remaining children were therefore eligible for the analysis and represent the study population.

The clinical records of patients were reviewed. A central histological review was performed by the SIOPEL group expert pathologists (A.Z. and J.K.) for all 15 patients who had histological material available for review (a diagnostic biopsy specimen in 14 patients and a resected primary tumour in one case). Conventional histological criteria for HB classification were used, as well as the new criteria proposed by Zimmerman.[9]

SIOPEL 1, 2 and 3 trials were based on the same treatment philosophy[5] of pre-operative chemotherapy, after an initial biopsy, for all patients (regardless of initial tumour resectability), followed by delayed definitive surgery and then further post-operative chemotherapy. In all three trials, patients were classified according to the pre-treatment extent of disease on imaging, known as the PRETEXT system.[4] It was named the 'grouping system' to distinguish it from the previously used staging systems. Patients were then further classified as having extra-hepatic disease (E), venous involvement (V), portal vein involvement (P) or metastatic disease (M).

In the SIOPEL 1 study, all patients received the same chemotherapy whereas in SIOPEL 2 and 3, patients were stratified into two risk groups, the standard-risk group (PRETEXT group I, II and III without extra-hepatic extension) and the high-risk group (PRETEXT IV and/or extra-hepatic extension). In SIOPEL 3, patients with AFP <100 ng/ml were included in the high-risk group.

In the SIOPEL 1 study, chemotherapy was based on cisplatinum (80 mg/m^2) combined with doxorubicin (60 mg/m^2), the so-called 'PLADO regimen', administered every 21 d up to a total of four courses before surgery followed by two other courses after surgery. In SIOPEL 2, patients with standard-risk HB received four courses of cisplatin (80 mg/m^2) before surgery and two additional courses after surgery whereas, in SIOPEL 3 study, they were randomised to receive either PLADO or cis-platinum alone. In both SIOPEL 2 and 3, chemotherapy for high-risk patients consisted of 15 d courses of carboplatin (500 mg/m^2) and doxorubicin (as above) alternating with cisplatin (as above), later known as 'Super PLADO'. Patients received seven pre-operative courses and three post-operative courses.

In the 3 SIOPEL studies, response was defined as complete (CR) if there was no evidence of disease on imaging and serum AFP was normal for the patient's age, as partial (PR) if there was any tumour volume shrinkage on imaging and a decrease in serum AFP >1 log below the initial measurement, stable disease (SD) was defined as no change in tumour volume and <1 log drop in serum AFP concentration and progressive disease (PD) was defined as an unequivocal increase in tumour size in one or more dimensions and/or unequivocal increase of the AFP concentration. In the case of tumours with a low diagnostic serum AFP value, a decrease in serum AFP was not used to assess response.

Overall survival (OS) was defined as the time interval between the date of diagnosis and the date of death, from any cause, or the date of the last follow-up. Survival curves were estimated using the Kaplan–Meier method.[10]

All three SIOPEL studies were reviewed by an Ethics committee according to the guidelines in each participating country at the time of the study and informed consent was obtained from the parents before including patients in the study.

3. Results

Relevant pre-treatment clinical and tumour characteristics, the treatment received and the outcome of the study population are summarised in Table 1. There were six girls and 15 boys aged 3–158 months (median 10 months): 12/21 patients were 12 months old or less at diagnosis and four children were older than five at diagnosis.

All but two patients had serum AFP levels measured at least twice prior to any treatment. The median AFP value was 12 ng/ml (0.37–75 ng/ml).

In 12 children, the AFP value at diagnosis was between 10 and 75 ng/ml: nine of them were one-year-old or younger at diagnosis. In 9 patients, the AFP value at diagnosis was below 10 ng/ml: four of them were younger than one year.

All patients had a biopsy at diagnosis and a histological analysis of the tumour was also performed after resection in nine cases. The diagnosis of HB was confirmed in all the

Table 1 – Patient characteristics and histology with outcome

SS/TN	Age (months)	Sex	AFP at diagnosis (ng/ml)	PRETEXT group	Extra-hepatic extension	Tumour focality	HBL histological subtype	Chemotherapy	Response	Surgery	Status/survival
1/232	7	F	4	IV	Lung and omental metastases	U	Foetal	PLADO	PD	No	DOD (1,7 months)
3/248	7	F	0.37	IV	No	MU	Foetal	SuperPLADO	PR	Macroscopic residue	ANED (6 years+)
2/118	32	F	10.4	III	No	U	SCUD	SuperPLADO	PD	No	DOD (4,4 months)
2/221	88	M	0.8	III	No	MU	SCUD	SuperPLADO	PR	Microscopic Residue	SRD (7 months)
2/240	12	M	19.5	IV	Lung metastases	MU	SCUD	SuperPLADO	PD	No	DOD (2 months)
3/9	7	M	14	III	No	U	SCUD	PLADO	PD	No	DOD (4,5 months)
3/169	10	F	30	II	No	U	SCUD	SuperPLADO	PD	Complete excision	DOD, relapse (4,2 months)
3/224	14	M	8	III	Lung metastases and cervical and mediastinal lymph node involvement	U	SCUD	SuperPLADO	PD	No	DOD (2,1 months)
3/338	138	M	12	III	Lung metastases	U	SCUD	SuperPLADO	PD	No	DOD (2,8 months)
3/179	6	M	17	IV	Lung metastases	MU	Mixed (foetal/ SCUD/LCUD)	SuperPLADO	PD	No	DOD (0,5 months)
3/158	130	F	1	III	No	U	SCUD (unusual variant)	SuperPLADO	PR	Microscopic residue	DOD, relapse (13 months)
3/143	8	M	3	III	No	U	MEM	SuperPLADO	PR	Microscopic residue	SRD (6,1 months)
1/101	158	M	2	III	No	U	PHST	PLADO	PR	Macroscopic residue	ANED (5 years+)
1/43	6	M	75	IV	No	U	HB NOS	PLADO	PD	No	DOD (1,3 months)
3/144	7	M	19	III	Para-aortic lymph nodes and intracardiac extension	MU	HB NOS	SuperPLADO	PD	No	DOD (2,4 months)
1/195	24	M	39	II	Abdominal node involvement	U	NCR	PLADO	PR	No	AWD (2 years+)
1/065	21	M	41	II	No	U	NCR	PLADO	PR	Complete excision	ANED (5 years+)
1/239	4	M	14	IV	Abdominal node involvement	MU	NCR	SuperPLADO	PD	No	DOD (0,5 months)
2/269	8	M	12	IV	No	MU	NCR	SuperPLADO	PR	Macroscopic residue	DOD (9 months)
3/388	39	M	3	IV	No	MU	NCR	SuperPLADO	PD	No	DOD (2 months)
3/481	3	F	5	III	No	U	NCR	SuperPLADO	PR	Complete excision	ANED (3 years+)

Abbreviation: SS/TN SIOPEL, Study/Trial number; M, male; F, female; MU, multifocal; U, unifocal; SCUD, small-cell undifferentiated; LCUD, large cell undifferentiated; MEM, mixed epithelial and mesenchymal; PHST, paediatric hepatic stromal tumour; HB NOS, hepatoblastoma, not otherwise specified; NCR, no central review; DOD, died of disease; ANED, alive with no evidence of disease; SRD, surgery-related death and AWD, alive with disease.

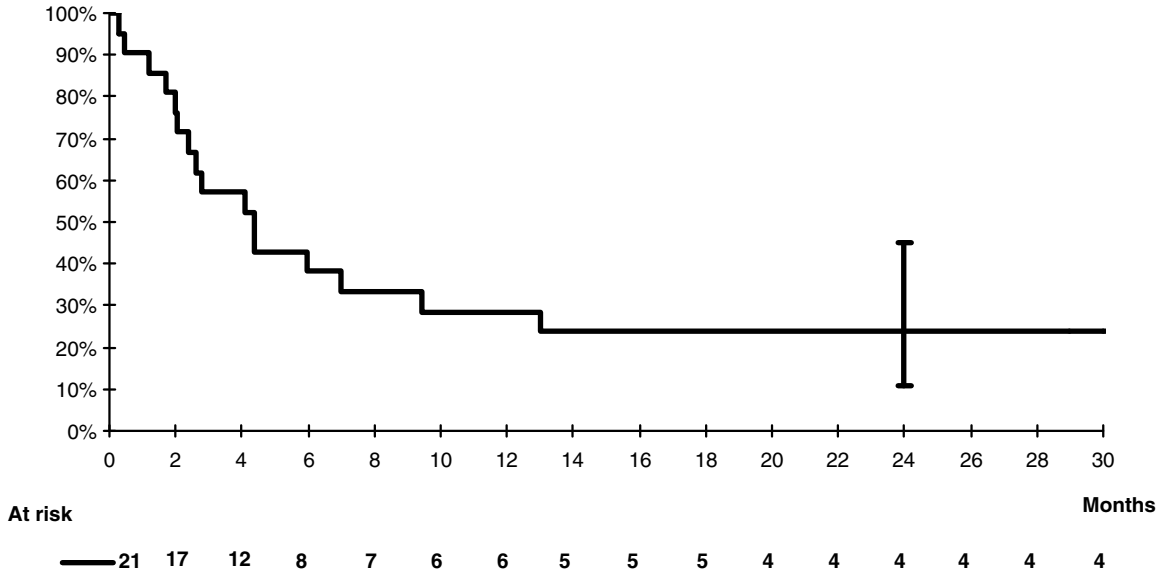

Fig. 1 – Survival curve of 21 patients with HB and AFP level <100 ng/ml at diagnosis.

15 cases which were centrally reviewed. However in six cases, there was a discrepancy between the subtype given by the local pathologist and the subtype given by the reference pathologist at review. Among the 15 cases reviewed, 2 patients were of the pure foetal histology. Nine patients had a partial or a predominantly small-cell undifferentiated histology. Two patients had a stromal–epithelial subtype (mixed epithelial and mesenchymal type in 1 patient and paediatric hepatic stromal tumour[9] in the other). Two tumours could not be further characterised and are therefore classified as 'HB not otherwise specified' – NOS. None of these cases had a rhabdoïd histology.

According to the PRETEXT grouping system, no tumour was PRETEXT I, three were PRETEXT II, 10 were PRETEXT III and eight were PRETEXT IV. The tumours were unifocal in 13 patients and multifocal in eight. Four patients had vascular involvement (hepatic vein (V+) involvement in 4 patients 2 of whom also had a portal vein (P+) involvement). Extra-hepatic loco-regional tumour extension was diagnosed in 4 patients (E+) with abdominal lymph node enlargement in 3 patients and omental spread in the other. Metastases (M+) were present in five children: they all had lung metastases and one also had mediastinal and cervical lymph node involvement.

All patients received pre-operative chemotherapy according to the contemporary SIOPEL trial. Only nine achieved a partial response (PR). The other 12 patients, all with unresectable disease, experienced early tumour progression (PD), in most cases within 15 weeks of starting chemotherapy. In 10 patients, including five with no extra-hepatic extension at diagnosis, primary PD was associated with the progression of extra-hepatic disease including lung metastases in nine and peritoneal implants in 1 patient. Delayed surgery was attempted in 9 patients (8 with a PR, after initial chemotherapy, and in 1 child with PD who required emergency surgery because the tumour was bleeding into the abdomen). All patients underwent a partial hepatectomy. Resection was microscopically complete in only 3 children and incomplete in six including 3 patients with microscopic residual disease and three with a macroscopic residual mass. Two patients died of surgery-related complications (haemoperitoneum and multiple organ failure, respectively). Post-operative chemotherapy was administered to 6 patients according to the relevant SIOPEL trial, whereas 1 patient received post-operative radiotherapy delivered to macroscopic residual disease and no chemotherapy. Three early relapses occurred at 4, 7 and 12 months after the diagnosis including two local relapses and a recurrent disease at both local and distant sites in 1 patient. After relapse or progression, second-line chemotherapy was attempted in 8 patients with various drugs including high-dose cyclophosphamide in two and irinotecan in one but none of them responded.

Overall, only 4/21 patients are alive without the evidence of disease and a median follow-up of 42 months since the diagnosis: 1 patient was alive with unresectable disease at the last follow-up and 16 children had died (11 during pre-surgical chemotherapy, two as a result of surgical complications and three after an early relapse). The two-year OS is 24% (confidence interval (CI): 10–45%) as shown in Fig. 1. Median survival was only 4.4 months (range 0.5 month–9 year).

4. Discussion

This study clearly identified children with HB and a low AFP level at diagnosis as a small subgroup of high-risk HB patients with more extensive disease at diagnosis and a high-risk of treatment failure.

Only 2.7% of all children enrolled onto the three consecutive trials run by the SIOPEL group in the 14-year period from 1990 to 2004, presented with a serum AFP value below 100 ng/ml at diagnosis. This contrasts to the incidence reported in other series. Von Schweinitz et al.[2] reported 4/37 (11%) cases

Table 2 – Comparison of clinical characteristics and outcome of patients included in this study with the entire population of patients in SIOPEL 1 and 2

	This study	SIOPEL 1	SIOPEL 2
Median age (months)	10	16.5	18
Male (%)	71	63	54
PRETEXT group III and IV (%)	79	54	51
Presence of P, V or E (%)	38	10	9
Multifocality (%)	38	14	20
Metastases (%)	23	20	17
Partial response to pre-operative CT (%)	42	82	96 (SR) 78 (HR)
3-year overall survival (%)	24	75	91 (SR) 53 (HR)

of 'low AFP' HB and Fuchs et al.[11] 7/69 (10.1%) patients with this subtype. Ortega et al.[12] reported 5/45 (11%) patients with a low AFP level, although their series included patients with hepatocellular carcinoma (HCC) as well as HB. In our series, possible false negative results were carefully excluded: 3 patients who presented with a low AFP value but who later exhibited a secondary rise in serum AFP were intentionally excluded. The presence of extremely high serum AFP concentration in some HB patients can generate erroneously low AFP results. This effect named 'Hook effect' is a well-recognised problem that can occur in assays of most tumour markers, including AFP.[13]

The diagnosis of HB was confirmed by the reference pathologist for all the cases submitted to central review. Due to the rarity of this entity, we decided to keep in the analysis the six cases whose histology has not been centrally reviewed in the present study and for whom the subtype had not been specified by the reference pathologist. The characteristics of those six cases were very similar to those of the 15 cases with histological material available for review.

Although patients with HB with a low AFP level at diagnosis represent a heterogeneous group, they share some characteristics which are very different from classic HB (Table 2).

The median age at diagnosis of the study population was 10 months, which is much lower than that reported for the overall population of children enrolled in the SIOPEL 1, 2 and 3 trials. The median age of the children enrolled in the SIOPEL 1, 2 and 3 trials was 16.5 months in SIOPEL 1[3] and 18 months in SIOPEL 2 and 3.

However, 'low AFP' HB does not occur exclusively in very young children. In fact, 4 patients in our series were older than 5 years at diagnosis (7, 10, 11 and 13-years-old, respectively). We cannot compare the 'very young' and 'older' children presenting with 'low AFP' HB, because the series is too small. It is nonetheless noteworthy that 3/4 'older' patients had a tumour confined to the liver and that two of them had a SCUD histology.

The predominance of boys is higher in this series than usually reported in the literature.[14] In this series of patients, the male/female ratio is 2.5/1 whereas it is 1.7/1 in SIOPEL 1 and 1.1/1 in SIOPEL 2.

Children suffering from HB with a low AFP level at diagnosis seem to present with extensive local and/or metastatic disease, more frequently than the other children. All but 3 of the patients in our series had a PRETEXT III or IV tumour, while in the SIOPEL 1 and 2 trials, only 54% and 51%, respectively, presented with advanced intra-hepatic disease.[3,5,7] More than a third of them also had multifocal disease, a finding which contrasts with the fact that tumour multifocality was reported in only 14% of the entire population of children enrolled in SIOPEL 1[7] and less than 20% in SIOPEL 2.[5] Eight children (38%) in this group of patients had evidence either of main portal vein invasion (P+) and/or involvement of all three hepatic veins (V+) and/or of extra-hepatic intra-abdominal tumour extension (E+). In SIOPEL 1 and 2, the corresponding figures for P, V and/or E positivity were 10% and 9%, respectively.[3,5] Two children in this series also presented with enlarged lymph nodes, as well as omental and peritoneal tumour seedings, findings that have not been reported so far in any other patients enrolled in the SIOPEL trials. Finally, 5 patients had lung metastases at diagnosis. This incidence of lung dissemination is higher than that reported in the general population of children with HB in which it accounts for 10–20% of the cases.[5,12] This tendency to disseminate was also obvious at relapse since in 14 patients in whom disease progressed or relapsed, 11 had hepatic and extra-hepatic relapses. This contrasts with the events described in the previous SIOPEL studies where most events were local progressions.[4,5]

In our series, the most striking feature is the high proportion of patients with a poor outcome. More than 50% of the patients experienced PD during chemotherapy and only 9 patients achieved a partial response. This 42% response rate markedly differs from the overall response rate of 82% reported in the SIOPEL 1 trial[3] and from the 96% and 78% response rates reported in the SIOPEL 2, Standard-risk and high-risk studies, respectively.[5] This poor response portended the very dismal OS observed, which was less than 24% at 2 years, with a median survival of 4.2 months. Orthotopic liver transplantation cannot be an option for these patients due to the very fast growing pattern of these tumours and the high proportion of patients with extra-hepatic disease.

The histological review revealed that these HB tumours were heterogeneous. The most important finding was the presence of the partial or predominant small-cell undifferentiated subtype (SCUD) in 9/15 (60%). This figure is much higher than that reported in other larger HB series where it ranges from 7.5% to 15%.[15,16] The young age at presentation and

the dismal prognosis seem to be two clinical features shared by the group of children presenting with HB with a low AFP level or the SCUD histology. In the series of 16 patients with a completely resected SCUD HB, reported by Haas et al.,[17] the average age was 11 months and six of them (37%) died of their disease at a median time of 5 months since the diagnosis and the recurrence rate was 63%. The authors did not provide any other data regarding the clinical profile of these patients allowing us to compare the SCUD HB they described and ours. The association of a low AFP serum concentration at diagnosis, SCUD histology and a worse prognosis has already been reported in smaller series.[15–17] In our study, an association between SCUD histology and low AFP is fairly convincing.

The data reported here clearly show that other histological variants of HB can present with a low AFP level. Indeed, in this series, there were two pure foetal HB, two HB of the stromal–epithelial subtype (mixed epithelial and mesenchymal type in one and a paediatric hepatic stromal tumour in the other).[9,11] Two other tumours could not be further classified and were thus considered 'HB-NOS'. As most of the histological material available for central review was obtained exclusively from the biopsy specimen and as the SCUD component of the neoplasm may be visible only in some but not all the tumour sections, according to the report by Haas et al. on SCUD HB,[17] the true incidence of a SCUD component may be underestimated in this series. Further investigations are needed to understand the spectrum of HB histology potentially presenting with a low AFP value and the actual relationship between this group of children and those affected by SCUD HB. Molecular biology studies are warranted to further classify those tumours which might prove to be distinct from HB with a different molecular signature and gene profile. For the time being, it can be firmly stated that patients presenting concurrently with a low serum AFP level and SCUD histology are at high-risk of treatment failure. The only 4 patients in this series, who are alive without disease, did not have a SCUD component in their tumour.

In conclusion, HB with a low AFP level are a rare subset of hepatic tumours. Even though they are heterogeneous tumours with different histological subtypes, they nonetheless exhibit some common characteristics such as widespread disease at presentation, chemoresistance and a poor outcome, which signal intrinsically aggressive genetic and biological tumour profiles. Clearly more investigations and large-scale cooperation among groups involved in HB research are warranted to further improve our understanding of these heterogeneous tumours.

Conflict of interest statement

None declared.

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