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Prospective Evaluation of Circulating Hepatocytes by Alpha-Fetoprotein mRNA in Humans During Liver Surgery

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Objective

The objective of this study was to analyze the specificity of detecting liver tumor cell dissemination by alpha-fetoprotein (AFP) mRNA in peripheral blood.

Summary Background Data

Alpha-fetoprotein mRNA has been used for the detection of circulating micrometastatic tumor foci of hepatocellular carcinoma (HCC); however, the interpretation of the results has been equivocal.

Methods

Sixty-four consecutive patients with malignant HCC (n = 20), liver metastases (n = 27), or nonmalignant (n = 17) liver diseases undergoing partial or total hepatectomy and orthotopic liver transplantation were included in this prospective study from January to July 1995. Peripheral blood samples were obtained before surgery, during surgery, and after surgery (range, 6–15 months). Total mRNA was extracted from nucleated cells, and cDNA synthesis and polymerase chain reaction amplification (nested polymerase chain reaction in one tube) were performed with specific AFP primers.

Results

Preoperative AFP mRNA was detected in 20 patients (17%), of which 5 of 20 had HCC. Intraoperative assessment showed positive AFP mRNA values in a total of 34 patients (53%) with various causes, of which 8 of 20 (40%) had HCC, 17 of 27 (63%) had other malignancies, and 9 of 17 (53%) had nonmalignant diseases. Recurrent tumor in patients with HCC occurred in four cases after surgery (range, 6–15 months) and did not correlate with AFP mRNA positivity before surgery, during surgery, or after surgery.

Conclusions

Alpha-fetoprotein mRNA in peripheral blood is not a specific marker of circulating micrometastases from HCC, especially in the context of surgical treatment of HCC.

The result with both orthotopic liver transplantation (OLT) and hepatic resection with the intent to cure patients with hepatocellular carcinoma (HCC) has been dismal because of the high rate of recurrence despite thorough preoperative screening for extrahepatic disease.[1,2] This high incidence of recurrence indicates that malignant hepatocytes had entered the circulation either before or during surgery, therefore, arguing for some type of adjuvant chemotherapy when surgery is required. Detection of small numbers of circulating cells and examination of tumor markers have advanced with the refinement of molecular biology techniques. These advances have been popularized more recently with attempts to detect circulating tumor cells before dissemination is clinically apparent.[3-10] Two markers for circulating tumoral liver cells have been proposed. One involves the albumin mRNA[11-13] and, more recently, the other involves the presence of AFP mRNA in peripheral blood, which has been reported to be more specific in patients with HCC.[14-18] Particularly, these markers have been positive in patients with a high grade of HCC or with extrahepatic metastases.[15,18] However, the accuracy of this "HCC-specific" gene transcript has yet to be determined.

Equivocal interpretations from the previous studies concerning markers for circulating HCC cells prompted our group to initiate a prospective study of AFP mRNA specificity in a large group of consecutive patients undergoing either partial or total hepatectomy and OLT for various tumoral and nontumoral liver diseases. Blood samples were taken before and at two different timepoints during surgery (during the exploratory phase and after hepatectomy) to elucidate the status of AFP mRNA in the circulation of patients with malignant or nonmalignant liver disease during the course of liver surgery. We hypothesized that liver surgery would be associated with a release of hepatocytes into the bloodstream that could be detected using AFP mRNA as the target gene transcript, therefore, questioning its potential for clinical use regarding therapeutic decisions such as intraoperative chemotherapy or in the immediate postoperative period.

PATIENTS AND METHODS

Study Population

Sixty-four consecutive patients with malignant or nonmalignant liver diseases undergoing partial (n = 45) or total hepatectomy and OLT (n = 19) were included in this prospective study from January to July 1995. There were 20 patients with HCC (19 men and 1 woman), 2 with cholangiocarcinoma, 25 with liver metastases (12 men and 13 women) from colorectal carcinoma (n = 19), breast cancer (n = 3), and other types of malignancy (n = 3). Thirteen patients without liver tumor confirmed by histologic analysis had cirrhosis (11 men and 2 women), caused by alcoholism in 8 patients, chronic viral hepatitis C infection in 1, alcoholic disease associated with viral hepatitis C in 2, primary biliary cirrhosis in 1, and cirrhosis from unknown origin in 1. Four patients had neither liver tumor nor cirrhosis but two cases of amyloid neuropathy, one case of porphyria, and one case of focal nodular hyperplasia. Diagnosis of HCC was made by ultrasonography or computed tomography, serum AFP, and confirmed by final pathology results after surgical resection. Size (*i.e.,* maximal diameter of tumor), number of nodules in the liver, and total volume of the tumor were calculated using imaging and volumetric scanning techniques, including intraoperative ultrasound. Final pathology results determined the grade of HCC according to Edmonson classification, the presence of portal vein invasion, and the Child score for cirrhosis. The control group included 28 normal, healthy volunteers without liver diseases (14 men, 14 women; mean age, 30 years).

The majority of patients with HCC (15/20, 75%) received neoadjuvant chemoembolization, and serum AFP levels were normal (<20 ng/mL) at the time of the hepatectomy in 12 (60%) of 20 patients. Fifteen (56%) of the 27 patients operated on for liver metastasis from other cancers received neoadjuvant chronomodulated chemotherapy according to our institutional protocol.[19]

Alpha-Fetoprotein mRNA Assay

Peripheral blood samples were obtained before surgery and during surgery at two different intervals: the first during the exploratory phase and the second after hepatectomy was completed, using ethylenediaminetetraacetic acid as an anticoagulant. All patients surviving the early postoperative period (n = 59) were observed 6 to 15 months after hepatectomy with blood samples obtained during routine clinic examination. Sensitivity of our assay was determined with human hepatocytes isolated from cadaveric multiple organ donors.[20]

Nucleated cells were isolated from peripheral blood using tetradecyltrimethylammonium bromide as described elsewhere,[21] and total RNA was extracted from the pellet or from cryopreserved liver tissues according to our technique reported previously.[22] Special attention was given to the choice of AFP primers to avoid cross-reaction between albumin and AFP mRNA because they present a 50% homology at the mRNA level. The primers

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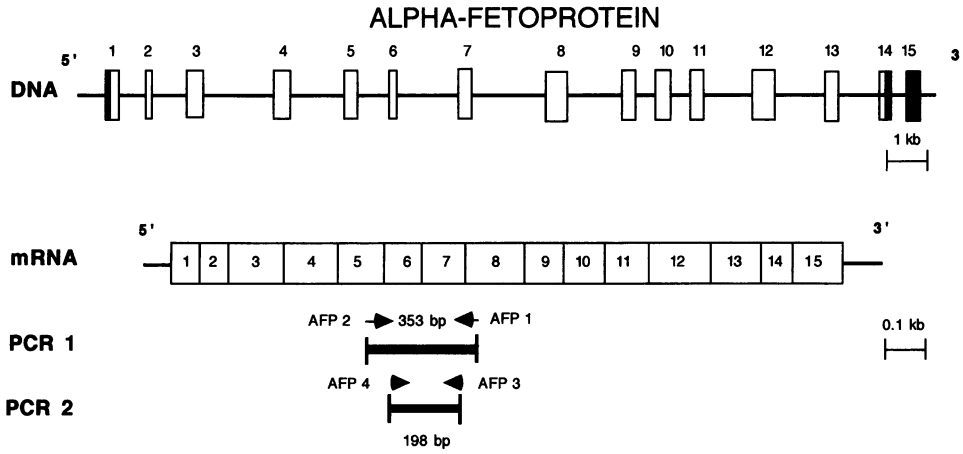

Figure 1. Representation of alpha-fetoprotein gene, alpha-fetoprotein gene transcript, and the position of primers used in reverse transcriptase–polymerase chain reaction.

were designed from the gene sequences of human serum alpha-fetoprotein.[23] To avoid false-positive results caused by DNA contamination, primers were selected in different exons of the AFP gene (Fig. 1). Sequences of primers used in the experiment were as follows. The sense primers were 5′ CAA TTC TTC TTT GGG CTG CTC GCT ATG AC 3′ (AFP 2) and 5′ ATG CAG TTG AAT GCT TCC AA 3′ (AFP 4), and the antisense primers were 5′ AGT GTC TTG TTG AGA ACA TAT GTA GGA CAT G 3′ (AFP 1) and 5′ CCA CAT CCA GGA CTA GTT TCT 3′ (AFP 3). The cDNA synthesis and polymerase chain reaction (PCR) amplification procedure (nested PCR in one tube) were performed as reported already by us.[22] Size of the amplified products of AFP mRNA was 198 base pairs.

Statistical Analysis

Statistical analysis was performed to determine if the influence of the indication for surgery (HCC *vs.* other indications), the surgical procedure itself (partial *vs.* total hepatectomy), and/or the use of neoadjuvant chemotherapy could affect detection of AFP mRNA in the circulation. The test results in the study group of HCC were correlated to the incidence of recurrence. Results are presented as mean ± standard deviation. Statistical analysis was performed with a statistical program (Statistica; StatSoft, Tulsa, OK) using the chi square test with a significance level set at 0.05.

RESULTS

Alpha-Fetoprotein mRNA Assay Sensitivity and Control Group

The sensitivity of our assay, determined in a dilution experiment (Fig. 2) using freshly isolated human hepatocytes (10^5 to 10^1) in 1 mL whole blood before RNA extraction, was approximately 1 hepatocyte for 10^5 peripheral mononuclear cells. Alpha-fetoprotein mRNA was not detected in the peripheral blood of 28 healthy subjects.

Preoperative Detection of Alpha-Fetoprotein mRNA

Before hepatectomy, AFP mRNA was detected in the blood of 11 (17%) of the 64 patients diagnosed with HCC (n = 5/20), cholangiocarcinoma (n = 1/2), colorectal carcinoma with liver metastasis (n = 4/25), and chronic active viral C hepatitis infection (n = 1/13). Patients with malignant disease and detectable AFP mRNA before hepatectomy (n = 10/47) had received chemotherapy, which was stopped 3 to 12 months before surgery (average, 6 months).

Intraoperative Detection of Alpha-Fetoprotein mRNA

During surgery, a total of 34 (53%) of 64 patients had detectable AFP mRNA at either 1 or both sampling inter-

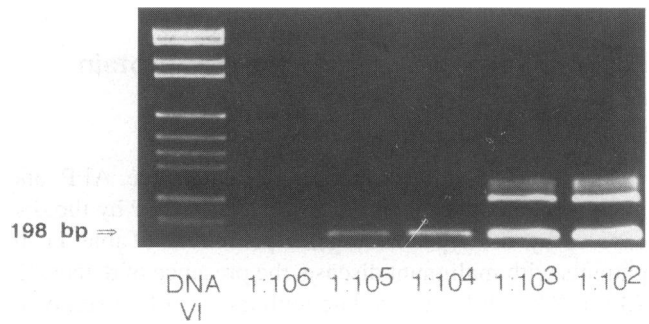

Figure 2. Sensitivity of the assay. Sensitivity of our assay was established using dilution of freshly isolated normal hepatocytes (10,^{5} 10,^{4} 10,^{3} 10,^{2} 10) in normal blood before total RNA extraction.

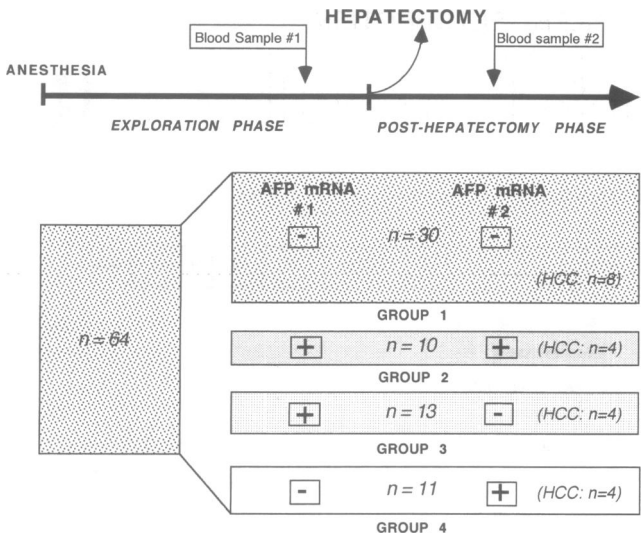

Figure 3. Schematic representation of intraoperative alpha-fetoprotein (AFP) mRNA profile in patients who are operated on. Group 1 = patients with no detectable AFP mRNA, group 2 = patients with detectable AFP mRNA at both blood samples, group 3 = patients with detectable AFP mRNA at the first blood sample only, group 4 = patients with detectable AFP mRNA at the second blood sample only.

vals. Alpha-fetoprotein mRNA was observed in 23 of the 64 patients during the exploration phase (first intraoperative blood sample), whereas 10 of these latter patients and 11 others were positive for AFP mRNA after hepatectomy (second intraoperative blood sample). Patients could be divided in four groups according to their intraoperative AFP mRNA profile (Fig. 3). In group 1, the largest (n = 30), patients had no detectable AFP mRNA during liver surgery, including 8 patients who received partial hepatectomy for HCC. In group 2 (n = 10, including 4 HCC), patients had detectable AFP mRNA at both the first and the second operative timepoints. In group 3 (n = 13, including 4 patients treated for HCC), patient results were positive at the first blood sampling and then negative in the second. Conversely, in group 4 (n = 11, including 4 patients treated for HCC), patient results were negative at the first blood sampling and positive after hepatectomy.

Correlations Between Alpha-Fetoprotein mRNA Test Results and Clinical Characteristics

The presence of detectable intraoperative AFP and mRNA was not influenced significantly either by the disease or by the type of surgical procedure (Table 1). In patients with malignant disease, the presence of detectable AFP mRNA did not correlate with the use of neoadjuvant chemotherapy (Table 1). The 20 patients with HCC included 4 patients receiving OLT and 16 having partial hepatectomy (Table 2). Forty percent of patients with HCC had more than one tumor foci, and the majority (n = 17, 85%) were encapsulated tumors. None of the tumors were of the fibrolamellar variety. Hepatocellular carcinoma was impossible to grade in 60% of patients because of tumor necrosis, whereas in the remaining patients, only three were classified as grade III or IV. Vascular invasion was present in 50% of the patients examined. No AFP mRNA was detected in 40% (n = 8) of patients who were operated for HCC (Table 2, Fig. 4). During surgery, in 40% of cases, AFP mRNA was detected in one of the two intraoperative samples, and four patients had detectable AFP mRNA at both sampling points. There was no evident relation between the presence of AFP mRNA in the blood during surgery and the serum level of the tumor marker AFP, the characteristics of the tumor (in particular the size and number of tumor foci), and the presence of vascular invasion.

Correlations Between Alpha-Fetoprotein mRNA Test Results and Clinical Outcome

Complete follow-up with peripheral blood sampling was performed 6 to 15 months after surgery. Eighteen (28%) of 64 patients had detectable AFP mRNA (1/17 nontumor causes, 7/27 secondary metastases, and 10/20 HCC) during the course of follow-up. The patient who had a signal for AFP mRNA without hepatic tumor had active chronic viral C hepatitis, whereas in the group of patients with metastatic tumor, only two of the four who had positive results before surgery remained with positive results 8 and 11 months after surgery, respectively. In the group of patients with HCC, three died in the immediate postoperative period, two from terminal liver failure and one from primary nonfunction after transplantation. These were three patients without detectable AFP mRNA during surgery (patient 20; Table 2) or only one positive test result during surgery (patients 16, 17; Table 2). The follow-up available for the 17 surviving patients ranges from 6 to 14 months (median, 9 months). Ten patients with HCC had positive results and seven had negative results for AFP mRNA signal after surgery. Among the five patients who had a signal for AFP mRNA before surgery, only one had a negative result 9 months after surgery (patient 1, Table 2). There were three patients without detectable AFP mRNA during surgery that remained with negative results (patients 1, 2, 11), whereas the results of three others became positive during surgery (patients 3, 12, 14). Two patients in the latter group had recurrent viral C hepatitis. In the group of patients that had detectable AFP mRNA during surgery, the results of seven remained positive (patients 4, 5, 6, 7, 10, 13, 18), whereas the results of four others became negative by subsequent testing (patients 8, 9, 15, 19). Finally, four patients[8–10,13]

Table 1. INFLUENCE OF INDICATION, TYPE OF SURGERY, AND PREOPERATIVE CHEMOTHERAPY ON INTRAOPERATIVE ALPHA-FETOPROTEIN mRNA IN PERIPHERAL BLOOD*

	Number of Patients (n = 64)	Alpha-Fetoprotein mRNA		
		Negative (n = 30)	Positive (n = 34)	
Indication				
Hepatocellular carcinoma	20	8 (40)	12 (60)	NS
Liver metastasis of other cancers	27	11 (41)	16 (59)	
Nonmalignant liver diseases	17	11 (65)	6 (35)	
Surgery				
Total hepatectomy and OLT	19	14 (74)	5 (26)	NS
Partial hepatectomy	45	17 (38)	28 (62)	
Chemotherapy				
Yes	28	10 (36)	18 (64)	NS
No	20	7 (35)	13 (65)	

OLT = orthotopic liver transplantation; NS = not significant.

* Patients were considered negative if no AFP mRNA was detected intraoperatively, positive if AFP and mRNA was detected in at least one of the two intraoperative samples. Chemotherapy was performed preoperatively in 28 of 47 patients with malignant liver disease. Statistical analysis was performed using the chi square test (significance set at $p < 0.05$). Values are number (%).

Table 2. HISTOLOGIC PARAMETERS OF PATIENTS OPERATED FOR HEPATOCELLULAR CARCINOMA

Patient Number	Preoperative AFP (ng/mL)	Chemoembolization	Number (size, cm) of Tumors*	Capsule	Grade†	Liver	Vascular Invasion	AFP mRNA‡				Recurrence
								Pre	1	2	Post	
1	<20	Yes	>3 (15)	No	II	NC	Yes	+	–	–	–	No
2	<20	No	1 (20)	Yes	I	NC	Yes	–	–	–	–	No
3	<20	Yes	2 (9)	Yes	II	NC	Yes	–	–	–	+	No
4	<20	Yes	1 (5)	Yes	N	C	Yes	+	+	+	+	No
5	215	Yes	1 (5)	Yes	N	NC	No	–	+	–	+	No
6	<20	Yes	3 (5)	Yes	N	NC	No	+	+	–	+	No
7	<20	No	1 (1.5)	Yes	N	C	No	+	–	+	+	No
8	10,350	Yes	1 (6)	Yes	III or IV	C	No	–	–	+	–	Yes
9	694	Yes	1 (4.5)	Yes	N	C	No	–	–	+	–	Yes
10	<20	Yes	2 (4)	Ruptured	III	NC	Yes	–	+	–	+	Yes
11	99	Yes	>3 (14)	Yes	II	NC	Yes	–	–	–	–	No
12	<20	Yes	2 (4)	Yes	N	C	No	–	–	–	+	No
13	2056	Yes	2 (7)	Yes	III	NC	No	–	+	+	+	Yes
14	593	Yes	1 (15)	Yes	N	NC	Yes	–	–	–	+	No
15	<20	Yes	2 (3)	No	N	C	No	–	–	+	–	No
16	40	No	1 (3)	No	II	NC	Yes	–	+	–	ND	Died
17	<20	No	1 (2)	Yes	N	C	No	–	–	–	ND	Died
18	<20	Yes	1 (6)	Yes	N	C	Yes	+	+	+	+	No
19	<20	Yes	1 (8)	Yes	N	NC	Yes	–	+	+	–	No
20	<20	Yes	1 (10)	Yes	N	C	No	–	–	–	ND	Died

AFP = Alpha-fetoprotein; N = necrosis; NC = noncirrhotic; C = cirrhotic; ND = not done.

* For multiple tumors, the size of the tumor indicated in the parentheses is the size of the largest one.

† Grade of the tumor was determined according to Edmonson. None of the patients studied had metastases to lymph nodes or distant organs.

‡ AFP mRNA was detected during partial hepatectomy or orthotopic liver transplantation (patients 7, 10, 11, and 12) at two time points (exploration phase, 1; after hepatectomy, 2) and 6–15 months later.

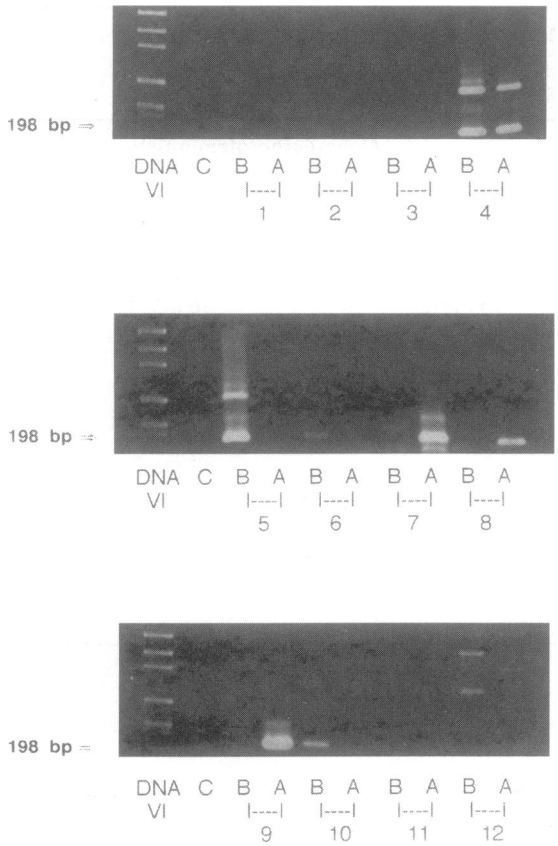

Figure 4. Electrophoresis pattern of 12 randomly chosen patients with hepatocellular carcinoma in our series of 20 cases. (A) After partial or total hepatectomy (second intraoperative blood sample) and (B) before partial or total hepatectomy (first intraoperative blood sample). Detailed characteristics of these patients are provided in Table 2. DNA VI = size marker, (C) negative control sample. Larger bands correspond to residual amplification products of the first polymerase chain reaction round.

had detectable recurrence by abdominal ultrasound, thoracoabdominal computed tomographic scan, bone scintiscan, or serum AFP levels. Of these four patients with recurrence, all had negative preoperative AFP mRNA values, but all had positive values shown during the intraoperative examination. In the immediate postoperative period, the test result was positive in only two of the patients with recurrent disease. There was no correlation between the incidence of recurrence and the test results before, during, and after surgery.

DISCUSSION

The results of this prospective study to evaluate AFP mRNA in peripheral blood lead us to conclude that this is not specific for circulating tumor foci. The use of this test, especially in the context of surgical treatment of HCC, cannot yet be recommended despite the suggestion of others in previous reports that AFP mRNA or albumin mRNA could be used clinically as a specific indicator of hematogenous dissemination of HCC cells.[11–18] We, as others,[14,15] examined albumin mRNA in the peripheral blood by PCR; however, its presence was shown in all control samples tested (n = 28, data not shown), whereas none of the control samples using AFP mRNA had tested positive. These results give credence to the concept of "illegitimate" transcription of the albumin gene in mononuclear cells.[14,15,24] Consequently, the detection of AFP gene transcripts seemed to be a valid method to identify abnormal liver cells in the circulation and, therefore, the possibility to visualize circulating HCC micrometastatic foci. None of our control patients had a positive signal for AFP mRNA, as also reported previously.[15]

Before surgery, we found a similar rate of detection in patients with HCC (25%) than in patients with other malignant liver diseases (20%). This is the first report documenting an association between detectable AFP mRNA in the circulation and liver metastases of other cancers. In the series of Komeda et al.,[18] none of the eight patients with metastatic liver cancer exhibited AFP mRNA in the blood, although another patient without cancer and chronic active viral C hepatitis had a signal for AFP mRNA before surgery. The presence of AFP mRNA already has been reported in cirrhosis (0%–15%) as well as acute and chronic hepatitis (0%–75%).[11,15–17] However, if only the preoperative test results of the nonmalignant patients (17 nonmalignant liver disease and 28 healthy control subjects) are considered, then only 1 of 45 was positive for AFP mRNA. The percentage of detectable AFP mRNA before surgery in patients with HCC (25%) was lower than that from previous results reported by others (36%–52%).[15,18] These differences cannot be explained by a lower sensitivity of our technique because a reverse transcription followed by nested PCR assay in one tube[25,26] is considered the most sensitive available (theoretically one abnormal cell for 10^6) with limited cross-contamination and is perfectly adapted to clinical laboratory use. In addition, when compared to methods involving preliminary isolation of cells,[12,15,17] our processing of blood samples is rapid, easy, and inexpensive. Furthermore, in our experience, techniques using Ficoll (Pharmacia, Uppsala, Sweden) cell extraction or dextran-based technique described by Matsumura et al.[16] obtained approximately ten times lower sensitivity than with tetradecyltrimethylammonium bromide (data not shown). Differences in AFP mRNA results in patients with HCC are more likely explained by the characteristics of patients examined, because in our report, only 3 patients (15%) had HCC grade III or IV, 12 other patients showed total tumor necrosis, thus rendering it impossible to grade, and no patient had known extrahepatic disease. This suggests that in these patients, few or no intact tumor cells would be circulating in the bloodstream. The fact that most patients

(75%) had received neoadjuvant arterial chemotherapy before surgery could explain why AFP serum levels were normal (<20 ng/mL) in many patients (65%). Although all of these factors individually might influence the potential for dissemination and subsequent detection of AFP mRNA, this has yet to be elucidated completely. In the series by Komeda et al.,[18] the presence of AFP mRNA in peripheral blood clearly was related to the stage of the disease (10% in stages I and II, 30% in stage III, and 77% in stage IV). However, the use of AFP mRNA as a marker of tumor dissemination would be useful if the tumor is at a stage when it is still considered curable, such as with stage I and II disease when AFP levels still are presumably at low levels in the group of patients examined in our series. The use of AFP mRNA in patients with late-stage (stage IV) disease seems elaborate considering that even if specific for metastases, this information most likely would be ascertained by more conventional clinical imaging methods. The subset of patients who theoretically would benefit would be at clinical stage I or II disease with positive AFP mRNA results, which then would alter the clinical therapeutic decision in the form of treatment rendered.

In our group of 64 consecutive patients undergoing either partial or total hepatectomy, detection of AFP mRNA in peripheral blood intraoperatively was possible in 53%, regardless of the disease. Detectable AFP mRNA could not be explained by the surgical indication (60% for HCC *vs.* 59% for liver metastases), and no significant differences were observed between partial or total hepatectomy (62% *vs.* 26%). In addition, the use of neoadjuvant chemotherapy (64% *vs.* 65%) and total or partial vascular exclusion of the liver during hepatectomy (data not shown) did not affect significantly the incidence of detecting AFP mRNA in peripheral blood. Our results suggest that liver manipulation clearly leads to hematogenous spread of cells from liver origin, which can be detected by AFP mRNA. The release of cancer cells has been shown during tumor resection by immunocytochemical techniques and PCR in breast cancer,[7] colorectal cancer,[27] and primary renal cancer.[28] Similarly, chemotherapy has been shown to induce a release of circulating AFP mRNA-positive cells in the circulation in small groups of patients with HCC.[18] Alpha-fetoprotein gene transcripts are found not only in HCC cells, but also in normal liver cells[11]; therefore, the malignant origin of the detected cells cannot be established or ruled out. Surprisingly, AFP mRNA signal fluctuates rapidly during the course of liver surgery. For example, patients in group 3 have a release of hepatocytes at the time of the first blood sample but not at the second endpoint, theoretically the most traumatic period. These results concur with other experimental[25,26] and clinical data[27,28] suggesting that release of abnormal cells in the circulation, either spontaneously or secondary to surgical manipulation, is an intermittent and transient phenomenon. As a result, detection of AFP mRNA might instead be dependent on the timing of the peripheral blood sampling, which further indicates the high likelihood of a "sampling error" during surgery.

During follow-up, 17 (27%) of 64 had positive AFP mRNA signals, of which 3 had chronic active viral C hepatitis. Follow-up results of patients operated on for breast cancer[7] or after chemotherapy for HCC[18] have shown that previously positive samples failed to show target gene expression 24 hours and 7 days after treatments, respectively. Analysis of HCC in our series shows ten with positive signals, whereas three had negative signals intraoperatively. Subsequently, only four patients with HCC had recurrent disease diagnosed; however, only two of these patients tested positive for AFP mRNA. The sensitivity and specificity of this test for clinical decisions, especially regarding therapy, were low (50% and 36%, respectively) with a predictive value of only 18%. This is contrary to previous studies because in only one report, disease recurrence occurred in three patients when albumin mRNA was measured in the peripheral blood of nine patients with HCC (stages II and IV) after OLT.[12] It cannot be overemphasized that reports of illegitimate transcription have been well documented with the use of albumin mRNA[14,15,24] since the test was described initially.[11,12] These results indicate that the malignant origin of detected circulating cells cannot be established by AFP mRNA that fluctuates over time, thus could represent a "sampling error." Therefore, the prognostic value of intraoperative circulating cancer cells shows both low sensibility and specificity.

The current study does reflect the inaccuracy of using the AFP mRNA as a biologic marker for detecting circulating tumor micrometastases in HCC and, therefore, the AFP gene cannot be considered an "HCC-specific" gene. Thus, suggesting different treatment options for patients with intraoperative detection of AFP mRNA cannot be advocated, and further studies are necessary to isolate certain genetic markers, which are specific for micrometastatic foci of HCC. Rather than the use of "tissue-specific" genes, potential approaches for the detection of micrometastases include the identification of "metastatic" genes, which are expressed only in metastatic cells such as the one coding for CD44, a glycosylated cell surface adhesion molecule, or the detection of an "HCC-specific" form of serum AFP. Until an accurate test is devised, it would be imprudent to attempt clinical application of AFP mRNA in the detection of micrometastatic foci.

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