Thrombosis and Haemostasis – © F. K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 60 (3) 468–470 (1988)

Coagulation Assays as Diagnostic Markers of Hepatocellular Carcinoma

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Key words

Coagulation assays – Hepatocellular carcinoma – Decarboxy-prothrombin – Proaccelerin

Summary

With the aim of improving the biological diagnosis of hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP), des-gamma-carboxyprothrombin (DCP) and factor V levels were assayed in 119 patients with HCC and 60 cirrhotic patients without HCC. Among the patients with HCC, increased levels of AFP (>300 ng/ml) and of DCP (>15 mU/ml) were observed in 36% and 69% of the cases, respectively. None of the 60 patients without HCC had increased AFP, and one had abnormal DCP; in this patient, DCP level returned to normal value after vitamin K_1 injection. No significant correlation was found between increased AFP and DCP, thus indicating that the two tests complement each other for the diagnosis. A factor V level higher than expected from the reduced prothrombin time test of the patient was detected in 50% of patients with HCC and only 7% of those without HCC. No correlation was found between increased factor V and abnormal AFP or DCP. The thrombin time, fibrinogen activity to antigen ratio, and polymerization index failed to differentiate between cirrhosis and HCC. We conclude that AFP, DCP and factor V may give complementary informations in the diagnosis of HCC, one of these markers at least being positive in 88% of the patients.

Introduction

Serum alpha-fetoprotein (AFP) is the most used biological marker in the diagnosis of hepatocellular carcinoma (HCC). However, significant increase of this marker, suggestive of HCC, is lacking in a marked percentage of cases (1, 2, 3). Coagulation tests have recently been proposed to improve the biological diagnosis of HCC. The increase in des-gamma-carboxyprothrombin (DCP), a a-gamma-carboxylated form of prothrombin (4), has been described in HCC (5, 6, 7) and the specificity of this marker was unproved after vitamin K_1 injection (8). The increase in factor V in HCC (9) was also found to be useful for HCC diagnosis. Furthermore, acquired dysfibrinogenemia was reported in various liver diseases (10), such as HCC (11) and cirrhosis (12); however, it has been suggested (13) that the prolongation of thrombin time due to an acquired dysfibrinogenemia was suggestive of the presence of HCC in cirrhotic patients. The aim of this study was to evaluate the real value of these coagulation assays for improving the biological diagnosis of HCC.

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Patients and Methods

Patients

One hundred nineteen patients with HCC were studied. Mean age was 61 years (range 27–88); 91 were male; 94 had cirrhosis (alcoholic: 80; post-hepatitic: 10; other etiologies: 4). In all cases, the diagnosis of HCC was histologically proven by biopsy or autopsy.

Samples from 60 patients having alcoholic cirrhosis without HCC were used as controls.

Methods

DCP, factors II and V assays and AFP were performed in fresh samples from the 119 HCC patients and in the 60 cirrhotic patients without HCC.

Plasma DCP assay was performed using a previously described method (6). This method assays the activity of DCP, using staphylocoagulase on undiluted adsorbed plasma. The thrombin-coagulase formed is measured on a chromogenic substrate, and the results are expressed in milliunits CNTS (arbitrary units) per milliliter (mU/ml) of increment of the optical density following the release of p-nitroaniline. After studying the plasma of numerous blood donors, the upper limit of normal was established to 15 mU/ml (6). A DCP level higher than 15 mU/ml was considered "positive".

Plasma factors II and V levels were assayed using previously described methods (14, 15). A "positive" factor V was arbitrarily defined either as a factor V level exceeding the factor II level by more than 30%, or as a normal (>75%) factor V associated with a decreased (<75%) factor II.

Serum AFP was assayed by radioimmunoassay (2). We considered "positive" an AFP level higher than 300 nanogram per milliliter (ng/ml), which was reported to be strongly in favor of HCC (1). Following the first sampling revealing an increased DCP level, 12 patients (11 HCC, 1 cirrhosis) received a slow intravenous injection of 20 mg of vitamin K_1. Further samplings for DCP assays were obtained 10 days or more after the vitamin K_1 injection.

The presence of dysfibrinogenemia was investigated in 38 HCC patients and in 47 cirrhotic patients without HCC by the determination of: 1) The thrombin time (TT) (15) with a control to 20 sec. The results obtained were separated into normal TT (maximum difference with control of 3 sec), moderatly prolonged TT (3 to 10 sec), prolonged TT (more than 10 sec). 2) The ratio of fibrinogen activity (16)/fibrinogen antigen (Mancini method). This ratio was 0.99 ± 0.09 in 15 control subjects. A ratio lower than 0.90 was considered abnormal. 3) Fibrinogen polymerisation test expressed by a polymerisation index corresponding to the ratio optical density × 100/fibrinogen antigen. The polymerisation index determined in 26 controls subjects was found to 14.2 ± 3.1. An index lower than 11 was considered abnormal.

Statistical analysis used: the Chi-2 test.

Results

The results of AFP, DCP, factor V assays in the 119 HCC patients and in the 60 cirrhotic patients without HCC are shown in Table 1. In HCC patients, the mean levels of DCP, factors II and V were respectively 60 ± 19 mU/ml, 54 ± 7% and 98 ± 25%; in cirrhotic patients, they were respectively 8 ± 3 mU/ml, 47% ± 8% and 43 ± 11%. The presence of an increased DCP and of an increased factor V was significantly more common in patients

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Table 1 AFP, DCP and factor V assays in HCC patients and in cirrhotic patients without HCC

	HCC patients	Cirrhotic patients without HCC	
No.	119	60	
Positive DCP	82 (68.9%)	1 (1.6%)	p <0.001
Positive AFP	43 (36.1%)	0 (0%)	p <0.001
Positive factor V	60 (50.4%)	4 (6.6%)	p <0.001
Positive DCP and AFP	30 (25.2%)	0 (0%)	p <0.001
Positive DCP and factor V	44 (37.0%)	0 (0%)	p <0.001
Positive AFP and factor V	22 (18.5%)	0 (0%)	p <0.001
Positive DCP, AFP, factor V	16 (13.4%)	0 (0%)	p <0.001
Negative DCP, AFP, factor V	14 (11.7%)	55 (91.6%)	p <0.001
Positive DCP and/or AFP	96 (80.6%)	1 (1.6%)	p <0.001
Positive DCP and/or factor V	93 (78.1%)	5 (8.3%)	p <0.001
Positive AFP and/or factor V	81 (68.0%)	4 (6.6%)	p <0.001
Positive DCP and/or AFP and/or factor V	105 (88.2%)	5 (8.3%)	p <0.001

Table 2 Thrombin time, fibrinogen activity/fibrinogen antigen ratio, polymerisation index of fibrinogen in HCC patients and in cirrhotic patients without HCC

	HCC patients	Cirrhotic patients without HCC	
No.	38	47	
Thrombin time*)			
– normal	15	23	
– moderatly prolonged	14	15	(NS)
– markedly prolonged	9	9	
Fibrinogen activity/fibrinogen antigen ratio*)			
>0.9	17	24	(NS)
<0.9	21	23	
Polymerisation index			
>11	10	13	(NS)
<11	28	34	

NS = Non significant difference (Chi-2 test).
*) See text for details.

with HCC than in cirrhotic controls (p <0.001). No significant relationship was found between positive AFP and positive DCP, between positive AFP and positive factor V, and between positive DCP and positive factor V. No difference was noted when comparing the group with cirrhosis and HCC versus that with HCC only.

In the 12 patients having received vitamin K_1 injection, the second sampling (10 days or more after injection) was found to be positive in the 11 HCC patients and negative in the cirrhotic patient.

The difference between the results of TT, fibrinogen activity/fibrinogen antigen ratio and polymerisation index in the 38 HCC patients and in the 47 cirrhotic patients was not significant for the 3 tests (Table 2).

Discussion

The existence of hepatocellular failure in patients with cirrhosis may be responsible for haemostasis disorders due to a quantitative defect of synthesis of coagulation factors. The increased DCP observed in HCC patients is independent of the hepatocellular failure. Its mechanism is due to an anomaly of the tumor cells to gamma-carboxylate the vitamin K dependent factors (17). This increase is not linked to a vitamin K deficiency (8) since it persists following vitamin K_1 injection. Indeed vitamin K_1 injection allows to distinguish increased levels of DCP due to HCC or to vitamin K deficiency. An increased DCP level which persists after vitamin K_1 injection seems to be specific for HCC (8). In our patients, DCP was positive in 69% of the HCC, whereas AFP was positive in only 36%. It may be noted that these sensitivities have been calculated using different limits for DCP (>15 mU/ml, i.e., the upper limit of normal) and AFP (>300 ng/ml). However, as indicated above, this was justified by the fact that these limits of positivity corresponded to similar specificity of the two tests (98% for DCP and 100% for AFP, respectively, Table 2).

Factor V is synthetized at least in part in the liver. Its plasma level is decreased in parenchymal liver diseases (18, 19, 20). An increased synthesis by tumor cells appears to be the most probable mechanism of the increased DCP levels in HCC. The lack of correlation between the increases in AFP and DCP or factor V is of interest because these three markers complement each other for the diagnosis of HCC, one of these markers at least being positive in 88.2% of patients with HCC.

Dysfibrinogenemias have been described in various hepatic disorders (12). Our results confirm that dysfibrinogenemia may be observed not only in HCC but also in cirrhotic patients without HCC. It therefore cannot be used as a HCC diagnosis marker. We conclude that DCP, factor V and II assays must be taken into account, along with AFP assay, for the diagnosis of HCC. The comparative interest of these 3 tests requires further studies to determine their value in the early detection of HCC in subjects at risk.

Acknowledgement

We thank A. V. Bendetowicz for her valuable technical collaboration.

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Received May 9, 1988 Accepted after revision August 5, 1988

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D. REINWEIN
P. C. SCRIBA
(Eds.)

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International Thyroid Symposium, October 18–21, 1984, Budapest

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