

## ASSOCIATION BETWEEN PROLONGED BLEEDING TIME AND GASTRO-INTESTINAL HEMORRHAGE IN 102 PATIENTS WITH LIVER CIRRHOSIS: RESULTS OF A RETROSPECTIVE STUDY

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### ABSTRACT

**Background.** Gastrointestinal bleeding is a frequent complication of liver cirrhosis (LC) and represents an important warning sign of imminent death. Platelet dysfunction is an abnormality occurring prevalently in severe liver failure, and could well predispose to bleeding.

**Methods.** One hundred and two patients with liver cirrhosis diagnosed by needle liver biopsy were studied. According to the Child-Pugh classification, 23 were A class, 42 B class and 37 C class cases. Prothrombin activity, aPTT, fibrinogen, FDPs, XDP and platelet count were measured in each patient; bleeding time was measured in all but 17 of them. Forty (39%) had experienced gastrointestinal bleeding during the last 3 years (2 A class, 12 B class, 26 C class).

**Results.** Patients with a history of previous gastrointestinal bleeding showed lower values for prothrombin activity and fibrinogen, and higher percentage of elevated FDP and XDP levels; moreover, they presented lower platelet counts and more prolonged bleeding times than patients without gastrointestinal blood loss.

**Conclusions.** While our findings confirm the relationship between hyperfibrinolysis and bleeding, the association between bleeding time prolongation and gastrointestinal blood loss suggests studying platelet function prospectively in LC in order to analyze its role, if any, in favoring hemorrhage activity.

Key words: liver cirrhosis, gastrointestinal hemorrhage, platelet function

Gastrointestinal bleeding is a frequent complication of liver cirrhosis (LC) and represents an important warning sign of imminent death.<sup>1-6</sup> Variceal size, increased intrahepatic gradient pressure and severe liver failure are considered risk factors, but the mechanism linking severe liver failure and gastrointestinal bleeding is still not clear.<sup>5</sup> Reduced activity of many clotting factors, which occurs

prevalently in severe liver failure, may be a favoring mechanism but equivocal results do not support a close relationship between clotting changes and bleeding.<sup>7-10</sup> On the contrary, a hyperfibrinolytic syndrome, which is also prevalent in decompensated liver cirrhosis, seems to be a precipitating factor.<sup>11-14</sup> Platelet dysfunction, which is another abnormality occurring prevalently in severe liver failure,

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could well predispose to bleeding;<sup>15</sup> however, only one retrospective study carried out on a small number of LC cases showed that patients with bleeding time (BT) > 8 min had a greater incidence of gastrointestinal hemorrhaging than those whose bleeding time was < 8 min.<sup>16</sup>

To further explore the relationship between bleeding time and gastrointestinal hemorrhage, we retrospectively studied 102 patients with different degrees of LC. Coagulation and fibrinolytic indexes were also investigated to assess their relationship to bleeding.

### *Patients and methods*

#### *Patients*

Between 1989 and 1992, we studied 102 consecutive patients [64 males (63%), 38 females (37%), aged 31 to 77 years], with LC diagnosed by needle liver biopsy. In the case of patients with severe liver failure, the diagnosis was supported by a previous liver biopsy. Patients with hepatocarcinoma, acute hepatitis or acute infections were excluded. All participants gave informed consent before entering the study. Standard treatment consisted of spironolactone, furosemide, ethacrynic acid, albumin, lactulose and non-absorbable antibiotics. None of the patients had been taking any drug known to affect platelet function for two weeks, nor had any of them been transfused for 4 weeks before the investigation. A complete clinical history, with particular reference to previous episodes of bleeding, was recorded for every patient admitted to the study; a complete physical examination was also performed in order to score liver failure. Patients were defined as class A (n = 23; 23%), B (n = 42; 41%), or C (n = 37; 36%) according to the Child-Pugh classification<sup>17-18</sup>. Of the 102 patients, 42 (41%) presented serological markers for hepatitis C virus (HCV), 20 (20%) for hepatitis B virus (HBV), 12 (12%) HBV+HCV; 13 (13%) were alcoholics, 4 (4%) cryptogenetic, 1 (1%) had biliary cirrhosis, and 10 (10%) were not defined.

#### *Bleeding time*

Skin bleeding was measured by following the technique described by Mielke et al.<sup>19</sup> using a Simplate II bleeding time device (General Diagnostics, New Jersey, USA). Two 5 mm long × 1 mm deep incisions were made at the

lateral aspect of the volar surface of the forearm, parallel to the antecubital crease. The bleeding time was determined on a skin area without superficial veins that had been disinfected with alcohol and subsequently dried.

A sphygmomanometer cuff inflated to 40 mmHg (5.3KPa) pressure was kept on the upper arm for the entire test period. Every 30 seconds the drops of blood coming from the incision were dried with absorbant paper. The average time required for the flow of blood to stop from each of the two cuts was taken as the bleeding time. This procedure was carried out by the same investigator at all times, between 8 and 9 a.m., after the patients had been fasting for 12 hours. A bleeding time of more than 10 minutes was considered abnormal, ten minutes being the upper limit observed in 25 healthy subjects matched for sex and age.

Further laboratory investigations were performed on each patient the same day the bleeding time test was done. These included platelet count, packed cell volume (PCV), total serum bilirubin and albumin concentrations, and a study of coagulation and the fibrinolytic system; a blood sample for the determination of these variables was taken after the bleeding time measurement.

#### *Coagulation study*

Between 8 and 9 a.m. a 2 mL blood sample was taken without stasis from the antecubital vein of patients who had been fasting for at least 12 hours, and was immediately transferred to a prewarmed tube containing 20 NIH thrombin for evaluation of fibrinogen degradation products (FDP).<sup>11</sup> Another sample (9 parts) was mixed with 1 part of 3.8 Na citrate and treated in order to study clotting factor activities, as reported below. Citrated blood samples were immediately centrifuged for 20' at 2000×g, and the supernatant stored at -70°C until use.

The following blood coagulation screening tests were performed on each sample:

- prothrombin activity was assessed by Normotest (Nyegaard & Co, Oslo, Norway; ref. val. 70-120%), which explores vitamin K-dependent factors and was used according to the manufacturer's instructions; intra- and interassay coefficients of variation were 4 and 5%, respectively;
- activated partial thromboplastin time (aPTT)

- was evaluated by Cephotest (Immuno-Diagnostics, Pisa, Italy; ref. val. 25-33 sec);
- plasma fibrinogen was studied according to the Clauss method<sup>20</sup> (Baldacci, Pisa; ref. val. 150-400 mg/dL) and by the radial immunodiffusion technique<sup>21</sup> (NOR-Partigen Fibrinogen, Behring Institute, Italy). A significant correlation was observed between fibrinogen levels measured by the clotting test and those measured by radial immunodiffusion ( $r = .80$ ;  $p = .0001$ ).
- The data reported in the results refers to fibrinogen measured by the clotting test. A Schnitger and Gross coagulometer was employed for the Normotest, aPTT and plasma clottable fibrinogen assays.<sup>22</sup>

#### Fibrinolytic system study

Serum levels of fibrinogen degradation products were measured by the Thrombo-Wellco test (Wellcome Diagnostics Ltd, Dartford, London, UK; ref. val.  $\leq 10$   $\mu\text{g/mL}$ ), a semi-quantitative test using a latex suspension coated with sheep anti-FDP globulin. Patients were considered positive for FDP if they had values greater than 10  $\mu\text{g/mL}$  in two consecutive controls performed within 10 days.<sup>23</sup>

D-dimer (XDP), which is an *in vivo* marker for thrombin and plasmin activation,<sup>24</sup> was evaluated in patient plasma by the ORTHO dimertest (ORTHO Diagnostics, Milan, Italy; ref. val.  $< 200$  ng/mL), a semi-quantitative test

using latex coated with anti-XDP monoclonal antibodies.

#### Statistical analysis

The study population was examined in relation to the presence or absence of previous bleeding. The statistical significance of differences between the two groups of patients for each variables measured was evaluated by the Mann-Whitney U test and by the chi-square test. The relationship among the variables under study was examined by Spearman's rank correlation test.<sup>5</sup>

Data are presented as mean  $\pm$  standard deviation and 95% confidence limits. A  $p < .05$  was regarded as statistically significant. All calculations were made using a StatView II (Abacus Concept, Berkeley, CA, USA) computer program.

#### Results

Table 1 reports the clinical characteristics and the incidence of previous gastrointestinal bleeding in our population.

Forty (39%) patients had suffered bleeding episodes during the previous 3 years (range from 2 to 36 months): 2 (5%) A class, 12 (30%) B class and 26 (65%) C class. Those with a previous history of gastrointestinal bleeding showed lower prothrombin activity and lower fibrinogen levels, lower serum albumin and PCV, and higher serum bilirubin concentrations (Table 2).

Systemic signs of hyperfibrinolysis discriminated patients with gastrointestinal blood loss; in fact, high values of D-dimer and FDP were observed in 55% and 60%, respectively, of patients who had hemorrhaged (Table 2). Platelet count was lower in bleeding patients (Table 3); 55% of hemorrhagic and 34% of non hemorrhagic patients had platelet counts  $< 100 \times 10^9/\text{L}$ . Bleeding time was significantly longer in those with gastrointestinal bleeding (Table 3). A higher percentage of bleeding patients (52%) than non bleeding ones (22%) showed a bleeding time  $\geq 10$  min, ( $p = .0001$ ) (Table 3). Bleeding time was inversely correlated with platelet count ( $r = -.60$ ;  $p = .0001$ ).

The association between bleeding time and gastrointestinal hemorrhage seems to be independent of the degree of liver failure: 70% B class and 76% C class patients had bleeding

Table 1. Clinical and etiological features of the whole series of cirrhotic patients.

Whole series <i>n</i> = 102			
age (yrs)	59 $\pm$ 10 (31-77)	<i>etiology</i>	
		HBV	20% ( <i>n</i> = 20)
males	63% ( <i>n</i> = 64)	HCV	41% ( <i>n</i> = 42)
		Alcoholic	13% ( <i>n</i> = 13)
		HBV+HCV	12% ( <i>n</i> = 12)
grade		Cryptogenetic	4% ( <i>n</i> = 4)
A	23% ( <i>n</i> = 23)	Biliary	1% ( <i>n</i> = 1)
B	41% ( <i>n</i> = 42)	Unknown	9% ( <i>n</i> = 10)
C	36% ( <i>n</i> = 37)		
Incidence of previous gastrointestinal bleeding			
39% ( <i>n</i> = 40)			

Table 2. Clinical and laboratory features of cirrhotic patients according to the presence of previous gastrointestinal bleeding.

<i>previous bleeding</i>	YES <i>n</i> = 40	<i>p</i> <	NO <i>n</i> = 62
Age (yrs) <sup>°§</sup>	58±9 (40-75)	NS	58±11 (56-62)
Males	24 (60%)	NS	40 (64%)
Grade A/B/C <sup>^</sup>	2/12/26	***	21/30/11
Normotest (%) <sup>°§</sup>	45±15 (42-52)	***	62±20 (57-67)
aPTT (sec) <sup>°§</sup>	31±7 (29-33)	NS	29±5 (27-30)
Fibrinogen (mg/dL) <sup>°§</sup>	175±60 (155-194)	***	230±72 (212-249)
Bilirubin (mg/dL) <sup>°§</sup>	5.0±5.6 (3.2-6.8)	***	1.8±1.4 (1.4-2.1)
Albumin (g/L) <sup>°§</sup>	3.2±0.7 (2.9-3.5)	*	3.7±0.6 (3.5-3.9)
PCV (%) <sup>°§</sup>	0.34±0.06 (0.32-0.36)	*	0.38±0.08 (0.36-0.40)
FDP > 10 µg/mL <sup>^</sup>	24 (60%)	*	20 (33%)
D-Dimer > 200 ng/mL <sup>^</sup>	22 (55%)	**	12 (19%)

<sup>°</sup>data are presented as mean±standard deviation; parentheses contain 95% confidence limits; <sup>§</sup>Mann-Whitney test; <sup>^</sup>Chi-square statistic.  
\**p* < .05; \*\**p* < .001; \*\*\**p* < .0001.

times ≥ 10 min and a clinical history of previous gastrointestinal hemorrhage.

### Discussion

This study lends further support to previous investigations showing that LC patients have a complex disturbance of hemostasis.<sup>7, 26, 27</sup> Our population, in fact, had systemic signs of reduced activity for many clotting factors, hyperfibrinolysis and platelet dysfunction. Bleeding was more frequent in C class patients, confirming that severe liver failure is an important risk factor.

The association between clotting changes and hemorrhage is not clearly defined, apart from the hyperfibrinolysis syndrome, which seems to facilitate gastrointestinal bleeding.<sup>7, 11</sup> Indeed LC patients with reduced euglobulin time lysis or high D-dimer values tended to bleed more than

those without systemic signs of hyperfibrinolysis.<sup>11-14, 28</sup> On the other hand, it is not certain that reduced clotting factor activity and platelet dysfunction are risk factors.<sup>7-10, 15</sup> Our methodology does not allow us to solve this problem fully because this study is retrospective. It is worth noting, however, that patients with gastrointestinal bleeding have significantly lower fibrinogen activity and vitamin K-dependent factor levels and, more interestingly, prolonged bleeding time. The relationship between platelet dysfunction and bleeding in LC is still controversial, mainly because there are no prospective studies indicating that prolonged bleeding time may be an independent risk factor. Our retrospective study shows that about 50% of LC patients with gastrointestinal blood loss have BT ≥ 10 min, suggesting a possible role for platelet dysfunction in favoring hemorrhage.

This potential role was particularly evident when the association between BT and hemorrhage was analyzed according to the severity of liver failure. We observed that B and C class patients with BT ≥ 10 min presented an almost identical percentage of gastrointestinal bleeding, indicating that platelet dysfunction may be an important factor favoring hemorrhage independently of the degree of liver failure.

The cause of prolonged bleeding time in LC is complex and probably depends on mechanisms both intrinsic and extrinsic to the platelet membrane.<sup>15</sup> It is certain, however,

Table 3. Platelet count and bleeding time of cirrhotic patients according to the presence of previous gastrointestinal bleeding.

<i>previous bleeding</i>	YES <i>n</i> = 40	<i>p</i> <	NO <i>n</i> = 62
Bleeding time (min) <sup>°§</sup>	14±7 (11-17)	**	8±3 (7-9)
Bleeding time ≥ 10' <sup>^</sup>	21 (52%)	**	14 (22%)
Plt count (×10 <sup>9</sup> /L) <sup>°§</sup>	100.3±48.4 (84.8-115.83)	*	130.4±79.1 (110.1±150.7)
Plt count < 100 (×10 <sup>9</sup> /L) <sup>^</sup>	22 (55%)	NS	21 (34%)

<sup>°</sup>data are presented as mean±standard deviation; parentheses contain 95% confidence limits; <sup>§</sup>Mann-Whitney test; <sup>^</sup>Chi-square statistic.  
\**p* < .05; \*\**p* < .0001.

that platelet dysfunction is due only in part to low platelet count. In fact, LC patients with low platelet counts may have normal BT, or viceversa.<sup>29, 30</sup>

In conclusion, this study shows that LC patients with a previous history of gastrointestinal bleeding suffer from complex hemostatic disturbances and platelet dysfunction. Because the relationship between hemorrhage and platelet function has never been examined prospectively in LC patients, our findings suggest that such studies should be planned to assess whether platelet dysfunction is an independent risk factor for gastrointestinal bleeding. This hypothesis is currently being investigated in a multicenter study that includes more than one hundred patients followed up prospectively for more than 12 months.

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