LIVER DISEASE

Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis

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Background and aims: Thrombosis of the small intrahepatic veins has been suggested to trigger liver tissue remodelling. We evaluated the prevalence of multiple thrombotic risk factors and their association with the extent of fibrosis in chronic viral hepatitis.

Methods: Ninety consecutive patients with chronic hepatitis B or C without malignancy, a history of venous thrombosis, or antiviral/immunosuppressive therapy within the last six months were included. Thrombophilic and coagulation factors were evaluated on the liver biopsy day.

Results: One or more thrombotic risk factors were found in 68% and ≥ 2 factors in 37% of patients. Higher necroinflammatory activity was independently associated with higher prothrombin time ($p=0.003$), alanine aminotransferase level ($p=0.011$), and histological staging ($p=0.018$). Patients with staging scores of 4–6 compared with those with scores of 0–3 more frequently had deficiency of protein C (24% v 3%; $p=0.007$), antithrombin III (28% v 5%; $p=0.005$), and plasminogen (19% v 2%; $p=0.03$), and a trend for more frequent activated protein C resistance (8% v 0%; $p=0.075$). The presence of ≥ 1 significant thrombotic risk factor was observed in 11/25 (44%) patients with staging scores of 4–6 and in 6/65 (9%) patients with scores of 0–3 ($p<0.001$), being the only variable independently associated with advanced staging (odds ratio 2.4, $p=0.02$).

Conclusions: Thrombotic risk factors are frequently detected in patients with chronic viral hepatitis and the presence of ≥ 1 significant factor is associated with more advanced fibrosis. Whether the association of such thrombophilic conditions with advanced fibrosis is a primary or secondary phenomenon and whether their development in combination with local inflammation accelerate the progression of liver fibrosis need further evaluation.

Chronic viral hepatitis can be a progressive disease. It is estimated that approximately 20–30% of patients with chronic hepatitis C progress to cirrhosis over a period of 20 years^{1,2} and about 15–20% of patients with chronic hepatitis B may do so within five years.^{3,4} Patients with established cirrhosis have a poor prognosis as they are at high risk of liver decompensation and/or the development of hepatocellular carcinoma (HCC).^{5–8} In chronic viral hepatitis, cirrhosis is considered to be the end result of a diffuse chronic necroinflammatory process and is characterised by extensive fibrosis and abnormal hepatic architecture with nodule formation.⁹ All recent histological classification systems of chronic viral hepatitis have separated necroinflammatory activity (referred to as grading) from fibrosis (often referred to as staging).^{10–12}

Hepatic fibrosis develops as a response to chronic liver damage from several causes but acute severe or even fulminant hepatitis often resolves without the development of fibrosis,¹³ which suggests that liver necroinflammation may not be sufficient to initiate production of permanent fibrosis. Although there have been many studies attempting to evaluate the rate of progression to cirrhosis and several factors have been associated with rapid or slow progression of fibrosis,^{1–4} it is very difficult to predict the course of the disease in individual patients. Thrombosis of the intrahepatic veins is frequently observed in cirrhosis and has been associated with its progression,¹⁴ while occlusion of small sized intrahepatic veins and sinusoids has been considered as a potential triggering factor of liver tissue remodelling.¹⁵ The involvement of various thrombotic risk factors in the pathogenesis of vein thrombosis is well established but their role in chronic viral hepatitis or cirrhosis has not been investigated. In the present study, we evaluated the prevalence of several genetic and acquired prothrombotic conditions in patients with chronic

hepatitis B or C as well as their possible association with necroinflammatory activity (grading) and extent of fibrosis (staging) of liver disease.

PATIENTS AND METHODS

Patient population

Ninety consecutive patients with chronic hepatitis B or C admitted to our department for a liver biopsy between March 2000 and February 2001 were included in this study. Patients with known HCC or any other malignancy, a history of venous thrombosis, or those who had received any form of antiviral and/or immunomodulatory therapy within the last six months were excluded. No patient was taking oral contraceptives or anticoagulation therapy.

Liver histology

All 90 patients had a percutaneous liver biopsy. There were no inadequate liver specimens, as defined previously on the basis of no portal tracts identified or the specimen size itself being too small to make a diagnosis. All liver biopsies were evaluated blindly by a single liver histopathologist (KP) and histological changes of chronic hepatitis were classified according to the classification system proposed by Ishak and colleagues.¹¹ The chronic hepatitis grading score (0–18), which represents necroinflammatory activity, was the sum of the piecemeal necrosis score (0–4), confluent necrosis score (0–6), focal lytic necrosis, apoptosis, and focal inflammation score (0–4), and

Abbreviations: HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl-transpeptidase; ULN, upper limit of normal.

Table 1 Epidemiological, haematological, biochemical, and histological characteristics of 90 patients with chronic hepatitis B or C

Characteristic	Chronic hepatitis B (n=39)	Chronic hepatitis C (n=51)	Total (n=90)
Sex (M/F)	30/9	34/17	64/26
Age (y)	50 (14)*	41 (13)*	45 (14)
Possible source of infection (n)	**	**	
Blood transfusion	1	19	20
Parenteral drug use	0	15	15
Intrafamilial contact	17	2	19
Health care work	0	1	1
Unknown	21	14	35
Haemoglobin (g/dl)	14.1 (1.3)	14.1 (1.6)	14.1 (1.5)
White blood cell count ($\times 10^3$ /l)	6.3 (1.6)***	7.6 (2.0)***	7.1 (2.0)
Platelet count ($\times 10^3$ /l)	182 (56)****	228 (64)****	208 (64)
Prothrombin time (s)	12.0 (1.0)	11.7 (0.8)	11.8 (0.9)
Partial thromboplastin time (s)	31 (4)	32 (5)	32 (4)
Fibrinogen level (mg/dl)	253 (48)	273 (70)	264 (62)
ALT (IU/l)	94 (24–490)	82 (24–365)	88 (24–490)
AST, (IU/l)	50 (19–213)	51 (19–189)	51 (19–213)
Alkaline phosphatase ($\times$ ULN)	0.6 (0.3–1.2)	0.6 (0.3–1.4)	0.6 (0.3–1.4)
GGT ($\times$ ULN)	0.5 (0.2–3.0)	0.7 (0.2–3.0)	0.5 (0.2–3.0)
Histological grading	7.1 (2.3)	6.8 (2.4)	6.9 (2.3)
Histological staging	3.0 (1.4)	2.8 (1.4)	2.9 (1.4)
Cirrhosis (staging=5–6) (n (%))	6 (15)	6 (12)	12 (13)

Quantitative values are expressed as mean (SD) or median (range).

Chronic hepatitis B versus chronic hepatitis C patients: * $p=0.001$; ** $p<0.001$; *** $p=0.003$; **** $p=0.001$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl-transpeptidase; ULN, upper limit of normal.

portal inflammation score (0–4). The chronic hepatitis staging score (0–6), which is referred to as the fibrosis score, was based on the degree and extent of fibrosis, architectural alterations, and development of cirrhosis. Advanced staging or severe fibrosis was considered a staging/fibrosis score of ≥ 4 .

Laboratory studies

Full blood count, including platelet count, prothrombin time, and international normalised ratio, activated partial thromboplastin time, fibrinogen levels, and liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and gamma-glutamyl-transpeptidase (GGT)) were performed using commercially available assays in all patients on the liver biopsy day. Venous blood samples were also collected from all patients on the liver biopsy day and plasma, serum, and EDTA blood samples from each patient were stored at -80°C until determination of the various thrombophilic and coagulation factors.

Anticardiolipin antibodies (IgG and IgM isotypes) and anti-beta2-glycoprotein I antibodies (IgG) were determined using commercially available enzyme immunoassays (Gull Diagnosti, Bad Hamburg, Germany). The presence of lupus anticoagulant was detected by three screening assays: APTT, dRVVT (Diagnostica Stago), and KCT (inhouse technique) and confirmed by an APTT using hexagonal phospholipids (Staclo-LA, Diagnostica Stago Asnieres-Sur-Seine, France). Activities of protein C, antithrombin III, and plasminogen were determined by chromogenic assays while free protein S was measured by a clotting assay (Diagnostica Stago). Factors VIII, IX, XI, and XII were also measured by one stage clotting assays (Diagnostica Stago). Activated protein C resistance was determined by original and modified assays (Behring-Dade, Marburg, Germany). The presence of the prothrombin 20210A mutation was detected by isolation of DNA from EDTA whole blood, in vitro amplification of coagulation factor II sequences, and subsequent detection of the mutation by allele specific hybridisation in microwells (ViennaLab, Vienna, Austria). The presence of the factor V Leiden mutation was also determined in DNA isolated from EDTA blood (ViennaLab) but only in patients with abnormal activated protein C resistance.

Statistical analysis

All data were analysed using the statistical package SPSS 10.0. Statistical analysis was performed using the *t* test or the Mann-Whitney test for comparisons of continuous variables between groups, and corrected χ^2 method or two tailed Fisher's exact test for qualitative data, where appropriate. Spearman correlation or linear regression analysis was used for evaluation of the relationships between several continuous variables and histological grading or staging, whenever they were considered as continuous variables. Multivariate analysis was performed using multiple regression or logistic regression models, where appropriate. A two tailed *p* value less than 0.05 was considered significant.

RESULTS

Epidemiological, haematological, biochemical, and histological characteristics of the 39 patients with chronic hepatitis B and 51 patients with chronic hepatitis C are presented in table 1. Patients with chronic hepatitis B compared with those with chronic hepatitis C were significantly older and had significantly lower white blood cell counts and platelet counts. Moreover, as expected, blood transfusions in the past and parenteral drug abuse were possible sources of infection almost exclusively in patients with chronic hepatitis C and intrafamilial contact in chronic hepatitis B cases. There was no significant difference in the severity of histological grading or staging between the two groups (table 1).

Levels of thrombophilic and coagulation factors as well as the prevalence of thrombotic risk factors in our patient population are presented in tables 2 and 3, respectively. Patients with chronic hepatitis B compared with those with chronic hepatitis C were found to have significantly higher serum levels of both IgG and IgM anticardiolipin antibodies as well as lower plasma levels of antithrombin III and plasminogen (table 2). Anti-beta2-glycoprotein I antibodies were not detected in any of the six patients with elevated levels of anticardiolipin antibodies. Lupus anticoagulant was detected in two more patients (one with chronic hepatitis B and one with chronic hepatitis C). There was no significant difference in the prevalence of any thrombotic risk factor between chronic

Table 2 Levels of thrombophilic and coagulation factors in 90 patients with chronic hepatitis B or C

Factor	Chronic hepatitis B	Chronic hepatitis C	Total	Normal range
IgG aCL antibodies (U/ml)	5.7 (1.7) (1.6–10.1)*	4.9 (1.8) (1.1–10.1)*	5.2 (1.8) (1.1–10.1)	<10
IgM aCL antibodies (U/ml)	7.3 (5.4) (1.4–23.4)**	5.3 (2.1) (1.0–11.5)**	6.2 (4.0) (1.4–23.4)	<10
Protein C levels (%)	95 (26) (50–166)	102 (24) (46–163)	99 (25) (46–166)	70–140
Protein S levels (%)	84 (25) (46–150)	77 (25) (30–130)	80 (25) (30–150)	65–140
Antithrombin III levels (%)	95 (17) (45–121)***	110 (14) (53–136)***	103 (17) (45–136)	80–140
Plasminogen levels (mg/dl)	91 (13) (63–116)****	98 (15) (65–134)****	95 (15) (63–134)	75–140
APC ratio	2.8 (0.4) (2.1–3.8)	2.7 (0.4) (2.1–3.6)	2.8 (0.4) (2.1–3.8)	>2.2
Factor VIII levels (%)	168 (67) (71–390)	165 (64) (68–391)	166 (65) (68–391)	60–150
Factor IX levels (%)	112 (32) (59–173)	125 (43) (49–200)	119 (39) (49–200)	>60
Factor XI levels (%)	97 (29) (50–171)	107 (43) (29–200)	103 (38) (29–200)	>60
Factor XII levels (%)	101 (37) (23–200)	113 (36) (43–200)	108 (36) (23–200)	>60

Quantitative values are expressed as mean (SD) (range).

aCL, anticardiolipin; APC, activated protein C.

Chronic hepatitis B versus chronic hepatitis C patients: *p=0.04; **p=0.02; ***p<0.001; **** p=0.01.

Table 3 Prevalence of thrombotic risk factors in 90 patients with chronic hepatitis B or C

Thrombotic risk factor	Chronic hepatitis B (n (%))	Chronic hepatitis C (n (%))	Total (n (%))
aCL antibodies elevated (IgG/IgM/IgG+IgM)	4 (10.2) (0/3/1)	2 (3.9) (1/1/0)	6 (6.7) (1/4/1)
Protein C deficiency	5 (12.8)	3 (5.9)	8 (8.9)
Protein S deficiency	8 (20.5)	19 (37.3)	27 (30.0)
Antithrombin III deficiency	9 (23.1)*	1 (2.0)*	10 (11.1)
Plasminogen deficiency	2 (5.1)	3 (5.9)	5 (5.6)
APC resistance†	1 (2.6)	1 (2.0)	2 (2.2)
Prothrombin 20210A mutation‡	4 (10.2)	2 (3.9)	6 (6.7)
Factor VIII levels elevated	23 (59.0)	28 (54.9)	51 (56.7)
≥1 thrombotic risk factor	25 (64.1)	36 (70.6)	61 (67.8)
≥2 thrombotic risk factors	16 (41.0)	17 (33.3)	33 (36.7)

aCL, anti-cardiolipin; APC, activated protein C.

†None of the two cases with APC resistance was found to have the factor V Leiden mutation.

‡All six patients were heterozygous for the prothrombin 20210A mutation.

Chronic hepatitis B versus chronic hepatitis C patients: *p=0.005.

hepatitis B and C cases, except for antithrombin III deficiency that was detected significantly more frequently in chronic hepatitis B than C patients (23% v 2%; p=0.005). Elevated factor VIII levels and protein S deficiency were the most common thrombotic factors detected in 57% and 30% of cases, respectively. In particular, protein S deficiency was found relatively more frequently in chronic hepatitis C than B patients (37% v 21%; p=0.14) while elevated anticardiolipin antibodies and the prothrombin 20210A mutation (only heterozygous cases) were detected in 10% and 4% (p=0.44) and protein C deficiency in 13% and 6% of chronic hepatitis B and C patients, respectively (p=0.44). Activated protein C resistance and plasminogen deficiency were detected in less than 3% and 6% of cases. No patient had the factor V Leiden mutation. At least one thrombotic risk factor was present in 68% and two or more thrombotic risk factors in 37% of our patients (table 3). Moreover, one or more coagulation factor deficiencies (<60%) were found in 16% of 90 patients without any significant difference between chronic hepatitis B and C cases (7/39 or 17.9% v 7/51 or 13.7%, respectively; p=0.80).

Higher necroinflammatory activity (histological grading as a continuous variable) was found to be significantly associated with older age (p=0.006), lower platelet count (p=0.02), more prolonged prothrombin time (p<0.001), higher ALT levels (p=0.007), higher AST levels (p<0.001), higher histological staging (p<0.001), protein C deficiency (p=0.04), and antithrombin III deficiency (p=0.02), while there was a trend towards an association with lower fibrinogen levels (p=0.07) and plasminogen deficiency (p=0.09). Multivariate analysis showed that higher necroinflammatory activity was independently associated only with more prolonged prothrombin

time (p=0.003), higher ALT levels (p=0.011), and higher histological staging (p=0.018).

Higher histological staging (as a continuous variable) was also significantly associated with older age (p<0.001), lower white blood cell count (p=0.024), lower platelet count (p<0.001), more prolonged prothrombin time (p<0.001), higher AST levels (p=0.02), higher GGT levels (p=0.01), higher histological grading (p<0.001), protein C deficiency (p<0.001), protein S deficiency (p=0.04), antithrombin III deficiency (p<0.001), elevated factor VIII levels (p=0.047), and plasminogen deficiency (p<0.001), and relatively associated with activated protein C resistance (p=0.06). Multivariate analysis showed that higher staging was independently associated only with higher GGT levels (p=0.013) and antithrombin III deficiency (p=0.01).

Associations of histological staging scores 4–6 (advanced) or scores 0–3 with patient characteristics and with levels of thrombophilic and coagulation factors and the presence of thrombotic risk factors are presented in tables 4 and 5, respectively. Patients with compared with those without advanced staging were significantly older (52 v 42 years; p=0.001), had a lower platelet count ($170 \pm 222 \times 10^3/l$; p=0.001), more prolonged prothrombin time (12.2 ± 11.7 s; p=0.02), higher AST levels (69 ± 47 IU/l; p=0.005), higher alkaline phosphatase levels ($0.7 \pm 0.6 \times$ upper limit of normal (ULN); p=0.023), higher GGT levels ($0.9 \pm 0.5 \times$ ULN; p=0.017), and higher grading (8.1 ± 6.5 ; p=0.002) (table 4). In addition, patients with advanced staging also had significantly more frequently protein C deficiency (24% v 3%; p=0.007), antithrombin III deficiency (28% v 5%; p=0.005), and plasminogen deficiency (19% v 2%; p=0.03), and relatively

Table 4 Epidemiological, haematological, biochemical, and histological characteristics of 90 patients with chronic hepatitis B or C in relation to their histological staging

Characteristic	Staging 0–3 (n=65)	Staging 4–6 (n=25)	p Value
Sex (M/F)	47/18	17/8	0.89
Age (y)	42 (13)	52 (14)	0.001
Possible source of infection (n)			0.60
Blood transfusion	15	4	
Parenteral drug use	10	5	
Intrafamilial contact	14	5	
Health care work	0	1	
Unknown	26	10	
Haemoglobin (g/dl)	14.1 (1.5)	14.0 (1.4)	0.74
White blood cell count ($\times 10^3$ /l)	7.3 (2.1)	6.5 (1.9)	0.11
Platelet count ($\times 10^3$ /l)	222 (59)	170 (63)	0.001
Prothrombin time (s)	11.7 (0.8)	12.2 (1.0)	0.02
Partial thromboplastin time (s)	32 (4)	30 (5)	0.16
Fibrinogen levels (mg/dl)	264 (65)	266 (56)	0.88
ALT (IU/l)	80 (24–490)	115 (25–230)	0.07
AST (IU/l)	47 (19–213)	69 (33–179)	0.005
Alkaline phosphatase ($\times$ ULN)	0.6 (0.3–1.4)	0.7 (0.3–1.2)	0.023
GGT ($\times$ ULN)	0.5 (0.2–2.9)	0.9 (0.2–3.0)	0.017
Histological grading	6.5 (2.2)	8.1 (2.3)	0.002
Piecemeal necrosis score	1.8 (0.8)	2.4 (0.8)	0.004
Confluent necrosis score	0.4 (0.7)	0.7 (0.8)	0.04
Focal inflammation score*	1.9 (0.6)	2.1 (0.6)	0.16
Portal inflammation score	2.4 (0.7)	3.0 (0.7)	0.001

Quantitative values are expressed as mean (SD) or median (range).

*Focal lytic necrosis, apoptosis, and focal inflammation score.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl- transpeptidase; ULN, upper limit of normal.

more frequently activated protein C resistance (8% *v* 0%; $p=0.075$). Elevated anticardiolipin antibody levels, prothrombin 20210A mutation, protein S deficiency, and elevated factor VIII levels were not found to be associated with advanced staging. Two or more thrombotic risk factors were present in 56% of patients with advanced staging and in only 29% of those with staging 1–3 (odds ratio 3.1, 95% confidence interval 1.2–8.0; $p=0.03$), while deficiency of one or more coagulation factors was not associated with staging (table 5).

One or more significant thrombotic risk factors (antithrombin III deficiency, protein C deficiency, plasminogen deficiency, activated protein C resistance) were present in 11 (44%) of 25 patients with staging 4–6 and in only 6 (9%) of 65 patients with staging 0–3 ($p<0.001$). Logistic regression analysis showed that the presence of at least one significant thrombotic risk factor was the only variable independently associated with advanced staging (adjusted odds ratio 2.4, 95% confidence interval 1.14–5.0; $p=0.02$).

DISCUSSION

Our study has evaluated for the first time the association between thrombotic risk factors, detected by comprehensive thrombophilic screening, and severity of necroinflammatory activity or fibrosis in patients with chronic viral hepatitis. At least one thrombotic risk factor was detected in 68% and two or more factors in 37% of patients with chronic hepatitis B or C. As protein C, protein S, antithrombin III, and plasminogen are produced by the liver, their levels may decrease in patients with chronic liver disease.¹⁶ This is usually considered to be the case in established cirrhosis where such anticoagulant factors decrease in parallel with reductions in the levels of coagulation factors.¹⁶ However, deficiency of coagulation factor VIII, IX, XI, or XII was found in only 16% of our patients and thus impairment of hepatocellular production cannot be exclusively to blame for the observed high prevalence of protein C, protein S, antithrombin III, and plasminogen deficiency. It should also be noted that patients with clinical signs of cirrhosis were excluded from our study and that only

13% of the patients included had a fibrosis score of 5 or 6, corresponding to the cirrhotic stage.

Thrombotic risk factors were found to be independently associated with the extent of fibrosis but not with the degree of necroinflammatory activity. In particular, antithrombin III deficiency was independently associated with more extensive fibrosis, and the presence of at least one of four significant thrombotic risk factors (antithrombin III, protein C, plasminogen deficiency, and activated protein C resistance) was the only variable associated with advanced staging of chronic hepatitis. The observed association between thrombotic risk factors and advanced staging supports the hypothesis of vascular obstruction for the histological progression of chronic viral hepatitis.^{14–15} Obliteration of small portal and hepatic veins due to thrombosis and phlebitis has been proposed as an important factor for the progression of chronic liver disease as it results in local hepatocyte death and the development of fibrosis (parenchymal extinction).^{14–15} In fact, in cirrhotic livers, obstruction of small intrahepatic portal and hepatic veins is observed almost invariably.^{17–18} Thus changes in the composition of the blood towards a hypercoagulable state in combination with changes in the endothelium of intrahepatic vessels and/or in intrahepatic blood flow, which often develop in chronic liver disease with fibrosis,^{19–20} certainly favour the development of thrombosis in intrahepatic veins. Thus one could hypothesise that patients with chronic viral hepatitis who carry specific genetic or develop more easily specific acquired thrombotic risk factors have a more rapid progression to more severe staging of chronic hepatitis. Alternatively, the higher prevalence of some thrombotic risk factors in patients with more severe staging may reflect greater impairment of anticoagulant production by patients with more advanced liver disease. Even if this is the case, however, the secondary development of anticoagulant deficiency favours the development of intrahepatic vein thrombosis and further parenchymal extinction.

Elevated levels of factor VIII and protein S deficiency were the two thrombotic risk factors that were most frequently detected in our population but none was found to be

Table 5 Levels of thrombophilic and coagulation factors or presence of thrombotic risk factors in 90 patients with chronic hepatitis B or C in relation to their histological staging

Factor	Staging 0–3 (n=65)	Staging 4–6 (n=25)	p Value
IgG aCL antibodies (U/ml)	5.1 (1.8)	5.6 (1.9)	0.21
IgM aCL antibodies (U/ml)	6.1 (4.1)	6.3 (3.7)	0.84
aCL antibodies elevated (n (%)) (IgG/IgM/IgG+IgM)	4 (6.2) (0/3/1)	2 (8.0) (1/1/0)	—
Protein C levels (%)	104 (23)	84 (25)	<0.001
Protein C deficiency (n (%))	2 (3.1)	6 (24.0)	0.007
Protein S levels (%)	84 (25)	71 (22)	0.02
Protein S deficiency (n (%))	16 (24.6)	11 (44.0)	0.12
Antithrombin III levels (%)	108 (14)	93 (19)	<0.001
Antithrombin III deficiency (n (%))	3 (4.8)	7 (28.0)	0.005
Plasminogen (mg/dl)	97 (14)	90 (14)	0.045
Plasminogen deficiency (n (%))	1 (1.5)	4 (19.0)	0.03
APC ratio	2.8 (0.4)	2.8 (0.4)	0.74
APC resistance (n (%))	0	2 (8.0)	0.075
Prothrombin 20210A mutation* (n (%))	5 (7.7)	1 (4.0)	0.88
Factor VIII levels (%)	157 (61)	192 (70)	0.02
Factor VIII levels elevated (n (%))	34 (52.3)	17 (68.0)	0.27
Factor IX levels (%)	121 (41)	121 (36)	0.95
Factor XI levels (%)	104 (38)	99 (38)	0.57
Factor XII levels (%)	106 (41)	111 (24)	0.61
≥ 1 coagulation factor deficiency (n (%))	12 (18.5)	2 (8.0)	0.33
≥ 1 significant thrombotic risk factor† (n (%))	6 (9.2)	11 (44.0)	<0.001
≥ 1 any thrombotic risk factor (n (%))	42 (64.6)	19 (76.0)	0.43
≥ 2 any thrombotic risk factors (n (%))	19 (29.2)	14 (56.0)	0.03

Quantitative values are expressed as mean (SD).

aCL, anticardiolipin; APC, activated protein C.

*All six patients were heterozygous for the prothrombin 20210A mutation.

†Significant thrombotic risk factors included protein C deficiency, antithrombin III deficiency, plasminogen deficiency, and APC resistance.

associated with staging of disease. Levels of factor VIII and Willebrand's factor are known to increase in chronic liver disease but this is usually due to a disproportionate increase in procoagulant antigens compared with functional coagulant active proteins of these two factors.^{16–21} Of protein S plasma concentrations, 60% are bound to a complement binding protein, which increases in inflammatory conditions, and only 40% represent the active free form of protein S, which is a cofactor of activated protein C and not a direct anticoagulant.^{16–22} Thus laboratory findings of elevated levels of factor VIII or protein S deficiency may not always reflect abnormal anticoagulant activity.

Antithrombin III, protein C, and plasminogen deficiency were the thrombotic risk factors that were found to be significantly associated with advanced staging while there was a trend towards an association with activated protein C resistance. In particular, antithrombin III or protein C deficiency as well as activated protein C resistance are considered to be strong thrombotic factors increasing the risk by approximately 8–10-fold.²³ In contrast, the presence of the prothrombin 20210A mutation, which was not associated with severity of fibrosis in our study, is generally considered to be a mild thrombotic risk factor increasing the risk by only 2–3-fold.²³ Finally, the presence of two or more of any of the thrombotic risk factors, which represents a condition with an increased risk of thrombosis,²³ was also significantly associated with advanced staging.

Increased levels of anticardiolipin but with no anti-beta2-glycoprotein I antibodies, which are usually needed for the development of thrombosis associated with positive anticardiolipin antibodies, or the presence of lupus anticoagulant, were not associated with histological staging in our study. Anticardiolipin antibodies have been reported to be present in up to 44% of patients with hepatitis C virus infection but their clinical significance remains controversial.²⁴ In chronic hepatitis C, such antibodies have sometimes been associated with the development of thrombocytopenia, portal hypertension, or

thrombotic events.^{24–26} Positive anticardiolipin antibodies were detected in only 4% and 10% of our chronic hepatitis C and B patients, respectively. None of our six patients with positive anticardiolipin antibodies had a history of thrombotic episodes or clinical signs of portal hypertension, only two had advanced histological staging, and only one had a relatively low platelet count ($120 \times 10^3/l$). Thus our data are in agreement with reports suggesting that anticardiolipin antibodies are positive in less than 5% of chronic hepatitis C patients and seem to represent an epiphenomenon without any clinical relevance.^{24–27}

The prevalence of thrombotic risk factors or of coagulation factor deficiency evaluated in this study did not significantly differ between chronic hepatitis B and C patients, except for antithrombin III deficiency which was observed in 23% and 2% in the two groups, respectively. This isolated difference may represent a specific effect of chronic hepatitis B virus infection or may be associated with more severe liver disease in chronic hepatitis B than C patients. Regarding liver disease severity, chronic hepatitis B compared with chronic hepatitis C patients had significantly lower white blood cell and platelet counts, but similar biochemical (aminotransferases levels) and histological (grading and staging) findings as well as a similar prevalence of all other thrombophilic or coagulation factors (tables 1, 3).

In conclusion, our data suggest that thrombotic risk factors are frequently detected in patients with chronic viral hepatitis and that the presence of at least one of the significant factors (antithrombin III, protein C, or plasminogen deficiency, and activated protein C resistance) is associated with more extensive fibrosis and advanced staging. Whether the association of such thrombophilic conditions with advanced staging of chronic viral hepatitis is a primary or secondary phenomenon and whether their development in combination with local inflammation accelerate the progression of liver fibrosis need further evaluation.

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