Annals of Gastroenterology (2011) **24,** 47-52

Platelet IgG antibodies are significantly increased in chronic liver disease

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Abstract

Background The aim of this study was to investigate the presence of IgG antiplatelet (anti-P) IgG antibodies in patients with chronic liver disease (CLD) of diverse but well defined etiology.

Methods One-hundred fifty-six consecutive patients with CLD (65 with chronic hepatitis B, 57 with chronic hepatitis C, 23 with alcoholic liver disease and 11 with primary biliary cirrhosis), and 240 healthy blood donors were investigated for the presence of anti-P antibodies.

Results Anti-P antibodies were present in 36.5% (57/156) of patients with CLD, and 2.9% (7/240) of controls (P=0.0001). In detail, anti-P antibodies were detected in 35.4% (23/65) of patients with chronic hepatitis B, 26.3% (15/57) of patients with chronic hepatitis C, 47.8% (11/23) of patients with alcoholic liver disease and 72.7% (8/11) of those with primary biliary cirrhosis. The study also demonstrated the significantly higher prevalence of anti-P antibodies in patients with cirrhosis (53.0%) than in non cirrhotic patients (26.4%, P=0.0018). The association of anti-P antibodies with thrombocytopenia was inconsistent.

Conclusions This study showed a high prevalence of anti-P IgG antibodies in patients with CLD compared to healthy controls.

Keywords antiplatelet antibodies, chronic liver disease, thrombocytopenia, chronic hepatitis, cirrhosis

Ann Gastroenterol 2011; 24 (1): 47-52

Introduction

Thrombocytopenia is a common finding in patients with chronic liver disease (CLD) [1]. More than one third of cirrhotic patients develop clinically apparent thrombocytopenia. In many cases, the severity of thrombocytopenia is directly related with the stage of CLD [2].

The cause of thrombocytopenia in CLD patients is complex. The sequestration of platelets in the splenic pool attributed to hypersplenism, the increased destruction of platelets and the reduced production of them in the bone marrow contribute to thrombocytopenia. The redistribution of platelets in the splenic pool is considered the most important cause of thrombocytopenia in CLD patients [3,4]. The increased destruction of circulating platelets is the second most important cause of thrombocytopenia and it is due to activation of low-grade disseminated intravascular coagulation [5] and increased destruction of platelets through autoimmune mechanisms [6,7] In fact, platelets isolated from patients with CLD of diverse etiology (such as chronic viral hepatitis, alcoholic hepatitis, alcoholic cirrhosis and primary biliary cirrhosis) had increased levels of bound immunoglobulins that were combined with high levels of circulating immune complexes in serum [2,7,8]. No correlation between the size of the spleen and the amount of platelet specific immunoglobulin was found, and this may partly explain the inconstant relationship between the size of the spleen and the degree of thrombocytopenia in patients with chronic liver disease [9].

Thrombocytopenia develops only if the increased production of platelets in the bone marrow cannot counterbalance the increased removal of platelets from the circulation [10]. Infections, drugs, toxins and deficiencies of nutrients can suppress the bone marrow and actually aggravate thrombocytopenia in CLD patients [11].

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Conflict of interest: None

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Received 4 October 2010; accepted 13 December 2010

Few previous studies have demonstrated the presence of true antiplatelet (anti-P) antibodies in CLD patients [12,13]. The aim of this study was to investigate the presence of IgG anti-P antibodies in patients with chronic liver diseases of diverse but well-defined etiology, since so far there is no well established data with regard to the prevalence of these antibodies among patients with liver diseases. In addition, this study tried to appreciate the influence of anti-P antibodies on the degree of thrombocytopenia in CLD patients.

Patients – Methods

Patients

One hundred and fifty six consecutive patients of the Outpatient Hepatology Clinic with chronic liver disease (CLD) and 240 healthy blood donors of comparable gender and age were investigated for the presence of anti-P antibodies [Table 1].

The mean age of patients with CLD was 50.96±13.10 years. Ninety four of them were males and the remaining 62 were females.

The 240 healthy controls of this study came from the Blood Bank of the University Hospital of Ioannina. They were randomly selected, except for preferring blood donors of older age, so that they would be comparable to the patients of the study. The mean age of the healthy controls was 45.06±7.26 years, 131 were males and 109 were females. The characteristics of all subgroups of the study are shown in Table 1.

To include a patient or control in the study, he should not have been a recipient of blood products within the last three years. Although there was not a specific exclusion criterion for decompensated cirrhotics in our study, we did not include patients who had received blood products. This was done to preclude confounding factors such as HLA antiplatelet antibodies related to transfusion. However, in any case, we used a method that could discriminate true anti-P antibodies from HLA related to transfusion anti-P as described below. In addition, we excluded patients who were receiving drugs related to thrombocytopenia. Platelet count measurements were done with an automatic analyzer and additionally to a blood smear that was extracted without anticoagulant to exclude false thrombocytopenia. All patients underwent routine haematological and biochemical tests and were tested for the viruses of hepatitis B, hepatitis C, hepatitis D, and HIV. Anti-nuclear and anti-smooth muscle antibodies, as well as cryoglobulins were measured in all CLD patients. In addition, all CLD patients underwent routinely an abdominal ultrasound examination with emphasis on liver and spleen. Splenomegaly was defined by palpation and ultrasound splenic index.

Methods

For the detection of the anti-platelet (anti-P) antibodies we used a commercially available method, the solid phase red cell adherence test (SPRCA test, P-Capture Ready Screen, Immucor, Norcross, USA). This method is designed for the laboratory detection of IgG anti-P antibodies [14-17]. Incubation of platelet monolayer of the wells of the kit with choroquine diphosphate for 1 hour at 37^0C can help the separation of true anti-P antibodies (against specific membrane components of platelets) from anti-HLA antibodies against platelets. In our study, every experiment with a positive anti-P result was repeated after incubation with a commercially prepared chloroquine solution and if only anti-P was positive again the serum sample was considered truly positive for anti-P antibodies. Some of the serum samples were also tested by the method of the platelet suspension immunofluorescence test (PSIF test) for confirmation. The PSIF test was performed according to the standard method of von der Borne [18].

Statistical analysis

Statistical comparisons were made using the Statistica (Statsoft Corporation) electronic package, version 5. For the

Table 1 Subgroups of the patients and controls of the study

Disease	No of patients	Mean age	Sex (M/F)
Chronic hepatitis B	45	46.00 ± 12.14	32/13
Chronic hepatitis C	41	47.70 ± 12.29	21/20
Cirrhosis due to hepatitis B	20	53.25 ± 9.16	12/8
Cirrhosis due to hepatitis C	16	64.06 ± 5.44	8/8
Alcoholic liver disease	23	59.08 ± 9.73	21/2
Primary biliary cirrhosis	11	58.09 ± 9.53	0/11
Controls	240	45.06 ± 7.26	131/109

comparison of prevalence of anti-P antibodies among the various groups we used methods for categorical data, such as X^2, paired Wilcoxon test and statistical correlation. For numerical data, we estimated the mean value and standard deviation and we used t-test for unpaired or paired samples as appropriate. Platelet count as a biological parameter follows a rather normal distribution, so t-test was considered appropriate for the comparisons.

Results

Comparison of the two used methods

The two used methods for the detection of anti-P antibodies were compared. All available serum samples (n=438) underwent SPRCA testing, while PSIF testing was used for detection of anti-P in 100 serum samples, which were randomly selected among the available serum samples. Both methods gave similar results, as shown in Table 2. To evaluate the variation of the SPRCA test, we re-evaluated 30 previously tested serum samples. The results of the repeated experiments were consistent with the initial results. Therefore both tested methods were found accurate, comparable and effective for the detection of anti-P antibodies, as previously reported [16-18]. The following results are given according to the SPRCA test, which was used on all occasions.

Prevalence of antiplatelet antibodies

Overall we found that anti-P antibodies were present in 36.53% (57/156) of patients with chronic liver disease (CLD) and 2.91% (7/240) of controls. The prevalence of anti-P was significantly higher in CLD patients than in controls (P=0.0001, Fisher's Exact test). These results are shown in Table 3.

We further analyzed the results in CLD patients. Anti-P were positive in 35.38% (23/65) of patients with chronic hepatitis B, 26.31% (15/57) of patients with chronic hepatitis C, 47.80% (11/23) of patients with alcoholic liver disease and 72.72% (8/11) of patients with primary biliary cirrhosis. Prevalence of anti-P was significantly higher in patients with primary biliary cirrhosis than in those with chronic hepatitis B (P=0.023) or those with chronic hepatitis C (P=0.0051).No other significantly different frequencies were found.

The frequencies of anti-P antibodies in the subgroups of CLD patients are shown in Table 4.

Table 2 Comparison of SPRCA test and PSIFT test. Results from 100 serum samples

Test results	anti-P
Positive in both methods	22
Positive only in PSIF test	0
Positive only in SPRCA test	2
Negative in both methods	76

Pearson correlation r=0.94 (P<0.001).

Table 3 Prevalence of antiplatelet antibodies in the main study groups

Study group	anti-P (+)
Chronic liver disease	36.53% (57/156)
Healthy controls	2.91% (7/240)

P=0.0001

Correlation of antiplatelet antibodies with the platelet count

The next step of the study included a correlation of anti-P with the platelet count in the various groups and subgroups of CLD patients. The mean platelet count results are presented in Table 5. In patients with chronic hepatitis B (n=65), the mean platelet count did not differ between patients with negative and positive anti-P [P=0.222]. The same results were found when we studied separately the subgroups of chronic hepatitis B patients with or without cirrhosis. In patients with chronic hepatitis C (n=57), the mean platelet count in patients with negative anti-P was significantly higher than in those with positive anti-P [P=0.002]. However, when we studied separately the subgroups of chronic hepatitis C patients with or without

Table 4 Prevalence of positive antiplatelet antibodies in the subgroups of patients with chronic liver disease

Disease	Total	No cirrhosis	Cirrhosis	P
Chronic hepatitis B	35.38% (23/65)	26.82% (11/41)	40% (8/20)	0.220
Chronic hepatitis C	26.31%(15/57)	19.51% (7/41)	43.75% (7/16)	0.065
Alcoholic liver disease	47.8% (11/23)	36.36% (4/11)	58.88% (7/12)	0.412
Primary biliary cirrhosis	72.72% (8/11)	60.00% (3/5)	83.33% (5/6)	0.391

Table 5 Mean platelet count in various groups of patients with chronic liver disease

Group – Disease	Mean platelet count	
Chronic hepatitis B – all patients	179,228±59,980 /mm3	
Chronic hepatitis C – all patients	183,120±63,864 /mm3	
Alcoholic liver disease	150,076±75,038 /mm3	
Primary biliary cirrhosis	153,900±82,384 / mm3	
*Chronic hepatitis B - no cirrhosis	190,179±44,207 /mm3	P=0.006
*Chronic hepatitis B - cirrhosis	124,071±47,769 /mm3	P=0.014
**Chronic hepatitis C – no cirrhosis	209,142±47,623 /mm3	
**Chronic hepatitis C - cirrhosis	122,400±55,892 /mm3	
¶Chronic liver disease – no cirrhosis	176,925±46,988 /mm3	
¶Chronic liver disease – cirrhosis	122,209±52,103 /mm3	

** P<0.01 between chronic hepatitis B patients with cirrhosis vs no cirrhosis*
*** P <0.05 between chronic hepatitis C patients with cirrhosis vs no cirrhosis*
¶ P<0.000 between all chronic liver disease patients with cirrhosis vs no cirrhosis

cirrhosis this difference became weaker and non-significant.

In patients with alcoholic liver disease (n=23), the mean platelet count in patients with negative anti-P did not differ from the mean platelet count in those with positive anti-P [P=0.755].

In patients with primary biliary cirrhosis (n=11) the mean platelet count was 153,900±82,384/mm3. Four of them had a platelet count below 100,000/mm3. All these four patients had histologically proven cirrhosis and 3 of the 4 had positive anti-P.

In total, the mean platelet count in patients with CLD (n=156) was 183,209±58,388/mm3 in those with negative anti-P (n=99) and 163,714±73,669/mm3 in those with positive anti-P (n=57) [P=0.098].

In addition, in CLD patients (n=156) the mean platelet count was 122,209±52,103 in those with cirrhosis (n=53) and 176,925±46,988/mm3 in those without cirrhosis (n=103) [P=0,000034]. There was a significantly higher frequency of anti-P antibodies in cirrhotic patients (53 %, 55/103) than in non-cirrhotic patients (26%, 14/53) [P=0.0018, Fisher's Exact test]. In conclusion, cirrhotic patients irrespectively of etiology of cirrhosis had a higher frequency of anti-P than non-cirrhotic patients.

Finally, a mean platelet count below 100,000/mm3 was found in 19 patients with CLD. Thirteen of them had positive anti-P (13/19, 68%). Four of 19 had chronic hepatitis B, 3 had chronic hepatitis C, 5 had alcoholic liver disease and 4 had primary biliary cirrhosis. Eighteen of these 19 patients had cirrhosis.

It is interesting to mention that splenomegaly during clinical examination or ultrasound evaluation was found in 17 of 156 CLD patients. Fourteen of these 17 had cirrhosis and 10 of 17 patients had positive anti-P. The mean platelet count in patients with splenomegaly was 93,117±33,175/mm3.

Discussion

This study examined the prevalence of anti-P antibodies in CLD patients in comparison with normal controls. The frequency of anti-P was studied in various groups and subgroups of CLD patients. We also investigated any correlation of anti-P antibodies with the mean platelet count in CLD patients. Finally, we found a significant coexistence of anti-P antibodies and thrombocytopenia in patients with CLD, but we did not demonstrate that these anti-P antibodies were the real pathogenetic cause of thrombocytopenia.

About two decades ago, pioneer investigators found increased levels of platelet-associated IgG in patients with advanced chronic liver disease and hypothesized that it could be related to the development of thrombocytopenia [19]. In contrast, they did not find increased levels of platelet-associated IgG in patients with acute hepatitis or with mild histological findings in liver biopsy. One year later, another study demonstrated high levels of platelet-associated IgG in a high percentage of patients with alcoholic or cryptogenic chronic liver disease [6]. The study correlated these findings with the presence of hypergammaglobulinemia and circulating immune complexes in cirrhotic patients, but failed to show any correlation between platelet-associated IgG and thrombocytopenia. On the contrary, in another study, Australian investigators found that the increased levels of platelet-associated IgG (75%) and circulating immune complexes (37.5%) were related to thrombocytopenia in patients with autoimmune or cryptogenic hepatitis [20].

Recently, newer studies with other precise methods (antigen capture assays with monoclonal antibodies) have been performed to detect anti-P antibodies in serum or on the surface of platelets. In a study using this method [12],

the authors found positive anti-P antibodies in 64% (23 of 36) of chronic liver disease patients of diverse etiology (e.g. alcoholic, viral). These anti-P antibodies were directed against the main platelet antigens which have been identified in common diseases with autoimmune thrombocytopenia (e.g. idiopathic autoimmune thrombocytopenia, systemic lupus erythematosus), namely the glycoprotein complexes GP-Ib/IX (83%) and GP-IIb/IIIa (51%). No direct correlation between anti-P antibodies and thrombocytopenia was shown in that study. Another recent study concluded that anti-P antibodies contribute to thrombocytopenia associated with chronic hepatitis C virus infection [21].

A very interesting study investigating the role of autoantibodies against the major platelet surface autoantigen GPIIb-IIIa in thrombocytopenia in cirrhotic patients has been published [22]. The same authors had previously established a convenient and sensitive assay for identifying patients with autoantibody-mediated thrombocytopenia by detecting circulating B cells that produce anti-GPIIb-IIIa antibodies [23,24]. In the recent study, circulating B-cells producing anti-GPIIb-IIIa antibodies as well as platelet-associated plasma anti-GPIIb-IIIa antibodies were examined in patients with liver cirrhosis, idiopathic thrombocytopenic purpura (ITP) and healthy controls. The frequency of anti-GPIIb-IIIa antibody-producing B-cells in patients with liver cirrhosis and ITP was significantly greater than in healthy controls and anti-GPIIb-IIIa antibodies were mainly present on the surfaces of circulating platelets rather than in the plasma in an unbound form. The authors concluded that the similar profile of anti-GPIIb-IIIa autoantibody response in patients with liver cirrhosis and ITP suggested that autoantibody-mediated platelet destruction might contribute at least in part to cirrhotic thrombocytopenia [22,25].

The present study included a high number of CLD patients of diverse etiology and examined the presence of anti-P antibodies in them, but also in healthy controls. Using a sensitive and commercially available assay (confirmed by another approved assay), it showed a high frequency of anti-P antibodies in CLD patients (36.53%, 57 of 156 patients). This frequency was much higher than that found in healthy controls (2.91%, 7 of 240). Anti-P antibodies were detected in all studied groups of CLD patients, but not in the healthy controls, showing specificity of the finding for CLD. Prevalence of anti-P did not differ significantly between patients with chronic hepatitis B or C and the results could be attributed to the hyperglobulinemia observed in patients with chronic viral hepatitis and especially with cirrhosis. The tendency for a lower platelet count in patients with chronic hepatitis C and positive anti-P antibodies compared to those with negative anti-P could mean that anti-P antibodies are active in those patients and contribute to the development of thrombocytopenia. But still, because of the compensatory mechanisms for platelet production in the bone marrow and the ample production of platelets in humans, the autoimmune destruction of platelets has limited clinical significance even in this clinical setting [21,26].

The accuracy of our findings was supported by using two different methods for the detection of antiplatelet antibodies; the traditional platelet suspension immunofluorescence test developed by van der Borne and a new sensitive commercially available assay, the solid phase red cell adherence test (SPRCA test, P-Capture Ready Screen, Immucor, Norcross, USA). This method is designed for the laboratory detection of IgG anti-P antibodies and has been found reliable and reproducible. Incubation of platelet monolayer of the wells of the kit with choroquine diphosphate for 1 hour at 37^0C enabled us to detect only true anti-P antibodies (against specific membrane components of platelets) and not HLA related antibodies against platelets.

The frequency of anti-P antibodies varied among different groups of patients with CLD. Anti-P antibodies were significantly more frequent in patients with primary biliary cirrhosis than in those with chronic viral hepatitis B or C. In addition, anti-P were significantly more frequent in cirrhotic than in non-cirrhotic patients.

In another study, anti-P were found in 40% of patients with primary biliary cirrhosis, but not in patients with alcoholic liver disease [13]. No clear correlation of anti-P with thrombocytopenia was proven. The same authors have found anti-P in only about 50% of patients with idiopathic thrombocytopenic purpura. The high specificity of their method with monoclonal antibodies may account for these rather low percentages. In addition, in that study, the frequency of anti-P on platelet surface was similar to the frequency of anti-P in serum.

In conclusion, most studies have demonstrated a high frequency of anti-P in patients with primary biliary cirrhosis (40-70%) and a lower but considerable frequency of anti-P in other groups of CLD. We have previously shown that treatment of viral hepatitis with interferon-a does not induce anti-P antibodies in most patients [8].

Our study showed, as expected, that cirrhotic patients had a lower mean platelet count than non-cirrhotic patients with CLD. In addition, cirrhotic patients had a statistically significant higher prevalence of anti-P antibodies than non-cirrhotic patients. Of the CLD patients (n=156) of the study with a platelet count below 100,000/mm^3 (n=19), 68% had positive anti-P antibodies and 94.7% had cirrhosis. In addition, 17 of 156 CLD patients (10.89%) had splenomegaly (on clinical examination or ultrasound splenic index). Finally, 82.35% of patients with splenomegaly had cirrhosis and 58.82% had positive anti-P antibodies.

The lack of permanent association between platelet count and the presence of anti-P antibodies could be attributed to the complex mechanism of thrombocytopenia in CLD patients. Anti-P antibodies may reduce the mean life span of platelets, but other factors, such as sequestration of platelets in the spleen and perhaps reduced production of platelets from megakaryocyte in the bone marrow are usually required in CLD patients for the development of clinically evident thrombocytopenia [27,28]. It has also been shown that patients with elevated platelet-associated IgG may have normal platelet counts [29]. A defective macrophage Fc-receptor function has been implicated in the reduced clearance of IgG sensitized

autologous cells in CLD patients with alcoholic cirrhosis [30]. In other words, reduced clearance of the IgG sensitized platelets by the reticuloendothelial system may partly counterbalance the effect of anti-P antibodies on platelet count [12,31,32]. In any case, the main platelet antigens for anti-P antibodies remain the glycoprotein complexes GP-Ib/IX and/or GP-IIb/IIIa [12,13,33].

In conclusion, this study demonstrated a high prevalence of anti-P antibodies in CLD patients. The latter prevalence was higher in certain groups of CLD patients, such as primary biliary cirrhosis, or in the presence of cirrhosis. These antibodies may contribute to the induction or aggravation of thrombocytopenia observed in cirrhotic patients.

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