

Thrombocytopenia in Chronic Hepatitis C

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Abstract

Background & Aims: Thrombocytopenia in patients with chronic hepatitis C may be the result of several factors: bone marrow inhibition, the decrease of liver thrombopoietin production and an autoimmune mechanism. Clinical variables such as age, gender, severity of liver disease and degree of viremia could influence the severity of platelet reduction. The goal of this study is to determine the prevalent mechanism of thrombocytopenia in patients with chronic hepatitis C and the clinical predictors of its severity. **Methods:** Eighty-one patients with chronic hepatitis C and thrombocytopenia were included. The viral inhibition on the bone marrow (central mechanism) was studied by performing bone marrow biopsy from the iliac crest. The presence of antiplatelet antibodies by ELISA assessed the peripheral mechanism. The clinical predictors included in the analysis were: age, gender, ALT level, liver fibrosis stage and HCV RNA. **Results:** Coexistence of a central and peripheral mechanism was found in the vast majority (93.3%) of patients with severe thrombocytopenia (< 100,000/ μ L) and in most patients (61.53%) with moderate thrombocytopenia (100,000- 125,000/ μ L). In patients with less severe thrombocytopenia (126,000-149,000/ μ L), autoimmune destruction was the sole mechanism (85%). Thrombocytopenia was significantly associated with ALT values, viral load and stage of fibrosis. **Conclusions:** Our data demonstrates that chronic hepatitis C is associated with a variable degree of thrombocytopenia. As the disease advances, the platelet count decreases and, in most cases, both mechanisms are involved. The stage of fibrosis is one of the major determinants of thrombocytopenia.

Key words

Thrombocytopenia – bone marrow inhibition – antiplatelet autoantibodies – liver fibrosis – HCV RNA.

Introduction

Chronic hepatitis C has a prevalence of 3.23% in Romania and is a major health problem. Most of the patients (95.5%) are affected by genotype 1 [1]. Among the haematological derangements in chronic hepatitis C, the decrease of platelet number seems to be the most common [2-4].

The thrombocytopenia has a negative impact on the evolution of the disease, mainly in the advanced stages, when the platelet number falls below 50,000/ μ L [5]. In patients with thrombocytopenia, the hemorrhagic risk is augmented by a concomitant decrease in the platelet function.

Patients with chronic hepatitis C infection may have thrombocytopenia on presentation or this may develop consequently on account of the initiation of the antiviral treatment. The chronic hepatitis C associated thrombocytopenia may be the result of three pathological processes (Fig.1). The goal of our study was to identify the most prevalent mechanism leading to thrombocytopenia prior to initiation of the antiviral therapy.

Methods

The present study included 81 patients (age range 18-65 years) with chronic hepatitis C and thrombocytopenia.

Thrombocytopenia was defined as a peripheral platelet count below 150,000/ μ L. Patients with compensated or decompensated cirrhosis were excluded by clinical, biological, endoscopic and imaging criteria.

After medical history and physical examination, the following laboratory data were obtained: complete blood count, coagulation parameters, liver function tests. The clinical data analyzed for possible association with the degree of thrombocytopenia were: age, gender, degree of liver impairment (ALT level and the fibrosis score) and viremia (HCV viral load).

In all patients, presence of hepatitis B surface antigen

Received: 10.08.2010 Accepted: 09.11.2010

J Gastrointest Liver Dis

December 2010 Vol.19 No 4, 381-385

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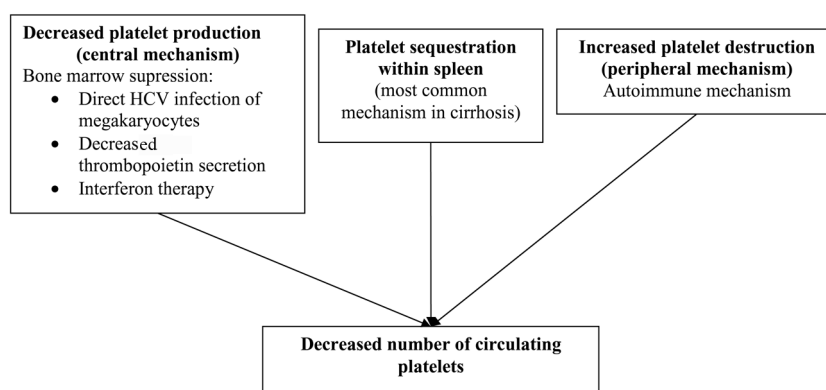


Fig 1. Mechanisms of thrombocytopenia in chronic hepatitis C [8].

(HBsAg), anti HCV antibodies and anti HIV antibodies was tested.

The liver involvement was evaluated with abdominal ultrasonography and percutaneous liver biopsy. Fibrosis was evaluated according to the Metavir score. Esogastroduodenoscopy was performed in selected cases, in order to exclude portal hypertension.

Bone marrow biopsy was performed at the level of the postero-superior iliac crest. The bone marrow was examined for megakaryocyte series (megakaryocytes hypoplasia), megakaryocyte function (thrombogenesis), and identification of any dysplastic changes. The presence of any of the above criteria indicated a central mechanism for thrombocytopenia.

We searched for the antiplatelet autoantibodies by 3rd generation ELISA tests. The following antibodies bound to the platelet surface were determined: anti IIb/IIIa glycoprotein, anti Ia/IX glycoprotein and anti Ia/IIa glycoprotein. The presence of any of these defined a positive result.

The degree of viremia was quantified with an HCV RNA by PCR assay. The viral load was quantified by an Amplicor HCV Monitor TM20 (Roche Molecular Systems, detection limit 50 IU/ml).

Depending on the severity of platelet reduction (normal range: 150,000 to 450,000 platelets/ μ L), the patients were divided into 3 groups: with severe thrombocytopenia: < 100,000/ μ L ; moderate thrombocytopenia: 100,000 - 125,000/ μ L and mild thrombocytopenia: 126,000 - 149,000/ μ L.

Statistical analysis

Linear regression was used to investigate the independent risk factors for thrombocytopenia. Means, presented as mean values \pm standard deviation, were compared using the nonparametric ANOVA test. The relationship between fibrosis and platelet counts was demonstrated with the Spearman's rank correlation test. The threshold of statistical significance was $p < 0.05$ and the correlation coefficient was $-1 < r < 1$; an r coefficient 0.34 - 0.66 indicates medium strength relationships and $r > 0.67$ indicates strong relationships. We used an EpiData v3.0 database with an EPI Info 2008-CDC

Atlanta statistical package and for the Spearman's rank correlation test SPSS statistical package.

Results

Eighty-one thrombocytopenic patients with hepatitis C were included in the study. The basic demographic and clinical characteristics are shown in Table I.

The 81 patients comprised 29 men (35.81%) and 52 women (64.19%).

Most of the patients had mild thrombocytopenia (Table I). The platelet count varied between 84,000/ μ L and 149,000/ μ L, with a mean value of 122,444.4/ μ L (95%CI: 118,270.7-126,598.1).

For each degree of thrombocytopenia, we divided

Table I. Basic characteristics of the 81 patients studied

Characteristics	
Age (years)*	44.98 \pm 10.20
Sex	
Male, n (%)	29 (35.81)
Female, n (%)	52 (64.19)
ALT (IU/L) *	72.01 \pm 22.95
HCV RNA*	2,780,360.11 \pm 2,002,004.16
Platelet counts, n (%)	
under 100,000/ μ L	15 (18.52)
between 100,000 - 125,000/ μ L	26 (32.09)
between 126,000 - 149,000/ μ L	40 (49.39)
BMB, n (%)	12 (14.81)
Autoantibody, n (%)	38 (46.91)
BMB + Autoantibody, n (%)	31 (38.28)
Fibrosis	
Stage 1, n (%)	2 (2.5)
Stage 2, n (%)	39 (48.1)
Stage 3, n (%)	40 (49.4)

* Mean \pm SD. ALT: alanine aminotransferase; BMB: bone marrow biopsy

the patients into subsets, based on the suspected causal mechanism: central, peripheral or both (Fig.2).

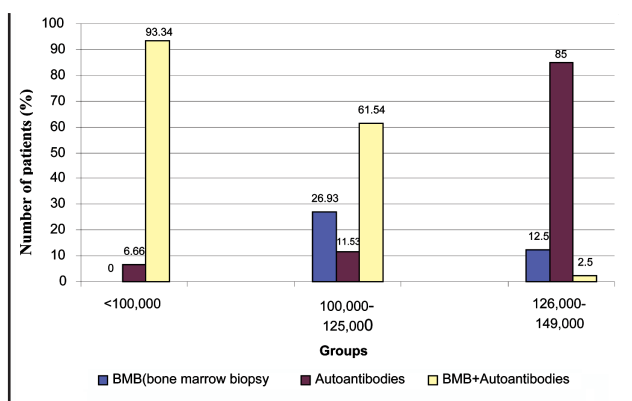


Fig 2. Classification of patients considering the platelet number and causes of thrombocytopenia.

In almost all patients (93.34%) presenting with severe thrombocytopenia the peripheral and central mechanism co-existed. For patients with moderate thrombocytopenia, 26.93% had only bone marrow inhibition, 11.53% had autoimmune destruction, while a majority (61.54%) demonstrated the presence of both mechanisms. In the mild thrombocytopenia group, the prevailing mechanism was peripheral.

Using the correlation analysis and the linear regression we searched for any statistically significant relationships between the platelet counts and age, ALT serum level, HCV RNA load, gender and stage of fibrosis.

The average age of our patients was 44.99 years (95% CI: 42.73 - 47.24) (range 23-63 years). A positive correlation was established between the presence of thrombocytopenia and age (correlation coefficient $r = 0.3741$, $p = 0.0004$).

Serum ALT values ranged from 34 IU/L to 121 IU/L, with an average value of 72.01 (95% CI: 66.94 – 77.09). The linear regression analysis revealed a statistically significant relationship between the platelet counts and the blood ALT level (correlation coefficient $r = 0.734$, $p = 0.000$).

The viral load varied from 20,874 IU/L to 9,780,672 IU/L with a mean value of 2,780,360.11 (95% CI: 2,337,680.6 – 3,223,039.6). There was a statistically significant relationship between the viral load and the platelet count (correlation coefficient $r = 0.529$, $p = 0.000$).

We found no correlation between the sex and platelet counts ($p = 0.86$) using the non parametric ANOVA test.

According to the Metavir score for liver fibrosis, most patients were in stage 2 and 3 (Table I). We evidenced a statistically significant inverse correlation between the platelet count and the stage of liver fibrosis ($\rho = -0.86485$, $p = 0.00$): subjects with low platelet counts had a significantly higher stage of fibrosis (Fig.3).

Discussion

Chronic hepatitis C virus infection may be accompanied

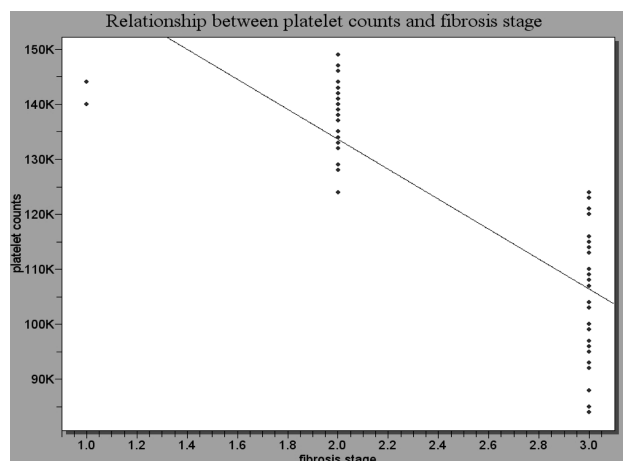


Fig 3. Correlation between platelet counts and stages of fibrosis.

by a variable degree of thrombocytopenia caused by a central and/or a peripheral (autoimmune) mechanism.

In patients with less than <100,000 platelets/ μ L both mechanisms (central and autoimmune) were involved in 93.33% of cases. In patients with 100,000-125,000 platelets/ μ L, both mechanisms were involved in 57.60% cases. In 85% of the patients with mild thrombocytopenia, the autoimmune mechanism was present.

The HCV infection can exert its effects on thrombogenesis by either a direct suppressive effect on the bone marrow, reducing the megakaryocyte production, or it can have a direct effect on the megakaryocytes, leading to low platelet production [7].

Weksler’s study shows that in chronic hepatitis C virus infected patients, without hypersplenism or evidence of antiplatelet autoantibodies, the alpha interferon therapy was followed by a significantly increased platelet number due to the considerable decrease in the viral load [8]. However, interferon also has a direct mielosuppressive effect which in itself can lead to thrombocytopenia, attenuating some of the positive effects [8].

Chronic alcohol abuse may be an additional factor, which contributes to medullary megakaryocyte inhibition [9], but this aspect was not evaluated in the present study.

The autoimmune mechanism of platelet destruction is similar to that of autoimmune thrombocytopenic purpura. First, binding of the virus to thrombocytes generates autoantibodies against the thrombocyte membrane antigens [8]. Over 90% of patients with chronic hepatitis C infection develop high levels of IgG associated with thrombocytes (PAIgG, platelet associated immunoglobulin G) [2]. The high levels of PAIgG seem to be directly related with the liver disease severity, suggesting that chronic hepatitis C is associated with major changes in the immune system [8, 10]. This type of IgG antibodies reacts with the glycoproteins on the thrombocytes membrane surface and “marks” them for autoimmune destruction. The macrophages in the spleen and liver are responsible for the accelerated seizure and destruction of the marked platelets [11, 12].

A recent study has demonstrated that platelet antibodies

are common in hepatitis C patients, but their detection does not assist in the diagnosis of immune thrombocytopenia [13]. In our study, the concomitant presence of the autoimmune mechanism produced more severe thrombocytopenia than the bone marrow inhibition alone.

Older age has a minimal influence on the platelet count in chronic hepatitis C patients. This can be explained by the fact that our study population was below the age of 65; age related myelodysplasia usually affects older groups.

There was a statistically significant relationship between the platelet count and the serum ALT levels. We found higher ALT levels in patients with a low platelet count; in these patients, hepatitis C virus is active and can generate thrombocytopenia by one of the mechanisms described above.

A significant correlation was found between the degree of thrombocytopenia and the level of viral load. The same association was reported recently by Chia-Yen Dai et al [14].

We found an unexpected high prevalence of female cases (64.19%) infected with HCV in our population study. Between 1969 and 1989, any medical contraception was illegal in Romania. Many women performed unsafe abortions; consequently, they developed bleeding complications requiring blood transfusions. This could explain our findings. We found no correlation between gender and platelet counts.

The stage of fibrosis was directly related to the decrease in platelet count. This fact was also pointed out by Giannini et al in 2002 [15]. In our study, the Spearman's rank correlation test ($\rho = -0.86485$) demonstrated an inverse relationship between the platelet counts and the stage of fibrosis. As the liver disease advances, the platelet count decreases and this fact may be related to a decrease in thrombopoietin (TPO) production in the hepatocytes. A decreased TPO could thus explain thrombocytopenia as an independent factor.

The TPO, a cytokine involved in the megakaryocyte activity regulation, is produced mainly in the liver and only in limited (reduced) amounts in the bone marrow and kidneys [16]. It plays a role in all the stages of formation and maturation of the megakaryocytes and also in platelets releasing [17]. In healthy individuals, the TPO level is inversely proportional to the platelet counts [16].

Thrombopoietin is a potent cytokine since it binds to the TPO receptor expressed on the surface of stem cells, megakaryocytes progenitor cells, megakaryocytes and platelets and regulates the megakaryocyte and platelet production.

Because TPO is produced mainly by hepatocytes, liver damage causes a decrease of TPO secretion followed by a decreased bone marrow megakaryocytes stimulation and therefore thrombocytopenia. The correlation between the stage of fibrosis in advanced liver disease and the level of serum TPO has been already demonstrated [18]. In the study performed by McHutchison et al [19] on compensated cirrhotic patients with thrombocytopenia, the patients received eltrombopag (a small-molecule nonpeptide oral

platelet growth factor that acts as a TPO receptor agonist) therapy, which raised the platelet count to a level that permitted the antiviral therapy initiation. Eltrombopag seems to be a promising therapy.

Conclusions

Several potential mechanisms can contribute to thrombocytopenia in chronic HCV infection, including accelerated platelet clearance due to an immune complex disease or a decreased platelet production due to direct marrow suppression.

Our data demonstrates that chronic hepatitis C may be associated with variable degrees of thrombocytopenia. In most cases, both a central (bone marrow suppression) and a peripheral (platelet antibodies) mechanisms are involved. There is no connection between gender and the severity of thrombocytopenia. There is a correlation between thrombocytopenia and the severity of liver disease (ALT and liver fibrosis stage) and the viral load (HCV RNA). Older age has a minimal influence.

More studies are required to further elucidate the pathogenesis of thrombocytopenia in these patients.

Conflicts of interest

None to declare.

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