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Human Platelet Antigen-3 Genotype Predicts Platelet Count in Patients with HCV Infection

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Abstract. *Background/Aim: A low platelet count is one of the most sensitive tests for cirrhosis detection in patients with hepatitis C virus (HCV) infection. We evaluated whether the human platelet antigen (HPA) genotype could predict platelet count in HCV-positive patients. Materials and Methods: We genotyped the HPA 1, 2, 3, 5 and 15 polymorphisms in consecutive patients with HCV infection. Results: Out of the 56 patients enrolled, 56.1% had liver cirrhosis. The mean platelet count was significantly lower in those with HPA1aa genotype than in those with HPA1ab/bb genotype. Platelet count did not differ among the other HPA polymorphisms. However, at logistic regression analysis, only the HPA3aa genotype and liver cirrhosis were independent predictors of a low platelet count. Conclusion: HPA3aa is an independent factor for a low platelet count in this cohort of patients with HCV chronic infection regardless of disease stage.*

Approximately 160 million people are estimated to be chronically-infected with hepatitis C virus (HCV) worldwide (1, 2). Once penetrated in the host, HCV causes acute hepatitis which is often asymptomatic (3-6). In most cases (60-80%), hepatitis becomes chronic (7-9). If left untreated chronic hepatitis progresses to liver cirrhosis in 25% of patients within 20-30 years (10-12). Each year, 6% of patients with cirrhosis develop decompensated disease or hepatocellular carcinoma (HCC) (13). Therefore, guidelines recommend ultrasound screening for HCC, and upper endoscopy to detect varices in HCV-infected patients with cirrhosis (14). Finally, cirrhosis is an urgent indication for anti-viral treatment (15) which, when successful, is associated with improved survival and a decreased decompensation rate (16-20). Therefore, it is essential to identify patients with cirrhosis among those with HCV infection.

Liver biopsy is the standard method for assessment of liver cirrhosis (21-25). However, liver biopsy is an invasive test and consequently it has a low but not null rate of complications (0.3-0.8%) and mortality (0.01-0.3%) (26-29). Moreover, liver biopsy has a non-negligible rate of false-negative results, up to 20% (22), and is not often performed in clinical practice (30-32).

Various non-invasive tools have been reported to predict liver cirrhosis in patients with chronic HCV infection (15, 33-48). Most of them are based on routinely-available laboratory parameters (15, 33-46, 48), and nearly all include platelet count. In fact, it is well-recognized that patients with cirrhosis have a low platelet count. This is mainly due to splenic sequestration and portal hypertension, although reduced hepatic thrombopoietin production has also been implicated in the platelet decrease observed in patients with cirrhosis (49). Moreover, platelet-associated glycoprotein-specific antibodies play a key role in HCV-related thrombocytopenia (50), and levels of anti-platelet antibodies differ according to polymorphisms in platelet glycoproteins (namely, human platelet antigens, HPA) (51). Little is known about regarding the association of HPA polymorphisms and platelet count in patients with HCV infection.

The aim of this study was to evaluate whether the HPA genotype could predict the platelet count in a cohort of patients with chronic hepatitis C and liver cirrhosis.

Materials and Methods

Patients. Consecutive patients with different stages of HCV infection admitted to the Department of Clinical Medicine and Surgery, Section of Infectious Diseases prospectively from January to June 2012, were enrolled in the present study. The inclusion criterion was a positive serum anti-HCV and HCV RNA test. Exclusion criteria were other

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Table I. *Laboratory results and distribution of the HPA genotype (n=56).*

Age (years)	65.5 (52.75-74)
Gender	
M	49.1%
F	50.9%
AST, U/l (IQR)	41.5 (23.75-75.75)
ALT, U/l (IQR)	34 (19-59.75)
Albumin, g/dl*	3.8±0.66
Platelets, elements/μl*	138,648±76,077
Hemoglobin, g/dl*	12.09±2.22
HCV RNA, UI/ml (IQR)	1,375,000 (784,500–4,832,500)
Cirrhosis	56.1%
HPA1 genotype	
aa	64.9%
ab	33.3%
bb	1.8%
HPA2 genotype	
aa	80.7%
ab	17.5%
bb	1.8%
HPA3 genotype	
aa	31.6%
ab	42.1%
bb	26.3%
HPA4 genotype	
aa	100%
HPA5 genotype	
aa	78.9%
ab	21.1%
HPA15 genotype	
aa	15.8%
ab	61.4%
bb	22.8%

For quantitative variables, data are provided as the median and IQR or, if marked with *, as the mean±standard deviation. HPA: Human platelet antigen; AST: aspartate transaminase; ALT: alanine transaminase; HCV RNA: hepatitis C virus ribonucleic acid. IQR: interquartile range.

causes of liver disease, HBV or HIV co-infection, HCC, prior liver transplantation, and incomplete data on blood counts or liver panel. Diagnosis of cirrhosis was based on laboratory or ultrasound examination as reported elsewhere (30). Signed informed consent was obtained in all cases.

Laboratory tests. Peripheral venous blood samples were collected in ethylenediaminetetracetic acid tubes. DNA was extracted from whole-blood samples using the QIAamp DNA blood mini kit (Quiagen, Milan, Italy), stored at −20˚C and analyzed with BLOODChip IDHPA based on XmapLuminexTechonology (Progenica-Grifols, Bilbao, Spain). Genomic DNA was amplified in multiplex (PCR) reaction using biotinylated dCTP. PCR products were denatured and hybridized onto oligonucleotide probes coupled to color-coded beads (Luminex Corporation, Austin, Texas, USA) and labeled with conjugate streptavidin. The bead signal was analyzed with a Luminex 100/200 flow cytometer. The data analysis software interprets the quantified signals and produces a file with the genotype, and the BLOODchipID Software (Luminex corporation, Austin, Texas, USA) converts the genotype into predicted HPA phenotype.

Table II. *Platelet count (elements/μl in patients according to HPA genotype (n=56).*

	aa	ab or bb	*p*-Value*
HPA1	118,114±60,940	176,474±87,732	0.006
HPA2	131,659±76,171	169,400±71,228	0.159
HPA3	135,722±74,577	140,111±77,822	0.844
HPA5	141,791±76,292	126,364±77,593	0.553
HPA15	115,333±86,685	143,311±73,973	0.318

Data are given as the mean±standard deviation. *Student's *t*-test.

Statistical analysis. The Kolmogorov-Smirnov test was used to check quantitative variables for Gaussian distribution. In the case of Gaussian distribution, data are reported as the mean±standard deviation (SD), otherwise they were reported as the median and interquartile range (IQR). In the case of Gaussian distribution, the Student's *t*-test for unpaired variables was applied, while the Mann-Whitney *U*-test was used in case of non-Gaussian distribution. The Chi-square test with Yates correction (or Fisher's exact test, where appropriate) was used for categorical variables. A $p<0.05$ at two-sided test was considered statistically significant.

To assess the role of HPA polymorphisms in the platelet level, all polymorphisms together with disease stage were included in a binary logistic regression analysis model using the forward conditional stepwise method. The cut-off values used for the stepwise method were: $p=0.05$ for entry into the model and $p=0.1$ for removal. The dependent variables was low platelet count defined as a platelet count less than 150,000/μl. All statistical analyses were performed with the Statistical Package for the Social Sciences version 19.0 (SPSS Inc. Chicago, IL, USA).

Results

Fifty-seven patients with chronic HCV infection were enrolled, out of these, 32 (56.1%) were affected by liver cirrhosis. Laboratory results and distribution of HPA genotypes are reported in Table I. Most patients were infected by HCV genotype 1 (69%) or 2 (21.4%). The mean platelet count (±SD) was significantly lower in those with genotype HPA1aa than in those with genotype HPA1ab or HPA1bb (118,114/μl±60,940 *vs*. 176,473/μl±87,731; $p=0.006$, Student's *t*-test). Platelet count did not differ among HPA2, HPA3, HPA5 and HPA15 genotypes (see Table II).

To evaluate whether HPA polymorphisms were independently related to platelet level, we included all these polymorphisms together with the stage of the disease (liver cirrhosis *versus* non-liver cirrhosis) in a logistic regression analysis with a low platelet count (using the usual cut-off of 150,000/μl), as dependent variable. Logistic regression analysis (Table III) showed that genotype HPA3 aa ($p=0.038$) and disease stage ($p<0.001$) were independent predictors of platelet count, while HPA1 polymorphism was not ($p=0.310$).

Due to conflicting results between univariate and multivariate analyses, we performed a separate analysis for the HPA3

Table III. *Logistic regression for low platelet count (<150,000/μl).*

	Regression coefficient	Standard error	Odds ratio (95%CI)	*p*-Value*
HPA3 aa *vs*. ab or bb	2.369	1.143	10.687 (1.137-100.464)	0.038
Cirrhosis *vs*. non cirrhosis	3.974	1.121	53.209 (5.917-478.475)	<0.001

polymorphism in patients with and without cirrhosis. Using the cut-off of 150,000/μl platelets, in patients with cirrhosis, all those with HPA3aa genotype had a low platelet count compared with 78% of those with HPA3ab or HPA3bb genotype (p=0.553). In patients without cirrhosis, 92.3% with HPA3ab or HPA3bb genotype had a high platelet count compared to only 58.3% of those with HPA3aa genotype (p=0.073).

Discussion

Our study shows that in a cohort of patients with HCV-related chronic liver disease, the HPA3aa genotype is associated with a low platelet count irrespective of the disease stage. However, at univariate analysis, the HPA3 polymorphism was not associated with platelet count. The discrepancy between univariate and logistic regression analyses can probably be explained by a previous study of our group showing that the HPA3aa genotype was significantly associated a reduced risk of liver cirrhosis (52) – a condition associated with a low platelet count (52). The sum of these two effects associated with the HPA3aa polymorphism (reduced risk of cirrhosis and reduced platelet count) is null at univariate analysis, as shown in a preliminary analysis of the present study (53), and confirmed herein. However, when disease stage was included in the logistic regression model, the HPA3 polymorphism exerted a significant effect. To our knowledge, this is the first study to evaluate the association of HPA polymorphisms with platelet count in patients with chronic HCV infection.

Most HPA polymorphisms are single-nucleotide polymorphisms in the genes encoding for membrane glycoproteins (GP). The platelet membrane protein GPIIb is the GP involved in HPA3 polymorphisms (51). This protein exerts various functions. It interacts with the endothelium (54) and, together with GPIIIa, has been implicated in the risk of myocardial infarction (55) and thrombosis (56). In fact, GPIIb/IIIa receptor antagonists are effective inhibitors of platelet aggregation (57). The same antagonists reduce platelet half-life (57). A study in dogs showed that platelets are sequestered in the spleen during exposure to GPIIb/IIIa receptor antagonists (57). However, the most well-recognized role of HPAs is their function as antigens for alloantibodies against human platelets involved in neonatal alloimmune thrombocytopenia, post-transfusion purpura and refractoriness to random donor platelets (51). A study of 50 HCV-positive patients showed that the frequency of platelet-specific antibodies was as high as 86.7% in patients with thrombocytopenia (50). The most likely target antigens of platelet antibodies were GP IIb/IIIa (30%), followed by GP IIIa (20.5%), GP IIb (13.3%), GPIb (13.3%) and GPIa (10%). Interestingly, the platelet count was inversely-correlated to the levels of platelet-specific antibodies and significantly paralleled the spleen size. The authors concluded that platelet-associated GP-specific antibodies are a mechanism that induces thrombocytopenia in patients with chronic HCV infection (50). Indeed, a complex viral/immune interaction has also been reported in autoimmune thrombocytopenia (58). Therefore, it is probable that HPA polymorphisms play a role in the different rate of immune-mediated platelet clearance that occurs in the spleen of HCV-positive patients. Studies correlating platelet levels and platelet-specific antibodies with the different HPA polymorphisms are now warranted to test this hypothesis.

Another interesting result of our study is that HPA3 affects platelet count to such a degree that its polymorphism can impair the potential of platelet count to discriminate between chronic hepatitis and liver cirrhosis. Platelet count is one of the most early and sensitive markers of cirrhosis, and it is included in most non-invasive scores devised to detect not only liver cirrhosis (44, 47), but also esophageal varices (one of the most common signs of portal hypertension) and their progression (30, 59-63). In fact, in our patients without cirrhosis, 41.7% of those with HPA3aa genotype had a low platelet count compared to 7.7% of those with HPA3ab or bb genotype.

In conclusion, the HPA3aa genotype was shown to be an independent factor for low platelet count in a cohort of patients with chronic HCV infection, regardless of the stage of their disease.

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