Published in final edited form as:

J Acquir Immune Defic Syndr. 2009 December ; 52(5): 595–599. doi:10.1097/QAI.0b013e3181b79aff.

Brief Report: Risk Factors for Thrombocytopenia in HIV-infected Persons in the Era of Potent Antiretroviral Therapy

Kristen M. Marks¹, Robin M.A. Clarke^{1,*}, James B. Busse², Andrew H. Talal³, and Marshall J. Glesby¹

¹Division of Infectious Diseases, Weill Cornell Medical College, New York, NY

²Division of Hematology and Oncology, Weill Cornell Medical, New York, NY

³Center for the Study of Hepatitis C and the Division of Gastroenterology and Hepatology, Weill Cornell Medical College, New York, NY

Abstract

Background—Prior to potent antiretroviral therapy, thrombocytopenia was observed frequently. Little is known about risk factors for or severity and consequences of thrombocytopenia since establishment of highly effective therapy for HIV.

Methods—We conducted a retrospective matched case-control study of HIV-infected adult outpatients with and without thrombocytopenia to elucidate the contribution of HIV viremia, hepatitis C infection, and other potential risk factors for thrombocytopenia. 73 cases with thrombocytopenia (platelet count $<100 \times 10^9/L$ persistent for >3 months) were matched by age, sex and first clinic visit with 73 non-thrombocytopenic controls. Risk factors and outcomes were assessed using conditional logistic regression.

Results—Nadir platelet counts in cases were $\leq 50 \times 10^9/L$ in 58% and $\leq 30 \times 10^9/L$ in 38%. In multivariate modeling, HIV RNA >400 copies/ml, HCV infection, and cirrhosis were significantly associated with thrombocytopenia with adjusted odds ratios of 5.3 (CI 1.6-17.1, $P=0.006$), 6.1 (CI 1.6-22.6, $P=0.007$), and 24.0 (CI 1.7-338, $P=0.019$), respectively. Thrombocytopenia was significantly associated with major bleeding events and non-bleeding related death.

Conclusions—Thrombocytopenia in the era of potent antiretroviral therapy is associated with HCV infection, cirrhosis, and uncontrolled HIV replication, as well as serious complications including major bleeding and death.

Keywords

thrombocytopenia; HIV; hepatitis C

Introduction

In the 1980s and early 1990s, the prevalence of thrombocytopenia was reported as approximately 5-15% in patients with HIV infection and 20-45% in patients with AIDS¹. The epidemiology of and risk factors for thrombocytopenia in HIV-infected individuals in the era

Reprints or correspondence: Dr. Kristen M. Marks, Division of Infectious Diseases, Weill Cornell Medical College, 525 East 68th Street, F24, New York, NY 10065, (212)746-6309, fax (212)746-8852, markskr@med.cornell.edu.

*Current affiliation: Department of Medicine, University of California Los Angeles Medical Center, Los Angeles, CA

Data presented orally at HIV & Liver Disease Conference, Jackson Hole, WY, September 25-27, 2008.

Potential conflicts of interest: none.

of potent HIV antiretroviral therapy has not been studied; however, thrombocytopenia remains an important issue because there is a risk of bleeding events and the presence of significant thrombocytopenia may complicate the treatment of HIV-related illnesses, particularly hepatitis C virus (HCV) infection. The presence of more severe thrombocytopenia may also predict decreased survival in HIV-infected patients². We conducted a retrospective case-control study of HIV-infected patients with and without thrombocytopenia receiving care at an urban HIV clinic to elucidate the contributions of HCV infection, HIV viremia, as well as other potential risk factors for thrombocytopenia in the era of potent antiretroviral therapy.

Patients and Methods

Study Population & Matching

This study included HIV-infected outpatients from the two HIV clinics (the Centers for Special Studies) of the New York Presbyterian Hospital-Weill Cornell Center. The most recent 100 adult patients seen prior to July 1, 2005, who had a complete blood count (CBC) demonstrating thrombocytopenia, defined as platelet counts $<100 \times 10^9/L$, were considered for inclusion in the study. The cutoff of $100 \times 10^9/L$ was recently selected by an international group standardizing definitions of immune thrombocytopenic purpura (ITP)³. Only patients with thrombocytopenia persisting for >3 consecutive months were eligible for the study. Patients with no outpatient medical records or thrombocytopenia resulting from interferon therapy, chemotherapy, or malignancy were excluded. Incident and prevalent cases of thrombocytopenia were included in the study. Controls matched 1:1 on the following criteria were generated randomly: age ± 5 years, same sex, and first appointment date ± 6 months. Controls without outpatient records were excluded.

Variables examined

Medical records were reviewed retrospectively to ascertain demographic, clinical, and laboratory characteristics at the time of detection of thrombocytopenia which was defined for the purpose of this study as onset of thrombocytopenia in incident cases or first clinic visit in prevalent cases. HIV- and HCV-associated variables included risk factor for transmission, years since HIV diagnosis, AIDS diagnosis, CD4 cell count and nadir, HIV RNA level (using a limit of detection of <400 or <50 copies/mL as available), antiretroviral regimen and duration, chronic HCV infection status (based on HCV antibody and HCV RNA results), and liver-related tests. Assessment of cirrhosis was based on provider documentation as well as imaging results (nodular liver or portal hypertension) or liver biopsy results describing histopathologic diagnosis of cirrhosis (Metavir Fibrosis Stage 4). Alcohol use was elucidated from the patient's medical record based on documentation provided by medical, psychiatric and social work providers and quantity interpreted as none, mild-moderate, heavy and qualified as past or current use. Outcomes including treatments for thrombocytopenia, major bleeding events (defined as gastrointestinal, intracranial, or events requiring hospitalization), and deaths during the duration of patient's follow up in the clinic were also recorded. The study was approved by the Institutional Review Board of the Committee on Human Rights in Research at Weill Cornell Medical College (New York, NY) and a waiver of need for informed consent was obtained.

Statistical Analysis

A sample size calculation estimated that 53 cases would provide 80% power to detect an odds ratio of 3.0 for the association between HCV infection and thrombocytopenia based on an estimated 40% prevalence of HCV infection in controls (alpha of 0.05). HIV RNA level (detectable/undetectable), HCV infection status and the other variables described above were compared between cases and controls using conditional logistic regression, and odds ratios with 95% confidence intervals were calculated. Variables with a skewed distribution were dichotomized around the upper limit of normal or the median depending on clinical relevance,

because transformation of data did not correct skewedness. Multivariate models were created using forward, stepwise conditional logistic regression; the criterion to enter the model was $P \leq 0.2$. Only covariates with P values of <0.05 in univariate analyses were considered in the multivariate analysis. Data were analyzed using Stata (version 9.0) software.

Results

Of the 100 patients identified with platelet counts $<100 \times 10^9/L$, 27 patients were excluded for the following reasons: thrombocytopenia did not persist for >3 months (6), no outpatient records existed (10), interferon-related (4), chemotherapy or malignancy-related (3), and age <18 years (4); this left 73 evaluable cases for whom 73 matched controls were generated.

Of the case patients, nadir platelet count was $\leq 50 \times 10^9/L$ in 58%, $\leq 30 \times 10^9/L$ in 38%, and $\leq 10 \times 10^9/L$ in 7%. The thrombocytopenia prevalence amongst 2298 outpatients who had CBC testing during the period from which the cases were obtained (May 2004-July 2005) was calculated at 3.2% (95% CI 2.5-4.0%). Of the 73 patients identified as thrombocytopenia cases, 43 (59%) were classified as incident cases of thrombocytopenia (platelet count was normal on preceding CBCs at our clinic) while 30 cases (41%) were defined as preexisting (prevalent) cases in which thrombocytopenia existed at the time of initial presentation to our clinic. Thrombocytopenia had not resolved at the time of last labs in 64% of cases. In these cases, the median duration of thrombocytopenia was 3.3 years (Interquartile range (IQR)=1.2-6.5 years), while it was 2.0 years (IQR=1.1-3.5) in cases with resolution of thrombocytopenia.

The table shows demographic characteristics of cases and controls at the time of onset of thrombocytopenia as well as the characteristics associated with thrombocytopenia on univariate analysis and multivariate analysis. HIV RNA detectability, HCV infection, and cirrhosis remained significantly associated with thrombocytopenia status after adjusting for the other model variables. In fact, 98% of case patients compared to 53% of controls had at least one of these risk factors, and all three were observed in 15% of cases compared to none of the controls. In patients without HCV infection or cirrhosis, detectable HIV RNA was strongly associated with thrombocytopenia case status (present in 21 of 22 cases versus 5 of 22 of controls with available HIV RNA data, OR =17.5 (CI 2.3-127.4) $p=0.006$). In contrast, in patients with known HCV infection or cirrhosis, HIV RNA detectability did not differ significantly between cases and controls.

Major bleeding events defined as gastrointestinal, intracranial, or events requiring hospitalization occurred in 13 cases compared to 2 controls (OR 6.5, CI 1.5-28.8; $P=0.014$). Amongst case patients, major bleeding events were not associated with nadir platelet count or duration of thrombocytopenia; however, there was an association with cirrhosis (OR = 11.5, CI 2.3-58.0, $P=0.003$) and a trend toward an association with HCV infection (OR = 3.92, CI 0.8-19.3, $P=0.09$). Compared to controls, cases with thrombocytopenia were more likely to suffer death from any cause or major bleeding events during their history of thrombocytopenia; however no fatal bleeding events occurred. Nine deaths occurred amongst cases compared to 0 in control subjects ($P=0.002$, chi-squared); control subjects, however, were more likely to have unknown vital status (7 cases vs. 16 controls, $P=0.04$, chi-squared).

Nineteen case patients (26%) were treated for thrombocytopenia with 7 documented as receiving >1 type of treatment. Treatments included: corticosteroids (received by 11 patients); platelet transfusions, IVIG, and anti-D (each received by 5); progesterone (3); splenectomy (2); and vincristine (1). In addition, 5 patients required packed red cell transfusion due to a major bleeding event.

Discussion

Our study demonstrates that thrombocytopenia remains a clinically significant problem in the current HIV treatment era. The prevalence of thrombocytopenia in HIV-infected outpatients detected in this study (approximately 3%) is decreased substantially compared to that seen during the era prior to potent antiretroviral therapy. Nevertheless among those thrombocytopenic in this study, more than half experienced a platelet count less than $50 \times 10^9/L$ with more than one-third experiencing a nadir below $30 \times 10^9/L$ and one-quarter requiring treatment. In addition to being severe, thrombocytopenia did not resolve quickly in most patients, often persisting for years.

As predicted, uncontrolled HIV and HCV replication, particularly associated with advanced liver disease, were strongly associated with thrombocytopenia in this study. Our data also indicate that it is HIV replication itself rather than advanced disease related to AIDS or low CD4 count that is associated with thrombocytopenia in the current era, as supported by epidemiologic, mechanistic and clinical data reported prior to the availability of potent antiretroviral therapy^{1,4-6}.

The strong association between HCV co-infection and thrombocytopenia in this study, likely explains why thrombocytopenia did not resolve with effective HIV treatment in some patients. Traditionally, the thrombocytopenia related to HCV has been attributed to cirrhosis and portal hypertension causing splenic sequestration of platelets, although other factors may also contribute. Advanced liver disease results in reduced liver production of thrombopoietin, the dominant humoral thrombopoietic factor⁷⁻⁹. Our study found a strong association of thrombocytopenia with cirrhosis (and splenomegaly), but also found an independent association with HCV infection suggesting that mechanisms other than advanced liver disease may play a role in HIV/HCV-coinfected patients. Chronic ITP associated with HCV has been described previously^{10,11}, and cryoglobulins¹² and anti-platelet autoantibodies¹³⁻¹⁵ have been detected in thrombocytopenic patients with HCV.

Deaths occurred more frequently in cases than controls, although no deaths were specifically attributed to bleeding. Thrombocytopenia has previously been identified as a poor prognostic marker for AIDS and death^{2,16}, however conflicting results have been described as well^{17,18}. Despite the absence of hemorrhagic deaths, major bleeding events occurred in 13 cases. Bleeding events were not associated with nadir platelet count or duration of thrombocytopenia but were associated with cirrhosis and trended toward an association with HCV infection. While coagulopathy or the presence of esophageal varices may explain this association of bleeding events with cirrhosis, an increased tendency to bleed at higher platelet counts has been described as a feature of HCV-related ITP without associated coagulopathy, possibly mediated by cryoglobulin-induced vasculitis¹².

Limitations of this study include its retrospective nature which introduces possible biases related to ascertainment, documentation and chart review. It is also possible that the presence of thrombocytopenia itself contributed to the diagnosis of cirrhosis or led to disproportionate testing to detect cirrhosis in cases compared to controls. However, an argument against this is that the cases also differed significantly in terms of other laboratory markers of cirrhosis, which were routinely assessed in all patients. In addition, both prevalent and incident cases were included in order to have a study population reflecting the spectrum of patients with thrombocytopenia in the current era. Because of this, cases displayed a variable duration of thrombocytopenia, including cases with thrombocytopenia predating their enrollment in the clinic. To assess the impact of these limitations, sensitivity analyses were performed (data not shown). The inclusion of only the incident cases in the multivariate models did not change the outcomes qualitatively, nor did excluding three subjects whose thrombocytopenia detection

predated 1996. Thus the results of this study appear to be generalizable to patients with thrombocytopenia whether it is newly diagnosed or longstanding. This study also predated the FDA approval of the first thrombopoietic agent, which has been shown to be an effective option for the treatment of refractory ITP.

In summary, thrombocytopenia still occurs frequently in HIV-infected patients and at levels that could predispose to bleeding and require treatment. Detectable HIV RNA, HCV infection, and cirrhosis are major independent risk factors associated with thrombocytopenia in the current antiretroviral era. When assessing patients with HIV infection, it is important to recognize that thrombocytopenia may be a sign of cirrhosis, particularly in HCV-coinfected patients, whether or not there is coincidentally detectable HIV RNA. Furthermore, in co-infected patients, milder degrees of thrombocytopenia may predispose to bleeding and preclude the use of pegylated interferon, which is of great relevance since a large burden of thrombocytopenia appears to be due to HCV infection and related liver disease. In patients without HCV infection or liver disease, detectable levels of HIV replication may account for most cases. The better understanding of the risk factors for and etiology of thrombocytopenia initiated here will help guide whether antiviral, immune-based or thrombopoietic therapies are the appropriate initial treatment strategy for HIV-infected individuals with thrombocytopenia in the future.

Acknowledgments

The authors thank the patients and physicians of the New York Presbyterian Hospital's Center for Special Studies for making this study possible as well as W.D. Johnson, Jr. and R.M. Gulick for their mentorship. We acknowledge Ximena Lavender for assistance with data entry.

Financial Support: Bristol-Myers Squibb Virology Fellows' Program to K.M.M.; National Institutes of Health/National Institute of Allergy and Infectious Diseases K23 AI065319 to K.M.M., K24AI078884 to M.J.G., NIH UL1 RR024996 to J.B.B. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

Reference List

1. Carbonara S, Fiorentino G, Serio G, et al. Response of severe HIV-associated thrombocytopenia to highly active antiretroviral therapy including protease inhibitors. *J Infect* 2001;42:251–256. [PubMed: 11545567]
2. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ciesielski CA. Surveillance for thrombocytopenia in persons infected with HIV: results from the multistate Adult and Adolescent Spectrum of Disease Project. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;14:374–379. [PubMed: 9111481]
3. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura (ITP) of adults and children: Report from an International Working Group. *Blood*. 2008e-pub
4. Servais J, Nkoghe D, Schmit JC, et al. HIV-associated hematologic disorders are correlated with plasma viral load and improve under highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001;28:221–225. [PubMed: 11694827]
5. Nardi MA, Liu LX, Karparkin S. GPIIIa-(49-66) is a major pathophysiologically relevant antigenic determinant for anti-platelet GPIIIa of HIV-1-related immunologic thrombocytopenia. *Proc Natl Acad Sci U S A* 1997;94:7589–7594. [PubMed: 9207136]
6. Oksenhendler E, Bierling P, Ferchal F, Clauvel JP, Seligmann M. Zidovudine for thrombocytopenic purpura related to human immunodeficiency virus (HIV) infection. *Ann Intern Med* 1989;110:365–368. [PubMed: 2916807]
7. Giannini E, Botta F, Borro P, et al. Relationship between thrombopoietin serum levels and liver function in patients with chronic liver disease related to hepatitis C virus infection. *Am J Gastroenterol* 2003;98:2516–2520. [PubMed: 14638357]

8. Giannini E, Borro P, Botta F, et al. Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. *J Hepatol* 2002;37:572–577. [PubMed: 12399221]
9. Adinolfi LE, Giordano MG, Andreana A, et al. Hepatic fibrosis plays a central role in the pathogenesis of thrombocytopenia in patients with chronic viral hepatitis. *Br J Haematol* 2001;113:590–595. [PubMed: 11380442]
10. Bauduer F, Marty F, Larrouy M, Ducout L. Immunologic thrombocytopenic purpura as presenting symptom of hepatitis C infection. *Am J Hematol* 1998;57:338–340. [PubMed: 9544980]
11. Pockros PJ, Duchini A, McMillan R, Nyberg LM, McHutchison J, Viernes E. Immune thrombocytopenic purpura in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 2002;97:2040–2045. [PubMed: 12190174]
12. Rajan SK, Espina BM, Liebman HA. Hepatitis C virus-related thrombocytopenia: clinical and laboratory characteristics compared with chronic immune thrombocytopenic purpura. *Br J Haematol* 2005;129:818–824. [PubMed: 15953010]
13. Sanjo A, Satoi J, Ohnishi A, Maruno J, Fukata M, Suzuki N. Role of elevated platelet-associated immunoglobulin G and hypersplenism in thrombocytopenia of chronic liver diseases. *J Gastroenterol Hepatol* 2003;18:638–644. [PubMed: 12753144]
14. Pereira J, Accatino L, Alfaro J, Brahm J, Hidalgo P, Mezzano D. Platelet autoantibodies in patients with chronic liver disease. *Am J Hematol* 1995;50:173–178. [PubMed: 7485078]
15. Kajiwarra E, Akagi K, Azuma K, Onoyama K, Fujishima M. Evidence for an immunological pathogenesis of thrombocytopenia in chronic liver disease. *Am J Gastroenterol* 1995;90:962–966. [PubMed: 7771431]
16. Pearce CL, Mack WJ, Levine AM, et al. Thrombocytopenia is a strong predictor of all-cause and AIDS-specific mortality in women with HIV: The women's interagency HIV study. *Blood* 2004;104:569A–570A.
17. Rieg G, Yeaman M, Lail AE, Donfield SM, Gomperts ED, Daar ES. Platelet count is associated with plasma HIV type 1 RNA and disease progression. *AIDS Res Hum Retroviruses* 2007;23:1257–1261. [PubMed: 17961113]
18. Holzman RS, Walsh CM, Karpatkin S. Risk for the acquired immunodeficiency syndrome among thrombocytopenic and nonthrombocytopenic homosexual men seropositive for the human immunodeficiency virus. *Ann Intern Med* 1987;106:383–386. [PubMed: 3468820]

Table

Univariate & Multivariate Analysis of Characteristics and Laboratory Values from Cases of Thrombocytopenia and Matched Controls

	Cases (n=73) ^a	Matched Controls (n=73) ^a	Univariate Matched Odds Ratio (95% CI) ^b	Multivariate Matched Odds Ratio (95% CI) ^c	P
Demographic Characteristics					
Male Sex (%)	52 (71.2)	52 (71.2)	<i>b</i>		
Age, years, mean (SD, range)	41.5 (7.75, 18-60)	41.2 (7.10, 23-57)	<i>b</i>		
Race (%)	22 (30.1)	34 (46.6)	Referent (Ref)		
Hispanic	29 (39.7)	21 (28.8)	2.47 (1.06-5.72)	0.036	
White	20 (27.4)	18 (24.7)	1.72 (0.72-4.1)	0.22	
HIV-related Characteristics					
Nadir CD4 median (IQ range), OR per 100 cells/mm ³	100 (141-195)	179 (71-280)	0.65 (0.48-0.89)	0.006	
CD4 count, <200 cells/mm ³	33/73 (45.2)	14/72 (19.4)	Ref		
201-500 cells/mm ³	29/73 (39.7)	32/72 (44.4)	0.41 (0.17-0.98)	0.045	
>500 cells/mm ³	11/73 (15.1)	26/72 (36.1)	0.20 (0.07-0.53)	0.001	
HIV RNA Viral Load >400-copies/ml (%)	44/65 (67.7)	25/66 (37.9)	2.9 (1.41-5.95)	0.004	0.006
History of ART (%)	67 (91.8%)	69 (94.5%)	0.67 (0.19-2.36)	0.53	
Taking cART during TP (%)	63 (86.3%)	61 (83.6%)	1.29 (0.48-3.45)	0.62	
TMP-SMX during TP (%)	42 (57.5)	25 (34.3)	3.43 (1.48-7.96)	0.004	
HIV Risk Factor MSM (%)	26 (35.6)	34 (46.6)	Ref		
Hetero (%)	14 (19.2)	23 (31.5)	0.77 (0.23-2.65)	0.69	
IVDU (%)	17 (23.3)	6 (8.2)	4.37 (1.31-14.60)	0.016	
IVDU/Hetero (%)	12 (16.4)	5 (6.8)	4.16 (1.05-16.53)	0.043	
Liver & HCV-related Characteristics					
Cirrhosis (%)	27 (37.0)	11 (1.4%)	27 (3.67-198.69)	0.001	0.019
HCV Infection (%)	46 (63.0)	15 (20.5%)	6 (2.53-14.24)	0.000	0.007
Splenomegaly (%)	32 (43.8)	2 (2.7%)	31 (4.23-227.08)	0.001	^d
Protein >8.0 g/dL (%)	30/73 (41.1)	18/72 (25.0)	2.0 (0.97-4.12)	0.061	
Albumin >4.0 g/dL (%)	29 (39.7)	46 (63.0)	0.41 (0.21-0.81)	0.010	
Total Bilirubin >1.3 mg/dL (%)	15 (20.5)	3 (4.1)	3.0 (1.41-6.38)	0.004	
ALT >45 U/L (%)	37/73 (50.7)	17/72 (23.6)	4.8 (1.83-12.58)	0.001	
Alkaline Phosphatase >95 U/L (%)	48 (65.8)	27 (37.0)	3.62 (1.66-7.93)	0.001	
Other Hematologic and Metabolic Characteristics					
Hemoglobin >13.4 g/dL (%)	33/73 (45.2)	43/69 (62.3)	0.39 (0.16-0.93)	0.034	
WBC >4.5K/ul (%)	24 (32.9)	36 (49.3)	0.45 (0.22-0.96)	0.039	
Other					
Total Cholesterol >=166 mg/dL (%)	22/71 (31.0)	44/72 (61.1)	0.30 (0.15-0.61)	0.001	

^a N=73 unless otherwise specified^b Variables used for matching of cases and controls^c n=98. Other variables significant in univariate model dropped out of multivariate model because they were not significant using conditional logistic regression with forward, stepwise regression with entry criterion into the model of $P \leq 0.2$.^d Because of collinearity, splenomegaly and cirrhosis could not be used in the same model. Replacing splenomegaly for cirrhosis did not change the model qualitatively.^e Because of collinearity, IVDU and hepatitis C could not be used in the same model.

Abbreviations: Odds ratio (OR), antiretroviral therapy (ART), combination antiretroviral therapy (cART), thrombocytopenia (TP), zidovudine (AZT), trimethoprim-sulfamethoxazole (TMP-SMX), men who have sex with men (MSM), heterosexual sex (hetero), intravenous drug use (IVDU), hepatitis C virus (HCV), aspartate aminotransferase (AST), alanine aminotransferase (ALT)