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ORIGINAL ARTICLE

Hematological manifestations of human immunodeficiency virus infection and the effect of highly active anti-retroviral therapy on cytopenia

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Background

The aim of this study is to investigate the hematological manifestations of human immunodeficiency virus (HIV) infection, the risk factors for cytopenia, and the effect of highly active anti-retroviral therapy (HAART) on cytopenia.

Methods

Medical records of patients treated for HIV at the Seoul National University Hospital from January 2005 to March 2010 were retrospectively reviewed. To determine the impact of HIV itself, we excluded HIV patients who had other conditions that could have resulted in hematological manifestations. Multiple logistic regression analyses were performed to identify risk factors for cytopenia.

Results

A total of 621 cases were investigated, and after exclusion, data of 472 patients were analyzed. The frequency of cytopenia was anemia, 3.0% (14/472); neutropenia, 10.0% (47/472); thrombocytopenia, 2.4% (12/472); lymphopenia, 25.7% (121/470); isolated cytopenia, 11.2% (53/472); and bicytopenia, 2.1% (10/472). The leading risk factor for cytopenia identified by multivariate logistic regression methods was AIDS status at initial presentation. After HAART, cytopenia was reversed in the majority of patients (thrombocytopenia, 100%; neutropenia, 91.1%; and anemia, 84.6%).

Conclusion

This study isolated the impact of HIV infection alone on hematologic manifestations and confirmed that these changes were reversible by HAART. Control of the HIV infection will have the main role in the management of hematological manifestations of the virus.

Key Words HAART, Hematologic manifestations, HIV infection, Risk factor

INTRODUCTION

Hematologic abnormalities are common in patients with advanced human immunodeficiency virus (HIV) infection [1] and can affect the outcomes of highly active anti-retroviral therapy (HAART), resulting in higher mortality [2-4]. Recently, it has been reported that CD4 lymphocyte counts, World Health Organization clinical stage, body weight, anemia status, total lymphocyte count, advanced age, and male sex are closely related to mortality in sub-Saharan Africa [5]. It is well known that CD4 lymphocyte counts are decreased during disease progression. However, other hematologic manifestations are not well known. Furthermore, country and ethnicity may also affect hematological manifestations [6].

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HIV itself can cause hematological manifestations. It has been shown that the viral gene products of HIV can indirectly influence survival and growth of hematopoietic progenitors [1]. Secondary factors such as the effects of medications, opportunistic infections, hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection, and liver cirrhosis also contribute to hematologic manifestations [2]. Previous studies have focused on hematologic manifestations without considering the etiology [7, 8]. In this study, we excluded these secondary factors, which may suppress bone marrow and focused only on the primary HIV-associated hematologic manifestations. The aim of this study was to investigate the hematological manifestations of HIV infection, to evaluate the risk factors for cytopenia, and to record the effect of HAART on cytopenia.

MATERIALS AND METHODS

1. Study population

We collected patient data retrospectively at the Seoul National University Hospital in South Korea. The enrollment period was from January 2005 to March 2010. Medical record of each patient was reviewed to identify eligible patients who were diagnosed as having HIV infection and had been followed for at least 1 month. Further analysis was done for those patients who met eligibility criteria after exclusion. The anti-retroviral therapy regimen, HAART, was managed by a board-certified infectious disease specialist.

2. Data collection and definition

Medical records were reviewed for demographic data, medication history, dates of anti-retroviral therapy, and past medical history. All available hematologic values determined from peripheral blood analysis were obtained for each patient at the beginning of enrollment. Total WBC count, absolute neutrophil count (ANC), lymphocyte count, Hb level, and platelet count were retrieved from the medical records. Plasma HIV RNA titers and CD4- and CD8-T-lymphocyte counts were also obtained. Laboratory data were obtained at 3 consecutive occasions, i.e., at baseline and then at 6 months and 3 years after initiation of HAART.

The definitions of cytopenia were as follows: thrombocytopenia was defined as platelet count below 100×10^9/L. Neutropenia was defined as ANC below 1.5×10^9/L. Anemia was defined as a Hb level below 10.0 g/L. Any patient with anemia, thrombocytopenia, or neutropenia was defined as a patient who had isolated cytopenia. Bicytopenia was defined as the state of a patient in whom any 2 of the 3 lineage cell counts (ANC, Hb, or platelets) were below the levels designated above. Hematological recovery after HAART was defined as status at which the cell counts would be higher than the cytopenia criteria.

Baseline prothrombin time (PT) with international normalized ratio, activated partial thromboplastin time (aPTT), fibrinogen level, and Venereal Disease Research Laboratory (VDRL) titers were also retrieved from the medical records. The VDRL results were categorized into 3 groups: negative; titer $\leq$1:32; and titer $>$1:32. AIDS was defined in this report when CD4 lymphocyte counts were lower than 200/μL. Viral load, $\log_{10}$ copies/mL, was categorized as <3.5, <4.5, <5, and ≥5, since each group can have each 100 number for even distribution.

3. Exclusion

Patients with any conditions other than HIV or AIDS that may cause bone marrow suppression or cytopenia were excluded. Patients who had received HAART previously were also excluded, because the reversal of cytopenia by HAART could mask the effects of HIV on the hematological status. Exclusion criteria can be summarized as follows: (I) patients with opportunistic infection or other signs of infectious illness such as diarrhea, fever, or rash; (II) patients with any malignancy such as lymphoma or patients who had received chemotherapeutic agents within 6 months prior to enrollment; (III) patients with medication history (e.g., antibiotics, ganciclovir, trimethoprim-sulfamethoxazole, anti-tuberculosis medications) known to induce cytopenia within 2 weeks prior to enrollment; (IV) patients with HBV or HCV co-infection or liver cirrhosis; and (V) patients with a history of previous HAART.

4. Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR) values. Multiple logistic regression methods were used to identify risk factors for cytopenia. Univariate analysis was performed for sex, age, progression to AIDS at the end of the study, AIDS at the initial stage, HIV viral load, $\log_{10}$ copies/mL grade, and VDRL titer with the occurrence of cytopenia. The results were reported as hazard ratio (HR) and 95% CI (confidence interval). Multivariate analysis was performed for variables that had P-values lower than 0.1. Hematologic reconstitution after HAART was evaluated after treatment. Statistical analyses were conducted using SPSS software (version 17.0; SPSS Inc., Chicago, IL). A value of $P<0.05$ was considered statistically significant.

5. Ethics statement

The study protocol was approved by the SNUH institutional review board (IRB; No. H-1008-045-326). Informed consent was waived by the IRB.

RESULTS

1. Patient characteristics

A total of 621 patients were initially registered from January 2005 to March 2010. Median follow-up duration was 39.99 months (IQR, 20.24-58.75). In this study, patients with any condition other than HIV or AIDS that could cause bone marrow suppression or cytopenia were excluded. The conditions of patients excluded on the basis of these criteria were described in the "Material and Methods"

Table 1. Baseline characteristics and values.

Characteristics	Study participants after exclusion (N=472)	Reference range (SI units)
Age, median years (IQR)	35 (27-44)	
Number of female patients (%)	41 (8.7%)	
CD4 cell count, median cells/μL (IQR)	300 (175-420)	
Number of immunologic AIDS (%)	134 (28.4%)	
Number of received HAART (%)	389 (82.4%)	
Viral load, $\log_{10}$ copies/mL (IQR)	4.43 (3.60-5.00)	
Absolute neutrophil count, median $\times 10^9$/L (IQR)	2.721 (1.992-3.609)	1.8-7.8$\times 10^9$/L
Hb, median g/L (IQR)	147 (134-157)	138-175 g/L
Platelets count, median $\times 10^9$/L (IQR)	209 (177-248)	150-450$\times 10^9$/L
Prothrombin time, median INR (IQR)	1.03 (0.97-1.09)	0.8-1.3 INR
Activated PT sec (IQR)	37.00 (33.53-41.70)	22-37 sec
Fibrinogen g/L (IQR)	3.95 (2.96-5.77)	2-4 g/L

Abbreviations: SI units, international system of units; IQR, interquartile range; HAART, highly active anti-retroviral therapy; INR, international normalized ratio; PT, prothrombin time.

Table 2. Risk factors for cytopenia (anemia, thrombocytopenia, or neutropenia) by multiple logistic regression methods.

Variable		Patients (N)	Univariate analysis by logistic regression model			Multivariate analysis by logistic regression model		
			HR	95% CI	*P*	HR	95% CI	*P*
Sex	Male	431	1.0	-	-			
	Female	41	0.757	0.388-1.476	0.414			
Route of transmission	Homosexual	208	1.0	-	0.791			
	Heterosexual	166	1.090	0.705-1.686	0.698			
	Blood transfusion	2	<0.0001	<0.0001	0.999			
	Unknown	96		0.478-1.398	0.461			
Age	<30	147	1.0	-	0.185			
	30-39	158	1.601	0.972-2.638	0.065			
	40-49	88	1.365	0.758-2.459	0.301			
	≥50	79	1.788	0.989-3.233	0.054			
AIDS status at the presentation	CD4 cell count, <200	134	8.894	5.642-14.020	<0.0001	8.520	5.166-14.052	<0.0001
	≥200	334	1.0			1.0		
Viral load, $\log_{10}$ copies/mL	<3.5	103	1.0	-	<0.0001	1.0	-	0.157
	<4.5	138	0.776	0.430-1.400	0.400	0.659	0.338-1.284	0.220
	<5.0	91	0.906	0.477-1.721	0.763	0.680	0.327-1.416	0.303
	≥5.0	116	2.588	1.469-4.560	0.001	1.226	0.621-2.421	0.556
VDRL titer	0	300	1.0	-	0.326			
	1 (<1:32)	93	0.742	0.445-1.238	0.254			
	2 (≥1:32)	43	0.657	0.318-1.358	0.257			

Abbreviations: HR, hazard ratio; CI, confidence interval; VDRL, Venereal Disease Research Laboratory test.

section. After exclusion, the total number of enrolled patients was 472 and data analysis was performed for these patients.

Baseline characteristics of the patients are listed in Table 1. The median age was 35 years (IQR, 27-44). The median CD4 lymphocyte count was 300/μL (IQR, 175-420), and the median $\log_{10}$ HIV RNA virus titer level/mL was 4.43 (IQR, 3.60-5.00) before initiation of anti-retroviral therapy. In 28.4% of patients, the CD4 lymphocyte count was below 200/μL, and this status was defined as immunologic AIDS. The percentage of patients who received HAART was 82.4%. Median baseline laboratory values are also shown in Table 1. Values for median WBC count, lymphocyte count, ANC, and Hb level were decreased compared to the reference range.

2. Coagulation abnormality

The median value of aPTT and fibrinogen was higher than the reference range. Only 2 patients in our cohort experienced venous thromboembolic events, and they both advanced to the AIDS stage. In addition, 2 patients had gastrointestinal bleeding, and 1 patient had intracranial hemorrhage. These events of thromboembolism and hemorrhage were not related to thrombocytopenia or coagulopathy.

Table 3. Rates of recovery after HAART.

Type of cytopenia	Recovery after HAART 6 months later (recovery/ total number [%])	Recovery after HAART 3 years later (recovery/ total number [%])
Neutropenia	41/45 (91.1%)	34/37 (91.9%)
Lymphopenia	88/105 (83.8%)	71/76 (93.4%)
Thrombocytopenia	9/9 (100%)	8/8 (100%)
Anemia	10/12 (83.3%)	9/9 (100%)
Isolated cytopenia	44/52 (84.6%)	42/46 (91.3%)
Bicytopenia	9/9 (100%)	8/8 (100%)

Abbreviation: HAART, highly active anti-retroviral therapy.

3. Frequency of cytopenia

The patients were divided according to the subtype and frequency of cytopenia. The frequency of cytopenia was anemia, 3.0% (14/472); neutropenia, 10.0% (47/472); thrombocytopenia, 2.4% (12/472); lymphopenia, 25.7% (121/470); isolated cytopenia, 11.2% (53/472); and bicytopenia, 2.1% (10/472).

4. Risk factors for cytopenia

We investigated the risk factors for cytopenia. Multiple logistic regression methods were used in both multivariate and univariate analyses. In univariate analysis, sex, age, route of transmission, AIDS status at baseline, HIV viral load ($\log_{10}$ copies/mL), and VDRL titer were included. AIDS status at baseline and HIV viral load ($\log_{10}$ copies/mL) were closely associated with cytopenia in the univariate analysis ($P<0.0001$). Significant variables (AIDS at the initial stage and HIV viral load grades [$\log_{10}$ copies/mL]) that had P-values lower than 0.1 were included in the multivariate analysis. In the multivariate analysis, only the initial AIDS status was significantly correlated with cytopenia ($P<0.0001$, HR=8.520, 95% CI=5.166-14.052). These results are described in detail in Table 2.

5. Effect of HAART

Remarkable hematologic recovery was observed after HAART in patients with cytopenia. Among 9 thrombocytopenia patients, 8 patients received HAART. Furthermore, HAART was given to 13 of 14 patients with anemia, 41 of 46 patients with neutropenia, and 105 of 121 patients with lymphopenia. The percentage of recovery of each cell lineage (thrombocytopenia, 100%; neutropenia, 91.1%; anemia, 84.6%; lymphopenia, 83.8%; isolated cytopenia, 84.6%; and bicytopenia, 100%) is shown in Table 3. Overall changes after 6 months and 3 years were established for each patient. After 6 months, 23 patients were lost during follow-up (anemia, 2; neutropenia, 1; thrombocytopenia, 2; lymphopenia, 16; isolated cytopenia, 1; bicytopenia, 1). Three years later, many patients were lost during follow-up. In Table 3, a denominator was used to describe the total number of patients after loss to follow-up.

DISCUSSION

In this study, the types and frequencies of cytopenia were examined. In the multivariate analysis, only AIDS status at baseline was significantly correlated with cytopenia. As in other studies, significant hematologic recovery occurred after HAART. Thromboembolic and hemorrhagic events occurred in 2 patients each in whom the infection had advanced to the AIDS stage. It has been reported that thromboembolism and hemorrhage are very rare and usually develop in the late stage of the disease [9, 10]. The incidence of hemorrhage in HIV patients is very low (8%), and intracranial hemorrhage has been reported only once in the literature [9]. Thus, our result is consistent with that of previous reports.

A low prevalence of cytopenia was reported in South Korea. A recent study reported that the prevalence of cytopenia in HIV patients receiving HAART in other countries was as follows: anemia, 12%; neutropenia, 14%; and thrombocytopenia, 7% [6]. This can be partially explained by the following reasons. First, the defining values for cytopenia in our study are lower than those in previous studies. Furthermore, the exclusion of patients with secondary causes of cytopenia from our cohort is likely to have reduced the prevalence of cytopenia significantly. The frequency of cytopenia was higher in the excluded patients than in the included patients (anemia, 8.1% [12/149]; neutropenia, 10.7% [16/149]; and thrombocytopenia, 4.7% [7/149]).

In our study, AIDS status at baseline was identified as the sole risk factor for cytopenia in the multivariate analysis. This finding supports the fact that bone marrow suppression and production defects are caused by HIV [1]. It is well known that the incidence and severity of cytopenia are generally correlated with the stage of HIV infection [7]. Therefore, this result is consistent with previous studies.

Our study also confirmed the reversal effect of HAART on thrombocytopenia, neutropenia, anemia, and lymphocytopenia, which again supports the fact that HIV infection causes bone marrow suppression and production defects [1]. Previous data by Sloand [11] suggests that HIV protease inhibitors may have direct effects on hematopoietic stem cells. Furthermore, it has been proven that HAART reduces HIV viral load and thereby the activity of immune effectors; e.g., the anti-proliferative activity by cytokines, and the concentration of transferrin receptor of neopterin are decreased. It has been demonstrated that the main mechanism of recovery from anemia by anti-retroviral therapy is the decline in the activity of immune effectors [12]. HAART decreases viral load, which may result in decreased activity of immune effectors, thus ameliorating anemia by reversing the anti-proliferative effects of cytokines. This suggests that immune reconstruction by control of the HIV viral load is the most important step in the management of HIV-related hematologic manifestations. Findings from previous studies have also demonstrated that HAART is the most effective means for recovery from thrombocytopenia [13]. Furthermore, the rate of reversal after HAART in our study was much higher

than that in previous studies [14]. In patients with HIV infection, hematological manifestations may be controlled primarily by HIV infection treatments such as HAART.

Compared with previous studies, patients in our study had certain unique characteristics. The median age was 35 years (IQR, 27-44), which was lower than the median age reported in studies from other countries (median age=38 years: IQR, 32-45) [4]. The median CD4 lymphocyte count was 295/μL (IQR, 178-410), which is higher than that reported in other countries. In addition, in 28.4% of patients, the infection had progressed to the AIDS status. This value was lower than that of studies from other countries (47.6%) and other Korean studies (37%) [15]. The higher CD4 count and lower percentage of AIDS patients was thought to be the result of the exclusion of patients with late-stage disease who had another infection and/or malignancy. South Korean HIV patients have specific characteristics. For instance, in HIV-infected Koreans, the primary route of reported HIV transmission is through sexual contact (99%), and the proportion of men is 91% [16]. Furthermore, until now, transmission through intravenous drug use has not been reported in South Korea. As a result, there were no patients in our study who had acquired HIV by intravenous drug use.

The strong points of this study are the lack of intravenous drug users and exclusion of patients with secondary causes of hematological impairments in HIV infection. Many preceding studies were conducted in heterogeneous groups that consisted of patients reporting various transmission routes, including intravenous drug use. Illicit use of intravenous drugs is associated with a reduction in the number and function of platelets even in non-HIV-infected individuals [17]. Other factors that may have influenced the hematological manifestations of HIV infection were also excluded from our study as much as possible. Therefore, our study is the first to investigate the impact of HIV infection itself on hematological manifestations in clinical situations.

Even though we reviewed all medical records in detail to exclude all other potential causes, it is possible that there were missed cases or incomplete medical records. Therefore, one of the limitations of our study could be the likelihood of incomplete exclusion. Another limitation is the tendency of retrospective data collection and small study populations to restrict statistical significances. Further randomized large-scale cohort studies are required in the near future.

In conclusion, on the basis of our study, the frequency of HIV-related cytopenia was very low in Korea. All types of HIV-related cytopenia were effectively reversed by HAART. Furthermore, the risk of cytopenia was related solely to the stage of HIV infection. Therefore, we propose that control of the HIV infection will be the core of the management of hematological manifestations of the virus.

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