

Thrombocytopenia according to Antiretroviral Drug Combinations, Viremia and CD4 Lymphocytes among HIV-Infected Patients in Cameroon A Snapshot from the City of Yaoundé

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Abstract

Background Thrombocytopenia is an abnormal decrease in blood platelets, which can affect the prognosis of people living with HIV (PLHIV). In order to limit the occurrence of this haematological disorder, we evaluated the frequency of thrombocytopenia according to antiretroviral drug combinations, viremia and the immune status of PLHIV. Methods A cross-sectional and analytical study was conducted from June-November 2016 among 310 PLHIV at the “Chantal BIYA” International Reference Centre for research on HIV/AIDS prevention and management (CIRCB), Yaoundé, Cameroon. Thrombocytopenia was assessed by blood count on Mindray BC 3000 plus, then categorized as mild (50,000-149,999 platelets/ μ L), moderate (20,000-49,999) and severe $<20,000$; HIV-1 viremia was measured by Abbott m2000RT and CD4 by BD FACS Calibur; treatment history was retrieved from medical records. Data were analysed using Graph Pad Prism.6, with $p < 0.05$ considered statistically significant. Results Median age was 40 [IQR: 33-49] years with, and 60.9% of participants being female. Up to 79.0% (245) were receiving antiretroviral therapy (ART); 54.5% had CD4 counts <500 cells/ mm^3 and 25.4% had viremia $>3 \log_{10}$ RNA/ml. Overall rate of thrombocytopenia was 19.0% (59/310), with 17.4% (54/310) mild, 1.6% (5/310) moderate and 0.0% severe. Following ART-exposure, rate of thrombocytopenia was 64.6% (42/65) versus 6.9% (17/245) in naïve versus treated patients respectively, $p < 0.0001$. Following ART regimens, rate of thrombocytopenia was 64.7% (11/17) versus 35.3% (6/17) among AZT-containing versus AZT-sparing regimens, $p = 0.02$. Following viral load ranges, rate of thrombocytopenia was 15.8% (20/130) in those with undetectable viral load, 11.0% (12/101) with viral loads 1.60-3.0 \log_{10} RNA/ml and 34.1% (27/79) with viral loads $>3 \log_{10}$ RNA/ml ($p = 0.03$; $r = -0.12$). As concerns CD4-count, rate of thrombocytopenia was 16.2% (42/259) in those with ≥ 200 CD4/ mm^3 versus 33.3% (17/51) with <200 CD4/ mm^3 ($p = 0.0003$; $r = 0.21$). After adjusting for age, sex, ART, viral load and CD4, only ART exposure was significantly associated with decreased risk of thrombopenia ($p < 0.0001$). Conclusions Thrombocytopenia occurs generally at mild-level among PLHIV in Cameroon, especially among ART-naïve, AZT-treated, high viremia and severe immune-compromised patients. Interestingly, ART coverage appears as an independent factor in preventing the occurrence of thrombocytopenia, especially for AZT-sparing treatment combinations in countries with similar features like Cameroon.

Background

In Cameroon, an estimated 560,000 people were living with HIV (PLHIV) and 34,000 AIDS-associated deaths were reported in 2014[1]. Global efforts in improved healthcare services of PLHIV resulted in a significant decline in the number of HIV/AIDS-related deaths and new infections. Of note, the number of AIDS-related deaths has declined by 42% worldwide between 2004 and 2014, with a greater decline in the sub-Saharan Africa region (48%) [1].

Within the frame of the clinical monitoring of PLHIV, haematological abnormalities are most often observed in addition to other complications [2]. These abnormalities are most often characterized by a decrease in peripheral blood cells. Specifically, a deficit in peripheral blood cells is likely to impair the clinical outcomes through anaemia, neutropenia and thrombocytopenia [2].

Thrombocytopenia is a condition characterized by an abnormally low amount of platelets in the blood. In clinical practice, this refers to a number of platelets less than 150,000 per mm³ of blood [3]. In the context of HIV infection, events of thrombocytopenia are likely due to either the drug-induced adverse events or the infection itself [4]. In this prospect, thrombocytopenia would therefore be suggestive of an increase in viremia, an alteration of the immune system and could also be due to the type of antiretroviral therapy (ART) [4].

In the western world, monitoring of HIV-infected patients is focused on regular measurements of viral load and CD4 lymphocytes, and this approach is becoming gradually accessible in resource-limited settings (RLS), including sub-Saharan Africa (SSA) [5]. With limited evidence on possible correlations/associations between thrombocytopenia and reference monitoring approaches of ART in SSA settings, it becomes relevant to generate findings that would guide on the occurrence of thrombocytopenia following exposure or non-exposure to ART, following type of ARV drug combinations, and following the viral dynamics and immune status of patients in SSA countries [6].

The aim of our study was to assess the burden of thrombocytopenia according to antiretroviral therapy (ART), viremia and CD4-lymphocytes count of PLHIV.

Methods

Study design and setting

A cross-sectional and analytical study was carried out among PLHIV in Yaoundé, Cameroon, monitored at the “Chantal BIYA” International Reference Centre for research on HIV/AIDS prevention and management (CIRCB). The CIRCB is a government institute of the Ministry of Public Health, in charge of research and reference clinical monitoring of HIV-infected patients, with participation in external quality assurance programs for HIV screening/diagnosis, viral load measurements, CD4 count, as well as biochemistry and haematological analysis (http://circb.cm/btc_circb/web/).

Following a consecutive sampling, a total of 310 participants were enrolled, based on the following criteria: (a) HIV-positive confirmed using the national algorithm in Cameroon; (b) providing of informed consent/assent for enrolment in the study; (c) documented treatment history. Non-inclusion was based on: events of pregnancy; co-infection with malaria; viral hepatitis B and C; or those who underwent blood transfusion during the past three months.

Laboratory procedures

At the medical units of CIRCB, the study information sheets were provided to each potential eligible patient and written informed consent/assent was obtaining from every participant. Sociodemographic data were obtained by data abstraction on a standard questionnaire for each participant. Clinical and therapeutic data were obtained from the medical records of each participant registered in the CIRCB database.

Briefly, 4 mL of whole blood was collected in a dry tube (for serological analysis) and 2 tubes of 4 mL of whole blood were collected in Ethylène diamine tetra acetic acid (EDTA) containing-tubes. The serum obtained from blood in the dry tubes was immediately used for carrying out the serological tests (HIV, Hepatitis B and C) by solid phase immunochromatography. Centrifugation of one EDTA tube was performed to obtain plasma which was then separated in two (2) 700 µL aliquots and stored at -20 ° C before analysis for viral load by Real Time PCR on the ABBOTT m2000RT platform as per the manufacturer's instructions (www.abbottmolecular.com/products/infectious-diseases/realtime-pcr/hiv-1-assay); the second EDTA tube was used to perform CD4 T lymphocyte typing using flow cytometry on Becton Dickinson's "FACS Calibur" according to the manufacturer's instructions (https://www.bdbiosciences.com/documents/BD_FACSCalibur_Brochure.pdf); the full blood count was performed on the "Mindray BC 3000 plus" automated system as per the manufacturer's instructions (<https://www.mindray.com/en/product/BC-3000Plus.html>) and the malaria diagnosis was done by fluorescence microscopy test as per the manufacturer's instructions (<http://www.cyto.purdue.edu/cdroms/cyto10a/sponsors/media/partec/cyscope.pdf>). The reliability and accuracy of the results were ensured by the systematic use of standard operational procedures and quality control panels.

Every potential case of thrombocytopenia from full blood count was confirmed by an observation of the blood smear. Thrombocytopenia was defined as a platelet count below $150 \times 10^3/\text{mm}^3$ [3], categorized as mild (50,000-149,999 platelets/ μL), moderate (20,000-49,999) and severe $<20,000$.

Statistical analysis

Data were recorded in an Excel spread sheet, and double-checked for data cleaning. The cleaned dataset was then analysed using the software Graph Pad prism version 6. The coefficient of correlation (r) was used to determine the existing relationships between the quantitative variables using the Spearman algorithm. Categorical data were analysed using the Mann-Whitney U test; and the significance threshold for statistical tests was set at 0.05.

Results

Characteristics of the study population

Out of 348 potential patients encountered, 29 had a positive plasmodium result and the remaining 09 had a positive viral hepatitis result (seven cases of hepatitis B and 2 cases of hepatitis C), which summed-up to 38 excluded cases. Thus, 310 patients were enrolled as study participants in the final dataset. Our study population consisted of 121 (39.03%) males and 189 (60.96%) females giving a female to male ratio of 2/1. Median age of participants was 40 [IQR: 33-49] years (Table 1). Regarding exposure to ART, 20.96% (65/310) were ART-naïve and 79.03% (245/310) were receiving ART as per national guidelines. Participants and by sex, viral load ≥ 3 Log copies/ml were slightly more prevalent in men versus women: 29.75% (36/121) versus 23.28% (44/189) respectively, $p=0.20$ (Figure 3).

Blood platelet levels in the study population

The mean blood platelet count was 217.64 ± 77.09 and ranged from 34.000 to 466.000/ μL . The prevalence of thrombocytopenia was 19.03% (59/310) with a predominance of mild thrombocytopenia 17.42% (54/310), followed by 1.6% (5/310) moderate, and no case (0.0%) of severe thrombocytopenia was found (Table 2).

Thrombocytopenia according to sex

According to sex, rate of thrombocytopenia was significantly higher among men as compared to women: 54.23% (32/59) versus 45.76% (27/59) respectively, $p=0.008$ (Figure 1).

Thrombocytopenia according to age

According to age distribution, the highest rate of thrombocytopenia was 59.32% (35/59) in adults aged between 19 to 45 years (Figure 2), without any significant difference ($p=0.39$).

Relationship between CD4 lymphocyte count and blood platelet count

According to CD4 T lymphocytes, mild thrombocytopenia was 27.77% among those with severe immunodeficiency, with a statistically significant difference ($p=0.003$) as compared to those with higher CD4 (Table 3). Furthermore, a weak positive and significant correlation was found between CD4 count and platelet count, $r=0.21$ (Figure 4).

Relationship between HIV viral load and platelet count

Up to 42.56% of thrombocytopenic patients were on viral load $>3\log_{10}$, a significant higher burden as compared to those with low-level viremia, $p=0.037$ (Table 4). Furthermore, a weak negative correlation between platelet count and viral load; $r=-0.12$ (Figure 5).

Relationship between ART-exposure and blood platelet count

Among naïve PLHIV, 64.6% (42/65) of cases of thrombocytopenia were observed compared to 6.9% (17/245) in ARV treated patients. Interestingly, depending on ART regimen, 64.7% (11/17) of thrombocytopenic patients were found among patients on AZT-containing HAART compared to 35.3% (6/17) non-AZT containing HAART, $p=0.02$ (Table 5).

Multi-regression analysis

After adjusting for age, sex, ART, viral load and CD4, only ART exposure was significantly associated with decreased risk of thrombocytopenia ($p<0.0001$), as shown in table 6.

Discussion

As the management of haematological disorders might be concerning in the course of HIV/AIDS, and for a better management of such potential burdens in settings where reference monitoring is restricted, it appears relevant to assess the burden of thrombocytopenia, to delineate possible determinants among PLHIV, and to propose measures to limit the occurrence of this haematological disorder among PLHIV in countries like Cameroon.

Using the target population of PLHIV in the city of Yaounde, women appeared predominant (60.96%), similar to the natural vulnerability to HIV and the epidemiological burden in the general population [6]. Our findings are therefore concordant to the study of Kouanfack *et al.*, in 2010 and Essomba *et al.*, in 2015 carried out in Cameroon (female predominance of 70.6% and 66.3% respectively) [7,8]. In the same line, the median age of 40 years is consistent with the sexually active population and the advent of ART that ensures longer lifespan to ART-experienced patients, as supported by Essomba *et al.* (43 years) [8].

In this target population of chronically infected Cameroonians, 19.03% of subjects had thrombocytopenia, almost all being mild disorders. Of note, the absence of severe thrombocytopenia can be explained by the fact that most of the patients were monitored within their routine care as outpatient, without emergency and most (79.0%) of them were on treatment against HIV infection, as appropriate ART also contributes to limiting the event of thrombocytopenia [14]. The rate of thrombocytopenia slightly differs from reports from Tene *et al.* (13.67% in 2014 within the same city) [9]. The slight difference could be attributed to lower representativeness in the previous study (n= 139 participants). On the other hand, our observation is close to that obtained by Taremwa *et al.*, in southwestern Uganda in 2015 (17.4%) [10] and 20% by Alaei *et al.*, in Iran around the year 2000 [11].

According to the distribution of thrombocytopenic cases by sex, men are more affected than women, as per knowledge on the variation of blood platelets by sex [12]. This observation could also be affected by the higher frequency of virological failure among men (29.75%) compared to women (23.28%) in our study thereby altering, if any effect, the platelet pipeline with high-level viremia [13].

Regarding the distribution of blood platelet count according to age, it has been found that platelet levels are low in children, high in adults and low in the elderly populations [13]. In our study population, adults had a higher thrombocytopenia rate (59.32% in people aged 19 to 45 years old) than children (3.38%) and the elderly (3.38%). This interpretation is limited by the low rate of children and elderly populations, which calls for investigation in this target population. Severely immune-compromised patients had 29% thrombocytopenia, with a significantly weak positive correlation between blood platelet count and CD4 count. This implies that decreasing CD4 cell count may be accompanied by a decrease in blood platelet count, similar to findings by Taremwa *et al.*, in Uganda [10]. On the same line, almost half (45.76%) of patients with high-level viremia ($\geq 3 \log_{10}$ copies/ml) were thrombocytopenic, indicating a significantly weak negative correlation between blood platelet count and plasma viral load. This may be attributed to the fact that an increase in viral activity could be accompanied by a decrease in blood platelet count, and consistent with the data of O'Bryan *et al.* in the United States [14]. As also reported by Mbanya *et al.*, in

2002, HIV infection can cause thrombocytopenia by various mechanisms including bone marrow destruction induced by viral activity, ART or immunological factors [15].

In our study, about 9% of people on ART had thrombocytopenia, slightly less than 13% obtained in Uganda [10], and higher than the 4.1% found in Ethiopia [17]. This suggest that ART might have a protective effect against thrombocytopenia, especially for those treated with AZT-sparing regimens (due to anaemic adverse effect of AZT) [18]. Among ART-naïve individuals, the burden of thrombocytopenia (37%) was slightly lower (27%) than data reported at the Yaoundé teaching Hospital (in the same township). O'Bryan *et al.*, in the United States also showed a decreased rate of thrombocytopenia in an HIV infected population moving from the pre-therapeutic to per-ART phase [14]. Of great clinical relevance, after adjusting for age, sex, ART, viral load and CD4, only ART exposure was significantly associated with decreased risk of thrombocytopenia. This result justifies the significance implication of ART in preventing risks of thrombocytopenia [19].

In assessing thrombocytopenia, we could not evaluate the root cause of platelet deficiency as either being of peripheral or central origin; also, our assessment is less representative of people at extreme age, thus calling for further investigations on haematological disorders in these target populations.

Conclusion

In this main city of the Centre Region of Cameroon, thrombocytopenia affects less than a quarter of PLHIV, with the majority having only mild thrombocytopenia. Thrombocytopenia occurs especially among ART-naïve patients, AZT-containing regimens, and slightly in the event of high-level viremia and severe immunodeficiency. Of great clinical relevance, after adjustment, ART coverage appears as an independent factor in preventing the occurrence of thrombocytopenia. However, an alternate therapeutic approach is needed for AZT-sparing treatment combinations in countries with similar features like Cameroon.

List Of Abbreviations

ARV: Antiretroviral;

AIDS: Acquired immunodeficiency syndrome;

ART: Antiretroviral therapy;

AZT: zidovudine;

CD4: Cluster of differentiation;

CHU: University Health Centre.

CIRCB: "Chantal BIYA" International Reference Centre for research on HIV/AIDS prevention and management

HAART: Highly active antiretroviral therapy

HIV: Human Immunodeficiency Virus;

PLHIV: People living with HIV;

Declarations

Ethics approval and consent to participate

This study obtained an ethical clearance from the Institutional Review Board of the University of Douala (N°CEI-UD/544/04/2016/T) and an administrative authorization from the CIRCB Directorate General. The participants freely provided their written informed consent forms, either in French or English language (with respect to the first language of the participant), while the minor participants provided their assent. For each participants aged <16 years, a written informed consent was obtained for parent or legal guardian, after providing of the study information. For purpose of confidentiality, data were processed by using specific identifiers and saved by a password encrypted computer.

Consent to publish

Not applicable

Disclosure statement

Authors declare that they have no financial, personal, or professional interests that could be construed to have influenced this manuscript.

Availability of data and materials

All datasets on which the conclusions of the manuscript are drawn are duly presented in the main paper and supplemental files.

Competing interests

Authors have no potential conflict or competing interests to declare.

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Authors' contributions

Conceived the study: Alex Durand Nka; Samuel Martin Sosso; Joseph Fokam; Elias Nchiwan Nukenine; Zélateur Aroga; Vittorio Colizzi; Alexis Ndjolo.

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Approved the final version of the submitted manuscript: All the authors.

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Tables

Table 1: Socio-demographic data

Variables	Frequency	Percentage (%)
Sex		
Male	121	39,03
Female	189	60,96
Age in years		
0-12	17	5,48
13-18	11	3,54
19-45	175	56,45
45-60	86	27,74
>60	21	6,77
Total	310	100

Table 2: Distribution of Blood platelet count in PLHIV

Blood platelet count (/ μ L)	Frequency	Mean \pm STD	Percentage (%)
Severe thrombocytopenia <20,000	0	0	0
Moderate thrombocytopenia (20 000 -50 000)	5	37.6 \pm 3.7	1.61
Mild Thrombocytopenia (50000 -150000)	54	119.3 \pm 25.0	17.42
Normal platelet count (150000 - 450000)	250	241.4 \pm 60.6	80.65
Thrombocytosis > 450000	1	466	0.32

STD: standard deviation

Table 3: Distribution of blood platelet count according to CD4 lymphocyte count

Blood platelet count (/ μ L)	CD4 lymphocyte count				Total
	< 200	[200 - 300]	[300 - 500]	> 500	
Severe thrombocytopenia (<20,000)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Moderate thrombocytopenia (20 000 - 50000)	2(40%)	1(20%)	1(20%)	1(20%)	5(100%)
Mild thrombocytopenia (50000 - 150000)	15(27.77%)	5 (9.25%)	13(24.07%)	21(38.88%)	54(100%)
Normal platelet count (150000 - 450000)	34(13.60%)	30 (12.00%)	68(27.70%)	118(47.2%)	250(100%)
Thrombocytosis > 450000	0 (0%)	0 (0%)	0 (0%)	1(100%)	1(100%)

Table 4: Distribution of blood platelet count by plasma viral load levels

Blood platelet count (/ μ L)	Plasma viral load			Total
	Not Detectable	1.60 log ₁₀ - 3.00 log ₁₀	>3,00 log ₁₀	
Severe thrombocytopenia	0 (0%)	0(0%)	0(0%)	0 (0%)
Moderate thrombocytopenia	1(20%)	0(0%)	4(80%)	5 (100%)
Mild thrombocytopenia	19(35.18%)	12 (22.22%)	23(42.59%)	54 (100%)
Normal platelet count	110 (44%)	88(35.2%)	52(20.8%)	250(100%)
Thrombocytosis	0(0%)	1(100%)	0(0%)	1(100%)

ART	Total(n)	Frequency (%)
Treatment with AZT	11	64.7
Treatment without AZT	6	35.3

p = 0.02

Table 5: repartition between ARV treatment and blood platelet count.

Thrombocytopenic cases on ART were more represented in those who were in therapeutic combinations containing AZT.

Table 6: Regression analysis of thrombocytopenia

Variable	Coefficient	95% confidence	Limits	Std Error	F-test	P-value
Age	-0.297	-0.805	0.211	0.258	1.3223	0.251083
Sex (F/M)	-3.495	-18.428	11.438	7.589	0.2121	0.645447
CD4	0.014	-0.011	0.038	0.012	1.2325	0.267801
Viral Load	0.000	0.000	0.000	0.000	1.5098	0.220118
ART Exposure (Naive/ART)	-114.561	-136.604	-92.519	11.201	104.5991	<0.000001
Constant	241.360	215.514	267.206	13.135	337.6706	<0.000001

After adjusting for age, sex, ART, viral load and CD4, only ART exposure was significantly associated with decreased risk of thrombocytopenia ($P < 0.0001$).

Figures

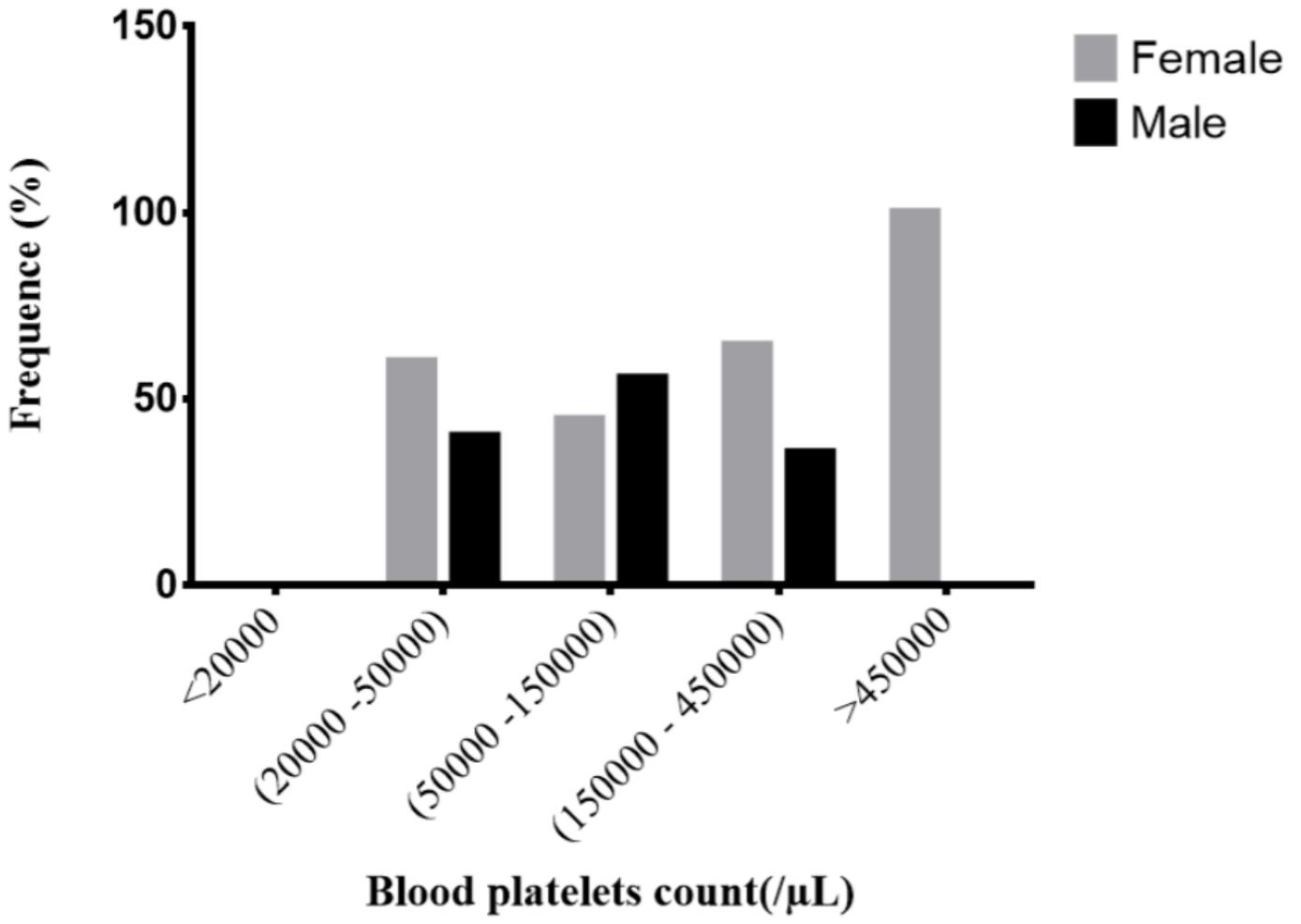


Figure 1

Distribution of blood platelets count by Sex

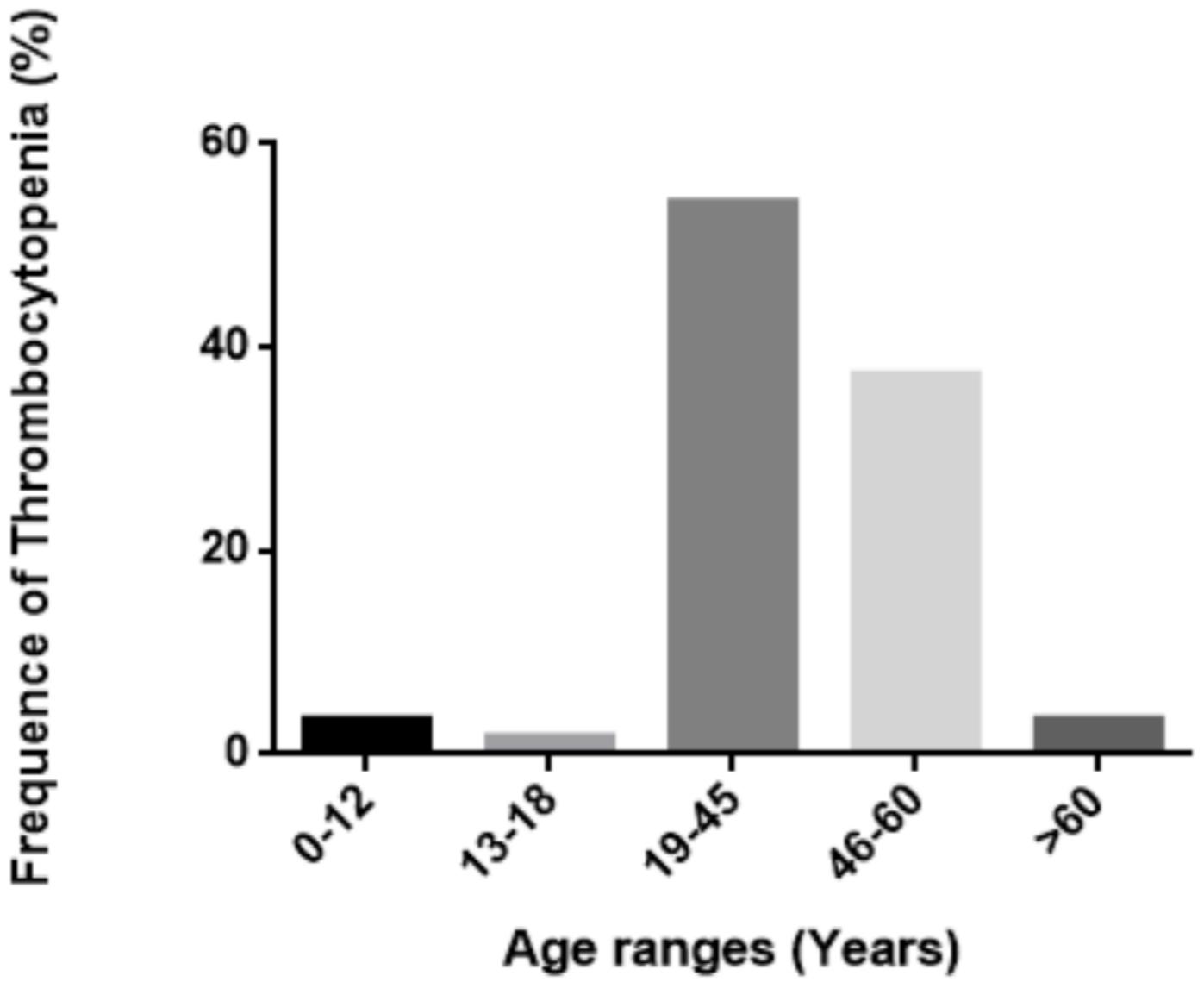


Figure 2

Distribution of blood platelet count by Age

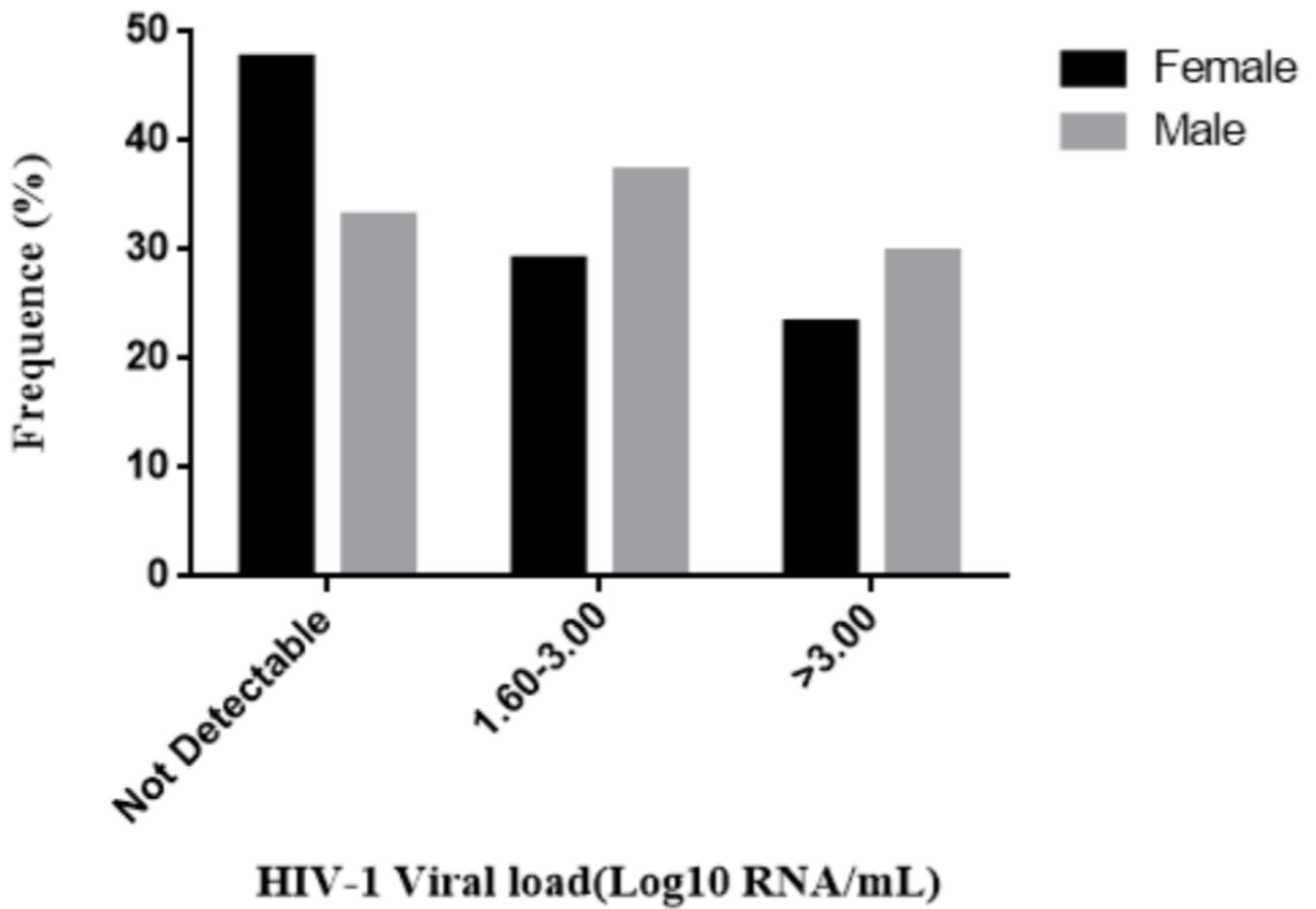


Figure 3

Distribution of HIV viral load by Sex

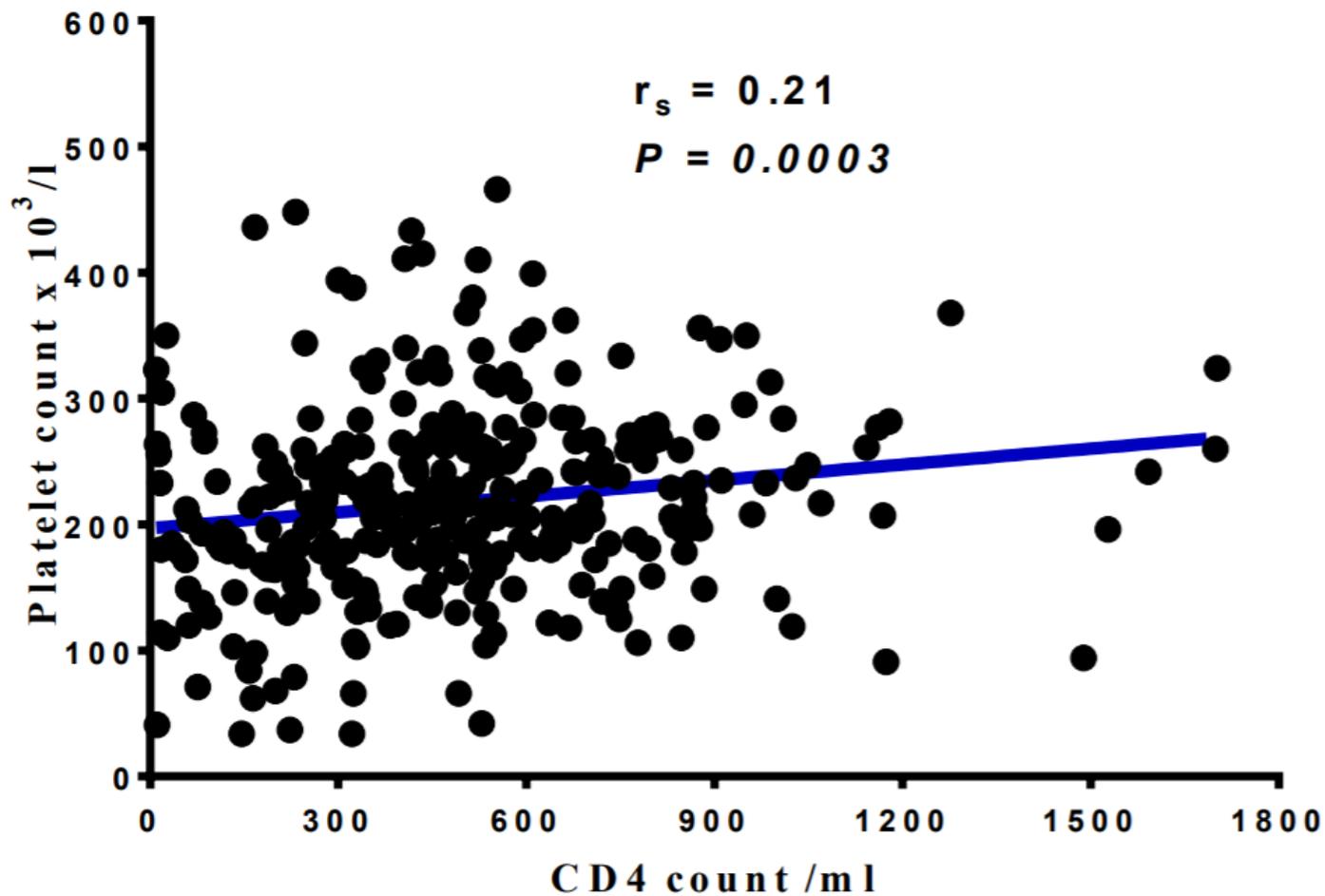


Figure 4

Correlation between blood platelet count and level of TCD4 lymphocytes. CD4: Cluster of differentiation 4.

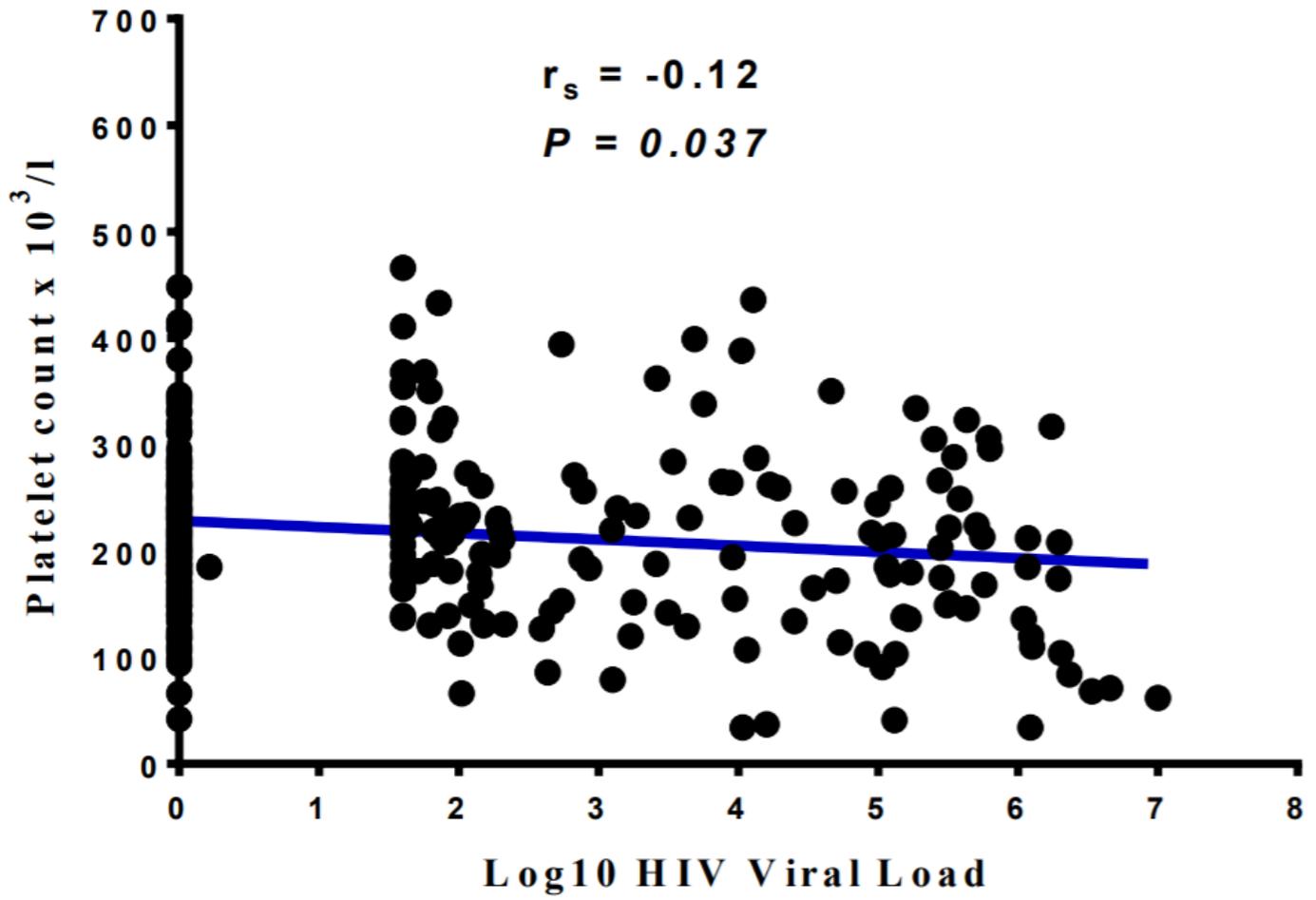


Figure 5

Correlation between blood platelet count and HIV viral load. HIV: Human immunodeficiency virus