International Journal of Infectious Diseases 15 (2011) e808–e811

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Hematological abnormalities in HIV-infected patients

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ARTICLE INFO

Article history:
Received 13 April 2011
Received in revised form 12 July 2011
Accepted 1 August 2011

Corresponding Editor: Mark Holodniy, California, USA

Keywords:
Anemia
Neutropenia
Thrombocytopenia
CD4 count
Viral load
Mortality

SUMMARY

Background: Anemia, neutropenia, and thrombocytopenia are commonly observed in HIV-infected patients. This study was undertaken to evaluate the prevalence of cytopenias and their association with CD4 count. Furthermore, the association of hemoglobin concentration with mortality was also investigated.

Methods: We reviewed the data of 701 HIV-infected patients followed at our institution. Blood cell counts, hemoglobin concentration, CD4 count, and viral load were recorded. We also recorded the mortality rate after 1 year in the groups with CD4 $<200/\mu l$ and $\geq 200/\mu l$ according to hemoglobin concentration.

Results: Of the total patients, 37.5% had anemia; 61.1% (110/180) were in the low CD4 group and 29.4% (153/521) were in the high CD4 group ($p < 0.01$). Mean neutrophil counts were 2.610×10^9/l and 3.204×10^9/l in the low CD4 and high CD4 groups, respectively ($p < 0.01$); mean platelet counts were 218.639×10^9/l and 234.807×10^9/l for the low CD4 and the high CD4 groups, respectively ($p = 0.03$). Patients whose hemoglobin concentration was below the median value had a higher death rate in both the low CD4 (14 vs. 4 deaths, $p = 0.013$) and high CD4 (8 vs. 1 death, $p = 0.0158$) groups.

Conclusions: We found an association between CD4 count and hemoglobin level, neutrophil count, and platelet count, and that anemia was independently associated with a higher mortality.

1. Introduction

The most important biomarkers of disease stage and progression in patients with an HIV infection are the CD4 count and HIV RNA concentration.[1–3] However, there are other factors that can influence or predict the prognosis.[4] Hematological abnormalities, such as anemia, neutropenia, and thrombocytopenia, are commonly observed in patients infected with HIV.[5,6] For this reason the total lymphocyte count, white blood cell count, and hematocrit or hemoglobin concentration have been proposed as alternative markers of the disease, especially for developing countries where financial resources are limited.[7] Nevertheless, the majority of these markers have not been adopted into routine use because of their supposedly poor correlation with disease progression.

Anemia is the most frequent hematological abnormality in patients infected with HIV, even in patients under highly active antiretroviral therapy (HAART), and has been associated with impaired quality of life,[8–10] accelerated progression of the disease, and a higher mortality rate.[11–15] Usually, anemia in an HIV-infected patient is normocytic and normochromic and is associated with a low reticulocyte count.[16] The causes of anemia are various. Its pathophysiology involves three mechanisms: (1) decreased red blood cell (RBC) production: opportunistic infection, direct effect of HIV infection itself, myelosuppressive medications, decreased production of erythropoietin, hypogonadism; (2) increased RBC destruction: autoimmune hemolytic anemia, thrombotic microangiopathy, disseminated intravascular coagulation; and (3) ineffective RBC production: folic acid and vitamin B12 deficiencies. Nutritional deficiencies such as vitamin and iron deficiencies are common in developing countries.[17] The degree of inflammation and its impact on hemoglobin levels could eventually be useful as a marker of disease status and prognosis.

Neutropenia as well as anemia is commonly observed in patients with HIV infection. Up to 70% of patients at advanced stages of AIDS present low neutrophil counts.[16] Worsening HIV disease, demonstrated by decreasing CD4 cells counts and increasing HIV-1 RNA levels, has been associated with the development of neutropenia.[18] Thrombocytopenia affects

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doi:10.1016/j.ijid.2011.08.001

approximately 40% of patients infected with HIV during the course of their illness, and low platelet counts can be the only hematological abnormality at presentation.[6] Isolated thrombocytopenia does not seem to affect the prognosis.[19,20]

The objective of this cross-sectional retrospective study was to evaluate the frequency of hematological abnormalities in HIV patients, and to evaluate the correlation of anemia, neutropenia, and thrombocytopenia with CD4 cell number, viral load, and with mortality. Furthermore, we aimed to evaluate the impact of zidovudine (ZDV) on hemoglobin concentration levels.

2. Methods

2.1. Patient population

We reviewed the data of 701 consecutive HIV-infected outpatients followed at our institution (Hospital das Clinicas de Ribeirão Preto da Universidade de São Paulo-USP) between August and November 2009. Patients had to have had at least one determination of hemoglobin level, CD4 count, and viral load (Table 1). We also recorded the number of deaths 1 year later. Patients under 16 were excluded from the analysis. We defined anemia as a hemoglobin concentration below 12.0 g/dl for women and below 14.0 g/dl for men. Data regarding co-infection with hepatitis C virus (HCV) were also recorded.

This study was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000, and was approved by the ethics committee of our institution (protocol number 13 418/2009). As this was a retrospective and cross-sectional analysis, the local committee exempted the authors from the need to apply for informed consent.

2.2. Blood cell counts

Peripheral blood cell counts were performed using an ABX Pentra 120 DX automated hematology analyzer (Horiba ABX, Montpellier, France). The analyses recorded were RBC count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), and white blood cell (WBC), lymphocyte, neutrophil, and platelet counts.

Table 1
Description of the patient population

Variables	Value[a]
Gender	
Male	432 (61.6%)
Female	269 (38.4%)
Age (years)	42 (16–79)
Exposure group	
Injection drug use	104 (14.8%)
Male homosexual	92 (13.1%)
Heterosexual	425 (60.6%)
Blood transfusion	21 (3.0%)
Maternal–fetal transmission	3 (0.4%)
Not defined	56 (8.0%)
Regular use of HAART	577 (82.3%)
CD4	
CD4 ≥200/μl	521 (74.3%)
CD4 <200/μl	180 (25.7%)
Undetectable viral load	458 (65.3%)
Hemoglobin (g/dl)	
Male	14.4 (6.4–20)
Female	12.6 (6.4–15.9)
Neutrophil count ($\times 10^9$/l)	2.800 (4.00–17.500)
Platelet count ($\times 10^9$/l)	227.000 (16.000–615.000)

HAART, highly active antiretroviral therapy.
[a] Values are *n* (%) or mean (range).

2.3. CD4 quantification

Absolute CD4 counts were obtained from a combination of the results from the hematology analyzer and from flow cytometry on a BD FACSCalibur cytometer (Becton Dickinson, San Jose, CA, USA). The reagents employed were the BD Multitest CD3/CD4/CD8/CD45 (Becton Dickinson, San Jose, CA, USA). In brief, specimens were stained for 15 min at room temperature with a cocktail of four monoclonal antibodies (CD4APC, CD8PE, CD45PerCP, CD3FITC). Erythrocytes were lysed by incubating with lysing solution (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) for 15 min at room temperature at a ratio of 1:9 (volume of sample:volume of lysing solution), and the specimens were analyzed. When combined with total lymphocyte counts from the hematology analyzer, the flow cytometric analyses produced T-cell CD4 counts.

2.4. HIV-1 viral load determination

HIV viral load was determined directly by a hybridization sandwich nucleic acid technique using the VERSANT® HIV-1 RNA 3.0 (bDNA) (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) protocol according to the manufacturer's instructions. Values less than 50 copies/ml were reported as undetectable. A negative control was included in each run.

2.5. Statistical analyses

Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Basic demographic characteristics of the study population were obtained, and comparisons were made between groups using unpaired *t*-tests, W^2 tests, and Wilcoxon rank sum tests, as appropriate. Comparison of categorical variables (clinical features or events) was performed using Fisher's exact test. Correlations between each hematological variable with viral load were tested using the Pearson correlation coefficient. The comparison of hemoglobin results according to gender was done using one-way analysis of variance (ANOVA). A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Groups according to CD4 number

The patients were divided into two groups according to their CD4 count: low CD4 (<200/μl) and high CD4 (≥200/μl). (At first, the patients were divided into three groups according to CD4 count: <200/μl, 200–500/μl, and ≥500/μl. However, the results of the hematological variables were not different between the last two groups, and for this reason they were merged.) The low CD4 group comprised 180 patients (25.7%) and the high CD4 group 521 patients (74.3%). There were no differences between the groups with regard to the types of antiretroviral drugs used (data not shown) or the time from diagnosis (CD4 <200/μl: 96 ± 75 months; CD4 ≥200/μl: 106 ± 61 months).

3.2. Hemoglobin concentration and prevalence of anemia

A total of 37.5% of patients (263/701) had anemia at the moment of evaluation. The prevalence of anemia was 33.1% (89/269) in female patients and 40.5% (175/432) in male patients ($p = 0.0544$). Furthermore, 61.1% of patients (110/180) had anemia in the low CD4 group and 29.4% (153/521) in the high CD4 group ($p < 0.01$). The mean ± standard deviation (SD) hemoglobin concentrations were 11.6 ± 2.07 g/dl and 12.8 ± 1.47 g/dl for female patients in the low and high CD4 cell count groups, respectively

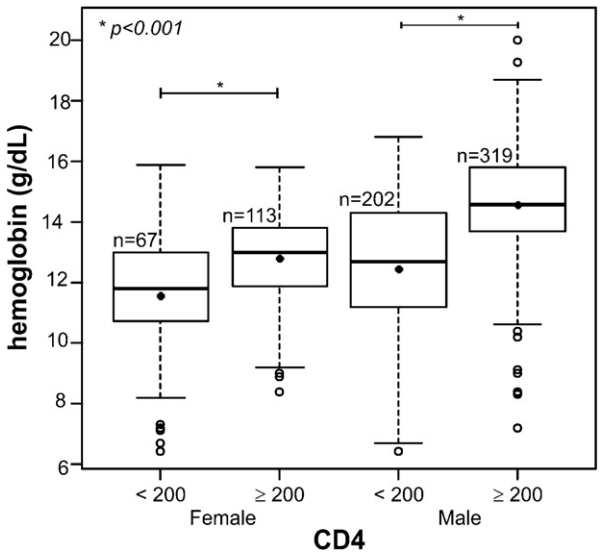

Figure 1. Hemoglobin concentration according to gender and CD4 groups.

($p < 0.001$), and 12.5 ± 2.36 g/dl and 14.6 ± 1.83 g/dl for male patients in the low and high CD4 cell count groups, respectively ($p < 0.001$) (Figure 1). Forty-five percent (119/263) of the anemic patients presented an MCV above 100 fl, and 8.4% (22/263) an MCV below 80 fl. As expected, microcytic anemia was more frequent in females (15.5% vs. 4.5%; $p = 0.0039$). There was no difference between the types of anemia (according to the MCV) in relation to CD4 groups (data not shown).

3.3. Neutrophil and platelet counts in the low and high CD4 cell groups

The mean ± SD neutrophil counts were $2.610 \pm 1.331 \times 10^9$/l and $3.204 \pm 1.690 \times 10^9$/l for the low CD4 and high CD4 groups, respectively ($p < 0.01$) (Figure 2A).

The mean ± SD platelet counts were $218.639 \pm 110.291 \times 10^9$/l and $234.807 \pm 79.080 \times 10^9$/l for the low CD4 and the high CD4 groups, respectively ($p = 0.03$) (Figure 2B).

3.4. Hematological variables and viral load

We did not find any correlation between the values of the hematological variables and viral load: hemoglobin concentration ($r = -0.18$), neutrophil count ($r = -0.09$), and platelet count ($r = -0.05$) (data not shown). However, as expected, an undetectable viral load was more frequently observed in the group of patients with CD4 cells >200/μl (75%) than in the group with CD4 cells < 200/μl (37%) ($p < 0.0001$).

3.5. Hemoglobin concentration and use of ZDV

Mean hemoglobin concentrations were 13.60 ± 2.35 g/dl for the group of patients using ZDV regularly (404/700, 57.7%) and 13.65 ± 2.00 g/dl for the group that did not use ZDV (296/700, 42.3%) ($p = 0.59$).

3.6. Anemia and co-infection with HCV

A total of 113 (16.1%) patients had a co-infection with HCV; 44 (38.9%) of them had anemia. Five hundred and eighty-eight (83.9%) patients did not have a co-infection with HCV, of whom 233 (39.6%) had anemia ($p = 0.9167$).

3.7. Anemia and survival

The groups of patients with low CD4 cells (180/701; 25.7%) and high CD4 cells (521/701; 74.3%) were further divided according to hemoglobin level. The patients whose hemoglobin concentration was less than the median value had a higher death rate after 1 year of follow-up than those whose hemoglobin concentration was on or above the median value, both in the low CD4 group (14 vs. 4 deaths, $p = 0.013$) and in the high CD4 group (8 vs. 1 death, $p = 0.0158$). Only six of 22 (27.3%) anemic patients who died were receiving vitamin supplementation (folate or vitamin B12). Infection was the cause of death in all eight anemic patients with CD4 ≥200/μl.

4. Discussion

The pathogenesis of anemia, neutropenia, and thrombocytopenia in HIV-infected patients is not fully understood, but is assumed to be multifactorial.[16] Anemia can occur at any phase of HIV infection and its prevalence and severity are correlated with progression of the disease.[11] The prevalence of anemia in our patients, defined as a hemoglobin concentration of less than 12 g/dl for women and 14 g/dl for men, was 37.7%, a value somewhat lower to that found by others.[12] Possible explanations for this finding could be the good adherence of our patients to medical follow-up and treatment (more than 80% of patients regularly using HAART drugs), and improvements in social and nutritional conditions that have occurred in Brazil, especially in our state, over recent decades. As expected, patients from the group with low CD4 counts presented a significantly increased rate of anemia compared to patients from the group with high CD4 counts

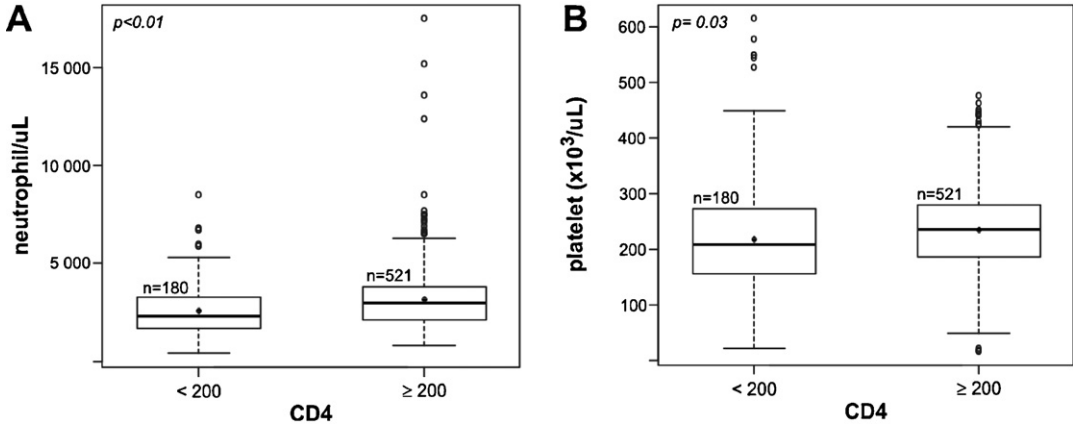

Figure 2. (A) Neutrophil and (B) platelet counts according to CD4 groups.

(61.1% vs. 29.4%). Also, we did not observe any difference in the prevalence of anemia between women (33.1%) and men (40.5%) ($p = 0.0544$). This result is in contrast with that found by others, who have observed a higher prevalence of anemia in women,[21] who are more liable to iron deficiency. However, in accordance with the above-mentioned reference, we did find a higher proportion of microcytic anemia in female patients, which could perhaps be explained by a higher propensity of this group of patients to have iron deficiency.

Furthermore, we did not observe any relationship between hemoglobin levels and the regular use or not of ZDV, a finding that differs from that obtained by others.[22] However, as mentioned above, all our patients were being treated with a HAART regimen; this could have to a certain degree counteracted the negative effects of ZDV on erythropoiesis. Others have shown that the use of ZDV is not associated with anemia when it is part of a HAART regimen.[14] Besides, at our institution, during the study period, ZDV was suspended as soon as anemia or a decrease in hemoglobin concentration was detected. Also, we did not observe any association between co-infection with HCV and anemia.

The mean neutrophil count was lower in the group of patients with low CD4 cell counts, a finding that is in accordance with the literature.[16,18] Similar results have been found for platelets. Patients with a more severe disease (CD4 $<200/\mu l$) presented a slightly lower platelet count. However, the difference between the two groups was of a lesser magnitude than that observed for neutrophils. This finding suggests that platelet level is not the most reliable surrogate marker for disease progression. It is known that a substantial percentage of patients present thrombocytopenia as the first clinical manifestation of HIV infection, when it is not expected until there is a significant degree of immunodeficiency.[23]

Finally, as expected, hemoglobin concentration was found to be a reliable predictor factor for disease progression. Patients with lower hemoglobin concentrations, irrespective of the CD4 count group, had a higher death rate after 1 year of follow-up, a finding that is in accordance with previous reports.[24–27] This finding confirms that hemoglobin concentration could be used as a reliable biomarker of the prognosis in HIV-infected patients, and that a therapeutic approach is imperative for patients with anemia.

In conclusion, in this study we found an association between CD4 count and hemoglobin level, neutrophil count, and platelet count. However, we did not observe any correlation between these variables and viral load. Moreover, hemoglobin concentration was strongly correlated with death rates for patients with both low and high CD4 counts, which warrants the use of this variable as a predictor factor for the prognosis, especially in countries with limited resources where routine enumeration of CD4 cells may not be feasible.

Acknowledgements

The authors thank Centro de Metodos Quantitativos (CEMEQ) of the Medical School of Ribeirão Preto, University of São Paulo, especially Juliana Boleta and Estela Cristina Carneseca, for the statistical analyses. The authors also thank Roberto Oliveira Freitas for assistance with informatics, and Sandra Navarro Bresciani for assistance with the formatting of figures.*Conflict of interest:* The authors declare that they have no conflict of interest to disclose.

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