

## ORIGINAL ARTICLE

# AUTOIMMUNE HEMOLYTIC ANEMIA IN HIV-INFECTED PATIENTS: A HOSPITAL BASED STUDY

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### Abstract

**Background:** The prevalence of anemia in HIV/ AIDS patients is high, with a multitude of possible etiologies; autoimmune hemolytic anemia (AIHA) in HIV/AIDS patients has been associated with a poor prognosis when treated with red cell transfusion. Our aim was to demonstrate the frequency of AIHA in a cohort of adult Nigerian HIV/AIDS patients and to see if the presence or not of AIHA is related to the severity of the disease with regards to the CD4 counts and the presence or absence of opportunistic infections.

**Method:** Ninety- eight adult patients with HIV infection were screened for the presence of AIHA using the packed cell volume (PCV), direct antiglobulin test (DAT) and reticulocyte count (RC).

**Results:** The frequency of AIHA was 3.06%, 36.74% of our study population were anemic; 11.22% had a positive DAT. Mean RC was 2.22 +/- 0.90 for all the patients. There was no statistically significant difference in the PCV of patients that had positive and negative DAT. There was no correlation between the presence of AIHA, use of ART, presence of opportunistic infections or CD4 counts.

**Conclusion:** We conclude that in spite of the low frequency of AIHA in HIV/AIDS patients, the fact that most patients will respond to standard treatment makes it imperative to screen HIV/AIDS patients with anemia for the presence of AIHA. Again since HIV/AIDS patients with AIHA may have a fatal reaction to red cell transfusion, we suggest that anemic patients with HIV/AIDS in non-emergency situations be screened for the presence of AIHA before receiving red cell transfusions when indicated.

**Key words:** Autoimmune, anemia, hemolysis, HIV/AIDS

### Résumé

**Culture générale:** Il y a trop de fréquence d'anémie parmi les malades sero-positifs ou les malades atteints de sida, souvent accompagnée d'un grand nombre d'étiologies: anémie hémolytique auto-immune (AHAI) parmi les malades sero-positifs est directement liée à une mauvaise pronostic, surtout quand on fait le traitement avec une transfusion de globule rouge. Notre objectif est de démontrer la fréquence de (AHAI) parmi un nombre d'adultes nigériens sero-positifs ou qui sont atteints du sida. C'est aussi de vérifier si la présence ou l'absence de (AHAI) est directement liée à la gravité de la maladie, de la maladie surtout quand il s'agit de la numération CD4 et de la présence ou absence des infections opportunistes.

**Modalité:** À l'aide de l'analyse directe antiglobuline (ADA) et la numération réticulocyte (NR) et le packed cell volume (PCV) ; quatre-vingt-dix-huit malades sero-positifs, ont passé un test de dépistage pour vérifier la présence de (AHAI)

**Résultat:** La fréquence de (AHAI) était 3.06%, 36.74% de notre population était anémique ; 11.22% était positif de l'analyse antiglobuline. Pour la numération réticulocyte, la moyenne était 2.22+/-0.90 pour tous les malades, on n'a trouvé aucune différence qui est statistiquement significatif parmi les malades

positif de (ADA) et négatif de (ADA). Il n'y avait aucun rapport entre la présence de AHAI, l'emploi de ART, présence des opportunistes ou la numération CD4.

**Conclusion:** Pour conclure, il faut souligner que, malgré la basse fréquence de AHAI dans les malades atteints du sida ou sero-positifs, tous les sero-positifs avec anémie doivent passer un test de dépistage, puisqu'il est évident que la plupart des malades réagissent positivement au traitement. Et en plus, nous proposons que désormais, avant la transfusion de globules rouges, tous les malades sero-positifs ou du sida, passent un test de dépistage afin de déterminer la présence de AHAI car parfois la prise de cette transfusion peut aboutir à la mort.

**Mots clés:** autoimmuno, anémie, hémolyse, SIDA\VIH

## Introduction

Infection with the human immunodeficiency virus (HIV) which causes the acquired immunodeficiency syndrome (AIDS) has remained a scourge in the developing countries of the world; recent research has shown that while only about 10% of the world's population lives in sub-Saharan Africa, the region is home to about 64% of people living with HIV/AIDS globally.<sup>1</sup> Nigeria has the largest population in Africa and an HIV/AIDS prevalence of about 5%.<sup>2</sup> In spite of the recent availability of antiretroviral therapy (ART) at subsidized rates in Nigeria, only a small fraction of patients are currently being treated. In fact, in 2005 only about 17% of persons in need of ART in sub-Saharan Africa received it.<sup>1</sup>

Anemia is prevalent among HIV/AIDS patients, it is said to be present in 10%-20% of patients at initial presentation and in over 70% over the course of the disease.<sup>3</sup> Anemia in HIV/AIDS has multiple etiologies, these include: decreased production following suppression of hemopoiesis by lymphoma cells, infections such as tuberculosis (TB) and inflammatory cytokines; disseminated intravascular coagulation, thrombotic thrombocytopenic purpura and immune and non-immune hemolysis; or ineffective production due to vitamin B12 or/ and folic acid deficiency; made worse by therapy with Zidovudine which is known to suppress erythropoiesis.<sup>3</sup> It is certain that the presence of anemia in people living with HIV worsens the prognosis since it is associated with progression to AIDS<sup>4</sup> and shorter survival times for HIV infected patients,<sup>5</sup> it has been shown that anemia is a predictor of poorer prognosis for HIV infected patients independent of the CD4 count.<sup>6</sup>

Despite the high frequency of anemia and a positive direct antiglobulin test (DAT) in these patients, autoimmune hemolytic anemia (AIHA) is less frequently diagnosed; this may be related to the frequent lack of reticulocytosis<sup>7</sup> which makes the diagnosis of AIHA more difficult, further complicated in our environment by the fact that autoimmune disorders are more common in Caucasians than the indigenous African population. This finding may be as a result of the endemicity of infectious and parasitic diseases in Africa which impair hosts T-cell immunity.<sup>8,9</sup> An earlier clinical and serological study

from Ethiopia confirmed that autoimmune diseases are rare in many parts of Africa and concluded that indigenous Africans that develop autoimmune diseases may have hereditary predisposition.<sup>10</sup> Though more recent reports show that some forms of autoimmune diseases may actually be more common in Africans than earlier thought.<sup>11</sup> The annual incidence of AIHA is estimated to be one per 75000-80000 population.<sup>12</sup> Autoimmune hemolytic anemia is characterized by binding of anti-erythrocyte autoantibodies to red cells and the subsequent destruction of the coated cells in the reticuloendothelial system.<sup>8</sup>

Anemia in patients with HIV infection may respond to treatment with ART, however, patients who continue to have symptomatic anemia while on ART may need additional intervention<sup>13</sup> such as erythropoietin.<sup>3</sup> Corticosteroids and immunoglobulin are considered the first line treatments for AIHA in immune competent patients, while splenectomy is reserved for those cases that do not respond.<sup>14</sup> In patients with HIV/AIDS and AIHA care must be taken in placing them on steroids as it may further worsen their immune suppression. Research has shown that HIV/AIDS patients with AIHA who receive red cell transfusions have an increased risk of thromboembolism which may be fatal.<sup>15</sup> People living with HIV/AIDS with resolved anemia have been shown to have a better prognosis than those with anemia or those with anemia that did not respond to therapy.<sup>16</sup>

The aim of our study was to demonstrate the frequency of AIHA among a cohort of adult HIV/AIDS patients attending the HIV clinic in UBTH; and to see if the presence or not of AIHA is related to the severity of the disease with regard to the CD4 counts of these patients and the presence or absence of opportunistic infections.

## Subjects and Methods

Ninety-eight unselected, consecutive, adult HIV positive patients attending the outpatient clinic for people living with HIV/AIDS at the University of Benin Teaching Hospital were screened for the presence of

AIHA using the packed cell volume (PCV), direct antiglobulin test (DAT) and the reticulocyte count (RC). They included: 48 males and fifty female patients. Only patients who gave informed consent were included in the study. For the purposes of this study, presence of AIHA was defined as a PCV less than 30%, positive DAT and a RC greater than 2.5%

Four milliliters of blood was withdrawn from each patient by clean venepuncture and placed in an EDTA bottle for use in estimation of PCV, RC and DAT. PCV, RC and DAT were carried out as previously described.<sup>17,18</sup> While the microhemocrit method and new methylene blue reagent were used for PCV and RC respectively; DAT was carried out as follows: the test cells were washed four times with a minimum of 3 ml of saline per wash, as much of the supernatant as possible was removed after each wash to achieve maximum dilution of residual serum and then made up to 3% suspension in saline. Two volumes of the antiglobulin reagent was added to two volumes of the 3% cell suspension, the test tube was immediately centrifuged after thorough mixing and then read for agglutination. The same procedure was repeated with Coomb's positive and negative cells as control for each batch.

Patients were interviewed and information entered onto a standardized data form, additional data such as the CD4 counts were extracted from the case notes, patients who had not done a CD4 count within the previous 4 weeks were excluded from the

study. The study was approved by the hospital's ethics committee.

Data collected in this study were analyzed on a computer with SPSS (Statistical Package for Social Sciences) software version 12.0. Means were compared using the Students' *t* test; level of significance was taken as  $P < .05$ . The average values are presented as mean  $\pm$  standard deviation (SD) unless otherwise stated.

## Results

Ninety-eight patients were involved in this study, made up of 48(48.97%) males and 50(51.02%) females respectively. Mean age for the study population was 37.43  $\pm$  7.93 years; age and sex distribution of the study population is shown in Table 1. Mean PCV was 31.62  $\pm$  5.94%. Three (3.06%) patients met the study criteria for AIHA; eight other patients had positive DAT, but did not meet the other study criteria for AIHA. Mean PCV was 24.71  $\pm$  3.30% in patients with AIHA compared with 32.15  $\pm$  5.77% in those without AIHA, this was statistically significant. Mean RC was 2.22  $\pm$  0.90% for all the patients; it was 6.77  $\pm$  0.45% in those positive for AIHA and 1.96  $\pm$  0.69% in those without AIHA, this was also statistically significant. Table 2 shows the result of laboratory parameters in different categories of the study population.

**Table 1.** Age and sex distribution of the study population

Age (years)	M (%)	F (%)	Total (%)
21-30	8 (8.16)	12 (12.25)	20(20.41)
31-40	28 (28.57)	23 (23.47)	51(52.04)
41-50	9 (9.18)	9 (9.18)	18(18.37)
51-60	3 (3.06)	6 (6.12)	9 (9.18)
Total	48 (48.97)	50 (51.02)	98(100)

**Table 2.** Laboratory parameters (mean $\pm$ SD) in different categories of the study population

Categories	PCV (%)	CD <sub>4</sub> (cells/ $\mu$ L)	RC
AIHA	24.71 $\pm$ 3.30	161.0 $\pm$ 37.57	6.77 $\pm$ 0.45
No AIHA	32.15 $\pm$ 5.77*	230.19 $\pm$ 119.97*	1.96 $\pm$ 0.69*
DAT positive	29.09 $\pm$ 6.67	153.73 $\pm$ 58.16	5.26 $\pm$ 2.18
DAT negative	31.62 $\pm$ 5.92	234.29 $\pm$ 119.96	2.22 $\pm$ 1.91**
PTB	31.88 $\pm$ 7.00	203.21 $\pm$ 137.26	2.09 $\pm$ 1.63
No PTB	31.54 $\pm$ 5.60	232.39 $\pm$ 110.17	2.24 $\pm$ 1.99

PCV: Packed cell volume; RC: reticulocyte count ; AIHA: autoimmune hemolytic anemia; DAT: direct antiglobulin test ; PTB: Pulmonary tuberculosis

\* $P < .05$  compared to AIHA

\*\*  $P < .05$  compared to DAT positive

Thirty-six (36.74%) patients had anemia using the study definition of PCV<30%. Eleven (11.22%) patients were positive for DAT, those that were DAT positive had a mean PCV of 29.09+/- 6.67% while those that were DAT negative had a mean PCV of 31.62+/- 5.92%, as shown in Table 2. Mean CD4 count was 225.25 +/- 117.31 cells/  $\mu$ L; it was 153.73+/- 58.16 cells/  $\mu$ L for those that were DAT positive and 234.29+/- 119.96 cells /  $\mu$ L for those that were negative;  $P>.05$ . Patients with AIHA had a mean CD4 count of 161.0+/- 37.57 cells/ $\mu$ L compared with 230.19 +/- 119.97 cells/  $\mu$ L in those without AIHA;  $P>.05$

Twenty-four (24.49%) patients had pulmonary tuberculosis (PTB) which was the only opportunistic infection found in the patients we studied. There was no statistically significant difference in CD4 counts between patients with PTB and those without: 203.21 +/- 137.26 cells/  $\mu$ L cells / compared with 232.39 +/- 110.17 cells/  $\mu$ L.

Eighty-one (82.65%) patients were already on ART, which consisted of: Stavudine, nevirapine and lamivudine. Eighty-eight (89.8%) patients were infected with HIV-1 virus, 10(10.20%) with both HIV 1 and 2 viruses. No patient was found with only the HIV-2 virus.

## Discussion

Anemia was present in 36.74% of patients in this study, this agrees with earlier studies which concluded that anemia was a frequent complication of HIV infection.<sup>16</sup>The frequency of AIHA in this study is 3.06%. This value agrees with previous studies which reported that AIHA is not frequently seen in the HIV/ AIDS patient and may be as a result of the difficulty in making a diagnosis as reticulocytosis is often absent in HIV/AIDS patients with AIHA,<sup>7</sup> the rather low frequency in our patients may also be related to the low frequency of autoimmune disorders in the black African.<sup>8</sup>

Eleven (11.22%) patients in the study had positive DAT, mean PCV among these patients was lower than the mean PCV among the DAT negative group, though this difference was not statistically significant, it is in agreement with previous studies which have shown that HIV/AIDS patients with positive DAT have lower PCV.<sup>19,20</sup>

Mean CD4 count was significantly lower in DAT positive patients compared to DAT negative patients. This may be as a result of the higher incidence of immune dysregulation in patients with progressive HIV/ AIDS, especially as it has been previously suggested that there may be serological anti-erythrocyte autoimmunity without hemolytic effects in a large majority of HIV/ AIDS patients.<sup>7</sup>

There was a statistically significant difference in mean PCV, CD4 and RC counts in patients that had

AIHA and those that did not. Also patients without AIHA had higher PCV and CD4 counts than those with AIHA, this may suggest that patients with a lower CD4 count are more likely to develop AIHA. However, the fact that there was no correlation between AIHA and the CD4 count and also between AIHA and the presence of PTB infection, further confirms the earlier finding that red cell autoantibodies may be present in HIV patients in the absence of features overt hemolysis.<sup>20</sup>

There was no correlation between PCV and RC in our study, this may be due to suppression of erythropoiesis by the HIV virus or ART drugs.

We conclude that in spite of the fact that AIHA may have a low prevalence in HIV/AIDS patients, the fact that most patients will respond to standard treatment and that those that do respond have a good prognosis makes it imperative to screen HIV/AIDS patients with anemia for the presence of AIHA. Again it has been reported previously that HIV/AIDS patients with AIHA may have a fatal reaction to red cell transfusion, it becomes imperative that anemic patients with HIV/AIDS in non-emergency situations be screened for the presence of AIHA before receiving red cell transfusions when indicated.

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