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HAART – Dependent CD4+ Lymphocyte Response Based on Pre-Therapeutic CD4 Lymphocyte Count in HIV-Infected Nigerians

[1]O. Erhabor, [1]O. A. Ejele and [2]E. K. Uko

Antiretroviral Therapy Programme, Departments of [1]Haematology University of Port Harcourt Teaching Hospital and [2]Haematology, University Of Calabar Teaching Hospital, Nigeria
Reprint requests to: Dr. O. Erhabor, Department of Haematology University of Port Harcourt Teaching Hospital, P. M. B. 6173 Port Harcourt, Nigeria. E-mail: n_osaro@yahoo.com

Abstract
Background: Increasing concerns related to cost, drug toxicity, pill burden, tolerability ability of patient to adhere to strict and complicated regimen and emergence of drug resistance has complicated the decision making process of when to start antiretroviral therapy. The present research is aimed at determining if there is any immunological advantage in initiating HAART at a pre-therapeutic CD4 count of > 350cells/µl rather than at 200-350 or < 200 cells/µl.
Methods: One hundred HIV-infected previously antiretroviral- naive individuals grouped under 3 $CD4^+$ lymphocyte count thresholds; < 200, 200 – 350 and > 350 cells/µl were randomized to take HAART of stavudine (40mg) lamivudine (150mg) and nevirapine (200mg) orally twice daily. CD4 lymphocyte count was determined serially every 8 weeks for an observation period of 48 weeks. CD4 lymphocyte count responses were compared statistically based on pre-therapeutic CD4 lymphocyte counts.
Results: The overall increase in CD4 lymphocyte count irrespective of baseline CD4 count was 122 cells/µl ($p < 0.01$). CD4 lymphocyte count response to 48 weeks HAART was significantly higher in patients initiating HAART at a pre-therapeutic CD4 count of <200 cells/µl (163 cells/µl) compared to 118 and 50 cells/µl respectively for those initiating at 200 – 350 and > 350 cells/µl respectively ($\chi^2 = 1.80$, $p < 0.05$). HIV-related morbidity of 3% was found among subjects who initiated HAART with a pre-therapeutic CD4 count of < 200 cells/µl. Steven -Johnson syndrome was the commonest adverse clinical event observed occurring in 15% of subjects.
Conclusion: Our study indicates that there is no long-term advantage in terms of CD4+ lymphocyte response in initiating HAART at a pre-therapeutic CD4 count of > 350 cells/µl rather than at 200 – 350 cells/µl. Our present study appears to support postponing the initiation of therapy in some patients until the CD4+ count approaches 200 cells/µl particularly in sub-Saharan Africa where drug accessibility and affordability constitutes a major challenge.

Key words: HAART, CD4+ lymphocyte, pre-therapeutic, HIV-infected, Nigerians

Résumé
Introduction : Des intérêts progressifs ayant rapport au coût, toxicité des drogues, charge de la pilule, capacité tolérable de patient pour observer les stricts et régime compliqué et émergence de la résistante aux drogues ont compliqué le processus de la prise de décision de quand on doit commencer la thérapie antirétrovirale. L'objet de cette recherche est de décider s'il y a quelque avantage immunilogique dans commencement de HAART dans le compte CD4 préthérapeutique de > 350 cellules/Ul plutot que en 200-350 cellules/Ul étaient randomisés afin de prendre HAART de stavudine (40mg) lamivudine (150mg) et nevirapine (200mg) par voie orale deux fois par jour. CD4 lymphocyte compte était décidé par série chaque 8 semaines pendant une période d'observation de 48 semaines. Compte de CD4 lymphocyte réponses ont été comparés de la base statistique sur CD4 pré-thérapeutique compte lymphocyte.
Résultats : *Dans* l'ensemble, l'augmentation dans le compte lymphocyte CD4 sans tenir compte de ligne de fuite compte CD4 était 122 cellules/Ul (P<0,01). Compte lymphocyts CD4 réponse aux 48 semaines HAART était sensiblement plus élevé chez des patients qui commencent l'HAART dans le compte CD4 préthérapeutique de <200 cellules/Ul (163 cellules/Ul) par rapport aux 118 et 50 cellules/Ul respectivement pour ceux qui commencent en 200 – 350 et > 350 cellules/Ul respectivement (X2 = 1,80,P <0,05). Le VIH lié à la morbidité de 3% était trouvé parmi des sujets qui ont commencé le HAART avec le compte CD4 pré-thérapeutique de <200 cellules/Ul. Syndrome de Steven-Johnson était un événement clinique opposé le plus ordinairement observé qui arrive chez 15% des sujets.

Conclusion : Notre étude montre qu'il n y a aucun avantage de longue durée au terme de la réponse CD4+ lymphocyte dans le commencement d'HAART au CD4 pré-thérapeutique compte du > 350 cellules/Ul plutôt que en 200-350 cellule/Ul. Cette étude semble supporter le ajournement de l'initiation de la thérapie chez quelques patients jusqu'au moment quand le compte CD4+ approche 200 cellules/Ul particulièrement en Afrique sud saharien ou accessibilité aux drogues et abordabilité constituent un défi majeur.

Mot clés : HAART, lymphocyte CD4+, pré-thérapeutique VIH infecte, Nigérians

Introduction

Highly active antiretroviral therapy (HAART) has changed the landscape of HIV- related care in the developed world with marked reduction in mortality and morbidity .[1] This possibility however is beyond the reach of a vast majority of HIV-infected in sub Saharan Africa.

Following the development of HAART, many physicians were quite aggressive in treating patients at virtually any stage of this human retroviral disease. [2] Increasing concern related to drug toxicities, pill burden, cost and ability of patients to adhere to strict and complicated regimens, have complicated the decision-making process for physicians and patients alike. [2] Despite promised price-reduction and increased availability of generic drugs in some countries, cost remains a major factor in deciding when to start therapy. Early intervention suggested that a higher proportion of patients achieved a viral load of < 500 cells/µl if started on HAART at a CD4+ count of >500 cells/µl and >350 cells/µl rather than at < 200 cells/µl. [3, 4] Long-term clinical outcomes data however are not available to fully endorse this approach. The British HIV association recommends that therapy be initiated once CD4+ cell count falls to 350 cells/µl. [5]

The argument for a conservation approach in the initiation of HAART is that most regimen are difficult to tolerate, non- adherence leads to the development of resistance, thus limiting future treatment options [6,7] and that considerable reconstitution of the immune system seems possible even in patients initiating HAART at a low CD4+ cell count. [8]

This current research effort is aimed at determining if there is any long-term advantage in terms of CD4+ lymphocyte count response in initiating HAART at a CD4+ count of > 350 cells/µl rather than at 200 – 350 cells/µl by comparing the CD4 count response in HIV-infected Nigerians initiating HAART of 2 nucleoside analogues (stavudine and lamivudine) and one non-nucleoside (nevirapine) at different baseline CD4+ cell count; <200 cells/µl, 200 – 350 cells/µ and >350 cells/µl.

Materials and Method

Subjects for this study included 100 consecutively recruited HIV-infected previously antiretroviral- naive patients aged 18 to 56 years, mean age 34.13 ± 8.45 years, made up of 53 males and 47 females, enrolled into the antiretroviral therapy programme at the Haematology Department of the University of Port Harcourt Teaching Hospital between February 2004 to March 2005. The hospital is a 500 bed tertiary health facility rendering specialist HIV/AIDS- related care in the cosmopolitan oil rich city of Port Harcourt in the Niger Delta geopolitical zone of Nigeria, and one of the designated centers for the Federal Government of Nigeria assisted antiretroviral therapy programme. Inclusion criteria included age > 18 years, HIV positivity, willingness to give informed consent, CD4 count of 100 – 500 cells/µl and previous antiretroviral naivety. Exclusion criteria included age < 18 years, previous antiretroviral use and pregnancy. Subjects were placed on highly active antiretroviral therapy of two nucleoside reverse transcriptase inhibitors (stavudine (40 mg) and lamivudine (150mg) and one non-nucleoside reverse transcriptase inhibitor, nevirapine (200mg) taken orally twice daily for an observational period of 48 weeks. The CD4+ lymphocyte counts were monitored serially every 8 weeks.

Specimen acquisition and laboratory method

Whole venous blood samples were collected by means of a 10 milliliters hypodermic syringe and needle into potassium EDTA anticoagulated tubes (5 milliliters) and plain tubes without anticoagulant (5 milliliters). Sera derived from the plain tubes were screened and confirmed for HIV-infection using a double enzyme linked immunosorbent assay (ELISA) of Immunocomb HIV I and II kits (Orgenics, Israel) and Genscreen HIV I and 2 kits (Bio, Rad, France) – both are enzyme immunoassay tests for the qualitative and differential diagnosis of HIV. The EDTA anticoagulant blood was used for CD4+ lymphocyte enumeration using the Dynal beads technique (Dynal Asa, Oslo, Norway), an alternative method to flow cytometry in resource – limited settings. Previous report [9] indicated that values from the alternative Dynal bead methods correlated positively and significantly with values from flow cytometry (r = 0.90). The Dynal beads technique uses paramagnetic polymer beads coated with anti – CD4+ monoclonal antibodies (mAbs) to capture and isolate CD4+ T-lymphocyte from whole blood.

Study design

This study included 100 HIV –infected previously antiretroviral- naive individuals who were grouped under 3 CD4+ lymphocyte thresholds; < 200, 200 – 350 and > 350 cells/µl (n= 48, 36, and 16) respectively and were randomized to take HAART of two nucleoside analogue (stavudine (40mg) and

lamivudine (150mg) and one non – nucleoside (Nevirapine) (200mg) twice daily. The CD4+ lymphocyte count was determined serially every 8 weeks for an observational period of 48 weeks. Thereafter the CD4+ lymphocyte count responses were compared based on the pre- HAART initiation CD4+ lymphocyte count and the results compared statistically.

Statistical analysis

Data were entered and analyzed using statistical software (Version 10 SPSS Inc. Chicago IL). Statistical analysis of mean and standard deviation were calculated. Chi-square analysis was used to assess the significance of baseline CD4+ lymphocyte count on response to HAART. A p-value of < 0.05 was considered significant for all statistical comparisons.

Results

The study population for this case study consisted of 100 HIV-infected who initiated therapy with baseline CD4 lymphocyte count of <200 cells/µl (n = 48), 200 – 350 (n = 36) and >350 cells/µl (n=16) made up of 53 males and 47 females, aged 18 – 56 years and mean age of 34.13±8.45 years .Patients were followed up for a median of 48 weeks from the date of initiation of HAART. CD4+ lymphocyte response in subjects was compared based on their pre-therapeutic baseline CD4 count value < 200, 200 – 350 and > 350 cells/µl. Table 1 shows the mean CD4+ lymphocyte value at baseline. On the average the CD4 cell count had increased by at least 122 cells/µl (from 235.60 ± 112.59 cells/µl to 357.22 ±69.73 cells/µl) after 48 weeks of HAART irrespective of the pre therapeutic CD4+ lymphocyte count.

Importantly, the absolute rise in CD4 count appeared to be significantly higher in subjects initiating HAART with a lower baseline CD4 count <200 cells/µl (from a baseline CD4+ count of 142.50 ± 28.2 cells/µl to 305.22 ± 12.95 cells/µl) representing a mean increase of 163 cells/µl compared to those initiating therapy at a baseline CD4+ count of 200-350 cells/µl (from baseline CD4+ count of 273.06 ± 45.00 cells/µl to 391.14 ± 31.60 cells/µl) representing a mean increase of 118 cells/µl and in subjects initiating therapy at a baseline CD4 count of >350 cells/µl(from a baseline CD4+ count of 430.63 ± 64.46 cells/µl to 480.63 ± 58.02 cells/µl) representing a mean increase of 50 cells/µl. There was a statistically significant variation in the therapy – dependent increase in CD4 lymphocyte count based on the pre-initiation CD4 lymphocyte count (χ^2= 117.1, $p < 0.05$) as shown in Table 2. The CD4+ lymphocyte count restored by HAART was evaluated as a prognostic indicator for a new adverse event and death. The incidence of HIV-related mortality observed in this study was three percent. Two of the subjects died within the first eight weeks of therapy while the third died before the end of the first 16 weeks. Mortality was found clustered among subjects who initiated HAART with a baseline CD4+ count of <200 cells/µl. Two of the subjects died of tuberculosis-related complications while the third died of pneumocystis carinni pneumonia-related complications. Severe potentially fatal Steven Johnson's syndrome was one of the commonest adverse clinical events observed in 15% of subjects on HAART. Ten percent of subjects developed Steven Johnson's syndrome within the first 8 weeks of therapy while 5% developed Steven Johnson's syndrome in the subsequent weeks of therapy.

Table 1: Pre-therapeutic CD4+ lymphocyte count

CD4+ Lymphocyte Count Threshold (Pre-therapeutic)	No.	Mean ± SD	Range
< 200	48	142.50 ± 28.2	114.3 – 170.6
200 – 300	36	273.06 ± 45.47	227.6 – 318.5
> 350	16	430.60 ± 68.50	362.1 – 499.1

Table 2: CD4+ lymphocyte count response to HAART based on pre-therapeutic CD4 lymphocyte count

Pre-therapeutic CD4+ count (cells/µl)	Baseline CD4+ count ($\bar{x}$ ± SD)	48 weeks post therapy CD4+ count ($\bar{x}$ ± SD)	Mean increase (cells/µl)	df	χ^2	p
< 200	142.50 ± 28.2	305.22 ± 12.95	163	2	117.1	0.05
200 – 300	273.06 ± 45.47	391.14 ± 31.60	118			
> 350	430.60 ± 68.50	480.63 ± 58.02	50			

Discussion

Following the development of HAART, many physicians were quiet aggressive in treating patients at virtually any stage of this human retroviral disease, almost regardless of CD4+ T-lymphocyte count. This study provides some evidence to help clinicians and patients to decide the optimal time to initiate HAART.

Our data showed that there is no long-term advantage in terms of CD4+ lymphocyte count response in initiating HAART at a CD4+ count of > 350 rather than at a CD4 count 200 – 350 and < 200 cells/µl. We observed a mean CD4+ rise of 163, 118 and 50 cells/µl respectively for subjects initiating HAART at a pre-therapeutic CD4+ count of < 200, 200 – 350 and > 350 cells/µl. This observation is consistent with previous report by Alessandro and co-workers[10] that there is no immunological advantage in initiating HAART at a CD4+ cell count of >350 cells/µl rather than at a CD4+ count of 200 – 350 or < 200 cells/µl. They observed a mean rise in CD4+ cell count of 280, 281 and 186 cells/µl respectively in patients initiating therapy with a baseline CD4+ count of < 200, 200 – 350 and > 350 cells/µl. The significant initial increase in CD4+ cell count following HAART may represent redistribution of CD4+ T cells from thymopoiesis as previously suggested. [11]

Our study indicated that HIV-infected patients initiating HAART with a low baseline CD4 lymphocyte count (< 200 cells/µl) might experience a robust CD4+ lymphocyte count response after 48 weeks, HAART. This finding is consistent with previous reports [11,12] which showed that considerable reconstitution of the immune system seems possible even in patients initiating HAART at a low CD4+ lymphocyte count. We found a uniformly high rate of disease progression and mortality clustered among HIV-infected patients initiating HAART at a baseline CD4 count of < 200 cells/µl. All the three cases of HIV-related mortality in this study were clustered in patients who initiated HAART at a low CD4+ lymphocyte count of < 200 cells/µl and who presented with cachexia at baseline with baseline body weight of < 45kg and body mass index (BMI) <20. Two of the patients died of tuberculosis –related complications while the third died of pneumocystis carinni pneumonia complications. This observation is consistent with previous report by Crowe and coworkers [13] who found disease progression and death clustered among HIV-infected patients initiating HAART at a low CD4+ lymphocyte count. Nevertheless, it seems that a reasonable conclusion would be to focus primarily on the CD4+ lymphocyte count in determining the optimal time to initiate antiretroviral therapy in many HIV-infected patients.

Severe Steven Johnson's syndrome characterized by oral erythema with associated constitutional symptoms of malaise, arthralgic fever and conjunctivitis was the commonest adverse clinical event observed in this study in 15% of HIV-infected subjects placed on a HAART of two-nucleoside analogue (stavudine and lamivudine) and one non-nucleoside (nevirapine). This observation is consistent with our previous report [14] which found a prevalence of Steven Johnson's syndrome in 9.3% of our cohort of 70 HIV-infected patients placed on short- term stavudine lamivudine and (nevirapine). This finding is also cordant with a previous report by D' Aquila and co-workers [15] who reported Steven Johnson's syndrome in 20% of patients who received HAART of zidovudine, didanosine and nevirapine compared to 9% in patients, who received zidovudine plus didanosine. The high incidence of Steven Johnson's syndrome may be as a result of presence of nevirapine in the regimen use.

Clinical guidelines are now suggesting a potential benefit in the conservative approach of initially withholding HAART for certain ART naive patients based on CD4+ lymphocyte count. [16] The argument for this conservative approach are; that there is limited access to antiretroviral therapy particularly in resource – limited settings in sub-Saharan Africa, cost implication of sustaining therapy on a long-term basis, the fact that antiretroviral regimen are difficult to tolerate, antiretroviral therapy for now is a life time therapy, the fact that non adherence to therapy may lead to the development of HIV resistance, that the long-term toxicity of antiretroviral drugs is not yet known coupled with the hope that more effective therapy may become available in future. [17-18]

In conclusion our study appears to support postponing the initiation of HAART for some HIV-infected patients until the CD4+ T-lymphocyte count approaches 200 cells/µl and that it may be important to regularly monitor the CD4+ lymphocyte counts of HIV-infected Nigerians allowing for therapy to be initiated when the potential benefit of therapy will outweigh possible toxicities and to ensure that the HIV- infected patients have the ability to remain on treatment on long-term basis particularly to prevent the development of drug resistance resulting from poor adherence due to cost or therapy related factors.

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