Clinical HIV and AIDS Journal

Evaluation of Procalcitonin Levels in HIV Seropositive Subjects in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

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Abstract

Background: Human Immunodeficiency virus (HIV) infection remains a major disease burden in global health of which the hallmark is the progressive depletion of CD4$^+$ cells thereby predisposing affected individuals to bacterial infections. A search for a suitable biomarker for bacterial sepsis in HIV sero-positive individuals becomes imperative. Procalcitonin a recently described biomarker may fit into this role.

Aim: This study assessed the risks of bacterial co-infections in HIV-seropositive individuals using procalcitonin as a biomarker.

Materials and methods: A total of 90 subjects were recruited for this study made up of 30 HIV sero-positive on HAART subjects (as test group 1), 30 HIV sero-positive naive individuals (as test group 2) and 30 HIV sero-negative individuals (as control group). HIV status was determined using the National algorithm involving serial testing by Determine, Uni-Gold and Stat-pak test kits while the CD4 count was evaluated using cyflow partec technique. Procalcitonin level was assessed using the ELISA technique.

Results: There was no significant difference in the mean serum level of procalcitonin in HIV sero-positive subjects, HIV sero-positive naïve individuals and HIV sero-positive subjects on HAART (p>0.05). In the post-hoc multivariate comparison there was no significant difference of procalcitonin levels in HIV sero-negative subjects compared to HIV sero-positive individuals on HAART (p>0.05) and in HIV sero-negative when compared to HIV sero-positive naïve subjects (p>0.05). However, there was a significant increase in the mean serum level of procalcitonin in HIV sero-positive subjects on ART compared with HIV sero-positive naïve subjects (p<0.05). Furthermore, there was a significant increase in the mean CD4$^+$ Count of HIV sero-positive Naïve subjects compared with the mean CD4$^+$ count of HIV sero-positive on ART (p<0.05). A non-significant negative correlation was observed between procalcitonin levels and CD4$^+$ count in HIV seropositive subjects on ART (r=-0.242; p=0.198).

Conclusion: We conclude that procalcitonin can aid in clinical differentiation of bacterial infections from other infections in HIV sero-positive patients and may also serve as a guide to physicians on decisions concerning commencement of antibiotic therapy especially in critically ill patients. However, procalcitonin needs to be further evaluated by follow-up studies with higher sample size in viral and bacterial related cases.

Keywords

Human Immunodeficiency Virus (HIV); Procalcitonin; Highly Active Antiretroviral Therapy (HAART); Cells of Differentiations (CD4+)

Introduction

The human immunodeficiency virus (HIV) is a lentivirus that replicates and predisposes the affected individuals to the acquired immunodeficiency syndrome (AIDS) [1,2]. It is therefore a disorder in humans with significant immune system failure with subsequent severe opportunistic infections and complications including cancers etc. HIV infection is mostly by blood transfusion of infected blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within the listed body fluids, the infective HIV form appears as both free viral particles and virus within infected immune cells from where they attack virtually all the

Article Information

DOI:	10.31021/chaj.20192110
Article Type:	Research Article
Journal Type:	Open Access
Volume:	2 **Issue:** 1
Manuscript ID:	CHAJ-2-110
Publisher:	Boffin Access Limited
Received Date:	12 June 2019
Accepted Date:	25 June 2019
Published Date:	30 June 2019

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Citation: Manafa PO, Umeilechukwu CV, Ekuma-Okereke O, Chukwuma GO, Ibe NC, et al. Evaluation of procalcitonin levels in HIV seropositive subjects in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. Clin HIV AIDS J. 2019 Jun;2(1):110

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body organs causing different metabolic derangements in addition to immune suppression [3,4]. The pool of infected individuals is maintained by 16,000 new infections daily and 6 million new infections yearly [5]. More than 3.5 million people in Nigeria are already infected. The 2003 National HIV sero–prevalence sentinel survey revealed that the prevalence rate for Nigeria was 5.0% with Anambra State having 3.8% prevalence.

The primary target for HIV infection is the helper T cells (specifically the $CD4^+$ T-cells), macrophages, and dendritic cells [6]. The most intriguing of HIV infection is the progressive depletion of $CD4^+$ T cells resulting to increased cellular destruction and manifestation of AIDS if not managed [6]. Using anti-HIV drugs in the treatment of individuals infected with HIV is termed antiretroviral therapy [7]. The therapy is a cocktail of at least three drugs that reduces or suppresses the replication of HIV. The combined therapy is popularly called Highly Active Antiretroviral Therapy (HAART). Using cocktail of medicines, confers the unique quality that can stop the HIV disease progression [7]. It has therefore been postulated that HIV epidemiology centers mainly on the immunological dysfunction which often predisposes the infected individuals to communicable diseases including mycobacterium tuberculosis (TB) [8,9].

Procalcitonin (PCT) is a 116-amino acid peptide, produced by the thyroid gland as a calcitonin precursor. Several reports has it that procalcitonin is one of the most reliable biomarker for severe bacterial infections and sepsis [10,11]. In 1993, the diagnostic importance of procalcitonin in differentiating bacterial infections and non-bacterial inflammatory conditions was first postulated by the French authors [10].

Reports have suggested that procalcitonin shows a favorable kinetic profile for clinical use as a biomarker; due to its prompt increase (i.e. 6 to 12 hours) upon stimulation. Circulating procalcitonin levels is also said halve daily when the infection is mediated by the host immune system or antibiotic therapy [12]. Serum procalcitonin concentrations is almost immeasurable in healthy individuals with a range of <0.05 ng/ml [13].

Procalcitonin is secreted in response to endotoxins (that is, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6) and its release is strongly correlates with the extent and severity of bacterial infections [14]. This implies that the serum level of procalcitonin serially increases as the intensity of the inflammatory response and the severity of infection increases [15,16]. In view of these diagnostic importance, procalcitonin has therefore demonstrated a high degree of specificity for bacterial infection, hence the aim for this study.

Justification of study

Human Immunodeficiency virus (HIV) infection remains a major health challenge worldwide due to its immune-destructive capacity. As the infections progresses, the more degenerating the immune cells hence resulting in susceptibility to bacterial co-infection [17].

Evaluation of procalcitonin level may therefore serve as a predictive tool on the risks of bacteria sepsis in HIV sero-positive individuals and thus a valuable prognostic addition in the management of subjects in this condition. Hence, this study was designed to assess the risks of bacteria sepsis in HIV sero-positive individuals using procalcitonin as a biomarker; achievable through the following objectives:

I. Evaluating the levels of procalcitonin in HIV sero-positive naive subjects and HIV sero-negative subjects.

II. Determining the levels of procalcitonin in HIV sero-positive individuals on HAART.

III. Assessing the $CD4^+$ count in HIV sero-positive subjects.

IV. Correlating the levels of procalcitonin with the $CD4^+$ count in HIV sero-positive individuals.

V. Correlating the levels of procalcitonin with age in HIV sero-positive subjects.

Materials and Methods

Study design

This was a case-controlled study designed to evaluate procalcitonin levels in HIV sero-positive subjects attending the HIV outpatients' clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria. A total of 90 subjects were recruited for this study which comprised 30 HIV sero-positive individuals on HAART (as test group 1), 30 HIV sero-positive naïve subjects diagnosed not later than 2months prior to study (as test group 2) and 30 HIV seronegative subjects (control group).

Inclusion and exclusion criteria

HIV seropositive naïve subjects diagnosed with infection not later than 2months prior to study, HIV sero-positive individuals on HAART and HIV sero-negative subjects, all within the age range of 18-60. However, the following were excluded from the study; pregnant women, anemic individuals, subjects with chronic diseases that may affect immune status, and individuals outside the age range of 18-60

Ethical approval

The ethical approval for this research was obtained from the ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state, Nigeria.

Specimen collection

Five (5) ml of whole blood was collected aseptically from each subject and dispensed into a plain container for the estimation of procalcitonin level. The specimen was centrifuged at 5,000 rpm for 5 minutes and the serum separated.

Methodology

HIV test

HIV testing was done using the National algorithm described by [18].

Principle of Determine HIV test kit

This is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. The sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient window site.

Principle of Uni-gold HIV test kit

Uni-Gold HIV test is a rapid immunoassay based on the immunochromatographic sandwich principle. Recombinant proteins representing the immune-dominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively, are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. During testing, two drops of serum, plasma or whole blood is added to the sample port, followed by two drops of wash solution and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device.

Principle of Stat-pak HIV test kit

This assay employs a combination of antibody binding protein, which is conjugated to colloidal gold dye particles, and antigens

to HIV1/2, which are bound to the membrane solid phase. The sample being tested and running buffer are added to the sample pad. The running buffer facilitates the lateral flow of the specimen through the membrane and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test area producing a pink/purple line. In the absence of HIV 1/2 antibodies, there is no pink/purple line in the test area. The sample continues to migrate along the membrane and produces a pink/purple line in the control area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimens and reagents have been applied properly and have migrated through the device.

CD4⁺ count determination

CD4⁺ was determined using cyflow partec machine, according to the method described by the manufacturer [19].

Principle of CD4⁺ count determination

The test principle is based on the detection and identification of fluorochrome-labeled (phytoerythrin, PE) cells as they pass through an optical detecting system, detectable by the angle of scatter incident laser and the wavelength of fluorescence emitted.

Estimation of Procalcitonin levels

The quantitative measurement of procalcitonin was estimated using the sandwich ELISA method as described by [20]

Test principle

The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to PCT. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. A biotinylated detection antibody specific for procalcitonin and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro-plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain procalcitonin biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm and the value is proportional to the concentration of procalcitonin. The concentration of PCT in the Samples can be calculated by comparing the OD of the samples to the standard curve.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). The results for were expressed as mean ± standard deviation. Values were deemed significant at $p<0.05$. Correlation studies were performed using the Pearson's correlation coefficient.

Results

There was no significant difference ($p>0.05$) in the mean serum levels of procalcitonin in HIV sero-positive subjects on ART, HIV sero-positive naïve individuals and HIV sero-negative subjects on HAART (Table 1). The post-hoc multivariate comparison test, also demonstrates that there was no significant difference in HIV sero-negative subjects compared to HIV sero-positive individuals on HAART ($p=0.457$) and HIV sero-negative subject when was compared with HIV sero-positive naïve subjects ($p=0.295$). Conversely, there was a significant increase ($P=0.075$) when the mean serum level of procalcitonin in HIV sero-positive subjects on ART was compared with that of HIV sero-positive naïve subjects.

There was a significant increase in the mean CD4+ Count of HIV sero-positive Naïve subjects compared with the mean CD4+ count of HIV sero-positive on ART ($p<0.05$) (Table 2).

A non-significant negative correlation (r= -0.242; $p=0.198$) was observed between procalcitonin level and CD4+ count in HIV seropositive subjects on ART while a non-significant positive correlation was observed between procalcitonin level and age in HIV sero-positive subjects on ART ($r_{=}$ 0.227; $p=0.228$) (Table 3). However, there was a non-significant negative correlation between procalcitonin level and duration of ART in sero-positive subjects (r=-0.061; $p=0.747$).

A non-significant positive correlation was observed between procalcitonin and CD4 count in HIV sero-positive naïve subjects (r=0.287; $p=0.125$) and between the serum level of procalcitonin and age in HIV sero-positive naïve subjects (r=0.074; $p=0.697$) (Table 4).

The mean serum levels of procalcitonin was significantly increased in HIV sero-negative male compared to HIV sero-negative females (Figure1).

The mean serum level of procalcitonin was significantly increased in HIV sero-positive males compared with HIV sero-positive females (Figure 2).

Groups Tests	Test Group 1 n=30	Test Group 2 n=30	Control n=30	F-value	*p*-value
Procalcitonin (ng/ml)	$1.31 \pm 1.15_{a,z}$	$0.96 \pm 0.34_{b,z}$	$1.16 \pm 0.56_{a,b}$	1.635	0.201

Keys: Test group 1 = HIV sero-positive subject on ART, Test group 2=HIV sero-positive naïve subjects, Mean difference is significant when p is <0.05. a=non-significant difference between HIV sero-positive subject on ART and control, b=non-significant between HIV sero-positive naïve subjects, z=significant difference between HIV sero-positive subject on ART and HIV sero-positive naïve subjects.

Table 1: Variations of the serum level of Procalcitonin in HIV seropositive subjects on ART (Test group 1), HIV sero-positive naïve subjects (Test group 2) and HIV seronegative subjects (control)

Groups	Mean ± SD	T-test	*p*-value
HIV subjects on ART (n=30)	666.00 ± 136.28		
		-2.277	0.026
HIV sero-positive naïve subjects (n=30)	737.53 ± 105.07		

Table 2: There was a significant increase in the mean CD4+ Count of HIV sero-positive Naïve subjects compared with the mean CD4+ count of HIV sero-positive on ART ($p<0.05$).

HIV Sero-Positive Subjects on ART		
Parameters	**r-value**	***p*-value**
Procalcitonin *vs.* CD4	-0.242	0.198
Procalcitonin *vs.* Age	0.227	0.228
Procalcitonin *vs.* ART Duration	-0.061	0.747

Table 3: Relationships between mean serum levels of procalcitonin, CD4+ Count, with Age and ART duration in HIV sero-positive subjects on ART

HIV Sero-Positive Naïve Subjects		
Parameters	**r-value**	***p*-value**
Procalcitonin *vs.* CD4	0.287	0.125
Procalcitonin *vs.* Age	0.074	0.697

Table 4: Association between procalcitonin level, CD4+ count and age in HIV sero-positive naïve subjects

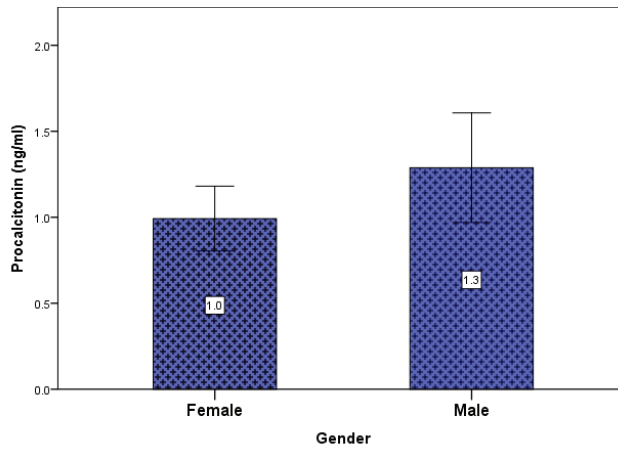

Figure 1: Mean serum levels of procalcitonin in HIV sero-negative female and male subjects

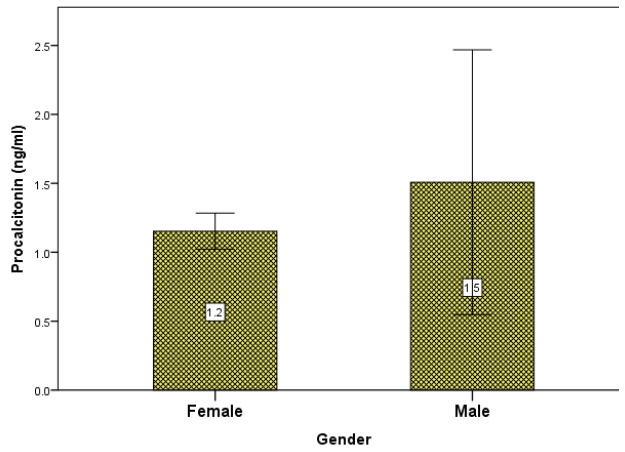

Figure 2: Mean serum levels of procalcitonin in HIV sero-positive female and HIV male subjects

Discussion

Human Immunodeficiency Virus (HIV) infection is a major health challenge in the modern world causing devastating adverse health conditions [21]. Among HIV-infected patients; bacterial co-infections predominate with high complication rate than in patients without immunodeficiency. Sepsis has a high rate of mortality in critically ill patients that is caused by bacterial infection [22]. Clinical signs and symptoms doesn't often reveal the source thus, very difficult to confirm the infection, especially in its early stage [23,24], in addition to being a promising biomarker for sepsis, Procalcitonin has also been demonstrated to play an important role in minimizing antibiotic exposure of critical care patients. Use of procalcitonin has since been advocated to assist in decision of bacterial infection [25]. However, every rise in procalcitonin is not indicative of infection, and non-infectious causes should be kept in mind [26].

In this study, there was no significant difference in the mean serum level of procalcitonin in HIV sero-positive subjects, HIV sero-positive naïve individuals and HIV sero-positive subjects on HAART. However, the mean serum level of procalcitonin in HIV sero-positive individuals on ART was non-significantly higher when compared with the procalcitonin level of HIV sero-positive naïve subjects and the control group. This implies that in those on HAART are slightly immnunological incompetent compared to the naïve individuals. This is evidently shown by the significantly elevated $CD4^+$ count in naïve HIV sero-positive subjects than those on HAART thereby suggesting that there could be a superimposed bacterial infection amongst the HIV sero-positive subjects on ART than HIV sero-positive naïve and HIV sero-negative individuals. This is congruent with a prospective, observational study carried out by [27] on procalcitonin in immune compromised patients. They found low baseline levels of procalcitonin, which did not differ significantly between infected and uninfected participants. Further studies conducted on HIV infected subjects have found out that procalcitonin does not increase that much with viral infection [23]. This is attributed to the production of interferon-α in viral infection which leads to suppression of tumor necrosis factor-α; a procalcitonin production stimulant [28]. Furthermore [29], found that serum procalcitonin levels were increased in their HIV-infected cohort but the test subjects were said to have pneumonia or tuberculosis [29].

In the multivariate analysis of our work also, the mean serum level of procalcitonin in HIV sero-positive subjects on HAART was significantly higher than the HIV sero-positive naïve subjects. However, there was no significant difference in HIV sero-negative subjects compared to HIV sero-positive individuals on HAART. The same pattern was observed when the mean serum levels of procalcitonin of HIV sero-negative was compared to HIV sero-positive naïve subjects. The significant higher level of procalcitonin in HIV subjects on ART than the naïve individuals further validates the fact that those on HAART are superimposed with bacterial infection that further deteriorates their immune system hence elevating procalcitonin which has become one of the promising sepsis biomarker. The findings of this study are in line with studies of [22,23], Procalcitonin is used by some to differentiate between viral and bacterial infections [28].

In developing countries such as Nigeria, bacterial infections are common most especially at immuoncompromised cases [30]. This study reported a significant increase in the mean CD4+ Count of HIV sero-positive Naïve subjects compared with the CD4+ count levels of HIV sero-positive on HAART. This clearly demonstrates the significant elevation of procalcitonin levels in HIV subjects that the naïve individuals thereby signifying that the HIV sero-positive subjects could be faced with unidentified bacterial infections. This is because, in the absence of viral infection, low CD4+ counts may be indicative of severe sepsis [31]. This is in one hand agreement with [31], who observed a statistical significant difference, in the CD4 count of HIV sero-positive naïve patients and HIV sero-positive patients on HAART, while it is in contrast with the report of [32] that stated that there was a statistically significant higher CD4 count in patients on HAART than HAART- naive patients. Although the CD^+ pool represent only a small fraction of the immune system, they often forms the first line of defense (response) during pathogenic attack [30]. As a result, a decrease in $CD4^+$ count is often associated with the significant immunodeficiency a characteristic symptom of AIDS [30]. A procalcitonin level on the other hand, has shown high degree of sensitivity for distinguishing invasive pneumococcal infections from viral infections in patients [33] thus, could be vital in the twin epidemics of HIV and tuberculosis (TB) by aiding in the clinical differentiation of bacterial TB from TB infections in HIV-seropositive patients. Procalcitonin levels have been studied as markers of bacterial lower respiratory tract infection [34].

Baseline procalcitonin levels were also correlated with independent variables. Our finding also revealed a negative correlation between CD4 count and serum procalcitoin, suggesting that procalcitonin maybe a useful marker in HIV/AIDS patients with superimposed bacteria infections. This finding is similar to the reports of [35] and [23]. However, there was a negative correlation between mean serum procalcitonin levels and duration of HAART in sero-positive subjects. This indicates the relative effect of HAART on immunologically associated infections (including the viral and bacterial infections). Thus, could be the reason behind the non-significant difference observed in the mean serum levels of procalcitonin in HIV sero-positive subjects, HIV sero-positive naïve individuals and HIV sero-positive subjects on HAART. This is because, in viral infection, there is production of alpha interferons, which inhibits synthesis of tumor necrosis factor that produces procalcitonin [28].

The mean serum level of procalcitonin was significantly elevated in men than in women both in HIV sero- negative subjects, HIV sero-positive subjects on HAART and HIV sero-positive naïve subjects. This gender difference does not seem to be indicative of a higher level of systemic inflammation in men. Clinical studies have shown sex disparities in sepsis incidence and outcome [36]. These discrepancies may result from not only study design and biological differences between men and women but also from issues related to gender, lifestyles, comorbidities, access to health care services and critical care investment [37].

In the light of these findings and from several other studies, we hypothesize that the monitoring success or failure of anti-infective therapies, especially in critically ill patients which has been unsatisfactory [38], could be aided by procalcitonin. This is because, procalcitonin have been independently shown to be elevated early in episodes of severe infections. In HIV infections for example, patients should have some clinical symptoms or signs of infection, over and above the diagnosis of HIV itself which could be differentiated or identified with procalcitonin, which has shown to be a highly sensitive marker of inflammation for the early detection of bacterial infections in immune-compromised individuals.

Conclusion

We conclude that procalcitonin can aid in the clinical differentiation of bacterial infections from other infections in HIV sero-positive patients and may also serve as a guide to physicians on decisions concerning commencement of antibiotic therapy especially in critically ill patients. However, procalcitonin needs to be further evaluated by follow-up studies with higher sample size in viral and bacterial related cases.

Conflicts of Interest

The authors boldly declare that there is no conflict of interest herein. This original research has neither been published nor being considered for publication elsewhere.

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