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Immuno-virological response and its association with inflammation marker C-reactive protein, Clinical and socio-demographic factors among HIV patients at least 12 months on antiretroviral therapy at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

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Acronyms and Abbreviations

1a:	d4t-3TC-NVP
1b:	d4t-3TC-EFV
1c:	AZT-3TC-NVP
1d:	AZT-3TC-EFV
1e:	TDF-3TC-EFV
1f:	TDF+3TC+NVP
1g:	ABC+3TC+EFV
2e:	AZT-3TC-LPV/r
2g:	TDF-3TC-LPV/r
2h:	TDF-3TC-ATV/r
2i:	ABC+3TC+LPV/r
3TC	Lamivudine
AHR:	Adjusted hazard Ratio
AIDS:	Acquired Immunodeficiency Syndrome
AOR:	Adjusted odds ratio
ART:	Antiretroviral therapy
BMI:	Body Mass Index
CD4:	Cluster of Differentiation4
CI:	Confidence Interval
COTRI:	Co-trimoxazole
CRP:	C - reactive protein
ddI:	didanosine
DHS:	Demographic Health Survey
EFV:	Efavirenz
FDRE:	Federal Democratic Republic of Ethiopia
FLUC:	Fluconazol
HAART:	Highly active antiretroviral therapy
HIV:	Human immunodeficiency virus
hs- CRP:	Highly sensitive C-reactive protein
IM:	Immunological
INH:	Isoniazid
IQR:	Inter Quartile Range

MOH:	Ministry of health
NA:	Nucleoside analogue
NNRTI:	Non- Nucleoside Analogue Reverse Transcriptase Inhibitor
NRTI:	Nucleoside Analogue Reverse Transcriptase Inhibitor
OI:	Opportunistic infection
OR:	Odds ratio
PI:	Protease Inhibitor
R.P.M:	Revolution Per Minute
SOP:	Standard operating procedure
TDF:	Tenofovir
TLC:	Total Leukocyte Count
VCT:	Voluntary Counseling and testing
VL:	Viral load
WHO:	World Health Organization
ZMH:	Zewditu Memorial Hospital

Abstract

Background: Highly active antiretroviral therapy (HAART), has significantly reduced the mortality of HIV infected population but, the morbidity attributed to it remained a serious concern. The magnitude and factors associated with Immuno-virologic responses to HAART are less investigated.

Objective: To assess Immuno-virologic response and its association with inflammation, Clinical and Socio-demographic factors after at least 12 months of HAART initiation among patients attending Zewditu Memorial Hospital, Addis Ababa, Ethiopia

Method: A hospital based prospective cross sectional as well as retrospective longitudinal study was conducted from January to June 2016 at Zewditu Memorial Hospital. Socio-demographic and clinical information was collected from ART database of 402 HIV patients who were on HAART for at least 12 months. Blood was collected from when participants come for their scheduled second follow up visit since ART initiation and examined for CD4 count and, viral load. Serum sample was separated for C-reactive protein determination. Descriptive statistics was used to determine the socio demographic characteristics and response rates. Binary and multiple logistic regressions were computed to assess associations between variables using SPSS version 20. P value less than 0.05 was considered as statistically significant.

Result: From the 402 participants 235(58.5%) were females. Majority 174(43.8) were in the age group of 30-39 years, attained high school 184(45.9%) and were married 193(48.0%). Most participants 159(39.6%) were on 1e regimen (TDF+3TC+EFV) and have normal BMI 224(55.7%). About 99.5% of them were on OI drugs. Concordance response was noted in 311(77.4%) participants while 16 (4.0%) failed both immunologically and virologically. Despite virological response, 55(13.7%) participants failed to restore CD4 count of at least 50 cells/ mm^3 after 12 months of HAART. Majority of CRP positive participants were in the unfavorable responder category. Participants having CD4>350 cells/ mm^3 (adjusted odds ratio = 0.06; 95% CI: 0.02-0.19) and viral load < 1000 copies/ml (adjusted odds ratio = 0.26; 95% CI: 0.13-0.55) at baseline were significantly associated factors of immuno-virological discordant responses

Conclusion: Individuals who had a baseline CD4 count above 350 cells/ mm^3 and baseline viral load below 1000 copies/ml were less likely to have discordant response. Though the concordance response rate is high (77.4%), the proportion of patients failing immunologically (13.7%) despite virologic response, signifies the importance of virologic monitoring of HAART responses. Simple markers like CRP could supplement monitoring of unfavorable responses.

Keywords: HIV, CD4, Viral-load, CRP, Concordant response, Discordant response

1. INTRODUCTION

1.1 BACKGROUND

Human immunodeficiency virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS) was discovered way back in 1983[1]. More than three decades since its discovery, HIV infection diagnosis, treatment and management has been a big challenge to the medical field. HIV infection is attributed to cause compromised T-cell and B-cell immunity, promote different malignancies and the infected individuals are at risk of acquiring other viral, bacterial, fungal and parasitic infections. HIV disease progression was found to be different in infected population. Where few HIV infected patients develop AIDS in less than 5 years and are called as early progressor, HIV infected population in whom development of AIDS is slow and who may remain normal up to 10 years are termed as late progressor. Those patients in whom the symptoms of AIDS are not seen even after 10-15 years are labeled as long term non-progressor [2].

The variability in disease progression is not well described. After the discovery of HAART, the mortality of HIV infected population has significantly reduced but the morbidity attributed to HAART has remained as a serious concern. From being a life threatening infection HIV has now become a chronic infection, where patients live their natural life years with the HAART therapy. Among the most significant changes in HIV infection and pathogenesis is the development of non-infectious complications attributed to the HIV infection, HAART therapy, other demographic factors and co-morbidities. Other factors that influence the disease pathogenesis and progression include chronic immune activation, drug resistance and toxic side effects of HAART therapy. Among these, immune activation plays a key role in the pathogenesis and progression of HIV infection [3].

UNAIDS estimated that there were on average 35.3 million people living with HIV at the end of 2014. Over 12 million have died over the last 20 years. Effective antiretroviral therapy has led to sustained HIV viral suppression and immunological recovery in patients who have been infected with the virus. The majority of HIV infections are in sub-Saharan Africa where 70% of the HIV population lives [4]. In Ethiopia an estimated 753,100 people are living with HIV with a

declining national HIV prevalence in 2011 which was estimated to be 1.5% according to the demographic and health survey (DHS) 2011 compared to 1.1% in 2015 (2014 Spectrum projection). Urban are more affected than rural areas while females are twice affected than male population with HIV. [5]

Ethiopia launched free ART in 2005. According to the National Guideline for Comprehensive HIV Prevention Care and Treatment of Federal Ministry of Health (FMOH), the eligibility of initiating HAART among adults and adolescents HIV infected patients was CD4 count $\leq$500 cells /mm^3 irrespective of WHO clinical stage. The first line comprise nucleoside reverse transcriptase inhibitors (NRTI) as a backbone (TDF+3TC) and one non-nucleoside reverse transcriptase inhibitors (NNRTI) (EFV) are maintained as preferred choice in adults, adolescence and children older than ten years. Whereas the recommended second line ART regimens for adult and adolescents include a boosted protease inhibitor (PI) plus two NRTIs (determined by the drug used as first line therapy) [6].

In HIV-infected individuals on ART, the decision on when to switch from first-line to second-line therapy is determined by treatment failure, and this can be measured in three ways: clinically, immunologically, and virologically [7]. Of the three, the viral load is an earliest marker to detect treatment failure [8], however, due to its cost ART monitoring in developing countries including Ethiopia is mainly based on CD4 counting until the recent effort to introduce routine viral load monitoring. Hence, this study is designed to determine the immunological and virological response to ART and to evaluate the utility of immunological response to predict virological failure and its correlation with CRP.

1.2 STATEMENT OF PROBLEM

Patients who start HAART have a rapid decrease in HIV viremia to undetectable levels within 6 months of treatment initiation and a gradual increase in CD4 cell count to levels approaching those seen among uninfected patients. However, HIV drug resistance and treatment failures are known to result in greater treatment complexity, cost increment as well as worsening of morbidity and mortality rates among treated persons [9]. For instance persistently low CD4 count despite antiretroviral treatment for at least a year has been reported by Jalg *et al* in South Africa [10]. The prognostic value of discordant immunologic (CD4 cell decrease) and virologic (plasma HIV RNA level increase) responses to antiretroviral treatment is not well known [11].

A retrospective cohort study was conducted by Agete *et al* on trends of Immune-virological response among HIV- infected patients receiving HAART at Hawassa, Southern Ethiopia. Depending on clinical practices the immuno-virological responses were categorized in to three groups through CD4+ cells count and VL level within 6-12 months after ART commencement; these are Concordant responders (VL<150copies/ml and CD4+cells increase ≥ 50/µl), Concordant non-responders (VL >150copies/ml and CD4+ cells increase < 50 cells/µl and Discordant responders which is sub divided as virological non-responders (VL >150copies/ml and CD4+ cells increase ≥ 50/µl) or immunological non-responders (VL <150copies/ml and CD4+ cells increase <50 cells/µl), in comparison with baseline values. The result showed that concordant positive responders were 62.8% and concordant negative responders were 8.1% at 12 month. Also the proportion of discordant responders' was 29.0%; among this proportion, patients with immunologic failure despite virologic response were 15.1%). In contrast patients with immunological response and virological non-response were 13.9%. Furthermore, concordant responders had significantly higher CD4+ cells and body weight. Misclassification of virological ART failure occurs frequently using WHO clinical and immunological criteria of ART failure for poor settings. A viral load test confirming virological ART failure is, therefore, advised to avoid unnecessary switching to second-line regimens [11]. This study tried to assess the magnitude of immuno-virologic responses and the role of inflammation and other clinical factors associated with non-responses.

1.3 SIGNIFICANCE OF THE STUDY

In Ethiopia HAART initiation and its monitoring is based on investigating immunological response frequently, but virologic responses were not routinely monitored due to resources limitation. So that, this study helps to emphasize on both immunological and virological responses of HIV patients on HAART. Furthermore, it provides information to investigate association of an inflammatory marker (CRP) with Imunologic and/or virologic failures. Therefore, this study generates information to guide clinicians to decide early drug shift before the development of HIV related complications and to minimize mortality rate of HIV infected patients.

In addition, the findings give direction for policy makers to revise ART care and treatment strategies to focus on virologic response by associating with immunologic response and the clinical utility of simple markers like CRP.

2. LITERATURE REVIEW

As indicated in the review by Misgena *et al,* WHO recommends clinical and immunological assessments as surrogates of plasma viral load (VL) to identify first-line treatment failures in resource-poor settings. However, immunological tools have poor sensitivity (20- 30%) and specificity (86-90%) to identify virologic failures that may lead to continuing with failed regimen or to unnecessary switch of regimen which could result in a more complex profile of resistance. There are three main types of immuno-virologic responders in clinical practice: concordant responders (40-60%), concordant non-responders (12- 27.3%), and discordant responders that include lack of CD4+ increases despite viral suppression (7-48%), and optimal CD4+ responses in the absence of viral suppression (5-23.8%). The risk of morbidity and mortality is higher in the concordant non-responders and discordant responders [12].

A study conducted in India by Ingole *et al* enrolled a total of 130 patients of whom 84 (64.6%) were less than 40 years of age and 86 (66.2%) were males. Of these, 63 (48.5%), 34 (26.2%), 29 (22.3%), and 4(3.1%) patients belonged to WHO clinical stages I, II, III, and IV, respectively. High baseline viral load was a significant risk factor for virological failure at 6 months and low baseline CD4 count was a significant risk factor for immunological failure at 12 months. At 6 months, 87 (79.81%), 7 (5.5%), 13 (11.92%), and 2 (1.83%) patients had concordant favorable, immunological only, virological only, and concordant unfavorable responses, respectively. At 12 months, 61 (69.3%), 9 (10.2%), 16 (18.2%), and 2 (2.3%) patients had concordant favorable, immuno-logical only, virological only, and concordant unfavorable responses, respectively. No significant difference was observed in age, gender, baseline WHO clinical stage, baseline CD4 counts, and baseline viral loads in concordant or discordant response of patients [7].

A dynamic and observational cohort study in Shenzhen showed that out of the 3099 patients surveyed, 2172 (70.1%) were included in the study. The median age was 33 years; 78.2% were male and the median follow-up time was 31 months (IQR, 26–38). A total of 81(3.7%) patients died, whereas 292 (13.4%) and 400(18.4%) patients experienced virological and immunological failures, respectively. Adjusted Cox regression analysis indicated that baseline viral load (HR=2.19, 95% CI 1.52 to 4.48 for patients with a baseline viral load greater than or equal to

1, 000,000 copies/mL compared to those with less than 10 000 copies/mL) and WHO stage (HR=4.16, 95% CI 2.01 to10.57 for patients in WHO stage IV compared with those in stage I) were significantly associated with virological failure. The strongest risk factors for immunological treatment failure were a low CD4 cell count (HR=0.46, 95% CI 0.32 to 0.66 for patients with CD4 cell counts of 50–99 cells/mm^3 compared to those with less than 50 cells/mm^3) and higher baseline WHO stage at treatment initiation (HR=2.15, 95% CI 1.38 to 3.34 for patients in WHO stage IV compared to those in stage I [13].

Paradoxical CD4 decline in HIV-infected patients with complete virus suppression taking tenofovir and didanosine were documented by Barrios A *et al* from Spain. In the study the outcomes were analyzed in 570 individuals according to treatment modality; the nucleoside analogue (NA) backbone (298with TDF +ddI, 88 with ddI, 44 with TDF, and 140 with neither ddI nor TDF); and the third agent used (378 with non-nucleoside analogues versus 192 with NA). Significant CD4+ T-cell declines were seen in patients taking ddI + TDF with respect to all other NA combinations, including ddI or TDF separately. Patients exposed to high ddI doses or taking a third NA showed more pronounced CD4 declines. Plasma levels of ddI correlated with the extent of CD4+ T-cell loss [14]. The study added evidence on how immunologic response varies by treatment types.

A study conducted in Nigeria by Anude *et al* on immuno-virologic outcomes and immuno-virologic discordance responses among adults alive and on anti-retroviral therapy at 12 months demonstrated factors associated with immuno-virologic success as well as failures. The study showed that virologic suppression rate (<400 copies/ml) was 76.7%, immunologic recovery rate (CD4 change from baseline ≥50 cells/mm^3) was 77.4% and immuno-virologic discordance rate was 33%. In multivariate logistic regression, virologic failure was associated with age <30 years (OR 1.79; 95% CI: 1.17-2.67, p=0.03), anemia (Hemoglobin < 10 g/dl) (OR 1.71; 95% CI: 1.22-2.61, p=0.03), poor adherence (OR 3.82; 95% CI: 2.17-5.97, p=0.001), and post-secondary education (OR 0.60; 95% CI: 0.30-0.86, p=0.02). Immunologic failure was associated with male gender (OR 1.46; 95% CI: 1.04-2.45, p=0.04), and age <30 years (OR 1.50; 95% CI: 1.11-2.39, p=0.03). Virologic failure with immunologic success (VL-/CD4+) was associated with anemia (OR 1.80; 95% CI: 1.13-2.88, p=0.03), poor adherence (OR 3.90; 95% CI: 1.92-8.24, p=0.001), and post-secondary education (OR 0.40; 95% CI: 0.22-0.68, p=0.005). Among the factors

identified, as shown by the adjusted odds ratios people with post-secondary education are less likely to have immunologic or virologic failure [15].

A study was conducted by Julg *et al* to assess the impact of age, gender, baseline CD4 cell count, hemoglobin, BMI, tuberculosis and other opportunistic co-infections, and frequencies of regimen change on CD4 cell recovery at 12 and 30 months and on overtime change in CD4 cells among 442 virologically suppressed HIV-1 Clade C infected South Africans. Despite adequate virological response 37% (95% CI:32%–42%) and 83% (95% CI:79%–86%) of patients on antiretroviral therapy failed to restore CD4 cell counts ≥200 cells/mm3 after 12 and ≥500 cells/mm3 after 30 months, respectively. Critical risk factors for inadequate recovery were older age and nadir CD4 cell count at ART initiation, while concurrent TB co-infection, BMI, baseline hemoglobin, gender and antiretroviral regimen were not significant risk factors for immunological failure despite virologic success. These data suggest that greater efforts are needed to identify and treat HAART-eligible patients prior to severe CD4 cell decline or achievement of advanced age [10].

A study conducted in Ghana by Annison *et al* on the immunological response of HIV-positive patients initiating HAART indicated that at baseline, women had higher CD4 count (mean of 77.4cells/µl), and mean age of participants was 40 years. The CD4 count increased from a mean baseline of 70.2 cells/µl to 229.2, 270.0, and 297.6 cells/µl at 6, 12, and 18 months of treatment respectively (P <0.0001 at each time point). There were no gender and age differences in treatment response. There was no difference in treatment response comparing those with CD4 <250 cells/µl and those whose CD4 count was between 250 and 350 cells/µl at baseline although patients with baseline CD4 count <250 cells/µl showed larger increases after 12 months of treatment. Out of 282 patients with pre-therapy CD4 count <250 cells/µl, 241 (85.5%) and 41 (14.5%) were adherents and non-adherents respectively. Mean rate of increase was 15.2 and 8.4 cells/µl/month in adherent and non-adherent patients respectively [16].

Sufka *et al* studied 30 HIV–positive patients, in 3 groups to investigate the mechanisms that underlie discordant CD4+cell/virus load (VL) responses in patients who receive highly active antiretroviral therapy. Discordant responders maintained CD4+ cell levels 1200/mm3 with stable or increasing trend, despite sustained VLs of 500–5000 copies/mL, for 12 years. Treatment-success patients had CD4+ cell counts 1200/mm3 with stable or increasing trend and VLs 150

copies/mL, for 12 years. Treatment-failure patients initially responded to HAART, followed by decreasing CD4+ cell counts and increasing VLs. These findings suggests that discordance responses may be related to enhanced HIV- directed immune response diminished cellular activation, Decreased viral replication capacity, presentation of non–syncytium-inducing virus strains((CCR5-tropic) viruses [17].

Another cross-sectional study by Govender *et al* enrolled a total of 4793 adults attending VCT of which 1062 (22%) tested positive. Of the 1062, 799 (75%) were ART naive and 348/799 (44%) were first-time HIV testers. Of this group of 348, 225 (65%) were female. Overall their median age, CD4 count and viral load was 34 years (IQR: 28-41), 364 (IQR: 238-542) cells/mm3 and 13,000 (IQR:2050-98171) copies/ml, respectively. Female first time HIV testers had higher CD4 counts (419 IQR: 262-582 vs. 303 IQR: 199-418 cells/mm3) and lower viral loads (9,100 vs. 34,000 copies/ml) compared to males. Of 183 participants with CD4 count less than 350 cells/mm3, 62 (34%) had viral load greater than 10,000 copies/ml [18].

Collazos J *et al* study revealed that women had higher CD4 cell counts ($P < 0.001$), lower viral load ($P < 0.001$) and more favourable clinical profile ($P < 0.001$) than men at baseline. Following treatment, antiretroviral drug-naive women had higher CD4 cell count ($P = 0.01$) over time than drug-naive men but similar virological responses ($P = 0.6$); among drug-experienced individuals, women had also better immunological ($P = 0.06$) and similar virological ($P = 0.3$) responses compared with men. Consequently, the viro-immunological profile was significantly more favourable in women at each time point. Sex was significantly associated with clinical ($P = 0.01$), virological ($P = 0.01$) and immunological ($P = 0.006$) responses to antiretroviral treatment in multivariate analyses after adjustment for other variables. The differences between genders were not explained by different adherence to therapy [19].

Study by Tan et al showed that from 404 patients, 70.5% experienced favorable concordant responses (CD4 cell count $[CD4]^+$/viral load $[VL]^+$: increase in CD4 count of ≥50 cells/μL and achievement of undetectable plasma HIV RNA level), 15.8% an immunologic response only ($CD4^+/VL^-$), 8.7% a virologic response only ($CD4^-/VL^+$), and 5.0% a concordant unfavorable response ($CD4^-/VL^-$). $CD4^+/VL^-$ and $CD4^-/VL^-$ were associated with nonwhite race in multivariate logistic regression models (adjusted OR = 2.83, 95% CI: 1.46 to 5.47 and adjusted OR = 6.50, 95% CI: 1.65 to 25.69, respectively) [20].

On the other hand, studies have tried to investigate the role of simple markers like CRP in predicting immunologic and virologic responses. For instance, a study conducted by RaMana *et al* on alternate biomarkers in HIV disease and evaluating their efficacy in predicting CD4+ T Cell counts and disease progression in resource poor settings in HAART era, tested selective hematological and serological biomarkers. The findings indicated CRP values of >1.2 and hematologic parameters like absolute Eosinophilic counts of more than 550 cells/mm3, Hemoglobin less than 10 g%, ESR more than 20 mm, and TLC of <1800 cells/mm3 could be helpful in predicting CD4 cell counts of < 350 and <200 cells/mm3 [21]. This study underscores about CRP value as inflammation marker to observe immune-virologic response among different groups of study participants.

The results of study by Khan *et al* concluded that there is a significant association of immune activation, as measured by hs-CRP levels with HIV disease progression [22]. Moreover a study conducted by Draina *et al* lends additional evidence for the role of CRP levels as marker of HIV disease progression. In this study, during the follow-up period, 56 women progressed to WHO stage 4 and 188 died, and a high maternal CRP concentration was significantly associated with a 2.26-fold (95% CI 1.64–3.12) greater risk of progression to stage 4 or death. In multivariate analyses among adults, a high maternal CRP concentration was significantly associated with a 1.55-fold (95% CI 1.08–2.23) greater risk of progression to stage 4 or death [23].

A cross-sectional study by Lau *et al* showed that associations between log10 CRP were correlated inversely with CD4 lymphocyte counts (r=−0.17;P.001) and directly with log10 HIVRNA levels (r=0.20;P.001). Levels of CRP of more than 2.3 mg/L were associated with a decreased time to the development of AIDS (relative time to AIDS, 0.36;P.001) compared with individuals with CRP levels of 1.2 mg/L or less, which remained significant after adjustment for CD4 lymphocyte counts and HIV RNA and hemoglobin concentrations. Levels of CRP significantly increased over time with mean slopes of 8.5% (95% confidence interval, 4.9%-12.2%) and 4.5% (95% confidence interval CI, 2.1%-6.9%) per year for individuals with and without progression to AIDS, respectively. Individuals had a geometric mean CRP level of 2.5 mg/L in the 6-month interval before progression to AIDS [24].

A study conducted in Hawassa, Ethiopia by Agete *et al*. described the trends of Immuno-virological response among HIV-infected patients receiving HAART. The mean CD4+ cells

count showed significant increment at 6, 12, 18 and 24 months after ART treatment among patients having VL<5 (log10) compared to those VL≥5 (p=0.04; 0.002; < 0.0001; 0.001, respectively). Females had better mean CD4 count throughout the 24 months, though did not reach to a statistically significant level. Also CD4 cells recovery of patients over 50 years of age was sluggish in relative to younger patients. CD4+ cells and body weight of concordant positive responders showed significant rising trend at 6, 12, 18, 24 months when compared to discordant responders as well as concordant non-responders (0.003 vs. 0.05; <0.0001 vs. 0.04; 0.001 vs.0.008; 0.001 vs.0.03, respectively) [11].

Most ART outcome studies in Ethiopia depend on Immunologic and clinical responses with limited studies measuring virologic responses. This is because the country is very recently introducing routine viral load monitoring and in the past years has been dependent on CD4 count to initiate as well as monitor ART responses due to the unaffordable cost of viral load determination. A retrospective cohort study conducted in selected health facilities of Kembata and Hadiya zones, Southern Nations, Nationalities and Peoples Region of Ethiopia for example showed poor survival being associated with low baseline CD4 count, poor adherence and low hemoglobin level at ART initiation. Thus, this study by Ayele *et al* recommended Initiation of ART at the early stages of the disease, before deterioration of the functional status of the patients and before the reduction of CD4counts and hemoglobin levels [25].

Moreover, a multicentre retrospective follow up study done by Tsegaye *et al* on predictors of treatment failure on second-line antiretroviral therapy among adults in northwest Ethiopia shows incidence rate of failure was 61.7/1000 person years. The mean±SD age of participants at switch was 36±8.9 years, the probability of failure at the end of 12and 24 months were 5.6% and 13.6%, respectively. Out of 67 total failures, 42 (62.7%) occurred in the first 2 years. The significant predictors of failure were found to be WHO clinical stage IV at switch (adjusted HR (AHR) 2.1, 95% CI 1.1 to 4.1); CD4 count <100 cells/mm^3 at switch (AHR 2.0, 95% CI 1.2 to 3.5); and weight change (AHR 0.92, 95% CI 0.88 to 0.95) [26].

A study conducted in Zewditu Memorial Hospital in Addis Ababa, Ethiopia by Derbe *et al* tried to examine immunological response among 887 HIV/AIDS patients after ART therapy. Among them 472 (53.2%) were female and 415 (46.8%) were male patients. None of them had any opportunistic infection during the time of follow up. The mean age of the study group was 36.76

(17-76 years). The mean baseline CD4 count was 81.40 cells/µl; the mean CD4 count at the 6th, 9th and 12th month was 191.65, 284 and 331 cells/µl, respectively. There was a good immune recovery at the 6th month of therapy from the baseline mean CD4+ T cell count of 81 cells/µl to 191.65 cells /ml, which was statistically significant ($p<0.0001$). This first remarkable rise was continued in the achieving in the mean CD4+count of 284cells/µl at the 9^{th} month of visit. Followed by relatively steady lower increase and approaching stable CD4+ T cell count and 12th months of visit [27]. Like most of the ART outcome studies in Ethiopia [25, 26], this study did not address virologic responses and hence rate of neither concordant nor discordant responses. Therefore, our study aimed to fill this gap of knowledge by studying immuno-virologic responses of patients attending this hospital which contains the first model ART center in the country.

3. Objective

3.1 General objective

To assess Immuno-virologic response and its association with inflammation marker C-reactive protein, clinical and socio-demographic factors after initiation of HAART at 12 months and above among patients attending Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine immunologic response at 12 months and above of HAART using CD4 count
- To determine virologic responses at 12 months and above of HAART using plasma viral load
- To determine the proportion of concordant and discordant immunologic and virologic responders
- To assess the level of inflammation as measured by CRP among the study participants
- To determine clinical factors associated with immuno-virologic responses.

4. Hypothesis

- The rates of immunologic and virologic concordant versus discordant responses are similar to reports from Southern part of Ethiopia and other African countries.
- There is a difference in CRP level among immunologic and virologic responders versus non-responders.

5. Materials and Methods

5.1 Study area

The research project was done at Zewditu Memorial Hospital in Addis Ababa, Ethiopia. Zewditu Memorial Hospital is one of the oldest hospitals in Ethiopia and is located in Kirkos sub-City with a bed capacity of 188 beds. It was built, owned and operated by the Seventh - day Adventist church but was nationalized during the Derg regime in about 1976. It is Ethiopia's leading hospital in the treatment of ART patients and where the first ART program of the country started in 2003. As the largest and model HIV clinic in the country, Zewditu offers comprehensive HIV and integrated treatment. Currently the center treats more than 6,500 HIV patients each month as 350 clients are being seen daily. The ART clinic is run by 3 physicians and 9 nurses, 2 Health officers, and 9 data clerks.

5.2 Study design and period

A hospital based prospective cross sectional as well as retrospective longitudinal study was conducted from January to June 2016 at Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

5.3 Population

5.3.1 Source population

The source population was adult ART patients attending at Zewditu Memorial Hospital.

5.3.2 Study population

Adult (≥18years) HIV Infected patients attending ART clinic of Zewditu Memorial Hospital who are taking HAART at least for 12 months and that fulfill the inclusion criteria.

5.4 Eligibility

5.4.1 Inclusion criteria

- Adult patients (≥18years) who are at least for 12 months on HAART during the study period and
- Patients who have baseline and follow up CD4 and load viral counts.

5.4.2 Exclusion criteria

- Individuals who are on ART follow up but refused to give informed consent for additional testing of CRP were excluded.

5.5 Study variables

5.5.1 Dependent variables

- Rate of discordant Immunologic and virologic response
- Rate of concordant Immunologic and virologic response
- CRP values
- Virologic Failure
- Immunologic Failure

5.5.2 Independent variable

- Age
- Sex
- Marital status
- Weight+ Height (BMI)
- OI Drugs
- HAART regimen
- CD4 level
- Viral load level

5.6 Sample size calculation and sampling technique

5.6.1 Sample size calculation

$$n=\frac{Z^2p(1-p)}{d^2}$$ where;

Z: Z score

P: Prevalence (since no similar study in Zewditu Memorial Hospital, discordance rate of 50% will be used to achieve maximum sample size)

D: Allowable marginal error

With Z =1.96, P=0.05 and d=0.05 the minimun sample size becomes 384.

So, we include 402 HAART experienced HIV infected adult patients who satisfay the inclusion criteria and viait the clinic during the study period.

5.6.2 Sampling technique

Conveient sampling technique was employed to collect data from those ART patients visiting the center for their second follow up and above (12 months and above on HAART) during the study period.

5.7 Data collection procedure

Socio-demographic information and other relevant possible risk factors of the study participants are routinely collected using data extraction sheet by trained data collectors. The ART clinic is a model center with a well supervised electronic data base system. Baseline data including CD4 count of patients fulfilling the inclusion and exclusion criteria was extracted from the data base using formats. Viral load testing is recently included as a routine ART monitoring test and it was analyzed at the regional laboratory in the current visit for those who were visiting the center for their second follow up during the study period. The participants were requested for their consent to give additional blood sample for serum CRP determination.

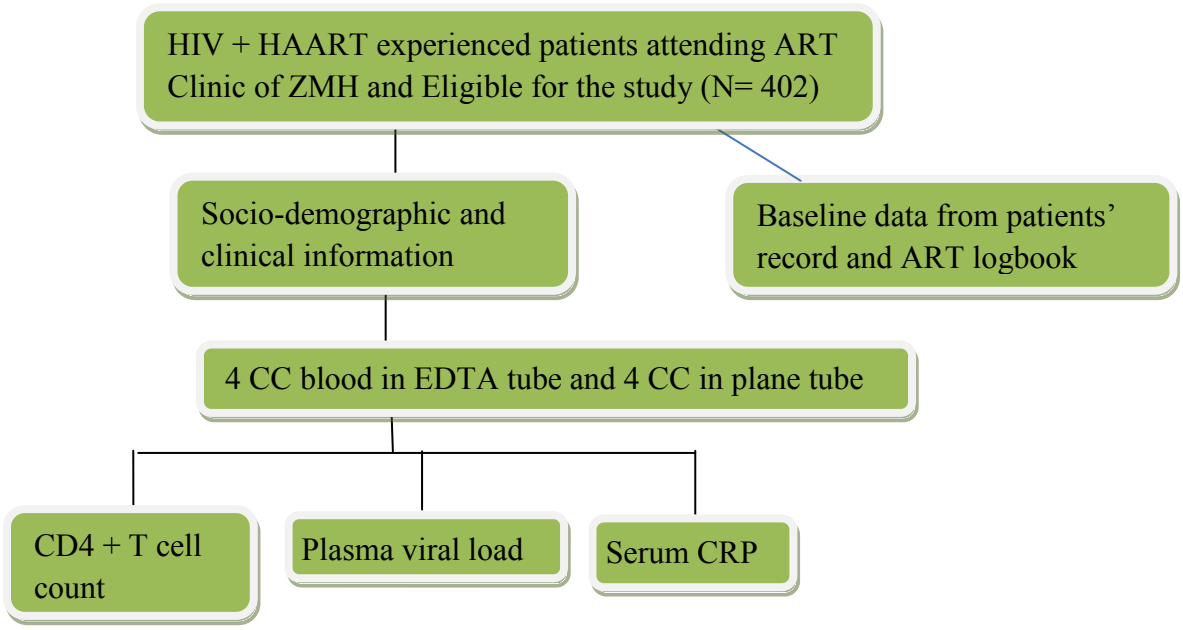

Figure 1- Work flow chart

5.8 Sample collection and analysis

Eight milliliter (8 ml) of venous blood was aseptically collected using plain and EDTA vacutainer tubes (4 ml in each tube) for the determination of CD4, Viral load and CRP. The CD4 count was done by BD FACSCalibur flow cytometer (BD Bioscience, San Jose CA). Viral load level was determined using m2000^{rt} viral load analyzer (ABBOTT, USA). Serum level of C reactive protein was determined using semi-quantitative method.

5.8.1 Flow Cytometry

When whole blood is added to the reagent Tru count tube containing BD Tri TEST CD_3 FITC /CD4 PE /CD45 PerCP, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocytes surface antigens. During acquisition, the cells travel past the laser beam (488nm Argon) and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals are detected by the instrument and provide information about cell's size, internal complexity, and relative fluorescence intensity. TriTEST reagents employ fluorescence triggering allowing direct fluorescence gating of the lymphocyte population to reduce interference of unlysed or nucleated red blood cells in the gate. The procedure is done using 50uL whole blood, which was collected in K_3EDTA tube, and added to the reagent tubes containing flurochrome labeled monoclonal antibodies. After incubating at room temperature for 15 minutes, 450 µl 1:10 diluted BD FACS lysing solution was added to each tube. The sample is incubated for 15 minutes in dark place at room temperature (20-25^{o}c). And the sample analyzed on the FACSCalibur then cells were acquired and analyzed using Mulitset software.

5.8.2 Viral Load determination

The Abbott Real Time HIV-1 assay uses RT-PCR to generate amplified product from the RNA genome of HIV-1 in clinical specimens. The test procedure started by thawing assay controls, sample, Internal Control, One bottle of mLysis Buffer, one vial of IC, and one RealTime HIV-1 Amplification Reagent Pack is used to support up to 24 reactions. The Abbott m Sample Preparation bottles were gently inverted to ensure a homogeneous solution. A calibrated precision pipette was used to add 500 µL of Internal Control to each bottle of mLysis Buffer. After mixing by gently inverting the container 5 to 10 times to minimize foaming, 100µl of mMicroparticles was added to each 12X75mm polypropylene tube. After vortexing 200µl of samples (specimens, controls and calibrators)

were transferred into a dedicated tube which contains 100µl of mMicroparticles and 2.5ml of mLysis using extended aerosol barrier pipette tips. Following incubation of the tubes in 50^0C heating block for 20 minutes, all the lysate from each tube was removed and 700µl of mWash 1 was added to each tube. The magnetic particles were resuspended and 700µl of mWash 1 was added for the second time After removing second wash of mWash2, 25µl of mElusion was added to each tube and incubated in 75^0C heating block for 20 minutes. After transferring 50µl of each sample eluate (supernatant) to the 96-well plate, the Abbott 96-Well Optical Reaction Plate was placed in the Abbott m2000rt instrument, and appropriate application file corresponding to the sample volume being tested was selected.

An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott m2000rt™ instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample.

5.8.3 C- reactive measurement

CRP-Latex/Determination of C- reactive protein slide test is a rapid slide agglutination procedure based on a modification of the latex fixation method developed for the direct detection and semi-quantification of C-reactive protein (CRP) in serum. We use the semi quantification one. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. After adding 50 µL of 0.9% saline solution into each of the circles of a card, 50 µL of specimen was added to the saline solution and mixed appropriately. Then 50 µL of the mixture was transferred to the saline solution in the second circle. The 2-fold serial dilutions were handled in a similar manner up to the six^{th} circle, and 50 µL was discarded from this circle. Sample dilutions were: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64. One drop of CRP-Latex Reagent was added to each circle next to the sample to be tested.

The slide was mixed and rotated using mechanical rotator (100 r.p.m.) for a period of 2minutes. The presence of a visible agglutination indicates an increase of the CRP level above the upper limit of the reference interval in the samples tested. The standard testing procedures is shown in the Annex 1.

5.9 Statistical Analysis

Data was entered, cleaned and analyzed using SPSS version 20 software. Descriptive analysis was employed to compute frequency, mean, median and standard deviations of continuous variables. Mean plus or minus standard deviation with 95% confidence interval (CI) was also be used for continuous variables and the difference in means was compared with independent-student-t-test. Binary and multiple logistic regressions were computed to assess associations between variables. Odds ratios with 95% CI and P value less than 0.05 were considered to determine statistically significant level.

5.10 Data Quality Assurance

Standard operating procedure (SOP) was followed during pre-analytical, analytical, and post analytical phases of the study. Routinely socio-demographic and clinical information is collected by experienced nurses in the clinic, so this minimizes technical errors and observer bias. All reagents were checked for their expiry date and we were making sure the reagents are prepared according to manufacturer's instructions. FACSCalibur accuracy is checked by approved control materials with three levels (low, medium and high). Moreover, the FACSCalibur is calibrated using calibrate beads and FACSComp software. For viral load analyzer, internal quality control was employed (negative, low positive and high positive). Positive and negative controls were run daily following the steps outlined in the Qualitative Test for CRP detrmination, in order to check the optimal reactivity of the reagent. Clear agglutination in the positive control assures quality.

5.11 Ethical considerations

Ethical clearance was obtained from research and ethics review committee of department of medical laboratory sciences. The proposal was also reviewed by ethical review committee of Addis Ababa Health Bureau. Written informed consent (signed or thumb print) was obtained from each participant after explaining the significance of the study as well as their right not to

participate in the study. Confidential identifier was used to code participant's identities. Results and any information regarding patients are kept confidential during and after the completion of the research project by password protecting electronic data and locking hard copy files.

5.12 Dissemination of Results

The result of the study will be presented to Addis Ababa University department of medical laboratory; and it will be disseminated Addis Ababa Health Bureau, Zewditu Memorial Hospital and other concerned bodies so that they work in collaboration to improve the health condition of the patients. Findings will be presented to the scientific community on conferences. Effort will also be made to publish the paper on peer reviewed journal.

5.13 Operational definitions

Immunological failure (IM-): According to National Guideline for Compressive HIV Prevention Care and Treatment of FDRE/MOH [18], CD4 count falls to baseline value at12 month of ART initiation (or below) or persistent CD4 level below100 cells/mm^3

Immunological response (IM+): is defined as a minimum absolute increase in the CD4 cell count of a minimum 50 cells/µl within 6-12 months after ART initiation.

Virological failure (VL-): According to National Guideline for Compressive HIV Prevention Care and Treatment of FDRE/MOH, plasma viral load above 1000 copies /ml after ≥6 months of ART initiation.

Virological response/ suppression (VL+): is defined as a VL become <150 copies/ml or undetectable after ≥6 months of ART initiation.

Concordant response: defined as both immunologic as well as virologic responses (IM+VL+)

Discordant response: defined as immunologic response and virologic failure (IM+VL-) or immunologic failure in the presence of virologic response (IM-VL+)

Concordant non-response: when there is both immunologic and virologic failure also can termed as unfavorable response.

OI: infections that occur more frequently and are more severe in individuals with weakened immune systems, including people with HIV.

6. Result

6.1Baseline characteristics of study participants

From a total of 402 study participants enrolled in this study, 235(58.5%) were females. Majority of study participants 174(43.8.8) were in the age group of 30-39, 184(45.9%) attained high school and 193(48.0%) were married. Based on the medical records, Of the study participants 159(39.6%) were on 1e regimen (TDF+3TC+EFV) and have normal BMI 224(55.7%). In addition, 99.5% of study participants were on OI drugs results are summarized in Table1.

Table 1 Socio-demographic and clinical features of HAART taking study participants at Zewditu Memorial Hospital ART clinic, Addis Ababa Jan -Jun, 2016 (n=402)

Variable	Category	Frequency (n)	%
Age(Years)	18-29	64	15.9
	30-39	174	43.3
	40-49	104	25.9
	>50	60	14.9
Sex	Male	167	41.5
	Female	235	58.5
WHO stage	I	74	18.4
	II	118	29.4
	III	148	36.8
	IV	62	15.4
Marital status	Married	193	48.0
	Divorced	51	12.7
	Single	107	26.6
	Widowed	51	12.7
Educational status (n=401)	Illiterate	48	12.0
	Elementary	90	22.4
	High School	184	45.9
	Certificate and above	79	19.7
Regimen type	1a	32	8.0
	1b	47	11.7
	1c	65	16.2
	1d	50	12.4
	1e	159	39.6
	1f	32	8.0
	1g	2	0.5
	2^{nd} line (2e,2g,2h,2i)	8	2.0
	Others(1^{st})	7	1.7
BMI*	Under weight	67	16.7
	Normal	224	55.7
	Over weight	92	22.9
	Obese	19	4.7
OI drug	COTRI	327	81.3
	COTRI+FLUC	1	0.3
	COTRI+INH	54	13.4
	COTR+INH+FLUC	1	0.3
	INH	17	4.2
	None	2	0.5

*Underweight = <18.5, Normal= 18.5-24.9, over weight= 25.0-29.9, obese= 30.0 and above

6.2. Changes in CD4+ T cell counts between baseline and follow up visits

The study revealed a remarkable change in the CD_4 count between baseline and at least 12 months of HAART. The change in the proportion of patients in the different CD_4 cell count categories between baseline and follow up was highest in the CD_4 category >350 cells/µl as evidenced by the large proportion of individuals moving to this group after being on HAART for at least 12 months (from 13.4% to 61.9%). On the other hand the number of study participants having very diminished CD_4 count (CD_4 count below 50cells/µl) decreased from 15.3% to 2.0% on follow up compared to baseline as indicated in table 2.

Table 2 Proportion of HAART taking study participants by CD_4 category at baseline and at least 12 month on HAART at Zewditu Memorial Hospital ART, clinic, Addis Ababa, 2016.

CD4 Category (cells/µl)	Baseline		≥ 12 month on HAART	
	Number	%	Number	%
<50	61	15.2	8	2.0
50-99	73	18.2	9	2.2
100-199	128	31.8	45	11.2
200-349	86	21.4	92	22.9
>350	54	13.4	248	61.9

The mean and standard deviation of CD4+ Tcell count at baseline and at least 12 months of HAART is displayed in Table 3. Using student t-test the change in mean CD_{4+} Tcell count between baseline and follow up (current) were statistically significant (P<0.05) for the different sex, age and BMI categories.

Table 3 Changes in CD4+ T cell counts (mean±SD) between baseline and follow up visits among patients attending Zewditu Memorial Hospital ART clinic, Addis Ababa, Ethiopia Jan -Jun, 2016 (n=402)

Variables	N	Baseline CD4+T cell (0 Month)	Current CD4+T cell (≥12 Months)	P value*
Sex				
Male	167	154 (151)	401(225)	P<0.0001
Female	235	212(159)	493(372)	P<0.0001
Age(Years)				
18-30	64	254(174)	398(181)	P<0.0001
31-40	174	184(152)	456(231)	P<0.0001
41-50	104	179(155)	504(514)	P<0.0001
>50	60	145(145)	426(210)	P<0.0001
BMI				
Under weight	67	190(168)	374(198)	P<0.001
Normal	224	182(155)	436(228)	P<0.0001
Over weight	92	195(149)	487(220)	P<0.0001
Obese	19	217(198)	458(257)	P=0.0002

*Student t test

6.3. Change in VL between baseline and follow up visits

Using cut off value of 1000 HIV RNA Copies/ml for viral load success, out of 402 study participants 65.9% (265/402) had viral load count <1000 HIV RNA Copies/ml at base line but during follow up 91.0% (366/402) of the participants had achieved viral Success(<1000 HIV RNA Copies/ml) . As indicated in Figure 2 majority of study participants positively responded to viral load.

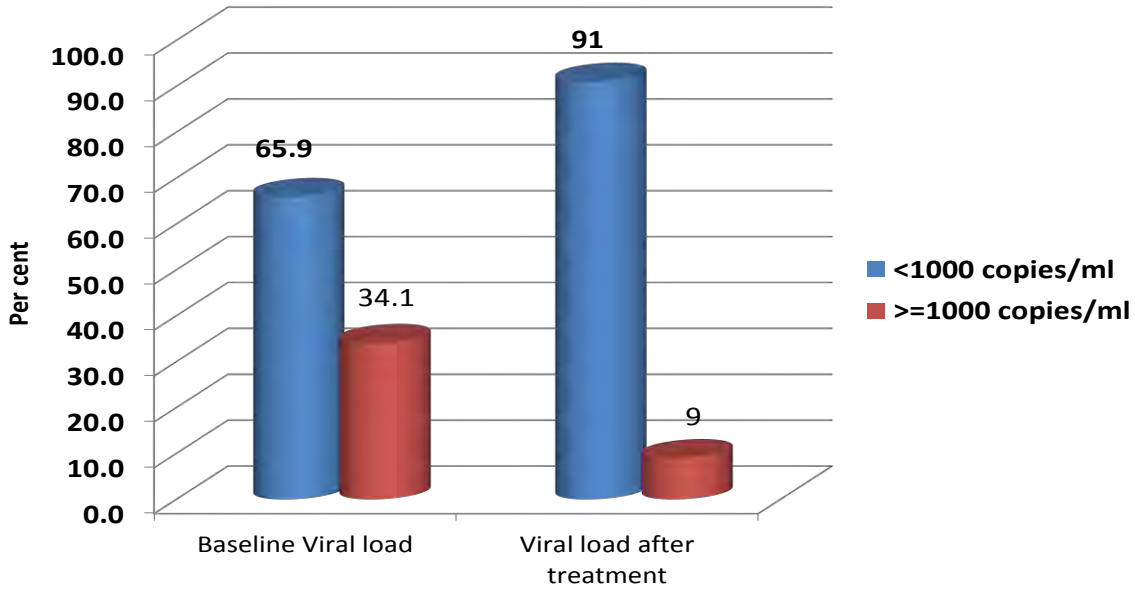

Figure 2 Proportion of patients by viral load category at baseline and after at least 12 month on HAART (follow up) at Zewditu Memorial Hospital ART clinic, Addis Ababa, 2016.

6.4. Immuno-virologic response

Analysis of immune-virologic response of study participants revealed that 311(77.4%) of them showed concordance positive response while 16 (4.0%) failed both immunologically as well as virologically. Despite virological response, 55(13.7%) of study participants failed to restore CD_4 count of at least 50 cells/uL after 12 months of HAART on the other hand Immunologically respond but virologicall failed to respond were 20 (4.9) as shown in figure 3.

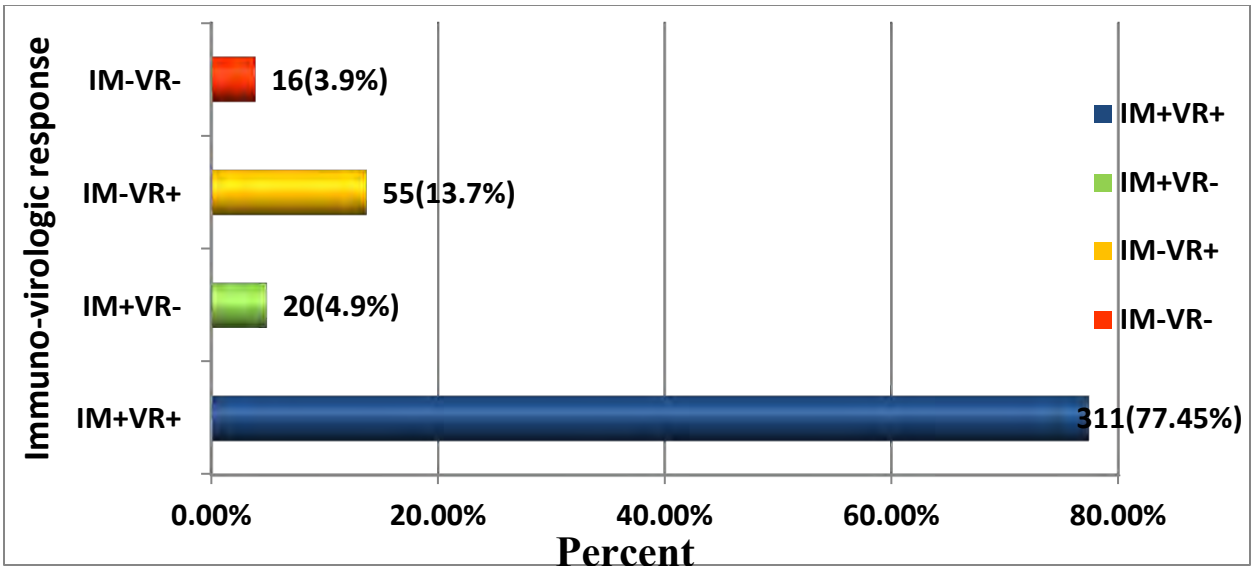

Figure 3 Immuno-virologic response of Patients attending Zewditu Memorial Hospital ART clinic, Addis Ababa, 2016.

6.5. Immuno-virologic response by marker of inflammation (CRP levels)

Positive CRP level among concordant unfavorable group, those who failed both Immunologically as well as virologically, were relatively higher than other categories of immune –virologicresponseasshowninfigure4.

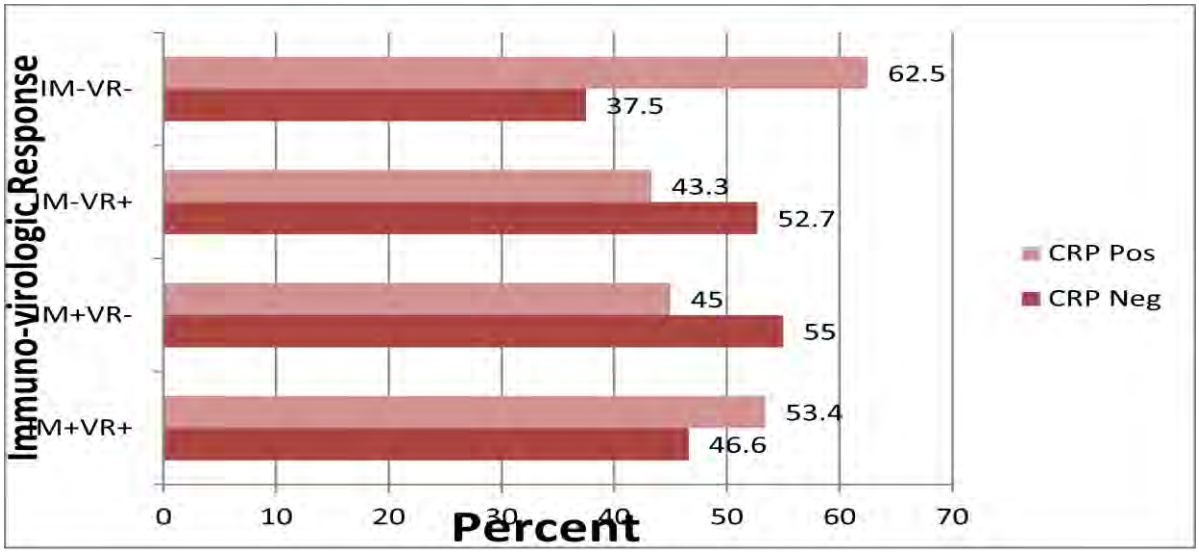

Figure 4 Immuno-virologic response and CRP level of Patients attending Zewditu Memorial Hospital , ART clinic, Addis Ababa, 2016.

6.6. Factors associated with immuno-virological discordant response.

On the multivariate analysis after adjusting for age, sex, WHO staging, BMI and CRP levels, being CD4 category >350 cells/mm^3 (adjusted odds ratio = 0.06; 95% CI: 0.02- 0.19) and being viral load <1000copies/ml (adjusted odds ratio = 0.26; 95% CI: 0.13-0.55) were significantly associated factors of immuno-virological discordant responses. As shown in Table 4, individuals who had a baseline CD4 count above 350 cells/ mm^3 and baseline viral load below 1000 copies per ml are less likely to have a discordant response (Table-4).

Table 4 Multivariate analysis of Factors associated with immuno-virological discordant response at Zewditu Memorial Hospital, ART clinic, 2016.

Explanatory variable	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Sex				
Male	1		1	
Female	1.42(0.89-2.28)	0.141	1.50(0.83-2.69)	0.173
Age(Years)				
18-30	1		1	
31-40	1.73(0.90-3.34)	0.098	1.082(0.49-2.34)	0.841
41-50	1.09(0.54-2.16)	0.802	0.93(0.40-2.16)	0.876
>50	2.39(0.98-5.81)	0.054	1.44(0.51-4.00)	0.483
BMI				
Under weight	1		1	
Normal	0.89(047-1.69)	0.740	1.01(0.50-2.05)	0.960
Over weight	2.09(0.91-4.78)	0.080	2.45(0.97-6.20)	0.057
Obese	0.87(0.27-2.81)	0.827	1.32(0.34-5.04)	0.684
CD4 baseline category (cells/ mm3)				
<50	1		1	
50-99	1.16(0.51-2.59)	0.715	0.71(0.29-1.72)	0.455
100-199	2.45(1.11-5.43)	0.026	0.89(0.34-2.31)	0.811
200-349	1.83(0.79-4.19)	0.153	0.63(0.22-1.78)	0.386
*>350	0.22(0.10-0.49)	0.000	0.06(0.02-0.19)	0.000
Baseline VL (copies/ml)				
≥1000	1		1	
*<1000	0.57(0.35-0.93)	0.025	0.26(0.13-0.55)	0.000
Baseline WHO stage				
Stage I	1		1	
Stage II	1.35(0.70-2.61)	0.357	1.02(0.47-2.20)	0.952
Stage III	2.29(1.17-4.48)	0.015	1.85(0.84-4.06)	0.125
Stage IV	1.03(0.49-2.16)	0.929	0.92(0.38-2.21)	0.858
CRP				
CRP negative	1		1	
CRP positive	0.95(0.52-1.65)		0.83(0.47-1.47)	0.536

*Among adjusted factors Baseline CD4 + T cell count of >350 and<1000 Viral load, VL (HIV RNA copies/ml) were statistically significant.

7. Discussion

This study describes the immuno-virologic response and its association with inflammation, clinical and other related factors after start of HAART. While HAART has dramatically changed treatment approach and increased longevity of HIV-infected individuals, various antiretroviral side effects are associated with different classes of antiretroviral combinations and response group.

The present study shows that change in CD4 were high after taking HAART for at least one year. This finding agrees with many studies including those from Ethiopia; a study from Hawassa reported that changes in CD4+ counts after HAART initiation were significantly increased [11]. Moreover a study by Derbe, *et al* in Zewditu Memorial Hospital also indicated that CD4 recovery was significantly increased after 12 month on HAART [27]. The positive response in this and earlier studies underscores the fact that HAART has considerably improved the immune recovery.

In line with CD4 recovery, this study also shows that numbers of study participants who have viral load counts below 1000 copies/ml were higher in follow up (91%) compared to baseline (65.9%). This outcome is supported by Geretti *et al* study in 2009 which revealed that 1906 (90%) of patients from a total of 2116, achieved undetectable viral load within 12 months after they started HAART [12]. A study by Wood *et al* in 2002 also indicated a <1 log10 decrease in plasma VL after one year therapy [28]. This is due to the fact that HAART inhibit viral replication. On the other hand, a study in Nigeria by Anude *et al* in 2013 which studied adult population, with comparable gender and age distribution, reported a relatively lower virologic response rate, which is 76.7% [15]. The possible explanation for the observed differences between ours (91%) and the Nigerian report could be variation in the cutoffs used to determine virologic responses. They used a viral load of 400 copies per ml to determine viral success [15] while we used 1000 copies per ml which is recommended by WHO as well as adopted by our country [6].

This study also shows evidence that Ethiopia is on the right track to achieve the global 90-90-90 target. UNAIDS set an ambitious 90–90–90 treatment target to help end the AIDS epidemic. According to this target by 2020, 90% of all people living with HIV will know their HIV status,

90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy, and by the same year, 90% of all people receiving antiretroviral therapy will have viral suppression. Our finding that 91% of the study population had viral suppression as compared to other sub-Saharan Africa which demonstrated 69%. Many countries are already on track to reach 90–90–90. Among then Botswana is set to achieve 90–90–90 by 2020, with 70.2% of people living with HIV from a large sample of people living in rural and peri-urban areas having already achieved viral suppression. In Rwanda, 86% of people living with HIV knew their HIV status in 2013, 63% were receiving antiretroviral therapy, and 82% of HIV treatment patients were virally suppressed. In Malawi also appear to be on track to reach 90–90–90, with 62% of people living with HIV in one rural district already virally suppressed. [29]The current study showed that majority of study participants (77.5%) had concordance immune-virologic response. This result supported by a study in southern Ethiopia by Hirigo *et al* which indicated that concordant positive responders were higher than other groups of responders with 60.5% at 6 months of treatment and this value was increased to 62.8% at 12 months [11]. Another study in Ethiopia also explained that majority of HAART users were concordant responders with 40-60% [27]. Compared to the earlier studies from Ethiopia, the concordance response rate in the current study is relatively higher. This could be evidence for good adherence of HAART in the study participants, who might have improved through time as the benefit of HAART is better understood in the society.

The discordant response rate in the current study is lower compared to the Nigerian study by Anude *et al*, [15]. The same explanation given for the virologic success rate difference holds true for the higher immuno-virologic discordant response rate they reported which is 33% versus the 18.6% in the current study.

In the current study, 13.7% of the participants failed immunologically despite viral success. This finding is slightly higher when compared to a study in Brazil by Casotti *et al* who documented 9% failure rate despite viral suppression [33]. The finding implies that had it not been for the introduction of routine viral load monitoring patients could have been exposed for unnecessary regimen change which will leave them with limited option for their future care. Thus, scaling up and sustaining of the current viral load monitoring in our country should be encouraged.

This study also presents that majority of CRP positive patients were in the concordant non responsive category. This group failed to respond to HAART both immunologically as well as virologically. This result is supported by Drain *et al* study which indicated that study participants who have high fold of CRP transferred to WHO stage four and they are at risk of death [23]. These might be due to the fact that higher hsCRP concentrations have been associated with lower CD4 counts and higher HIV viral RNA load among HIV infected individuals.

It has been shown that inflammatory markers directly correlate with morbidity and mortality in HIV infected patients. The inflammatory process, which is a correlate of T-cell activation, is much more pronounced when the virus is not suppressed [34]. As also seen in the current study, 62.5% of the patients who demonstrated both immunologic as well as virologic failure actually had elevated levels of CRP.

The current study tried to determine factors associated with discordant responses against HAART. Accordingly, participants having CD4 category >350 cells/cumm and viral load count < 1000copies/ml at baseline were less likely to have discordant responses. Jung *et al* identified old age and baseline CD4 count as critical risk factors for inadequate recovery despite adequate viral suppression [10]. Carlos *et al*, on the other hand, found that higher baseline CD4 count and lower HIV RNA level were associated with poor immunologic response to HAART in women with virologic suppression for at least 6 months [35]. The authors hypothesized that if the magnitude of reduction of plasma viral load under HAART is positively correlated to CD4 cell recovery, then even fully suppressive HAART in an individual with a lower baseline viral load could have less impact on CD4 cell recovery than in an individual with a high pre-suppression viral load. Similarly if the baseline CD4 cell count level is the result of HIV viral activity, then a lower CD4 cell count could represent more HIV viral activity and thus greater potential impact of viral suppression with HAART [36]. Similarly a study done in South Africa by Muzah *et al***,** showed that **o**n multivariate analysis, baseline CD4 cell count $\geq$ 200 cells/mm^3 (AOR 3.02; 95% CI 2.08 – 4.38; $p<0.001$) significantly associated with the development of discordant immune response [36].

8. Strength and Limitation of the study

8.1 Strength of the study

- This study categorize the study participant into four different group based on immunologic and virologic response, investigate their CRP level.
- Viral load and CD4 test was done in nationally accredited laboratory.
- The study is done in Zewditu memorial Hospital ART clinic, which is a model ART center in Ethiopia with well-organized data and resource setting.

8.2 Limitation of the study

- Base line CRP test is not done
- CRP testing is semi quantitative
- hs- CRP is not done

9. Conclusion and Recommendation

9.1 Conclusion

In this study, good CD4 cells recovery in response to HAART was documented in majority of follow-up cases. Moreover during follow up majority of study participant were not having significant viral load count, which has good viral suppression (91%) after treatment. This shows our country is on the right track in meeting the UNAIDS ambitious target of 90-90-90 by 2020 with regards to viral suppression. About 77.4% of patients respond concordantly both immunologically and virologically. Still remarkable proportion showed discordant responses. In line with this, majority of CRP positive study participant were in unfavorable responder category.

9.2 Recommendation

- Strengthen and scale up the current routine viral load monitoring throughout the country as viral load and CD4 testing is important to categorize and to decide immediate drug switch to second line if the patient is immunologically and/or virologically failed. Thus, it avoid unnecessary drug switching based on Immunological failure
- Using CRP test gives hint to indicate immuno-virologic failure in resources poor setting.
- This study supports CRP testing starting from base line as routine ART monitoring test which gives indication to early immuno-virologic response.

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ANNEXES

Annex-1 Laboratory methods

1.Sop for CD3/CD4 Positive Cell Count Determination

Purpose	To enumerate the absolute and percentage of lymphocytes that are CD4 + T-lymphocyte by lysing whole blood using FACScalibur machine.

Principle	When whole blood is added to the reagent, the flurochrome-labeled antibodies in the reagent bind specifically to leucocytes surface antigens. During acquisition, the cells travel past the laser beam (488nm Argon and 635nm Red diode) and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals are detected by the instrument provide information about cell's size, internal complexity, and relative fluorescence intensity. TriTEST reagents employ fluorescence triggering allowing direct fluorescencen gating of the lymphocyte population to reduce interference of unlysed or nucleated red blood cells in the gate[30].

Sample

Sample type	**Amount required**	**Transport and Storage**	**Stability**
Whole blood with anticoagulant of K_2/K_3 EDTA	4-5ml **N.B:-** Not less than 1/3 ml of the standard collection tube.	Room Temperature (20^{O}c - 25^{O}c).	72 hours before preparing(staining) & 24 hours after preparing(staining)

Materials

Reagents and supplies
Name
1.Tri TEST CD3/CD45/CD4 + TRU COUNT
2. BD CaliBRITE™3
3.. FACSlysing solution
4. FACS flow
5. FACs Clean
6. FACS rinse
7. TruCOUNT™Tubes

Equipment
1. FACS Calibur flow cytometer
2. Apple computer with monitor
3. HP laserjet printer
4. Racks
5. Sample mixer
6. Vortex
7. Precision adjustable micropipette
8. Supply tank
9. Waste reservoir
10. Refrigerator

Reagentspreparation

- If FACS clean is not available Make 1:3 dilution of bleach(%) with distilled water
- Diluted FACS Lysing solution Make 1:10 dilution with distilled water

Reagents stability and storage

- Diluted bleach stable for two days and store at room temperature.
- Reagent stable until expiry date and store at 2-8^{O}c
- Tru count tube store at room temperature.
- BD Tri TEST CD3FITC /CD4PE /CD45PerCP stable until expiry date and store at 2-8^{O}c
- FACS lysing solution stable until expiry date and store at room temperature
- Diluted FACS Lysing solution stable for one days and store at Room temperature
- FACS Flow stable until expiry date and store at room temperature
- FACS Clean stable until expiry date and store at room temperature

FACS caliber calibration procedure:

To calibrate use -BD calibrate 3 beads

-BD calibrate APC beads (only for 4 colour set up)

-Sheath fluid

1. Label tube one A and tube two B.

2. Vortex the stock vials of the BD calibrates beads to thoroughly suspend them.

3. Add 1ml of sheath fluid to tube A and 3ml to tube B.

4. use the following table to choose set up that you are to run in BD FAcscomp software.

Set up	Unlabeled tube A	Mixed tube B
Three color lyse/No wash	Unlabeled beads	Unlabeled ,FITC,PE ,Percp
Four color lyse/No wash	Unlabeled and APC beads	Unlabeled, FITC, PE, Percp and APC Beads.

5. Add one free falling drop from each of the BD calibrate beads based on the information in each column.

6. Cap the tube and mix by gently inversion.

7. Select BD FAcscomp from the display. And click accept.

8. Click lyse /No wash under assay selection if not selected.

9. Enter each BD calibrate beads lot ID as it appears if you use calibrator with different lot from previously set lot.

10. Gently mix tube A and install in to the cytometer sample injection port.

11. Set the flow rate to HI and press the RUN button on the cytometer.

12. In the software MENU click the RUN and start to begin acquiring event.

13. For 4 colour lyse/No wash APC beads are detected.

15. Gently mix tube B install in to the cytometer sample injection port and click START.

16. Remove the tube B from the cytometer.

17. Install a tube of D. water in to the sip and select STANDBY on the cytometer

Procedure	**Step**	**Action**
	1	First Calibrate the machine before run the sample
	2	Label each BD TruCount tube with the sample Identification number that is correlated with laboratory unique ID(barcode) of the sample.
	3	Pipette 20µl of BD Tritest CD3/CD4/CD45 in the bottom of each trucount tubes.
	4	Pipette 50µl of respective well-mixed K_3EDTA Blood in to the bottom of each trucount tube. Cap the tube and vortex gently to mix for at least 15 seconds.
	5	Incubate for 15 min. in the dark at room temperature (20-25^{o}c)
	6	Add 450 µl 1:10 diluted BD FACS lysing solutions to each tube.

	7	Incubate for 15 minutes in the dark at room temperature (20-25^{o}c). The sample is now ready to analyzed on the FACSCalibur. Then enter the barcode of each sample sequentially on the multiset software which is attached to the facscalibur machine at sample ID column and save.
	8	Run the samples within 24 hours.
	9	After finishing the test copy the export file from DATA-2 and paste on Polytech to export the results to LIS.
	10	Check that whether all results are exported to LIS or not by using sample ID or barcode and review the result. If there is a result <20cells/μL or >1000 repeat the test by new preparation to confirm the result. Accept the result if it is not discordant with the first one and file both results print out.
	11	Lastly print and attach result summary.

Calculation

Absolute count from the machine is determined by ratio:

$$\frac{\text{Observed counts from the population of interest}}{\text{Observer reference bead count}} \times \frac{\text{Reference bead count}}{\mu\text{L whole blood}} = \text{absolute count (cells/}\mu\text{L) of popul. interest}$$

Daily CD4 QC in house run

Select three samples

High ---------- result greater than 1000.

Normal ------- result between 500-600.

Low----------result 100-200

1. Store at appropriate temperature (20^{O}c - 25^{O}c).
2. Run each sample using sample testing procedure in the next day.
3. Compare the result with the previous day result.
4. The allowable difference should be within $\pm$7.02%

When does we use in house control?

Note: Use in house control when True COUNT controls andMultiCHEK controls are not available. But it is possible to use either tru count control or MultiCHEK control). In some cases it may be difficult to get all the three levels of inhouse controls. In this circumstance it is possible to use the available ones.

Limitations and Interference

1. Blood and Control bead delivery must be performed by reverse pipetting.

2. Sample must be collected in K3 EDTA vacutainer brand collection tubes

3. Don't store whole blood longer than 48 hours before preparing..

4.Do not refrigerate whole blood.

5. Do not dilute whole or use any volume other than 50 µL.

6. Store prepared samples at RT (20-25ºc) in the dark and run within 24 hrs.

7. Store the Trites Antibody at 2-8ºC. Do not use the reagent after the expiration date.

8. Do not freeze the reagent.

9. Do not expose the reagent to direct light.

10. Do not expose the reagent to direct light during incubation with cell.

11. Open the pouch only after it has reached room temperature.

12. Carefully reseal the pouch immediately after removing a tube.

13. Examine the desiccant each time you open the pouch. If the desiccant has turned from blue to Lavender, discard the remaining tubes.

14. Use tubes within one hour after removal the foil pouch.

15. Do not use the Tru-count tubes beyond the expiration date indicating on the packing.

16. Do not use the reagent if you observe any change in appearance.

17. Take care to avoid the reagent from microbial contamination, which can cause erroneous results.

18. Never pipette by mouth.

19. Wear suitable protective clothes and gloves.

20. Pipette must be calibrated to deliver 50 µL of sample.

21 Do not mix multiple lots of tubes in the same assay.

22. It is critical to use the bead count shown on the current lot of TruCOUNT tubes.

23. Do not use previously fixed and stored patient specimen.

24. Whole blood sample refrigerated before staining can give aberrant result.

25. Sample obtained from patient taking immunosuppressive drug can yield poor result.

26. Blast cell can interfere with test result

Result Interpretation	The sample printout reports percent and absolute counts for $CD3^+$, $CD4^+$andCD45$^+$ T lymphocytes.

Biological reference interval

Analyte	Reference range	Unit
CD3+ Cell	55-84	**cells/µL**
CD4+ Cell	410-1590	**cells/µL**
CD_3 average	690-2540	**cells/µL**

Clinical Utility	To monitor disease progression, establish decision points for initiating therapy and to monitor the effects of therapy in HIV infected individuals.

Maintenance

Step	Action
1	Daily Cleaning
2	Weekly cleaning
3	Monthly Cleaning
4	Periodic cleaning

Safety Precautions

- When laser radiation is open do not stare in to the beam.
- Follow National health and safety guideline

Sample disposal procedure:

1. Refer the sample disposal log and separate samples that its retention time is completed.
2. Prepare 0.5 % bleach solution.
3. Add 500µL of 0.5 % bleach solution to each blood specimen and stained sample and cap.
4. Wait for 30 minutes.
5. Put in to biohazard bag.
6. Pack and send to incinerator

Reference

1. BDFacscaliber - Instruction for use
2. BD FACSCalibur Manual (Safety and limitation)

2. Viral load determination

(SOP for HIV-1 Determination on the ABBOTT m2000rt Real-time Instrument)

Purpose	This procedure provides instructions about Abbott Real Time HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RTPCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals.

Principle	The Abbott RealTime HIV-1 assay consists of three reagent kits: • Abbott RealTime HIV-1 Amplification Reagent Kit • Abbott RealTime HIV-1 Control Kit • Abbott RealTime HIV-1 Calibrator Kit The Abbott RealTime HIV-1 assay uses RT-PCR to generate amplified product from the RNA genome of HIV-1 in clinical specimens. An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott m2000rt™ instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample. **Amplification** During the amplification reaction on the Abbott m2000rt, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostablerTth DNA polymerase. First, the HIV-1 and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is

extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of both targets (HIV-1 and IC) takes place simultaneously in the same reaction.

The target sequence for the Abbott RealTime HIV-1 assay is in the pol region of the HIV-1 genome. This region is highly conserved. The primers are designed to hybridize to the pol region with the fewest possible mismatches among various subtypes.

The IC target sequence is derived from the hydroxypyruvatereductase gene from the pumpkin plant, Cucurbitapepo, and is delivered in an Armored RNA® particle that has been diluted in negative human plasma.

Detection

During the read cycles of amplification on the Abbott m2000rt, the temperature is lowered further to allow fluorescent detection of amplification products as the HIV-1 and IC probes anneal to their targets (real-time fluorescence detection). The HIV-1 probe has a fluorescent moiety that is covalently linked to the 5′ end. A short oligonucleotide (quencher oligonucleotide) is complementary to the 5′ end of the HIV-1 probe and has a quencher molecule at its 3′ end. In the absence of HIV-1 target, the HIV-1 probe fluorescence is quenched through hybridization to the quencher oligonucleotide. In the presence of the HIV-1 target sequence, the HIV-1 probe preferentially hybridizes to the target sequence, dissociating from the quencher oligonucleotide, allowing fluorescent detection.

The IC probe is a single-stranded DNA oligonucleotide with a fluorophore at the 5′ end and a quencher at the 3′ end. In the absence of IC target sequences, probe fluorescence is quenched. In the presence of IC target sequences, probe hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection.

The HIV-1 and IC specific probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample[31].

Sample Human plasma specimens (collected in ACD-A or EDTA tubes) may be used with the Abbott Real Time HIV-1 assay.

Sample type	Amount required	Transport and Storage
Plasma	Minimum 1.5µl	For One day at 2 – 8^{o}C or 2 weeks at -20 - -40^{o}C or at -70^{o}C for longer time.

Limitations:

Lipemic or hemolyzed sample will affect the test.

Materials

Reagents:

A. **Abbott RealTime HIV-1 Amplification Reagent Kit**

- Abbott RealTime HIV-1 Internal Control (4 vials, 1.2 mL per vial)
- Abbott RealTime HIV-1 Amplification Reagent Pack (4 packs, 24 tests/pack)

- 1 bottle (0.141 mL) ThermostablerTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.
- 1 bottle (1.10 mL) HIV-1 Oligonucleotide Reagent. < 0.1% synthetic oligonucleotides (4 primers, 2 probes, and 1 quencher oligonucleotide), and < 0.3% dNTPs in a buffered solution with a reference dye. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
- 1 bottle (0.40 mL) Activation Reagent. 30 mM manganese chloride solution. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

B. Abbott RealTime HIV-1 Control Kit

- ❖ Abbott RealTime HIV-1 Negative Control (8 vials, 1.8 mL per vial)
- ❖ Abbott RealTime HIV-1 Low Positive Control (8 vials, 1.8 mL per vial)
- ❖ Abbott RealTime HIV-1 High Positive Control (8 vials, 1.8 mL per vial).

C. Abbott RealTime HIV-1 Calibrator Kit

- ❖ Abbott RealTime HIV-1 Calibrator A (12 vials, 1.8 mL per vial).
- ❖ Abbott RealTime HIV-1 Calibrator B (12 vials, 1.8 mL per vial).

D. Abbott RealTimeSample Preparation System (4 x 24 Preps)

- ❖ mLysis Buffer
- ❖ mMicroparticles
- ❖ mWash 1 Buffer
- ❖ mWash 2 Buffer
- ❖ mElution Buffer

Storage instruction for reagents

Abbott RealTime HIV-1 Amplification Reagent Kit

- The Abbott RealTime HIV-1 Amplification Reagent Pack and Internal Control vials must be stored at -10°C or colder when not in use. Care must be taken to separate the Abbott RealTimeHIV-1 Amplification Reagent Pack that is in use from direct contact with samples, calibrator and controls.

Abbott RealTime HIV-1 Control Kit

- The Abbott RealTime HIV-1 Negative and Positive Controls must be stored at -10°C or colder.

Abbott RealTime HIV-1 Calibrator Kit

- The Abbott RealTime HIV-1 Calibrator A and Calibrator B must be stored at -10°C or colder.

Abbott RealTimeSample Preparation System

- Abbott RealTimeSample Preparation System must be stored at room temperature.

Quality Control of Reagents

Indication of instability or deterioration of reagents

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary.

Equipment :

- Abbott m2000rt instrument
- Vortex mixer
- Biological safety cabinet approved for working with infectious materials(separated for reagent and sample preparation)
- Plate Centrifuge
- Heat block
- Calibrated Pipettes capable of delivering 20-1000 µL
- StrataCooler96 Base and Lid (store at -20°C)
- Repeater Pipette
- 12x75mm Magnetic rack, red
- 1.5 ml Magnetic rack, blue
- Non-magnetic racks
- Thermometer

Supplies:

- Abbott m Sample Preparation System (4 x 24 Preps)
- 5 mL Reaction Vessels
- Aerosol Barrier Pipette Tips for 20-1000 µL pipettes
- 10.0 mm to 16 mm sample tubes
- 200 µL and 1000 µL Disposable Tips
- Abbott Optical Adhesive Covers
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base
- Master Mix Vial
- 200 mL Reagent Vessels
- Abbott 96-Deep-Well Plate
- Abbott 96-Well Optical Reaction Plate
- single-use RNase/DNase-free tube
- Sterile disposable 5ml pipette
- Combi Tips Plus
- Adapter for 25ml Combi Tips Plus

- 12x75mm tube and cap
- Sealable plastic bags
- RNase-free water (Eppendorf or equivalent)
- 1.7 mL RNase-free Microcentrifuge Tubes
- Cotton Tip Applicators (Puritan or equivalent)
- 70% ethanol alcohol
- Pure Acetone alcohol
- Sucker/ Pater pipette

  †Note: These three items are used in the procedure for Monitoring the Laboratory for the Presence of Contamination.

Maintenance

Clean the Thermal Block

Caution: Do not remove the instrument casing. There isno components inside the m2000rt system that can safely service yourself. If you suspect a problem, contact an Abbott laboratories service representative.

NOTE: Do not use compressed air to clean the thermal block.

NOTE: Perform the contamination check procedure to prepare the instrument for cleaning the thermal block.

Warning: Instrument parts could be contaminated with potential infectious materials. So:

- Observe basic biohazard precautions
- Wear appropriate protective equipment such as glove, lab coats, and protective eye wear.

This procedure explains how to eliminate contaminants from the thermal block of the m2000rt system.

Assemble Required Materials

- Pipette
- 99% Ethanol alcohol solution

- Cotton or nylon swabs and lint-free cloths
- Distilled water
- Powder free gloves
- Thin screwdriver

NOTE: Wear powder-free gloves when you handle the thermal block.

Procedure

1. Perform the contamination check.
2. Power OFF and unplug the m2000rt system. Allow it to cool for 15 minutes.

Caution: Hot surface. During instrument operation, the thermal block can be heated as high as 100°C. Before performing the following procedure, be sure to wait until the thermal block reaches room temperature.

3. Open the access door to the m2000rt system.
    a) Insert a thin screwdriver in to the keyhole located on the edge of the access door, and press carefully until you hear the door latch release.
    b) Open access door.
4. Move the heated cover to the block of the instrument.
5. Open the tray drawer.
6. Remove the plate holder the instrument.
7. Close the tray drawer.
8. Clear the identified wells of the thermal block using a small volume of deionized water :
    a) pipette a small volume of the deionized water in to each well.
    b) Pipette the water up and down several times to rinse the well.
    c) Pipette the water to a waste breaker.
    d) Using a cotton or nylon swab, scrub (or clean) inside each well.
    e) Using a lint-free cloth, absorb the excess water.
9. Open the tray drawer.
10. Replace the plate holder.
11. Close the tray drawer.
12. Pull the heater cover to the front of the instrument and close the access door

NOTE: ensure the heater cover is pulled all the way to the front of the instrument.

13. Plug in and power ON the m2000rt system.
14. Perform the contamination check to verify the identified wells have been cleaned.
15. On successful contamination check procedure, perform a Background calibration.
16. If contamination check fails, repeat the entire procedure (starting with step 1) using 99% EtOH solution.
17. If the contamination check continues to fail, contact your area Abbott Customer Service.

Calibration

A calibration curve is required to quantify the HIV-1 RNA concentration of specimens and controls.

Two assay calibrators are run in replicates of three to generate a calibration curve (HIV-1 concentration versus the threshold cycle [Ct] at which a reactive level of fluorescent signal is detected). The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of HIV-1 RNA in a sample is calculated from the stored calibration curve. Results are automatically reported on the m2000rt workstation.

Follow the procedure for sample extraction, master mix addition, amplification and detection protocols as stated in the Abbott m2000rt Operations Manual.

Once an Abbott RealTime HIV-1 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- An Abbott RealTime HIV-1 Amplification Reagent Kit with a new lot number is used.
- An Abbott RealTime HIV-1 application file for a different sample volume is used.
- Equipment maintenance is performed.

For a detailed description of how to perform an Assay Calibration refer to the Abbott m2000sp and m2000rt Operations Manuals, Operating Instructions sections.

Procedure

1. Turn on the 50°C and 75°C heating blocks. Thaw assay controls, samples and Internal Control at 15-30°C or at 2-8°C. Thaw calibrators at 15-30°C or at 2-8°C only if performing a calibration run.

   Once thawed, assay controls, sample, Internal Control, and calibrators can be stored at 2-

8°C for up to 24 hours before use.

Vortex each assay calibrator and each control three times for 2-3 seconds before use. Ensure that the content of each vial is at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.

2. Thaw amplification reagents at 15-30°C or at 2-8°C and store at 2-8°C until required for the amplification master mix procedure.

- Once thawed the amplification reagents can be stored at 2-8°C for up to 24 hours if not used immediately.

NOTE: Use one bottle of mLysis Buffer, one vial of IC, and one RealTime HIV-1 Amplification Reagent Pack to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions. A maximum of 48 reactions can be performed per assay.

3. Gently invert the Abbott m Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.

4. Vortex each Internal Control three times for 2-3 seconds before use.

5. Use a calibrated precision pipette to add 500 µL of Internal Control to each bottle of mLysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.

6. A total of 48 samples can be processed in each run. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 45 specimens to be processed per assay.

- The Abbott RealTime HIV-1 assay minimum sample volume and associated rack requirements on the Abbott m2000sp are:

If frozen, thaw specimens at 15-30°C or at 2-8°C. Once thawed, specimens can be stored at 2-8°C for up to 6 hours if not processed immediately.

Vortex each specimen three times for 2-3 seconds before loading on the Abbott m2000sp worktable. Specimens showing particulate matter or turbidity should be clarified by centrifugation at 2,000 g for 5 minutes prior to testing. Aliquot each specimen into clean tubes or vials if necessary. Avoid touching the inside of the cap when opening tubes.

7. Place the low and high positive controls, the negative control, the calibrators, if applicable, and the patient specimens into the Abbott m2000sp sample rack.

8. Gently rock back and forth the mMicroparticles until particles are in suspension. There should be no settled particles in the bottom of the bottle. And add 100µl of mMicroparticles to each 12X75mm polypropylene tube.
9. Mix mLysis buffer (with the internal control added) gently by inverting 5 to 10 times. And add 2.5ml of mLysis by using repeater pipettor.
10. Vortex and transfer 200µl of samples (specimens, controls and calibrators) in a dedicated tubes which contains 100µl of mMicroparticles and 2.5ml of mLysis using extended aerosol barrier pipette tips.
11. Place the tubes in 50^0C heating block for 20 minutes.
12. Remove and place the tubes in magnetic capture stand for 2 min.
13. Carefully remove all the lysate from each tube using sterile disposable serological pipettes.
14. Add 700µl of mWash 1 to each tube and resuspend the magnetic particles by aspiration. And transfer wash fluid and particles to a labelled 1.5 ml screw top tube.
15. Carefully remove mWash1 from each tube.
16. Add 700µl of mWash 1 for the second time to each tube and resuspend the magnetic particles by aspiration. And carefully remove mWash1 from each tube.
17. Repeat the above washing procedure using mWash2. And centrifuge the sample at the second wash of mWash2 for 5Sec.
18. After removing second wash of mWash2 add 25µl of mElusion to each tube and resuspend the magnetic particles by aspiration.
19. Place the tubes in 75^0C heating block for 20 minutes.
20. Remove the tubes from the heating block and add 63µl of mWash2 to each tube and place them on magnetic rack.
21. Transfer 50µl of each sample eluate(supernatant) to the 96-well plate on the strata cooler which contain 50µl of master mix. And seal the Abbott 96-Well Optical Reaction Plate using Optical adhesive cover.
22. Centrifuge the 96-well plate in the splash free support base for 5min at 5,000xg.
23. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTime HIV-1 protocol, as described in the Abbott m2000rt Operations Manual, Operating Instructions section.

POST PROCESSING PROCEDURES

1. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose it along with the gloves used to handle the plate.

Clean the Splash Free Support Base before next use.

Calculation

The concentration of viral HIV-1 RNA in a sample or control is calculated from the stored calibration curve. The Abbott m2000rt instrument automatically reports the results on the Abbott m2000rt workstation. Assay results reported in Copies/mL (but it can also be reported in Log [Copies/mL], International Units (IU)/mL, or Log [IU/mL]; (1 IU = 0.58 copies, 1 copy = 1.74 IU))

Result Interpretation

Sample Volume	Result	Interpretation
0.2 mL	Not Detected	Target not detected
	<150 Copies/ml	Detected
	150to10 million Copies/ml	Detected
	>10 million Copies/mL	> ULQ

Expected Values

The upper limit of quantitation (ULQ) for the Abbott RealTime HIV-1 assay is 10 million copies/mL, and the lower limit of quantification is equivalent to the LOD (40 copies/mL for the 0.6 mL sample volume procedure, 75 copies/mL for the 0.5 mL sample volume procedure, and 150 copies/mL for the 0.2 mL sample volume procedure).

Limitations

- **FOR IN VITRO DIAGNOSTIC USE ONLY.**

• Optimal performance of this test requires appropriate specimen collection, handling, preparation, and storage.

• Human plasma specimens (collected in ACD-A or EDTA tubes) may be used with the Abbott RealTime HIV-1 assay.

• Use of the Abbott RealTime HIV-1 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott m1000 System, the

Abbott m2000sp and the Abbott m2000rt instruments.

• The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.

• A specimen with a result of Not Detected cannot be presumed to be negative for HIV-1 RNA.

• As with any diagnostic test, results from the Abbott RealTime HIV-1 assay should be interpreted in conjunction with other clinical and laboratory findings.

Control

A negative control, a low positive control, and a high positive control are included in each test in order to evaluate run validity. The lot specific values for the low positive control and high positive control are specified on each Abbott RealTime HIV-1 Control Kit insert and must be enteredinto the assay test order when a run is performed.

An error control flag is displayed when a control result is out of range. Refer to the Abbott m2000rt Operations Manual for an explanation of the corrective actions for the error control flag. If negative and/or all positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation. If the negative control and at least one positive level control pass/are in the range, there is no need of repeating the test (release the result).

The presence of HIV-1 must not be detected in the negative control. HIV-1 detected in the negative control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott m2000rt instrument and repeat sample processing for controls and specimens following the Procedural Precautions.

Special Safety Precautions

The Abbott Real Time HIV-1 assay is only for use with plasma specimens that have been handled and stored in capped tubes. During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent

introduction of ribonucleases (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.

Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the RealTime reagents used in the amplification step become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

Use at leasttwo dedicated areaswithin the laboratory for performing the Abbott RealTime HIV-1 assay with the Abbott m2000rtSystem:

1. The Reagent Preparation Area is dedicated to combine the Abbott RealTime HIV-1 amplification reagent components to create the amplification master mix and transfer aliquots of the master mix to the reaction plate. Laboratory coats, pipettes, pipette tips, and vortexes used in the Reagent Preparation area must remain in that area and not be moved to either the Sample Preparation Area or the Amplification Area.
2. The Sample Preparation Area is dedicated to processing samples (specimens, Abbott RealTime HIV-1 Controls, and Calibrators), and to adding processed samples, controls, and calibrators to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to either the Reagent Preparation Area or the Amplification Area. Do not bring amplification product into the Sample Preparation Area.
3. The Amplification Area is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to either the reagent Preparation area or the Sample Preparation Area. Only two dedicated areas, Sample Preparation Area and Amplification Area, are recommended when the Abbott m2000rt is used.

Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.

Do not use kits or reagents beyond expiration date.

Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, calibrators, or specimens.

Refer to the m2000rt Operations Manuals for instrument cleaning procedures. If the Abbott m2000rt instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott m2000rt Operations Manual along with the gloves used to handle the plate.

Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous materials in accordance with local, state, and federal regulations. All materials should be handled in a manner that minimizes the chance of potential contamination of the work area. Note: Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only one time. Clean and disinfect spills of specimens and reagents as stated in the m2000rt Operations Manuals.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNase contamination, cross-contamination between samples, and inhibition:

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching

the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.

- Change aerosol barrier pipette tips between all manual liquid transfers.

The Abbott mSample Preparation System (4 x 24 Preps) reagents are single use only. Use new reagent troughs or vessels, reaction vessels, and newly opened reagents for every new Abbott RealTime HIV-1 assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott m2000sp Operations Manual and the Abbott Sample Preparation System (4 x 24 Preps) product information sheet.

3. C-Reactive Protein measurement

SOPs for C - reactive protein/ CRP-Latex Determination of

C- reactive protein slide test.

PRINCIPLE

CRP-Latex Test is a rapid slide agglutination procedure based on a modification of the latex fixation method developed for the direct detection and semi-quantitation of C-reactive protein (CRP) in serum. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of a visible agglutination indicates an increase of the CRP level above the upper limit of the reference interval in the samples tested.[32]

REAGENT COMPOSITION

R- CRP-Latex Reagent - Suspension of polystyrene latex particles coated with specific anti-human C-reactive protein antibodies in a buffered saline solution. Contains 0.95 g/L of sodium azide.

CONTROL (positive) - Human serum with a CRP concentration > 15 mg/L. Contains 0.95 g/L of sodium azide.

CONTROL(negative)-Animal serum with a maximum concentration of human CRP of 1 mg/L. Contains 0.95 g/L of sodium azide.

Precautions: Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and antiHCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.

Warning: The reagents in this kit contain sodium azide. Do not allow contact with skin or mucous membranes.

PACKAGING CONTENTS

REF 2410005, kit 50 tests. 1 vial CRP-Latex Reagent, 1x1 mL Positive control, 1x1 mLNegative control, 3 Test cards and 1x50 disposable stirrers.

REF 2410010, kit 100 tests. 2 vials CRP-Latex Reagent, 1x1 mL Positive control, 1x1 mLNegative control, 3 Test cards and 2x50 disposable stirrers.

STORAGE AND STABILITY -Store at 2-8ºC. Do not freeze. Frozen reagents could change the functionality of the test. Reagent and Controls are stable until the expiry date stated on the label.

REAGENT PREPARATION -Reagent and Controls are ready to use.

SAMPLES -Use fresh, clear serum collected by centrifuging clotted blood.

After the clear serum has been separated it may be stored at 2-8ºC forupto one week or longer periods at –20ºC or the sample must be frozen.

If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 72 hours after collection.

As in all serological tests, hemolytic or contaminated serum must not be used. **Do not use plasma!**

MATERIAL REQUIRED - Automatic pipettes.

- Saline solution (0.9% NaCl, only for semi-quantitation procedure).
- Mechanical rotator, adjustable at 100 r.p.m.
- Laboratory alarm clock.

PROCEDURE

A. Qualitative Test

1. Bring the test reagents and samples to room temperature (Note 1).
2. Suspend the Reagent vial gently. Aspirate dropper several times to obtain a thorough mixing.

3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of 2 minutes (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

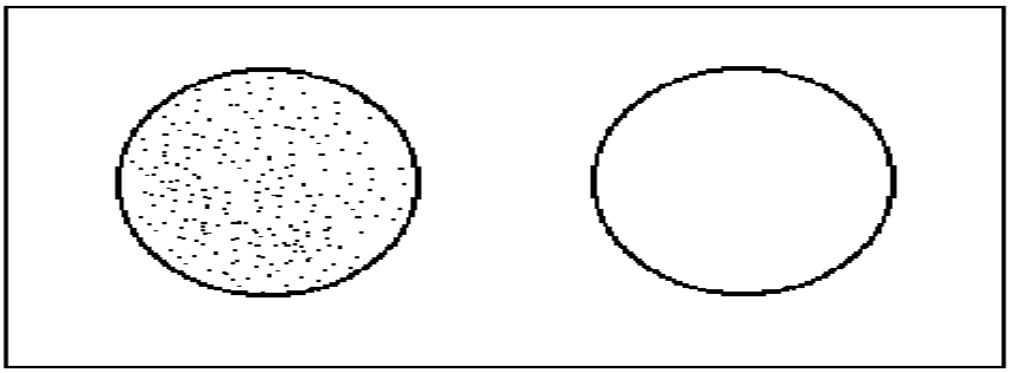
Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control (Note 3).

Reactive: Any degree of agglutination visible macroscopically (Note 4).

EVALUATION OF RESULTS

POSITIVE- A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the CRP Negative Control.

NEGATIVE- A negative reaction is indicated by a uniform milky suspension with noagglutination as observed with the CRP Negative Control.



Positive Negative

Semi-quantitative test evaluation

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution showing a positive reaction. The titer of the serum is the reciprocal of the highest

dilution which exhibits a positive reaction. For example, if the last positive reaction is found in the 1:8 dilutions, the titer of the sample is 8.

Test validity

CRP Positive and Negative Control should be included in each test batch.

Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the CRP Negative Control and agglutination with large aggregates is observed with the CRP Positive Control.

A. **Semi-quantitative Test**

1. For each specimen to be tested place with an automatic pipette 50 µL of 0.9% saline solution into each of the circles of a card. Do not spread diluent.
2. To circle one add 50 µL of specimen to the saline solution and, using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 50 µL of the mixture to the saline solution in the second circle.
3. Continue with the 2-fold serial dilutions in a similar manner up to the sixth circle, and discard 50 µL from this circle. Final sample dilutions will be: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64.(Test each dilution as described in steps 4-7 for the Qualitative)
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of 2minutes (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilutionshould be negative.If the highest dilution tested is reactive repeat the test starting witha preliminary 1:16 dilution. Use a 1:50 dilution of negative controlserum in 0.9% saline solution to the replace the 0.9% saline solution in the new 2-fold dilution series. The approximate CRP level (mg/L) present in the sample may be obtained multiplying the titer of the last positive dilution by the minimum detectable unit (analytical sensitivity).

NOTES

1. The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25ºC.
2. Delays in reading the results may result in over-estimation of the CRP concentration.
3. When CRP contents of the serum is in excess, prozoning effect may result in false negative reactions with undiluted serum. The test may be repeated using 10 µL of sample. In case of positivity, use the titration procedure above.
4. The strength of the agglutination reaction is not indicative of the CRP concentration in the samples tested.

QUALITY CONTROL

Positive and negative controls should be run daily following the steps outlined in the Qualitative Test, in order to check the optimal reactivity of the reagent. The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit.

EXPECTED VALUES

While the C-reactive protein concentration is generally below 5 mg/L in the sera of healthy adults, in a number of disease states these values often exceeded within 4 to 8 hours after an acute event and reach levels up to 500 mg/L. Since an elevated CRP level is always associated with pathological changes, determination of CRP is of great value in diagnosis, treatment and monitoring of inflammatory conditions.

CLINICAL SIGNIFICANCE

C-reactive protein is an acute phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial and virus infections, inflammation, and malignant neoplasia. CRP contributes to non-specific defense by complement activation and accelerating phagocytosis. CRP testing has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings.

LIMITATIONS OF PROCEDURE

The presence of rheumatoid factors (RF) in a serum sample may cause false positive reactions. Weak or negative reactions may occur with marked antigenexcess (prozone effect).

ANNEX 2 Information Sheet (English version)

Title of the Research Project: Immuno-virologic response and its association with inflammation, Clinical and other Factors at 12 months of HAART initiation among patients attending the Antiretroviral therapy clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia

Name of Investigator: Mekdes Solomon (BSc, Msc candidate)

Name of the Organization: Addis Ababa University, College of Health Science, Department of Clinical Laboratory Science.

Introduction

You are invited to participate in a study to be conducted by MSC student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. It investigate immuno-virologic response and its association with inflammation as measured by C-reactive protein after 12 months of HAART initiation using CD4 and Viral load count of patients attending Zewditu Memorial Hospital ART clinic. After the result of the study is disseminated, strategies will be designed to prevent and control the predisposing factors. Moreover, it will also be a useful reference for drug choice. Please read the following statements and ask any unclear points before you agree to participate.

Participation in the study is exclusively voluntary. If you are not willing to participate in the study or if you want to withdraw even after deciding to participate, there will be no consequences and you will get all the services provided in the hospital with no problem. . If you decide to participate, you have to sign the consent form and you can get a copy of this information sheet.

Participation in this study is exclusively voluntarily. If you are not interested to participate to you or if you once decide to participate and want to draw from participation at any time, there will be no consequences and you will get all the services provided in the hospital with no problems. If you decide to participate, you have to sign the consent form and may obtain a copy of this information sheet.

What is expected from you as a participant of the study?

As a participant of this study you are expected to give 3-4 ml blood. In addition you are expected to give answers for some questions about yours health and socio demographic

conditions. You need to know that the results might be discussed with appropriate individuals out of this hospital. But your name, address and phone number will not be disclosed to anyone and to be more precise, identification code will be used in such conditions.

How long participation will take you?

You will spend 20-35 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

What are the risks of participating in this study?

There are no anticipated risks to your participation except minor discomfort during venipuncture because well experienced professionals will collect blood samples.

How the information is to be kept confidential?

All information that you give and the results from your specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What are the benefits from participation?

Since this study is MSc student research, there will not be payment for participants. But your participation is important for studying the prevalence and associated factors of anemia which will be useful in the improvement of management of HIV positive patients.

What are your rights as a participant of this study?

You can ask any question questions for further explanation. The principal investigator and the data collectors are responsible to clear any doubt you may have during participation. You have the right to get the results of the analysis.

What can I do if I have a problem or a question?

Please forward any question or problems you may encounter during this study to

Mekdes Solomon
Department of medical laboratory science
School of Allied health sciences

College of health sciences

Addis Ababa University

Mob: +251-9111- 881521

Email:mekdess2013@gmail.com

Agree to participate?

- ☐ Yes
- ☐ No

ANNEX-3 Information sheet (Amharic version)

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መግ ቢያ

የ ጥና ቱር ዕ ስ ፡ - ኢሚዩኖ-ቫይሮሎጊክ ምላሽ ከ ሲ-ሪአክቲቭ ፕሮቲን ጋር ያለው ግንኙነት በ 12 ወር የፀረ ኤች-አይ-ቪ መድሀኒት እየወሰዱ በነበሩ የዘውዲቱ ኤ-ር-ቲ ክሊኒክ ታካሚዎች የሚደረግ ጥናት.

እ ር ስ ዎ በ ዚህ ጥና ትላ ይየ ሚኖሮ ትተሳ ትፎ መሉበ መሉ በ ን ፈ ቃደ ኝነ ትላ ይየ ተመሰ ረተ ነ ዉ፡ ፡ በ ዚህ ጥና ትዉስ ጥላ ለ መሳ ተፍ ወይ ምለ መሳ ተፍ ከ ወሰ ኑ በ ኋላ ለ ማቋ ረ ጥየ ሚወስ ኑ ቢሆን ምእ ን ኩዋ በ ዚህ ሆስ ፒታልየ ሚሰ ጠዉማን ኛ ወምአ ገ ልግ ሎት አ ይቋ ረ ጥም፡ ፡ በ ጥና ቱለ መሳ ተፍየ ሚስ ማመክ ሆነ የ ስ ምምነ ትቅጹላ ይበ ጹ ሁፍ ወይምበ ጣት ፊ ር ማማስ ቀ መጥ ይጠበ ቅ ዎ ታል፡ ፡ ከ ፈለ ጉ ይህ ን ን መረ ጃ አ ን ድቅ ጅለ ራስ ዎሊያ ስ ቀ ሩ ይች ላ ሉ፡ ፡

የ ጥና ቱተሳ ታፊ በ መሆኖየ ሚጠበ ቅ ቦ ትምን ድን ነ ው?

በ ዚህ ጥና ትለ መሳ ተፍየ ሚስ ማመክ ሆነ የ ደ ምና መና ለ መስ ጠት መስ ማማት ይጠበ ቅ ብዎ ታል፡ ፡ ይሁን እ ን ጅይ ህ አ ይነ ቱ መረ ጃየ ር ስ ዎን ማን ነ ትየ ሚገ ልጡ መረ ጃ ዎች ን ማለ ትም ስ ም፤ አ ድራሻ ና የ ስ ልክ ቁ ጥ ር የ መሳ ሰ ሉ ት ን መረ ጃ ዎች ን አ ይ ጨምር ም፡ ፡ ይልቁን ምለ ዚህ አ ገ ልግ ሎት ብቻየ ሚወሰ ና ለ ማወቅ የ ሚያ ስ ችል መለ ያ ቁ ጥ ር ጥቅ ምላ ይእ ን ዲወሰ ይደ ረ ጋ ል፡ ፡ በ ተጨማሪ ምስ ለ ር ስ ዎአ ጠቃላ ይየ ጠና ሁኔ ታለ ሚቀ ር ቡአ ን ዳ ን ድተጨ ማሪ ጥያ ቄ ዎች መልስ መስ ጠት ይጠበ ቅ ብዎ ታል፡ ፡

በ ዚህ ጥና ት መሳ ተፍ ምን ያ ህ ል ጊ ዜ ይፈ ጃ ል?

የ ተዘ ጋ ጀ ዉን መጠይቅ ለ መመላ ት፤ የ ስ ምምነ ትቅጹላ ይለ መፈ ረ ምና ና መና ለ መስ ጠት ከ 20-25 ደ ቂ ቃያ ስ ፈ ል ጋ ል፡ ፡

በዚህ ጥናት መሳተፍየ ሚያስከትላቸዉቸግሮቸምን ድን ናቸዉ?

ናመና በሚሰበሰብበትወቅትምን ምአይነ ትየ ከፋችግርአያጋጥምዎትምምክን ያቱምና መና ወየ ሚወሰደው Gምድባላቸወየ ጠናባለመያዎችበመሆኑነ ው። ፡

የእኔየህክምና መረጃበሚስጥርተጠብቆመቆየ ትየ ሚቸለዉእንዴትነ ዉ?

የሰጠትማን ኛወምመረጃናከተወሰደወና መና ላይየ ተገኘወየ ላቦራቶሪ ውጤትየ ሚወሰ ወሰ ጥናቱአላማብቻ ነ ው። ፡ ይህንን ማህደርሊያገኙየ ሚቸሉትየ ተወሰኑየ ጥናቱተባባሪሰራተኞችብቻና ቸው። ፡ ከዚህ ምበላ ይስለእርሶያለ ውን ማን ኛወምመረጃየ ተለየ የ ይለ ፍቃልባላ ወየ ኮ ምፒውተርየ መረጃማህደር ውስ ጥእን ዲ ቀመጥይደረገ ል፡ ፡

በዚህ ጥናት መሳተፍየ ሚያስገኛቸውጥቅሞችምን ድን ናቸው?

ይህ ጥናት የ ማስተርስ ዲግሪ መመረቂያ ፅሁፍእንደመሆኑ መጠን ለተሳታፊዎችገንዘብአይሰጥም፡ ፡ ሆኖም ከጥናቱየ ሚገኘውመረጃየ ኤችአይቪህመማን ንህክምና ለማሻሻልአስተዋፅዎያደርጋል፡ ፡ በተጨማሪ ምት ኩረትያልተሰጣቸውን ኢንፌክሽየ ስበሽታዎችን ለ ማህበረሰቡማስገንዘብ፡ ፡

የዚህ ጥናት ተሳታፊመብቱምን ድን ነ ው?

ከዚህ ምበ ተጨማሪ ጥናቱበ ተመለ ከተማን ኛወን ምአይነ ትጥያቄየ መጠየ ቅናገለጻየ ማግኘትመብትአለዎት ፡ ፡ የላብራቶሪ ምር መራውጤቱን ምበነ ጻ ማግኘትይችላሉ፡ ፡

ጥያቄካለኝወይምችግር ቢያጋጥመኝ ምን ማድረግይገ ባል?

ይህንን ጥና ትበ ተመለ ከተወይምከ ዚህ ጥና ትጋር በ ተዛ መደ መልኩስለ ሚያጋጥመድን ገ ተኛአደጋዎችወይም ጥያቄካለዎትበ ሚመለ ከተውአ ድራሻይጠቀሙ፡ ፡

መቅደስ ሰለሞን

የ ህክምና ላባራቶሪ ሳይንስት/ክፍል

የ አላይድጤና ሳይንስት/ቤት

የ ጠና ሳይንስ ኮሌጅ

አዲስአበባዩኒ ቨርሲቲ

ሞባ ይል +251-9111- 881521

ኢሜይል mekdess2013@gmail.com

ለ መሳ ተ ፍ ይ ስ ማ ማ ሉ ?

እ ስ ማ ማ ለ ሁ □ አ ል ስ ማ ማ ም □

ANNEX-4 Consent Form (English version)

Code number----------

Name of the participant----------

I have been informed about the study which is aimed at Immuno-virologic response and its association with inflammation, Clinical and other Factors at 12 months of HAART initiation among patients attending the Antiretroviral therapy clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia. For this study blood sample is required from a participant. The aims of the study and possible risks were explained to me as well.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make withdrawal from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my blood sample for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my sample measured for free via the counselor nurse.

Participant's signature /finger print ----------

Name of Data collectors ---------- signature---------- Date----------

Please direct any questions or problems you may encounter during this study to:

Mekdes Solomon

Department of medical laboratory science

School of Allied health science

College of health sciences

Addis Ababa University

Mob: +251-9111- 881521

Email: mekdess2013@gmail.com

For additional information, please contact Addis Ababa University, College of Health Science institutional review board (IRB) office at:

Tell. +251-11-8-96-13-96

Fax +251-11-5-51-1-51-30-99

P.O. Box 9086, Addis Ababa, Ethiopia

Email: aau.mf.irb@yahoo.com

ANNEX-5 Consent Form (Amharic version)

<u>የ ተሳ ታፊ ዎችስ ምምነ ትማረ ጋገ ጫቅ ጽ</u>

የ ሚስ ጥር ቁጥር ----------

የ ተሳ ታፊ ወስ ም ----------

እኔ ስ ሜክ ላ ይየ ተጠቀ ሰ ውተሳ ታፊ በ ዓ ዋቂነ ትየ ዕ ድሜክ ልልበ ሚገ ኙየ ኤድስ ቫ ይረ ስ ተጠቂ ግለ ሰ ቦች ላ ይ ለ ሚሰ ራወኢ ሚዩኖ-ቫይሮሎጊክ ምላሽ ክ ሲ-ሪአክቲቭ ፕሮቲን ጋር ያለው ግንኙነት በ 12 ወር የፀረ ኤች-አይ-ቪ መድሀኒት እየወሰዱ በነበሩ የዘውዲቱ ኤ-ር-ቲ ክሊኒክ ታካሚዎች የሚደረግ ጥናት ላይ በቂ ገለፃ ተደርጎልኛል

ለ ጥና ቱምክ እኔ የ ተወሰ ደየ ደ ምና መና እን ደ ሚያ ስ ፈ ልግ ተገ ልጾ ልኛ ል ፡ ፡ የ ጥና ቱን ምአ ላ ማዎች በ ሚገ ባ ተረ ድቻ ለ ሁ ፡ ፡

በ መጠይ ቁላ ይየ ገ ለ ጽኳ ቸውመረ ጃ ዎች በ መሉ በ ሚስ ጥር የ ተጠበ ቁእን ደ ሚሆኑ ተነ ግሮ ኛ ል ፡ ፡ በ ጥና ቱላ ይ ያ ለ መሳ ተፍ ና ማን ኛ ውን ም መረ ጃ ያ ለ መስ ጠት እን ዲሁ ም በ ማን ኛ ውም ጊዜ ከ ጥና ቱራ ሴን የ ማግ ለ ል መብ ቴየ ተ ጠበ ቀእን ደ ሆነ ተገ ልጾ ልኛ ል ፡ ፡

ስ ለ ዚህ ለ ዚህ ጥና ት መረ ጃ ና የ ስ ምምነ ት ቃሌን የ ሰ ጠሁ ት በ አ ጠቃላ ይሁኔ ታውን በ መረ ዳ ት ና በ ፍጹ ምፍ ቃደ ኛነ ት ነ ው ፡ ፡ የ ምስ ጠወምና መና ለ ምር ምር ብቻእን ደ ሚወጪ ምተረ ድቻ ለ ው ፡ ፡ በ ተጨማሪ ምጥ ያ ቄ ለ መጠየ ቅ ተፈ ቅዶ ልኝ ለ ማወቅ የ ፈ ለ ኩት ን ያ ህ ል ማብራሪ ያ አ ግኝ ቻ ለ ሁ ፡ ፡ የ ዚህ ጥና ት ተሳ ታፊ በ መሆኔ የ ማገ ኘ ውጥ ቅ ምየ ሁሉን ምምር መራ ወጡት በ ነ ጻ ማግ ኘ ትእን ደ ሆነ ተረ ድቻ ለ ሁ ፡ ፡

የ ተሳ ታፊ ወፈ ር ማ /የ ጣት አ ሻ ራ ----------

የ ምስ ክ ር መሉ ስ ምፊ ር ማ

1. ---------- ----------
2. ---------- ----------
3. ---------- ----------

(የ ስ ምምነ ትቅጹን ማን በ ብለ ማይችሉተሳ ታፊዎች)

የ መረ ጃስ ብሳ ቢውስ ም ---------- ፊር ማ ---------- ቀን ----------

--

<u>ጥና ቱን የ ሚያ ካ ሂ ደ ዉስ ዉማረ ጋ ገ ጫ</u>

ይህ ን ጥና ትበ ተመለ ከ ተወይ ምከ ዚህ ጥና ትጋ ር በ ተዛ መደ መል ኩስ ለ ሚያ ጋ ጥመድን ገ ተኛ አ ደ ጋ ዎች ወይ ምጥ ያ ቄ ካ ሎ ዎ ትበ ሚከ ተለ ወአ ድራ ሻ ይጠቁ መን ፡ ፡

መቅደስ ሰለሞን

የ ሕክ ምና ላ ቦ ራ ቶ ሪ ሳ ይ ን ስ ት/ክ ፍል

የ አ ላ ይድጠና ሳ ይ ን ስ ት/ቤት

የ ጠና ሳ ይ ን ስ ኮ ሌጅ

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ስ .ቁ +251-11-8-96-13-96

ፋ ክ ስ +251-11-5-51-1-51-30-99

ኢ ሜይ ል : aau.mf.irb@yahoo.com

ANNEX-6 Data extraction sheet

ID	Age	Sex	Marital Status	Wgt	Hgt	Educa Status	OI Exp	OI Drug	Baseline Result		HAART Regm'n	HAART Duration	WHO Stage	Current Result		
									CD4	VL				CD4	VL	CRP

Collected by _____ **Signature** _____

Date _____

Declaration

I, the undersigned, declare that this M.Sc thesis my original work has not been presented for a degree in this or another university that all source of materials used for the thesis have been duly acknowledged.

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Place: Addis Ababa, Ethiopia.

This thesis has been submitted with our approval as advisors.

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