Original article

Immunological recovery time of adult AIDS patients on ART: A case study at Felege-Hiwot Referral Hospital, Bahir-Dar, Ethiopia

Bezalem Eshetu Yirdaw[1], Eshetu Wencheko[1]

Abstract

Background: Antiretroviral Treatment (ART) has improved the immunological recovery of HIV patients. However, the extent of immunological recovery over time is not easy to predict because it depends on a number of factors.

Objectives: The purpose of this study was to estimate time to immunological recovery of AIDS patients under ART.

Methods: A sample of 387 patients was taken from patients' records at Bahir-Dar Felege-Hiwot Referral Hospital from June 2006 to August 2013. The Kaplan-Meier method and Cox proportional hazard model were applied to describe and analyze the data.

Results: Associated with a relatively short immunological recovery time were the categories: high CD4 count, weight 45kgs and higher, WHO stages I, II and III, and being non-anemic. The respective adjusted hazard ratios are: 1.11 for a 50 cells/µl increase in the baseline CD4 count, 1.13 for a 5kgs increase of baseline weight, 1.88, 1.67, 1.64, respectively, for WHO stages I, II, III, and 1.34 for not-anemic patients. Associated with a *delayed* immunological recovery, on the other hand, were male gender, advanced age, total WBC count <1200, and regimen change. The adjusted hazard ratios are: 0.73 for males, 0.92 for a 5-years increase in age, 0.77 for patients with total WBC count <1200 cells/mm^3, 0.69 among patients who changed regimen.

Conclusion: The major predictors of time to immunological recovery of HIV/AIDS patients were gender, age, anemia status, baseline CD4 count, baseline WBC count, baseline weight, baseline WHO stage and regimen change. Immunological recovery experience was found to be statistically not significant among groups classified by, marital status, knowledge of ART, residence, voluntary counseling and testing, educational level, regimen type, condom use, adherence to treatment, partner's HIV status, risk factors and functional status. [*Ethiop. J. Health Dev.* 2014;28(2):126-135]

Introduction

On a global scale, the HIV epidemic has stabilized, although with unacceptably high levels of new HIV infections and AIDS deaths. According to the United Nations on HIV/AIDS (UNAIDS) report from 2013, an estimated 34 million people worldwide were living with HIV in 2011 among which 23,500,000 people were living in sub-Saharan Africa. The adult HIV infection was about 30.7 million and the remaining 3.3 million patients were under the age of 15 years. Specifically, an estimated 2.5 million became newly infected with HIV and overall 1.7 million people died due to AIDS in 2011. In the same year, an estimated 1.8 million people were newly infected with HIV in sub-Saharan Africa. The epidemic in sub-Saharan Africa is highly diverse and especially more severe in southern Africa (1).

Antiretroviral therapy has been proved to improve the lives of HIV infected persons by reducing HIV/AIDS-related mortality and morbidity (2, 3). There was an estimated 25% reduction in new infections in sub-Saharan Africa in 2011 (a total of 1.8 million new infections was observed) compared to 2001 (2.4 million new infections) (2), and 27% fewer infections in 2010 compared to 1996 after introduction of ART (3). In 2012 some 9.7 million of the 34 million people living with HIV (PLWH) were estimated to be on ART in low- and medium-income countries, which is a six-fold increase from 2005 when only 1.3 million people were receiving ART. Between 2003 and 2010 there was an increase in ART coverage from 20% to 47% for low- and middle-income countries (2). By 2010 WHO has planned to put 9.8 million people on ART with the goal of providing universal access to HIV care and ART (1).

HIV infection has changed from a fatal condition to a manageable chronic illness mainly due to the development of ART. The goals of this therapy are: to improve survival; to reduce HIV associated morbidity and mortality; to increase the quality of life; to restore immune function and to achieve maximal and sustained suppression of viral replication (4).

To reduce the mortality and morbidity rate caused by the HIV/AIDS epidemic, different initiatives were taken by international organizations and donors. One of the initiatives was the launch of WHO's '3 by 5' initiative in 2003. It was estimated that 3 million HIV patients in the world would have access to ART by 2005. The initiative enabled many sub-Saharan African countries to establish

[1]Department of Statistics, College of Natural Sciences, Addis Ababa University, Ethiopia.

national ART programs. By the end of 2005, an estimated 1.3 million people in low- and middle-income countries had access to treatment, which is about 20% of those estimated to be in need. The WHO target of providing access to ART for 3 million people by 2005 was not achieved. But in mid-2005, the WHO target had already been overtaken by an even more motivated aim. In July 2005, the G8 group of industrialized countries committed to the goal of achieving "as close as possible to universal access to treatment for all those who need it [ART] by 2010" (5). This program is called UNIVERSAL access 2010. Ethiopia is one of the countries which benefited from this program. To address the problem of provision of a fair access to ART implementation program, the government of Ethiopia launched the free ART program in January 2005. In 2011 a total of 249,174 adults (of which 86% were eligible) were on ART (6).

Although the provision of ART has been started at Felege-Hiwot Referral Hospital in 2005, little research has been undertaken to predict/estimate the time to immunological recovery of patients. Hence, this study has been conducted with the aim to provide empirical findings and information with respect to the subject under consideration.

Methods

The study was based on data obtained from Felege-Hiwot Referral Hospital, in Bahir-Dar City of the Amhara region, Ethiopia. The city is located approximately 578 kilometers northwest of Addis Ababa at latitude and longitude of 11° 36'N, 37° 23'E with an elevation of 1,840 meters above sea level. The ART clinic of the hospital was established in 2003. The clinic started to provide free ART service to patients in 2005. At the time when the current study was undertaken at the beginning of 2014, the clinic had three physicians, three nurses and four data clerks.

An important piece of information worth mentioning is that the ARV first line drugs approved by the Ministry of Health of Ethiopia were NRTI and NNRTIs (d4T, 3TC, AZT, EFV and NVP). These drugs are combined to form the following four first line regimens taken by more than 95% of adult HIV/AIDS patients in the Felege-Hiwot Referral Hospital: (d4T/3TC/NVP (1a), d4T/3TC/EFV(1b), AZT/3TC/NVP(1c) and AZT/3TC/EFV(1d).

The target population of patients under follow-up from June 2006 to August 2013 were adults; that is, 15 years and older HIV patients. The study reviewed patient intake forms and follow-up cards prepared by the Ministry of Health of Ethiopia to be uniformly used by clinicians to identify and document clinical and laboratory measurements. An important justification for carrying out this research is that the region has experienced, and still experiences, the highest prevalence of HIV in Ethiopia. The secondary data used in this study were obtained from intake forms and patient follow-up cohort on the basis of a questionnaire designed to extract those variables considered in this study. Adult patients under ART were included irrespective of their treatment category during the study period. Patients on ART whose diagnosis duration was missing, who did not have at least two follow-up CD4 measures, and whose date of death was missing were excluded.

There were a total of 11,040 patients in the sampling frame, which is the list of all patients who received ART from the hospital from June 2006 to August 2013. Each patient had a chart/record with a distinctive identification number called ART unique identification number. A systematic random sampling method has been adopted as an appropriate technique to select a representative sample of patients based on their ART identification number as in a previous similar study at Adama Referral Hospital, Oromiya Regional State, Ethiopia (7). Accordingly, in the current study a sample size of 387 was obtained (using the proportion of death, a statistical level of significance $\alpha = 0.05$, and an absolute precision factor of $d = 0.03$).

Approval to access patient data was obtained from the Medical Director of the Hospital upon a written request made by the Ethical Clearance Committee of the College of Natural Sciences, Addis Ababa University. The authors have kept the personal data of the patients confidential. Integrity and medical ethical standards were adhered to according to the laws of the country.

In this study we used STATA (for description and model fitting) and SPSS (for model diagnostics).

The response variable in this study is time to recovery in months (with status of CD4 cells count where time is the duration measured to attain an increase of 100 cells over baseline =1, censored=0). The predictor variables considered in the study (all at the initiation of ART by patients) were: age, place of residence (urban, rural), marital status (never married, married, divorced/widowed), level of education (no education, primary, secondary and higher), voluntary counseling and testing (yes, no), partner's HIV status (positive, negative, unknown), knowledge of ART (yes, no), CD4 count, weight (in kg), total WBC count (<1200, ≥1200), WHO clinical stage (stage I, stage II, stage III, stage IV), functional status (bed-ridden, ambulatory, working), risk factor (alcohol intake, soft and hard drug use, tobacco use) (yes, no), condom use (yes, no), TB status (positive, negative), anemia status (normal, severe/mild/moderate), ARV regimen (D4T-3TC-NVP, D4T-3TC-EFV, AZT-3TC-NVP, AZT-3TC-EFV, TDF-3TC-EFV,TDF-3TC-NVP), regimen change (yes, no), and adherence to treatment (yes, no), and gender.

Results

Description and Descriptive Statistics:

There were 387 patients in the cohort study; out of which, 54 (14%) died, and the remaining 333 (86%) were censored. There were 236 females and 151 males. Out of the dead, 25 (46.3%) were females and 29 (53.7%) males. Out of the 54 deceased 21 (38.9%) were bed-ridden, 14 (25.9%) were ambulatory, and 19 (35.2%) were working. There were 89 (23.3%) TB-positive and 293 (76.7%) TB-negative patients; out of those who died 24 (44.4%) were TB-negative and 30 (55.6%) were TB-positive. Among the dead, 38 (70.4%) were anemic and 16 (29.6%) were non-anemic. The average CD4 count at the start of treatment for the dead patients was 128.29, with a maximum of 322; censored average CD4 count was 164.38, with a maximum of 497. There were 65 (16.8%), 95 (24.5%), 176 (45.5%) and 51 (13.2%) patients, respectively, in WHO stages I, II, III and IV. Among the dead 5 (9.3%), 11 (20.4%), 18 (33.3%), and 20 (37%) were in WHO stages I, II, III and IV, respectively. About 55% of the deceased patients used condom; 37 (84.1%) were exposed to risk factors (smoking tobacco, drinking alcohol and using hard/soft drugs). There were 233 (60.2%) patients whose baseline WBC count was ≥1200 cells/mm^3 and 154 (39.8%) patients with WBC count < 1200 cells/mm^3. Among the dead; 29 (53.7%) had WBC < 1200 cells/mm^3 and 25 (46.3%) had WBC count ≥1200 cells/mm^3; 20 (37%) had weight < 45kgs and 34 (63%) had weight 45kgs and higher.

The following comparisons have been made based on Kaplan-Meier survival estimates and results in Tables 1 and 2. The Kaplan-Meier survivor estimates for the two genders show that females had a relatively shorter median recovery time than males. The median recovery time was shorter for working patients than for ambulatory and bed-ridden patients. Baseline CD4 count >200cell/µl, TB-negative, total WBC count ≥1200 cell/mm^3 and baseline weight ≥45kgs were associated with short immunological recovery time. Low baseline CD4 count ≤200cell/µl, TB-positive status, WHO stage IV status, and being anemic contributed to delay of recovery. Patients who did not change regimen had shorter recovery time than those who changed regimen. Proper adherence to ART contributed to faster recovery than fair and poor adherence. The estimated Kaplan-Meier curves for residence, condom use, educational level, voluntary counseling and testing (VCT), age, partner's HIV status, marital status, risk factors, knowledge of ART and regimen type did not provide conclusive comparisons because the curves for the different categories of the respective variables cross each other; the conclusion, in these cases, is that recovery time is not associated with the groups formed for these variables at 5% level of significance.

Table 1: **Summary of immunological recovery time by categories for socio-demographic and risk behavior variables at Felege-Hiwot Referral Hospital during 2006-2013.**

Variables	Median immunological recovery time (in months)	95% CI for median time	Log-rank p-value
Age			0.139
15-39	9	7.98-10.02	
>=40	12	8.47-15.52	
Gender			0.002*
Female	9	7.84-10.13	
Male	12	8.38-15.61	
Marital status			0.811
Never married	10	6.16-13.84	
Married	10	7.92-12.087	
Divorced/ widowed	10	7.83-12.17	
Educational level			0.168
No education	10	7.42-12.58	
Primary	9	7.29-10.70	
Secondary and higher	11	8.35-13.65	
Functional Status			0.002*
Bed-ridden	20	8.14-31.86	
Ambulatory	10	7.57-12.43	
Working	9	7.85-10.15	
Residence			0.941
Rural	10	8.43-11.56	
Urban	9	6.73-11.26	
Partners HIV status			0.212
Negative	14	11.28-16.72	
Positive	11	5.93-16.07	
Unknown	10	8.78-11.21	
VCT			0.052
No	9	6.95-11.05	
Yes	10	8.40-11.59	
Risk Factors			0.934
No	10	8.15-11.85	
Yes	10	8.00-11.99	

***** = The association is significant at α=0.05.

Table 2: **Summary of immunological recovery time by categories for health variables at Felege-Hiwot Referral Hospital during 2006-2013.**

Variables	Median immunological recovery time (in months)	95% CI for median time	Log-rank p-value
TB Status			0.001*
Negative	10	8.77-11.22	
Positive	12	7.21-16.78	
Baseline CD4 count			0.041*
<200	11	9.71-12.28	
>= 200	6	5.34-6.66	
Baseline weight			0.002*
<45	15	12.04-17.96	
>= 45	9	7.98-10.02	
WHO clinical stage			0.006*
Stage I	10	7.76-12.23	
Stage II	9	7.26-10.74	
Stage III	10	8.29-11.71	
Stage IV	16	10.27-21.73	
Baseline WBC count			0.001*
<1200	13	10.55-15.44	
>=1200	9	7.91-10.09	
Knowledge of ART			0.498
No	15	7.04-22.96	
Yes	10	8.73-11.27	
Anemia status			0.000*
Normal	9	7.92-10.08	
Severe/mild/moderate	15	9.66-20.34	
Regimen Type			0.222
D4T-3TC-NVP	10	7.68-12.32	
D4T-3TC-EFV	8	5.97-10.02	
AZT-3TC-NVP	9	6.26-11.74	
AZT-3TC-EFV	11	8.56-13.44	
TDF-3TC-NVP	10	5.06-14.94	
TDF-3TC-EFV	11	3.81-18.19	
Others	15	0.00-30.59	
Condom use			0.445
Never	10	8.31-11.68	
Sometimes/always	9	5.86-12.14	
Regimen change			0.003*
No	9	7.79-10.20	
Yes	11	8.74-13.26	
Adherence			0.003*
Proper	10	8.69-11.30	
Fair	15	8.17-21.82	
Poor	24	10.34-33.78	

*** =** The association is significant at α=0.05.

Results based on Cox proportional Hazard Model:

***Single covariate analysis*:** Time to immunological recovery is significantly related with gender, functional status, TB status, anemia status, baseline CD4 count, WHO clinical stage, baseline weight, baseline WBC count, adherence to ART and regimen change. The covariates age, residence, educational level, condom use, regimen types, marital status, VCT, partner's HIV status, risk factor and knowledge of ART are not statistically significant at 5% level of significance.

***Multiple covariate analysis*:** The empirical results in this subsection are displayed in Table 3; adjusted hazard ratios (aHR) are used to interpret the results. We would like to point out that there were no indications of confounding between any of explanatory variables in the model and the outcome. Also, there were no interactions. After adjusting for other covariates, it was found that the chance to attain immunological recovery for male patients was about 26.7% lower than for female patients (aHR=0.73, CI= 0.570-0.940). The adjusted hazard ratio for a 50 cells/µl increase in baseline CD4 count is exp(0.002*50) (CI 1.002-1.22). That is, for a 50 CD4 cell/µl increase of baseline CD4 count, the immunological recovery rate increased by 11%. For a 5kgs increase of baseline weight, immunological recovery increased by 13% (aHR=1.13, CI=1.058-1.213). The rate of attaining immunological recovery decreased by 8% for a 5-year increase in age over the immediate next younger category (aHR=0.92, CI=0.862-0.988). For non-anemic patients the rate of recovery was 34.4% higher than for patients with severe/moderate anemia status (aHR=1.34, CI=1.020-

1.780). Immunological recovery for patients with total WBC count <1200 cells/mm^3 was about 23% lower than for patients with total WBC count of ≥ 1200 cells/mm^3 (aHR=0.77, CI=0.600-0.980). Patients in WHO stages I, II and III, respectively, had 88% (aHR=1.88, CI=1.170-3.030), 67% (aHR= 1.67, CI=1.070-2.580), and 64% higher rates of recovery relative to patients who were in WHO stage IV. Immunological recovery for patients who changed regimen was 31% lower than for those who adhered to regimen prescription (aHR=0.69, CI=0.540-0.870).

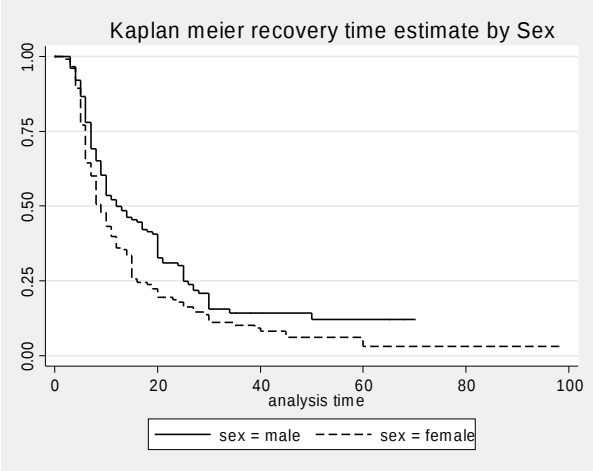
Table 3: **Multiple covariate analysis of socio-demographic and health variables about time to immunological recovery of adult HIV patients on ART at Felege-Hiwot Referral Hospital during 2006-2013.**

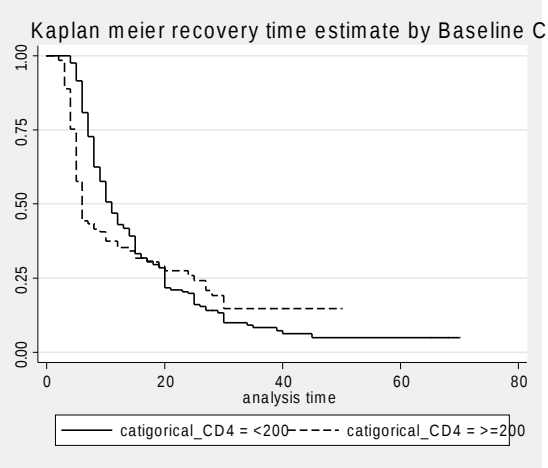
Variable	Df	$\hat{\beta}_i$	SE	Wald	Sig.	aHR	95% CI for HR
Gender							
Male	1	-0.311	0.128	5.886	0.015	0.733	0.57-0.94
Female (*reference*)						1	
Age	1	-0.016	0.007	4.461	0.035	0.984	0.97-0.99
Baseline CD4 count	1	0.002	0.001	14.123	0.000	1.020	1.01-1.04
Baseline weight	1	0.025	0.007	14.272	0.000	1.026	1.01-1.04
Baseline WHO stage							
Stage I	1	0.633	0.242	6.825	0.009	1.884	1.17-3.03
Stage II	1	0.510	0.224	5.175	0.023	1.665	1.07-2.58
Stage III	1	0.496	0.208	5.663	0.017	1.642	1.09-2.47
Stage IV (*reference*)						1	
Baseline WBC							
<1200	1	-0.264	0.126	4.436	0.035	0.768	0.60-0.98
≥1200 (*reference*)						1	
Anemia status							
Normal	1	0.295	0.143	4.256	0.039	1.344	1.02-1.78
Severe/moderate (*reference*)						1	
Regimen change							
Yes	1	-0.373	0.121	9.562	0.002	0.688	0.54-0.87
No (*reference*)						1	

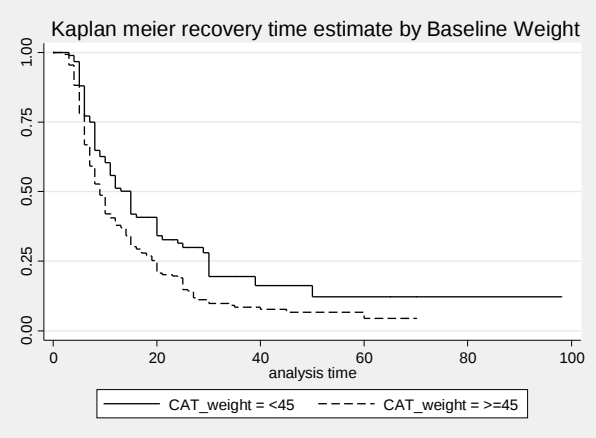
Df: Degrees of freedom, SE: Std. Error, CI: Conf. Interval.

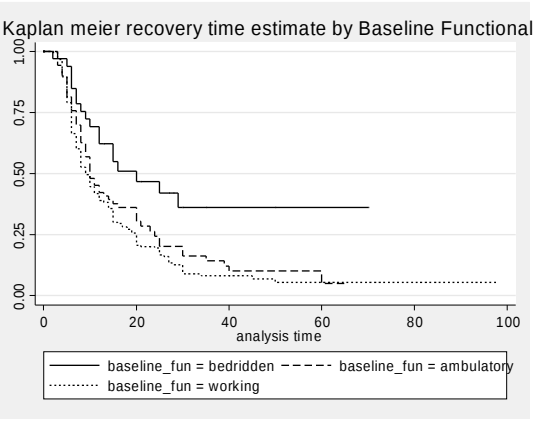
Residual plots can be used to check the validity of the linearity assumption as well as to check for the presence of influential and outlier observations. Plots of residuals (not given here to save space) depicted observations with spikes which are suspected to have undue influence on the parameter estimates. Removal of these and refitting the remaining observations to check their influence proved that that there were no significant changes in the model estimates, thereby leading to the conclusion that they are not as such influential outliers, and therefore, can be retained in the model. Again graphs/plots (not given here) show no definite pattern in the scatter plots, and the smoothed curve is almost a horizontal line through the origin – also an indication of linearity in the covariates. The likelihood ratio test (chi-square=69.79, p< .000) and score test (chi-square=68.549, p< .000) show that the model fit is good, i.e. significant at 5% level. Tests for confounding and first-degree interactions showed that there were no problems in those regards. After the above checks, we took the model with the significant covariates in Table 3 as appropriate. Hence the subsequent discussion in the following section will be based on this final model.

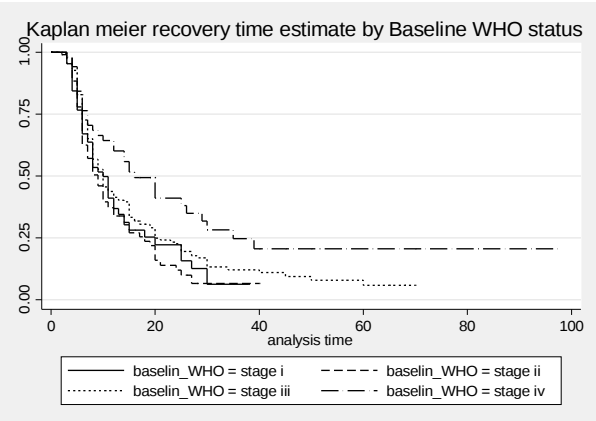
Kaplan-Meier survival curves

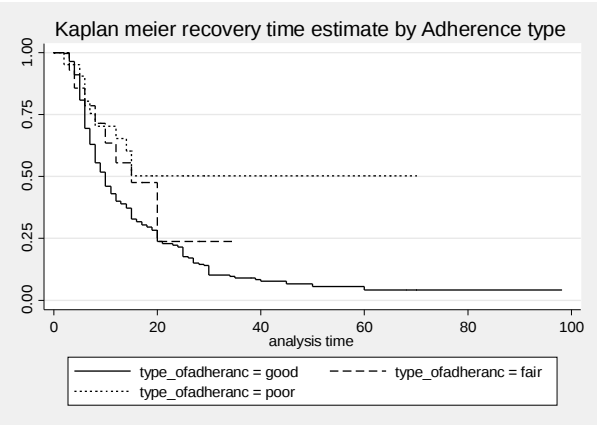


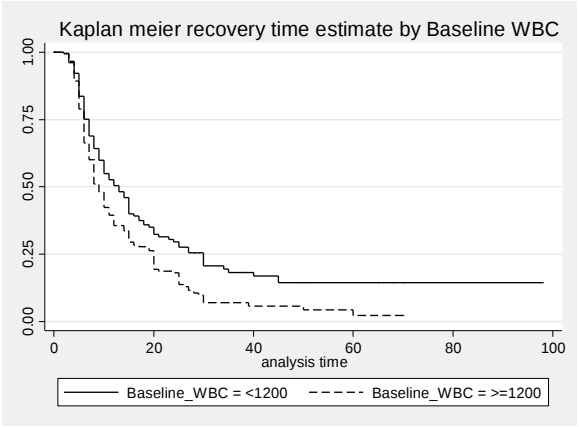


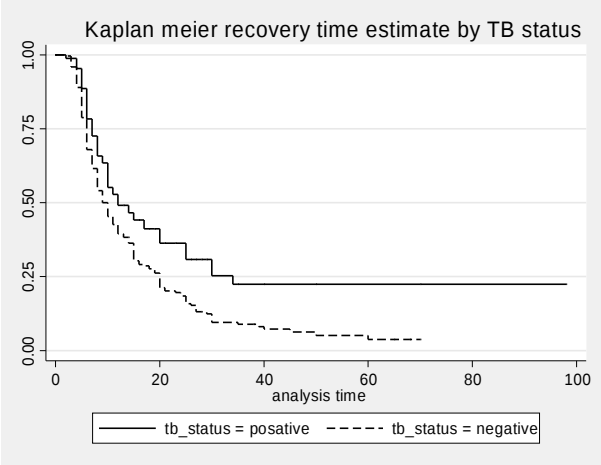


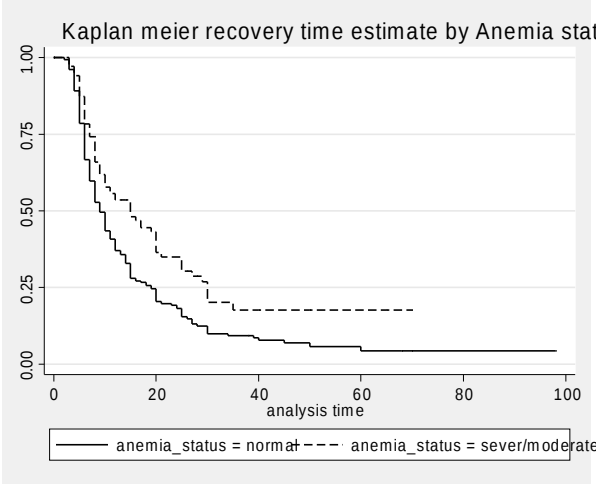


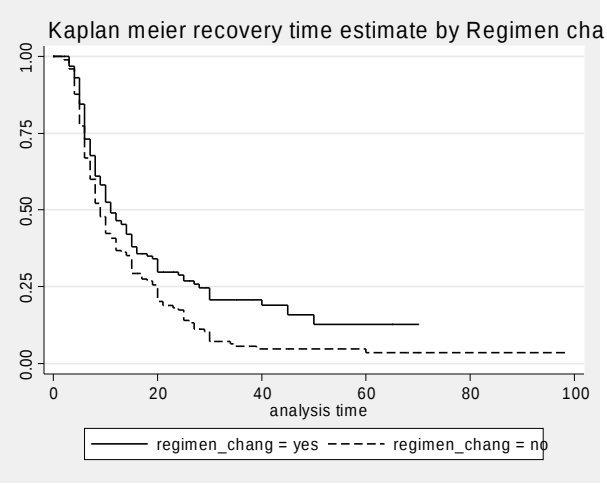


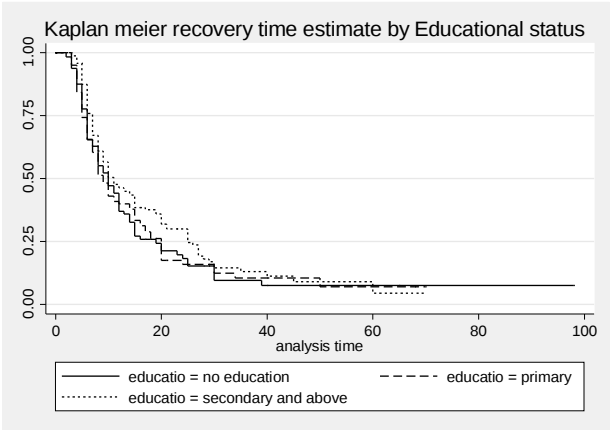












Discussion

With respect to CD4 count, a multi-country study in sub-Saharan Africa has shown that median CD4 cell count increased from a baseline of 97 cells/ml to 261 cells/ml at 48 weeks (8). Reports from an HIV/ART Swiss cohort study showed that over one-third of patients failed to attain an absolute CD4 count of 500 cells/µl during a follow-up period of 4 years (9, 10). It was reported that poor survival and long recovery were associated with patients who started ART at low CD4 count (11-14). It

was also found that immunological and virological recovery was low among patients who started ART at low baseline CD4 count; the greatest risk was among patients having CD4 count <50 cells/µl. It is recommended to start ART before CD4 cell count falls below 350 cell/µl (15). It has been shown that age, baseline CD4 count and initial viral load were found to be inversely associated with early CD4 response to suppressive ART (16). Brian *et al*. (17) and Castagna *et al*. (18) found that immunological recovery is largely dependent on baseline CD4 count, and thus the timing of ART initiation is important in order to maximize the CD4+ T-cell response to ART. A study from southern Ethiopia found that sustained treatment is significantly associated with improvements in CD4 count and weight (19). Another study in Zewditu Hospital, Addis Ababa, Ethiopia, showed that immunological recovery depends on baseline CD4 count (20). The current study found that, for a 50 CD4 *cells/*μl increase in the baseline CD4 count, the rate of attaining immunological recovery increased by 11%.

With regards to body weight, we found that for a 5-kilogram increase in baseline weight, the rate of attaining immunological recovery increased by 13%. This result agrees with an earlier finding reported in (21).

Our results show that for patients who were in WHO stages I, II and III, respectively, the rates of attaining immunological recovery were about 88%, 67%, and 64%, respectively, higher than for patients in WHO stage IV. This result agrees with the findings of Farias *et al*. (22).

Our findings showed that the rate of attaining immunological recovery for non-anemic patients was about 34% higher than that of patients with severe and mild levels of anemia. This result is in agreement with a study from England (23) and another from USA (24), showing that anemia is a very significant predictor of immunological recovery in AIDS patients.

The current study found that the rate of attaining immunological recovery for male patients is about 27% shorter than that of female patients, thereby concurring with the finding of Derbel *et al*. (20) and Puthanakit *et al*. (25). As relates to the significance of age, some studies from Europe and the USA reported a low immune response to ART in older patients (26-28). Although data related to the effect of age in Africa are very scarce, it has been found that poor ART response was observed among older patients (29). A study in West Africa showed that among HIV-infected adults immunological response after 12 months of ART was very poor in elderly patients (30). Our study showed that the rate of attaining immunological recovery decreased by 8% for a 5 year increase in age coming to the same conclusion as in (31).

A significant association was observed between total WBC count < 1200 cells/mm^3 and subsequent poor immunological recovery, disease progression or mortality. Variables that favored a good immunological recovery process after ART initiation were baseline CD4 count (> 200 cells/μl) and baseline total WBC count $\geq$1200 cells/mm^3 (17). It was also found that decline in WBC count and weight loss are associated with increased mortality and morbidity among patients on ART (32). We found that the rate of attaining immunological recovery for patients with WBC count < 1200 cells/mm^3 was about 23% lower than for patients with a total WBC count ≥1200 cells/mm^3.

This study found that the rate of attaining immunological recovery for patients who changed regimen was about 31% lower than those patients who did not change regimen. This result agrees with the finding of Balestre, *et al*. (30).

Conclusion:

The major predictors of time to immunological recovery of HIV/AIDS patients were gender, age, anemia status, baseline CD4 count, baseline WBC count, baseline weight, baseline WHO stage and regimen change. Recovery time for females was shorter than for males. Being TB- and anemia-positive, low baseline CD4 count, low baseline WBC count, low weight and advanced age deter immunological recovery.

Acknowledgement

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