Review article

The pattern of immunologic and virologic responses to Highly Active Antiretroviral Treatment (HAART): Does success bring further challenges?

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Abstract

Background: Since the advent of HAART, there is a significant reduction in opportunistic Infections (OIs), morbidity, mortality and HIV transmission. However, the low antiretroviral Therapy (ART) coverage in resource-limited countries (42%) and the presence of globally 500-800 thousand patients on first-line having to required switch to second-line drugs in 2010 are some concerns. Other challenges related to HAART include: lifelong therapy, failed treatment response, optimal time to start treatment and switching regimens, drug interaction, toxicity, cardiovascular risks, drug resistance, lost to follow-up, immune reconstitution inflammatory syndrome (IRIS), early mortality, and lack of restoration of solid immunity against HIV. To achieve the goals of ART, national ART programmes focus on the vital patient monitoring systems including clinical, immunologic, virologic, adherence, lost to follow-up and mortality.

Objectives: This review is aimed at addressing the profile of immunovirological responses to HAART and the factors associated with, with a special emphasis on the drawbacks of immunologic assessment to diagnose virologic failures.

Main findings: 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 continue 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 immunovirologic 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%), whereby the risk of morbidity and mortality is higher in the concordant non-responders and discordant responders.

Conclusions: ART benefits a substantial number of HIV patients even in resource-poor settings. Since clinico-immunological assessments have lower performance in diagnosing virologic failures, moving towards the availability of VL testing to confirm treatment failures, if not pre-HAART resistance testing, is a logical and timely approach for resource limited countries like Ethiopia where the long-term effect of the roll-out ART is not well investigated. However, the high cost and technical demand of VL testing, lack of experience of health professionals, weak infrastructure and health care system, the unavailability and high costs of second-line drugs could be the major challenges during expansion of VL testing. Moreover, longitudinal studies on long-term effects of HAART, and surveys focused on transmitted or acquired HIV drug resistance, and Early Warning Indicators are highly pertinent.
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Introduction

HIV/AIDS remains to be a global challenge since its discovery (1). At the end of 2008, 33.4 million people were living with HIV, 2.7 million newly infected, and 2 million deaths occurred due to HIV/AIDS worldwide (2). In Ethiopia, HIV prevalence of the adult population in 2007 is estimated to be 2.1% (Urban 7.7%, Rural 0.9 %), and the number of people living with HIV is 977,394, including 64,813 children (3).

Despite the absence of curative therapy for HIV/AIDS, highly active antiretroviral therapy (HAART) reduces OIs, HIV transmission, morbidity and mortality at global level (4, 5). Mean survival of those on HAART is estimated to be 13 years (6), although the high rates of deaths among lost to follow-ups should be considered for accurate estimate of survival (7). Moreover, since outcome of HAART at population level depends on the time of starting ART, uptake, adherence, pre-treatment and co-infections, survival following HAART might not be uniform in all HIV infected groups (8,9).

Since the launching of "3 by 5" global initiative (10), > 4.7 million people were on ART worldwide at the end of 2008, although only 42% of the 9.5 million people in need of ART had an access in the resource limited countries (5). In Ethiopia, where 3880 patients were getting free ART in three sites before the start of free ART in 2005, >167,000 (~53%) of the adults requiring ART were getting the service in 517 health facilities as of October 2009 (11). Of those on ART in Ethiopia, 99% were on first line regimens with a retention rate of 74% (11), while retention rate in other African countries was 75% and 67% at 12 and at 24 months, respectively (5).

With all the success stories of HAART, there are challenges which could compromise the goals of ART

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including failed/incomplete treatment responses (12), drug interaction and toxicity (13), drug resistance (14), lost to follow-up (5), and early mortality (15). Moreover, the presence of 500-800 thousand patients that require switching to second-line drugs in 2010 (5), and where this number could increase gradually with the expansion of ART services, indicates another challenge in terms of availability and increment of cost of the second-line therapy per patient (16).

In summary, whereas the positive impact of ART is remarkable, in view of the patient monitoring system in resource-poor countries which is exclusively dependent on clinico-immunological methods, which lacks sensitivity/specificity to detect virological failures, accurate diagnosis and management of treatment failures could be a major challenge in the era of rapid ART expansion (17). The focus of the current review is therefore to highlight the profile of immunologic and virologic responses to HAART and the risk factors associated with treatment failures, with special emphasis to the limitations of the immunologic based patient monitoring.

Methods

This review comprises published articles in Ethiopia, in developing and the developed world which are related to HIV/AIDS infection, ART and the treatment outcomes as measured by immunological and virologic responses with special emphasis on the limitations of the immunological based monitoring of patients on ART. The review process was done through a desk review and online search with more focus to publications of the past five years.

Results

Monitoring of treatment responses in patients on HAART

Although, the primary goal of HAART is to suppress plasma HIV-1 RNA level (viral load, VL) below the level of detection within three to six months of starting therapy and to maintain it for the rest of the patient's life (18), there are other important goals of HARRT including restoring and preserveing immunologic function, reduceing HIV-related morbidity and mortality, improving quality of life and reducing vertical transmission (17).

Since the discovery of Zidovudine (ZDV) in 1987 (19), more than 30 ARV drugs have been made available (20). Although it varies in different guidelines, according to WHO (21), first-line ART includes at least two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) analog; while second-line ART includes two NRTIs boosted with protease inhibitors, preferably Ritonavir. The preferred first line regimens in Ethiopia includes Tenofovir (TDF) + Emtricitabine (FTC) + Efavirenz (EFV); ZDV+ Lamivudine (3TC)+ EFV; or ZDV+3TC+ Nevirapine (NVP) (21).

Although, it varies in different settings, ART should be started when CD4+ count is 201-350, or <= 200/µl for developed and resource-poor settings, respectively (21). However, given the observed lower morbidity, mortality and fewer adverse events associated with the initiation of HAART at higher CD4 cell counts (23, 24), WHO recommends CD4+ count <= 350 cells/µl to initiate ART as a universal guideline (5).

Times of initiating, replacing and stopping therapy are the most critical questions during ART (21). Based on the basics that progression of HIV infection is affected by the synergetic effect of immunological and virological along with host factors (race, genetics, age, gender, mode of transmission, co-infections, nutrition, pregnancy, psychosocial factors) (25), CD4+ count and VL measurements are golden prognostic biomarkers of HIV/AIDS disease progression (26). In settings where measurements of CD4+ count and VL level are limited, total lymphocyte count, hemoglobin (Hgb) and body mass index (BMI) are recommended as simple markers for disease progression (25, 27).

Therefore, taking resource constraints into account, and that virological failures precede immunological failures, then comes clinical failure, WHO guidelines recommend clinical and immunological (CD4+ count) assessments as surrogates for VL to monitor patients on ART in resource limited settings, unlike to that in high income countries where VL is done three to four times a year (21). However, the sequential nature of treatment failure which is not strongly evidence based and may take years to happen is the major drawback of clinicoimmunological assessment based patient monitoring (17). Evidence from models showed an average of five years from the first evidence of virological failure until 50% of patients progress to WHO stage III (3). Likewise, CD4+ counts correlate with the level of VL at group level but not at individual level (28). Thus, immunologic markers have poor sensitivity (20%-33%), specificity (86%-90%), with 21% and 91% positive and negative predictive values, respectively, to identify virologic failures which could lead to continue to treat patients with failed regimen or to unnecessary switch of regimens (29, 30). Thus higher morbidity, mortality and more complex profile of resistance were observed in settings where virologic assessment is not available (31).

Definitions of ART failures

The criteria to define ART failures are not uniform. According to WHO (21), there are three definitions: clinical failure when there is a recurrent WHO stage 4; immunologic failure when CD4 falls to below the pre-therapy baseline, or below 50% of the on- peak value, or is persistently < 100 cells/mm; virologic failure when plasma VL >10000 copies/ml; Virologic success when VL is < 400 or 50 copies/ml (depending on the type of the assay) after six months of treatment (21). According to a recent WHO guideline, which recommends VL to be

done every six months, treatment failure is defined as persistent VL > 5000 copies/ml (5). Although not well defined, VL cut-off > 10000 copies/ml to define treatment failures is linked with subsequent decline in CD4+ cell count (32) and clinical progression (33).

Others define immunologic failure as an increase of CD4+ cells/ul < 50 at 6-12 months (34); < 100 at 12-24 months (35, 36), or < 500 at 4-5 years (37) irrespective of viral suppression. Virologic failure was defined as a primary failure where VL does not decrease to < 50 copies/ml on two different occasions after six months on ART; and secondary failure (viral rebound) where there is VL >50 copies/ml confirmed (21).

Putting together, the variation in defining the cut-off values of treatment failures indicates the need of research and programmatic data to better understand the profile of immunovirological responses to HAART, which might differ in different countries.

The profile of immunologic and virologic responses to HAART

Immunologic responses (CD4+ recovery)

Without therapy, the average decline rate of CD4 cells/µl ("CD4 slope") is estimated to be 50 cells per year, and the average VL level ranges from 30,000 to 50,000 copies/ml (26). CD4+ recovery following HAART, which is due to redistribution of the cells from tissues, regeneration of naïve T cells, or due to the reduction of immune activation mediated cell death (apoptosis) (37), occurs as a two phase process: In the first phase of two months on ART, rapid increase of CD4+ cells occurs; and in the second phase of the third month and onwards on ART, CD4+ count increase slows down but persists over time (38). Overall, the long-term shape of CD4+ count after HAART depends on the baseline CD4+ count, control of viral replication overtime, the stage of the disease at baseline, duration on treatment (39, 40), as well as on baseline patient factors including higher HIV RNA level, co-morbidities, presence of drug resistant viruses, sub-optimal pharmacokinetics, and potency of the ARV regimen (17). The time required to reach to normal value of CD4+ counts ranges from two to eight years (24, 41).

Immunological failure and the risk factors

Complete immune recovery following HAART is not observed in any of the patients. Absent or modest improvements in CD4+ counts did occur in 5–27% of the patients on HAART that achieved plasma HIV-1 RNA suppression (42,43,44) which has clinical implications. Higher relative risk of progression to AIDS; and AIDS and non-AIDS related mortalities were reported among discordant responders as compared to those virologic and immunologic concordant responders (45). Whereas there is no clear understanding on how to assess immunologic failures with regard to time after HAART, questions such as the clinical risks and the possible treatment for immunological failures would be a concern for the health care workers in the ART clinic (46).

Risk factors for failure or incomplete immune recovery includ the degree of CD4+ decline before and at the initiation of the treatment (the steeper the decline the steeper the rise), the rate of decline in viral-load (47), old age (17, 47), co-infection (e.g. HCV, HIV-2, HTLV-1, HTLV-2), medications (ZDV, TDF+DDI), and persistent immune activation (17). However, others have reported no difference in immunological response related to baseline viral load, HIV risk factor, sex, HCV co-infection and HAART regimen (24). Several explanations have been given about the mechanisms by which inadequate immune CD4+ recovery occurred in response to HAART. These included, myelosuppressive effects of ARV drugs (e.g. ZDV) (48), thymic involution related to old age (49), and abnormal cell death (apoptosis) due to higher immune activation related to higher background risk of endemic infections (50).

Virologic responses

VL level is an excellent indicator of the degree of viral replication in the immune system, progression to AIDS, morbidity, mortality, and HIV transmission. VL level predicts also treatment success/failure faster than CD4+ counts and also resolves discordant clinico-immunological responses, so that it is an important tool to protect 1st and 2nd line regimens from unnecessary switch whereby resistance risk, is reduce because of all these factors, therefore, VL measurement has been considered as a golden standard to monitor patients on ART (51). However, whereas CD4+ level which measures the strength of the immune system is the best biomarker of when to start treatment (5), VL test is less necessary before initiating ART as it rarely informs when to start ART (21).

Even though not always true, the minimum change in VL after treatment to be considered statistically significant (2 standard deviations) is a threefold or a 0.5 $\log_{10}$ copies/ml change (52). Virologic response has been reported therefore to decrease at week 72 and disappeared after 96 weeks of treatment (53). It has been observed also that 75-90.7% of treatment-naïve patients reached undetectable viral load by 12 months on ART, while it was reduced to 72% after 24 months (53). The proportion of treatment naïve patients with viral rebound was 9.4% after one year, and 20.1-20.6 % after 2 years, while it was 35·7–40·1% after 2 years of pretreated patients (54,55).

Virological failure and the risk factors

The risk factors for virological failure includes sex (although reports are controversial) (19, 56), old age, poor adherence, previous exposure to ART, lower base line CD4+ count, OIs, TB after ART, persistent lower VL, insufficient CD4+ cell gain, clinical symptoms, lower weight than baseline, and emergence of drug resistant viruses (57,58). Digestive symptoms and poor adherence to ART were also reported as risk factors for

low ARV plasma concentrations (59), which in turn results in sub-optimal virlogical responses.

Discordant/Concordant immunovirological responses

Besides the independent immunologic and virologic failures (12), concordant/discordant responses are another challenge during ART. Although the frequency of concordant/discordant immunovirologic responders depends on the definition (cut-off values) of immunologic and virologic responses, there are three immunovirological responders in clinical practices: 1) Concordant responders ($VL^+/CD4^+$) (40-60%), 2) Concordant non-responders ($VL^-/CD4^-$) (12-27.3%), and 3) discordant responders which is sub divided as immunological non-responders (lack of CD4 increases despite viral suppression ($VL^+/CD4^-$), (7%-48%), and immunological responders (good CD4+ responses in the absence of viral suppression, $VL^-/CD4^+$) (5%-18%) (15, 51, 59, 61, 62).

Whereas discordant results complicate the interaction between virological and CD4+ count response (61), and cause more challenges to the health care providers during patient management and monitoring (51), higher risk of clinical progression and mortality was observed in discordant responders as compared to complete response (62,63).

The risk factors for discordant/concordant ART responses

The mechanisms of discordant response ($VL^+/CD4^-$, $VL^-/CD4^+$) are not fully understood (36). Among the risk factors for $VL^+/CD4^-$ were lower baseline CD4+ count (50-100/µl), higher baseline VL (100,000 copies/ml), HAART composed of three NRTIs, the use of lamivudine (3TC)/zidovudine (ZDV), didanosine/tenofovir (DDI/TDF), poor adherence, advanced age, and being ARV naïve (63). The factors, which contribute $VL^-/CD4^+$ include sexual transmission of HIV, absence of clinical progression, lower baseline CD4 counts, higher baseline VL, low-level viral rebound during the first year after achieving undetectable VL, younger age, pretreatment and saquinavir regimen (63), use of 3TC/ZDV, ddI/3TC, or ddI/stavudine, ritonavir-boosted protease inhibitor-(PI) based regimen (36, 64), and treatment compliance (53).

Evidences showed that the frequency and risk factors for discordant responses to HAART in developing and developed countries were comparable (64). However, the studies are different in terms of study design, inclusion/exclusion criteria, ethnicity, ART experience, sample size, ARV regimen, length of follow-up, and the definitions, which results in the variations of results related to the factors associated with discordant responses. Therefore, longer follow-up studies are highly pertinent to assess the pattern as well as the long-term impact of concordant/discordant responses treatment outcomes and the risk factors associated with in the context of local settings (64).

HAART and TB/HIV co-infection

Co-infection with TB/HIV complicates pathogenesis, epidemiology, clinical presentation, diagnosis, treatment, prevention aspects of one or the other. Whereas 11% of all HIV/AIDS -related adult mortality are attributed to TB, 39% of all TB related deaths are attributable to HIV (64).

Patients from TB endemic areas present themselves to the health facilities for TB/HIV diagnosis and simultaneous treatment. Even though the optimal interval between starting TB treatment and ART remains to be determined (65), the objectives for initiating early ART in patients on anti-TB treatment are to reduce the risk of HIV related morbidity/mortality and improved sputum smear conversion; while the factors for differing ART includes high pill burden, poor adherence, impaired tolerability, drug with-drug interactions, toxicity, and morbidity and mortality because of TBIRIS (66).

According to WHO guidelines (21), ART should be initiated within 2 to 8 weeks of anti-TB treatment in those with CD4+ count < 200 cells/ul; in the continuation phase of anti-TB treatment in those with CD4+ cell counts 200-350 cells/ul; but with great urgency in those highly immunocompromised patients. However, in cases a person needs TB and HIV treatment concurrently, first line treatment options include ZDV/3TC or d4T/3TC plus either an NNRTI or ABC (5).

Overall, HAART restores host immune response specific to *Mycobacterium tuberculosis (MTB)*, reduces incidence of TB, and improves survival, while anti-TB treatment in TB/HIV co-infected patients on the other hand minimizes the negative effects of TB on the course of HIV and reduces the transmission of MTB (59, 67, 68, 69).

Whereas the long- term impact of HAART on TB control is dependent, in part, on the rate and extent of *MTB* specific immune restoration (68), TB disease prevalence at baseline, and incidence rate during the initial months of ART are higher on those enrolled for ART, which results in higher morbidity, mortality and complicates the delivery of ART (Fig 1) (68, 70, 71).

Specific strategy is therefore required to reduce the impact of TB in the era of ART.WHO has recommended the '3Is' strategy that incorporates intensified case finding, infection control and isoniazed preventive therapy to reduce the burden of TB in people living with HIV (72). However, the scenario of intensive case finding is also greatly affected by the screening strategy applied, immunodeficiency and the diagnostic tests available (64). In this regard, the fact that most diseases are sputum smear negative and culture-positive (73) is also a major challenge.

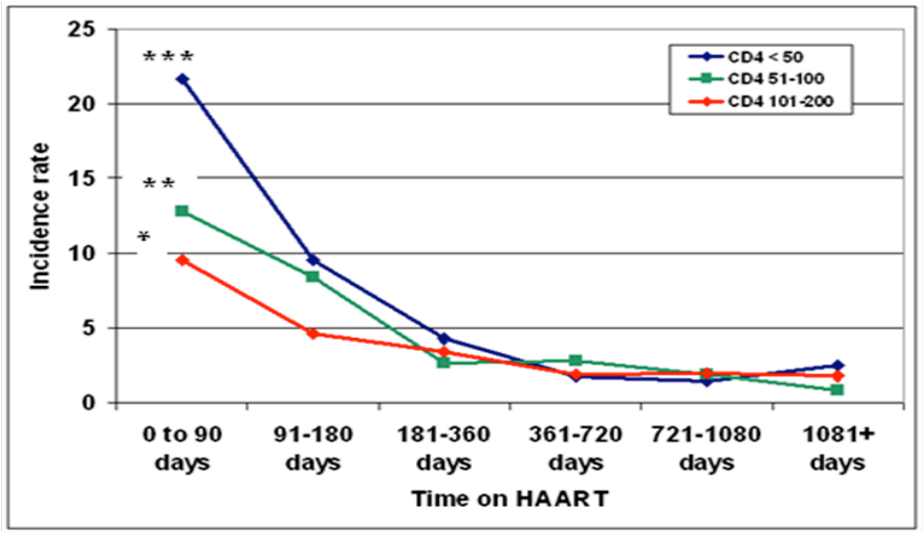

Figure 1: **The incidence of TB in HIV infected patients on HAART relative to duration of HAART (days) and CD4+ counts (***: CD4+ < 50; **: CD4+ 51-100; *: CD4+ 101-200 (adapted from 63)**

Furthermore, whereas concurrent treatments with anti-TB and HAART improves survival of the patients (74), there are also reported complications during the dual treatment including drug interactions, increased risk of treatment interruptions, high pill burden, shared toxicities, and paradoxical TB Induced Reconstitution Inflammatory Syndrome (TBIRIS) (65,75). Nevirapine concentrations are frequently sub-therapeutic in patients on rifampicin-based TB treatment, which may result in inferior virological outcomes (65), while others have reported virological responses in TB/HIV patients to be similar with those who did not have TB (70).

Discussion

This review has summarized the success of HAART at national and continental level on one hand, but also the major challenges observed related to the parameters which have been implemented to monitor the patients on HAART. The key questions addressed in this review, therefore, includes: the profile of immunologic and virologic responses to HAART, immunologic and virologic failures and the factors associated with; Discordant/Concordant immunovirologic responses, with special emphasis to the potential limitations of applying clinical and immunologic parameters for monitoring of patients on HAART in resource limited settings like in Ethiopia.

Immunologic responses to HAART

Overall, CD4+ recovery post-HAART occurs in two phases: in the first phase of the two months, rapid recovery of the CD4+ cells took place mainly due to the release of the sequestered cells in the body tissue; whereas from the third month onwards (the second phase), the rate of CD4+ recovery is slow and the factors contributed for the increase in cell number includes re-generating of new cells from bone marrow, and reduction of programmed cell death (37, 38). Moreover, although the rate of CD4+ recovery depends on several factors including baseline CD4+ count, control of viral replication overtime, the stage of the disease at baseline, duration on treatment (17, 40), the time required for CD4+ cells to reach the normal values ranges from two to eight years (24, 41). However, the fact that there is significant reduction in the frequency of OIs, and in morbidity and mortality, irrespective of the slow recovery rate as well as low absolute number of CD4+ cells post-HAART, raises a research question "how strong is the restoration of the functional immune response specific to variety of OIs post-HAART irrespective the low number of CD4+ cells in the periphery?"

Moreover, based on the observation that absent or modest improvements in CD4+ response occurs in 5–27% of the patients on HAART (42,43,44), where the risk factors included rate of CD4+ and VL decline, (47), old age (17, 47), co-infection, medications, immune activation (17), and apoptosis (50), there are two major un-resolved challenges remained: a) absence of clear understanding on how to assess immunologic failures with regard to the duration of time after HAART initiation, and the clinical risks as well as the possible treatment for immunological failures (46); and b) absence of defined cut-off values to determine immunologic failures.

Furthermore, whereas clinical and immunological parameters are recommended as a proxy for VL test to evaluate the response of HAART for resource poor settings (21), the occurrence of higher morbidity, mortality and complex profile of resistance in settings where VL testing is not available (31), which could be due to the poor sensitivity (20%-33%), and specificity (86%-90%), of immunologic markers to identify virologic failures (29, 30), implies the timely need to

incorporate VL testing to confirm treatment failures, in settings like in Ethiopia where free access to ART is expanded.

Virologic responses to HAART

Whereas CD4+ count, but not VL test (21), is the best biomarker for initiation ART (5), VL measurement is a golden standard to monitor patients on ART (51). However, higher cost, the need of trained human resource and infrastructure remains to be the major obstacle for the resource limited settings to implement VL test, indicating the need of simple and cheap point of care (POC) VL assay for poor countries. Overall, although virologic success observed in 75-90.7% of the patients by 12 month on HAART, a decrease in the proportion of those with successful virologic response, and an increase in viral rebound, as the time on HAART increased, has been reported (54,55). Moreover, the absence of defined cut-off values for virologic failure, like that in immunological failure, remains to be a major challenge when applying VL test for patient monitoring.

Discordant/Concordant immunovirologic responses to HAART

Despite the independent immunologic and virologic failures post-HAART, discordant immunovirologic responses ($VL^+/CD4^-$, $VL^-/CD4^+$) which is known to be associated with higher risk of clinical progression and mortality (62,63), and causes more challenges to the health care providers during patient management and monitoring (51), is another significant challenge during the monitoring of patients on HAART. Therefore, whereas the mechanisms of discordant immunovirologic responses are not fully understood (36), local follow-up studies are highly pertinent to assess the magnitude and long-term effect, as well as the risk factors associated with discordant immunovirologic responses

HAART and TB/HIV co-infection

Whereas TB is the most common OI in HIV patients (64), the summary of the literatures in this review have shown HAART restores the host immune response specific to TB, reduces incidence of TB, morbidity and mortality related to TB (68, 69). However, time to start ART, higher incidence of TB in HIV positive on HAART than that of HIV negatives (indicated incomplete immune restoration specific to TB), toxicities, TBIRIS, and drug interactions, has been reported as major obstacles (65,75).

Therefore, in TB/HIV clinics where simultaneous treatment with anti-TB and ART are provided, awareness of the health professionals is highly essential for the comprehensive and effective management of the TB/HIV patients. Furthermore, future researches aimed to address better treatment strategies of TB/HIV patients, the evolution of immune restoration specific to TB, and TBIRIS, are highly relevant.

Summary

HAART restores host immune responses, decreases risk of OI, morbidity and mortality globally. The un-resolved questions related to HAART have to do with variations in the treatment outcomes (countries, Ethnic), cut-off values for immunovirological failures, discordant results; drug resistance, toxicity, drug interactions, and early mortality.

Since clinical and immunological assessments lack sensitivity/specificity to diagnose virologic failures, complications in the patient monitoring in the extensive ART expansion that might occur in the resource poor settings, where patient monitoring is dependent on clinico-immunological parameters. Incorporating VL testing to confirm treatment failures, as well as genotyping testing for treatment failed samples, if not pre-HAART resistance testing, should be part of the immediate plan. Furthermore, considering the rapid expansion of ART where the long-term effect of which is not well investigated, and the research data on ART are predominantly from the developed world, local research data from well defined cohorts of patients on long-term HAART, which can complement data from randomized clinical trials (76) are highly pertinent and timely. Likewise, routine national surveys for transmitted and acquired ARV drug resistance and early warning indicators (EWI) should also be implemented in parallel to the speeded up expansion of ART.

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