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# Performance of Immunological Response in Predicting Virological Failure

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## Abstract

In HIV-infected individuals on antiretroviral therapy (ART), the decision on when to switch from first-line to second-line therapy is dictated by treatment failure, and this can be measured in three ways: clinically, immunologically, and virologically. While viral load (VL) decreases and CD4 cell increases typically occur together after starting ART, discordant responses may be seen. Hence the current study was designed to determine the immunological and virological response to ART and to evaluate the utility of immunological response to predict virological failure. All treatment-naive HIV-positive individuals aged >18 years who were eligible for ART were enrolled and assessed at baseline, 6 months, and 12 months clinically and by CD4 cell count and viral load estimations. The patients were categorized as showing concordant favorable (CF), immunological only (IO), virological only (VO), and concordant unfavorable responses (CU). The efficiency of immunological failure to predict virological failure was analyzed across various levels of virological failure (VL > 50, > 500, and > 5,000 copies/ml). At 6 months, 87(79.81%), 7(5.5%), 13 (11.92%), and 2 (1.83%) patients and at 12 months 61(69.3%), 9(10.2%), 16 (18.2%), and 2 (2.3%) patients had CF, IO, VO, and CU responses, respectively. Immunological failure criteria had a very low sensitivity (11.1–40%) and positive predictive value (8.3–25%) to predict virological failure. Immunological criteria do not accurately predict virological failure resulting in significant misclassification of therapeutic responses. There is an urgent need for inclusion of viral load testing in the initiation and monitoring of ART.

## Introduction

In HIV-infected individuals on antiretroviral therapy (ART), the decision on when to switch from first-line to second-line therapy is critical. If the decision is made too early the months or years of potential further survival benefit from any remaining first-line effectiveness is lost; if it is made too late, the effectiveness of second-line therapy may be compromised and the patient is put at additional and appreciable risk of death. The time of switching is dictated by treatment failure, and this can be measured in three ways: clinically, by disease progression and WHO staging; immunologically, using trends in CD4 counts over time; and virologically, by measuring plasma HIV-1 RNA levels (HIV viral loads).[1]

In the developed countries periodic CD4 count and viral load assessment are recommended for monitoring the patient after initiation of ART and treatment failure is defined as viral load greater than 50 copies/ml (polymerase chain reaction) or 75 copies/ml (branched DNA).[2,3]

However, the National AIDS Control Organisation (NACO) in India recommends clinical and immunological monitoring of patients once started on ART. Viral load measurement is not recommended for decision making for the initiation or regular monitoring of ART.[4]

While viral load decreases and CD4 cell increases typically occur together after starting ART, this does not always happen. Some people who achieve full suppression of HIV do not see much improvement in their CD4 cell counts (virological only responders), while others experience good CD4 cell recovery despite continued detectable HIV replication (immunological only responders). These so-called "discordant" responses tend to occur more often in highly treatment-experienced patients with drug-resistant HIV. Also, individuals with discordant responses on ART consistently do worse than individuals with complete responses (concordant favorable), yet generally do better than those with no response (concordant unfavorable).[5] Various authors have reported that CD4 cell count monitoring does not accurately identify individuals with virological failure among patients taking ART.[6,7]

Therefore, we designed a study to determine the immunological and virological response to first-line ART regimen,

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to evaluate the utility of immunological failure criteria to predict virological failure, and to identify the factors associated with immunological and virological failure.

## Materials and Methods

Our hospital offers a wide range of services including voluntary counseling and testing (VCT), treatment and referral services, monitoring of treatment response with CD4 cell counts, follow-up, and supportive care of HIV-infected persons. ART is provided free of cost to all HIV-infected individuals in need of treatment based on the NACO guidelines.[4] Data were extracted from the "Evaluation of incidence and risk factors for hyperlactemia and lactic acidosis in patients receiving HAART in a tertiary referral center in Mumbai, India" study that was conducted after Institutional Ethics Committee approval from May 2008 to March 2010. This study had three arms: prospective, cross-sectional, and symptomatic. Data from the prospective arm were extracted and analyzed in the current study. All treatment-naive HIV-positive individuals, aged 18 years and older, who had CD4 cell counts $<250$ cells/$\mu$l or WHO stage III or stage IV disease, who were eligible for ART were enrolled in the study after obtaining written informed consent. Demographic data were noted down for these patients. ART was started in all these patients and was a combination of two nucleoside reverse transcriptase inhibitors (NRTI) and a nonnucleoside reverse transcriptase inhibitor (NNRTI). None of the patients discontinued therapy for more than 1 month at any time during the study period.

All patients started on ART had baseline (measured less than 2 weeks before starting therapy) clinical assessment, CD4 cell count, and viral load estimations. Following initiation of therapy, patients were scheduled for a follow-up visits at 6 months and 12 months. Clinical assessment, CD4 cell counts, and the viral loads were measured at 6 months (5–7 month window) and 12 months (11–13 month window) after commencing ART to assess the response. Plasma HIV-1 RNA was measured using quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR; COBAS TaqMan HIV-1 test Roche Molecular Systems, Pleasanton, CA) with a lower limit of detection as 47 HIV-1 RNA copies/ml. For CD4 cell count, the samples were prepared and run on a Flow cytometer (FACS Calibur, Beckton Dickinson Biosciences, Franklin Lakes, NJ) according to the manufacturer's instructions.

World Health Organization (WHO) guidelines[1] define virological nonresponders as viral load $>5{,}000$ copies/ml. Immunological nonresponders are defined as CD4 count below 100 cells/$\mu$l after 6 months of therapy; a return to, or a fall below, the pretherapy CD4 baseline after 6 months of therapy; or a 50% decline from the on-treatment peak CD4 value (if known). Clinical nonresponders are defined as new or recurrent WHO stage 4 condition. Accordingly, the patients in the current study were categorized as showing concordant favorable response (CF), immunological only response (IO), virological only response (VO), and concordant unfavorable response (CU). The immunological and virological response to therapy was analyzed using the Friedman test (nonparametric repeated measures ANOVA). The association between factors such as age, gender, clinical staging, baseline CD4 counts, baseline viral load, and virological and immunological nonresponse was examined using the chi square test (or Fisher's exact test as applicable). A probability ($p$) of $<0.05$ was considered statistically significant. The efficiency of immunological failure to predict virological failure was analyzed across various levels of virological failure (VL $>50$ copies/ml, VL $>500$ copies/ml, and VL $>5{,}000$ copies/ml).

## Results

A total of 130 patients were enrolled in the study 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. In all, 109 (83.85%) patients had CD4 counts less than 200 cells/$\mu$l and 91 (70%) patients had viral load more than $10^5$ copies/ml; 109 (83.85%) patients were followed up at 6 months and 88 (67.7%) patients were followed up at 12 months (Table 1).

For the 88 patients who were followed up for 1 year, the median CD4 counts at baseline, 6 months, and 12 months were 127 cells/$\mu$l (IQR: 78–178), 227 cells/$\mu$l (IQR: 161–317), and 264 cells/$\mu$l (IQR: 165–338), respectively, and the difference was statistically significant ($p<0.001$). The median viral loads in these patients at baseline, 6 months, and 12 months were 20,661 copies/ml (IQR: 86,769–500,352), 177 copies/ml (IQR: 47–392), and $<47$ copies/ml (IQR: $<47$– 354), respectively, and the difference was statistically significant ($p<0.001$). All the patients responded clinically at 6 months; however, six patients developed a new stage IV disease at 12 months. There was no significant difference in the proportion of individuals with virological failure and immunological failure when participants were stratified by age ($\geq$ or $<40$

Table 1. Characteristics of Patients Analyzed at Baseline, 6 Months, and 12 Months of Starting Antiretroviral Therapy

| *Parameter* | *Baseline* (n = *130*) | *Follow-up at 6 months* (n = *109*) | *Follow-up at 12 months* (n = *88*) |
|---|---|---|---|
| Age group (years) | | | |
| $<40$ | 84 | 70 | 54 |
| $\geq 40$ | 46 | 39 | 34 |
| Gender | | | |
| Male | 86 | 74 | 59 |
| Female | 44 | 35 | 29 |
| Clinical staging | | | |
| I | 63 | 101 | 77 |
| II | 34 | 6 | 3 |
| III | 29 | 2 | 2 |
| IV | 4 | 0 | 6 |
| CD4 counts (cells/$\mu$l) | | | |
| $<50$ | 19 | 4 | 1 |
| 50–199 | 90 | 47 | 30 |
| $\geq 200$ | 21 | 58 | 57 |
| HIV viral load (copies/ml) | | | |
| $<50$ | 5 | 37 | 60 |
| 50–1,000 | 2 | 60 | 11 |
| 1,000–5,000 | 3 | 3 | 6 |
| 5,000–10,000 | 2 | 1 | 1 |
| 10,000–100,000 | 27 | 4 | 6 |
| $\geq 100{,}000$ | 91 | 4 | 4 |

Table 2. Predictive Value of Different Factors in Identifying Immunological and Virological Nonresponders

| Parameter at baseline | At first follow-up (6 months) INR | p value | VNR | p value | At second follow-up (12 months) INR | p value | VNR | p value |
|---|---|---|---|---|---|---|---|---|
| Age group (years) | | | | | | | | |
| <40 | 10 | 1 | 3 | 0.132 | 10 | 0.596 | 6 | 0.525 |
| ≥40 | 5 | | 5 | | 8 | | 6 | |
| Gender | | | | | | | | |
| Male | 9 | 0.555 | 6 | 1 | 9 | 0.098 | 8 | 1 |
| Female | 6 | | 2 | | 9 | | 4 | |
| Clinical staging | | | | | | | | |
| I | 8 | 0.844 | 4 | 0.673 | 12 | 0.173 | 3 | 0.846 |
| II | 3 | | 1 | | 3 | | 5 | |
| III and IV | 4 | | 3 | | 3 | | 4 | |
| CD4 counts (cells/μl) | | | | | | | | |
| <50 | 0 | 0.181 | 2 | 0.427 | 2 | 0.022 | 3 | 0.1 |
| 50–199 | 12 | | 6 | | 9 | | 9 | |
| ≥200 | 3 | | 0 | | 7 | | 0 | |
| HIV viral load (copies/ml) | | | | | | | | |
| <10,000 | 2 | 0.564 | 1 | 0.0126 | 3 | 0.6445 | 1 | 0.6001 |
| 10,000–100,000 | 4 | | 5 | | 4 | | 1 | |
| ≥100,000 | 9 | | 3 | | 11 | | 9 | |

INR, immunological nonresponders; VNR, virological nonresponders.

years) or gender. However, 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 (Table 2).

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, immunological only, virological only, and concordant unfavorable responses, respectively (Table 3). 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.

Table 3. Change in Concordancy/Discordancy Status Between First and Second Follow-Up

| First follow-up (6 months) | Second follow-up (12 months) CF | IO | VO | CU | LFU | Total |
|---|---|---|---|---|---|---|
| CF | 52 | 5 | 13 | 2 | 15 | 87 |
| IO | 3 | 4 | 0 | 0 | 0 | 7 |
| VO | 5 | 0 | 3 | 0 | 5 | 13 |
| CU | 1 | 0 | 0 | 0 | 1 | 2 |
| LFU | 0 | 0 | 0 | 0 | 21 | 21 |
| Total | 61 | 9 | 16 | 2 | 42 | 130 |

CF, concordant favorable responders; IO, immunological only responders; VO, virological only responders; CU, concordant unfavorable responders; LFU, lost to follow-up.

Table 4. Performance of Immunological Failure Criteria to Predict Various Levels of Virological Failure Among Patients Receiving First-Line Antiretroviral Therapy

| | CF | IO | VO | CU | SENS | SPEC | Efficiency | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|
| VL > 5000 copies/ml | | | | | | | | | |
| 6 months | 87 | 7 | 13 | 2 | 13.3 | 92.5 | 81.7 | 22.2 | 87 |
| 12 months | 61 | 9 | 16 | 2 | 11.1 | 87.1 | 71.6 | 18.2 | 79.2 |
| VL > 500 copies/ml | | | | | | | | | |
| 6 months | 78 | 16 | 13 | 2 | 13.3 | 83 | 73.4 | 11.1 | 85.7 |
| 12 months | 55 | 15 | 13 | 5 | 27.8 | 78.6 | 68.9 | 25 | 80.9 |
| VL > 50 copies/ml | | | | | | | | | |
| 6 months | 28 | 66 | 9 | 6 | 40 | 29.8 | 31.2 | 8.3 | 75.7 |
| 12 months | 47 | 23 | 12 | 6 | 33.3 | 67.1 | 60.2 | 20.7 | 79.7 |

CF, concordant favorable responders; IO, immunological only responders; VO, virological only responders; CU, concordant unfavorable responders; VL, viral load; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value.

Immunological failure criteria had a very low sensitivity (11.1–40%) and positive predictive value (8.3–25%) to predict virological failure (at various levels) among patients receiving first-line ART (Table 4).

## Discussion

A large majority of HIV-infected individuals respond favorably to first-line ART within the first year of treatment, therefore monitoring ART response is used mainly to identify the minority of patients who fail to respond to ART or develop subsequent treatment failure after an initial response to therapy has occurred.[8]

Of the 130 individuals who were started on ART during the study period, 86 (66.2%) were male and 84 (64.6%) were less than 40 years of age, which is similar to that reported by others.[7,9,10] Various authors[9,11] have reported that older age is a risk factor for treatment failure, which was not seen in the present study. Of 33 (25.38%) patients were in WHO clinical stage III and IV, 109 (83.85%) patients had CD4 counts less than 200 cells/μl and 118 (90.77%) patients had viral load greater than 10,000 copies/ml at baseline. This proves that virological failure is the first to occur followed by immunological failure and lastly clinical failure. Hence, though the patient might be clinically asymptomatic it is imperative that the patient is regularly monitored immunologically as well as virologically.

Twenty-one (16.15%) patients were lost to follow-up at 6 months and a further 21 (19.27%) were lost to follow-up at 12 months. In spite of repeated efforts, these patients did not come back and we could not obtain any further information about them. Hence, response to treatment was assessed in only 88 patients. The high loss to follow-up was possibly due to the shifting of residence of the patient, transferring the patient to a newly started ART center near his place of residence, drug toxicity, drug intolerance, and death.

Various authors[6,7,9,12] have reported the median baseline CD4 cell count from 74 to 153 cells/μl, which was similar to our study. Follow-up CD4 count testing at 6 months and 12 months showed a median CD4 cell count increase of 100 cells/μl and 137 cells/μl, respectively, from baseline and the difference was statistically significant ($p < 0.001$). Similarly, the median baseline viral load showed a significant decrease at 6 months

and 12 months. In all, 15 (13.76%)/9(8.23%) and 18 (20.45%)/11 (12.5%) patients did not respond immunologically/virologically at 6 months and 12 months of treatment, respectively (Table 2). Similar findings have been reported by other authors.[9,10,12] Protease inhibitor (PI)-based regimens have been reported to have a higher success rate compared to NNRTI-based regimens probably because the HIV replicative capacity is higher in patients on NNRTI-based regimens than in patients receiving PI-based regimens, perhaps reflecting different barriers to selection of resistant virus.[13–15] We could not make any useful comparisons of treatment outcomes on the basis of regimen type because all our patients were on NNRTI-based regimen.

The failure of treatment cannot be diagnosed on the basis of clinical criteria in the first 6 months of ART. Clinical events that occur before the first 6 months of therapy often represent IRIS and not failure.[4] In the present study, the majority of patients improved clinically at 6 months. However, six patients developed a new stage IV disease at 12 months follow-up. At 6 months, all these six patients responded immunologically and two of them responded virologically. At 12 months, all six were virological nonresponders and four of them were immunological nonresponders.

The WHO treatment failure criteria have been proposed with the goal of identifying individuals who are not responding adequately to treatment.[1] The number of people living with HIV in resource-limited countries, who will fail first-line treatment and benefit from regimen switching, will steadily increase in the coming years. The diagnosis of treatment failure in many settings is challenging because of limited access to plasma HIV RNA testing. Discordant immunological and virological responses at 3 to 9 months after HAART initiation play important roles in predicting long-term clinical outcomes in treatment-naive patients.[10] Tuboi *et al.*[16] reported that discordant immunological and virological responses are associated with intermediate risk of death compared with concordant response. Studies in industrialized countries have shown that a discordant response to therapy occurs in 20–40% of treated patients, with isolated immunological response being slightly more common than isolated virological response.[13]

In the present study, 20 (18.35%) and 25 (28.41%) patients had discordant responses (IO or VO) at 6 months and 12 months, respectively (Table 3). In India, Ganesh Anusuya *et al.* reported that 21.1% of HIV patients on ART had a virological only response.[17] Similar findings have also been reported by other authors.[10–13,18] Patients developing immunological failure in the absence of virological failure would have been switched to a second-line regimen if only the immunological monitoring criteria were applied. It would have negative consequence for such patients because they would be prematurely switched off a regimen that was effectively controlling viral replication. Conversely, the clinical consequences of the late identification of the virological failure patients who did not develop immunological failure are unknown but would allow greater time for viruses in these patients to accumulate multiple drug resistance mutations. In high-income countries, HIV resistance testing is used to guide changes in treatment regimens. It has been argued that this approach is neither practical nor necessary in resource-limited settings because regimen changes usually involve replacement of all three drugs in the initial regimen (usually from a nonnucleoside reverse transcriptase inhibitor-based to a protease inhibitor-based regimen), with the expectation that further accumulation of resistance mutations may have limited impact on the success of second-line regimens.[7]

Risk factors for immunologic-only response include younger age, a lower baseline CD4 count, higher baseline viral load, poor adherence to therapy, and antiretroviral drug resistance. A virological-only response is associated with increasing age, low baseline CD4 count, and low viral load.[5,11,13,18] Similarly in the present study, 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 (Table 2). This highlights the importance of both CD4 count estimation and measurement of plasma HIV-1 RNA levels not only for making decisions regarding the starting of ART but also in making decisions regarding the switching of therapy. Also, it is implied that we should not wait until CD4 counts fall to a very low level before initiating ART to ensure a good response to therapy.

Of those discordant at 6 months 63% were concordant at 12 months. Later improvement in CD4 counts seen in some patients categorized early as having a suboptimal CD4 response might have been a consequence of a continued, albeit slow recovery of immune response on ART.

The threshold used to define a good virological response has varied from 50 to 10,000 copies/ml or a 1 $\log_{10}$ copies/ml decrease from baseline.[1,2,19–21] The criteria used to define treatment failure (not achieving viral load measurements $<5{,}000$ copies/ml) as recommended by WHO were relatively conservative, given that current ART guidelines in industrialized countries use failure to achieve viral load measurements of $<50$ copies/ml as evidence of treatment failure. NACO recommends viral load measurement $>10{,}000$ copies/ml as treatment failure in its latest guideline.[8] Immunological failure had a very low sensitivity (11.1–40%) and positive predictive value (8.3–25%) and good specificity (29.8–2.5%) and negative predictive value (75.7–87%) for predicting virological failure according to all the three study levels (viral load thresholds of $>5{,}000$ copies/ml, $>500$ copies/ml, or $>50$ copies/ml). Similar findings have been reported by Reynolds *et al.* (sensitivity/specificity/PPV/NPV—23%/90%/21%/91%).[12] This analysis has shown that using immunological criteria to predict which patients have not achieved virological suppression results in significant misclassification of therapeutic responses. Health professionals with only CD4 cell count monitoring available to assess ART treatment failure should therefore interpret these values and the WHO/NACO monitoring guidelines quite cautiously. We are concerned that the low sensitivity of immunological failure criteria to predict virological failure could result in prolonged undetected virological failure. Prolonged virological failure in the presence of ongoing drug pressure could result in a significant accumulation of resistance mutations, which could ultimately limit second-line treatment options.

Our analysis has several limitations. We examined changes only within a 6- or 12-month period to look for correlations with VL status. It is possible that CD4 changes over longer periods, which correspond to more prolonged periods of inadequate VL suppression, may perform better. Also, because of the modest sample size, we may have had insufficient statistical power to differentiate the effects between the two discordant groups. Furthermore, all cohort studies of ART

response are compromised to some degree by loss of subjects attributable to toxicity and drug intolerance as well as loss to study follow-up.

To conclude, the majority of patients respond to first-line ART immunologically and virologically. Using immunological criteria to predict which patient has not achieved virological suppression results in significant misclassification of therapeutic responses. Early, unnecessary switching to second-line treatments incurs additional expense from increased drug costs and also limits the treatment duration of critically important first-line regimens. In discordant responders assessment at 12 months may be preferred given the number of slow responders. There is an urgent need for the availability of viral load testing in initiation as well as monitoring of ART. Also, the development of standardized and universally accepted definitions of virological failure is necessary to allow meaningful therapeutic interventions.

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### Author Disclosure Statement

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