ORIGINAL ARTICLE

Risk factors for discordant immune response among HIV-infected patients initiating antiretroviral therapy: A retrospective cohort study

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Background. The therapeutic goal of antiretroviral therapy (ART) is sustained immune recovery and viral suppression. However, some patients experience poor CD4 cell count responses despite achieving viral suppression. Such discordant immune responses have been associated with poor clinical outcomes.
Objective. We aimed to determine the prevalence of discordant immune response and explore associated factors in a retrospective cohort of patients attending 2 large public sector clinics, during the 6 months following ART initiation.
Methods. Data were analysed from 810 HIV-infected adults initiated on first-line HAART at 2 clinics in Johannesburg, between 1 November 2008 and 31 December 2009. Multivariate logistic regression models were used to estimate adjusted odds ratios (AORs) to determine associations between discordant immune response and clinical and demographic factors.
Results. At ART initiation, 65% (n=592) of participants were female, with a mean age of 38.5 years. Median baseline CD4 cell count was 155 cells/mm^3, 70% (n=645) of patients had a haemoglobin level >11 g/dl and 88% (n=803) were initiated on stavudine-lamivudine-efavirenz/nevirapine (D4T-3TC-EFV/NVP). Six months after ART initiation, 24% (n=220) of patients had a discordant immune response and 7% (n=67) a discordant virological response. On multivariate analysis, baseline CD cell count ≥200 cells/mm^3 (AOR 3.02; 95% confidence interval (CI) 2.08 - 4.38; p<0.001) and moderate anaemia (8.0 - 9.4 g/dl) at baseline (AOR 2.30; 95% CI 1.25 - 4.59; p=0.007) were independently associated with the development of discordant immune response, after adjustment for education level, World Health Organization (WHO) clinical stage and ART regimen.
Conclusions. Discordant immune response following ART initiation was common and associated with baseline anaemia and CD4 cell count in our cohort. Intensive monitoring of at-risk individuals may improve clinical outcomes.

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HIV infection is typically associated with progressive CD4 cell depletion and consequent immunodeficiency.[1-3] The introduction of antiretroviral therapy (ART) has seen a decline in the morbidity and mortality associated with HIV infection.[4] This is a consequence of the ability of ART to suppress HIV viraemia to undetectable levels and allow immune restoration, resulting in an increase in circulating CD4 cells.[5] In clinical practice, however, not all patients receiving ART

achieve the desired concordant response of viral suppression with CD4 cell count increase.[6] As many as 20 - 40% of patients on ART do not show a significant increase in CD4 cell count despite viral suppression.[6] This phenomenon is referred to as discordant immune response and is associated with an increased risk of developing an AIDS event or death.[6-9]

Discordant immune response may arise either as a result of failed immune reconstitution or the excessive destruction of CD4 cells.[10] The reconstitution of peripheral CD4 cells is a biphasic process with an initial rapid increase of memory CD4 cells, succeeded by a slow increase in naive CD4 cells.[11,12] The second rise in CD4 cells may be due to cellular expansion or sustained cell survival in the periphery, as well as the central regeneration of cells by the thymus.[10]

Despite the relative frequency of discordant immune response following ART initiation, data on the prevalence of this phenomenen and associated factors are still limited in South Africa (SA), as well as in other low- and middle-income countries, where treatment is primarily nucleoside reverse transcriptase inhibitor (NRTI)-based.[13] In these settings patients often initiate treatment at advanced stages of immunosuppression and have co-morbidities that compromise treatment response.[14] The lack of knowledge about this subgroup may contribute to inadequate clinical management, as current HIV treatment guidelines do not provide specific applicable guidance. In this retrospective study we describe the prevalence of, and factors associated with, discordant immune response in a cohort of patients from 2 large public sector clinics in SA in the first 6 months after ART initiation.

Methods

We retrospectively analysed data from 810 HIV-infected patients aged >16 years who initiated ART at 2 district comprehensive care management and treatment centres in Ekurhuleni, Gauteng Province, from 1 November 2008 to 31 December 2009. Ekurhuleni, the largest district in the province, has a population of nearly 3 million people, and an HIV prevalence of 31.5%.[15] Patients were included in the study if they were ART-naive at the time of treatment initiation, and were maintained on a standard first-line ART regimen[16] for at least 3 months following treatment initiation.

Ethical approval and study permission were obtained from the Human Research Ethics Committee of the University of the Witwatersrand and the Ekurhuleni Ethical Panel.

Data collection

Patient demographics and contact information were recorded at the commencement visit to the clinic. At ensuing visits, the patients' weight, reports of any symptoms and new diagnoses were recorded. Data were collated by trained data capturers after each visit. Results of CD4 cell count, plasma HIV viral load (PVL), full blood count and liver function tests (LFTs) were recorded upon receipt. All data were maintained in the patient health management database Therapy-Edge. STATA software (version 11) was used for data analysis.

Socio-demographic characteristics (age, gender, education level, occupation status and alcohol use/smoking), medical history including prior pulmonary tuberculosis (TB) and ART information were extracted from Therapy-Edge, as were physical examination findings such as body mass index (BMI), liver function and haemoglobin (Hb) values. Hb, LFTs and CD4 cell count were measured at ART initiation (i.e. baseline) and at 6 months post-ART initiation, with only PVL measured at 6 months.

Outcome assessment

Viral suppression was defined as a PVL <400 copies/ml at 6 months after ART initiation.[16] Immune reconstitution was defined as an absolute increase in the CD4 cell count value of 50 cells/mm^3 at an average of 6 months after ART initiation. A discordant immune response was defined as a failure of immune reconstitution (increase in CD4 cell count <50 cells/mm^3) within 3 - 6 months of ART initiation, in the presence of viral suppression (PVL <400 copies/ml). Measurements within 3 - 6 months of ART initiation were used, in accordance with the 6-month follow-up recommended in national guidelines.[16]

Statistical methods

Descriptive statistics, using means and standard deviations for continuous variables, and frequencies for categorical variables, were used to report sample characteristics. Associations between the main outcome and potential explanatory factors were assessed using variate logistic regression, using odds ratios (ORs) and 95% confidence intervals (CIs) to express the measure of association. Factors that were significant at $p \leq 0.2$ in univariate analysis were considered for inclusion in multivariate logistic regression models. Final models were derived using forward selection and backward elimination techniques. The final model was adjusted for education level, World Health Organization (WHO) clinical stage, baseline CD4 cell count, and ART regimen, and adjusted ORs (AORs) were presented. Baseline WHO clinical stage was included in the final model regardless of statistical significance in the univariate analysis because of the link between HIV clinical disease and outcome. These models were tested using the Hosmer-Lemeshow goodness-of-fit test. Interactions between all significant variables in the model were also investigated. Collinearity was tested in all the regression models. A sensitivity analysis to demonstrate the robustness of the study findings to variation in definition of CD4 cell count response was also conducted using a 30% increase from baseline CD4 cell count as the desired response.

Results

A total of 6 460 adults enrolled in the ART programme at the 2 clinics; 4 581 (80%) were excluded due to an absent baseline/6-month follow-up CD4 cell count and/or PVL, and a further 962 were excluded due to a lack of additional information (e.g. ART regimen or baseline laboratory values). Of the remaining 917 eligible, 810 were included for analysis. The remainder were excluded as they had discordant virological responses (i.e. immune reconstitution in the absence of viral suppression) or were non-responders (no change in CD4 cell count or PVL).

Prevalence of discordant immune response on ART

Within the cohort, 220 (24%) experienced a discordant immune response within 6 months of ART initiation. At baseline, the mean CD4 cell count in the discordant group was 218 cells/mm^3 (SD ±168), compared with 137 cells/mm^3 (SD ±85) in the group with a concordant response to treatment.

Factors associated with a discordant immune response

Baseline characteristics of discordant and concordant immune responders were

Table 1. Cohort characteristics with logistic regression analysis of factors associated with discordant v. concordant immune response

Characteristic	Total (*N*=810)	Discordant response *n* (%)*	OR (95% CI)	*p*-value‡	AOR (95% CI)	*p*-value‡
Age (years), mean (±SD)	220	39.5 (±8.9)	1.02 (1.00 - 1.04)	0.028	1.02 (1.00 - 1.04)	0.031
Gender						
Female	138	138 (63)	1			
Male	82	82 (37)	1.18 (0.85 - 1.66)	0.323		
ART						
D4T-3TC-NVP/EFV	188	188 (86)	1			
AZT-3TC-NVP/EFV	27	27 (12)	1.91 (1.14 - 3.20)	0.014		
TDF-3TC-NVP/EFV	5	5 (2)	0.78 (0.29 - 2.15)	0.639		
Baseline CD4 cell count (cells/mm^3)						
<200	122	122 (55)	1		1	
>200	98	98 (45)	2.90 (2.08 - 4.03)	0.0001	3.02 (2.08 - 4.38)	0.001
WHO clinical stage						
1	164	164 (75)	1			
2	4	4 (2)	2.12 (0.56 - 7.98)	0.268		
3	52	52 (23)	0.91 (0.63 - 1.31)	0.616		
Hb (g/dl)						
Normal (≥11)	162	162 (74)	1		1	
Mild anaemia (9.5 - 10.9)	31	31 (14)	0.76 (0.49 - 1.19)	0.230	0.88 (0.54 - 1.45)	0.629
Moderate anaemia (8 - 9.4)	23	23 (11)	1.55 (0.90 - 2.68)	0.117	2.30 (1.26 - 4.19)	0.007
Severe anaemia (≤7.9)	2	2 (1)†(2)	0.20 (0.05 - 0.88)	0.032	0.36 (0.08 - 1.63)	0.188
History of TB						
No	192	192 (87)	1			
Yes	28	28 (13)	1.26 (0.78 - 2.04)	0.335		
Smoking						
No	147	147 (79)	1			
Yes	38	38 (21)†(35)	0.99 (0.65 - 1.50)	0.948		
Alcohol						
No	139	139 (77)	1			
Yes	41	41 (23)†(40)	1.11 (0.73 - 1.67)	0.630		
BMI						
Normal (18.50 - 24.99)	93	93 (54)	1			
Underweight (<18.50)	15	15 (9)	0.94 (0.50 - 1.79)	0.854		
Overweight (25.00 - 29.99)	34	34 (20)	0.58 (0.37 - 0.91)	0.017		
Obese (≥30.00)	30	30 (17)†(48)	0.88 (0.54 - 1.41)	0.594		
Occupation status						
Unemployed	128	128 (64)	1			
Employed	71	71 (36)†(21)	1.25 (0.88 - 1.76)	0.210		
Education level						
Illiterate	9	9 (5)	1			
Primary	33	33 (17)	0.48 (0.18 - 1.24)	0.124		
Secondary	149	149 (78)	0.49 (0.20 - 1.19)	0.116		
Tertiary	1	1 (1)†(28)	0.10 (0.01 - 0.93)	0.043		
AST (IU/l)						
Normal	136	136 (64)	1			
Above normal	77	77 (36)†(7)	1.16 (0.83 - 1.61)	0.379		
ALT (IU/l)						
Normal	209	209 (95)	1			
Above normal	10	10 (5)†(1)	0.83 (0.40 - 1.71)	0.605		

ART = antiretroviral therapy; D4T = stavudine; 3TC = lamivudine; EFV/NVP = evafirenz/nevaripine; AZT = zidovudine; TDF = tenofovir; WHO = World Health Organization; Hb = haemoglobin; TB = tuberculosis; BMI = body mass index; ALT = alanine aminotransferase; AST = aspartate transaminase.

*Data are expressed as *n* (%) for categorical variables and mean (±SD) for continuous variables.

†Missing values.

‡*p*-values were obtained using χ^2 test and Student's *t*-test.

Table 2. Uni- and multivariate analysis for sensitivity of factors associated with discordant immune response at 6 months after ART initiation

	OR (CI)	*p*-value	AOR (CI)	*p*-value
Age (years)	1.02 (1.00 - 1.04)	0.0590	1.02 (1.00 - 1.04)	0.0300
Baseline CD4 cell count (cells/mm^3)				
<200	1		1	
>200	5.51 (3.91 - 7.77)	0.0001	5.31 (3.71 - 7.61)	0.0001

OR = odds ratio; AOR = adjusted odds ratio; CI = confidence interval.

compared (Table 1). Compared with concordant immune responders, patients with discordant immune responses were more likely to be older, to have initiated ART at a higher baseline CD4 cell count, to have been initiated onto zidovudine or tenofovir-containing ART regimens, and to have significantly different Hb levels and moderate anaemia at the start of ART. No significant differences were observed between the groups in terms of gender, occupational status, education level, history of tuberculosis, smoking or alcohol use, BMI, WHO clinical stage, or aspartate transaminase (AST) and alanine transaminase (ALT) levels.

In the final model, a discordant immune response was found to be associated with increasing age (AOR 1.02; CI 1.00 - 1.04; p=0.031), initiating treatment at a CD4 cell count >200 cells/mm^3 (AOR 3.02; CI 2.08 - 4.38; p<0.0001), and the presence of moderate anaemia (Hb 8.0 - 9.4 g/dl) (AOR 2.3; CI 1.26 - 4.19; p=0.007), after adjusting for baseline education, WHO clinical stage, CD4 cell count and ART regimen (Table 1). No significant interactions were found between the significant variables in the final model, which was deemed adequate using the Hosmer-Lemeshow goodness-of-fit test (p=0.416).

The results of the sensitivity analysis, conducted by considering a 30% increase from baseline CD4 cell count as the desired response, are summarised in Table 2. The univariate analysis produced similar findings to those in the primary analysis. Only baseline Hb level failed as a significant factor in the development of discordant response using the modified outcome definition.

Discussion

Despite an adequate virological response, 24% of patients did not achieve an adequate immune response at 6 months after ART initiation. Increasing age, initiating ART at a CD4 cell count >200 cells/mm^3, and initiating ART with moderate anaemia were associated with failure to achieve optimal immune restoration. No associations between discordant immune response and gender, BMI or ART regimen were observed, although these were identified as risk factors in other studies.[17,18]

The findings concerning age were consistent with findings from other studies, where increasing age was associated with poor immune recovery.[5] In the North American AIDS Cohort Collaboration on Research Design (NA-ACCORD) study, data from 19 cohorts and 12 196 participants showed that increasing age was associated with a lower chance of achieving an increase in CD4 cell count >100 cells/mm^3 at 24 months following ART initiation.[19] This has been linked to the observation that thymus activity, which is largely responsible for immune restoration, decreases with ageing.[10]

The literature is conflicting regarding the association of baseline CD4 cell count with discordant immune response outcome. Findings similar to ours emerged from a cohort of 4 810 patients initiating ART in the Antiretroviral Therapy in Low-Income Countries (ART-LINC) study.[20] The association between ART initiation with a baseline CD4 cell count >200 cells/mm^3 and the development of discordant immune response can be explained by the nonlinear nature of CD4 cell count increases after ART initiation across the different baseline CD4 cell count strata:[4] starting treatment at higher CD4 cell counts limits the scope for further increases.[21] These findings are important as treatment programmes increase the CD4 cell count threshold for ART initiation.

In contrast to our findings, several studies conducted in resource-rich settings have shown that low baseline CD4 cell count is associated with discordant immune response.[4] This is biologically plausible given that a low nadir pre-treatment CD4 cell count is thought to be suggestive of more extensive depletion of CD4 cells in the gut-associated lymphoid tissue during acute HIV infection, and may be delayed or refractory to reconstitution with ART.[22] Genetic variability has been investigated as a possible modulator of immunological recovery, and may explain the divergent associations in the existing literature.

These data suggest that moderate anaemia at baseline is associated with failure to achieve immune recovery at 6-month follow-up – an association that has not been documented previously. The aetiology of anaemia in HIV infection is multifactorial, but is commonly due to underproduction of erythrocytes by the bone marrow stem cells.[23] These stem cells are also responsible for the production of CD4 cells through the myeloid precursor cell.[24] Poor production of myeloid precursor cells can therefore result in decreased production of both CD4 cells and erythrocytes. In addition, the erythrocytes of HIV-infected individuals may experience membrane changes which result in decreased pliability and premature destruction.[25] The same applies to CD4 cells.[25] These mechanisms of reduced stem cell activity and membrane changes could explain the association between anaemia when starting ART and a subsequent discordant immune response. However, this finding should be interpreted with caution, as it was not significant in the sensitivity analysis when the definition of immune response was altered.

Study strengths and limitations

Although the sample size was relatively small, it was likely to be representative of patients in routine clinical care in Gauteng Province. The validity of the results may have been limited by the high proportion of missing data. Patterns in missing data could have resulted in non-differential misclassification of patients, as a consequence of inaccurate measuring of outcomes and subsequent bias. However, such errors could have been evenly distributed among the groups and data were noted to be missing at random (analysis not shown).

The outcomes were measured between 3 and 6 months after ART initiation; it is therefore possible that factors associated with discordant immune response may have varied with longer periods of treatment.

Conclusion

The findings of this study suggest that a significant proportion of patients initiating ART in SA do not achieve an optimal immune response after an average of 6 months on ART, despite virological suppression. Significant factors associated with the development of a discordant immune response were increasing age, baseline CD4 cell count >200 cells/mm^3, and an Hb level of 8.0 - 9.4 g/dl. While further studies are required in local populations to examine these associations, these data may assist in the early identification of patients that are likely to have discordant immune responses on ART.

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