ORIGINAL RESEARCH ARTICLE

Immune reconstitution inflammatory syndrome in HIV-infected patients with and without prior tuberculosis

S Ramesh Kumar MBBS*, **Narendran Gopalan** MBBS DTRD (DNB)*, **Paru Patrawalla** MD†, **Pradeep Menon** MBBS DPH*, **Kenneth Mayer** MD‡ **and Soumya Swaminathan** MD*

*Tuberculosis Research Centre (ICMR), Chennai, India; †Boston Medical Center/Boston University, Boston, MA; ‡Miriam Hospital/Brown University, Providence, RI, USA

Summary: We conducted a nested case-control study in a cohort of patients initiating antiretroviral therapy (ART) to identify risk factors and common manifestations of immune reconstitution inflammatory syndrome (IRIS) and to validate the Robertson criteria for IRIS prediction. HIV-infected patients at the Tuberculosis Research Centre clinics, Chennai and Madurai, India, initiating ART between July 2004 and June 2005 were prospectively studied. Of 97 patients (62% men, median age 32 years, median CD4 count 63 cells/μL) included, 34 developed IRIS. IRIS was more common in patients with a prior history of tuberculosis (74% versus 52%, $P = 0.04$), median time to development was 46 days and the sensitivity and specificity of the Robertson criteria to predict IRIS were 91% and 22%, respectively. In this population, IRIS was a common event, more so among patients with prior tuberculosis, and neither the rate of CD4 increase nor the Robertson criteria were useful in predicting its development.

Keywords: HIV, immune reconstitution inflammatory syndrome, IRIS, Robertson score tuberculosis, TB, antiretroviral therapy, India

INTRODUCTION

The immune reconstitution inflammatory syndrome (IRIS) is a spectrum of clinical signs and symptoms occurring in HIV-infected persons resulting from the restored ability to mount an inflammatory response associated with immune recovery following antiretroviral therapy (ART). The consensus criteria for diagnosis of IRIS developed by the International Network for the Study of HIV-associated IRIS (INSHI) include (a) clinical or virological response to ART, (b) clinical deterioration of an infectious or inflammatory condition temporally related to ART initiation and (c) symptoms which cannot be explained by the expected clinical course of a previously recognized and successfully treated infection, medication side-effect, toxicity, treatment failure or complete non-adherence.[1] Typically, IRIS occurs within 2–12 weeks after the initiation of ART, although it may present later.[2] The incidence of IRIS has ranged from 10 to 40% in various studies and is higher among patients with low CD4 cell counts and high viral loads.[2] Ratnam *et al.*[3] found that the strongest independent predictors of IRIS were younger age, CD4+ cell percentage of less than 10% and a CD4/CD8 ratio <0.15, and observed that the majority of IRIS events were dermatological. In another study of 180 HIV-infected patients who received ART, 32% of patients developed IRIS and those patients with IRIS were significantly more likely to have initiated ART nearer to the time of diagnosis of their opportunistic infection (OI), to have been antiretroviral naïve at time of diagnosis of their OI, and to have a more rapid initial fall of in HIV-1 RNA level in response to ART.[4]

Tuberculosis (TB) is probably the most common manifestation of IRIS and most reports have been from South Africa.[5–8] The clinical manifestations associated with IRIS in India have been relatively less well-described,[9] particularly the influence of previously diagnosed TB on its incidence and presentation.

At the Tuberculosis Research Centres (TRCs) in Chennai and Madurai, south India, patients enrolled in clinical trials for the treatment and prevention of tuberculosis (NCT00376012 and NCT00351702) were followed regularly with clinical and laboratory monitoring. Consecutive patients initiating ART between July 2004 and June 2005 (when free ART became available through the government) were included in this nested case-control study. We compared the clinical and immunological features of patients who developed IRIS with those who did not. We made attempts to identify any pre-existing risk factors that might predict the occurrence of IRIS, particularly a prior history of TB. Further, we used this group of patients to validate a scoring system/algorithm developed by Robertson *et al.*[10] to predict IRIS. The Robertson score was based on a data-set derived from a predominantly Caucasian homosexual men population and its utility has not yet been tested in a resource-poor, high TB-burden, predominantly heterosexual epidemic setting. One previous report from Thailand suggested that this score may not be accurate in this region.

Correspondence to: Dr S Swaminathan, Department of Clinical Research, Tuberculosis Research Centre (ICMR), Mayor V.R. Ramanathan Road, Chetput, Chennai-600 031, India
Email: doctorsoumya@yahoo.com

METHODOLOGY

The study population consisted of participants enrolled in two different randomized clinical trials (RCT) performed at the TRC in Chennai and Madurai, India, between July 2000 and June 2005. The first trial ($n = 712$) compared the efficacy of two regimens for latent TB infection (LTBI) treatment: a six-month daily regimen of isoniazid (INH) and ethambutol (EMB) versus a three-year course of daily INH alone (in lieu of lifelong therapy). HIV-infected individuals without evidence of active TB were recruited for this trial, regardless of baseline CD4 count or tuberculin skin test results. Active TB was ruled out if patients were asymptomatic with a normal chest X-ray and had three negative *Mycobacterium tuberculosis* sputum cultures. The second trial ($n = 327$) compared the efficacy of treating active pulmonary TB with a six- versus a nine-month course of anti-TB treatment. Antiretroviral- and TB treatment-naïve HIV-infected patients with newly diagnosed pulmonary TB were recruited. Active TB was suspected based on history, physical examination and chest X-ray, and was confirmed by a positive acid-fast bacillus smear and/or culture for *M. tuberculosis*. All these patients were undergoing follow-up and were referred for ART initiation if eligible as soon as the Indian government rolled out its free ART programme in April 2004. The first 100 consecutive patients initiating ART were included in this study – patients who developed IRIS were the cases and those who did not develop IRIS were the controls.

The ART regimens used were zidovudine or stavudine (d4T) along with lamivudine (3TC) and nevirapine (NVP) or efavirenz, and the criteria for ART initiation were as per National AIDS Control Organization (NACO) guidelines at that time (World Health Organization [WHO] clinical stage 3 and 4 or CD4 count less than 200 cells/μL).[11] Patients were seen in the clinic every month and blood investigations performed every six months or sooner if clinically indicated. Other investigations such as chest X-ray, abdominal ultrasound, sputum, blood or urine cultures and cerebrospinal fluid examination were performed when indicated. Detailed records were maintained with physician notes and results of all tests in the source documents. Specialist opinion was obtained where necessary by referring patients to the nearest tertiary government medical college hospital. Case management was as per standard guidelines and outcomes were documented. A retrospective review of case records was undertaken by one of the authors (PP) and any events that occurred after ART initiation within one year were noted. A second physician (GN) independently examined the records and only those events that were picked by both as IRIS events were considered true cases. All the clinical manifestations of IRIS were documented.

IRIS was diagnosed using the following criteria (based on Robertson *et al.*):[10]

(1) Appearance of symptoms after starting ART among previously asymptomatic patients;
(2) Absence of a positive culture of the causative organism involved in IRIS at the time of symptoms;
(3) Rapid response to anti-inflammatory drugs; and
(4) Absence of drug-resistant organisms at baseline

Statistical analysis

Data were entered into Excel and analysed using SPSS (Chicago, IL, USA, version 13). Categorical variables were compared with the chi-squared test and continuous variables with the *t*-test or Wilcoxon rank sum test. A P value <0.05 was considered significant. Sensitivity and specificity of the Robertson score were calculated using data from all patients (those with and without IRIS).

RESULTS

In total, 97 patients had complete data available and were included in this analysis. A total of 34 patients (cases) developed IRIS, while 63 patients (controls) had no occurrence of IRIS. Age and sex ratios were similar between cases and controls; controls weighed more and had higher CD4 counts at baseline. Ninety seven percent of patients in the IRIS group and 84% in the non-IRIS group were treated with a d4T/3TC/NVP regimen, which is the standard regimen in India (Table 1). Seventy four percent of IRIS patients had a previous history of TB treatment and >70% had a history of three or more OIs; this was significantly more than controls.

The median duration from ART initiation to developing IRIS was 45.5 days (15.5–86.5) and 35% developed IRIS within 30 days. The range of CD4 counts at ART initiation was 8–189 cells/μL; CD4 was lower among patients developing IRIS and remained lower than controls at six months.

Table 2 describes the clinical manifestations of IRIS observed in this group of patients. Mucocutaneous manifestations were the most common, followed by an extra-pulmonary TB. To examine differences in CD4 response between early and later IRIS events, patients were classified into three groups, based on the time of IRIS presentation after starting ART: 0–30 days, 31–90 days and more than 90 days. Figure 1 shows the CD4 count at 0, 6 and 12 months in the three IRIS groups and controls. While baseline CD4 count was significantly lower among all IRIS groups compared with controls, the rate of change of CD4 count was not different between groups (Table 3).

Haemoglobin (Hb), CD8 T-cell count and occurrence and number of OIs prior to ART initiation were used to calculate the Robertson score for each patient. Among the 34 patients who developed IRIS, 31 had a Robertson score of 1 or greater while three patients had a score less than 1. Among patients who did not develop IRIS, 49 had a score of 1 or greater while 14 had a score of <1. The sensitivity of the Robertson criteria to predict IRIS was 91% and the specificity was 22%. Table 4 shows the distribution of Robertson scores in the IRIS and non-IRIS groups.

DISCUSSION

The introduction of ART has dramatically improved outcomes for persons living with HIV/AIDS and successful suppression of viral replication is followed by an increase in CD4+ lymphocytes and a partial recovery of T-cell-specific immune responses. However, some HIV-infected patients have clinical deterioration early in the course of ART; the event has been termed immune reconstitution inflammatory syndrome.

WHO reports the incidence of IRIS to be 10% among all patients initiating ART and up to 25% among patients initiating ART with a CD4 cell count below 50 cells/μL.[2] The incidence of IRIS in our cohort of patients with advanced HIV disease was 33% and was higher among those with a previous history of TB treatment. IRIS has been reported in 8–43% of patients co-infected with HIV and TB disease.[12] One-third of cases in the South African cohort had an initial diagnosis of pulmonary TB but later

Table 1 **Demographic, immunological and clinical characteristics of IRIS versus non-IRIS patients at enrollment (*N* = 97)**

	IRIS *N* = 34	Non-IRIS *N* = 63	*P* value
Demographics			
Gender			
Men (%)	62	64	NS
Women (%)	38	36	
Median age (years)	32 (28–36.3)	34 (29–38)	NS
Median weight (kg)	44.5 (40.8–52.2)	50.0 (42.4–56.3)	NS
ART regimens (%)			
*SLN	97	84	
ZLN	3	8	
SLE		8	
History of TB (%)	74	52	0.04
Median time from ART initiation to IRIS (days)	45.5 (15.5–86.5)		
Median time from TB diagnosis to ART (days)	288 (213–430)	294 (169–780)	NS
IRIS (%)			
0–30 days	36		
31–90 days	41		
>90 days	23		
CD4 at ART initiation (range), cells/μL	8–189		
At ART initiation			
Median haemoglobin (g/dL)	10.7 (8.9–12.2)	11.2 (9.7–12.3)	NS
Median CD4+ cell count (cells/μL)	63 (36–90.5)	105 (54–165.8)	0.002
Median CD4%	6 (3.5–9)	8 (5–11)	NS
Median CD8+ cell count (cells/μL)	472 (342–851.5)	724 (441.8–1037.3)	NS
Median CD8%	57 (47–66)	56 (45.3–63)	NS
CD4/CD8 ratio	0.11 (0.05–0.20)	0.16 (0.12–0.22)	NS
AST	30 (23–48)	26 (21–38)	NS
ALT	24 (16–30)	20 (12–31)	NS
Number of opportunistic infections:			
2 or fewer	23	46	0.01
3–5	59	51	
>5	18	3	
CD4 count (cells/μL)			
At 6 months	273 (152–374)	331 (225–434)	0.04
At 12 months	290 (216–402)	338 (250–482)	NS
CD8 count (cells/μL)			
At 6 months	792 (492–1484)	1144 (785–1568)	0.04
At 12 months	918 (714–1324)	1155 (838–1526)	0.05

All values are median (IQR) unless specified otherwise
*ART = antiretroviral therapy; SLN = Stavudine (d4T), Lamivudine (3TC), Nevirapine (NVP); ZLN = Zidovudine (ZDV, 3TC, NVP); SLE = d4T, 3TC, Efavirenz; TB = tuberculosis; IRIS = immune reconstitution inflammatory syndrome

Table 2 **Clinical manifestations of immune reconstitution inflammatory syndrome (IRIS) and days to IRIS event**

Description	%	CD4 at ART initiation, median (IQR)	Days to IRIS median (range)
Pulmonary tuberculosis	8.8	90 (60–91)	45 (17–78)
Extrapulmonary tuberculosis	20.6	66 (34–155)	66 (32–142)
PCP/pneumonitis	17.6	71 (27–103)	13 (8–120)
Mucocutaneous (herpes, PPD and fungal)	35.2	62 (32–117)	47 (21–94)
CMV retinitis	5.9	56 (48–63)	94 (32–155)
Cryptococcal meningitis	5.9	44 (18–70)	28 (16–39)
Mycobacterium avium complex	5.9	31 (12–50)	39 (14–63)

ART = antiretroviral therapy; PCP = Pneumocystis jiroveci pneumonia; CMV = cytomegalovirus; PPD = pruritic papular dermatitis

developed both pulmonary and intra-abdominal manifestations of IRIS during ART. Further, the risk of TB-associated IRIS is very high for those with low baseline CD4 counts initiating ART early in the course of anti-TB treatment.[5]

Interestingly, the main types of IRIS observed in our study were extra pulmonary TB and mucocutaneous lesions including herpes zoster, herpes simplex, pruritic papular dermatitis and other fungal skin infections. In our population, herpes zoster was one of the most common lesions to occur after initiation of ART and produced severe discomfort and pain. Therefore, it is important for physicians to be aware of this and to counsel patients accordingly. Pulmonary TB was less common than expected perhaps because patients in this study had all been previously screened, with over half having been treated for TB. All the patients improved with symptomatic management and no mortality was observed.

The most important factor predicting the likelihood of IRIS is considered to be a low baseline CD4 cell count. In our study, the median CD4+ cell count at ART initiation for the IRIS cases was 63 cells/μL, while the median CD4+ cell count for the controls was 105 cells/μL. In general, patients in resource-poor settings tend to initiate ART at an advanced stage of the disease and most have low CD4 counts at initiation. Therefore, this may not be a useful predictor for IRIS development in this population. Viral loads are not available in most settings though a trend towards higher HIV-1 RNA levels in patients who developed IRIS after starting ART has been described.[13] Other predictors of IRIS include initiating ART close to the time of diagnosis of an OI, being antiretroviral-naïve at the time of diagnosis of an OI and having a more rapid initial decrease in the HIV-1 RNA level in response to ART than in patients with higher counts.[2] In this study, there was no difference in the time between TB diagnosis and ART between the two groups and the lag was quite long. This may be because free ART became available only from April 2004 while this cohort was established in 2001 and many patients, although eligible, could not access ART in a timely manner.

The observation that the increase in CD4 counts at six months of initiating ART was greater in the non-IRIS group than in the IRIS group was surprising, as many previous studies have described a rapid increase in CD4 as a risk factor for IRIS. This suggests that inflammatory pathways other than an increased number of CD4 lymphocytes are involved in the development of IRIS.[14] Some studies suggest that circulating IL-6 levels prior to ART may be associated with IRIS and it is hypothesized that proinflammatory cytokines produced excessively in response to systemic bacterial LPS may lead to clinical deterioration and 'paradoxical worsening' of inflammatory responses against both infectious and non-infectious microbial antigens.[15] A greater number of prior OIs in the IRIS group may signify the presence of significant residual antigens that could confer an increased risk for immune reconstitution syndrome.[10] In our study, multiple prior OIs were present more often in cases than controls.

Robertson *et al.*[10] developed a statistical model to predict IRIS, that was based on the number of prior OIs, the patient's CD8+ T-cell count and Hb level prior to initiation of ART. A

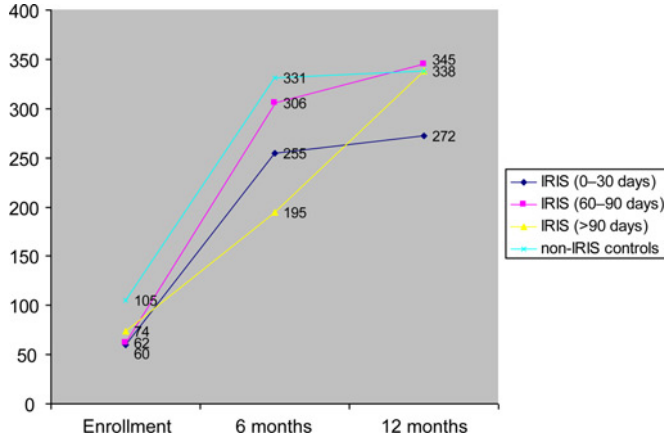

Figure 1 **Change in CD4 count in the three immune reconstitution inflammatory syndrome (IRIS) groups and controls at six and 12 months after antiretroviral therapy initiation**

score of 0 was given if the patient had ≤1 OI; 1 if the patient had >1 OI, a baseline CD8+ T-cell count >468.5 cells/μL and a baseline Hb level >10.2 g/dL; 2 if a patient had >1 OI, a baseline CD8+ T-cell count >468.5 cells/μL and a baseline Hb level ≤10.2 g/dL; 3 if patient had >1 OI, a baseline CD8+ T-cell count of ≤468.5 cells/μL and a baseline Hb level >13.6 g/dL; and 4 if a patient had >1 OI, a baseline CD8+ T-cell count of >468.5 cells/μL and a baseline Hb level ≤13.6 g/dL. A score of greater than or equal to 1 in the predominantly Caucasian, homosexual population studied by the authors was proposed as being predictive for the development of IRIS. However, Zhou and Ditangco[16] reported that the model did not accurately predict IRIS among Asian HIV-infected patients (enrolled in a prospective cohort in 15 sites in the Asia Pacific region) and proposed that it might be related to the different epidemiology of HIV-related illnesses, in particular, the greater rates of TB among their population. In our study, the Robertson score had a sensitivity of 91% with a score of > or equal to 1 in 31 out of 34 cases. However, because a large proportion of controls also had prior OIs, the specificity was only 22%. This scoring system is therefore unlikely to be useful in this geographic region and further research is required to define more accurate predictive models.

The INSHI is an international collaborative network of investigators interested in IRIS.[17] The purpose of the collaboration is to harmonize ongoing and future studies in utilizing similar case definitions and measurements to enable cross-study comparisons. A key focus of INSHI is to facilitate collaborations among international investigators to enhance future IRIS research in prospective cohort studies and randomized trials bringing together expertise in epidemiology, immunology and basic science to enhance IRIS-related research. Future studies should focus on evaluating the INSHI criteria for diagnosing IRIS as well as TB-associated IRIS and studying risk factors and treatment strategies. The issue of whether ART should be initiated early or late, considering the complications of AIDS versus the management of IRIS is debatable. Current guidelines are mostly in favour of initiating ART earlier.[12] Patients in the South African cohort who initiated ART in the first month of TB treatment had a 70-fold greater risk of IRIS than patients starting ART beyond three months of TB treatment.[5] However, delaying ART is associated with unacceptably high levels of mortality.[12] Mortality due to IRIS has previously been reported to be very low, and in our study there was no mortality due to IRIS.

The limitations of our study include the relatively small sample size, the advanced disease status of this cohort and the lack of immunological investigations other than CD4 and CD8 counts. However, the detailed clinical characterization of the cohort, the good follow-up and record of intercurrent illnesses are the main strengths.

In conclusion, we have found that among patients starting antiretroviral treatment in south India, IRIS is a fairly common event especially among patients with a previous history of TB. A prediction model for IRIS suggested by Robertson *et al.* had poor specificity as did the rate of rise in CD4 count. Perhaps the best strategy to reduce the incidence of IRIS would be to initiate antiretroviral treatment at higher CD4 counts before patients have had too many OIs.

Table 3 **CD4 count change in patients developing immune reconstitution inflammatory syndrome (IRIS) at various time points after ART and those who did not**

	CD4 at enrollment (cells/μL)	CD4 at six months (cells/μL)	CD4 at 12 months (cells/μL)
IRIS (0–30 days) (*N*=12)	60 (30–75)	255 (174–378)	272 (216–315)
IRIS (30–90 days) (*N* = 14)	62 (20–101)	306 (118–430)	345 (201–607)
IRIS (>90 days) (*N* = 8)	74 (44–108)	195 (136–286)	338 (238–379)
Non-IRIS controls (*N* = 63)	105 (55–166)	331 (225–434)	338 (250–482)

Table 4 **Distribution of Robertson scores in immune reconstitution inflammatory syndrome (IRIS) and non-IRIS groups**

Score	IRIS (*n* = 34)	Non-IRIS (*n* = 63)
0	3 (8.8%)	14 (22.2%)
1	9 (26.5%)	20 (31.8%)
2	6 (17.6%)	13 (20.6%)
3	2 (5.9%)	0 (0%)
4	14 (41.2%)	16 (25.4%)

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