International Journal of Infectious Diseases 98 (2020) 261–267

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Characteristics of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome and its influence on tuberculosis treatment outcomes in persons living with HIV

Gopalan Narendran[a], Keerthana Jyotheeswaran[a,1], Thirumaran Senguttuvan[a,1], Caian L. Vinhaes[b,c,d,1], Ramesh K. Santhanakrishnan[a], Tamizhselvan Manoharan[a], Anbhalagan Selvaraj[a], Padmapriyadarsini Chandrasekaran[a,2], Pradeep A. Menon[a,2], Kannabiran P. Bhavani[a,2], Devarajulu Reddy[a,2], Ravichandran Narayanan[e], Balaji Subramanyam[a], Sekhar Sathyavelu[f], Raja Krishnaraja[e], Pownraj Kalirajan[a], Dhanalakshmi Angamuthu[a], Stella Mary Susaimuthu[a], Ranjit R.K. Ganesan[g], Srikanth P. Tripathy[a], Soumya Swaminathan[h], Bruno B. Andrade[b,c,d,i,j,k,*]

[a] National Institute for Research in Tuberculosis, Indian Council of Medical Research Chennai, Tamil Nadu, India
[b] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil
[c] Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador, Brazil
[d] Curso de Medicina, Faculdade de Tecnologia e Ciências (UniFTC), Salvador, Brazil
[e] Government Hospital of Thoracic Medicine, Tambaram Sanatorium, Chennai, Tamil Nadu, India
[f] Rajiv Gandhi Government General Hospital, Park Town, Chennai, Tamil Nadu, India
[g] Government Rajaji Hospital, Madurai, Tamil Nadu, India
[h] World Health Organization, Geneva, Switzerland
[i] Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Brazil
[j] Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, Brazil
[k] Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

ARTICLE INFO

Article history:
Received 21 May 2020
Received in revised form 27 June 2020
Accepted 29 June 2020

Keywords:
HIV
Tuberculosis
Antiretroviral therapy
Anti-TB treatment
IRIS
Paradoxical reaction

ABSTRACT

Objective: The influence of tuberculosis (TB)-immune reconstitution inflammatory syndrome (IRIS) on TB treatment outcomes and its risk factors were investigated among people with human immunodeficiency virus (HIV) and co-infected with TB.
Methods: Newly diagnosed, culture-confirmed, pulmonary TB patients with HIV and enrolled in a clinical trial (NCT00933790) were retrospectively analysed for IRIS occurrence. Risk factors and TB outcomes (up to 18 months after initiation of anti-TB treatment [ATT]) were compared between people who experienced IRIS (IRIS group) and those who did not (non-IRIS group).
Results: TB-IRIS occurred in 82 of 292 (28%) participants. Significant baseline risk factors predisposing to TB-IRIS occurrence in univariate analysis were: lower $CD4^+$ T-cell count, CD4/CD8 ratio, haemoglobin levels, presence of extra-pulmonary TB focus, and higher HIV viral load; the last two retained significance in the multivariate analysis. After 2 months of ATT commencement, sputum smear conversion was documented in 45 of 80 (56.2%) vs. 124 of 194 (63.9%) ($p = 0.23$), culture conversion was in 75 of 80 (93.7%) vs. 178 of 194 (91.7%) ($p = 0.57$) and the median decline in viral load (log_{10}copies/mm^3) was 2.7 in the IRIS vs. 1.1 in the non-IRIS groups ($p < 0.0001$), respectively. An unfavourable response to TB therapy was detected in 17 of 82 (20.7%) and 28 of 210 (13.3%) in the IRIS and non-IRIS groups, respectively ($p = 0.14$).

* Corresponding author at: Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, no. 121, Candeal, Salvador, Bahia 40269-710, Brazil.
E-mail address: bruno.andrade@fiocruz.br (B.B. Andrade).
[1] KJ, TS and CLV contributed equally to the work.
[2] PC, PAM, KPB and DR contributed equally to the work.

https://doi.org/10.1016/j.ijid.2020.06.097

Conclusions: TB-IRIS frequently occurred in people with advanced HIV infection and in those who presented with extra-pulmonary TB lesions, without influencing subsequent TB treatment outcomes.

Introduction

Paradoxical tuberculosis (TB)-immune reconstitution inflammatory syndrome (TB-IRIS) is the apparent clinical or radiological deterioration seen in pre-existing lesions or emergence of new TB lesions after anti-retroviral therapy (ART) initiation among human immunodeficiency virus (HIV)-TB coinfected patients, despite temporary improvement with anti-TB treatment (ATT) and effective virological suppression (Gopalan et al., 2014; Lawn et al., 2005; Lawn et al., 2007). The dynamics of this phenomenon that frequents concomitant therapy, especially in the scenario of advanced HIV and early ART initiation, is explained by the cytokine storm that becomes unleashed with ART (Bourgarit et al., 2006; Tadokera et al., 2011). Important risk factors predisposing to IRIS occurrence include lower levels of $CD4^+$ T-cell count (French et al., 2004; Haddow et al., 2012; Murdoch et al., 2008; Worodria et al., 2012), CD4/CD8 ratio (Breton et al., 2004), haemoglobin (Haddow et al., 2012; Worodria et al., 2012), higher viral load (Haddow et al., 2012; Narendran et al., 2013), and shorter ATT-ART interval (Meintjes et al., 2008; Naidoo et al., 2012; Narendran et al., 2013). It has been demonstrated that there are two prerequisites that are necessary for IRIS occurrence: (i) a long-standing infection and (ii) an advanced immunodeficiency status. Both hamper pathogenic clearance, leading to tissue accumulation and dissemination of antigens (Barber et al., 2012). IRIS is likely to occur with the reconstitution of pathogen-specific responses against these TB antigens, whether dead or alive, brought about by starting ART (Cevaal et al., 2019; Sereti et al., 2010). Thus, IRIS is most likely to manifest within the first 3 months of starting ART (French et al., 2004; Lawn et al., 2005; Meintjes et al., 2008; Namale et al., 2015).

Tuberculosis not only hails as the commonest opportunistic infection in people with HIV but also accounts for the largest number of IRIS cases (Haddow et al., 2012; Namale et al., 2015). A recent systematic review of 7789 HIV-TB co-infected patients reported a pooled IRIS incidence of 18% (95% CI 16–21%), with pulmonary lesions followed by lymph node enlargements as frequent presentations of IRIS (Namale et al., 2015). Many studies have clearly documented the survival advantage of commencing ART early, despite the increased risk of TB-associated IRIS, with the severity and duration intensifying with a shorter ATT-to-ART interval (Naidoo et al., 2012; Sereti et al., 2010). Polymorphisms in the genes encoding for the enzyme leukotriene A4 hydroxylase may also play a role in severity and duration of TB-IRIS (Narendran et al., 2016). A prospective study in India conducted in a cohort of both ATT and ART-naïve patients who were sequentially started on ATT followed by ART showed the TB-IRIS incidence to be up to 54% (Narendran et al., 2013). Even though the role of ART on TB and HIV outcomes has been well described (Abay et al., 2015; Chareesil et al., 2019; Kumarasamy et al., 2013; Naidoo et al., 2012), there is a dearth of information on the influence of TB-IRIS, per se, on subsequent TB treatment outcomes. Therefore, the current study analysed data from a randomised clinical trial (RCT), comprising a well-characterised cohort of people with HIV with newly diagnosed, sputum culture-confirmed pulmonary TB, to effectively describe TB-IRIS characteristics and also evaluate the influence of TB-IRIS on subsequent TB outcomes.

Methods

Study subjects

An RCT (NCT00933790)–conducted at the Indian Council of Medical Research, National Institute for Research in Tuberculosis (ICMR-NIRT), formerly Tuberculosis Research Centre, South India, between 2009–2016 – provided the data required for analysis in the current study (Gopalan et al., 2018). Study participants were enrolled if: aged ≥18 years, with newly diagnosed, sputum culture-confirmed (solid media), rifampicin-sensitive TB (<128 μg, by the MIC method), and with ATT-naïve HIV. They had to be willing to participate in TB treatment under supervision and signed a written and dated informed consent. Approval was obtained from the Institutional Human Ethics Committee.

Investigations

Laboratory investigations included: two rapid tests for HIV (TriDot-J Mitra M/s and Retroquic Qualpro diagnostics, India); Hepatitis B (HEPA card, Diagnostic enterprises, India); Hepatitis C (HCV tri-DOT, Diagnostic enterprises, India); full blood count (automated haematology analyser ABX, France); $CD4^+$ T-cell count (FACS count flow cytometer, Becton Dickinson, USA); quantification of plasma HIV-1 viral load (Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, Germany); liver and renal function tests; and random plasma glucose (automated analyser, Olympus Corporation, Tokyo, Japan). A standard chest X-ray with poster-oanterior view was taken and reviewed by two independent readers from a group of four chest physicians. All investigations except ELISA for HIV-1 and HIV-2 were periodically repeated at (i) baseline, (ii) at IRIS event (IRIS cases) or 2 months after starting ART (in non-IRIS controls), (iii) at the end of 6 months of ATT, and iv) at 18 months after ATT commencement and whenever clinically indicated. Sputum smears were collected every month up to 18 months (three specimens during the treatment period and two during follow-up, i.e. 7–18th months and whenever clinically indicated), examined by fluorescence microscopy, processed by the modified Petroff's method, and cultured on Lowenstein-Jensen medium (LJ), with species identification and drug susceptibility testing performed, if positive, on LJ culture (Bryan and Allen, 1968; Canetti et al., 1969).

TB treatment details and evaluation of TB outcome

Standard four drug ATT–comprising ethambutol (E), rifampicin (R), pyrazinamide (Z) and isoniazid (H)–was started in the first 2 months of the induction phase (intensive phase) followed by H and R for 4 months (in the continuation phase); it was administered daily or intermittently as per trial randomisation (Gopalan et al., 2018). The three regimens were daily ($2EHRZ_7/4HR_7$), partly daily ($2EHRZ_7/4HR_3$) and fully intermittent ($2EHRZ_3/4HR_3$). Drug intake was directly observed for the entire 6-month treatment period, except during weekends. The ART regimen consisted of either stavudine (30/40 mg BD) or zidovudine (300 mg BD) initially, or tenofovir (300 mg OD) at a later date, given along with lamivudine (300 mg OD/150 mg BD) and efavirenz (600 mg OD), initiated as an in-patient for an initial 2 weeks, as per prevailing national guidelines (India, 2007, 2013). TB treatment outcomes were

classified as favourable if sputum cultures collected during the last 2 months of TB treatment and follow-up were available and negative for *Mycobacterium tuberculosis* (*M. tuberculosis*). Unfavourable responses in this study included failures, recurrences and death during treatment, as previously published (Gopalan et al., 2018).

TB-IRIS diagnosis and treatment

Diagnosis of IRIS was confirmed by using the INSHI-2008 consensus definition (Meintjes et al., 2008) fulfilling additional criteria of a 0.5-$\log_{10}$ decline in HIV viral load values, with *M. tuberculosis* culture from sputum being negative or downgraded from baseline around the IRIS event and/or any other biological specimen collected at the IRIS event being negative for *M. tuberculosis* (Narendran et al., 2013). A panel of independent physicians was presented with clinical records of patients manifesting signs and symptoms suggestive of paradoxical IRIS, supplemented with relevant investigations. Routine work-up of febrile episodes, to rule out other endemic causes of fever, was performed. Those who fulfilled the above-mentioned criteria were labelled as IRIS cases. A separate endpoint review committee was formed for adjudicating the TB outcomes. Non-steroidal anti-inflammatory drugs were initially prescribed for a few days for those experiencing IRIS phenomena, followed by prednisolone at a dose of 0.5–2 mg/kg body weight and tapered over a 2-to-8-week period, depending on the severity and response and on a case-by-case basis, as previously described (Narendran et al., 2013, Swaminathan et al., 2010). Parenteral steroids were only administered in severe cases, with dexamethasone starting 8 mg twice a day, tapered for 1–2 weeks and then to oral steroids for the next 4–6 weeks.

Statistical analysis

TB-IRIS incidence was estimated and its characteristics revisited. The entire cohort was subdivided into two groups: those experiencing TB-IRIS (TB-IRIS group) and those with an uneventful immune recovery (non-IRIS group). Baseline (pre-treatment) risk factors for IRIS occurrence were compared. To identify the possible predisposing factors associated with TB-IRIS occurrence, univariate analysis was performed with calculations of odds ratio (OR) values, including important determinants such as: $CD4^+$ T-cell count, CD4/CD8 ratio values, haemoglobin levels, presence/absence of other opportunistic infections, presence/absence of extrapulmonary focus of TB lesion, ATT regimen, extent of radiological involvement (fewer or more than three zones) on chest X-ray, sputum acid fast bacilli (AFB) smear and culture for *M. tuberculosis* (grades of <2 and >2+), and ATT-to-ART interval. All proportions were tested for significance using the Pearson's Chi-square test, while the Mann–Whitney *U* test was used for continuous variables, taking a *p*-value <5% as the significance threshold level. A multivariate binary logistic regression model was constructed taking significant factors (i.e. $p < 0.05$) into consideration. Kaplan–Meier curves were plotted for each stratum of $CD4^+$ T-cell count at baseline, to calculate the IRIS-free survival probability in weeks, with values censored at 24 weeks. The mean survival estimates were compared using the log-rank test. Factors determining TB outcomes at 2, 6 and 18 months post-ATT commencement were evaluated to ascertain if there was a difference that existed between the IRIS and non-IRIS groups. An 18-month observational period was chosen because most TB recurrences occurred within the first year after completion of ATT (Leone et al., 2010). All statistical analyses were performed using IBM SPSS Version 25.0 software. As part of this study, findings were presented as a poster at the 50th Union World Conference on Lung Health, India (Narendran and Chandrasekaran, 2020).

Results

Incidence and characteristics of TB-IRIS

The parent RCT included 331 patients, of which 292 were culture-confirmed, rifampicin-sensitive pulmonary TB cases (by solid cultures). In this TB-IRIS study, 292 were included in analysis to diagnose IRIS and segregate multidrug-resistant TB (MDR-TB) cases. Furthermore, 82 (28%) patients developed paradoxical TB-IRIS, with pulmonary lesions in 30 and extra pulmonary diseases in 52. The incident curve of TB-IRIS peaked in the first 15 days after ART and began to fade after 45 days, exhibiting a similar temporal trend in both types of lesions (Figure 1). Extra pulmonary manifestations predominated over pulmonary deterioration with lymph node enlargement being the commonest IRIS manifestation (33/82), followed by pulmonary infiltrates (22/82). The incidence of IRIS was 73/257 (28.4%) among ART-naïve patients who started ATT first followed by ART initiation, compared with 9/45 (20%) among newly diagnosed TB patients who were already on ART and subsequently started ATT ($p = 0.279$). Of note, the latter group experienced IRIS only after both therapies were started.

Predisposing factors of paradoxical TB-IRIS

Tables 1 and 2 show comparisons of the baseline demographic characteristics between the subgroups of patients who further developed IRIS and those who had uneventful follow-up. Baseline data were comparable with respect to age, frequency of males, weight, and ATT-to-ART interval duration. In the univariate analysis, there were significantly lower $CD4^+$ T-cell counts, lower CD4/CD8 ratio values, lower haemoglobin concentrations in peripheral blood, with higher HIV viral loads and a greater frequency of detected extra-pulmonary TB foci present in the IRIS group when compared with the non-IRIS group (Table 1). IRIS cases were 48/184 and 34/98 ($p = 0.03$) among those undertaking daily and intermittent ATT regimens during the intensive phase of therapy ($p = 0.03$). In a multivariate binary logistic regression, low baseline $CD4^+$ T-cell count (OR 2.3, 95% CI 1.2–4.2; $p = 0.009$) and presence of extra-pulmonary TB focus at baseline (OR 3.58, 95% CI 2.0–6.3; $p < 0.0001$) remained independently associated with TB-IRIS (Table 2). Using Kaplan Meier survival plots, it was deduced that the tendency or risk for IRIS occurrence was approximately doubled if the pre-ART $CD4^+$ T-cell count was <100 cells/mm^3, whereas such risk was significantly reduced in patients with $CD4^+$ T-cell count >350 cells/mm^3 ($p < 0.001$) (Figure 2).

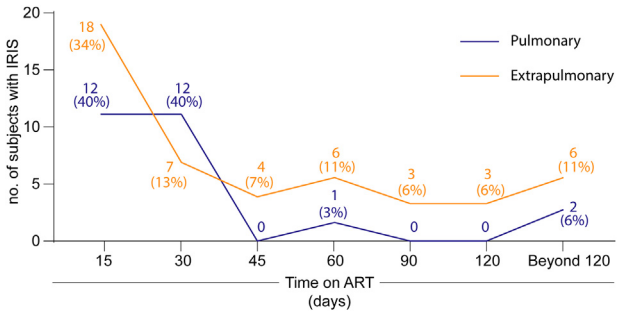

Figure 1. Temporal distribution of IRIS cases according to site of lesion after starting ART (in days). Eight patients (three with pulmonary and five with extrapulmonary TB) had IRIS with ATT itself before starting ART. Those who had IRIS after ART are depicted in the graph. Of those who developed IRIS within the first month of starting ART, 43/49 cases had culture negative while six had cultures downgraded from 3+ to 1+ or few colonies, confirming response to ATT.

Table 1
Characteristics of the study population at baseline.

Characteristic	TB-IRIS $n = 82$	Non-IRIS $n = 210$	p-Value
Age (years)	39 (32–45)	38 (33–44)	0.7
Weight (kg)	42 (37–48)	43 (37–49)	0.2
ATT-to-ART Interval (days)	17 (2–45)	16 (9–33)	0.8
$CD4^+$ T-cell count, per μL	92 (45–175)	159 (90–287)	<0.0001
CD4/CD8 ratio	0.17 (0.08–0.36)	0.24 (0.15–0.41)	0.001
Plasma HIV RNA – $\log_{10}$ copies/mL	5.5 (5.2–5.8)	5.2 (4.0–5.7)	<0.00001
Haemoglobin (g/dL)	9.2 (7.2–10.6)	9.6 (8.4–11.5)	0.026

Data, except for proportions, represent medians and interquartile ranges.
The Mann–Whitney *U* test was used to compare continuous variables between the groups and the distributions of age whereas Fisher's exact test was used to compare frequency distribution.

Table 2
Predisposing factors associated with TB-IRIS.

Parameter (numbers/percentage)	TB-IRIS $n = 82$	Non-IRIS $n = 210$	OR (95% CI)	p-value
Male	68 (83)	160 (76)	1.15 (0.78–2.92)	0.2
Extra-pulmonary TB focus present	48 (59)	57 (27)	3.78 (2.22–6.46)	<0.0001
Chest X-ray >3 zones	51 (62)	146 (69)	0.72 (0.42–1.23)	0.23
On daily ATT regimen	27 (32)	71 (33)	0.96 (0.55–1.65)	0.88
Opportunistic infection present	24 (29)	44 (21)	1.56 (0.87–2.78)	0.13
AFB smear grade >2+	37 (45)	107 (50)	0.79 (0.47–1.32)	0.37
Mycobacterium tuberculosis culture grade >2+	58 (71)	142 (67)	1.15 (0.63–2.01)	0.60

Data represent distribution of possible risk factors associated with TB-IRIS between groups.
A logistic regression model was performed to assess the odds ratios (OR) and 95% confidence intervals (CIs).

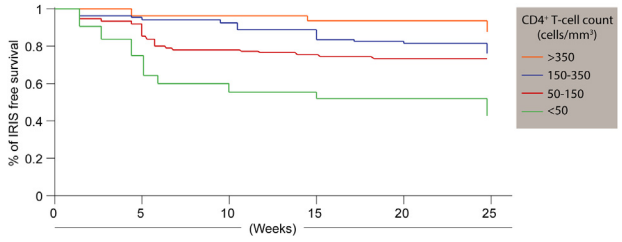

Figure 2. Kaplan–Meier survival curves depicting IRIS–free survival probability, among HIV-TB coinfected patients categorised by baseline $CD4^+$ T-cell count values (cells/μL).

Steroid treatment in IRIS

Among IRIS cases, 59/82 (72%) required treatment with steroids. Twenty-three did not require steroids, with nine of them having a cold abscess arising from the lymph nodes, requiring only incision and drainage. Forty-four patients required oral steroids (prednisolone) whereas 15 of them required initial parenteral steroids (dexamethasone) for 7–12 days followed by oral steroids tapered over 3–4 weeks (see Methods for more details). Of the 15 patients who required parental dexamethasone, 10 had lesions in the central nervous system, two were abdominal IRIS, one had multi-organ dysfunction syndrome, and two had bulky mediastinal disease. Of these 15 cases, four (two with central nervous system involvement and two with abdominal involvement) had to temporarily stop their ART, which was resumed after IRIS subsided under cover of steroids as inpatients in the hospital.

Impact of IRIS on TB outcomes

At the end of 2 months of ATT, frequency of smear conversion and culture conversion, which are considered surrogate markers of subsequent TB treatment outcomes, was between the distinct clinical groups (TB-IRIS vs. non-IRIS) (Table 3). Regarding immune restoration after ART: the median decline in HIV viral load values was significantly greater ($p = 0.0001$), whereas the increase in $CD4^+$ T-cell counts was slightly higher in the IRIS group ($p = 0.08$) when compared with the non-IRIS group. At the end of 6 months of ATT, as well as at 18 months after starting ATT, the frequency of unfavourable TB treatment outcomes (failure to TB therapy, mortality, recurrences) was similar between the clinical groups (Table 3).

Table 3
Response to TB therapy and immune recovery in both study groups at different study timepoints.

Parameter/Outcome	TB-IRIS $n = 82$	Non-IRIS $n = 210$	p-value
TB culture negative at 2 months (%)[#]	75 (94)	178 (92)	0.57
Smear negative for AFB at 2 months (%)[#]	45 (56)	124 (64)	0.23
Log-10 reduction in HIV viral load from baseline to IRIS or month 2 of ART, median (IQR)	2.7 (2.0–3.2)	1.1 (0–2.5)	< 0.0001
$CD4^+$ T cell count increase from baseline to IRIS or month 2 of ART, median (IQR)	114 (17–189)	75 (8–158)	0.08
Unfavourable TB outcomes (%)	19 (23.1%)	35 (16.6%)	0.14
TB treatment failure (end of Rx – 6 months)	4	7	
Deaths (at 6 months)	6	12	
TB recurrence (in up to 18 months of follow up)	7	9	
Deaths during follow-up	2	7	

The Mann–Whitney *U* test was used to compare continuous variables between the groups and the distributions of age whereas Fisher's exact test was used to compare frequency distribution.
[#] Available sputum specimen at 2 months was 80 and 190 in IRIS and non-IRIS, respectively. Rx, treatment.

Discussion

It is believed that this is probably the first report of a sequential comparison of TB characteristics, between IRIS and non-IRIS groups, studied from pre-treatment (before ATT administration) until 18 months after ATT, to evaluate the possible impact of TB-IRIS on TB outcomes. With adherence ensured and drug-resistant TB ruled out early, IRIS was diagnosed with better precision. Tuberculosis leads to an expressive increase in immune activation, as recently described in an Indian population (Oliveira-de-Souza et al., 2019). Sputum culture-confirmed pulmonary TB itself signifies a more severe clinical form, in the context of advanced HIV. The heightened levels of inflammatory markers in both pulmonary and extrapulmonary forms justify the need to explore the dynamics of TB-HIV coinfection when dually infected patients experience TB-IRIS (Vinhaes et al., 2019).

The current findings demonstrated that the occurrence of TB-IRIS had no impact on TB outcome at any point in time from IRIS occurrence until 18 months after ATT, despite important baseline differences that existed between the IRIS and non-IRIS groups. It was hypothesised that the dysregulated immune regulation of IRIS, although it results in exaggerated immune activation, becomes reduced by anti-inflammatory agents, making it inconsequential in the protracted course of TB therapy. This study also supports the shift from switching to a daily ATT regimen during the intensive phase, as it probably offers protection against IRIS breakdown due to greater antigenic clearance, as evidenced by a higher culture conversion, as described in the parent RCT (Gopalan et al., 2018). However, the logistic regression analysis indicated that the effect of ATT regimen on risk of IRIS was nullified due to more important factors such as advanced HIV and extrapulmonary TB, which remained significantly associated with IRIS, independent of other factors included in the adjusted model. Thus, other determinants aside from ATT regimen played a more significant role in determining the risk of IRIS.

It is equally surprising that this robust recovery of the immune responses in the IRIS group did not translate into higher rates of smear or culture conversion in sputum. As clear evidence exists on the benefit of steroids in reducing IRIS-associated mortality and the duration of hospitalisation, even when lower doses were used (Meintjes et al., 2010; Swaminathan et al., 2010), steroids were prescribed for treating IRIS episodes. The clinically reassuring fact is that steroid therapy in IRIS did not influence the occurrence of unfavourable TB treatment outcomes or sputum positivity at the end of treatment. This was observed regardless of the fact that steroids were started early in the course of ATT, despite advanced HIV and sputum culture positivity for *M. tuberculosis* when ART was provided concomitantly.

In the current study, the higher decline in HIV viral load and an increase in $CD4^+$ T-cell count observed in the IRIS group when compared with the non-IRIS group, around the end of the intensive phase of TB treatment, could have been the reason for IRIS to occur. An earlier report by Breton et al. demonstrated a significant increase in CD4/CD8 ratio values in the IRIS group, with a modest increase in $CD4^+$ T-cell count and decline in HIV viral load, probably due to a smaller number of patients who were investigated (Breton et al., 2004). On the contrary, the studies by Chareseel et al. and Kumarasamy et al. found no difference in increase of $CD4^+$ T-cell count or decline in HIV viral load at 6 and 12 months after ART initiation among IRIS and non-IRIS patients (Chareesil et al., 2019; Kumarasamy et al., 2013).

The following characteristics of the study design (Namale et al., 2015; Narendran et al., 2013) probably contributed to a higher incidence and detection rate of IRIS in the current study: (i) routine inpatient hospitalisation during the initial 2 weeks of ART initiation, as mandated by National HIV India, (ii) directly observed therapy for TB and (iii) confirmed pulmonary TB with advanced HIV. Luetkemeyer et al. found a four-fold risk of IRIS (12.6% vs. 3.2%) among confirmed TB cases compared with probable TB cases (Luetkemeyer et al., 2014). It is well known that IRIS incidence surges during the initial months of ART (Blanc et al., 2011; Lawn et al., 2005; Namale et al., 2015). In the current analysis, patients on ART diagnosed with pulmonary TB and started on ATT had a similar incidence of TB-IRIS as those who were initiated on ATT first and subsequently started on ART.

In this study, the extra-pulmonary TB focus, apart from low $CD4^+$ T-cell count, proved to be an important clinical parameter predisposing to a four-fold risk of IRIS. Presence of an extra-pulmonary TB focus, as a potential surrogate marker for persistent antigenemia and disseminated TB, has been reported in various studies (Meintjes et al., 2010; Namale et al., 2015), potentiating the likelihood of IRIS occurrence up to eight times (Manosuthi et al., 2009).

The factors predisposing to IRIS that were identified in this study–such as lower $CD4^+$ T-cell count, CD4/CD8 ratio, low haemoglobin, higher HIV viral load, and presence of extra-pulmonary TB–are all direct or indirect surrogates of advanced HIV (Haddow et al., 2012; Lawn et al., 2007; Namale et al., 2015; Worodria et al., 2012). This fact supports the World Health Organization's (WHO) policy of 'test and treat', which can play a major role in mitigating IRIS occurrence (WHO, 2015). Early ART initiation due to a change in guidelines and that some of the patients were already on ART while diagnosed with TB could have dampened the influence of a shorter ATT-to-ART interval as a predisposing factor for IRIS in the current retrospective analysis compared with a previous prospective study (Narendran et al., 2013).

The main strength of this retrospective study was the selection of a population from a clinical trial cohort, which was well characterised, analysing only rifampicin-sensitive pulmonary TB patients, which immediately excluded drug-resistant TB. Of note, MDR-TB is reported as the closest mimic of TB-IRIS (Meintjes et al., 2009). Apart from using the INSHI criteria, the accuracy of TB-IRIS diagnosis in the present study was enhanced by the inclusion of additional criteria such as at least a 0.5 log decline in HIV viral load and sputum cultures, which had to be either downgraded or negative, around the time of IRIS diagnosis (Narendran et al., 2013). Patients were followed up for a considerable period of 18 months, with close monitoring to help to increase the validity of the findings.

This study had some limitations. At first glance, the study may have been slightly underpowered due to the relatively small sample size. Nevertheless, the validity of the findings was still significant, considering the fact that this unique study could not have been prospectively performed and IRIS was precisely confirmed based on stringent criteria, as described above, to avoid misdiagnosis of MDR-TB cases. Because the study only included pulmonary TB patients with sputum culture positive for *M. tuberculosis* it was not possible to extrapolate the findings to exclusive extra-pulmonary TB cases or cohorts of smear-negative TB patients. No extensive active diagnostic workup for extra-pulmonary involvement of pulmonary TB was performed at baseline. Hence, there is some uncertainty as to whether the extra-pulmonary foci, which erupted at the time of IRIS, was new or simply an unmasking type. Although all patients requiring steroid therapy started treatment at a dose of 0.5–1 mg/kg body weight, the duration of steroid therapy varied from patient to patient, as per the discretion of the treating physician, and heterogeneity did exist. However, there was no recurrence of TB-IRIS in any of the patients on stopping steroid therapy, except in one patient who had a multifocal presentation (Narendran et al., 2019).

Conclusions

In conclusion, IRIS occurrence or its containment with steroids had no influence on subsequent TB outcomes when studied progressively from IRIS occurrence to 18 months after ATT. Advanced immunodeficiency, especially in the presence of an extra-pulmonary TB focus at pre-ART, followed by rapid decline in HIV viral load after ART initiation predisposed to higher frequency of IRIS.

Ethics approval and consent to participate

All clinical investigations were performed following the principles outlined in the Declaration of Helsinki. Ethics approval and waiver of consent to participate was approved by the Institutional Human Ethics Committee of the Indian Council of Medical Research, National Institute for Research in Tuberculosis, India. The parent clinical trial (NCT0933790) was also approved by the Institutional Human Ethics Committee of the Indian Council of Medical Research and all study participants signed informed consent before enrolling into the study.

Consent for publication

Consent for publication not applicable.

Contributions

Conceived and designed the study: GN, BBA, TS, SS; performed the experiments: GN, BBA, SA, SMS, SB; physicians and research staff involved in screening, recruitment, management and follow up: SRK, CPP, PAM, PKB, DR, NR, RKR, AD, SMS, SPT, SS; helped in monitoring ART and inpatient hospitalisation: NR, SS, RKR, RRKG, SeS; data capture, quality assurance, data cleaning and analysis: GN, BBA, KJ, TS, TM, CLV, KP, DL, SS; wrote the paper: GN, ABB, KJ, TS, CLV.

Data availability statement

The datasets generated during and/or analysed during the current study are available from Dr Gopalan Narendran on reasonable request.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

We sincerely acknowledge the valuable work of Irini Sereti (National Institutes of Health [NIH], USA), Brian Porter (NIH), Alan Sher (NIH), Lis Antonelli (Fundação Oswaldo Cruz, Brazil), Cynthia Lassnoff (NIH), Christopher Whalen (NIH), Michael Duvenhage (NIH) and Drs Thomas Nutman, Subash Babu and Aleyamma Thomas from NIH who provided valuable input and periodical guidance. We deeply appreciate the work of the team members at the National Institute of Research in Tuberculosis (NIRT), India: Lokeshwaran Nithyananthan, Solomon Priyakumar, Rajasekaran Subramani and Ramesh Babu, for clinical procedures; Syed Hissar, Kannan, Murugesan, Sudha Subramanium, Luke Elizabeth Hanna for lab support. We also thank the entire team of medical officers and nursing staff of NIRT-Chennai, NIRT-Madurai and the Government Hospital of Thoracic Medicine. We finally acknowledge the excellent secretarial assistance given by Sanathanakumar Natarajan and Maheshwari Theyagarajan (NIRT). This retrospective study did not receive specific funding. The work from BBA was supported by the Intramural Research programme of the Fundação Oswaldo Cruz (FIOCRUZ), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; senior scientist fellowship). CLV received fellowship from CNPq. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abay SM, Deribe K, Reda AA, Biadgilign S, Datiko D, Assefa T, et al. The effect of early initiation of antiretroviral therapy in TB/HIV-coinfected patients: a systematic review and meta-analysis. J Int Assoc Provid AIDS Care 2015;14(6):560–70.

Barber DL, Andrade BB, Sereti I, Sher A. Immune reconstitution inflammatory syndrome: the trouble with immunity when you had none. Nat Rev Microbiol 2012;10(2):150–6.

Blanc FX, Sok T, Laureillard D, Borand L, Rekacewicz C, Nerrienet E, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med 2011;365(16):1471–81.

Bourgarit A, Carcelain G, Martinez V, Lascoux C, Delcey V, Gicquel B, et al. Explosion of tuberculin-specific Th1-responses induces immune restoration syndrome in tuberculosis and HIV co-infected patients. AIDS 2006;20(2):F1–7.

Breton G, Duval X, Estellat C, Poaletti X, Bonnet D, Mvondo D, et al. Determinants of immune reconstitution inflammatory syndrome in HIV type 1-infected patients with tuberculosis after initiation of antiretroviral therapy. Clin Infect Dis 2004;39(11):1709–12.

Bryan W, Allen FJB. Mycobacteria: isolation, identification and sensitivity testing. London: Oxford Butterworths; 1968.

Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 1969;41(1):21–43.

Cevaal PM, Bekker LG, Hermans S. TB-IRIS pathogenesis and new strategies for intervention: insights from related inflammatory disorders. Tuberculosis (Edinb) 2019;118:101863.

Chareesil C, Werarak P, Angkasekwinai N. Incidence and outcomes of tuberculosis-associated immune reconstitution inflammatory syndrome (IRIS) following antiretroviral therapy (ART) in HIV-infected patients. J Med Assoc Thai 2019;102(8):893–8.

French MA, Price P, Stone SF. Immune restoration disease after antiretroviral therapy. AIDS 2004;18(12):1615–27.

G Narendran NP, P Chandrasekaran. Paradoxical tuberculosis immune reconstitution inflammatory syndrome (TB-IRIS) in HIV with culture positive rifampicin-sensitive pulmonary TB: risk factors and influence on TB outcome 2020. Available from: https://hyderabad.worldlunghealth.org/wp-content/uploads/2019/11/20191101_UNION2019_Abstracts_Final.pdf.

Gopalan N, Andrade BB, Swaminathan S. Tuberculosis-immune reconstitution inflammatory syndrome in HIV: from pathogenesis to prediction. Expert Rev Clin Immunol 2014;10(5):631–45.

Gopalan N, Santhanakrishnan RK, Palaniappan AN, Menon PA, Lakshman S, Chandrasekaran P, et al. Daily vs intermittent antituberculosis therapy for pulmonary tuberculosis in patients with HIV: a randomized clinical trial. JAMA Intern Med 2018;178(4):485–93.

Haddow LJ, Moosa MY, Mosam A, Moodley P, Parboosing R, Easterbrook PJ. Incidence, clinical spectrum, risk factors and impact of HIV-associated immune reconstitution inflammatory syndrome in South Africa. PLoS ONE 2012;7(11):e40623.

India Go. National AIDS Control Organisation. Antiretroviral Therapy Guidelines for HIV-Infected Adults and Adolescents Including Post-exposure Prophylaxis. NACO. In: Welfare MoHeF, editor. India 2007.

India Go. National AIDS Control Organisation.Antiretroviral Therapy Guidelines for HIV-Infected Adults and Adolescents. In: Welfare MoHF, editor. 2013.

Kumarasamy N, Venkatesh KK, Vignesh R, Devaleenal B, Poongulali S, Yepthomi T, et al. Clinical outcomes among HIV/tuberculosis-coinfected patients developing immune reconstitution inflammatory syndrome after HAART initiation in South India. J Int Assoc Provid AIDS Care 2013;12(1):28–31.

Lawn SD, Bekker LG, Miller RF. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. Lancet Infect Dis 2005;5(6):361–73.

Lawn SD, Myer L, Bekker LG, Wood R. Tuberculosis-associated immune reconstitution disease: incidence, risk factors and impact in an antiretroviral treatment service in South Africa. AIDS 2007;21(3):335–41.

Leone S, Nicastri E, Giglio S, Narciso P, Ippolito G, Acone N. Immune reconstitution inflammatory syndrome associated with Mycobacterium tuberculosis infection: a systematic review. Int J Infect Dis 2010;14(4):e283–91.

Luetkemeyer AF, Kendall MA, Nyirenda M, Wu X, Ive P, Benson CA, et al. Tuberculosis immune reconstitution inflammatory syndrome in A5221 STRIDE: timing, severity, and implications for HIV-TB programs. J Acquir Immune Defic Syndr 2014;65(4):423–8.

Manosuthi W, Van Tieu H, Mankatitham W, Lueangniyomkul A, Ananworanich J, Avihingsanon A, et al. Clinical case definition and manifestations of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. AIDS 2009;23(18):2467–71.

Meintjes G, Lawn SD, Scano F, Maartens G, French MA, Worodria W, et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. Lancet Infect Dis 2008;8(8):516–23.
Meintjes G, Rangaka MX, Maartens G, Rebe K, Morroni C, Pepper DJ, et al. Novel relationship between tuberculosis immune reconstitution inflammatory syndrome and antitubercular drug resistance. Clin Infect Dis 2009;48(5):667–76.
Meintjes G, Wilkinson RJ, Morroni C, Pepper DJ, Rebe K, Rangaka MX, et al. Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. AIDS 2010;24(15):2381–90.
Murdoch DM, Venter WD, Feldman C, Van Rie A. Incidence and risk factors for the immune reconstitution inflammatory syndrome in HIV patients in South Africa: a prospective study. AIDS 2008;22(5):601–10.
Naidoo K, Yende-Zuma N, Padayatchi N, Naidoo K, Jithoo N, Nair G, et al. The immune reconstitution inflammatory syndrome after antiretroviral therapy initiation in patients with tuberculosis: findings from the SAPiT trial. Ann Intern Med 2012;157(5):313–24.
Namale PE, Abdullahi LH, Fine S, Kamkuemah M, Wilkinson RJ, Meintjes G. Paradoxical TB-IRIS in HIV-infected adults: a systematic review and meta-analysis. Future Microbiol 2015;10(6):1077–99.
Narendran G, Andrade BB, Porter BO, Chandrasekhar C, Venkatesan P, Menon PA, et al. Paradoxical tuberculosis immune reconstitution inflammatory syndrome (TB-IRIS) in HIV patients with culture confirmed pulmonary tuberculosis in India and the potential role of IL-6 in prediction. PLOS ONE 2013;8(5):e63541.
Narendran G, Kavitha D, Karunaianantham R, Gil-Santana L, Almeida-Junior JL, Reddy SD, et al. Role of LTA4H polymorphism in tuberculosis-associated immune reconstitution inflammatory syndrome occurrence and clinical severity in patients infected with HIV. PLOS ONE 2016;11(9):e0163298.
Narendran G, Oliveira-de-Souza D, Vinhaes CL, Akrami K, Fukutani KF, Banu K, et al. Multifocal tuberculosis-associated immune reconstitution inflammatory syndrome – a case report of a complicated scenario. BMC Infect Dis 2019;19(1):529.
Oliveira-de-Souza D, Vinhaes CL, Arriaga MB, Kumar NP, Cubillos-Angulo JM, Shi R, et al. Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations. Sci Rep 2019;9(1):8002.
Sereti I, Rodger AJ, French MA. Biomarkers in immune reconstitution inflammatory syndrome: signals from pathogenesis. Curr Opin HIV AIDS 2010;5(6):504–10.
Swaminathan S, Narendran G, Venkatesan P, Iliayas S, Santhanakrishnan R, Menon PA, et al. Efficacy of a 6-month versus 9-month intermittent treatment regimen in HIV-infected patients with tuberculosis: a randomized clinical trial. Am J Respir Crit Care Med 2010;181(7):743–51.
Tadokera R, Meintjes G, Skolimowska KH, Wilkinson KA, Matthews K, Seldon R, et al. Hypercytokinaemia accompanies HIV-tuberculosis immune reconstitution inflammatory syndrome. Eur Respir J 2011;37(5):1248–59.
Vinhaes CL, Oliveira-de-Souza D, Silveira-Mattos PS, Nogueira B, Shi R, Wei W, et al. Changes in inflammatory protein and lipid mediator profiles persist after antitubercular treatment of pulmonary and extrapulmonary tuberculosis: a prospective cohort study. Cytokine 2019;123:154759.
WHO. Treat all people living with HIV, offer antiretrovirals as additional prevention choice for people at “substantial” risk. 2015 Available from: https://www.who.int/mediacentre/news/releases/2015/hiv-treat-all-recommendation/en/.
Worodria W, Menten J, Massinga-Loembe M, Mazakpwe D, Bagenda D, Koole O, et al. Clinical spectrum, risk factors and outcome of immune reconstitution inflammatory syndrome in patients with tuberculosis-HIV coinfection. Antivir Ther 2012;17(5):841–8.