Prolonged tuberculosis-associated immune reconstitution inflammatory syndrome: characteristics and risk factors

by

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DECLARATION

I, Tasnim Mohammed Bana hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Authors' contributions

Tasnim Bana was responsible for writing the literature review, study database development and all data collection, basic statistical analysis using GraphPad Prism, formulation of figures and tables for the manuscript and writing up of the 1st draft of the manuscript then involved in finalising the manuscript

Maia Lesosky was responsible for the statistical analyses and production of figures and tables for the manuscript.

Dominique Pepper, Helen van der Plas, Charlotte Schutz and Rene Goliath were responsible for clinical recruitment and follow up of patients in the initial studies and primary data acquisition.

Chelsea Morroni was involved in database development and statistical analyses in original studies.

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Graeme Meintjes was responsible for supervision of the first author, primary data acquisition in the 3 previous studies & finalising the manuscript for publication.

Abstract

Background: In a proportion of patients with HIV-associated tuberculosis who develop paradoxical immune reconstitution inflammatory syndrome (IRIS), the clinical course of IRIS is prolonged necessitating substantial health care utilization for diagnostic and therapeutic interventions. This phenomenon has not been systematically studied to date. We aimed to determine the proportion of patients with prolonged TB-IRIS, as well as the clinical characteristics and risk factors for prolonged TB-IRIS.

Methods: We pooled data from 2 prospective observational studies and a randomized controlled trial that enrolled patients with paradoxical TB-IRIS using the same diagnostic approach and clinical case definitions in Cape Town, South Africa. Prolonged TB-IRIS was defined as TB-IRIS symptoms lasting > 90 days. Risk factors for TB-IRIS were analysed using Wilcoxon rank sum test, Fisher's exact test, multivariate logistic regression and Cox proportional hazards model. In a separate set of analyses, risk factors for relapsing after a 4-week course of prednisone for treatment of TB-IRIS were analysed.

Results: Two-hundred and sixteen patients with TB-IRIS were included. The median duration of TB-IRIS symptoms was 71.0 days (IQR=41.0-113.2). In 73/181 patients with sufficient follow-up (40.3%) IRIS duration was > 90 days. Six patients (3.3%) had IRIS duration > 1 year, mainly with nodal involvement. In univariate logistic regression analysis the following were significantly associated with IRIS duration > 90 days: lymph node involvement at initial TB diagnosis (p=0.02), drug-resistant TB (p=0.02), lymph node TB-IRIS (p=0.0005) and not being hospitalized at time of TB-IRIS diagnosis (p=0.004). The association with lymph node TB-IRIS (p=0.02) and hospitalization status (p=0.05) remained significant in the multivariate logistic regression model. In the Cox proportional hazards

model, IRIS lymph node involvement was independently associated with lower hazards of IRIS resolution (HR 0.55, 95%CI=0.38-0.78). In univariate analysis those patients with lesser reductions in liver function abnormalities during prednisone treatment had a higher risk of relapse after stopping prednisone, but no significant associations remained in multivariate analysis.

Conclusions:

Around 40% of patients with TB-IRIS have symptoms for more than 90 days. Lymph node IRIS involvement is an independent risk factor for a prolonged course and in the small proportion of patients (3%) with symptoms more than one year this usually manifests with lymph node involvement. Whether earlier recognition and treatment of lymph node TB-IRIS could reduce the risk of prolonged TB-IRIS needs to be evaluated.

Trial registration: The randomized controlled trial was registered with Current Controlled Trials ISRCTN21322548.

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PART A:

Literature Review

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Literature Review: HIV-associated tuberculosis, tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) and prolonged TB-IRIS

Introduction

The objectives of this review were to evaluate the published literature for reported cases of a prolonged course of tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS), its incidence, predictors, clinical manifestations, treatment and outcomes. We defined prolonged paradoxical TB-IRIS as TB-IRIS symptoms lasting more than 90 days. This was chosen because previous studies have reported a median duration of TB-IRIS of 1-3 months [1-7].

There are indeed very few instances in the literature where a prolonged course of TB-IRIS has been described. In fact, there is a general limitation in the literature with regard to the reporting of this enigmatic clinical phenomenon, in that most studies to date have had a small study population, were cases series or single case reports, and were retrospective. I have, in addition, endeavoured to contextualize this clinical entity, by commencing with an overview of the literature on TB-IRIS in general and have included in this review a concise summary of the literature pertaining to the following aspects:

1. The burden of HIV associated TB in sub-Saharan Africa
2. The Immune reconstitution inflammatory syndrome (IRIS): types and, associated pathologies
3. Paradoxical Tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS):
    - Incidence, clinical course and manifestations
    - Spectrum of clinical manifestations
    - Morbidity and mortality related to TB-IRIS
    - Immunopathogenesis

- Diagnosis and case definitions
- Validation of INSHI (International Network for the Study of HIV-associated IRIS) case definitions
- Risk factors for paradoxical TB-IRIS
- Treatment of TB-IRIS

4. Prolonged TB-IRIS

5. Conclusion

1. The burden of HIV associated TB in Sub-Saharan Africa

The Human Immunodeficiency Virus (HIV) and tuberculosis (TB) are leading global causes of mortality and morbidity [8]. Sub-Saharan Africa bears the brunt of the dual epidemic, accounting for approximately 78% of the estimated world burden in 2013 as reported by the World Health Organisation [9] People living with HIV are 29 times more likely to develop active TB disease than persons without HIV [9]. Highly active antiretroviral therapy (ART) dramatically decreases the mortality and the incidence of opportunistic infections in patients with advanced HIV infection, including those co-infected with TB [8].

2. The Immune Reconstitution Inflammatory Syndrome (IRIS)

The immune restoration associated with ART however may result in initial clinical deterioration due to immunopathological reactions in patients with underlying opportunistic infections [10]. The immune reconstitution inflammatory syndrome (IRIS), (also known as immune reconstitution

syndrome, immune reconstitution disease, or immune restoration disease), is a widely recognised phenomenon that can complicate ART and results from the rapid restoration of pathogen-specific immune responses to opportunistic infections [11]. It may cause either worsening symptoms of an already diagnosed infection which showed initial improvement on appropriate treatment prior to ART termed “paradoxical IRIS” or it may cause new symptoms of a subclinical infection to manifest which is referred to as “unmasking IRIS”. Both entities are often accompanied by a marked inflammatory response. IRIS has been associated with a wide spectrum of pathologies including infections with mycobacteria (both tuberculosis and non-tuberculous mycobacteria), viruses, fungi; cancers such as Kaposi’s Sarcoma and non-Hodgkins lymphoma; Progressive Multifocal Leukoencephalopathy (PML) as well as non-AIDS illnesses such as sarcoidosis and rheumatic diseases [12]. The most commonly occurring IRIS phenomena in our context are those related to TB and deep fungal infections particularly cryptococcal meningitis [11].

IRIS typically occurs in the early weeks of ART with several studies reporting a median duration to onset of symptoms of a few weeks after commencing ART [1, 13, 14] but this varies depending on the cause of IRIS. A relatively large prospective observational study reported TB-IRIS symptom onset at a median of 14 days (interquartile range, 7–25 days) after the initiation of combination ART in a series of 100 patients with suspected IRIS, already on TB treatment prior to ART initiation [15].

3. Paradoxical Tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS)

Incidence, clinical course and manifestations

Given the very high burden of TB in patients infected with HIV in many low and middle income countries [8], many patients entering ART programmes already have a diagnosis of tuberculosis or develop tuberculosis following ART initiation. Locally, Lawn et al. [16] reported that 238 (25%) of

944 patients attending a community-based ART programme in South Africa were receiving tuberculosis treatment at ART initiation. Sharma et al. [17] reported that 237 (38%) of 627 patients studied had TB at initiation of ART at a tertiary centre in India.

TB-IRIS complicates early ART in a considerable proportion of HIV-infected patients started on therapy in resource-limited countries. Paradoxical TB-IRIS is reported to occur in 8 – 54% of patients starting ART while on anti-tubercular therapy [11, 18-19]. The typical clinical course of this entity is that an untreated HIV infected individual, with newly diagnosed TB is initiated on anti-tuberculosis therapy resulting in clinical improvement and stabilization. However, with the subsequent introduction of ART, IRIS presents as the development of recurrent, worsening or new symptoms or signs of TB such as fever, respiratory symptoms, lymph node enlargement often with abscess formation, or new or deteriorating radiological manifestations, within the early weeks of ART [11].

TB-IRIS may have a wide range of clinical manifestations often with involvement of multiple organ systems, reflecting widespread dissemination of *Mycobacterium tuberculosis* in those with profound immunosuppression [20]. In addition to those previously mentioned, patients may present with new or worsening serositis (pleural effusions, ascites or pericardial effusions), psoas abscesses or other cold abscesses and central nervous system involvement (e.g. meningitis, enlarging tuberculomas). Another common manifestation is that of a granulomatous hepatitis (Hepatic TB-IRIS) [21-22] which usually presents as a tender hepatomegaly associated with elevated serum concentrations of predominantly the bile canalicular hepatic enzymes but without evidence of biliary duct dilatation or obstruction on ultrasound. This phenomenon may occur independently but most often accompanies TB-IRIS at another anatomical site eg. respiratory involvement. It is important to recognize this clinical entity which can sometimes be misdiagnosed as drug induced liver injury leading to unnecessary interruptions in ART and TB treatment. Other causes of liver enzyme elevation need to be excluded prior to diagnosis of hepatic TB-IRIS such as exacerbation of Hepatitis B and C.

Morbidity and mortality related to TB-IRIS

TB-IRIS causes substantial morbidity, often resulting in hospitalization and / or the need for diagnostic and therapeutic procedures [2, 23]. Burman et al. [2] reported that in their study 24% of patients required surgical drainage of nodal or soft tissue abscesses, 44% required needle aspirations and strikingly 16% of patients required recurrent aspirations of suppurative lymphadenopathy with up to 108 aspirations being performed on one of the patients in the study. Mortality related to TB-IRIS is said to be infrequent ranging between 2.5-4% of patients [3, 12]. A systematic review of IRIS by Muller et al. published in 2010 [12] reported a mortality of 3.2% in patients with TB-IRIS. Patients with neurological TB-IRIS however represent an important exception in that their presentation may be particularly severe with a wide range of manifestations such as meningitis, intracranial tuberculomata, tuberculous brain abscesses, radiculomyelopathy and spinal epidural abscesses [24-26]. Pepper et al. [26] reported a combined mortality and loss to follow-up of 30% amongst patients with neurological TB-IRIS in their case series.

Immunopathogenesis

The currently proposed immunopathogenic mechanisms suggest that TB-IRIS arises as a result of rapid change in the innate response with consequent influence on the acquired response. TB-IRIS is associated with dysregulated inflammatory responses demonstrating an elevation in both effector and regulatory cytokines whose release is influenced by *Mycobacterium tuberculosis* antigen load in patients. The pattern of cytokine elevation seen in TB-IRIS suggests significant involvement of the innate immunity. Serum levels of CRP have been noted to be higher in both unmasking and paradoxical TB-IRIS cases than non-IRIS controls at baseline, and the CRP levels increase further at the time of TB-IRIS development. Antigen-specific CD4+ T cell restoration and expansion have been shown in certain studies to have a significant association with TB-IRIS but findings in the literature vary, a possibility being that CD4+ T-cell expansion is a consequence, rather than the cause of the

syndrome. Although increases in several cellular and immunological factors have been found to be associated with TB-IRIS, their predictive value is not well established. It is hoped that improved understanding of the interconnected network of innate and adaptive immunity in TB-IRIS pathogenesis will lead to the identification of biomarkers that can be used in prediction and diagnosis as well as recognizing new targets for therapeutic interventions.

Diagnosis and case definitions

Making the diagnosis of TB-IRIS can be challenging given the absence of a diagnostic test, or validated biomarker. Prior to 2008 diagnosis was based on expert opinion case definitions published in the literature. The most widely used definitions were those proposed by French et al. in 2004 [28], who described major and minor criteria for the diagnosis of IRIS in general (i.e. not disease specific) and in 2006, there were 2 other proposals for case definitions published. Shelburne et al. [29] also described general diagnostic criteria for IRIS, while Colebunders et al. [30] published a case definition specific for TB-IRIS describing criteria for suspected cases and confirmed TB-IRIS cases. These criteria included a combination of clinical features, radiological findings as well as immunological and virological laboratory parameters, with a fall in HIV-1 RNA concentration and/or a rise in the CD4+ T cell count being a core part of confirming the diagnosis. Due to the high cost of the immunological and virological tests these criteria are rendered largely impractical for use in resource-limited settings where the highest burden of opportunistic diseases and IRIS exist. Subsequently the development of consensus case definitions for TB-IRIS by the International Network for the Study of HIV-associated IRIS (INSHI) [11], has been instrumental in facilitating clinical diagnosis in addition to providing for standardisation of research definitions to enable greater understanding and insight into this condition. These criteria emerged from a consensus meeting in Kampala, Uganda where 97 TB-IRIS researchers from 16 countries on six continents were in

attendance and included delegates from a variety of disciplines and representatives from the World Health Organization (WHO).

These criteria are particularly beneficial in resource-constrained environments where access to expensive laboratory parameters and radiological investigations are often limited and are not a pre-requisite for diagnosis in the INSHI criteria. The authors of the INSHI document proposed that omission of these laboratory parameters would not substantially compromise case definitions for TB-IRIS since IRIS frequently develops shortly after initiation of ART and before any measurable increase in peripheral blood CD4 cell count. Furthermore, most ART-naïve patients adhering to therapy have substantial reductions in viral load in the early months of treatment [11]. Therefore the inclusion of viral load changes in case definitions is largely redundant in the context of a patient adherent to therapy.

Validation of INSHI case definitions

Several studies have subsequently addressed validation of these case definitions with both retrospective [17, 31], and prospectively collected data [32]. Sharma et al. conducted a retrospective study in India using the consensus case-definition in 627 HIV infected patients initiating ART and concluded that it provides a useful tool in resource-limited settings for the diagnosis of paradoxical TB-IRIS [17]. Haddow et al. prospectively studied 498 HIV infected patients commencing ART. Patients were followed up over a 24 week period and all possible TB-IRIS events were assessed using both consensus expert opinion and INSHI case definition. The authors reported good agreement between the 2 diagnostic modalities for both paradoxical and unmasking TB-IRIS, supporting the use of the INSHI case definition in clinical practice and research [32].

Risk factors for paradoxical TB-IRIS

A number of risk factors have been identified. These include a low baseline CD4 count prior to ART

[1, 14], particularly a CD4 count < 50 cells/uL [5-6], a marked rise in CD4 count with commencement of ART [1, 13], and a short duration between the start of TB treatment and the introduction of ART [2, 14, 29] as well as TB factors such as the presence of extra-pulmonary or disseminated TB at the time of TB diagnosis [1-2, 4, 13].

Treatment of TB-IRIS

Older studies employed a variety of strategies, either on their own or in combination, in attempt to manage this condition, including the interruption of ART, the use of non steroidal anti-inflammatory drugs (NSAID), glucocorticoid therapy as well as a wait and see approach [13]. Studies specifically addressing the treatment of paradoxical TB-IRIS are rare and based almost exclusively on expert opinion, except for the randomized placebo controlled trial of prednisone for the treatment of paradoxical TB-IRIS conducted by Meintjes et al. which demonstrated that a 4-week course of prednisone reduced the need for hospitalization and outpatient therapeutic procedures and resulted in more rapid improvements in symptoms, radiography, markers of inflammation, performance, and quality of life [23].

It is important to investigate for other causes of clinical deterioration prior to glucocorticoid therapy, among them being poor adherence to TB therapy, drug toxicity, other opportunistic infections (particularly in patients with smear negative TB or extra-pulmonary TB where the initial TB diagnosis was not microbiologically confirmed) and failure of TB treatment due to drug resistance [11]. Undiagnosed rifampin-resistant TB has been reported to be present in up to 10% of patients that present with paradoxical TB-IRIS [15].

4. Prolonged Tuberculosis-associated immune reconstitution inflammatory syndrome

With regards to the reporting of TB-IRIS in the literature in general, Leone et al. in their systematic

review of IRIS associated with *Mycobacterium tuberculosis* between 1990 and 2007, noted the limitation in the literature in that most of the original manuscripts had a small population, were cases series or single case reports, and were retrospective. The pooled number of patients in that systematic review was 238 patients from 51 articles, with the largest study reporting on 21 TB-IRIS cases [10].

The natural history or full clinical course of TB-IRIS has not been thoroughly described. While most cases are self-limiting, a proportion of patients, will require hospital admission and / or recurrent diagnostic and therapeutic procedures to manage their TB-IRIS manifestations. This is particularly the case for the subgroup of patients who experience a protracted clinical course of TB-IRIS. The median duration of symptoms in several observational studies has been reported to be 1-3 months [1-7] but in a subset of patients the condition may last for a considerably longer period of time [1-2, 6-7, 11].

There are several instances in the literature where a prolonged course of mycobacterial IRIS is described and most cases have been reported in relation to a different infection with non-tuberculous mycobacteria (NTM). Phillips et al. [33] reported on 51 patients with NTM IRIS and the median duration of IRIS symptoms was 6 months (range: 0–27 months) with ongoing symptoms at last follow-up in 5 patients (median duration of follow up of 29 months). Riddell et al. [34] described the long-term outcomes of Mycobacterium avium complex (MAC) IRIS in 20 patients: 16 (80%) responded to treatment and were disease free after a mean of 17.4 months of therapy for MAC IRIS. Four patients had persistent or relapsing disease despite 27 months of treatment.

Prolonged TB-IRIS has not been systematically studied but presents one of the most important challenges for treatment programmes and clinicians in the management of HIV and TB co-infection. While there are case reports of patients with a prolonged course of TB-IRIS this phenomenon has not

been well characterized to date. The Table below summarises studies that reported TB-IRIS duration or described prolonged TB-IRIS cases. In a case series by Olalla et al. [7], who looked retrospectively at 33 patients who were HIV infected and had proven mycobacterial disease (7 MAC and 26 TB), 9 patients in the group had paradoxical reactions (27%; 8 had TB and 1 MAC). The length of the paradoxical response was very variable (between 19 days and more than 395 days), with a median of 57 days, but it was longer in those patients whose paradoxical responses manifested the appearance or enlargement of lymph nodes (median duration 195 days). Huyst et al. [35] reported a case with 2 episodes of IRIS associated with *Mycobacterium tuberculosis*, the first episode occurring soon after initiation of ART and the second episode occurring almost 4 years after commencement of ART when the patient's CD4 lymphocyte count was around 300 cells/ul and she was virologically suppressed at the time.

In the more recent literature, the largest body of data with regard to TB-IRIS, including the reporting of IRIS durations, has emerged from the clinical trials exploring the optimal timing of ART in patients with HIV-TB co-infection. Luetkemeyer et al. in the STRIDE trial [5] reported an overall median TB-IRIS duration of 87 days (IQR 44-139 days) with a median of 92 days (IQR 46-134 days) in the subset of patients that received early ART (within 2 weeks after TB treatment initiation), and a median duration of 104 days (IQR 46-136) in patients with a CD4 cell count ≤ 50 cells/uL. This demonstrated that a significant number of patients (around 50%) in the trial experienced TB-IRIS symptoms that lasted longer than 90 days, which we defined as a prolonged course of IRIS in our study.

Laureillard et al. [3] reporting on TB-IRIS in the CAMELIA (Cambodian early versus late initiation of ART) study found a median TB-IRIS duration of 7.4 weeks (IQR 4-19.8 weeks), amongst the largest cohort of TB-IRIS patients published to date (n=155). Locally, Naidoo et al [6] in a secondary

analysis of the SAPIT (Starting Antiretroviral Therapy at Three Points in Tuberculosis) trial conducted in Durban reported on 80 patients with TB-IRIS. They found a median duration of TB-IRIS of 70.5 days in the early integrated ART arm (initiation of ART within 4 weeks of TB treatment initiation) compared to a median TB-IRIS duration of 34 days in the late integrated arm (initiation of ART within 4 weeks of completion of the intensive phase of tuberculosis treatment) of their study. They concluded that the early integrated group demonstrated significantly higher IRIS rates, longer time to IRIS resolution, and more severe cases of IRIS requiring hospitalization.

The overall recommendation that has emerged as a result of these 3 trials is that ART initiation as early as 2 weeks after commencement of TB treatment is strongly advocated in HIV-TB co-infected patients who are severely immunocompromised especially in those with a CD4 count less than 50 cells/uL, due to the survival benefit which outweighs the increased risk of TB-IRIS associated with early ART. Fear of TB-IRIS in this group of patients should therefore not be an impediment to early ART in resource limited, high burden settings [3] but patients need to be monitored closely in the first months of ART for the complications of TB-IRIS to enable appropriate management of this condition which can sometimes be severe, and in rare cases fatal.

Other reports on prolonged cases of TB-IRIS in the literature are very few and tend to be single cases or small numbers of patients within TB-IRIS studies that reported IRIS duration. Burman et al. [2] reported on 25 patients that developed TB-IRIS within their study. Forty percent (n=10) had a TB-IRIS duration of more than 90 days (range 91-511 days) and 54% of these patients manifested with suppurative lymphadenopathy. Breton et al. [13] looked at different strategies in the management of 34 patients with TB-IRIS and found an initial median IRIS duration of only 20 days (IQR 15-33), however 32% of patients relapsed with TB-IRIS symptoms in their series with 4 long term relapsing cases with durations of 14 months, 20 months, 3 years and 4 years. The authors did not comment on the TB-IRIS manifestations in these prolonged cases. In a study from Brazil by Serra et al. [36] the

mean TB-IRIS duration was 91 ± 30 days (n=10) and interestingly in this study, the sole clinical manifestation was lymph node enlargement. Michaelidis et al. [1] reported a median TB-IRIS duration of 2.53 months (range 0.53–14.97 months) with 1 patient manifesting with a discharging psoas abscess for 15 months and 80% (n=14) presenting with lymphadenopathy.

5. Conclusion

There are several limitations in the literature with regard to the description of TB-IRIS with most studies being retrospective and reporting on a small population of patients. The median duration of TB-IRIS symptoms reported across studies has been between 1-3 months. The aims of our study were to determine the proportion of patients that presented with a prolonged course of TB-IRIS which we defined as lasting greater than 90 days duration; to describe their clinical characteristics and to identify risk factors. This phenomenon is infrequently reported in the literature and yet may be an important aspect in the natural history and clinical course of the disease and represents a key management challenge in HIV programmes dealing with large numbers of patients with HIV-associated TB.

Table: Summary of studies that have reported TB-IRIS duration or prolonged cases

Study	TB-IRIS cases	Duration TB-IRIS	Prolonged cases reported	Treatment	Mortality n (%)	TB-IRIS manifestations
Michaelidis [1]	14	Median 2.53 (0.53–14.97) months	1 patient had a discharging psoas abscess for 15 months	Steroids 79%, IL-2 and G-CSF [1] in 1 patient	Not reported	Lymphadenopathy 80%, fever 57%
Burman [2]	25	Median 60 d (IQR 45-98)	10 pts (40%) TB-IRIS duration >90 d range 91-511d Four pts with duration 100-511 d required recurrent aspiration of cold abscesses – up to 108 aspirations	Steroids 4, needle aspiration 11 , surgical drainage 6	1 (4%)	Fever 64%, suppurative adenopathy 52%, increased pulmonary infiltrates 40%
Laureillard (CAMELIA) [3]	155	Median 7.4 weeks (IQR 4-19.8)	Not reported	NSAIDs 36%, steroids 38%	6 (3.9%)	Lymphadenopathy 77%, fever 68%, new or worsening CXR 53%
Luetkemeyer (STRIDE) [5]	61	Median 87 d (IQR 44-139)	Median IRIS duration for earlier ART group 92 d (IQR 46-134) n=42 & CD4 ≥ 50 group 104 d (46- 136) n=28	Steroids 54% Invasive procedures 34%	No deaths	lymphadenopathy 59%, constitutional symptoms 54%, and radiographic changes 41%.
SAPiT [6]	80	70.5 d (IQR 42-151) Early integrated group, 34 d (IQR 24-118) Late integrated group	2 unresolved TB-IRIS cases by end of follow up at 18 months	Steroids 10%	2 (2.5%)	Respiratory symptoms 86%, lymphadenopathy 22.5%
Olalla [7]	9	Median 57 days (range 19 to >395 days)	Paradoxical responses manifesting lymphadenopathies: median duration 195 days	Steroids 22% NSAIDS 22%	No deaths	Lymphadenopathy 67%, fever 67%, pulmonary infiltrates 22%, meningitis 11%
Breton [13]	34	Median 20 d (IQR 15–33) But 32% relapsed with TB-IRIS symptoms	4 long term relapses of TB-IRIS with durations: 14 months, 20 months, 3 years & 4 years	NSAIDs 3%, ART interruption 38%, ART interruption + steroids 9%, steroids alone 24%	Not reported	fever 88%, lymphadenopathy 65%, pulmonary involvement 23%
Marais [24]	16	The median total duration of corticosteroid treatment in TBM-IRIS patients was 109 days (IQR, 69–141)	Nil	Steroids 100% ART interruption in 1 patient [2]	2 (12.5%) all cause 4 (25%)	Neurological
Serra [36]	10	Mean 91±30 d	1 relapse reported, duration not stated	Steroids 80%, NSAIDs 20%	No deaths	Lymph node enlargement was the sole clinical manifestation

1 = Interleukin-2 and granulocyte macrophage colony-stimulating factor were administered to the patient with the discharging psoas abscess for 15 months with resultant resolution.
2 = ART was interrupted during TBM-IRIS in 1 patient because of brainstem involvement. This patient made a full recovery and had no recurrent symptoms after recommencement of ART under prednisone cover.

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Prolonged tuberculosis-associated immune reconstitution inflammatory syndrome: characteristics and risk factors

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Abstract

Background: In a proportion of patients with HIV-associated tuberculosis who develop paradoxical immune reconstitution inflammatory syndrome (IRIS), the clinical course of IRIS is prolonged necessitating substantial health care utilization for diagnostic and therapeutic interventions. This phenomenon has not been systematically studied to date. We aimed to determine the proportion of patients with prolonged TB-IRIS, as well as the clinical characteristics and risk factors for prolonged TB-IRIS.

Methods: We pooled data from 2 prospective observational studies and a randomized controlled trial that enrolled patients with paradoxical TB-IRIS using the same diagnostic approach and clinical case definitions in Cape Town, South Africa. Prolonged TB-IRIS was defined as TB-IRIS symptoms lasting > 90 days. Risk factors for TB-IRIS were analysed using Wilcoxon rank sum test, Fisher's exact test, multivariate logistic regression and Cox proportional hazards model. In a separate set of analyses, risk factors for relapsing after a 4-week course of prednisone for treatment of TB-IRIS were analysed.

Results: Two-hundred and sixteen patients with TB-IRIS were included. The median duration of TB-IRIS symptoms was 71.0 days (IQR=41.0-113.2). In 73/181 patients with sufficient follow-up (40.3%) IRIS duration was > 90 days. Six patients (3.3%) had IRIS duration > 1 year, mainly with nodal involvement. In univariate logistic regression analysis the following were significantly associated with IRIS duration > 90 days: lymph node involvement at initial TB diagnosis (p=0.02), drug-resistant TB (p=0.02), lymph node TB-IRIS (p=0.0005) and not being hospitalized at time of TB-IRIS diagnosis (p=0.004). The association with lymph node TB-IRIS (p=0.02) and hospitalization status (p=0.05) remained significant in the multivariate logistic regression model. In the Cox proportional hazards model, IRIS lymph node involvement was independently associated

with lower hazards of IRIS resolution (HR 0.55, 95%CI=0.38-0.78). In univariate analysis those patients with lesser reductions in liver function abnormalities during prednisone treatment had a higher risk of relapse after stopping prednisone, but no significant associations remained in multivariate analysis.

Conclusions:

Around 40% of patients with TB-IRIS have symptoms for more than 90 days. Lymph node IRIS involvement is an independent risk factor for a prolonged course and in the small proportion of patients (3%) with symptoms for more than one year this usually manifests as lymph node involvement. Whether earlier recognition and treatment of lymph node TB-IRIS could reduce the risk of prolonged TB-IRIS needs to be evaluated.

Trial registration: The randomized controlled trial was registered with Current Controlled Trials ISRCTN21322548.

Introduction

Paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is an immunopathological reaction occurring in 4-54% patients who start antiretroviral therapy (ART) while on treatment for tuberculosis (TB) [1-3]. Mortality due to TB-IRIS is infrequent [4, 5]. However, TB-IRIS results in substantial morbidity, necessitating hospitalisation and other health care utilisation for diagnostic and therapeutic procedures [6, 7], particularly when TB-IRIS has a protracted clinical course. The median duration of TB-IRIS symptoms reported from several observational studies and clinical trials has been 1-3 months [4, 6-11]. However, the condition may last considerably longer, even more than one year in certain cases [2, 6, 9-12].

The risk factors and clinical features of those patients who develop a prolonged course of TB-IRIS symptoms have not been systematically studied. We conducted this study to determine what proportion of TB-IRIS patients experienced a prolonged clinical course (defined as symptoms lasting > 90 days), their clinical characteristics, outcomes, and risk factors for this phenomenon. In a second set of analyses we determined predictors of relapse of TB-IRIS symptoms after stopping a 4-week course of prednisone treatment in the context of a randomized placebo-controlled trial (RCT) of prednisone for the treatment of TB-IRIS [13]. The rationale for including this second set of analyses was that patients who relapse after a course of prednisone may have a more refractory form of TB-IRIS that may share common risk factors with prolonged TB-IRIS.

Methods

Study design

We pooled data from 3 prospective studies of TB-IRIS that our group conducted in Cape Town, South Africa between 2005 and 2010 [13-15]. Data from eligible TB-IRIS cases assessed and followed up in these studies were included in this combined *post hoc* analysis. One of these studies was a randomized controlled trial (RCT) and the other 2 were prospective observational cohort studies. The RCT was conducted at GF Jooste Hospital with enrollment between June 2005 and December 2007. This was a secondary-level university-affiliated hospital serving communities with high HIV/TB co-infection rates. Patients were generally started on TB treatment and ART in primary care clinics and referred to the hospital with suspected TB-IRIS. Those enrolled in this trial (n=110) had paradoxical TB-IRIS that fulfilled a study case definition. The treatment intervention tested was a 4-week course of prednisone (1.5 mg/kg/day for 2 weeks followed by 0.75mg/kg/day for a further 2 weeks). The trial was placebo-controlled with 1:1 randomisation. Trial design allowed patients who deteriorated on or relapsed after stopping study drug to be commenced on open-label prednisone at clinician discretion. The trial duration was 12 weeks, but if patients still had TB-IRIS symptoms at 12 weeks follow-up was prolonged until TB-IRIS had resolved [13].

The first observational cohort study was also conducted at GF Jooste Hospital contemporaneously with the RCT [14]. Sixty-one patients with TB-IRIS who were seen prior to the RCT or were ineligible for the RCT (for example because of neurological TB-IRIS) were enrolled and followed up as outpatients until resolution of TB-IRIS symptoms between February 2005 and July 2006. The second observational cohort study was conducted at Brooklyn Chest TB Hospital, also in Cape Town, with recruitment between May 2009 and November 2010 [15]]. This was a study of ART-naïve patients who required hospitalization for the treatment for HIV-associated TB and who started

ART while in hospital and were followed for at least 12 weeks. Forty-seven TB-IRIS cases were diagnosed in this study and patients were followed for longer than 12 weeks if TB-IRIS was ongoing.

TB was diagnosed microbiologically (smear or culture) or using WHO guidelines for the diagnosis of smear-negative pulmonary and extrapulmonary TB [16]. Patients were treated according to national guidelines for TB and HIV. During the study periods TB cases received 6 months of therapy (rifampin, isoniazid, pyrazinamide, and ethambutol for 2 months, followed by rifampin and isoniazid for 4 months). The retreatment regimen included the addition of streptomycin for the first 2 months of an 8-month regimen. Routine TB drug susceptibility testing (DST) was not performed for new TB cases. Patients receiving retreatment and patients not responding to TB treatment may have had DST performed. DST was only performed for rifampin, isoniazid, and ethambutol using culture-based techniques as previously described [14]. When drug resistant TB was diagnosed a standard multi-drug resistant TB regimen was prescribed. First-line ART in South Africa was stavudine, lamivudine, and either nevirapine or efavirenz. Efavirenz was preferred for patients receiving rifampin-based TB treatment. Patients with a CD4 cell count <200 cells/μl and/or World Health Organization stage 4 disease were eligible to commence ART. In April 2010, the guidelines changed and tenofovir replaced stavudine, and TB patients were eligible for ART with a CD4 count < 350 cells/μl. Laboratory test were performed by the National Health Laboratory Services (NHLS).

Inclusion and exclusion criteria

All patients included in this study fulfilled the International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition for paradoxical TB-IRIS [2] with one exception. Patients with rifampicin resistant TB were included in the first set of analyses of prolonged TB-IRIS. We have previously described an overlap of TB-IRIS and rifampicin-resistant TB [14] and for this reason when a patient with drug resistant TB was also considered to have TB-IRIS they were not excluded

from the analyses of prolonged TB-IRIS as we wished to determine if underlying drug resistant TB was a potential risk factor for a more prolonged course of TB-IRIS despite appropriate treatment. However, rifampicin resistant TB was an exclusion criterion for the prednisone RCT, therefore the second set of analyses of patients relapsing after stopping prednisone did not include patients with known rifampicin resistance.

Study definitions

Pulmonary TB-IRIS was defined as new, recurrent or worsening respiratory symptoms and/or a worsening chest radiograph pulmonary infiltrate. Lymph node TB-IRIS was defined as enlarging peripheral lymph nodes on clinical examination, or thoracic nodes on chest radiograph or abdominal nodes on ultrasound or CT scan. Abdominal TB-IRIS was defined by abdominal symptoms at the time of TB-IRIS (eg. abdominal pain), clinical hepatomegaly or features of abdominal TB on ultrasound (eg. lymphadenopathy). Multi-organ TB-IRIS was defined by the presence of TB-IRIS symptoms, clinical signs or radiological/ultrasound abnormalities that involved more than 1 organ system (eg. pulmonary and nodal). These definitions relate to any features present at TB-IRIS presentation or that occurred at any time during the course of the TB-IRIS episode.

Prolonged paradoxical TB-IRIS was defined as TB-IRIS symptoms lasting more than 90 days. This was chosen because previous studies have reported a median duration of TB-IRIS of 1-3 months [4, 6-11]. IRIS symptom onset date was as reported by the patient. IRIS end date was recorded as date of resolution of all symptoms attributed to TB-IRIS. If a patient was asymptomatic, but had only a persistent pulmonary infiltrate, tachycardia or hepatomegaly this did not constitute ongoing IRIS. In patients manifesting with lymph node enlargement due to TB-IRIS, IRIS resolution date was recorded when patients complained of no symptoms related to lymph nodes, and nodes had reduced in size by more than half of initial size or when node(s) were less than 2cm in maximal diameter. The

term procedure was used to denote all invasive diagnostic (eg. lumbar puncture) and therapeutic (eg. aspiration of pus collection) procedures.

Data collection

Clinical data regarding TB presentation and diagnosis, HIV history, TB-IRIS symptoms, corticosteroid treatment and duration, as well as laboratory results were extracted from the trial databases using a standard data extraction form. When necessary the source notes were reviewed to extract other relevant data. Data was entered onto a Microsoft Excel® spreadsheet.

Statistical analyses

Data analysis was performed using GraphPad Prism and R 3.0. Median and interquartile range (IQR) are presented for continuous variables and frequency and percentage for categorical variables. Changes in explanatory variables between follow up time points and baseline were calculated as follow up value minus baseline value. Duration was calculated in days. P-values were estimated from Fisher's exact test (for categorical variables) and the Wilcoxon rank sum test (for continuous variables). P-values ≤ 0.05 were taken to be statistically significant.

The first set of analyses (prolonged duration of TB-IRIS) was performed combining the 3 study databases (Figure 1). The duration of TB-IRIS symptoms was described for these patients. The clinical features of those patients with prolonged TB-IRIS (> 90 days and > 1 year) were described. Thereafter, factors associated with prolonged TB-IRIS were analysed in univariate analysis, followed by multivariate logistic regression analysis. The multivariate logistic regression model included all variables with p-value < 0.1 by univariate analysis and certain pre-specified adjustment variables (eg. gender). In the event of multiple variables measuring the same process alternate models were tested. A Cox proportions hazards model was developed in order to assess variables independently

associated with time to resolution of TB-IRIS. A competing risk analysis accounting for mortality was also performed. For the analyses of factors associated with prolonged TB-IRIS (>90 days) patients who were lost to follow up or died prior to 90 days and before IRIS resolution were not included in the univariate and multivariate logistic regression models. They were included in the Cox proportional hazards model and censored at death or loss to follow-up if these occurred before IRIS resolution. They were similarly included in the competing risks analysis.

In the second set of analyses (relapse after stopping prednisone) only patients randomised to the prednisone arm of the RCT were included. In this RCT we noted that while patients had more rapid symptom improvement with prednisone compared with placebo, a subset of patients relapsed after completing a 4-week course of prednisone. Many of these patients required further corticosteroid therapy for several months to control the symptoms of IRIS: the median duration of open label prednisone being 84 days [13]. The outcome of interest in these analyses was relapse of TB-IRIS symptoms after stopping 4 weeks of prednisone, such that the clinician took the decision to restart open label prednisone. Factors associated with TB-IRIS relapse were analysed in univariate analysis and a multivariate logistic regression model was developed using the same criteria described above.

Ethical approval

This study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 300/2012). All three individual studies had also received approval from this ethics committee.

Results

Two-hundred and sixteen patients were diagnosed with paradoxical TB-IRIS across the 3 studies. One-hundred and eighty one patients were included in the initial analyses that compared patients with IRIS duration ≤ 90 days versus > 90 days. Reasons for exclusion are shown in Figure 1. In the Cox proportional hazards and competing risks models 212 patients were included (in 4 patients the ART start date was not known). The characteristics of the 212 patients are summarized in Table 1.

Among the 181 participants included in the initial analyses, 116 (64.1%) were female and median age was 31 years (IQR=27-36). The median CD4 count prior to ART was 53 cells/μl (IQR=29-94) and at first TB-IRIS assessment was 117 cells/μl (IQR=71–218). The TB diagnosis was made by culture of *Mycobacterium tuberculosis* in 97 (53.6%), positive smear for acid-fast bacilli in 41 (22.6%) and clinical/radiological diagnosis in 43 (23.8%). One-hundred and fifteen patients (63.5%) had extra-pulmonary involvement at TB diagnosis. Median duration from starting TB treatment to ART was 56.0 days (IQR=31.0–81.3). Median duration from ART to TB-IRIS symptom onset was 13.5 days (IQR=7.0-19.3 days). The most frequent organ systems involved by TB-IRIS were: abdominal (n=140, 77.3%), pulmonary (n=72, 39.8%) and lymph nodes (n=71, 39.2%). In 150 patients (82.9%) there was multi-system involvement. At the time of TB-IRIS diagnosis among the 181 patients, 83 (45.9%) were hospitalized (for 5 patients this data was missing).

The median duration of TB-IRIS symptoms was 71.0 days (IQR=41.0-113.2) for those who had a known TB-IRIS start and resolution date (n=172, this excluded all those who died or were lost to follow-up prior to TB-IRIS resolution even if > 90 days). In the 181 patients, IRIS duration was longer than 90 days in 73 (40.3%) and 6 (3.3%) had IRIS duration of over one year. The distribution of duration shown in Supplementary Figure 1.

One-hundred and twenty seven of the 216 patients (58.7%) received prednisone treatment. 111 of the 181 patients (61.3%) received prednisone for a median 42 days (IQR=28-91). This included prednisone received as study drug or open-label prednisone in the RCT and as TB-IRIS treatment in the observational cohorts. In the RCT and in clinical practice standard practice was to start prednisone at a dose of 1.5mg/kg/day. Outside of the RCT, clinician practice was to taper prednisone according to symptom response.

Of the 216 patients, 16 (7.4%) were known to have died a median of 33.5 days after TB-IRIS onset (IQR=28-61.5). 14 patients died within 90 days and 2 patients died after 90 days. TB-IRIS was considered the main cause of death in 5 of the 16 deaths. The most frequent other cause of death among the TB-IRIS cases was sepsis (n=5).

Characteristics of patients with prolonged TB-IRIS

In the 73 patients with TB-IRIS lasting > 90 days the following features were present either at presentation or at some time during the course of their TB-IRIS: lymph node involvement in 40 (54.8%), pulmonary involvement in 23 (31.5%), abdominal involvement in 59 (80.8%) and pleural, pericardial effusions or ascites in 18 (24.7%). In 64 (87.7%) TB-IRIS was multi-system. There were 2 deaths among the patients with prolonged TB-IRIS (>90 days). The characteristics, treatment and clinical course of the 6 patients who had TB-IRIS symptom duration > 1 year are summarized in Table 2.

Factors associated with prolonged TB-IRIS

Comparisons between those with prolonged IRIS and those with IRIS duration ≤ 90 days are shown in Table 3. In univariate statistical comparisons the following variables were significantly associated

with IRIS duration > 90 days: drug-resistant TB, lymph node involvement at initial TB diagnosis, lymph node TB-IRIS and not being hospitalized at the time of TB-IRIS diagnosis. Treatment with corticosteroids was not associated with prolonged TB-IRIS nor did duration between TB treatment and ART initiation influence the development of prolonged TB-IRIS.

In univariate logistic regression analysis (Table 4; unadjusted results) the following variables were significantly associated with IRIS duration > 90 days: lymph node involvement at initial TB diagnosis (OR 2.36, 95%CI=1.18-4.76; p=0.02), drug-resistant TB (OR 4.13, 95%CI=1.32-15.56, p=0.02) and lymph node TB-IRIS (OR 3.01, 95%CI=1.63-5.65; p=0.0005). Hospitalization at the time of TB-IRIS diagnosis was associated with TB-IRIS resolving before 90 days (OR 0.40, 95%CI=0.22-0.75; p=0.004) as was cohort (OR for observational compared to RCT 0.29, 95%CI=0.12-0.62, p=0.002; and OR for BCH compared to RCT 0.06, 95%CI=0.01-0.18; p<0.0001). Hospitalisation and not cohort were included in the multivariate models due to perfect separation if both were included. In the multivariate logistic regression model (Table 3; adjusted results) lymph node enlargement at time of TB-IRIS demonstrated a statistically significant association with IRIS duration > 90 days (aOR 2.27, 95%CI=1.13-4.59; p=0.02). Hospitalization was associated with an IRIS duration ≤ 90 days (aOR 0.5, 95%CI=0.25-0.99; p=0.05). Drug-resistant TB demonstrated a trend towards but did not reach statistical significance in association with prolonged TB-IRIS (aOR 3.26, 95%CI=0.97-12.99; p = 0.07).

Due to the potential bias around the BCH cohort and hospitalization (as all patients in the BCH cohort were admitted to a TB hospital from the time of starting ART whereas in the other 2 studies patients were only admitted to a general hospital for shorter periods based on severity of illness at the time), a sensitivity analysis was performed excluding all individuals in the BCH cohort. Both univariate and multivariate regression models were assessed (See appendix 3: Supplementary

Table 1) and although, as expected, the number of variables with statistically significant associations decreased, the direction and size of the effects remained largely the same. However, hospitalization was no longer associated with prolonged TB-IRIS (aOR 1.16, 95%CI=0.53-2.57, p=0.71).

The Cox proportional hazards model (Table 5) included 212 patients for whom the TB-IRIS start date was known and a multivariate model was run based on predictors of interest as indicated by previous analyses and pre-specified variables. Predictors included were: initial TB lymph node involvement, TB-IRIS lymph node involvement, TB-IRIS pulmonary involvement, drug-resistant TB, as well as age, gender and hospitalisation. IRIS lymph node involvement was statistically significantly associated with a lower hazard of IRIS resolution (HR 0.55, 95%CI=0.38-0.78; p = 0.0009) in both the full model and the model run without the BCH cohort (HR 0.60, 95%CI=0.40-0.89; p = 0.01, See appendix 3: Supplementary Table 2). Additionally, competing risks models were analysed to account for the competing risk of death and IRIS resolution and the model demonstrated similar findings (See appendix 3: Supplementary Table 3). In Supplementary Figure 2, the Kaplan-Meier plot demonstrates more rapid TB-IRIS resolution for those without lymph node TB-IRIS compared to those with TB-IRIS with lymph node involvement (p < 0.0001; log-rank test).

Factors associated with TB-IRIS relapse after stopping prednisone in RCT

Those participants who completed 4 weeks of prednisone in the RCT as study drug who then developed a significant relapse of TB-IRIS symptoms after stopping, could be re-initiated on open label prednisone at study clinician discretion and in these analyses this was the endpoint of interest. Reasons for exclusion from these analyses are shown in Figure 2. Of the 43 RCT participants who completed 4 weeks of study prednisone 9 (20.9%) relapsed with TB-IRIS symptoms and were started on open-label prednisone. The median duration to commencing open-label prednisone after stopping study drug prednisone was 27 days (IQR=24-46).

Because there were a relatively small number of patients in this analysis, it was largely exploratory. The following variables were significantly associated with relapse in univariate analysis (Table 6 and Supplementary Figure 3): lower reduction in alkaline phosphatase from 0 to 4 weeks (p=0.03) and lower reduction in ALT from from 0 to 2 weeks (p=0.03) and 0 to 4 weeks (p=0.04). The following variables showed a non-significant trend towards being associated: lower reduction in alkaline phosphatase from 0 to 2 weeks (p=0.06), higher alkaline phosphatase concentration at week 2 (p=0.08), longer duration from TB-IRIS to trial enrolment (and starting prednisone) (p=0.10), lower Karnofsky score at week 2 (p=0.09) and higher pulse rate at week 4 (p=0.09). The pulse rate at week 4 in those who relapsed was a median116 beats/minute (IQR=99-118) versus 97 (IQR=80-107) in those who did not relapse. In a multivariate logistic regression model (data not shown) none of the variables remained significantly associated with relapse.

Discussion

In this combined analysis that included patients enrolled in 3 previously reported studies the duration of paradoxical TB-IRIS symptoms was a median of 71 days, with 40.3% of patients experiencing TB-IRIS symptoms lasting > 90 days. Six patients (3.3%) experienced a very protracted course of TB-IRIS with symptoms > 1 year duration. TB-IRIS lymph node involvement was independently associated with a longer duration of symptoms.

The TB-IRIS case fatality rate in the combined cohort was 7.4%. This is higher than that reported in a meta-analysis (3.2%) [5], and this may reflect that our studies were conducted at a hospital level with patients having more severe clinical disease. Five of the 16 deaths were attributed to TB-IRIS (2.3%). In the CAMELIA trial mortality amongst the 155 TB-IRIS cases was higher than in our study (23 deaths among the 155 patients (14.8%) with 6 deaths attributed to TB-IRIS (3.9%)), likely a consequence of the advanced immunosuppression at ART initiation in the CAMELIA participants (median CD4 = 27 cells/μl) [4].

Hospitalization was associated with an IRIS duration ≤ 90 days (aOR 0.5, 95%CI=0.25-0.99; p=0.05). One possibility is that these findings were due to a cohort effect. In the BCH cohort of hospitalized TB-IRIS patients, because of close prospective follow up during early ART, TB-IRIS was diagnosed early and treated promptly with steroids when necessary thereby potentially reducing duration. This close follow-up may also have resulted in diagnosis of milder cases who may inherently have a shorter duration.

Abdominal manifestations were the most frequent IRIS clinical feature in our study, which has not been reported in previous studies [17]. This was in part related to the permissive definition we used

for abdominal IRIS which included any patient who had TB-IRIS fulfilling INSHI criteria and who had any abdominal symptom (eg. abdominal pain or vomiting), hepatomegaly on clinical examination or abdominal ultrasound features of TB at the time of TB-IRIS. In a previous report, that included patients in this study, we reported over 50% of TB-IRIS patients had hepatomegaly and many had cholestatic liver function derangement at TB-IRIS presentation [14].

In the 6 patients who had TB-IRIS lasting > 1 year the prolonged features were nodal enlargement and suppuration and abscess formation, including cerebellar abscesses in 1 case. These patients received prolonged corticosteroids and 4 had one or more relapses when steroids were tapered or interrupted. However, in all of them steroids were eventually stopped long before IRIS resolution because of the clinical impression that they were no longer providing benefit and concerns regarding cumulative toxicity. The longest duration of steroid therapy was 308 days, and the longest duration of TB-IRIS was almost 4 years.

Prolonged IRIS represents a key management challenge in ART programmes. Prolonged IRIS has best been described in patients with *Mycobacterium avium* complex (MAC) and other non-tuberculous mycobacterial (NTM) infections. Phillips [18] reported 51 patients with NTM IRIS in whom the median duration of IRIS symptoms was 6 months with a range of 0–27 months. Riddell [19] reported the long-term outcomes of MAC-IRIS for 20 patients: 16 responded to treatment and were disease free after a mean of 17.4 months of therapy for MAC IRIS, whereas 4 patients had persistent or relapsing disease despite 27 months of treatment. For paradoxical TB-IRIS several studies have reported on the duration of symptoms and cases with a prolonged course have previously been described. The median duration of TB-IRIS symptoms reported across studies has been between 40 and 90 days [4, 6-9, 11]. In the SAPiT trial those patients who started ART within 4 weeks of TB treatment and developed TB-IRIS had a significantly longer duration of TB-IRIS

(median 71 days) compared to TB-IRIS cases who started ART 8-12 weeks after TB treatment (median 34 days) [10]. Unlike in the SAPiT and CAMELIA trials, most patients in our study had a long duration between starting TB treatment and commencing ART, median duration was 56.0 days (IQR=31.0–81.3)) In the CAMELIA trial, 155 cases of TB-IRIS were diagnosed, 77% with nodal involvement and 38% were treated with corticosteroids and 36% with non-steroidal anti-inflammatory drugs. The median duration of IRIS was 7.4 weeks (IQR=4-19.8) [4]. Ollala *et al* [11] reported a very variable duration of mycobacterial IRIS (between 19 days and more than 395 days, median of 57 days), and duration was longer in those patients whose paradoxical responses manifested the appearance or growth of lymphadenopathies (median 195 days) similar to our study. Huyst *et al* [20] reported a case with 2 episodes of IRIS associated with TB; the first episode occurring soon after initiation of ART and the second episode occurring almost 4 years later. Similar to our findings, Burman *et al* reported 10/25 (40%) patients with TB-IRIS with a duration > 90 days. In 2 patients the duration of TB-IRIS was > 1 year and one required 108 aspirations of the IRIS lymph nodes [6]. Breton *et al* reported 4 among 34 patients who had long term relapses lasting 14 months, 20 months, 3 and 4 years [12]. Naidoo *et al* reported 2 cases of TB-IRIS unresolved after 18 months of trial follow-up in the SAPiT trial [10] and Michailidis *et al* a case with a discharging psoas abscess for 15 months [9].

The risk factors most consistently implicated in the development of TB-IRIS itself have been a low pre-ART CD4 count, short duration between starting TB treatment and ART and extra-pulmonary TB [21]. Neither of the first two were found to be associated with prolonged TB-IRIS whereas TB-IRIS lymph node involvement (an extrapulmonary manifestation) was, suggesting there is a common risk factor for developing TB-IRIS itself and for a prolonged course of TB-IRIS. In the CAMELIA trial mediastinal adenopathy was a risk factor for TB-IRIS [4] and in a Brazilian observational study superficial adenopathy was a TB-IRIS risk factor [22]. Nodal TB is also associated with a high

incidence of paradoxical reactions in HIV-negative TB patients [23]. Multiple lines of evidence therefore suggest TB in a nodal location increases the risk for inflammatory reactions and in our study a longer duration of these.

In a separate analysis of factors associated with relapse of TB-IRIS symptoms after completing a 4-week course of prednisone a lesser decrease in alkaline phosphatase and ALT on prednisone were significantly associated with relapse in univariate anlaysis. It is possible that these biomarkers reflect TB-IRIS liver involvement, which is common [14, 24], and their persistent elevation reflects ongoing granulomatous inflammation in the liver and this may predict that TB-IRIS systemic inflammatory process will flare up on stopping prednisone. Monocytes produce alkaline phosphatase during differentiation [25] and this is another potential source given their activation in TB-IRIS [26]. These findings are preliminary and need to be confirmed in larger cohorts. If validated, it may be possible to use alkaline phosphatase as a biomarker in assisting decisions regarding when to withdraw corticosteroid treatment in TB-IRIS. Interestingly pulse rate at week 4, likely reflecting systemic inflammatory state, was higher in patients who relapsed but this did not reach statistical significance. We had hypothesised that CRP, which decreased rapidly within the first 2 weeks of starting prednisone treatment for TB-IRIS [13] would predict relapse, but this was not the case.

A limitation of this study is that it included patients enrolled in 3 separate studies introducing heterogeneity. One of the studies was an RCT, and 2 were observational studies. The RCT and 1 of the observational studies were done at a referral hospital and the other observational study at an inpatient TB hospital. This weakness was mitigated by the use of a similar diagnostic approach and case definition for TB-IRIS across the 3 studies. Also, the studies were all conducted by the same research group. Management protocols for TB-IRIS (apart from during the intervention period of the RCT) were similar across studies. Given that 2 of the studies were conducted at a referral hospital,

this may have excluded milder cases of TB-IRIS who were not referred from primary care, biasing the findings towards patients with more severe TB-IRIS. The location of the studies may also have contributed to loss to follow-up: loss to follow-up was defined as loss to follow-up at the referral or TB hospital and did not necessarily mean the patient was also lost to follow-up at their primary care HIV or TB clinic. In the analyses we included multiple variables and no correction for multiple comparisons was conducted. The analysis of relapse after completing 4 weeks of prednisone was limited by small sample size and analyses were therefore exploratory. Consistent with INSHI case definitions, some overlap existed in the definitions of lymph node TB-IRIS and abdominal TB-IRIS (with abdominal lymphadenopathy).

A strength of this study overall is the large number of patients with paradoxical TB-IRIS included. This is to our knowledge the largest number of TB-IRIS patients to be included in a single analysis, and this allowed us to present important and novel findings with respect to the clinical course of TB-IRIS and factors contributing. All patients fulfilled the INSHI case definition, apart from the inclusion of certain patients with drug resistant TB who were also considered to have TB–IRIS.

In conclusion, around 40% of patients with TB-IRIS have a clinical course of symptoms lasting longer than 90 days. Lymph node IRIS involvement is an independent risk factor for a prolonged course. In the small proportion of patients (3%) with symptoms > one year this manifests with lymphadenitis or abscesses. Whether earlier recognition and treatment of lymph node TB-IRIS could reduce the risk for prolonged TB-IRIS needs to be evaluated in future studies.

Acknowledgements

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We are appreciative of the support of the clinical and administrative staff of the Provincial Government of the Western Cape of South Africa.

Table 1: Baseline and TB-IRIS characteristics of the 212 patients included in Cox proportional hazards model

Variable	Median (interquartile range) or number (%)
Gender (n=212) Female Male	 138 (65.1%) 74 (34.9%)
Age (years) (n=212)	31 (27, 36)
WHO stage (n=212) 3 4	 71 (33.5%) 141 (66.5%)
CD4 count prior to ART (cells/μl) (n=199)	52 (28, 92.5)
CD4 count at TB-IRIS (cells/μl) (n=95)	116 (71, 209.5)
Previous TB (n=211)	55 (25.9%)
Drug-resistant TB (n=212)[1]	21 (10%)
Initial TB diagnosis with extra-pulmonary (n=212)	134 (63.2%)
Initial TB diagnosis with lymph node involvement (n=212)	50 (23.6%)
Duration from TB treatment to ART (days)(n=211)	56 (31, 83)
Duration from ART to IRIS onset (days) (n=211)	14 (7, 21)
Hospitalised at time of IRIS diagnosis (n=206)	107 (50.5%)
IRIS lymph node involvement (n=212)	80 (37.7%)
IRIS pulmonary involvement (n=212)	83 (39.2%)
IRIS meningitis (n=209)	10 (4.7%)
IRIS abdominal involvement (n=212)	154 (72.6%)
IRIS multisystem involvement (n=212)	166 (78.3%)
Pulse rate at IRIS (n=134)	120 (107.3, 132)
C-reactive protein (mg/l) (n=195)	103 (63, 158.5)
Haemoglobin (g/dl) (n=136)	9.1 (8, 10.4)
White cell count (x 10^9/l) (n=132)	5.7 (4.1, 8.1)
ALT (IU/l) (n=180)	34 (22, 51)
Alkaline phosphatase (IU/l) (n=164)	159 (107, 274.8)
Corticosteroid treatment for TB-IRIS	125 (59.0%)

Footnote for Table 1:

Pulse rate and laboratory values refer to the value obtained when the patient was first assessed with TB-IRIS symptoms, unless otherwise stated. Numbers in brackets after the variable name refer to the number of patients for whom that variable was available.

[1] Among 21 patients with drug resistant TB 4 had rifampicin mono-resistance, 3 had isoniazid mono-resistance and 14 had resistance to rifampicin and isoniazid.

Abbreviations: ALT = alanine transferase, ART = antiretroviral therapy, IRIS = immune reconstitution inflammatory syndrome, TB = tuberculosis, WHO = World Health Organisation.

Table 2: Characteristics, treatment and clinical course for 6 patients in whom TB-IRIS duration was longer than 1 year

	Age at IRIS (years)	Pre-ART CD4 count (cells/μl)	Initial TB diagnosis	Initial IRIS features	Duration of TB-IRIS (days)	Duration of steroid treatment (days)	Features that persisted > 1 year
1	35	78	Extra-pulmonary TB (nodal)	Nodal	746 (IRIS ongoing at last visit)	70 [1]	Cerebellar tuberculomas / abscesses[2]
2	34	160	Extra-pulmonary TB (nodal and miliary)	Nodal	824 (IRIS ongoing at last visit)	98 [1]	Supraclavicular nodes and large cold abscess
3	56	65	Extra-pulmonary TB (miliary)	Nodal	426	0	Cervical and submandibular nodes
4 [3]	26	44	Extra-pulmonary TB (abdominal)	Abdominal	462	168 [1]	Axillary and cervical nodes, abdominal wall cold abscesses, mastitis
5	36	39	Extra-pulmonary TB (nodal and abdominal)	Nodal	1362	308 [1]	Multiple suppurating cervical nodes, psoas abscesses and abdominal pus collections [4]
6	39	80	Extra-pulmonary TB (nodal)	Nodal	519	137	Lymph nodes and cold abscesses

Footnote for Table 2:

[1] Patients 1, 2, 4 and 5 experienced recurrence of their TB-IRIS symptoms on tapering of steroids and required re-escalation of their steroid doses on 1 or more occasions.
[2] The initial TB-IRIS episode involved lymph nodes and resolved on prednisone then patient re-presented several months later with new neurological manifestations and multiple rim-enhancing lesions in the cerebellum. Brain abscess resection tissue cultured drug-susceptible *Mycobacterium tuberculosis (MTB).*
[3] Patient found to have rifampicin mono-resistant *MTB* (urine culture sent at diagnosis) at TB-IRIS diagnosis. She was commenced on prednisone after commencement of appropriate treatment for rifampicin mono-resistant TB.
[4] Patient still had residual left iliac fossa mass on abdominal imaging at TB-IRIS resolution that was decreasing in size and was asymptomatic.

Abbreviations: IRIS = immune reconstitution inflammatory syndrome, TB = tuberculosis.

Table 3: Factors associated with prolonged course of paradoxical TB-IRIS (defined as TB-IRIS symptoms lasting longer than 90 days) (n=181)

	TB-IRIS symptoms > 90 days (n=73) (40.3%)	**TB-IRIS symptoms ≤ 90 days (n=108) (59.7%)**	**p-value for comparison**
Female gender Male gender	50 (68.5) 23 (31.5)	66 (61.1) 42 (38.9)	0.39
Age (years)	31 (26, 35)	31 (27, 37)	0.32
WHO stage (n=181) 3 4	 22 (30.1) 51 (69.9)	 40 (37) 68 (63)	0.42
CD4 count prior to ART (cells/μl) (n=170)	57.5 (29, 91.2)	52 (29.2, 101)	0.77
CD4 count at TB-IRIS (cells/μl) (n=89)	117 (71.7, 193.5)	116 (69, 262.5)	0.77
Previous TB (n=180)	15 (20.6)	30 (28)	0.34
Drug-resistant TB (n=181)[1]	10 (13.7)	4 (3.7)	0.02
Initial TB diagnosis with extra-pulmonary features (n=181)	49 (67.1)	66 (61.1)	0.5
Initial TB diagnosis with lymph node involvement (n=178)	25 (34.2)	19 (18.1)	0.02
Duration from TB treatment to ART (days)(n=181)	58 (33, 84)	56 (31, 79.5)	0.8
Duration from ART to IRIS onset (days) (n=181)	13 (7, 23)	14 (7, 17)	0.51
Hospitalised at time of IRIS diagnosis (n=176)	25 (34.2)	58 (56.3)	0.01
IRIS lymph node involvement (n=181)	40 (54.8)	31 (28.7)	< 0.0001
IRIS pulmonary involvement (n=181)	23 (31.5)	49 (45.4)	0.07
IRIS meningitis (n=181)	1 (1.4)	5 (4.7)	0.42
IRIS abdominal involvement (n=181)	59 (80.8)	81 (75)	0.37
IRIS multisystem involvement (n=181)	64 (87.7)	86 (79.6)	0.17
Pulse rate at IRIS (n=120)	121 (105, 133)	119 (108, 130)	0.96
C-reactive protein (mg/l) (n=172)	111 (74, 157.3)	95.5 (46.6, 165.7)	0.14
Haemoglobin (g/dl) (n=113)	9 (8, 10.1)	9.1 (8, 10.6)	0.62
White cell count	5.9 (4.2, 7.2)	5.98 (4.38, 8.62)	0.33

(x 10^9/l) (n=109)			
ALT (IU/l) (n=157)	40 (25, 60)	34.5 (22, 47.7)	0.13
Alkaline phosphatase (IU/l) (n=147)	164 (113.5, 261.2)	164 (95, 278)	0.46
Corticosteroid treatment for TB-IRIS	48 (65.8%)	63 (58.3%)	0.35

Footnote for Table 3:

Medians (interquartile range) or number (%) are shown. Categorical variables were compared using Fisher's exact test and continuous variables using Wilcoxon rank sum test. Pulse rate and laboratory values refer to the value obtained when the patient was first assessed with TB-IRIS symptoms, unless otherwise stated. Numbers in brackets after the variable name refer to the number of patients for whom that variable was available.

[1] Among 14 patients with drug resistant TB 4 had rifampicin mono-resistance, 2 had isoniazid mono-resistance and 8 had resistance to rifampicin and isoniazid. The reference group is combined culture negative, culture not done and drug sensitive.

Abbreviations: ALT = alanine transferase, ART = antiretroviral therapy, IRIS = immune reconstitution inflammatory syndrome, TB = tuberculosis, WHO = World Health Organisation.

Table 4: Odds ratios for univariate (unadjusted) and multivariate (adjusted) logistic regression models predicting the development of prolonged TB-IRIS

	Unadjusted OR (95% CI)	**Unadjusted p-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Age (per 1 year increase)	0.97 (0.93-1.01)	0.16	0.98 (0.94-1.05)	0.48
Male gender	0.72 (0.38-1.35)	0.31	0.80 (0.39-1.63)	0.54
Initial TB diagnosis with lymph node involvement	2.36 (1.18-4.76)	0.02	1.79 (0.81-3.98)	0.15
Drug-resistant TB	4.13 (1.32-15.56)	0.02	3.26 (0.97-12.99)	0.07
Hospitalised at time of IRIS diagnosis	0.40 (0.22-0.75)	0.004	0.5 (0.25-0.99)	0.05
IRIS lymph node involvement	3.01 (1.63-5.65)	0.0005	2.27 (1.13-4.59)	0.02
IRIS pulmonary involvement	0.54 (0.29-1.02)	0.06	0.65 (0.32-1.30)	0.23
BCH cohort[1]	0.06 (0.01-0.18)	< 0.0001	Not included	
Observational cohort[1]	0.29 (0.12-0.62)	0.002	Not included	

Footnote for Table 4:

[1] Reference cohort was randomized controlled trial

Abbreviations: BCH=Brooklyn Chest Hospital, IRIS = immune reconstitution inflammatory syndrome, TB = tuberculosis

Table 5: Cox proportional hazards model predicting time to resolution of TB- IRIS symptoms

	Adjusted HR (95% CI)	**p-value**
Age (per 1 year increase)	0.99 (0.97-1.01)	0.51
Male gender	1.22 (0.86-1.74)	0.27
Initial TB diagnosis with lymph node involvement	0.75 (0.50-1.15)	0.19
Drug-resistant TB	0.60 (0.34-1.08)	0.09
Hospitalised at time of IRIS diagnosis	1.28 (0.92-1.78)	0.14
IRIS pulmonary involvement	1.29 (0.92-1.81)	0.14
IRIS lymph node involvement	0.55 (0.38-0.78)	0.0009

Footnote for Table 5:

Abbreviations: IRIS = immune reconstitution inflammatory syndrome, TB = tuberculosis

Table 6: Factors associated with relapse of paradoxical TB-IRIS after discontinuing 28 days of prednisone in the randomised controlled trial (n=43)

	Relapse (n=9) (21%)	**No Relapse (n=34) (79%)**	**p-value for comparison**
Age (years) (n=43)	34 (30, 37)	31 (28.2, 34.7)	0.49
Gender (n=43)			
Female	8 (88.9%)	22 (64.7%)	
Male	1 (11.1%)	12 (35.3%)	0.32
WHO stage (n=43)			
Stage 3	3 (33.3%)	19 (56%)	
Stage 4	6 (66.7%)	15 (44%)	0.41
CD4 pre ART (cells/ul) (n=43)	57 (39, 93)	55.5 (30, 107)	0.99
CD4 at enrolment (cells/ul) (n=40)	118 (93.5, 183)	141.5 (81.7, 242)	0.58
CD4 at week 4 (cells/ul) (n=36)	134 (85.5, 240)	170 (84, 246)	1.00
Change in CD4 0-4 weeks (n=34)	0 (-17.5, 91)	17 (-19, 73.5)	0.92
VL at enrolment (IU/ml) (n=33)	470 (243, 790)	515 (145, 1325)	0.89
VL at week 4 (IU/ml) (n=21)	339.5 (49, 872)	49 (49, 71)	0.44
Previous TB (n=43)	2 (22.2%)	9 (26.5%)	1.00
Extra-pulmonary TB at diagnosis (n=43)	6 (66.6%)	14 (41.2%)	0.32
Duration TB Rx to ART in days (n=43)	60 (35, 77)	65 (44, 85.5)	0.79
Duration ART to IRIS in days (n=42)	12 (7, 28)	14 (7, 21)	0.99
Duration IRIS to enrolment/steroids in days (n=42)	18 (14, 29)	13 (7, 16)	0.10
TB IRIS manifestations (n=43)			
New/recurrent lymphadenopathy	4 (44.4%)	12 (35.3%)	0.91
Or cold abscess			0.47
New/worsening pulmonary infiltrate	3 (33.3%)	11 (32.4%)	1.00
New/worsening serous effusion	1 (11.1%)	5 (14.7%)	1.00
Recurrent symptoms & consistent radiography, but without baseline radiography available for comparison	4 (44.4%)	14 (41.2%)	1.00
Abdominal	8 (88.9%)	28 (82.3%)	1.00
Multi-organ involvement	8 (88.9%)	30 (88.2%)	1.00
Karnofsky score at enrolment (n=42)	50 (30, 72.5)	70 (52.5, 80)	0.14
Karnofsky score week 2 (n=39)	85 (60, 90)	90 (80, 90)	0.09
Karnofsky score week 4 (n=39)	90 (70, 95)	90 (90, 100)	0.24
Hospitalised at enrolment (n=43)	2 (22.2%)	7 (20.6%)	1.00
Pulse rate at enrolment (n=43)	121 (105, 132)	116 (107, 130)	0.66
Pulse rate week 2 (n=43)	114 (91, 120)	104 (87.7, 119)	0.68
Pulse rate week 4 (n=43)	116 (99, 118)	97 (80, 107)	0.09
CRP at enrolment (mg/l) (n=43)	118 (74, 128)	93 (42.5, 152.5)	0.51
CRP week 2 (mg/l) (n=41)	37 (26, 47)	29 (10.7, 48.7)	0.49
CRP week 4 (mg/l) (n=42)	38 (22, 54)	26 (17, 49)	0.52
Change in CRP 0-4 wk (n=42)	-64 (-70, -27)	-44 (-95, -20)	1.00
Haemoglobin at enrolment (g/dl) (n=42)	9.4 (7.8, 10.3)	9.1 (8.5, 10.2)	0.94
WCC at enrolment (x 10^9/l) (n=42)	5.2 (3.7, 6.7)	4.7 (3.2, 7.3)	0.87
Neutrophils at enrolment (x 10^9/l) (n=40)	4.2 (3.2, 5.3)	3.7 (2.4, 5.3)	0.75
Alk Phos at enrolment (IU/l) (n=43)	165 (135, 257)	155 (97, 327)	0.72
Alk Phos at week 2 (IU/l) (n=31)	240 (212, 287)	133 (95, 225)	0.08

Alk Phos at week 4 (IU/l) (n=32)	247 (191, 316)	123 (77, 228)	0.13
Change in Alk Phos 0-2 wk (n=31)	+21.5 (-59, 94)	-53 (-115, -29.5)	0.06
Change in Alk Phos 0-4 wk (n=32)	+40 (-22, 86.5)	-42 (-101, -17)	0.03
ALT at enrolment (IU/l) (n=43)	40 (25, 58)	35.5 (19, 52)	0.71
ALT week 2 (IU/l) (n=27)	51 (32, 79.5)	41 (25.5, 62)	0.52
ALT week 4 (IU/l) (n=21)	36.5 (29, 69)	34 (30, 50.5)	0.61
Change in ALT 0-2 wk (n=27)	28 (4.5, 31.5)	-0.5 (-13.5, 6.5)	0.03
Change in ALT 0-4 wk (n=21)	+15 (3, 21)	-3 (-12, 1.5)	0.04
Clinical signs (n=43)			
Fever	4 (44.4%)	8 (23.5%)	0.41
Hepatomegaly	7 (77.8%)	26 (76.5%)	1.00

Footnote for Table 6:

Medians (interquartile range) or number (%) are shown. Categorical variables were compared using Fisher's exact test and continuous variables using Wilcoxon rank sum test. Numbers in brackets after the variable refer to the number of patients for whom that variable was available. Demographic and clinical variables were available for all patients. Enrolment, week 2 and week 4 in this table refer to those scheduled visits during the clinical trial.

Abbreviations: Alk Phos = alkaline phosphatase, ALT = alanine transferase, ART = antiretroviral therapy, CRP = C-reactive protein, IRIS = immune reconstitution inflammatory syndrome, Rx=therapy, TB = tuberculosis, VL = HIV viral load, WCC = white cell count, WHO = World Health Organisation.

Legends for Figures:

Figure 1: Inclusion and exclusion of patients in the prolonged TB-IRIS analyses

The total study population included 216 TB-IRIS patients from 3 prior studies (1 randomised controlled trial and 2 prospective observational cohort studies). The analyses comparing those who experienced a prolonged course of TB IRIS symptoms (defined as lasting more than 90 days) to those that did not experience a prolonged course of TB IRIS included 181 patients. Reasons for exclusion of 35 patients from these analyses are shown.

Abbreviations: ART = antiretroviral therapy, BCH = Brooklyn Chest Hospital, IRIS = immune reconstitution inflammatory syndrome, RCT = randomised controlled trial, TB = tuberculosis.

Figure 2: Inclusion of participants in the analysis of relapse after completing a 4-week course of prednisone

This analysis included participants who were randomised to receive prednisone in the randomised controlled trial (RCT). Patients who relapsed with TB-IRIS symptoms after stopping 28 days of prednisone and were initiated on open label prednisone were compared to those who did not. Patients were excluded if they had an alternative reason for clinical deterioration or if they died before 4 weeks or switched to open label prednisone before completing 4 weeks of prednisone as study drug because of clinical deterioration during this time. Four patients were excluded from this analysis because they were found to have rifampicin resistant TB.

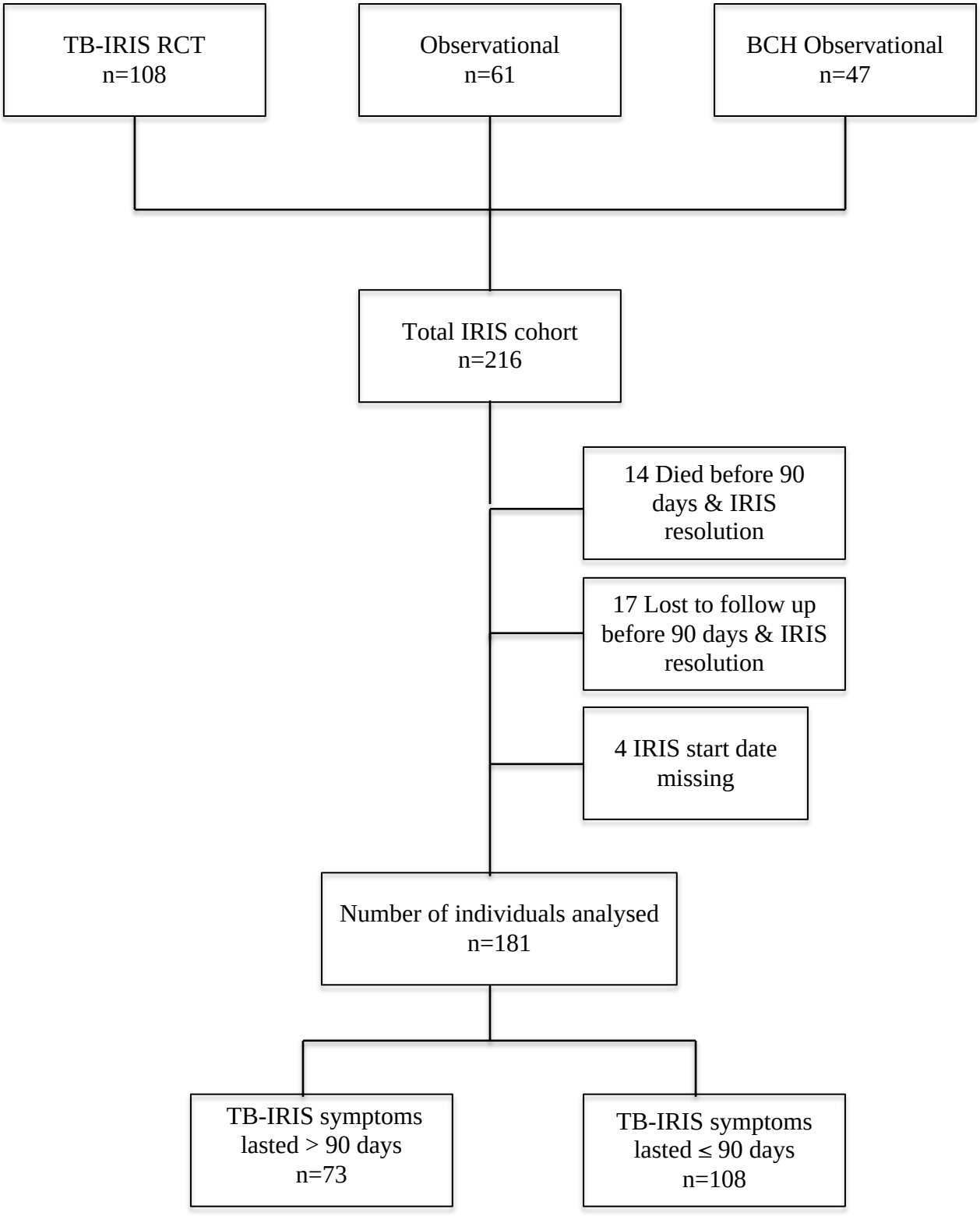

TB-IRIS RCT
n=108
Observational
n=61
BCH Observational
n=47
Total IRIS cohort
n=216
14 Died before 90 days & IRIS resolution
17 Lost to follow up before 90 days & IRIS resolution
4 IRIS start date missing
Number of individuals analysed
n=181
TB-IRIS symptoms lasted > 90 days
n=73
TB-IRIS symptoms lasted ≤ 90 days
n=108

Figure 1

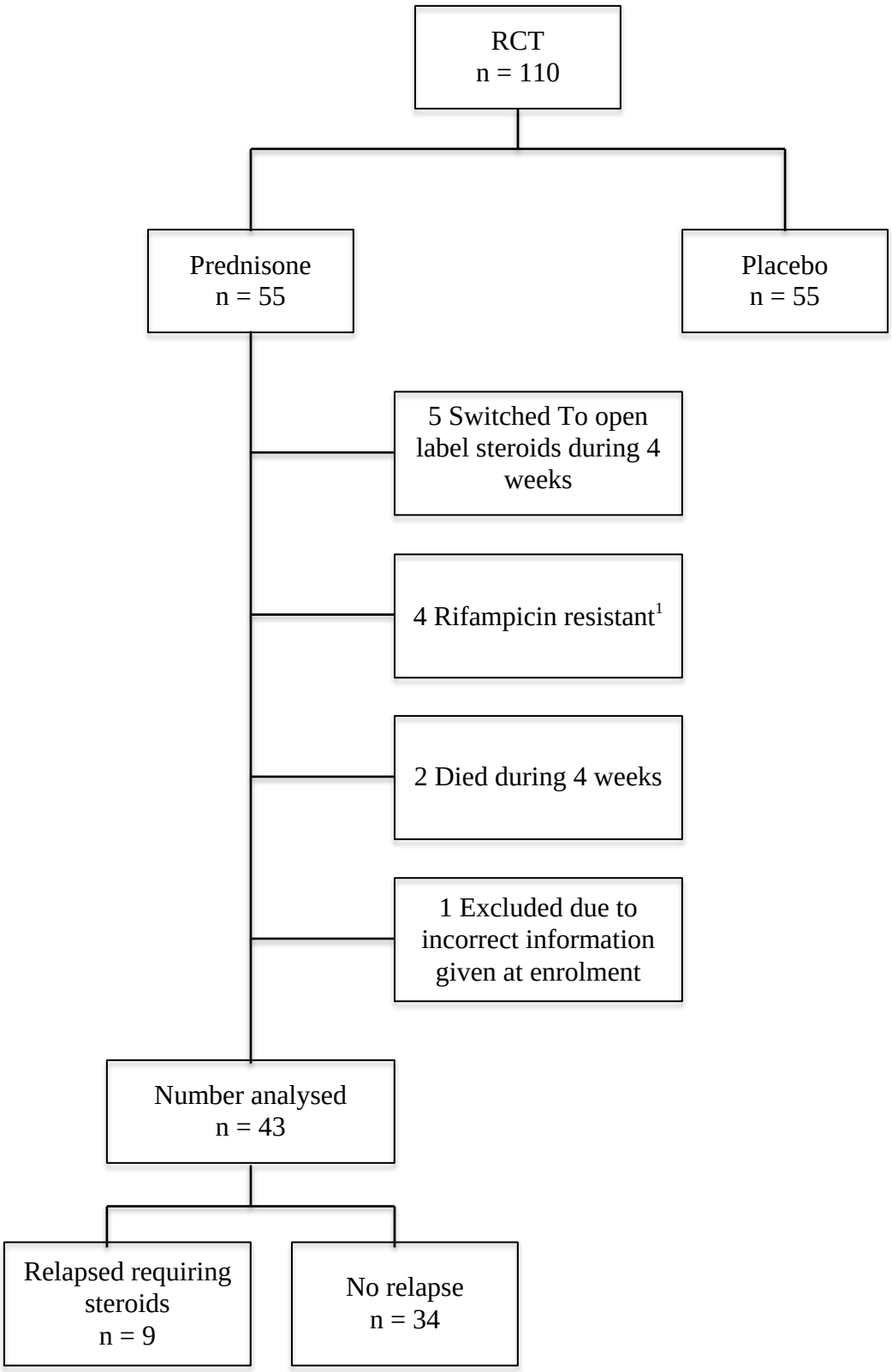

RCT
n = 110
Prednisone
n = 55
Placebo
n = 55
5 Switched To open label steroids during 4 weeks
4 Rifampicin resistant[1]
2 Died during 4 weeks
1 Excluded due to incorrect information given at enrolment
Number analysed
n = 43
Relapsed requiring steroids
n = 9
No relapse
n = 34

Figure 2

Legends for Supplementary Figures

Supplementary Figure 1: Distribution of TB-IRIS duration

This graph shows the number and proportion of patients who had a duration of TB-IRIS symptoms within each of the specified duration categories (in days) displayed on the x-axis. 172 patients who had a known TB-IRIS start and end date were included.

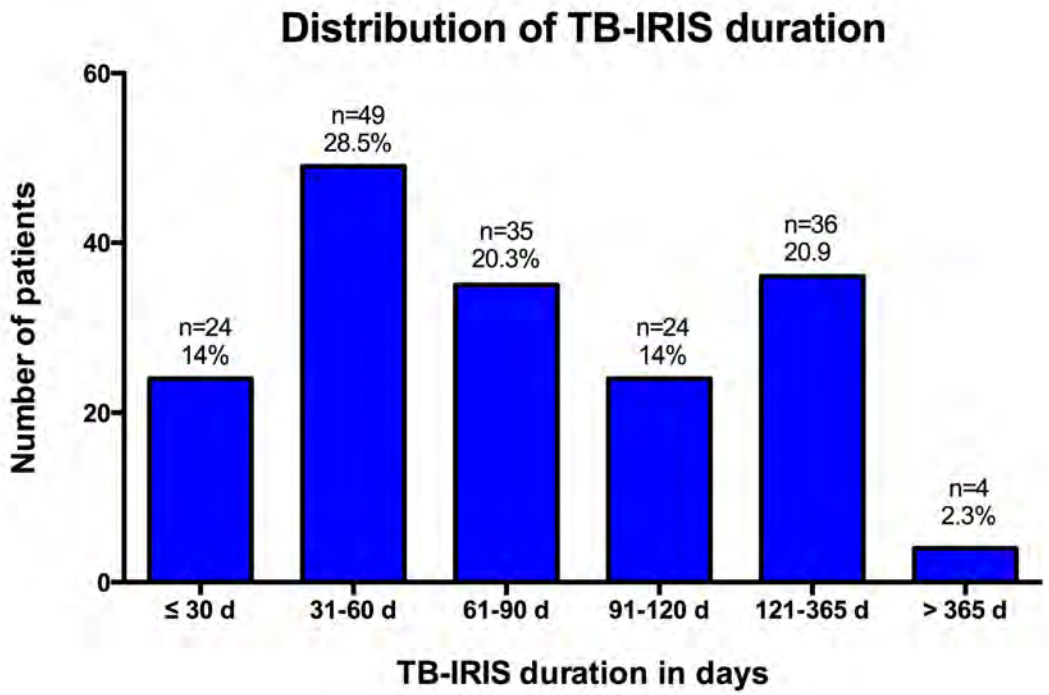
Supplementary Figure 2: Kaplan-Meier plot showing time to TB-IRIS resolution

The graph shows the comparison between patients who had TB-IRIS lymph node involvement to those patients who did not in terms of time to TB-IRIS resolution. Resolution was more rapid in those without lymph node involvement ($p<0.0001$, log-rank test).

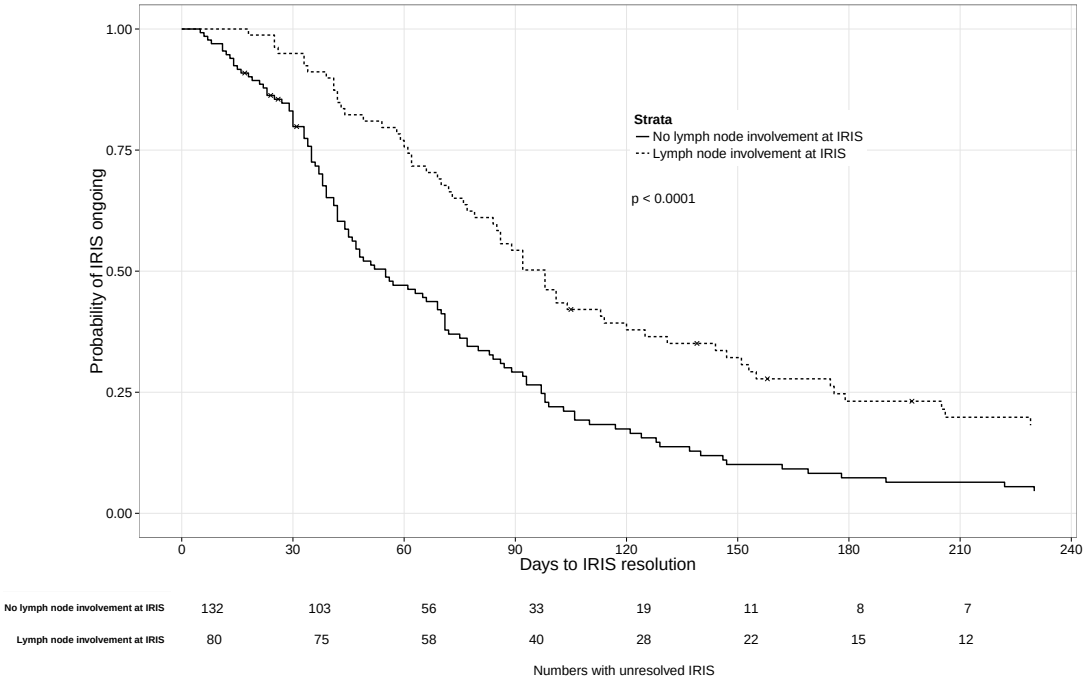
Supplementary Figure 3: Alkaline phosphatase and alanine transferase concentrations during the 4-week course of prednisone on the RCT, comparing those who relapsed with TB-IRIS symptoms after stopping prednisone to those who did not

In univariate analysis, lower reduction in alkaline phosphatase from 0 to 4 weeks ($p=0.03$) and lower reduction in ALT from 0 to 4 weeks ($p=0.04$) were significantly associated with subsequent relapse (see Table 5).

Supplementary Figure 1



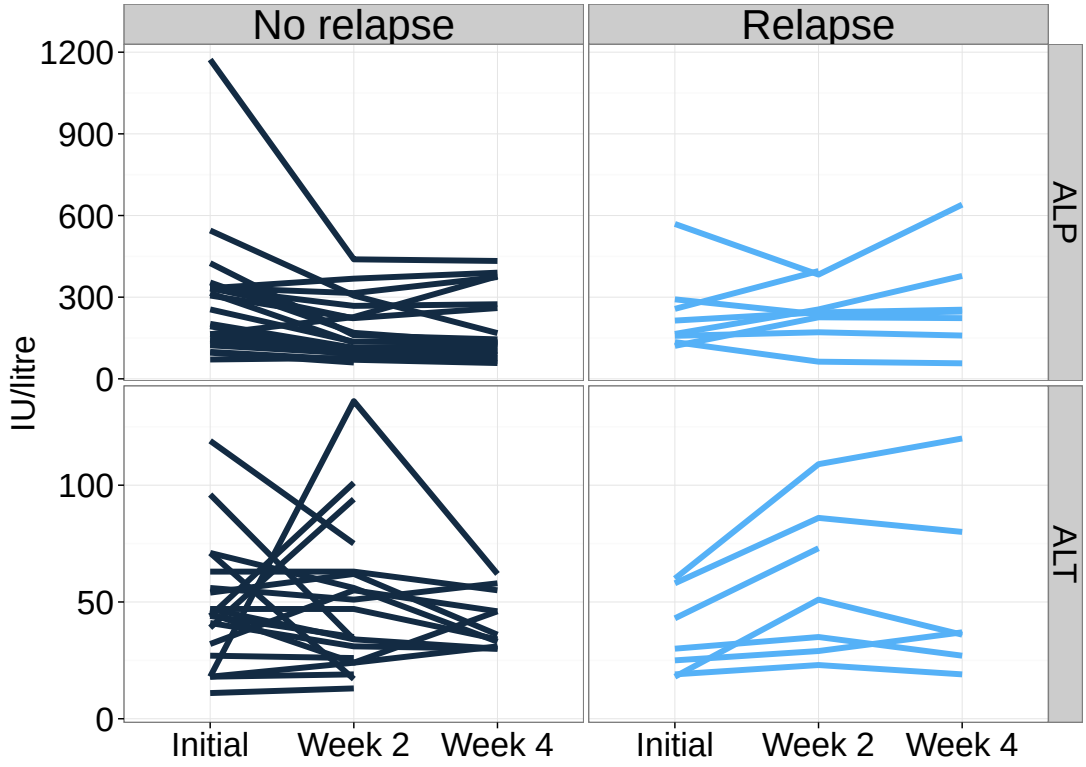
Supplementary Figure 2



No lymph node involvement at IRIS	132	103	56	33	19	11	8	7
Lymph node involvement at IRIS	80	75	58	40	28	22	15	12

Numbers with unresolved IRIS

Supplementary Figure 3



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PART C:

Appendices

Appendix 1: Ethics approval

Appendix 2: Guidelines for journal submission to BioMed Central Infectious Diseases

Appendix 3: Additional analyses not included in manuscript

Appendix 4: Data capture sheet

Appendix 1: Ethics Approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

13 June 2012

HREC REF: 300/2012

Dr T Bana
c/o A/Prof G Meintjes
Department of Medicine

Dear Dr Bana

PROJECT TITLE: PROLONGED TUBERCULOSIS-ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME: INCIDENCE, PREDICTORS, OUTCOMES

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th June 2013

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

s.thomas

Appendix 2: BioMed Central Infectious Diseases submission guidelines.

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- Title page
- Abstract

- Keywords
- Background
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- List of abbreviations used (if any)
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- Authors' contributions
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The title page should:

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2. Put all files necessary for viewing the mini-website within the folder, or sub-folders.
3. Ensure that all links are relative (ie "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters.
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article.

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- Please do not format the text in multiple columns.
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Units

SI units should be used throughout (liter and molar are permitted, however).

Appendix 3: Additional analyses not included in manuscript

Supplementary Table 1: Odds ratios for univariate (unadjusted) and multivariate (adjusted) logistic regression models predicting the development of prolonged TB-IRIS excluding BCH Cohort

	Unadjusted OR (95% CI)	**Unadjusted p-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Age (per 1 year increase)	0.98 (0.93, 1.03)	0.36	0.99 (0.94, 1.05)	0.723
Male gender	0.70 (0.34, 1.40)	0.31	0.63 (0.28, 1.41)	0.264
Initial TB diagnosis with lymph node involvement	2.36 (1.08, 5.39)	0.03	1.79 (0.75, 4.46)	0.20
Drug-resistant TB	2.79 (0.88, 10.60)	0.10	2.31 (0.69, 9.11)	0.19
Hospitalised at time of IRIS diagnosis	0.98 (0.48, 2.03)	0.96	1.16 (0.53, 2.57)	0.71
IRIS lymph node involvement	2.33 (1.19, 4.64)	0.01	1.90 (0.89, 4.09)	0.10
IRIS pulmonary involvement	0.59 (0.29, 1.17)	0.13	0.61 (0.28, 1.32)	0.21

Due to the potential bias around the BCH cohort and hospitalization a sensitivity analysis was performed excluding all individuals in the BCH cohort. Both univariate and multivariate regression models were assessed as shown in the table above and although, as expected, the number of variables with statistically significant associations decreased, the direction and size of the effects remained largely the same. However, hospitalization was no longer associated with prolonged TB-IRIS (aOR 1.16, 95%CI=0.53-2.57, p=0.71).

Supplementary Table 2: Cox proportional hazards model predicting time to resolution of TB- IRIS symptoms excluding BCH cohort

	Adjusted HR (95% CI)	**p-value**
Age (per 1 year increase)	0.99 (0.96-1.01)	0.36
Male gender	1.42 (0.92-2.18)	0.11
Initial TB diagnosis with lymph node involvement	0.72 (0.44-1.17)	0.18
Drug-resistant TB	0.65 (0.36-1.20)	0.17
Hospitalised at time of IRIS diagnosis	0.93 (0.62-1.40)	0.73
IRIS pulmonary involvement	1.23 (0.82-1.83)	0.32
IRIS lymph node involvement	0.60 (0.40-0.89)	0.01

In the Cox proportional hazards model, even when the BCH cohort was excluded (results in above table), IRIS lymph node involvement remained statistically significantly associated with a lower likelihood of IRIS resolution (HR 0.60, 95%CI=0.40-0.89; p = 0.01).

Supplementary Table 3: Competing risks model of time to TB-IRIS resolution (with competing risk of death)

	Mortality		TB-IRIS resolution	
Characteristic	**Adjusted HR (95% CI)**	**p-value**	**Adjusted HR (95% CI)**	**p-value**
IRIS pulmonary involvement	0.81 (0.29-2.32)	0.70	1.29 (0.92-1.81)	0.13
IRIS lymph node involvement	0.37 (0.11-1.18	0.09	0.55 (0.38-0.78)	0.0009
Initial TB diagnosis with lymph node involvement	2.13 (0.72-6.26)	0.17	0.75 (0.49-1.15)	0.19
Hospitalised at time of IRIS diagnosis	9.51 (2.05-44.04)	0.004	1.28 (0.92-1.78)	0.14
Age (per 1 year increase)	1.04 (0.99-1.10)	0.14	0.99 (0.97-1.01)	0.51
Male gender	0.32 (0.09-1.14)	0.08	1.22 (0.86-1.74)	0.27
Drug-resistant TB	1.96 (0.42-9.22)	0.39	0.60 (0.34-1.08)	0.09

Competing risks models were analysed to account for the competing risk of death and IRIS resolution. The model demonstrated similar findings to the Cox proportional hazards model as demonstrated in the table above.

Appendix 4: Data capture sheet

IRIS_No	RCT_No	Rel_req_ster	Age	Gender	WHO_Stage	CD4_preART

Prev_TB	DR_TB	Initital_TB_	EPTB_LN	EPTB_pl	EPTB_abd	EPTB_pericard

EPTB_other	methodTB_	dur_tbRx_art	dur_art_iris	dur_iris_ster	dur_ster	ster_dose1

ster_dose2	hosp.iris_	Kscore_wk0	Kscore_wk2	Kscore_wk4	iris_symp	iris_LN

iris_absc	iris_LN_absc	iris_pulm	iris_eff.ascites	iris_type_eff	iris_hapatitis	iris_BM

iris_skin	iris_mening	iris_SOL	iris_mening_SOL	iris_hepmeg	iris_splen

iris_peritonism	iris_fever	iris_cxr_infil_0baseline	iris_Pulmonary_infiltrate	iris_LN0baseline

lymph.nodes	iris_LN0baseline_site	iris_other_but0baseline	iris_multi	iris_nodalVSnon_nodal

iris_pulmVSnon_pulm	iris_abd	iris.12wk	start_OLS	dur_sterOLS	dur_all_steroids

randomisation.category	dose_OLS	re.escalation_streoids	PR_base	PR_2wk	PR_4wk

num_asp	type_asp	crp_base	crp_2wk	crp_4wk	Hb_base	wcc_base	neutr_base

ALT_base	ALT_2wk	ALT_4wk	ALP_base	ALP_2wk	ALP_4wk	art_stop	CD4_wk0

CD4_wk4	VL_wk0	VL_wk4	Dataset_No	Descr_featur	X_.ALP_0_4wk	X_.ALP_0_2wk

X_CRP_0_2wk	X_CRP_0_4wk	X_PR_0_2wk	X_PR_0_4wk	temp.1	temp.2	date_last_visit_old

date_last_visit	onset_art	onset_TBRx	onset_RCT	onset_iris	stop_steroid	iris_stopped

CD4_wk0_old	CD4_wk4_old	VL_wk0_old	VL_wk4_old	alp_0_2	alp_2_4	alp_0_4

diff_tbRx_art	diff_art_iris	diff_iris_ster	diff_ster	diff_iris	diff_cd4_art_iris	aa

ltf.outcome	steroids	dur_iris	censor.yn	prolong.iris	DR.tb	cd4_0_4	alt_0_4

crp_0_4	dead	ongoing	yr.long	cohort